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Synthesis of Mycobacterial Wax Esters and related compounds

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Synthesis of Mycobacterial Wax Esters and related compounds

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By

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Contents

List of abbreviations

AIDS	Acquired Immune Deficiency Syndrome
Aq	Aqueous
Bn	Benzyl
Br	Broad
CID-MS	Collision-induced dissociation mass spectrometry
cm^{-1}	Per centimetre
CoA	Coenzyme A
d	Doublet
dd	Double doublet
dt	Doublet of triplet
DCM	Dichloromethane
DEAD	Diethyl azodicarboxylate
DIBAL-H	Di-isobutylaluminium Hydride
D.W	Disttled water
DMAP	4-Dimethylaminopyridine
DMF	*N,N*-Dimethylformamide
dt	Doublet of triplet
EDCI	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
EI	Electron Impact
ELISA	Enzyme-linked immunosorbent assay
eq	Equivalent
Ether	Diethyl ether
g	Gram
GC	Gas Chromatography
H	Hour
^{1}H	Proton
HIV	Human Immunodeficiency Virus
HRMS	High Resolution Mass Spectra
HMPA	Hexamethylphosphoramide
HPLC	High Performance Liquid Chromatography
Hz	Hertz

IMS	Industrial Methylated Spirit
IR	Infrared
J	Coupling constant
L	Litre
LDA	Lithium *N,N*-Diisopropylamide
LiHMDS	Lithium *bis*(trimethylsilyl)amide
M	*meta*-
M	Multiplet
MAC	*Mycobacterium avium* complex
MALDI	Matrix-Assisted Laser Desorption/Ionization
MALDI-TOF	Matrix Assisted Laser Desorption Ionization Time-Of-Flight
MCPA	*m*-chloroperoxybenzoic acid
MHz	Mega Hertz
mmol	Millimole
mol. equiv.	Molar equivalents
M.P	Melting point
MS	Mass spectrometry
m	Multiplet
NBS	*N*-Bromosuccinimide
NMR	Nuclear Magnetic Resonance
PCC	Pyridinium Chlorochromate
PCR	Polymerase chain reaction
Petrol	Petroleum spirit (boiling point 40 to 60 ^{o}C)
PPTS	Pyridinium *p*-toluenesulfonate
ppm	Parts per million
q	Quartet
r.t	Room temperature
S	Singlet
SAM	S-Adenosyl-L-Methionine
Sat	Saturated
NaHMDS	Sodium *bis*(trimethylsilyl)amide
T	Triplet
TB	Tuberculosis

TBAF	Tetra-*n*-butylammoniunflouride
TBAH	Tetra-*n*-butylammoniunhydroxide
TBDMS	Tert-Butyldimethylsilyl
TBDPS	Tert-Butyldiphenylsilyl
TDM	Trehalose dimycolate
THF	Tetrahydrofuran
THP	Tetrahydropyran
TLC	Thin-Layer-Chromatography
TMM	Trehalose monomycolate
TNF	Tumor necrosis factor
TsCl	*p*-Toluenesulfonyl chloride
v.br.	Very broad
WHO	World Health Organisation

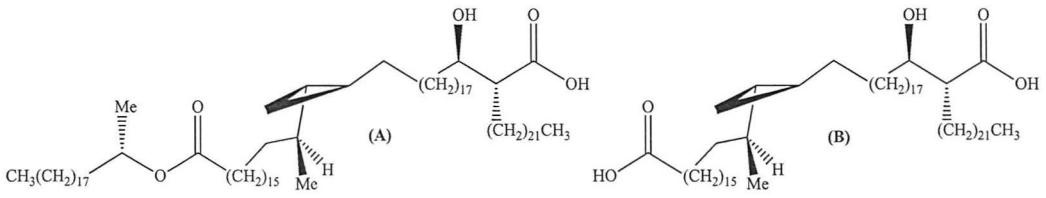
Abstract

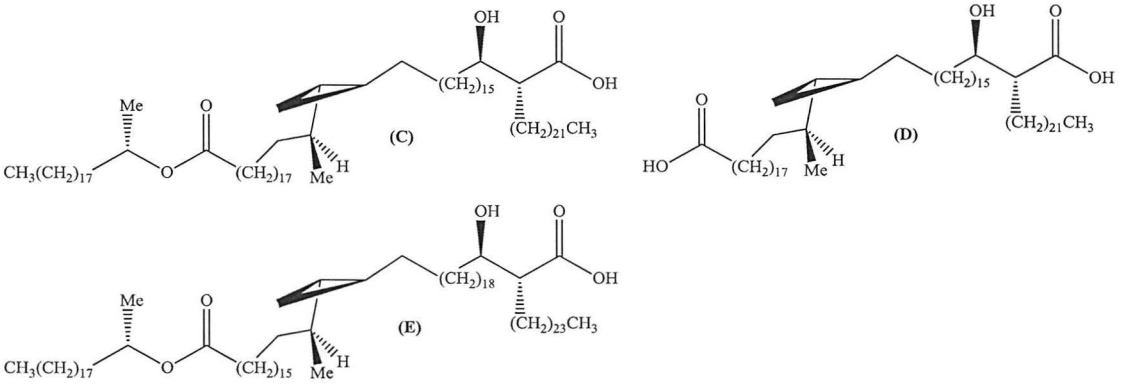
Mycobacteria are present in a wide range of environments. They contain characteristic complex mixtures of mycolic acids in the cell wall together with other lipids. The high resistance of mycobacteria to the majority of antibiotics, therapeutic agents and disinfectants is thought to be related to the unique structure of the mycobacterial cell wall. Mycolic acids are high molecular weight α-alkyl-branched ß-hydroxy long chain fatty acids (60-90 carbon atoms). Different species of *Mycobacteria* may produce different classes of mycolic acids including α-, methoxy, keto, and wax ester mycolic acids, each present as mixtures of homologues. These may contain different functional groups such as ester, keto, methoxy, hydroxyl, and alkene.

The most reported lipids present in the cell wall are sugar esters, e.g mycolyl trehalose esters. These mycolic acids and mycolyl trehalose esters show very interesting toxic and immunological properties; these offer considerable potential for application in the detection, control, and treatment of mycobacterial infection, and also in developing sensors for detection of disease. This study will seek to explore these potentials.

This project involved the synthesis of several mycolic acids, wax esters and trehalose esters. The biological activities of these synthetic compounds would be studied, particularly their suitability as specific antigens to detect mycobacterial infections in serodiagnostic assays. The objectives of the project are discussed in four parts.

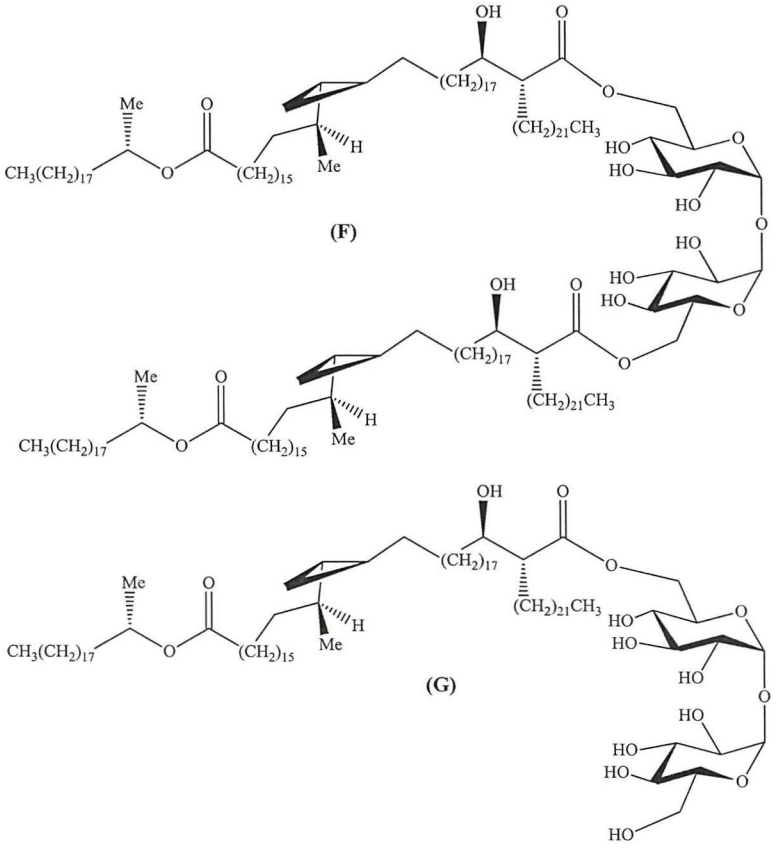
The first part of this project involved the first synthesis of wax ester **(A)** and its corresponding ω-carboxymycolic acid **(B),** one component of the complex mixture isolated from *Mycobacterium avium*. Once the synthesis had been optimised, synthesis of the wax ester **(C)** and its corresponding ω-carboxymycolic acid **(D)** were also achieved, the latter compounds being isolated from *Mycobacterium gordonae*. In addition the wax ester **(E)** was also prepared introducing longer chain lengths than the natural wax ester. This was to study whether or not the chain length has any effect on the biological activities.

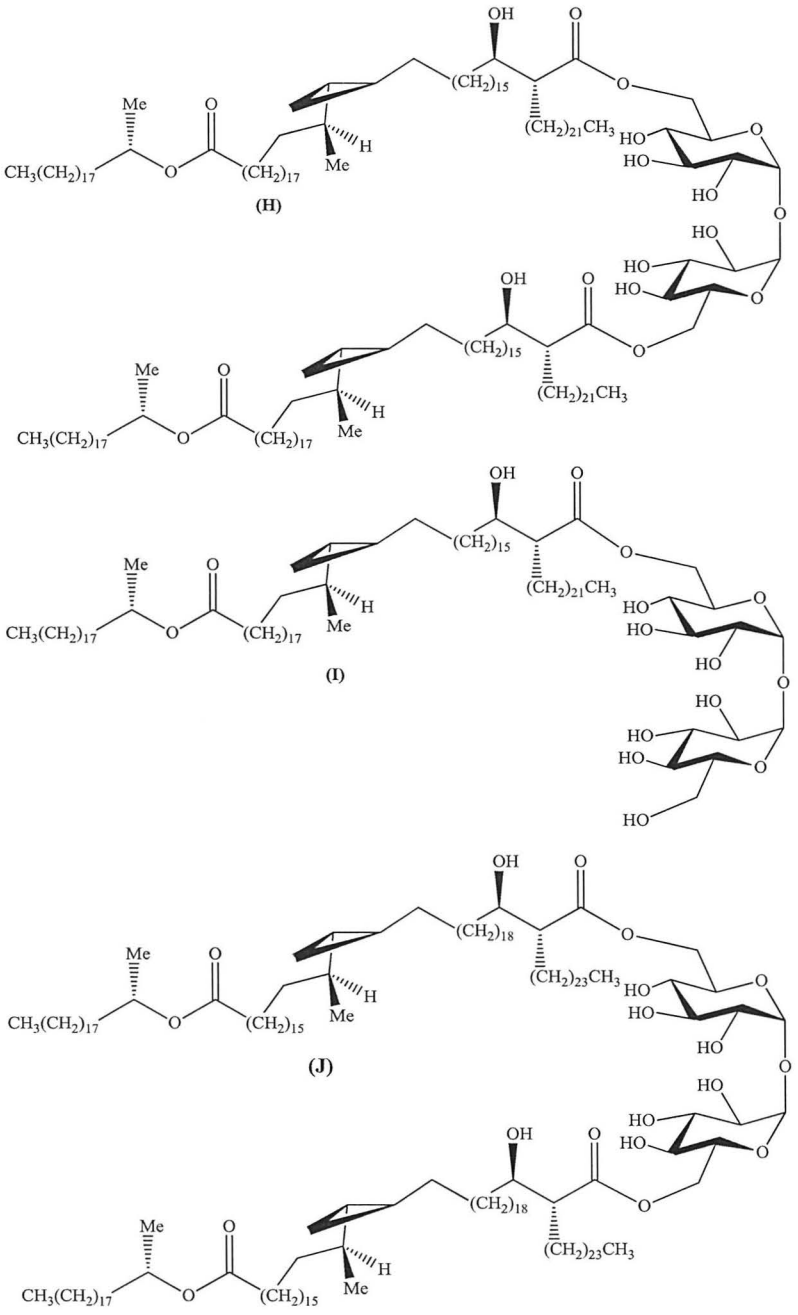




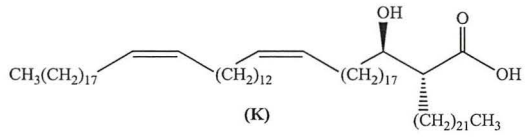
The second part of this project involved the synthesis of novel tehalose esters of the above wax esters. Firstly, the syntheses of trehalose dimycolate (TDM) **(F)** and trehalose monomycolate (TMM) **(G)** of the *M.avium* wax ester were achieved successfully, followed by the synthesis of TDM **(H)** and TMM **(I)** of the *M. gordonae* wax ester. In addition the syntheses of the trehalose esters of the non-natural wax ester (**E**) were also attempted, however only the TDM **(J)** was obtained.

These wax esters and their corresponding trehalose esters would be used as a specific antigens to distinguish the *M.avium* or *M.gordonae* from *M.tuberculosis* specifically in serodiagnostic assays (ELISA).





The third part of this study involved the synthesis of diene and alkene mycolic acids **(K)** and **(L)**, followed by the synthesis of their trehalose esters (**M**, **N**, **O**, **P**). These compounds would be tested for their immunological properties (TNF-α cytokine stimulation), as well as the determination of their specificity and sensitivity in ELISA assays to detect mycobacterial infection.



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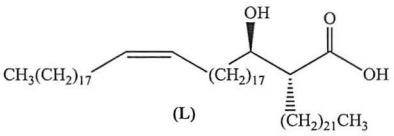

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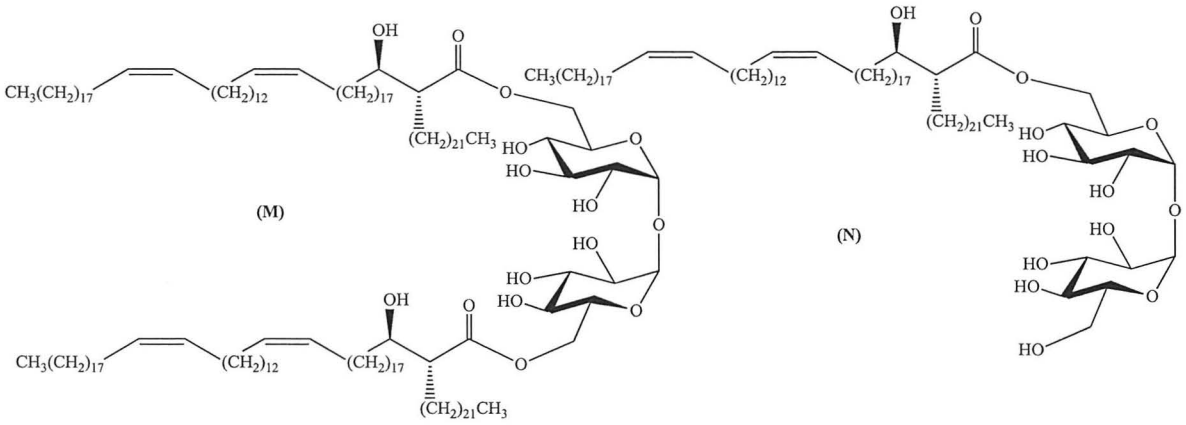

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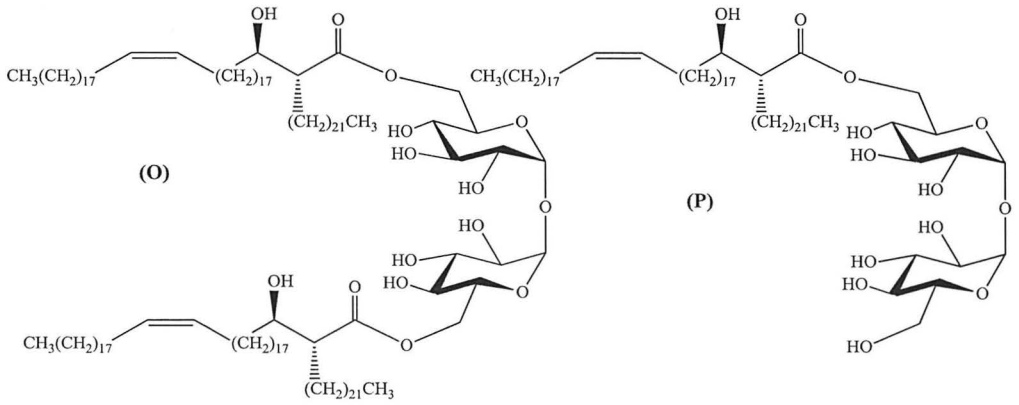
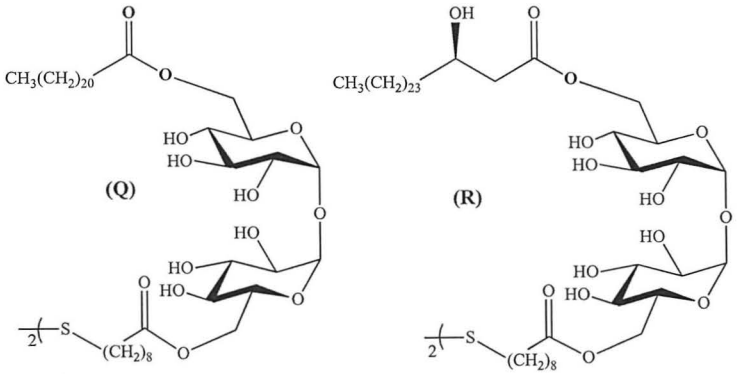


(O)

(P)

The fourth part entailed the first synthesis of disulfide and thiol modified trehalose esters (cord factors). This was attempted firstly, by synthesizing the simpler model disulfide trehalose esters **(Q)** and **(R)**. After the success in making those model compounds, the disulfide trehalose ester of α-mycolic acid **(S)** and its corresponding thiol **(T)** were prepared successfully. These compounds were designed to be coated onto a gold surface or gold nanoparticles to form a self-assembled monolayers, and then used as specific antigens to bind with TB-antibodies for the early detection of TB in biosensors.



(Q)

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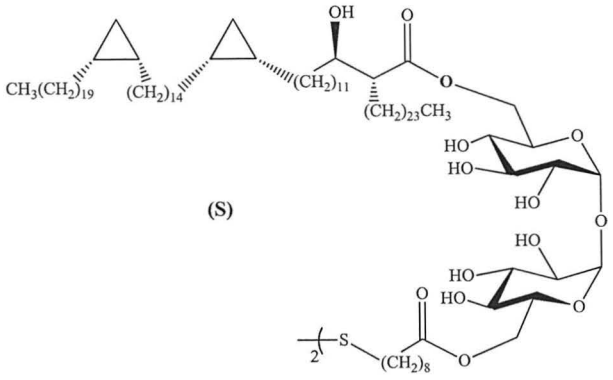

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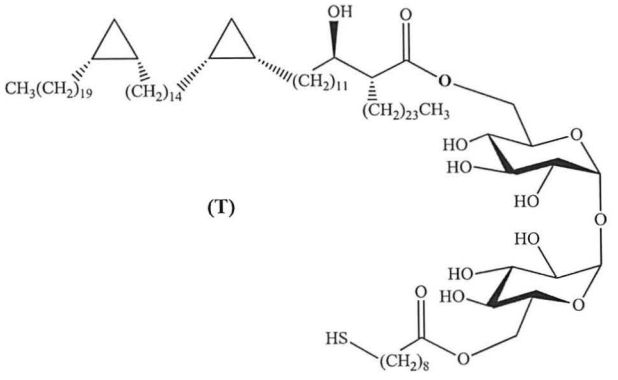


(T)

Chapter 1

1. Introduction

1.1 Tuberculosis

Tuberculosis (TB) is a disease of the lungs caused by an infection with the germ *Mycobacterium tuberculosis*. Nowadays it 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.[1] TB has an ancient history and it is believed to have originated in Africa as far back as 35000 years ago.[1,2,3] Studies have demonstrated the presence of TB in Egyptian mummies over 5,000 years old, and in a 9000 years old human skeleton in the Eastern Mediterranean.[1] In the 18th and19th centuries, TB spread over 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.[4] In the 20th century, the disease was slowly controlled as the standard of living and health care improved, and several medicines were discovered, such as streptomycin in 1944, and isoniazid in 1952, which are now used to treat TB.[4]

Around 1990, the number of TB cases increased and around 14,000 cases were reported in 2005 in the United States alone, because there were more people with HIV infection.[5] People who are HIV positive are more prone to get TB. The World Health Organization (WHO) estimated around 1.4 million cases of infection with TB in the world in 2004, and around 33% of deaths in the world occurred in South-East Asia.[6] In 2012, the WHO estimated 8.6 million new TB cases and 1.3 million deaths.[7] Figure 1 shows the WHO estimation for new TB cases per 100,000 populations in 2012. Regions such as Africa, Asia have a high rate of new TB cases of about 500 /100,000 of the population.[7]

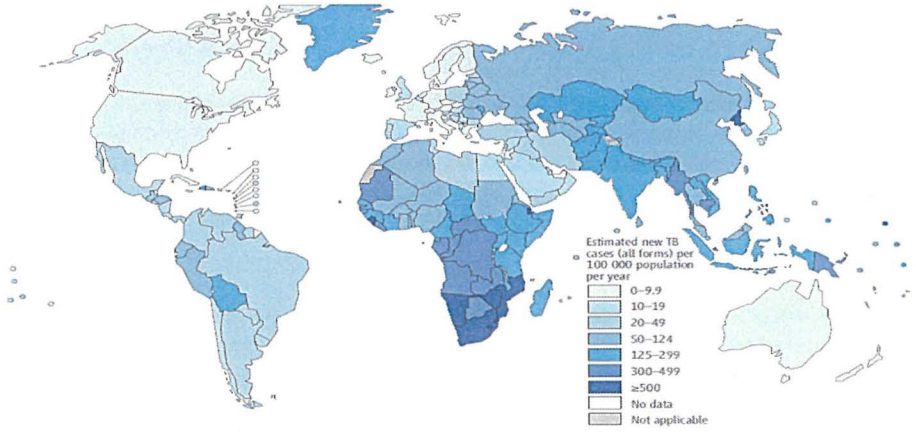


Figure 1: Estimated number of new TB cases in 2012.[7]

TB is usually seen in hostels, prisons, and areas where immigrants arrive from countries with higher rates of TB. The bacteria are spread in the air easily by talking, coughing, breathing or exchange of blood fluids. The infection of a person only requires a small number of the germs in the air to be inhaled. The symptoms of TB are fever, perpetual cough, weight loss, night sweats, swollen glands, pain while breathing, and no response to antibiotics. The symptoms of TB in an infected person may not appear immediately, and in some cases the person who infects another with TB may themselves be without symptoms. This is called latent TB, but when the immune system become weak the bacteria will start to spread through the blood, and then the infection will become serious.

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, because the HIV virus weakens the immune system, causing the TB bacteria to be more harmful. WHO reports show that the chance of developing active TB with a HIV positive patient is ten times more compared to a HIV negative patient with latent TB.[8] Moreover, if the patient stays untreated, active TB could cause a rapid death; therefore early diagnosis of TB is important, as recent studies showed that late diagnosis of TB co-infected HIV patients is the causative agent of death of 48-85% of these cases, which means at least one of three patients with HIV.[9] The WHO stated 1-1.2 million HIV-TB co-infected cases occur in African region countries,[7] and this is thought to be related to poor health services in these countries. Figure 2 shows worldwide estimated distribution of TB patients co-infected with HIV. As can be seen, the co-infection rate is highest in the African region.[7]

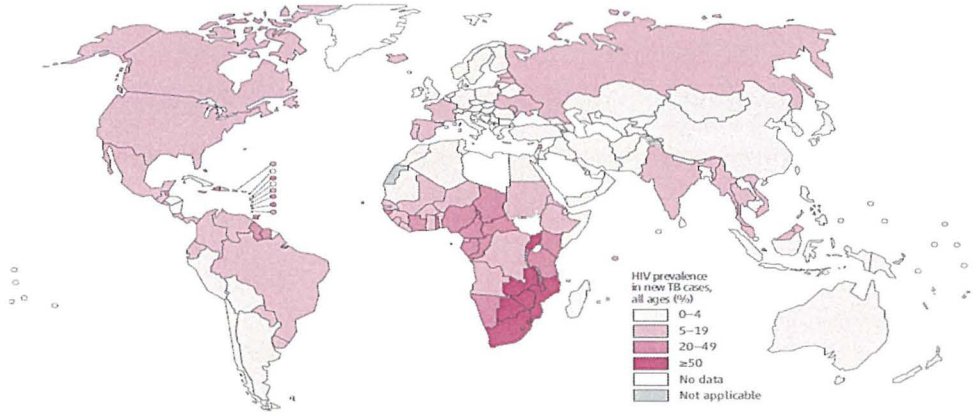


Figure 2: Estimated number of new HIV-TB co-infection cases in 2012.[7]

1.2 Mycobacteria

Mycobacteria are a genus of bacteria which can cause fatal diseases to both humans and animals. There are over 70 *Mycobacterium* species; they can be divided into two classes: Tuberculosis Mycobacteria such as *Mycobacterium tuberculosis*, *Mycobacterium bovis, Mycobacterium africanum, Mycobactterium microti, Mycobacterium caprae or Mycobacterium canettii*; non-tuberculosis mycobacteria (NTM) comprise over 90 species and the most common ones associated with disease are *Mycobacterium avium* complex (MAC), *Mycobacterium gordonae*, *Mycobacterium fortuitum, Mycobacterium smegmatis*.[10]

1.2.1 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 the tuberculosis disease in both of humans and animals.[11]

The most common in the family of tuberculosis mycobacteria is *M.tuberculosis.* MTB is classified as an acid fast gram-positive bacterium, small rod-shaped, approximately (1-4.4, 0.3-0.6 μm) in size **(Figure 3)**.[12]

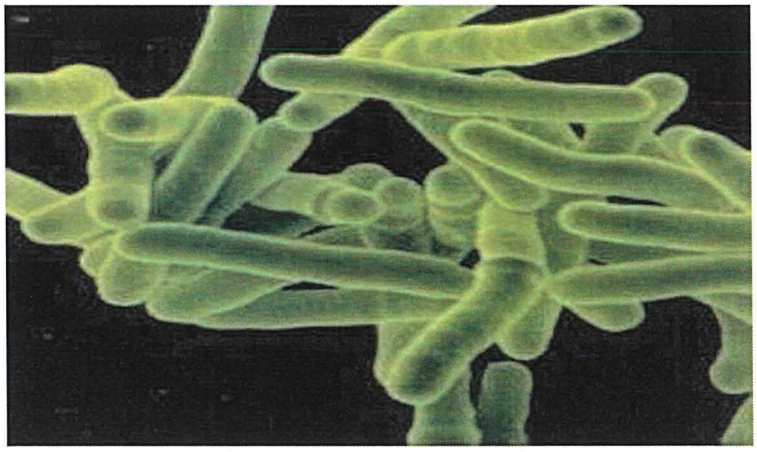


Figure 3: Scanning electron micrograph of *MTb.*[12]

MTB can survive in dry conditions for a long time, and is very resistant to the environmental conditions.

1.2.2 Non-Tuberculosis Mycobacteria (NTM)

Non-tuberculosis mycobacteria, also known as environmental mycobacteria, are small, rod shaped bacilli which enter the human body through environmental sources such as natural water, soils, foods, and water pipes of the distribution system. The reason for the survival of these bacteria in water pipes is due to their resistance to chlorine in water.[13] NTM species do not cause tuberculosis, but have the ability to cause other diseases to both human and animals such as skin disease, disseminated disease, and pulmonary disease in HIV negative patients.[10] Unlike tuberculosis, NTM are not transmitted from one person to another, the organism being acquired exclusively from environmental sources.[14] 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.[15] 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.[16] Similar reports also showed the prevalence of pulmonary NTM infection in other countries.[17,18]

1.2.2.1 *Mycobacterium smegmatis*

M. smegmatis is a rapidly growing acid-fast mycobacterium,[19] which is typically 3.0 to 5.0 μm long with a bacillus shape belonging to the family of environmental NTM. It was found and isolated by Lustgarten in 1884. 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.[20] *M. smegmatis* is found to belong to the same genus as *M.tuberculosis*, but it is not pathogenic; therefore it has been used for many research experiments as a model instead of *M. tuberculosis* and *M. bovis*.[21,22]

1.2.2.2 *Mycobacterium fortuitum*

M. fortuitum is another type of NTM, acid-fast, rapidly growing mycobacterium,[23] 1-3 μm x 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.[24,25,26,27] The treatment of *M.fortuitum* infection is not easy like any other mycobacterial diseases and requires a long duration of therapy. Amikacin and Ofloxacin have shown activity against *M. fortuitum*.[28]

1.2.2.3 *Mycobacterium gordonae*

M.gordonae is classed as a slow growing NTM, first discovered by Ruth E. Gordon. *M. gordonae* may 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 to humans, but surprisingly, recent reports showed cases of significant disease caused by *M. gordonae* infection such as pulmonary disease, disseminated infection, and genitourinary disease.[29,30,31]

Among these *M.gordonae* infections, those which entail co-infection with HIV require special consideration. Reports indicate that *M.gordonae* was isolated from the blood of HIV infected patients and caused serious health complications.[32,33] In UK, *M.gordonae* is classed as the second most common non-tuberculosis mycobacterium, with the rate of reported infections increased from 1 report in 1995 to 153 reports in 2006, with these cases being found to occur mainly in patients over 60 years of age.[15]

1.2.2.4 *Mycobacterium avium*

M.avium is the most frequently encountered NTM associated with both human and animal disease. They are rod-shaped, and slow growing.[34] The organism can grow at 45°C, and in a medium with a pH of 5.5.[35] 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.[36]

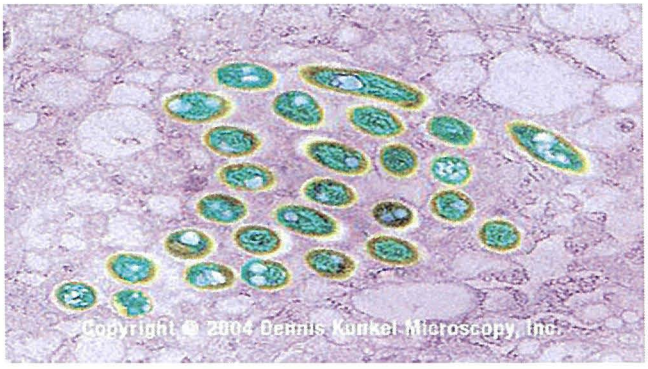


Figure 4: Transmission electron micrograph of *M. avium-intracellulare* in tissue.[37]

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.[38,39] There are three subspecies of *M.avium* including *M.avium* subsp., *M. avium paratuberculosis* and *M.intracellulare* collectively known as *M.avium* complex (MAC).[14,40,41]

M.avium can cause Johne's disease (paratuberculosis), which affects ruminant animals such as cattle and goats. This leads to weight loss, decreased milk production and diarrhea.[42,43,44] It is also believed that *M.avium* causes Crohn's disease in humans; it is a gastrointestinal tract inflammation which can lead to various symptoms like abdominal pain, diarrhea, weight loss, ulcers and bleeding.[44] Crohn's disease has a worldwide distribution. The incidence of the disease has increased in Western countries and particularly in UK in the last decade. It has been found in children aged between 1-16 years old.[45] Recent accurate population data for the disease is not available but on the basis of previous data, it has been predicted there would be 86880 Crohn's disease patients in Britain in 2002, increasing by 4980 cases per year.[46] Studies showed that, there is a probability that the same *M.avium* subspecies causes both Crohn's disease in human and Johne's disease in ruminant animals. It has been found that Crohn's disease mycobacteria are identical to Johne's disease mycobacteria (paratuberculosis).[44] The use of DNA probes and the *in situ* hybridization method have confirmed a link between paratuberculosis in animals and Crohn's disease in human.[44,47] Morever, *M.avium* has been linked to other inflammatory diseases; it has been reported *M. avium* causes lymphadenitis (lymph node inflammation) in children.[48]

M.avium infection is increasing throughout the world. In UK reports of *M. avium-intracellulare* infection increased from 0.4 to 1.2/100,000 population between the years 1995-2006. Out of the total NTM infection reports, there would be 43% of *M. avium-intracellulare*.[15]

A report in the United States stated that the epidemiology of non-tuberculosis mycobacterial lung disease has changed.[49] Later studies in the United States suggest that most of the reported cases of pulmonary non-tuberculosis mycobacterial lung infections were linked to MAC infection. Furthermore, there were only 20/100,000 of the population cases recorded in 1997, while this number increased to 47 cases in 2007.[50]

1.2.3 *M.avium* complex (MAC) and HIV/AIDS

MAC constitutes the leading infection in most immunocompromised patients such as HIV positive patients; those with low CD4 cell counts (less than 50 cells/mm^3) are also at risk. Nowadays it believed that MAC is one of the most pathogenic diseases in HIV-positive patients, and can affect almost all of them in the later stages of the disease. MAC is a worldwide disease, has been reported in many countries, including most European countries, and the United States.[51]

The infection occurs by ingestion or inhalation of the bacteria originating from environmental sources.[38,39] It is very unlikely to be transmitted between persons. The chance of exposure of MAC is high because the organism can be found in various environmental sources. Healthy people can also catch the organism, but it is only active in immunocompromised peoples. The organism enters the host body through either the respiratory system or gastrointestinal tract, then crosses the tissue of the infected area and enters the blood, by which it spreads throughout the entire body, but mainly affects reticuloendothelial organs.[52] For this reason the disease is called `disseminated MAC infection`. The symptoms of MAC-HIV co-infection resemble TB-HIV co-infection; they include fever, weight loss, sweating, abdominal pain, diarrhea and anaemia.

Like most mycobacterial species, treatment of MAC is very difficult and requires sets of anti-mycobacterial drugs for long periods of time. MAC shows high resistance to the majority of anti-mycobacterial drugs; this is thought to be related to the high lipid content of the cell wall, which is covered by hydrophobic waxy outer layer. Reports indicate the resistance of *M. avium* to Isoniazid (INH). Isoniazid is a potent anti-mycobacterial drug; however, it is 100-times less effective against *M. avium*, while it is very effective against *M.*TB.[53] The most used drugs to treat MAC are clarithromycin, azithromycin, ethambutol, clofazimine, isoniazid and rifabutin.[54]

In the last two decades, MAC was the most identified pathogen diagnosed in HIV positive patients. The rate of incidence of MAC-HIV co-infection increases with the period of HIV infection, with the rate reaching 40 % two years after HIV diagnosis.[55] Furthermore the chance of surviving a MAC-HIV co-infected patient decrease to 20 % after 9 months of diagnosis with MAC bacteria.[55]

One of the complications of MAC-HIV co-infection is that most HIV positive patients are infected with at least two different strains of *M.avium.*[56] This increases the risk of treatment complication.[56]

MAC-HIV co-infection is higher than M.TB-HIV co-infection. In 1985, Good studied 212 HIV positive patients in the United States; results showed that over 80 % of diagnosed mycobacteria were MAC, whereas only 9% were *M.*TB.[57] This finding indicates that MAC is the most frequent pathogen in HIV-positive patients. Another interesting observation of MAC-HIV co-infection is the higher frequency of the disease in Caucasians compared with Haitians, and Africa.[54] Instead there is a high incidence of TB-HIV co-infection in African countries. A very recent report in 2013 confirms the uncommon peritonitis infection due to MAC in a HIV positive patient.[58] Peritonitis is

the inflammation of the thin tissue that lines the inside of the abdomen, covering the abdominal organs.

Undoubtedly, MAC-HIV co-infection is increasing throughout the world, but unfortunately the true population data for the prevalence of MAC-HIV co-infection is not known, probably because there is HIV co-infection with other mycobacteria such as tuberculosis mycobacteria.

1.3 The Mycobacterial cell wall

A high resistance to the majority of antibiotics, therapeutic agents and disinfectants is a big problem in the eradication of mycobacterial organisms. This resistance is thought to be related to the unique structure of the mycobacterial cell wall, containing large amounts of lipid cell envelope.[59]

The cell wall of Mycobacterium species consists of three different components, plasma membrane, the cell wall (inner layer), and the outer layer (capsule) **(Figure 5)**.

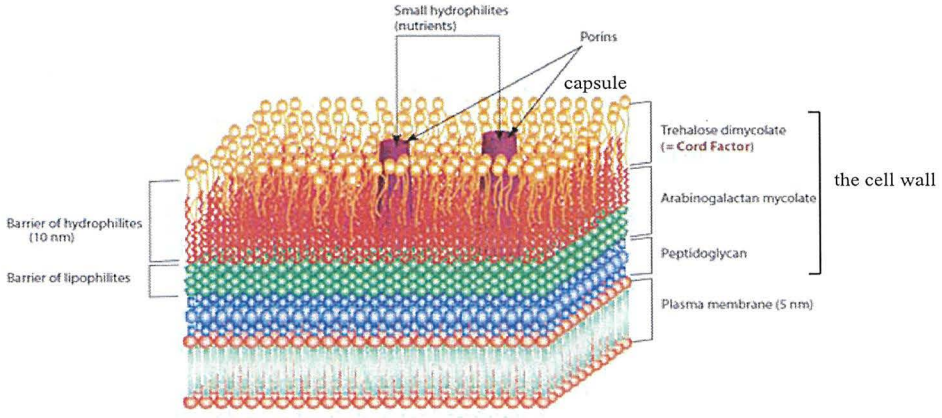


Figure 5: A model of the *mycobacterium* cell wall.[60]

The main components of the capsule outer layer are polysaccharides and proteins with low amounts of lipids.[61] The plasma membrane is 5 nm thick, and its composition is similar to that in other organisms. The cell wall (inner layer) consists of three linked macromolecules: peptidoglycan (PG), arabinogalactan (AG), and large group of mycolic acids linked to arabinogalactan (Figure 6: Mycolic acids are α-alkyl branched ß-hydroxy long chain fatty acids (60-90 carbon atoms), normally contains two intra-chain functional groups labelled as [X], [Y]).[59] The peptidoglycan consists of N-acetylglucosamine (NAG), and N-acetylated muramic acid (NAM) saccharides.[62,63] The arabinogalactan is a complex heteropolysaccharide consisting of D-arabinose in a

furanose form joined to a linear D-galactose units.[64,65,66] These three parts are linked together producing a tightly packed bilayer. Figure 6 shows a model of the linked molecules of the cell wall.

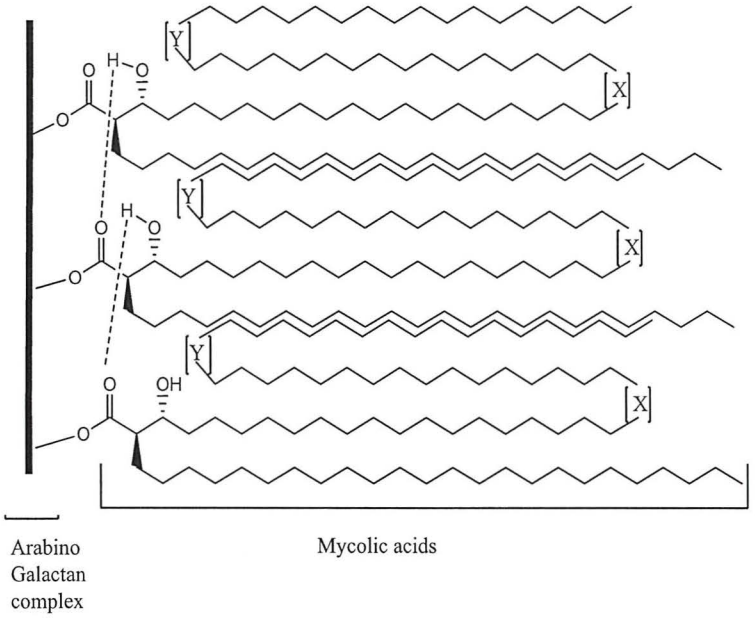


Figure 6: Schematic representation of inner layer of the *Mycobacterial* cell wall.

Together, mycolic acids (MA), arabinogalactan (AG) and peptidoglycan (PG) are known as the mycoylarabinogalactanpeptidoglycan complex (mAPG).[65]

Moreover, though the main component of the cell wall is mycolic acids, there are also large amounts of very complicated lipid amounting to 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 lipoarabinomannan (LAM), phospholipids, but the most reported lipids with interesting toxic and immunological properties are trehalose esters (cord factors), which are trehalose esterified with mycolic acids.[67,68,69,70] There is also a specific glycolipid produced by *M.avium* complex called Glycopeptidolipids (GPL), which is found to be strongly antigenic.[71] GPL serves as a protective barrier against the host cell-mediated immune response in the phagosome.[72]

1.4 Mycolic acids

Mycolic acids are complex hydroxylated high molecular weight α-alkyl branched ß-hydroxy long chain fatty acids with high carbon numbers (60-90 atoms). They were first reported by Anderson *et al.* about 70 years ago, and defined as being hydroxy acids.[73,74] They are main components of the cell wall of all of mycobacterial species. The first

mycolic acid structures were published by Minnikin *et al* in 1967.[75,76,77,78] The structure of mycolic acids can be divided in to two parts. The main part is called the meromycolate moiety and the second part is called the mycolic motif. The mycolic motif part contains the α-alkyl β-hydroxy fatty acid, which is similar in mycolic acids except for a slight variation in the chain length of the α-alkyl position. The main part is the meromycolate moiety, which normally has two intra-chain functional groups in the distal and proximal positions labelled as [X], [Y] **(Figure 7)**. The proximal position can be *cis* or *trans* cyclopropanes, or double bonds, while the distal positions can be the cyclopropanes, *cis* or *trans* double bonds, epoxy group, methoxy group, carbonyl group, hydroxyl group or ester group **(Figure 7)**.

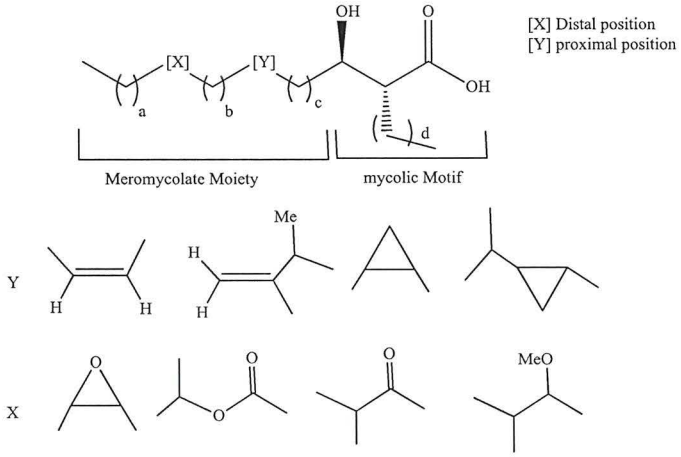


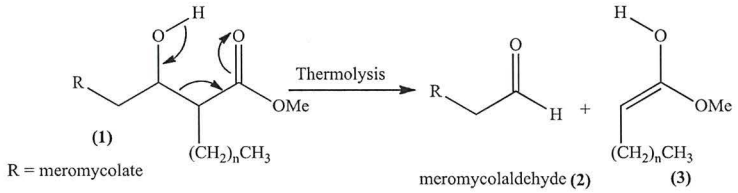
Figure 7: General structure of mycolic acids and various functional groups.

The chemical complexity of the mycobacterial cell wall is not only the consequence of the presence of different mycolic acids, but also the presence of a mixture of different homologues of those mycolic acids presenting different carbon chain lengths.

In the cell wall of *M. tuberculosis* over 500 individual mycolic acids with closely related chemical structures have been recognised; they are present as well as in other species of mycobacteria.[79] In the early years, the isolation of mycolic acids and separation from the mixture of various structures in the cell wall was the main problem in identifying their structures. Not only is isolation of individual mycolic acids difficult, but also the isolation and determination of a single compound from a mixture of similar mycolic acids with different carbon chain lengths. For this reason highly developed analytical techniques were required in order to determine the correct structures of these series of mycolic acids.

Over the last fifty years, by using new analytical techniques such as two dimensional TLC, HPLC, GC, MS, and NMR it has been less difficult to separate and identify several mycolic acids.[80,81,82,83,84] Mass spectrometry (MS) and Nuclear magnetic resonance (NMR) had the most significant impact upon the elucidation of mycolic acids structures, with MS providing accurate molecular weights of the mycolic acids and NMR allowing for the determination of the proximal or distal functional groups.[77,78] However the most powerful technique associated with the development of the structural understanding of mycolic acids is the combination of techniques such as mass spectrometry with either chromatographic techniques or NMR.[85,86 77,78]

In early studies, mycolic acids were analysed by GC using high injector temperatures, which produced a mero-compound as a mixture of homologues. When the high injector temperatures (300-350 °C) were applied, thermal cleavage of mycolic acid occurred and methyl esters with C_{22}, C_{24} or C_{26} chains were detected, but with low injector temperatures (235 °C or less) no mycolic acid cleavage occurred **(Scheme 1)**.[87]

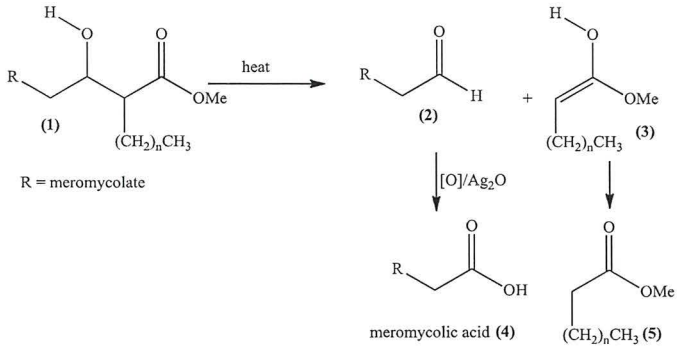


Scheme 1: Thermal cleavage of mycolic acids

Mass spectrometry (MS) has also been widely used to determine structure including the chain length and functional group positions of mycolic acids.[78,88] In particular, electron impact mass spectrometry (EI-MS) has been used to establish the position of cyclopropanes of mycolic acids.[88]

Recently, MALDI-TOF mass spectroscopy (Matrix-Assisted Laser Desorption/ Ionization Time-of-Flight) was used as a very fast, accurate and low sample consuming technique to determine the accurate molecular mass of mycolic acid; this could also be used to determine the exact numbers of carbons atoms, in addition to the presence of double bonds, and cyclopropane rings, which all led to a much clearer understanding of mycolic acid structure. Laval *et al.* used MALDI-TOF for the first time to analyse different classes of mycolic acids isolated from different mycobacterial strains.[89] Watanabe *et al.*[90,91] used NMR and MALDI spectrometry to study mycolic acids (major and minor components) and the ratio of different functional group mycolic acids. Furthermore the collision-induced dissociation mass spectrometry (CID-MS) was

employed to establish the functional group positions and chain length in the meromycolate moiety of wide range of individual mycobacterial strains. This was performed by pyrolysis of mycolate esters **(1)** at 300 °C to produce meromycolaldehydes **(2)** followed by oxidation using silver oxide (Ag_2O) to give the corresponding meromycolic acids **(4) (Scheme 2)**.[91]



Scheme 2: Pyrolysis and oxidation of mycolic acids

Different kinds of mycobacteria may produce a variety of mycolic acids. The major mycolic acid components of *M.tuberculosis* were completely characterized by Watanabe *et al.* using NMR spectroscopy and mass spectrometry techniques. The major kinds of *M.tuberculosis* mycolic acids include α-mycolic acids, which contain two cyclopropane rings in the *cis*-configuration **(6)**; keto-mycolic acids, which contain one cyclopropane ring in a *cis*-configuration **(7)**, or α-methyl-*trans*-cyclopropane **(8)**, and the methoxy mycolic acids **(9,10)** bearing the same configurations as the keto-mycolic acids **(Figure 8)**.

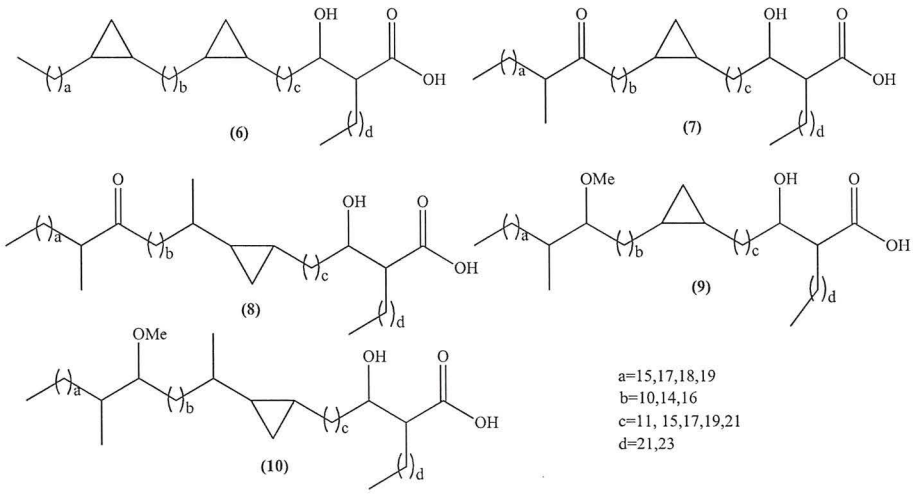


Figure 8: Major types of mycolic acids from *M. tuberculosis*

Different species of *Mycobacteria* may produce different classes of mycolic acids; thus *M. smegmatis* possesses different sets of mycolic acid named as α'- and α-mycolic acids with one or two double bonds, either in the *cis* or the *trans*-configuration. The α'-mycolic acids **(11)** contain one double bond in the *cis*-configuration and an overall carbons numbers 60, which is shorter chain length than to mycolic acids found in *M.tuberculosis*. The α-mycolic acids contains two double bonds either both in *cis*-configuration **(12)**, or the distal position in *cis*-configuration and the proximal position in *trans*-configuration **(13)**.[89,92,93] Diene mycolic acids have also been found in *Mycobacterium brumae* which contain two double bonds both in the *trans*- configuration **(14)**.[94] However diene methoxy mycolic acids have been isolated from *Mycobacterium alvei*; these include ω-1-methoxy-mycolic acids in two different configurations **(15, 16)**.[95] The presence of epoxy mycolic acids were also reported in *M.fortuitum* **(17-20)** **(Figure 9)**.[89,92]

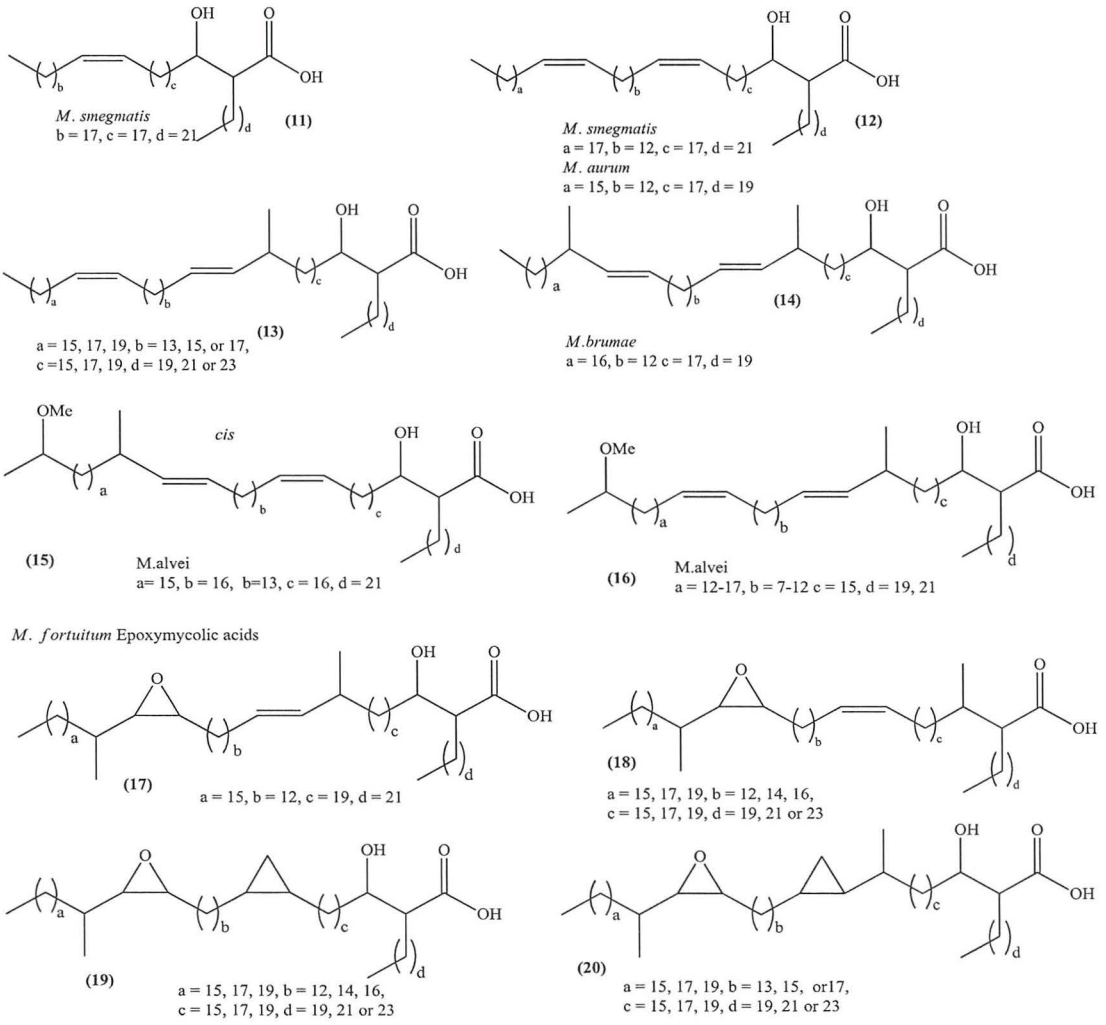


Figure 9: Mycolic acids from other mycobacteria

Not only mycobacteria possess mycolic acids, but also some other bacterial species, such as *Segniliparus* belonging to the genus *Segniliparus rotundus* and *Segniliparus rugosus*. These possess non-oxygenated mycolic acids with a high degree of unsaturation.[96,97] In regard to mycolic acids **(21, 22),** similar to those isolated from mycobacteria; *Segniliparus* species produce very long chain mycolic acids named "α^+ mycolic acids" **(23)** with an overall carbon chain number of 100 and containing three double bonds **(Figure 10)**. Scanning electron microscopy showed that these mycolic acids pack into a typical membrane form; these might attract interest to understand the packing and folding in the cell membrane.[96, 97]

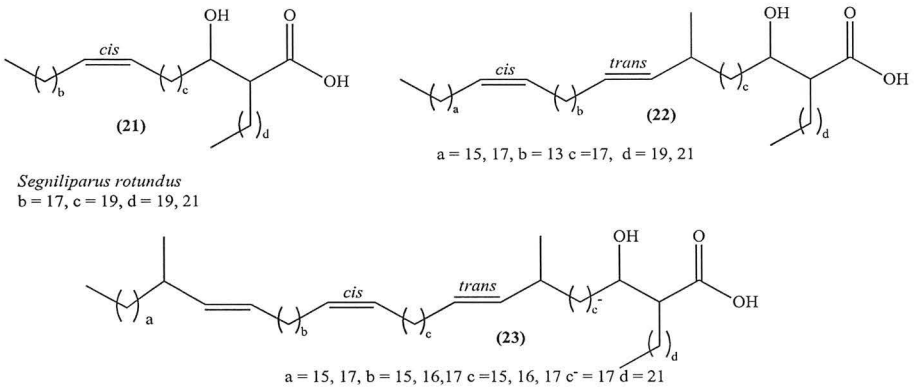


Figure 10: *Segniliparus* genus mycolic acids.

1.4.1 Wax ester mycolic acids

Wax esters **(24)** appear to be derived from a keto-mycolic acid by a Baeyer-Villiger type oxidation reaction.[98,99] They are partially characterised as **(24)** or the corresponding diacids **(25) (Figure 11)**.

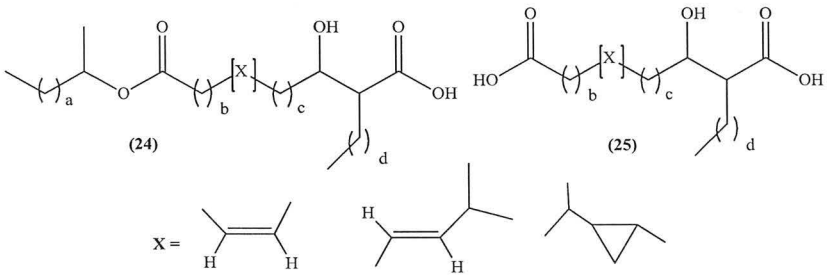


Figure 11: General structure of mycobacterial wax esters

The history of mycobacterial wax esters goes back about seventy years, when Anderson studied the *Mycobacterium* lipid wax esters as well as mycolic acids.[100,101,102] The isolation of these waxes was done by treating the cell residue of the bacteria with dilute hydrochloric acid, followed by extraction with ether. Saponification of the waxes led to

long chain, high molecular weight diacids and two long chain alcohols.[102] Anderson reported the isolation of a diacid ($[\alpha]_D$= +5.3) and long chain secondary alcohols mainly d-2-eicosanol, from avian tubercle bacilli (*Mycobacterium avium*).[101] Anderson had also named the di-acid as γ-mycolic acids. In a later study, Anderson reported the isolation of diacids and long chain alcohols from "*Timothy bacillus*", later named as *Mycobacterium phlei*. The diacids gave specific rotation $[\alpha]_D$= +6.1 in $CHCl_3$, and the two alcohols were d-2-eicosanol ($[\alpha]_D$= +3.5 in $CHCl_3$), and d-2-octadecanol ($[\alpha]_D$= +5.7 in $CHCl_3$).[102] Usually, mycobacterial wax esters have been analysed as the eicosanol-2 and octadecanol-2 and corresponding free di-acid after hydrolysis.[103,104] In 1970, Laneelle isolated wax esters, and their corresponding di-acids from *M. paratuberculosis*.[104] The use of two dimensional TLC has also provided additional evidence of the presence of wax esters in other mycobacteria.[105]

The composition of mycobacterial wax esters remained unclear until recent years. Finally, by the use of chromatography and mass spectroscopy in association with NMR spectroscopy, mycobacterial wax esters have been characterized more clearly. Wax esters and their corresponding di-acids isolated from *M.aurum* contain one double bond either in *cis* or *trans* configuration **(27a, 27b, 28a, 28b)**.[106] Furthermore, different wax esters were isolated from *M. avium-M. intracellulare* **(29)** and *M.gordonae* **(30)** which include the α-methyl *trans*-cyclopropane unit **(Figure 12)**.[107,108]

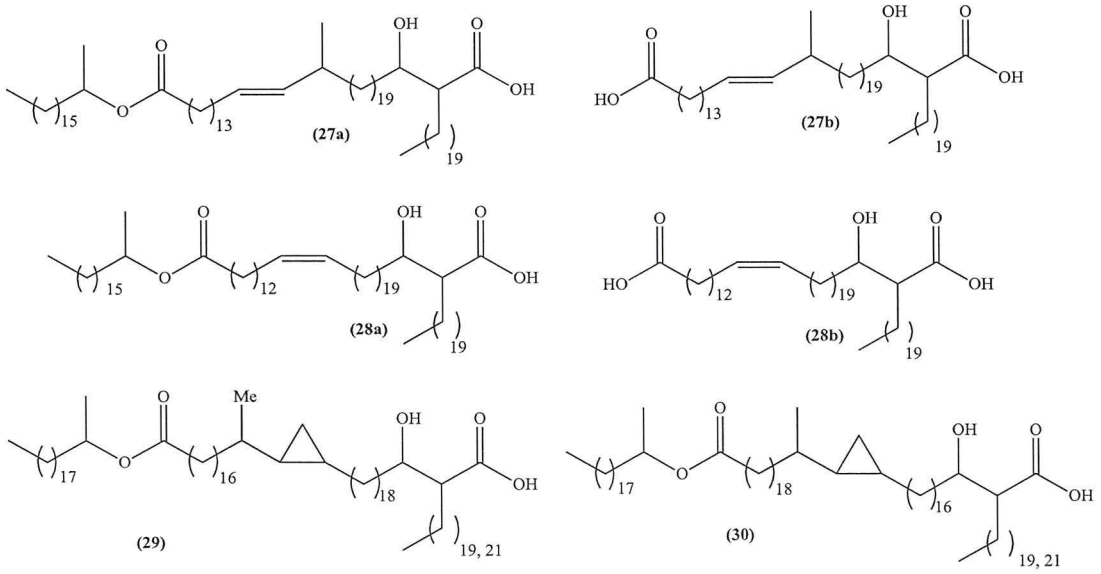


Figure 12: *Mycobacterial* wax esters

These mycobacterial species, e.g. *M.avium,* can be differentiated from *M.tuberculosis* based on the wax ester content in the cell wall. *M.avium* possesses wax ester but not

methoxy-mycolic acids, while *M.tuberculosis* possesses methoxy-mycolic acids but not wax esters, while keto- and α- mycolic acids are present in both species.

1.4.2 Stereochemistry of mycolic acids

Mycolic acids contain more than one set of chiral centre, which makes it difficult to determine the correct absolute configuration. The determination of the stereochemistry of mycolic acids has been the subject of many studies. The stereochemistry of the two chiral centres of β-hydroxy-acid fragment was determined to be *R,R*- in all mycolic acids.[75,76,77,78,109,110]

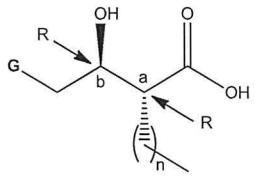


Figure 13: The chiral centres in the β-hydroxy fatty acid fragment

The stereochemistry of the chiral centres in the meromycolate part is difficult to determine because it contains several sets of chiral centres. It is believed that the methyl branch adjacent to the hydroxy (**31**), methoxy (**32**), keto (**33**), and wax ester groups **(34)** in the mycolic acids is in the *S*-configuration. The hydroxyl (**31**) and the methoxy (**32**) at the distal group have also been assigned to be in the *S*-configuration.[77,111,112] The wax ester **(34)** is derived from keto-mycolic acids through enzymatic oxidation via a Baeyer-villiger type reaction.[98,99] The methyl branch next to the *trans*-alkene unit (**36**) and *trans*-epoxy unit **(35)** are found to be in *R*-configuration,[113] however the stereochemistry of the epoxide ring **(35)** was reported in *S,S*-configuration **(Figure 16)**.[114]

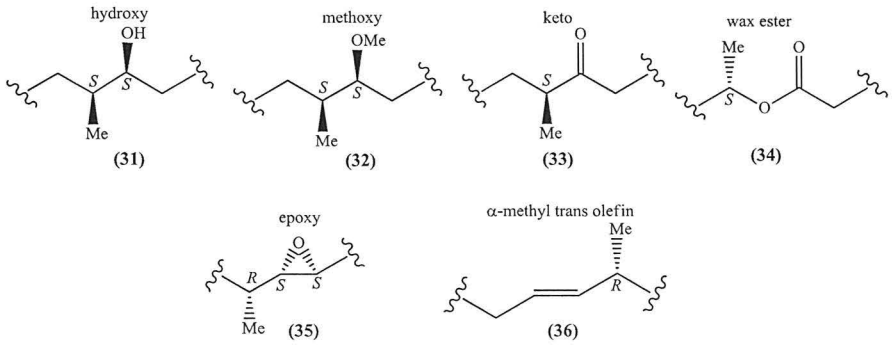


Figure 14: The strereochemistry of chiral centres of the mycolic acids

There are two more chiral groups in the mycolic acids; *cis*-cyclopropane and α-methyl-*trans*-cyclopropane. The easiest method for the determination of the chiralities of these

chiral groups is to convert the mycolic acids into simpler compounds by thermolysis. In this way the stereochemistry of the simple compounds could be more easily studied because of the existence of only one set of chiral centre. A recent study determined the absolute stereochemistry of the α-methyl-*trans*-cyclopropane.[115]

The α-methyl-*trans*-cyclopropane unit has been synthesized in three different configurations including the enantiomers **(37)** and **(38),** and the diasteromer **(39)** **(Figure 15).**

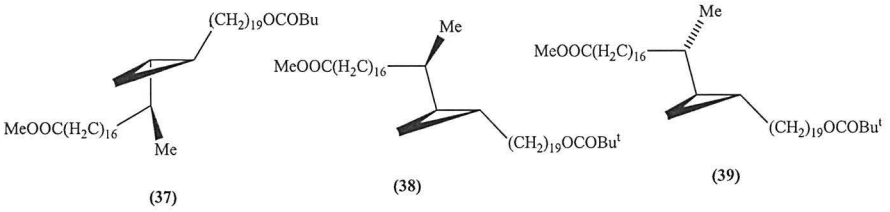


Figure 15: *α*-Methyl-*trans*-cyclopropane wax esters in different stereochemistry

The stereochemistry of the α-methyl-cyclopropane can be determined by comparison of the signals in the proton NMR with the natural mixture **(Figure 16)**.

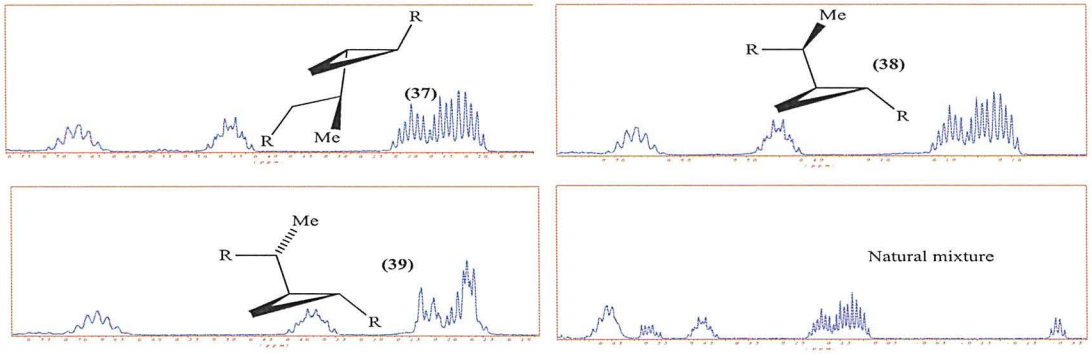


Figure 16: Cyclopropane region of proton NMR of **(37, 38,** and **39)** and *M. avium* natural mixture of the dimethyl ester of an ω-carboxy mycolic acid

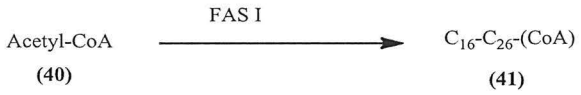
The proton NMR signals for the compound **(37)** and its enantiomer **(38)** are visually identical to each other, and also identical to the natural sample, while the signals for compound **(39)** were significantly different to those of its diastereoisomers **(37)** and **(38)**, and also to the natural compound. In addition compound **(38)** has a specific rotation $[\alpha]_D = -3.87$ (c= 1.16 ($CHCl_3$), while compound **(37)** showed $[\alpha]_D = +3.7$ (c= 1.06 ($CHCl_3$), which is close to the value of the natural material reported by Anderson *et al*, which showed $[\alpha]_D = +5.3$ in ($CHCl_3$).[101] On this basis, the compound **(37)** has the same stereochemistry as the natural material, which is (*S,R,S*).

1.4.3 Biosynthesis of mycolic acids

Understanding the biosynthesis of the mycolic acids is of utmost importance in finding new anti-mycobacterial drugs because most of the drugs relate to the inhibition of mycolic acid biosynthesis.[116] In recent years a series of studies have been conducted about the enzymes controlling the biosynthesis.

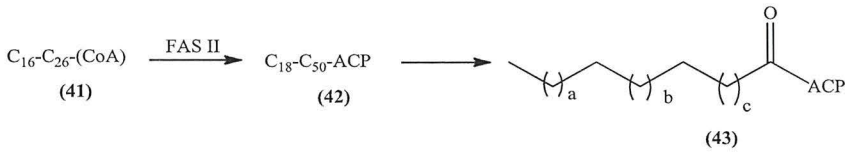
The biosynthesis commonly includes three major steps (achieved in three systems).

Firstly the mycobacterial FAS-I system (fatty acid synthase I) extends the acetyl group by two carbon units from acetyl-CoA **(40)** and also produces up to C_{16}-C_{26} chain length fatty acids **(41)** by elongating the short alkyl chain of mycolic acids **(Scheme 3)**.[117]



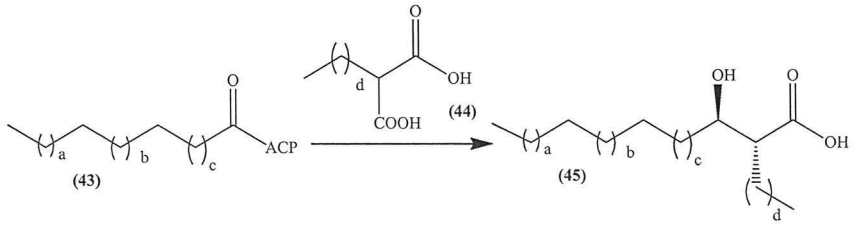
Scheme 3: FAS I system

Secondly, the mycobacterial FAS II system is used for further chain extension, up to 50 carbon units in the synthesis of meroacids **(42)**, and modifications to form different functional groups **(43) (Scheme 4)**.[117,118] The available anti-tuberculosis drugs such as thiolactomycin, isoniazed, ethionamide and prothionamide are inhibitors of the enzymes involved in the FAS II system.[53,119,120]



Scheme 4: FAS II system

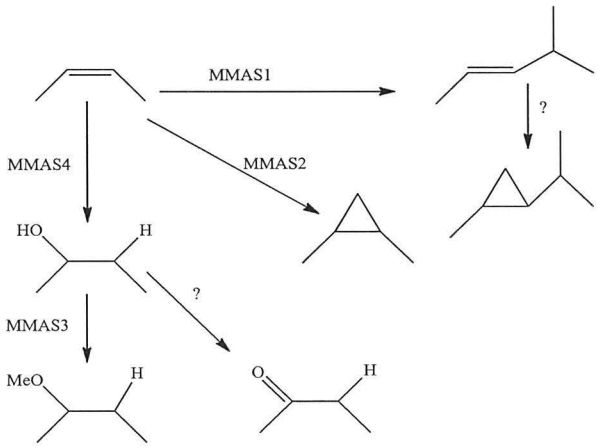
Thirdly, there is a condensation step in the biosynthetic pathway, to give the mycolic acid. The long chain fatty acid obtained from the FAS I, FAS II system undergoes condensation. It is believed to be a Claisen condensation between meromycolate (**43**) and alkyl dicarboxylic acid (**44**), with the occurrence of some more modifications like desaturation, cyclopropanation, methylation, elongation and formation of the other functional groups to give the mycolic acid **(45) (Scheme 5)**.[121]



Scheme 5: Condensation step

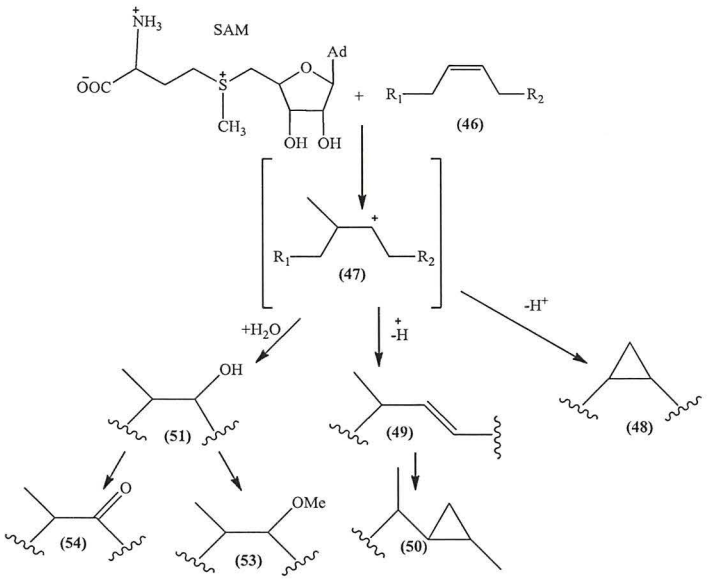
There are several modifications carried out in the final step of the biosynthesis, but cyclopropanation and methylation have always been more important than the introduction of other functional groups. This will be described below.

Yuan and Barry established the mechanism of the biosynthesis of methoxy and cyclopropane mycolic acids in *M.tuberculosis*.[122] Parallel studies reported the isolation of the genes or the enzymes (cma1, cma2) for the *cis-trans* cyclopropanation and four genes MMAS1-4 involved in methyl transfer.[122,123,124] MMAS-1 split the pathway either to *cis*-mycolate or *trans*-mycolate precursors. MMAS-2 appears to act upon the proximal *cis*-olefin and results in *cis*-cyclopropane formation. MMAS-4 can transform the olefinic meromycolate in the distal position into a secondary hydroxyl group with an adjacent methyl branch. Finally MMAS-3 converts the newly formed hydroxyl group to methoxy, or keto compounds **(Scheme 6)**.[122,124]



Scheme 6: The proposed mechanism of cyclopropanation

Meromycolic unit is the formation of a double bond. The MMAS-4 contains a typical SAM binding site,[122] and methylation of the *cis*-double bond of mycolic acid **(46)** occurs by transferring the methyl group from S-adenosyl methionine (SAM) to give the carbenium ion intermediate **(47)**. The ion could react through three processes. Deprotonation leads to either the *cis*-cyclopropane ring **(48)** or methyl *trans*-double bond **(49)**, and also could be the precursor of the *trans*-cyclopropane mycolic acid **(50)**. Addition of water to the intermediate gives alcohol **(51)**, and this would then lead to methoxy or keto mycolic acids **(52, 53) (Scheme 7)**.[122,124]

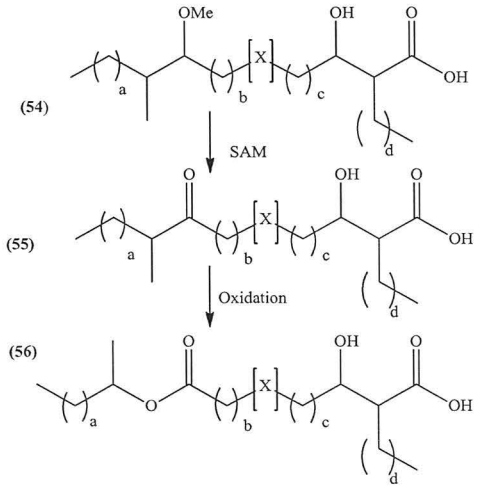


Scheme 7: Biosynthesis of mycolic acid functional groups

1.4.4 Biosynthesis of wax ester mycolic acids

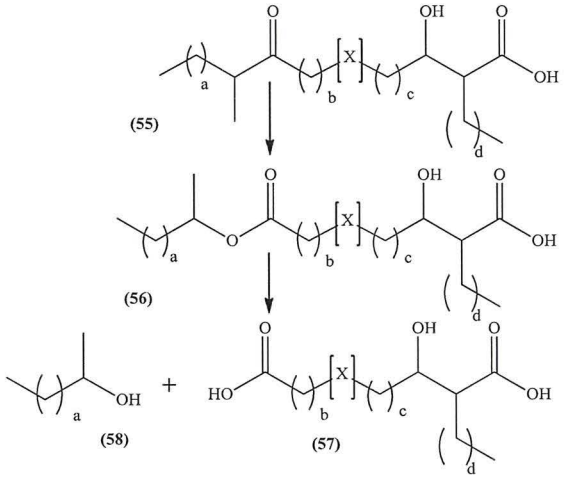
Oxidation reactions are one of the most important enzyme catalyzed reactions for maintenance of life in organisms, and significant amongst this type of reactions is the "Baeyer-Villiger type oxidation". It is observed in several organisms to convert steroids via this type of oxidation reaction.[125,126]

Baeyer-Villiger type oxidation is also involved in the biosynthesis of mycobacterial wax esters. It is thought that wax ester mycolic acids are derived from keto-mycolic acids through Baeyer-Villiger type oxidation reaction.[98,99,104] It was suggested that the biosynthesis of the wax esters originated from methoxy mycolic acids, firstly the conversion of a methoxy **(54)** into a keto-mycolic acid precursor **(55)** occurs, followed by conversion into the wax ester **(56)** by the oxidation reaction **(Scheme 8)**.[99,127]



Scheme 8: Hypothetical pathway of wax ester biosynthesis

This hypothesis was later strengthened by the work of Yano *et al.*[128] which involved incorporation of ^{18}O from an $^{18}O_2$ into the alcohols. It showed that when the labelled wax esters **(56)** were hydrolysed to di-carboxy acid **(57)** and alcohol **(58)** only the oxygen atom of the alcohol was labelled **(Scheme 9).**



Scheme 9: The incorporation of ^{18}O to study the biosynthesis of wax esters

At the present time little is known about the biosynthesis of wax esters. The biosynthetic pathway needs to be further investigated, not only based on structural analogies but also the study of the genes involved in this pathway in order to clearly understand the biosynthesis.

1.5 Synthesis of Mycolic acids

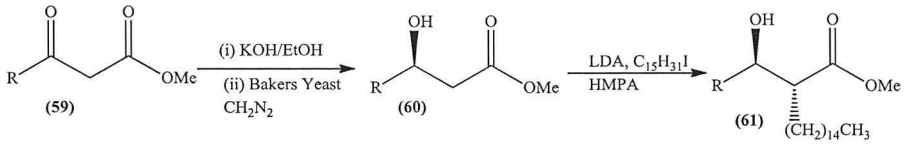
The synthesis of mycolic acids is of the utmost importance in understanding their role in the mycobacterial cell wall, the identification of natural mycolic acids and to study the stereochemistry of the chiral centres present in the mycolic acids. In addition, the synthetic mycolic acids assist the process of clarifying the biosynthetic origin of mycolic acids, and in the development of anti-mycobacterial agents. Furthermore, synthetic mycolic acids are important in the diagnosis of mycobacterial diseases, e.g., TB, MAC. In order to synthesize mycolic acids, usually the meromycolate moiety and mycolic motif are typically synthesized individually, prior to being coupled to each other to produce the complete mycolic acid.

1.5.1 Synthesis of mycolic motif

The mycolic motif part is similar in mycolic acids except for a slight variation in the chain length of α-alkyl position. The α-alkyl β-hydroxy fatty acid is present in *R,R*-

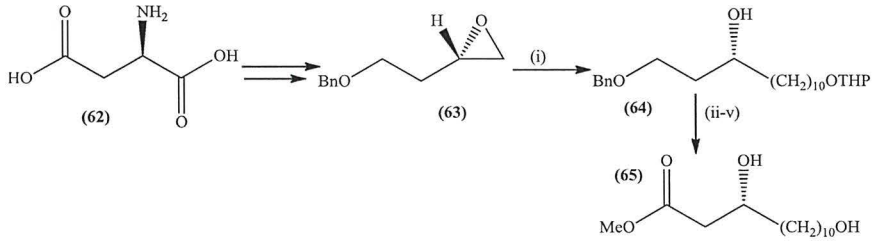
configuration. The synthesis of mycolic motif was first reported by Lederer *et al.* in 1952,[129] by condensation between two fatty acid molecules. Further studies were also carried out based on Claisen condensation, but all the attempts produced a mixture of diasteromers.[130,131]

The major problem with the synthesis of mycolic acids is the insertion of the chiral centres with the correct stereochemistry. Utaka *et al.*[132] used a stereo-selective chiral reduction of β-keto ester **(59)** employing Baker's yeast type reduction to produce β-hydroxy-ester **(60).** The α-position was then alkylated by a Fräter reaction to produce the α-alkyl-β-hydroxy carboxylate (**61**) with the correct absolute stereochemistry.[133]



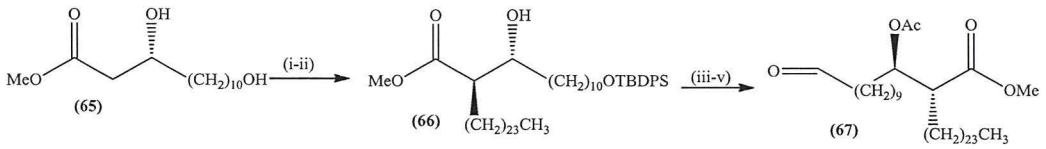
Scheme 10: The mycolic motif synthesis, Utaka *et al.*[132]

The approach of Baird, Al Dulayymi *et al.*[134] was subsequently used to synthesize the mycolic motif part originating from *R*-aspartic acid **(62)**. Ring opening of epoxide **(63)** with a Grignard reagent gave protected diol **(64)**, then after another four steps (protection, debenzylation, oxidation, esterfication) led to the diol **(65) (Scheme 11).**



Scheme 11: Ring opening of epoxide **(63)** (i) $BrMg(CH_2)_9OTHP$, CuI, 2h (ii) Imidazole, DMF, TBDMSCl; (iii) H_2, Pd/C, MeOH; (iv) $NaIO_4$,$RuCl_3.H_2O$, CCl_4; (v) MeOH, H_2SO_4.

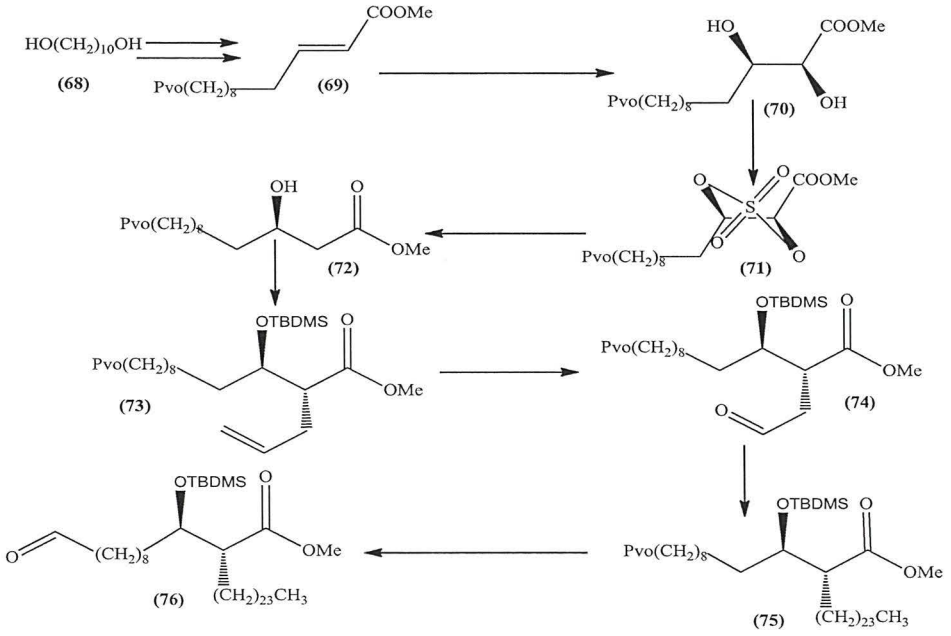
Again, protecting the diol **(65)** with the *tert*-butyldiphenylsilyl group and Fräter alkylation with 1-iodotetracosane (prepared by coupling of dodecyl-magnesium bromide with 12-bromododecanal then iodination) gave the α-alkyl-β-hydroxy ester **(66)**. After the secondary hydroxyl group was protected with acetate, the primary hydroxyl group was deprotected and oxidized to give the mycolic motif aldehyde **(67) (Scheme 12).**



Scheme 12: (i) TBDPSCl, DMAP, Et_3N; (ii) LDA, $CH_3(CH_2)_{23}I$, HMPA; (iii) Ac_2O, pyridine; (iv) TBAF, (v) PCC.

Koza *et al.*[135] also prepared the mycolic motif by a method similar starting from L-aspartic acid as a cheap and easy to scale up material (see Chapter 2).

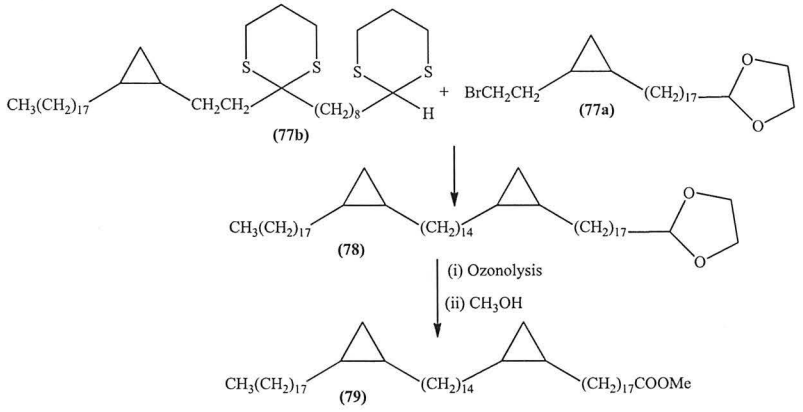
Toschi *et.al.*[136] used 1,10-decandiol in a different method to prepare the mycolic motif. The diol **(68)** was firstly converted to the *E*-α,β-unsaturated ester **(69)** in four steps, and then into diol **(70)** by Sharpless asymmetric dihydroxylation. The diol **(70)** was converted into sulphate **(71)**, and then hydrolysed to produce protected β-hydroxy ester **(72)**. Fräter allylation at the α-position on the ester **(72)** followed by silyl protection gave the alkene **(73),** which was then oxidized with OsO_4 to give aldehyde **(74)**. The Julia olefination coupling reaction was used to chain extend the aldehyde **(74)**, followed by hydrogenation to give **(75).** Finally the compound **(75)** was deprotected and oxidized to give the desired mycolic motif aldehyde **(76) (Scheme 13)**.



Scheme 13: Toschi *et.al.* synthesis of the mycolic motif.

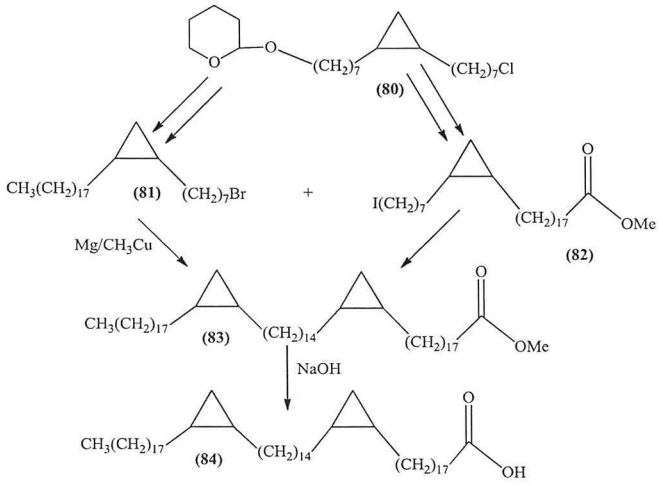
1.5.2 Synthesis of meromycolic acids

In 1977, Gensler *et al.*[137] 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 synthesis steps. Coupling of the different units of **(77a)** and **(77b)** gave a structure **(78)** which had the same carbon number as a meromycolic acid. Finally, this compound was transformed into the desired product **(79)** by desulphurisation and ozonolysis **(Scheme 14)**.



Scheme 14: The first approach of Gensler *et.al*

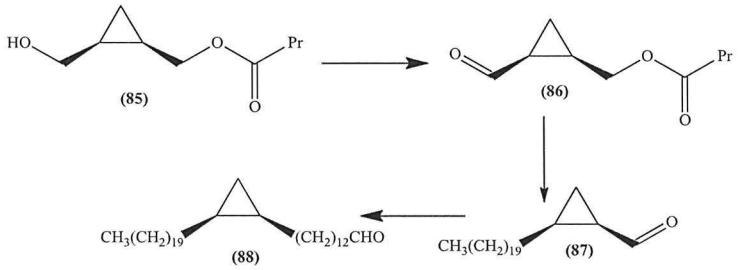
Subsequently, Gensler *et al.*[138] proposed a method involved the conversion of compound **(80)** to the alkyl bromide **(81)** and alkyl iodide **(82)** in several steps. The methyl meromycolate **(83)** was prepared by combining the different portions by using the Grignard reagent obtained from the alkyl bromide **(81)**, which formed a complex with the methyl copper and was coupled with the alkyl iodide **(82)** to give methyl meromycolate **(83)** as a mixture of four stereoisomeric di-*cis*-cyclopropanes. This mixture was then hydrolyzed to give meromycolic acid **(84) (Scheme 15)**.



Scheme 15: Second Gensler *et.al.* approach

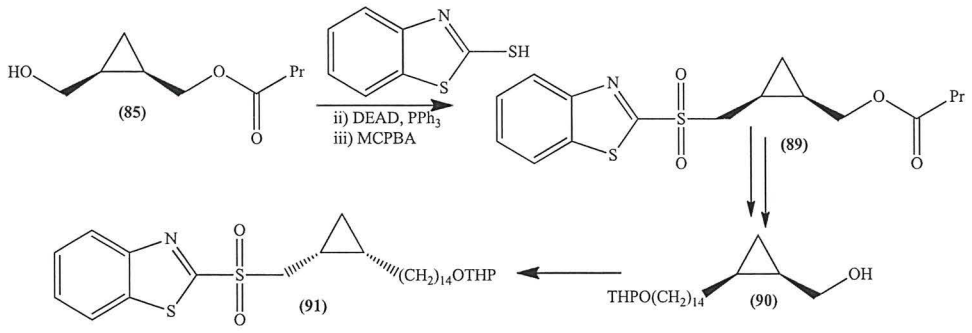
This method is shorter and could be more easily scaled up, but suffers from low yield and the uncontrolled stereochemistry.

Recently, another route for the synthesis of a single enantiomer of meromycolic acid was reported by Baird *et al.*[139] Again the acid is obtained by the coupling of two units, which were previously prepared using a combination of Julia and Wittig reactions. In this method the key step is the Julia reaction between the two cyclopropane units **(88)** and **(91)**. The aldehyde **(88)** was prepared starting from the anhydride of cyclopropane-*cis*-1,2-dicarboxylic acid which was converted into a single enantiomer **(85)**.[140] The cyclopropane intermediate with one protected hydroxyl group (**85**), was oxidised to an aldehyde **(86)**, and then the first Wittig reaction led to a mixture of *Z* and *E* isomers, which were saturated with diimide. Reduction of the ester, followed by oxidation gave the aldehyde **(87).** The C_{12} phosphonium salt was now linked to the aldehyde **(87)** in a Wittig reaction, and after the several steps (similar to those shown above) the chiral aldehyde **(88)** was obtained **(Scheme 16)**.



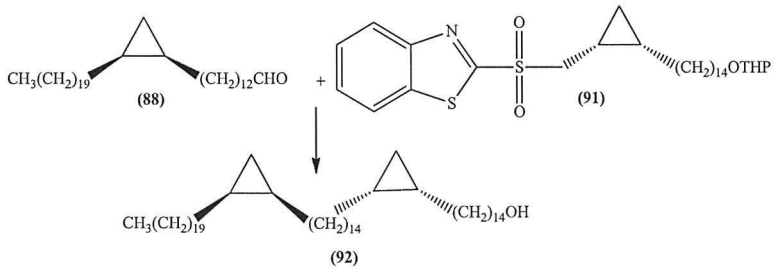
Scheme 16: The synthesis of first cyclopropane unit **(88)**

A second cyclopropane unit was prepared as a sulphone **(91)** again starting from compound **(85).** Reaction with benzothiazole, diethyl azodicarboxylate (DEAD), and then with triphenylphosphine led to a sulphide compound, then oxidation with *m*-chloro-perbenzoic acid (MCPBA) gave the sulphone **(89)**. The chain elongation by coupling with 13-tetrahydroopyranyloxy-tridecanal in a Julia reaction gave a mixture of *E* and *Z*-alkenes, which were saturated and reduced to alcohol **(90)**. The alcohol **(90)** was converted into the required sulphone **(91)** under the same conditions shown above **(Scheme 17)**.



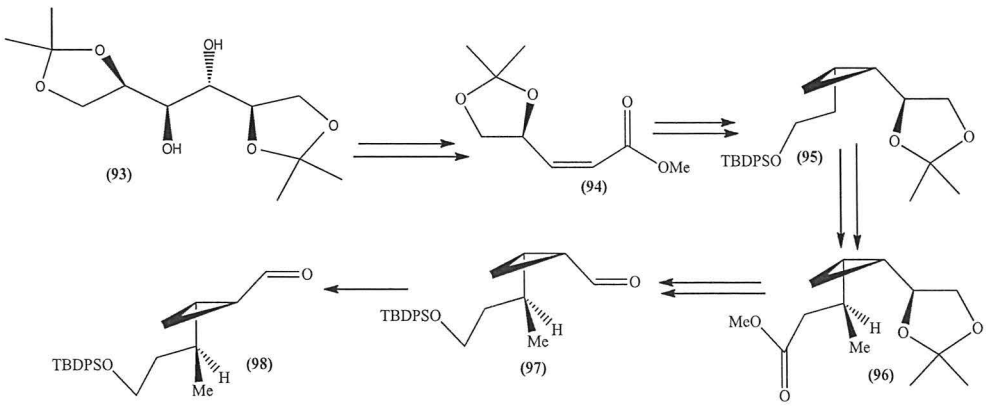
Scheme 17: The synthesis of second cyclopropane unit **(91)**

The cyclopropane units **(88)** and **(91)** were coupled in a Julia olefination reaction in the presence of base, which gave a mixture of *E*- and *Z*-alkenes. Saturation of the alkene followed by deprotection and gave the desired product **(92) (Scheme 18)**.



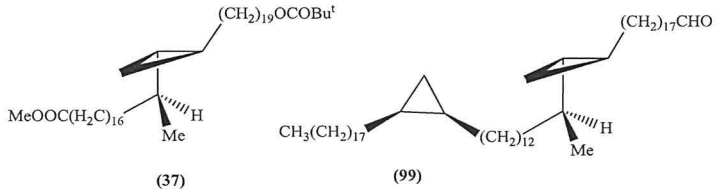
Scheme 18: *Al Dulayymi et.al.* method of the α-meromycolic motif synthesis

The synthesis of the α-methyl-*trans*-cyclopropane unit in meromycolic acids has also reported by Baird, Al Dulayymi *et al.*[115] The synthesis started with protected D-mannitol **(93)** which after oxidation and Wittig reaction led to alkene **(94)**, which was then, through several steps including a Simmons-Smith cyclopropanation reaction, converted into the optically active *cis*-1,2-disubstituted cyclopropane **(95)**. Several steps were carried out including methylation, oxidative cleavage, and epimerization to obtain α-methyl-*trans*-cyclopropane aldehyde **(98)** (This will be discussed in detail in Chapter 2).



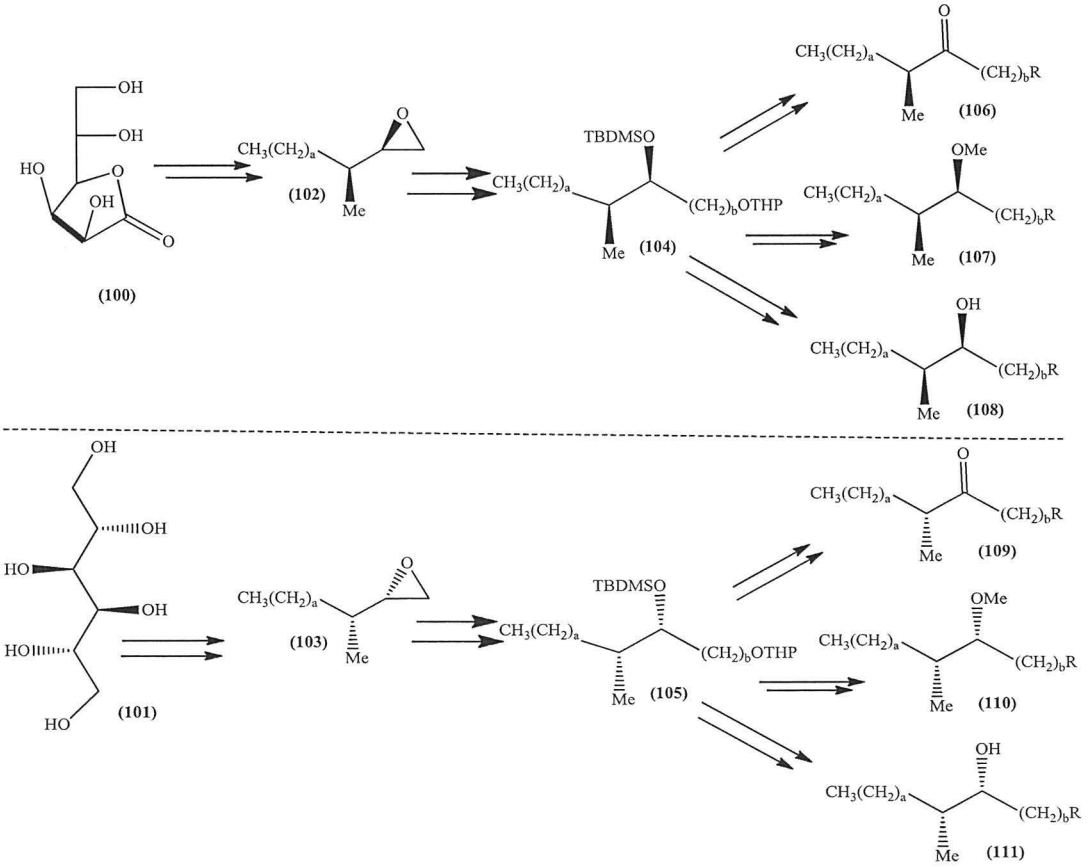
Scheme 19: *Al Dulayymi et al.* method of α-methyl-*trans*-cyclopropane synthesis

The α-methyl-*trans*-cyclopropane unit **(98)** was used to prepare different meromycolates such as **(37)** and **(99)** **(Scheme 20)**.[115, 141]



Scheme 20: The synthesis of meromycolate fragments containing an α-methyl-*trans*-cyclopropane

Baird, Al Dulayymi *et al.*[142] reported the synthetic route to the oxygenated units present in the structure of meromycolic acids. The preparation of those bearing *S,S* configuration originate from *L*-ascorbic acid **(100)**, while those of *R, R* configuration from D-mannitol **(101)**. The procedure is a multi-step synthesis to obtain the key intermediates **(104)** and **(105),** from which it is possible to prepare keto, methoxy and hydroxyl units **(106-111)** **(Scheme 21)**.

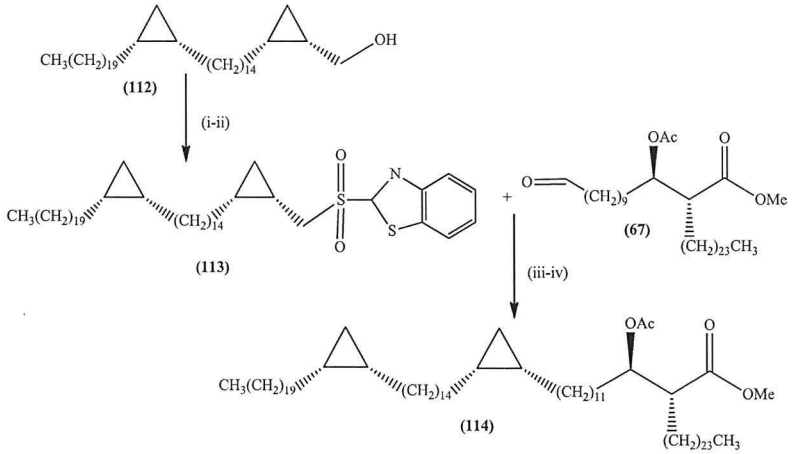


Scheme 21: Baird, Al Dulayymi *et al.* method to prepare *S,S* and *R,R* configuration of oxygenated mycolic acid

1.5.3 Synthesis of complete mycolic acids

The approach of Baird, Al Dulayymi *et al.*[134] was subsequently used to prepare a complete mycolic acid, by linking the mycolic motif to the meromycolic acid.

The single enantiomer of mero-mycolic acid **(112)** could be obtained using the same methods as for the meromycolate **(92) (Scheme 18).** The final step of the synthesis of the complete mycolic acid **(114)** was a Julia olefination reaction between meromycolate **(113)** and the aldehyde **(67)**, followed by hydrogenation **(Scheme 22)**.



Scheme 22: (i) Benzothiazole, DEAD, PPh_3, (ii) MCPBA, (iii) LiHMDS, THF, (iv) dipotassium azodicarboxylate, CH_3COOH, MeOH, THF

Later, Baird *et al.* synthesized the free α-mycolic acid **(115)** with the same stereochemistry as the above protected α-mycolic acid **(114)**,[143] followed by the synthesis of another three α-mycolic acids **(116-118)** with different a stereochemistry **(Figure 17).**[144, 145]

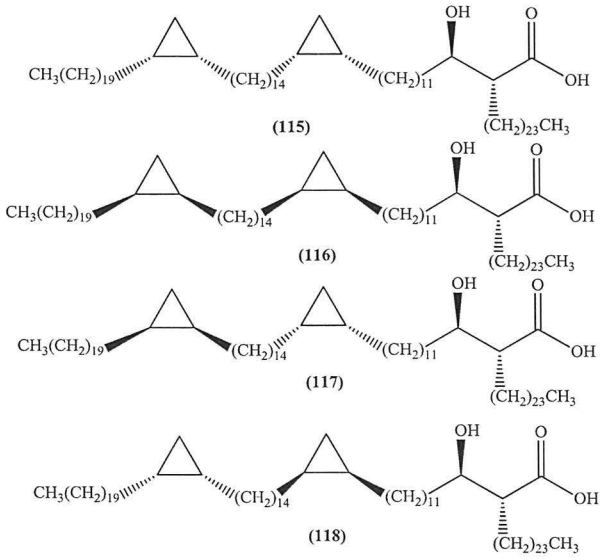


Figure 17: Synthesis of free α-mycolic acids with different stereochemistry.

After the optimisation of this first enantioselective synthesis, it was possible to prepare other single enantiomers of different mycolic acids in different stereochemistries, functional groups and chain lengths.

Methoxy mycolic of *M.Tuberculosis* acids have also been synthesized by Baird *et al.* in different stereochemistries containing either the *cis* or α-methyl-*trans*-cyclopropane **(Figure 18)**.[142,146,147]

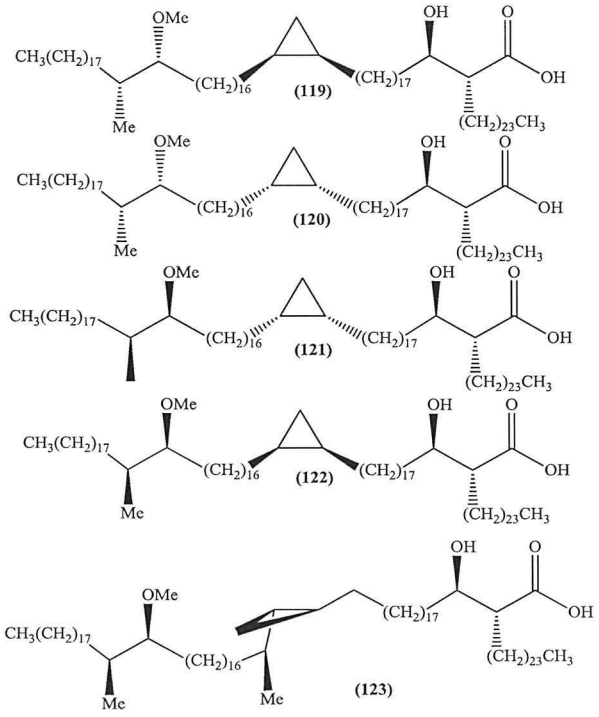
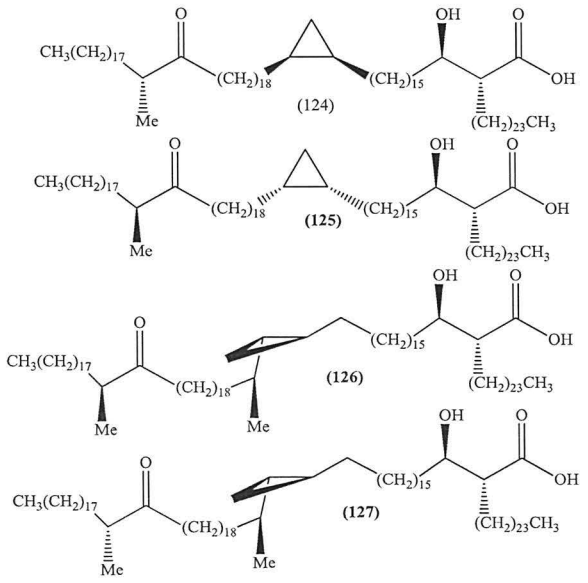


Figure 18: Synthetic methoxy mycolic acids

A set of hydroxy and keto-mycolic acids has also been prepared by the same group.[135,146,148] **(Figure 19)**



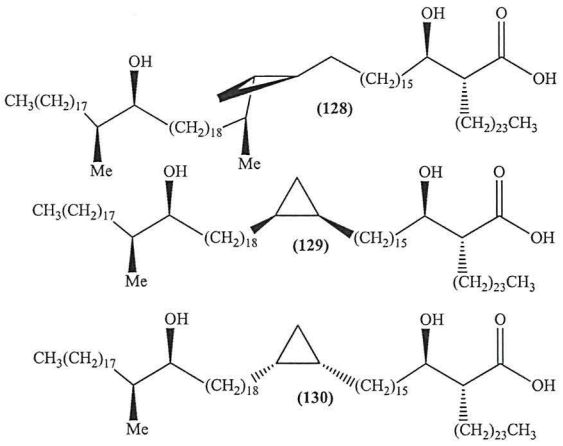


Figure 19: Synthesized hydroxy and keto-mycolic acids

Baird *et al.* have not only prepared single enantiomers of the cyclopropanated mycolic acids, but also mycolic acids bearing other functionalities such as the epoxy mycolic acids **(131)** and **(132)** containing an (*R*)-α-methyl-*trans*-alkene at the proximal position and the *cis* alkene mycolic acid **(133).** These have been found in *M. fortuitum* and *M. smegmatis.*[114, 149] **(Figure 20)**

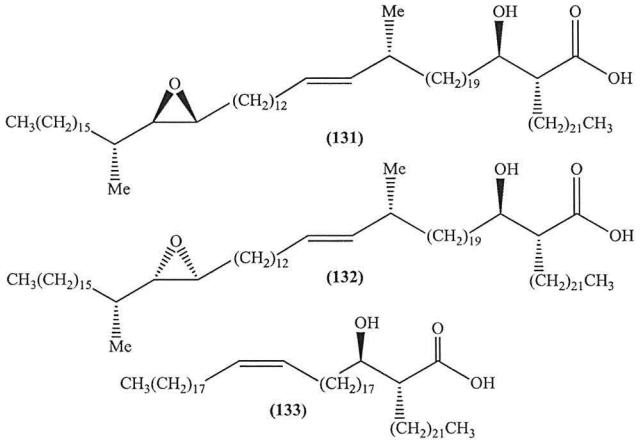


Figure 20: Synthesized epoxy and *cis* alkene mycolic acids

1.6 Trehalose esters (cord factors)

Trehalose is a non-reducing disaccharide in which the two glucose units are linked by an α,α-1,1-glycosidic linkage. The mycobacterial cell wall contains high levels of the free trehalose and esterified trehalose. However trehalose is also present in a wide variety of organisms including fungi, insects, invertebrates, and in plants.[150,151] Most bacteria use trehalose solely as a general osmoprotectant or thermoprotectant.[152] Another function of trehalose is as a stress protectant on exposure to heat, desiccation, or heavy metals.[153]

The most interesting trehalose compounds can be found in the outer cell membrane of mycobacteria as a complex glycolipid named "cord factor" which is a trehalose linked to mycolic acids to form trehalose-6,6-dimycolate (TDM, **134**) or to form trehalose monomycolate (TMM, **135**) **(Figure 21).**

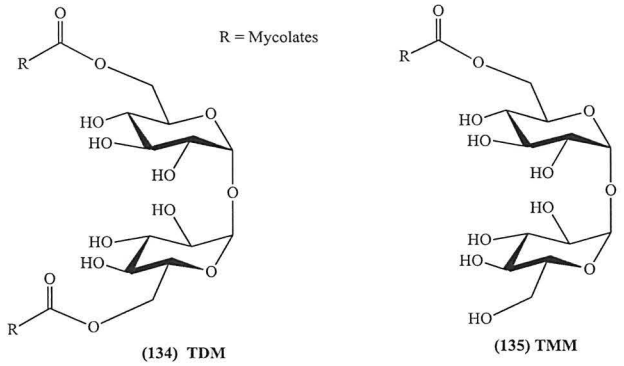


Figure 21: General structure of cord factors

The name cord factor has been used in the early papers, as far back as 1947.[154] Cord factors are a toxic lipid component from the mycobacterial cell wall that can be extracted using an appropriate solvent.[154,155] In 1956, the structure of *M.tb* cord factor was identified by Noll *et al.,*[70] and they proved that mycolic acids are esterified to trehalose to form trehalose-6,6-dimycolate (TDM, **136**) **(Figure (21))**. When the cord factor was subjected to alkaline hydrolysis free mycolic acid and non-reducing carbohydrate moiety were produced. They also proved that the structure of the carbohydrate moiety was α,α-trehalose. This was confirmed in two ways, firstly the acid hydrolysis of the carbohydrate moiety produced D-glucose, secondly the carbohydrate moiety was acetylated to give a crystalline acetate which was identified as α,α-trehalose octa-acetate. Furthermore, they proved that mycolic acids are linked with the trehalose in the 6,6 positions, by obtaining 2,3,4-tri-*O*-methyl-glucose **(137)** after hydrolysis of the methylated cord factor **(Figure 22)**.[70]

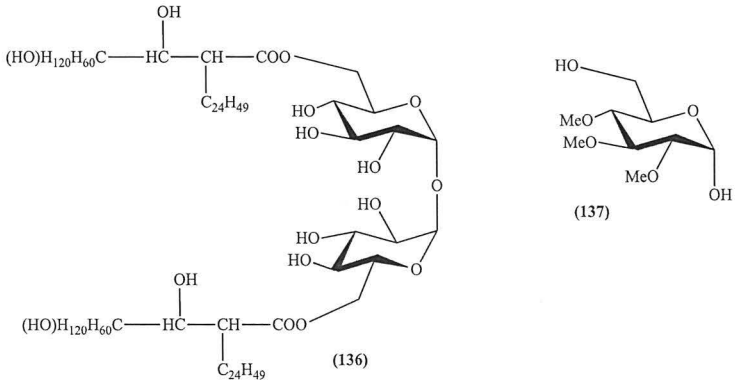


Figure 22: Proposed structure of trehalose 6,6-dimycolate by Noll *et al.*

Ioneda *et al.* reported the isolation of a glycolipid from *Corynebacterium diphtheria* containing the trehalose di-ester of corynomycolic acid and corynomycolenic acid.[156,157] In a later study, glycolipids were isolated from *M. fortuitum* and analysed by mass spectrometry and were found to comprise an asymmetric trehalose di-ester.[158]

In 1976, Prome *et al.*[159] isolated and identified mixtures of cord factors found in *M. phlei*. Two types of trehalose monomycolate (TMM) were found; the first one comprises α-mycolic acid and the second one was the trehalose mono wax ester. Another three types of trehaose dimycolate (TDM) were also found, one conaining two α-mycolic acids, one containing two wax esters, and one having one residue of each type.[159]

Recently MALDI-TOF mass spectroscopy (Matrix-Assisted Laser Desorption/ Ionization Time-of-Flight) was used to determine the accurate molecular masses of mycolic acids in addition to those of the trehalose dimycolate (TDM) and trehalose monomycolate (TMM) from several species of mycobacteria.[160,161] The advantage of MALDI-TOF analysis is its ability to determine the molecular weight of the molecules in excess of 3000, while no molecular ion peak could be detected for such species using other mass spectroscopy techniques. This led to a much clearer understanding of cord factor composition. For the first time, the MALDI-TOF analysis was used to identify the wax ester cord factors from *M. avium-intracellular* group, *Mycobacterium phlei, and Mycobacterium flavescens*. The wax ester TMM showed ions C_{83}, C_{85}, C_{87} for *M.avium-intracelluler* and C_{78}, C_{79}, C_{80}, C_{81}, C_{82}, C_{83} for *M. phlei* and *M.flavescens*. The corresponding wax esters TDM were also identified.[160, 161]

1.6.1 Known biological activities of cord factors

Mycobcterial cord factors are known as very interesting compounds due to their biological activity, which has been the subject of many investigations. Studies showed that these components of mycobacteria have immune activity. TDM are necessary for the survival of the mycobacteria inside macrophages.[162] TDMs can also induce cytokine production in the host's immune system (IL-1β, IL-6, and TNF) inside macrophages.[163] Early studies showed that cord factors can be used as an adjuvant (adjuvants are agents can enhance the immune response to an antigen) against immunological problems. Meyer and Azuma in 1975 discovered that the cell wall components of mycobacteria show adjuvant activity.[164] A study by Saito confirmed that mycobacterial cord factor was a good adjuvant and that it can enhance the immune system in mice and rats through antibody production. It can also cause delayed hypersensivity.[165]

Antitumor activity is another role of cord factor. For example cord factor (TDM) has been used for the treatment of cancer in animals.[166,167] Moreover, the treatment of mice with cord factor has also lead to resistance against influenza virus and different bacterial species, such as *Salmonella typhi* and *Salmonella typhimurium*.[168,169]

Recently the synthetic cord factors (TDM, TMM of methoxy and α-mycolic acids) were tested on the mouse macrophage cell line RAW 264.7 to study their activity in stimulating cytokine and chemokine production. It was found that the level of TNF-alpha production induced by synthetic α-TDM was three times higher than the commercially produced TDM sample, while the remaining synthetic TDMs and TMMs were found to exhibit a lower activity than the natural *M.tb*-TDM.[170] It has also been found that the level of chemokine MCP-1 production induced by α-TDM was twice more than the production for commercial TDM sample, while the other synthetic cord factors demonstrated an equivalent level of MCP-1 production compared to the commercially produced TDM sample.

The structure of the mycolic acids has an effect on the biological activities of cord factors. Studies confirmed that synthetic free mycolic acids from *M. tuberculosis* show different antigenic properties.[171,172] As cord factors and mycolic acids demonstrate many interesting biological properties such as their effects on the immune system, in diagnosis and controlling a number of diseases, the enantioselective synthesis of these compounds is important to allow these effects to be understood.

1.6.2 Biosynthesis of cord factors

In 1977, Takayama *et al.*[173] proposed that the small amounts of free mycolic acids and trehalose dimycolate formed by *M. tuberculosis* are probably produced by mycolyl acetyl trehalose (MAT), and transferred to the cell wall, as shown in **Figure 23**.

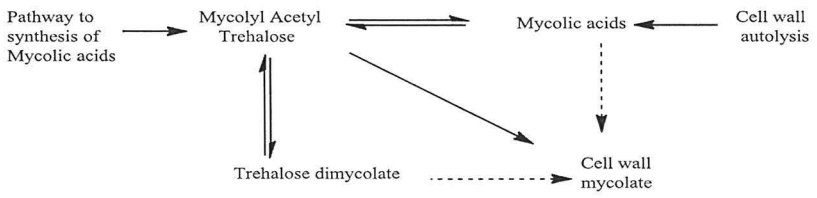


Figure 23: Proposed biosynthesis pathway of cord factor by Takayama *et al.*[173]

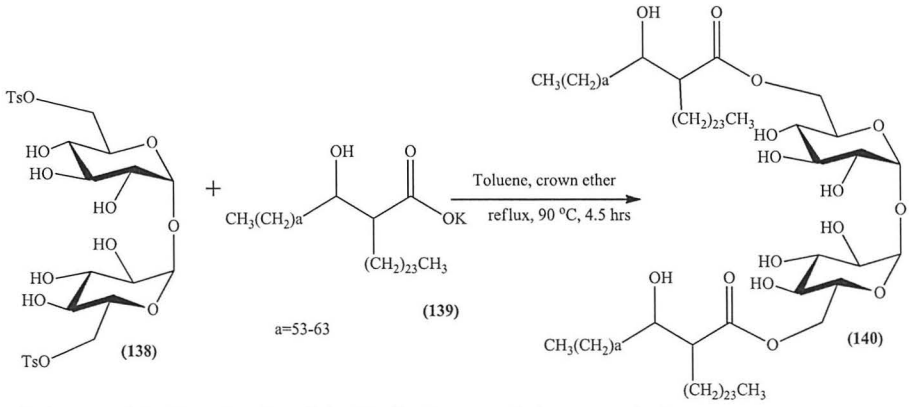
In recent years the biosynthesis of cord factors has been much more clearly understood. It has been found that mycobacteria can synthesize trehalose through three pathways.

The first is from glucose-6-phosphate and UDP-glucose catalyzed by trehalose-6-phosphate synthase (OtsA) and trehalose-6-phosphate phosphatase (OtsB2).[174] The second is from glycogen, by isomerizing the α-(1,4)-glucose polymers to α-(1,1)-trehalose, via the activity of maltooligosyltrehalose synthase (TreY) and maltooligosyltrehalohydrolase (TreZ). In the third pathway, trehalose is produced from maltose directly by the action of trehalose synthase (TreS) gen.[152] These pathways were found in both rapid and slow growing mycobacteria.[174]

Cord factor biosynthesis starts from inside the mycobacterial cell (cytoplasm), when mycolic acids are transferred to 6-0-mycolyl-β-D-manno-pyranosyl-l-monophosphoryl-3,7,11,15,19,23,27-heptamethyl-(2*Z*,6*E*,1*OE*)-octacosa-trien-l-ol,[175] and then transferred to trehalose 6-phosphate by mycolyltransferase II to form TMM-phosphate.[176] After removal of the phosphate group by 6-phosphate phosphatise, the TMM produced is transported to the outside of the membrane to produce TDM. The mycolyltransferases of the antigen 85 complex are responsible for transferring the mycolic acid of TMM to another molecule of TMM to produce the TDM.[176,177]

1.6.3 Synthesis of cord factors

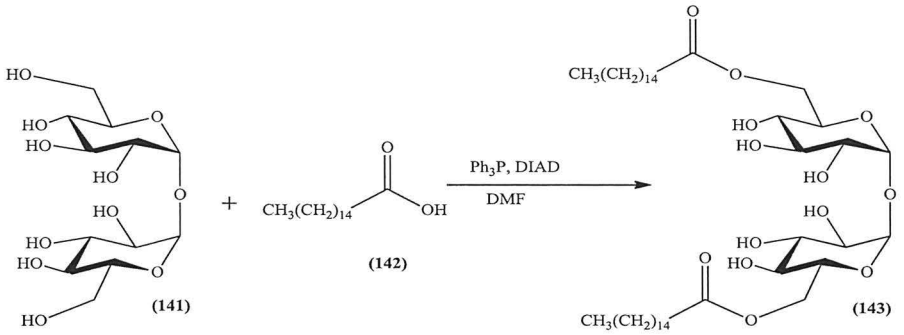
From the literature, it can be seen that many attempts have been made to secure a practicable route to trehalose esters. Polonsky *et al.*[178] involved protected the primary alcohols of unprotected trehalose with toluenesulfonyl groups and then reacted with the potassium salt of acid **(139)** in DMF. This produced the 6,6-trehalose diester **(140)** but in low yield. Replacing the DMF with toluene and refluxing at 90 ° C in the presence of a catalytic amount of crown ether improved the yield **(Scheme 23).**



Scheme 23: Synthesis of 6, 6'-di-*O*-mycolyl-α,α-trehalose

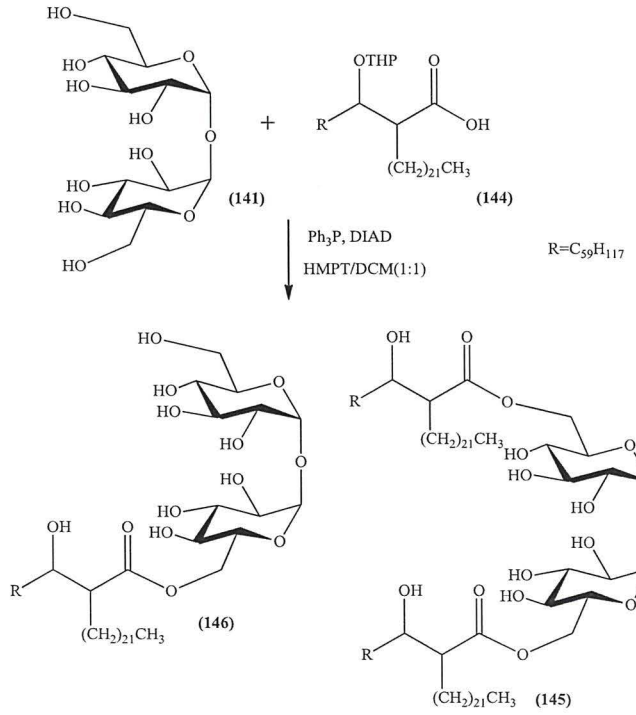
Later approaches to trehalose esters were based on the prior protection of the secondary hydroxyl groups of trehalose with benzyl or silyl groups and then coupling with

carboxylic acids or their corresponding salts.[179,180] However, a Mitsunobu reaction was also employed to esterify unprotected trehalose in reasonable yields. These will be described below. Bottle and Jenkins used this reaction as a mild procedure to synthesize a trehalose diester, linking the free unprotected sugar **(141)** with palmitic acid **(142)** using di-isopropyl azodicarboxylate (DIAD), triphenylphosphine and DMF as solvent **(Scheme 24)**.[181]



Scheme 24: Trehalose dipalmitate synthesis by a Mitsunobu reaction.[181]

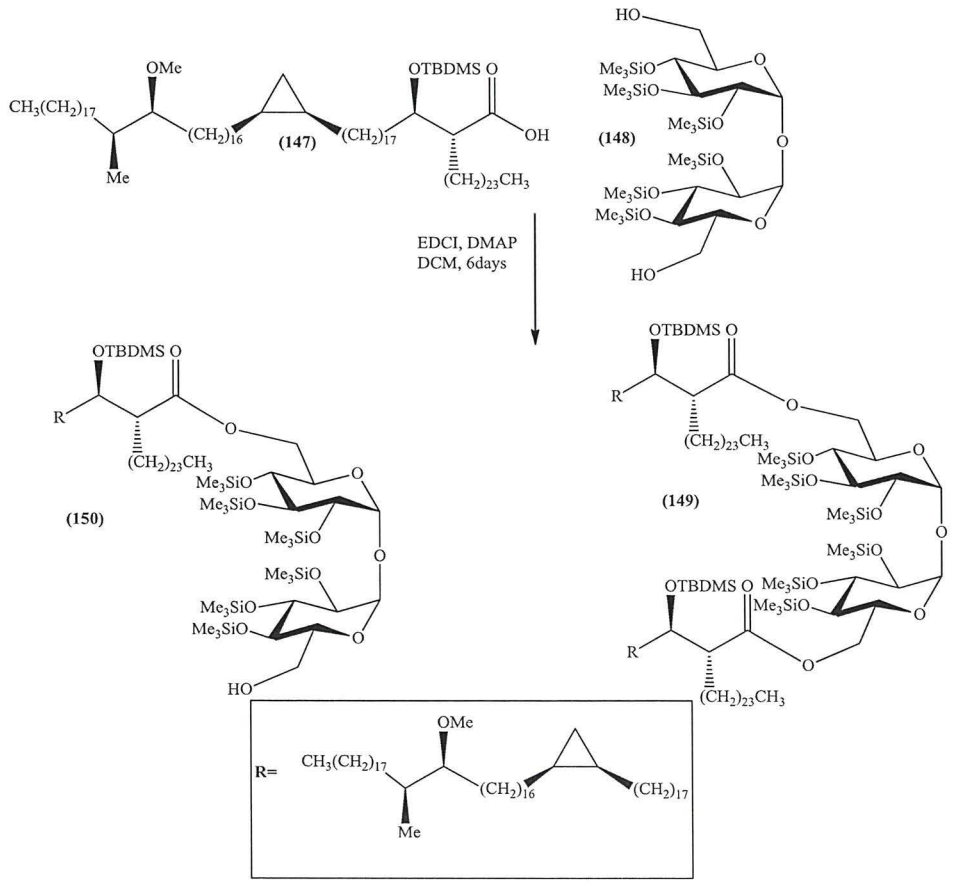
The above procedure could not be applied to mycolic acids, due the occurrence of β-elimination. Therefore, it was modified by the protection of the β-hyroxyl group of the mycolic acid with THP, then coupling with unprotected trehalose **(141)** to give both the TDM **(145)** and TMM **(146) (Scheme 25)**.[182]



Scheme 25: TDM, TMM synthesis by Mitsunobu reaction.[182]

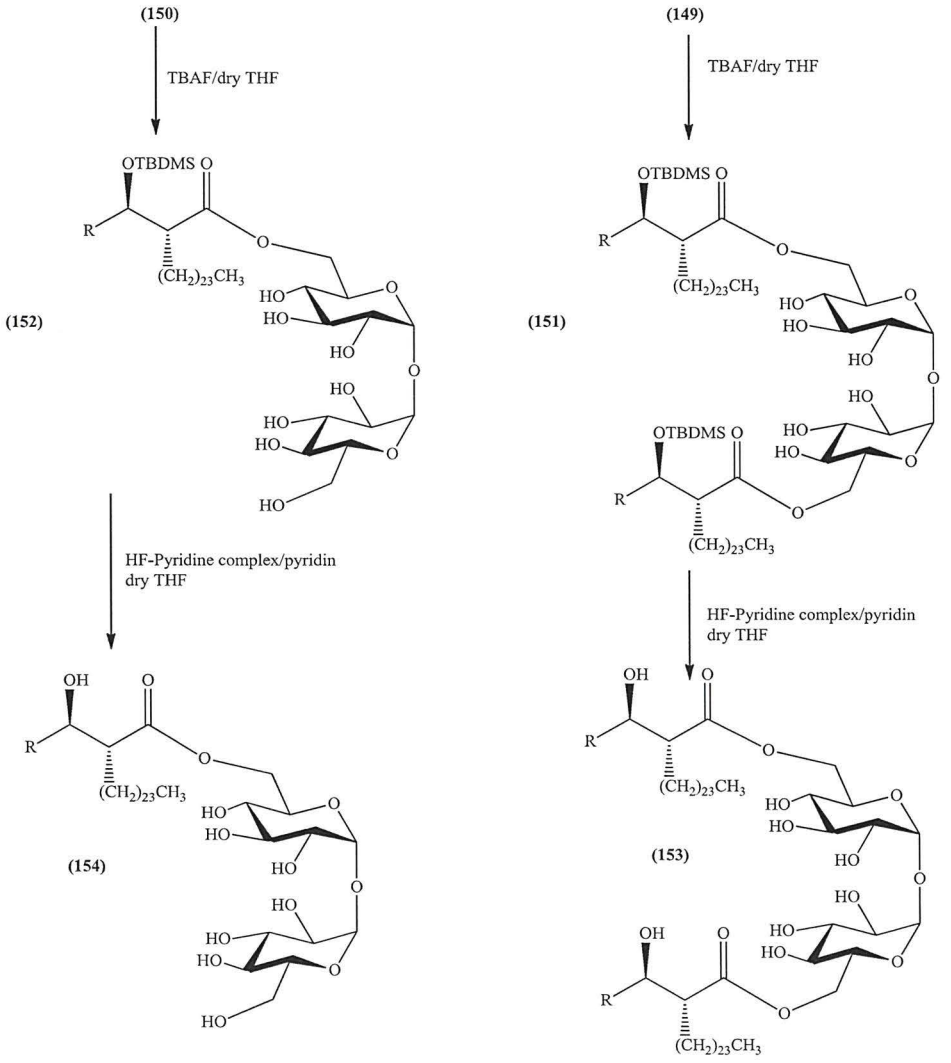
In early attempts, Tocanne and Toubiana prepared silyl protected trehalose to synthesize cord factors.[183,184] Their synthesis starts with the trimethylsilyl protection of the secondary hydroxyl group of the trehalose, and then the primary hydroxyl group was substituted by iodine, to serve as a good leaving group. Finally the esterification of protected trehalose with the potassium salt of a natural mycolic acid was carried out, followed by silyl deprotection to give TDM. Later, Data and Takayama, reported another method; by coupling silyl protected corynomycolic acid with trehalose using dicyclohexylcarbodiimide (DCC) and 4-dimethylamino-pyridine (DMAP).[185]

The first synthesis of cord factors employing synthetic enantiopure mycolic acids was published in 2009 by the Baird group.[170] This was achieved through the reaction of TBDMS protected mycolic acid **(147)** with trimethylsilyl protected trehalose **(148)** using 1-(3-dimethylaminopropyl-3-ethylcarbodiimide hydrochloride (EDCI) and 4-dimethylamino pyridine (DMAP), in the presence of 4 °A molecular sieves in dichloromethane and stirring for six days at room temperature. The protected TDM **(149)** and TMM **(150),** were obtained **(Scheme 26)**.



Scheme 26: Esterfication of protected methoxy mycolic acid with protected trehalose

Both **(149)** and **(150)** were deprotected in two steps, firstly involving the removal of the trimethylsilyl groups from the trehalose using tetrabutylammonium fluoride (TBAF) to give **(151)** and **(152)** respectively; secondly, the removal of the TBDMS group from the alcohol in the β-position of the mycolic acid using hydrogen fluoride-pyridine complex and pyridine in dry THF at 43 °C for 17 hrs, leading to the TDM, TMM **(153)** and **(154)** respectively **(Scheme 27)**.



Scheme 27: Silyl deprotections of TDM and TMM.[170]

Following the same protocol, Baird *et al.*[170] were able to synthesize cord factors (TDM, TMM) of α-mycolic acid **(116)**, and subsequently a set of new mycolic acid cord factors have been prepared.

1.7 TB detection

There are a number of available methods for detecting TB infection, which include the Tuberculin Skin Test (TST), X-ray imaging of an infected organ, interferon γ, culture, polymerase chain reaction (PCR), and smear microscopy. These available tests all meet the WHO's standards, but they still have disadvantages such as being time consuming, high cost, interference by BCG vaccination, incorrect results, and their inability to distinguish between latent and active TB. There are some newly available tests such as serodiagnostic assays includes ELISA and biosensor detection which are simple, faster, less expensive, and give better results (these will be described below).[186,187]

1.7.1 ELISA

Enzyme Linked Immunosorbent Assay (ELISA) is a method to detect many infectious diseases such as TB.[186] The principle of the ELISA method is based on the detection of the antibodies in an infected sample through antibody–antigen reaction which produces a detectable signal. A brief description of the methodology of this system is summarised in five steps.

(1) An antigen is attached to the surface of the ELISA plastic plate.

(2) The free unbound sites of the plate are blocked with a protein such as bovine serum albumin or casein.

(3) The sample containing antibody is applied inorder to bind to the antigen, and any excess unbound antibodies are washed away.

(4) Secondary antibody linked enzyme is applied to bind to the first antibody; again any unbound secondary antibody is washed away.

(5) In the final step, a substance containing enzyme is added, and produces a detectable signal (colour change, absorbance, *etc*...) **(Figure 24)**.

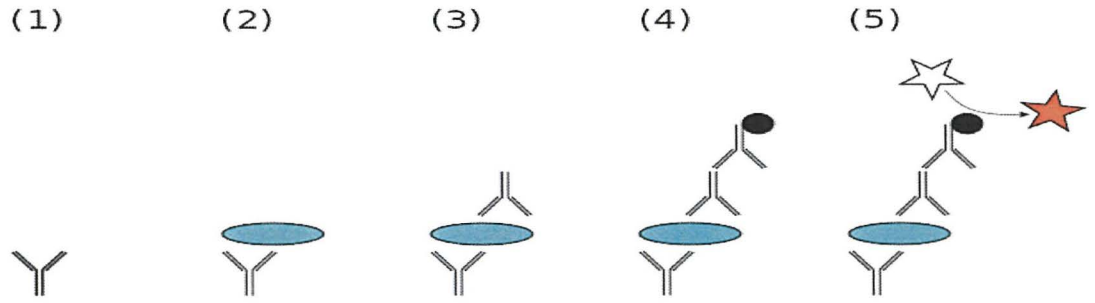


Figure 24: Schematic procedure of ELISA.[188]

Engvall and Perlmann first developed this system and call it ELISA.[189] It is known as a cheap, fast and safe immunoassay system. It was developed as a replacement system of the old radioimmunoassay which is required the use of antigens and antibodies linked with short half-life radioactive isotopes.

ELISA has been used to study the antigenicity of natural mycolic acids and their corresponding cord factors in order to detect TB, and other mycobacteria diseases.[186, 190,191,192,193] Pan *et al.*[192] showed that the anti-cord factor IgG antibody in TB patients recognizes mycolic acid structures in cord factors. Yano *et al.* [193] have also reported that the natural TDM isolated from *M.avium* is more antigenic than natural TDM from *M.tuberculosis* toward the sera of *M.avium* infected patients, and the opposite results were seen for the natural TDM from *M.tuberculosis.*

Schleicher *et al.*[191] used natural mixture of mycolic acids as antigens to detect TB in the sera of both TB infected and TB-HIV co-infected patients. Their findings confirmed that a high level of binding of mycolic acid to antibodies was found in TB positive serum samples, and the converse is true of TB negative serum samples. In addition this remained unchanged between HIV-positive and HIV-negative samples.

The results of this study were not satisfactory and recorded only 51% sensitivity and 63% specificity.[191] Sensitivity is defined as the proportion of actual positive samples correctly detected by the antigen to the total TB-positive sera, while specificity is the proportion of actual negative samples correctly detected by the antigen to the total TB-negative sera.

Later, a study by Beukes *et al.*[171] used synthetic *M. tuberculosis* mycolic acids to detect antibodies of active TB with those who are HIV positive. Three different classes of synthetic mycolic acids were used for this study. It has been found that methoxy, keto-mycolic acids were more antigenic than α-mycolic acid. Furthermore it has been found that free mycolic acids were highly recognized by antibodies compared to their corresponding methyl ester mycolic acids. This finding shows the role of the carboxylic acid functional group in the antibody binding to mycolic acids.[171]

1.7.2 Biosensor Detection of TB

The principle of this method depends on the binding of the host antibody to specific antigens such as mycolic acids, cord factors or their corresponding thiolated compounds. Thanyani *et al.* developed IAsys (interaction assay system) affinity biosensor for the detection of TB.[187] The method involves the binding of natural mycolic acids as antigens to the antibodies on the surface of a glass cuvette and then the measurement of the

refractive index by the IAsys biosensor at different time intervals. The change in the refractive index correlates with the amount of antigen-antibody binding.[187]

An electrochemical biosensor was developed by Mathebula *et al.*[194] to distinguish between TB-positive and TB-negative serum samples. The method is based on the binding of mycolic acids with the previously formed self-assembled mono-layer (SAM) of *N*-(2-mercaptoethyl)octadecanamide on a gold electrode. The distinction between TB-positive and TB-negative can be made by measuring the difference in impedance signals generated using electro impedance spectroscopy (EIS). The same group conducted further work toward the development of a better electrochemical biosensor based on gold coated with mycolic acids.[195] An optoelectronic biosensor was also developed by Sliva *et al.*[196] to identify *M. tuberculosis.* The method involves the use of gold nanoparticles coated to thiol modified oligonucleotides in the presence of amplified DNA samples derived from *M. tuberculosis*, and then identification of mycobacteria based on the colour changes detected by a colorimetric method.[196]

1.8 Thiol modified mycolic acids

Although the synthetic, natural mycolic acids and their cord factors can be used as an antigen on the serodiagnostic assays includes ELISA and biosensors for TB detection, the sensitivity and specificity is still not high enough to meet the WHO standards. Therefore there is a need for modification in the structure of the antigens to improve the sensitivity and specificity of the assays. It has been suggested that gold coated (Au-S bond) to thiol modified mycolic acids or their corresponding cord factors might have the potential to detect TB better than mycolic acids.

Thirty years ago, Nuzzo *et al.*[197] reported for the first time the formation of self-assembled monolayers of alkyl disulfides on a gold surface.

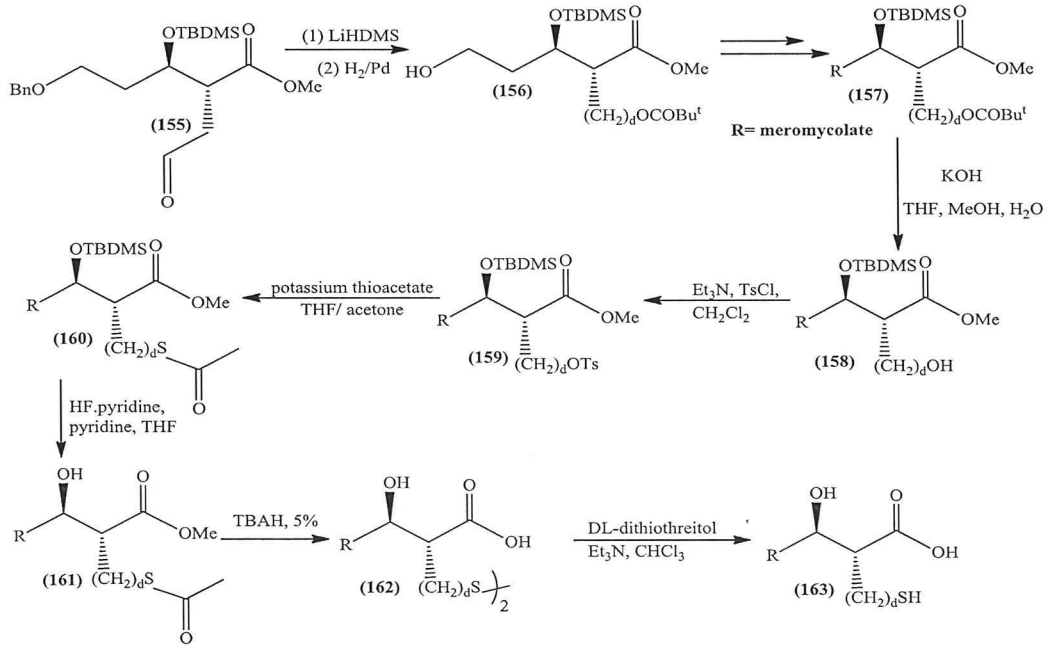
A recent study by Bourg *et al.*[198] used X-ray photoelectron spectroscopy (XPS) to study the Au-S bond. It has been found that the thiol group has an affinity to coat on the surface of gold to form self-assembled monolayers of Au-S bond on planar gold (2D) and on gold nanoparticles (3D). The same study also established that the oxidation state of Au is 0 in both the 2D and 3D self-assembled monolayers.[198]

It has been proved that both thiols and disulfides can bind to the gold surface to form a Au-S bond.[199] Furthermore, the thiols and disulphides have a specific affinity for gold regardless of the presence of other functional groups present in the thiol or disulphide structure, and the same is true for gold due to it is inactivity towards functional groups

(e.g. COOR, COOH, X, CN, NH_2, OH, CO).[200,201] This property could be important to allow for the coating of multifunctional thiolated compounds on a gold surface e.g. thiol modified mycolic acid or cord factors.

The first synthesis of thiol modified mycolic acids was reported by Baird *et al.* in 2013.[202] The first approach involved the synthesis of the thiol modified mycolic motif part with a C14 α-alkyl chain length. The second approach involved the synthesis of the full thiolated mycolic acids (α, methoxy mycolic acids). The generalised synthetic route to the thiolated mycolic acids is described below.

The aldehyde (**155**) was prepared using similar methods to those described before. The a modified Julia-Kocienski coupling reaction was selected to extend the α-alkyl chain and install a terminal tertiary butyl ester group; catalytic hydrogentation of the *E/Z*-mixture of alkenes obtained and debenzylation gave the modified mycolic motif compound (**156**). In this stage, the compound **(156)** could be linked to different meromycolate units to give **(157)** using methods described earlier**.** Hydrolysis of the terminal tertiary butyl ester of the α-alkyl chain of compound **(157)**, followed by tosylation gave the ester (**159**), which was then converted into the thioacetate (**160**) using potassium thioacetate. Silyl group deprotection was achieved by using HF.pyridine complex and pyridine to give (**161**), which was then, hydrolysed using 5% aq. tetrabutyl ammonium hydroxide, which gave the disulfide (**162**) instead of the desired thiol (**163**). Finally "Cleland's reagent" dithiothreitol (DDT) was used to cleave the disulfide (**162**) to give the desired thiolated mycolic acid (**163**) **(Scheme 28)**.



Scheme 28: Synthesis of thiolated mycolic acids

Chapter 2

Synthesis of mycobacterial wax esters and corresponding ω-carboxy mycolic acids

2.1 The aims:

The aims of this chapter are to discuss the first synthesis of two wax esters **(164)**, **(166)** and their corresponding ω-carboxy mycolic acids **(165)**, **(167)** with the right chain length and stereochemistry to match the natural compounds, which have been isolated form *M.avium* and *M.gordonae* **(Figure 25)**. The synthetic wax esters and their corresponding ω-carboxy mycolic acids would be used as specific antigens to distinguish *M.avium* or *M.gordonae* from *M.tuberculosis* specifically in serodiagnostic assays. In addition another wax ester **(168)** was also prepared with longer chain lengths than those reported from *M.avium* **(164)** and *M.gordonae* **(166)**, in order to study whether the chain length has any effect on the antigenicity **(Figure25)**.

2.1.1 Overview

The symptoms of *M.avium* infection are similar to *M.tuberculosis*, but the treatment is different, so a selective distinction between *M.avium* and *M.tuberculosis* is required. The major difference between the constituents of *M.avium* and *M.tuberculosis* is that the former possesses wax ester but not methoxy-mycolic acids, while the latter possesses methoxy-mycolic acids but not wax esters. Therefore, these synthetic wax esters could be used to distinguish between these two infections.

In two different studies, *M.avium* wax ester **(164)** was isolated and analysed by Minikin *et al*, and *M.gordonae* wax ester **(166)** by Astola *et al*.[107,108] Interestingly, it was found that in the structure of *M.avium* wax ester **(164)**, the chain length on the right hand side of the α-methyl-*trans*-cyclopropane is eighteen carbon units, and on the left hand side is sixteen carbon units, while opposite chain length was found for *M.gordonae* wax ester **(166)**.

Both of the *M.avium* and *M.gordonae* wax esters were synthesized in this work in order to study the effect of the chain length on the biological activities and to test their suitability as specific antigens to detect mycobacterial infections in serodiagnostic assays. For the above reason, another wax ester **(168)** was also prepared containing nineteen carbon units on the right hand side of the α-methyl-*trans*-cyclopropane, sixteen

carbon units on the left hand side, and C23 carbon unit on the α-alkyl chain instead of C21.

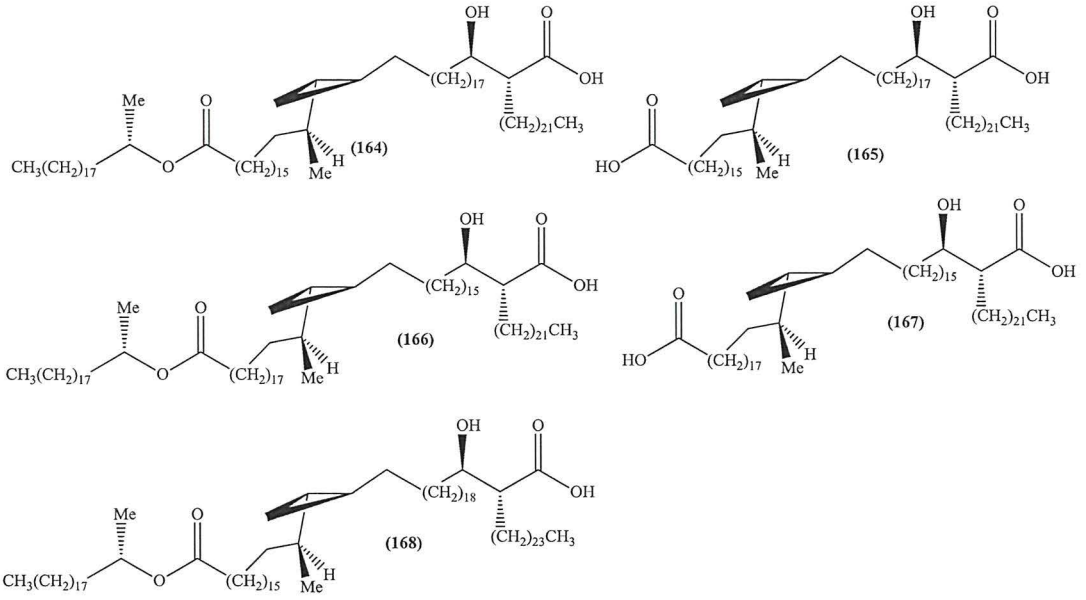
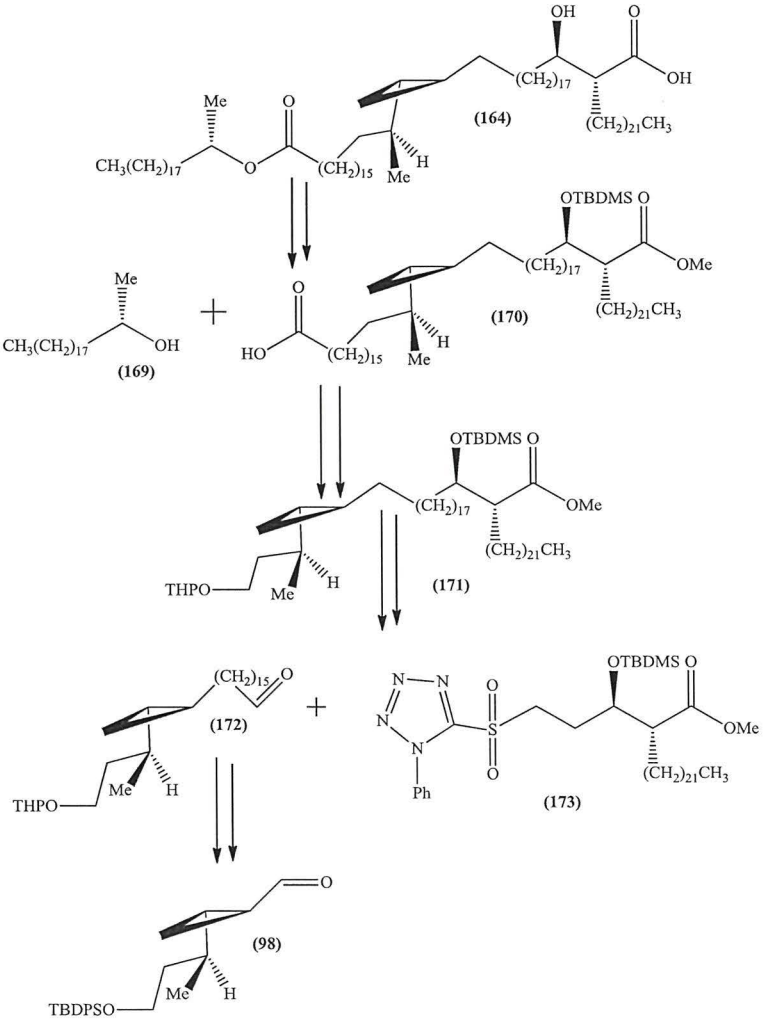


Figure 25: Synthesized wax esters and their corresponding ω-carboxy mycolic acids

2.2 Synthesis of *M.avium* wax ester and its corresponding ω-carboxy mycolic acid

The synthetic route of *M.avium* wax ester can be summarised in the following steps. The building block is the chain extended α-methyl-*trans*-cyclopropane aldehyde unit **(172)**. Chain extension of both of the sides of **(172)**, firstly the right side with mycolic motif sulphone **(173),** secondly the left side with acid chain to give **(170)**. Finally, esterification of **(170)** with (*S*)-eicosan-2-ol **(169)** gave the complete wax ester **(164)** **(Scheme 29)**.

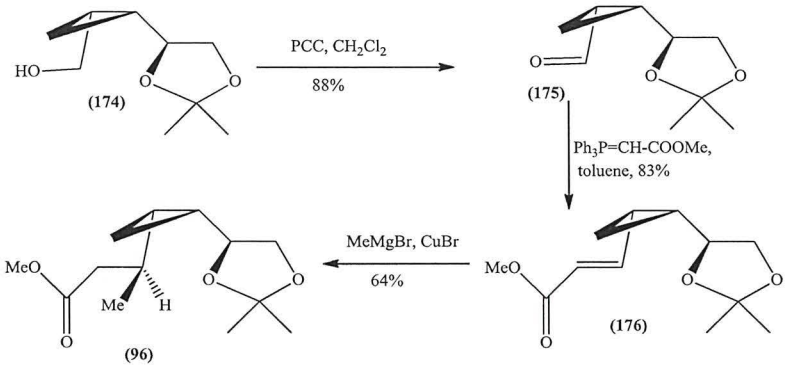


Scheme 29: Retrosynthetic route to the complete wax ester **(164)**

2.2.1 Synthesis of α-methyl *trans*-cyclopropane aldehyde (98)

The alcohol **(174)** obtained as described in the literature,[203] was provided by Dr.Al-dulayymi.

Oxidation of the alcohol **(174)** was performed with PCC (pyridinium chlorochromate) to give **(175)**. A Wittig reaction was then carried out on the aldehyde **(175)** with methoxycarbonylmethylenetriphenyl phosphorane in toluene leading to the α,β-unsaturated ester **(176)**. Methylation of **(176)** using methyl magnesium bromide and copper bromide in THF at –40 ^{0}C gave **(96)** **(Scheme 30)**.[115,141]



Scheme 30: Synthesis of the α-methyl-*cis*-cyclopropane

Addition of the methyl group formed a new carbon-carbon bond at the β-position of the ester adjacent to the *cis*-cyclopropane, which was achieved by a diastereoselective Michael addition.[204] The new carbon-carbon bond was formed between an organocopper reagent (obtained from methylmagnesium bromide and copper bromide) and the α,β-unsaturated ester. The magnesium is coordinated to both sides with two oxygen atoms of the carbonyl and the dioxolane ring, which allows for the new C-C bond to form with the α,β-unsaturated ester in high stereoselectivity, resulting in the formation of the mainly one isomer of α-methyl -*cis*-cyclopropane **(96)**. The stereochemistry of the α-methyl-*cis*-cyclopropane unit **(96)** was proved earlier by Al-dulayymi *et al.*[115] The mechanism is shown below **(Figure 26)**.

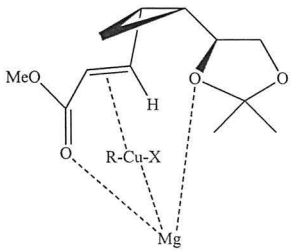
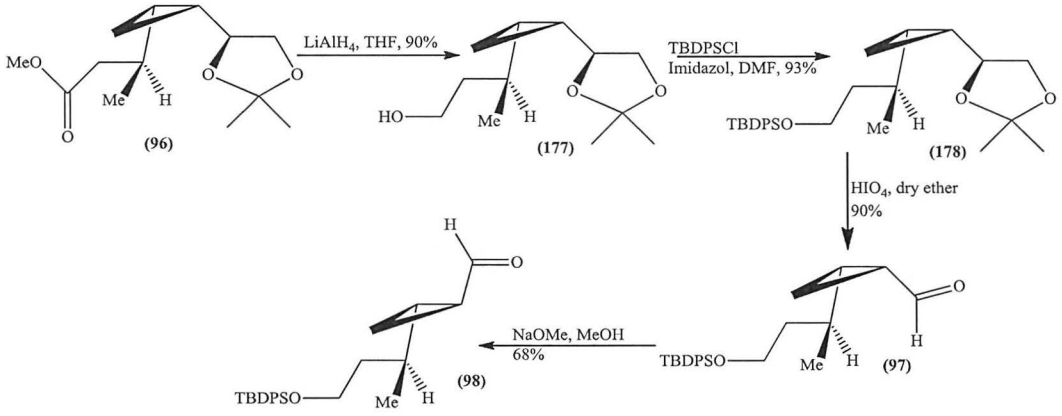


Figure 26: The mechanism of stereoselective methylation via Michael addition reaction

The α-methyl-*cis*-cyclopropane **(96)** was reduced using lithium aluminium hydride in dry THF giving alcohol **(177)**, followed by protection with *tert*-butyldiphenylsilyl chloride in DMF in the presence of imidazole gave compound **(178)**. This silyl protecting group was selected due to the need for its survival in the acid medium required in the next step. Periodic acid was used for deprotection of the acetal **(178)** and cleavage of the resulting diol to give the aldehyde **(97)** in 95% yield. Finally the aldehyde **(97)** was epimerised with sodium methoxide in MeOH under reflux for 69 hrs to give the *trans* aldehyde **(98) (Scheme 31)**.



Scheme 31: Synthesis of the α-methyl-*trans*-cyclopropane aldehyde

All of the above compounds showed identical analysis data (NMR, specific rotation) to those in the literature, and the experimental procedures are given in the appendix.[115]

The mechanism of the epimerisation involves the attack of methoxide ion at the hydrogen adjacent to the aldehyde giving an anion **(179)**, and then rearrangement to the more stable configuration, thermodynamically lower in energy (*trans*) **(180)**. The process is slow in cyclopropanes because of the increased strain in the planar intermediate **(Figure 27)**.

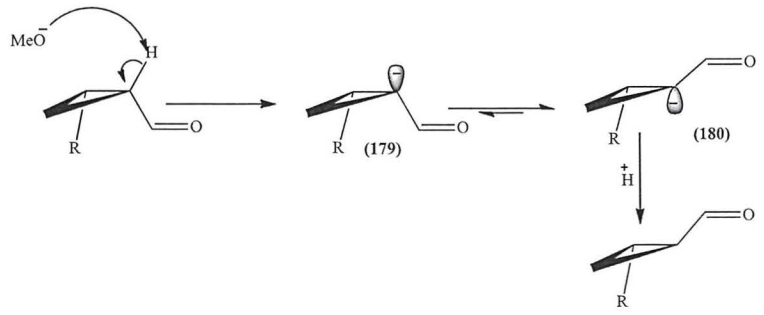
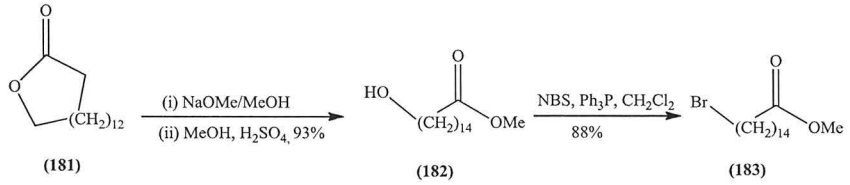


Figure 27: Mechanism of epimerization

The epimerisation was an important step because the natural wax ester contains α-methyl-*trans*-cyclopropane. This was proved earlier by Al-dulayymi *et al.*[115]

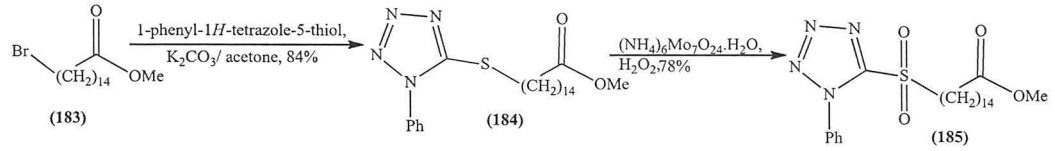
2.2.2 Synthesis of sulfone (185) for chain extension

The α-methyl-*trans*-cyclopropane (**98**) needs to be chain extended through a Julia-Kocienski reaction with the fourteen carbon chain sulfone **(185)**. The sulfone **(185)** was prepared starting with pentadecanolide **(181)**. Ring opening of **(181)** was achieved by adding it to freshly prepared sodium methoxide solution to give (**182**). The proton NMR showed a singlet at 3.67 ppm representing the methyl ester protons. A triplet was seen at 3.66 ppm (*J* 6.6 Hz) corresponding to the two protons adjacent to the hydroxyl group, and another triplet at 2.31 ppm (*J* 7.55 Hz) corresponding to another two protons next to the carbonyl group. The remaining protons appeared as two multiplets, the first one at 1.64 ppm integrated for four protons, the other at 1.36–1.25 ppm integrated for twenty protons. The carbon NMR spectrum showed the signals of the quaternary carbon at 174.4 ppm, the carbon bearing the hydroxyl group at 63.11 ppm, while the methyl ester carbon appeared 51.45 ppm. The next step was bromination using triphenylphosphine and *N*-bromosuccinimide in dichloromethane to give bromoester (**183**). The proton NMR spectrum of **(183)** showed a triplet at 3.41 ppm (*J* 6.6 Hz) integrating to two protons corresponding to the methylene next to bromine **(Scheme 32)**.[205]



Scheme 32: Ring opening of pentadecanolide

The bromo-ester **(183)** was converted into sulphide (**184**) using 1-phenyl-1*H*-tetrazol-5-thiol with potassium carbonate for 16 h at room temperature. The proton NMR spectrum showed the signals of the phenyl group as a multiplet at 7.74–7.59 ppm corresponding to the five aromatic protons, and the two protons next to the sulfanyl group appeared as a triplet at 3.40 ppm (*J* 7.4 Hz). The carbon NMR showed phenyl group signals at 133.8, 130.7, 129.8, 123.9 ppm. The last step was the oxidation of the sulphide **(184)** using hydrogen peroxide with ammonium molybdate to prepare sulphone **(185)** (**Scheme 33**).[206]



Scheme 33: Synthesis of the sulfone **(181)**

Evidence for formation of the sulfone **(185)** was that the two protons (H_a and $H_{a'}$) next to the sulphone group appeared as a distorted triplet split in to two patterns, which means that the protons are not magnetically equivalent **(Figure 28)**. This signal observed for the sulfone **(185)** is an example of the AA'BB' system in Newman projection. A and A' and B and B' are not magnetically equivalent, where H_A shows a *cis*- splitting to H_B and *trans*- splitting to $H_{B'}$ **(Figure 28)**.

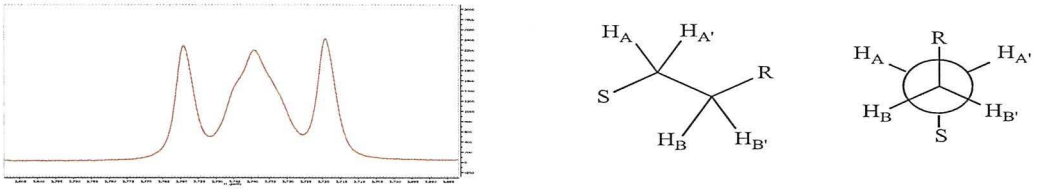
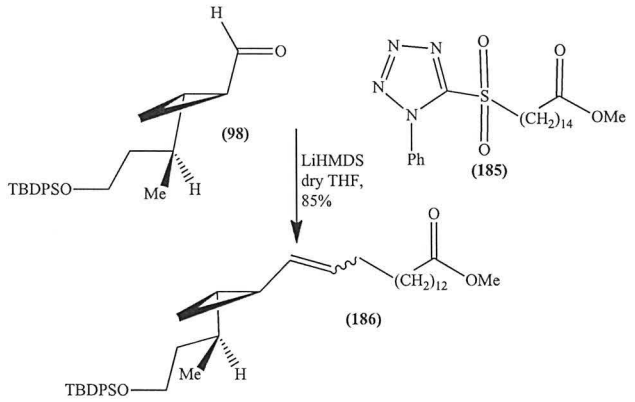


Figure 28: The characteristic signal of the two protons next to the sulfonyl group

2.2.3 Julia-Kocienski reaction between aldehyde (98) and sulphone (185)

The sulphone **(185)** was coupled with aldehyde **(98)** in a Julia-Kocienski reaction using lithium *bis*(trimethylsilylamide) (LiHMDS) in dry THF to give a mixture of *E/Z*-alkenes **(186) (Scheme 34)**.



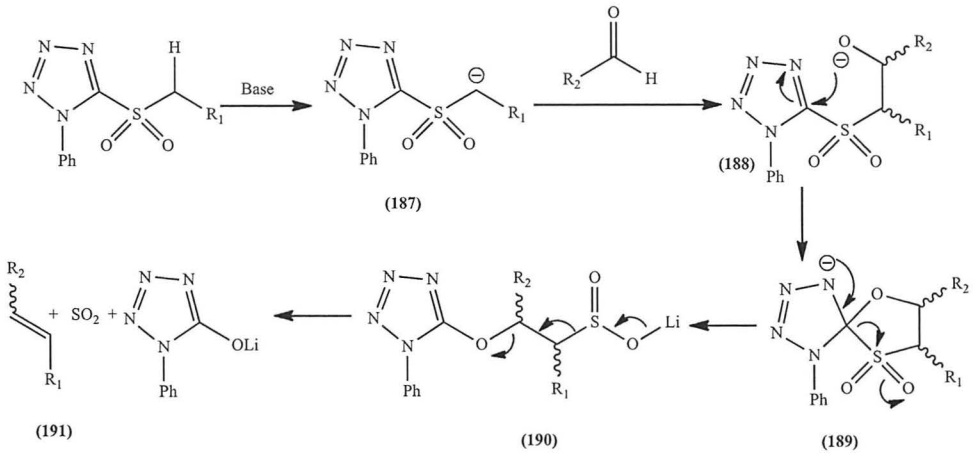
Scheme 34: Chain extension of the aldehyde (**98**) by a Julia-Kocienski reaction

2.2.3.1 An overview of the Julia-Kocienski olefination reaction

The coupling reaction between aldehydes and phenyl sulphones was first described by Marc Julia in 1973.[207] Later, the reaction was modified by Kocienski and Lythgoe, and named the Julia-Kocienski olefination reaction.[208] The reaction was then further modified by Sylvestre Julia.[209] They replaced the use of phenylsulfones with a new heteroarylsulfones, since then named as the modified Julia-Kocienski olefination reaction.

The mechanism of the reaction starts with the reaction of metallated sulfone (**187**) with the aldehyde to form a β-alkoxysulfone intermediate (**188**). Due to the instability of the

newly formed intermediate, it undergoes a Smiles rearrangement via the intermediate (**189**) with the transfer of the negative charge from sulfur to oxygen. Finally elimination of sulphur dioxide and lithium 1-phenyl-1*H*-tetrazolone from (**190**) gives the alkene (**191**) as a mixture of *E*- and *Z*-isomers (**Scheme 35**).



Scheme 35: Mechanism of the modified Julia-Kocienski reaction

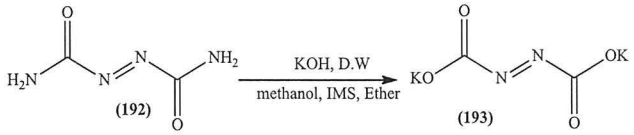
Julia coupling reactions are the best method for preparation of the long chain alkenes in the present work because the sulphonyl compound is stable and easy to prepare, and because of the ease of purification of the alkenes produced. Furthermore the by-product of the Julia coupling reaction (lithium 1-phenyl-1*H*-tetrazolone) 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.

2.2.4 Hydrogenation of the double bond

The most common procedure employed for the hydrogenation of double bonds is the use of palladium on carbon as a catalyst and an atmosphere of hydrogen gas. This was not used for the hydrogenation of cyclopropane containing compounds, because this might both hydrogenate the double bond and open the cyclopropane ring. Therefore the use of milder conditions for hydrogenation of the double bond was preferred. Di-imide (HN=NH) is found to be a very mild agent for hydrogenation, and can be generated in *situ* from the oxidation of hydrazine or decarboxylation of potassium azodicarboxylate.[210,211,212]

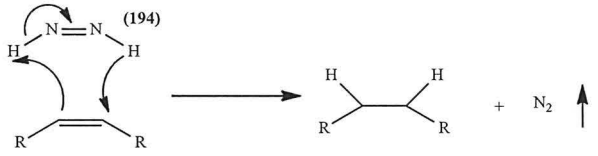
Dipotassium azodicarboxylate (**193**) was selected as a di-imide source for hydrogenation of the double bond of the compound (**186**), and for the hydrogenation of all of the cyclopropane containing alkenes prepared throughout this work. It was prepared by

addition of azodicarbonamide (**192**) in portions to a stirred solution of potassium hydroxide in de-ionised water (D.W) at 0 °C to give an unstable bright yellow solid compound after work up, which was kept in the freezer **(Scheme 36)**.



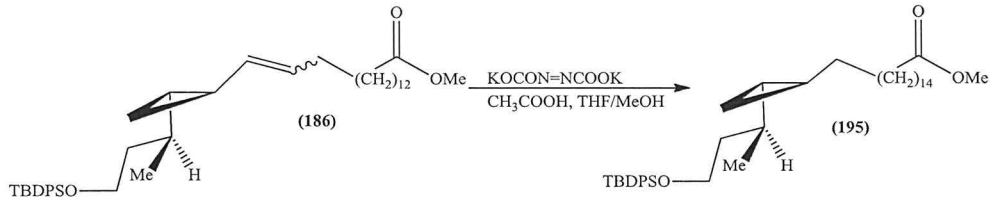
Scheme 36: Preparation of dipotassium azodicarboxylate

The di–imide **(194)** was generated from dipotassium azodicarboxylate **(193)** by decarboxylation after protonation in the presence of glacial acetic acid and methanol. The general mechanism of hydrogenation using di-imide is shown below **(Scheme 37)**.



Scheme 37: The mechanism of hydrogenation by di-imide

The mixture of *E/Z*-alkenes **(186)** was dissolved in 20:1 THF/methanol, and then dipotassium azodicarboxylate was added at 5 °C. A solution of acetic acid:THF (1:1) was added over 72 hrs. to give ester **(195) (Scheme 38)**.



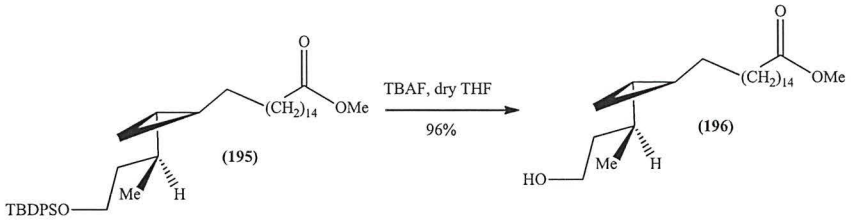
Scheme 38: Hydrogenation of a cyclopropane alkene with di-imide

The proton NMR confirmed the disappearance of olefin signals, and showed a broad multiplet at 7.68 ppm integrating to four protons and another multiplet at 7.42 ppm integrating for the other six protons of the diphenyl group. A multiplet was seen at 3.77–3.71 ppm integrating to two protons corresponding to the methylene next to the protecting group, and a singlet at 3.67 ppm for the methoxy ester. The two protons next to the carbonyl group appeared as a triplet at 2.30 ppm (*J* 7.55 Hz). A broad multiplet appeared at 1.43–1.26 ppm corresponding to the long chain of methylene groups. The *tert*-butyl protons of the protecting group showed a singlet at 1.05 ppm integrating for nine protons. The α-methyl cyclopropane protons appeared as three multiplets at 0.89,

0.42, 0.13 ppm respectively. The carbon NMR spectrum showed the signals of the carbonyl carbon at 174.2 ppm.

2.2.5 Silyl deprotection

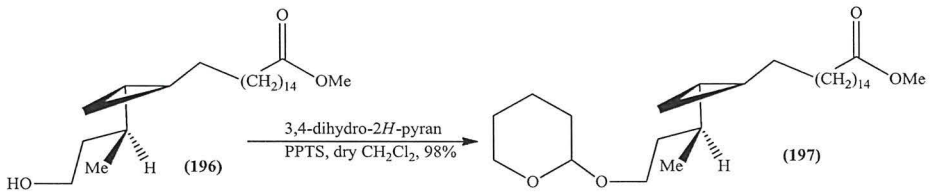
Removal of the protecting group from **(195)** was achieved using tetra-*n*-butyl ammonium fluoride (TBAF) in dry THF to give the alcohol **(196)** in 96% yield **(Scheme 39)**. The disappearance of the signals of the protecting group in the proton and carbon NMR spectrum confirmed the success of the reaction. The two protons next to the hydroxyl group appeared as a multiplet at 3.78–3.69 ppm.



Scheme 39: Silyl deprotection of **(195)**

2.2.6 Re-protection with a dihydropyranyl group

The alcohol **(196)** was re-protected with 3,4-dihydro-2*H*-pyran in dichloromethane in the presence of pyridinium-p-toluene–sulfonate (PPTS) as the ester **(197)** in 98% yield **(Scheme 40)**.[213] The reason for the removal of the silyl protecting group from the compound **(196)**, and then re-protecting it with different protecting group is the presence of another silyl protecting group present in the structure of mycolic motif part that will be linked in later steps.

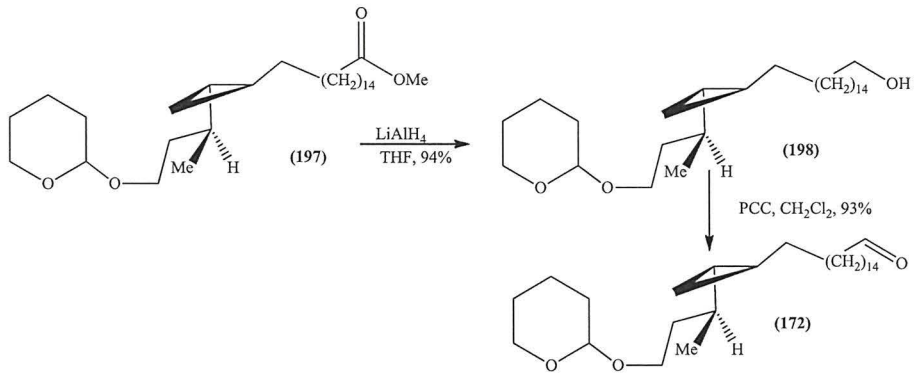


Scheme 40: Protection the alcohol with a THP group

The proton NMR showed the signals of the THP group starting from broad multiplet at 4.57 ppm integrated for one proton corresponding to the acetal proton of the THP group, and a multiplet at 3.88 ppm integrated for two protons adjacent to the oxygen of the THP ring, while the remaining signals of the THP group appeared between 1.69–1.53 ppm. Carbon NMR also showed THP signals at 99.0, 98.9 ppm for the carbon of the acetal in both stereoisomers.

2.2.7 Reduction and oxidation

The protected methyl ester **(197)** was reduced with lithium aluminium hydride in THF, and after quenching with sat. aq. solution of sodium sulfate decahydrate, gave alcohol **(198) (Scheme 41)**.



Scheme 41: Preparation of the chain extended α-methyl-*trans*-cyclopropane aldehyde

The proton NMR spectrum of compound **(198)** confirmed the disappearance of the sharp singlet belonging to the methoxy ester, and instead showed a triplet at 3.64 ppm (*J* 6.6 Hz) for the two protons next to the alcohol. The carbon NMR spectrum also showed a signal at 63 ppm for the carbon bonded to the hydroxyl group.

The alcohol **(198)** was oxidised with pyridinium chlorochromate (PCC) in dichloromethane, which gave aldehyde **(172) (Scheme 41)**. PCC is a mild oxidizing agent and widely used for the oxidation of primary alcohols to aldehydes without further oxidation to carboxylic acids. The oxidation occurs under neutral conditions, as the produced hydrochloric acid is neutralised by pyridinium ions. This has advantage of not removing the existing acid labile THP group on the left side of the cyclopropane.

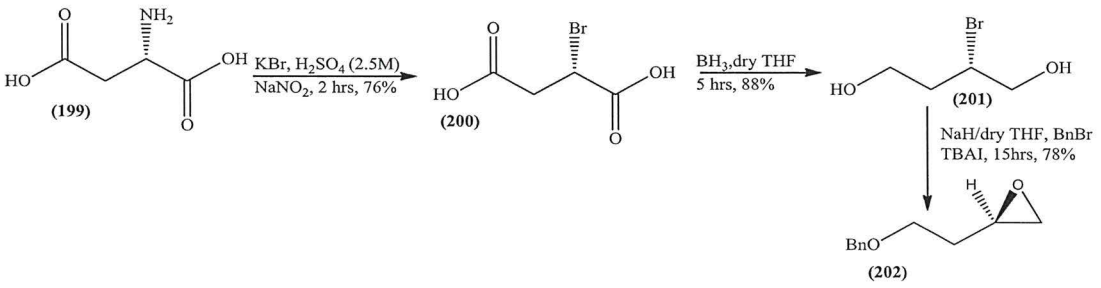
The proton NMR for the aldehyde **(172)** showed a triplet at 9.77 ppm (*J* 1.9 Hz) for the aldehyde proton, and a doublet of triplets at 2.42 ppm (*J* 1.9, 7.4 Hz) integrating for two protons next to the carbonyl group. The most significant signal of the THP group was a broad multiplet at 4.61–4.53 ppm integrating for one proton corresponding to the acetal proton. Another multiplet was seen at 3.56–3.40 ppm corresponding to the methylene adjacent to the protecting group. A doublet was seen at 0.96 ppm (*J* 6.9 Hz) for the methyl next to cyclopropane, while the cyclopropane protons appeared as three multiplets at 0.91–0.78, 0.59–0.41, 0.29–0.10 ppm respectively. The carbon NMR signal showed the carbon for the aldehyde at 203 ppm, and the THP acetal carbon at 99.0, 98.9 ppm for the both stereoisomers.

2.2.8 Synthesis of mycolic motif sulfone (173)

2.2.8.1 Synthesis of β-hydroxy ester (206)

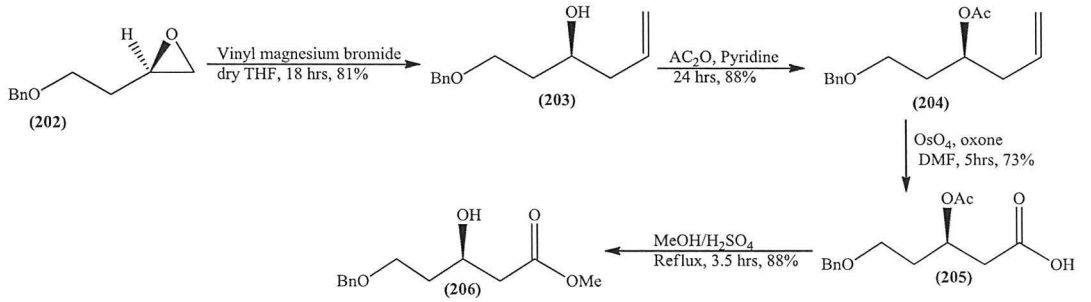
The mycolic motif part is similar in all mycolic acids, containing the α-alkyl β-hydroxy acid fragment, except for a slight variation in the chain length at the α-alkyl position. Several methods have been reported for the synthesis of the mycolic motif part, but that of Koza *et al.* is found the most successful.[135] L-aspartic acid is the starting material in this approach.

L-Aspartic acid **(199)** was converted into bromosuccinic acid **(200)** in a nucleophilic substitution reaction of a diazonium salt using potassium bromide, sulphuric acid, and sodium nitrite solution. The next step was the reduction of bromo–succinic acid **(200)** to (*S*)-2-bromo-1,4-butanediol **(201)** using BH_3 (borane in THF) at –10 °C. The diol **(201)** was converted successfully into epoxide **(202)** using sodium hydride at –10 °C, which was then protected with a benzyl group in a one pot reaction **(Scheme 42)**.[214]



Scheme 42: The synthesis of the epoxide **(202)**

Pursuing the synthetic route for another four steps including ring opening of epoxide **(202)** in a Grignard reaction, acetylation, oxidative cleavage using osmium tetroxide (OsO_4), and esterfication using methanol and acid, gave **(206) (Scheme 43)**.

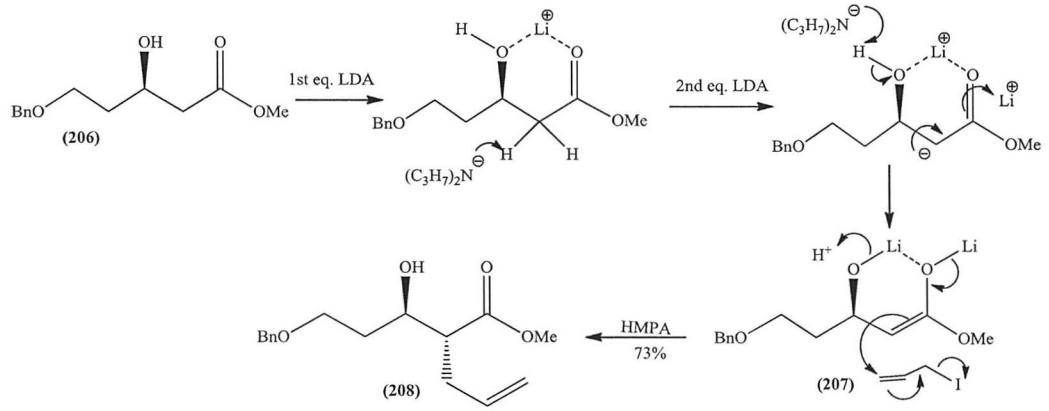


Scheme 43: Synthesis of β-hydroxy ester **(206)**

All of the above compounds showed identical NMR data to those given in the literature, and the experimental procedures are given in the appendix.[135]

2.2.8.2 The addition of α-alkyl chain (Fräter allylation)

The Fräter allylation reaction is widely used to allylate the α-position of the β-hydroxy ester.[215,216] In this work, the α-position of the 3-hydroxy pentanoate **(206)** was allylated to give the α-alkyl-β-hydroxy fragment in the (*R,R*)-configuration **(208) (Scheme 44)**. The first step is the generation of LDA (2 mol. eq) in *situ* by reacting di-isopropylamine with BuLi at –20 °C in dry THF. The β-hydroxy ester in dry THF was added to LDA solution at –55 °C and stirred between –55 °C to –50 °C for 1.5 hr. for the generation of the stable chelated *Z*-enolate (**207**). The reaction mixture was allowed to warm up to – 25 °C, and then cooled to –65 °C and allyl iodide and HMPA in dry THF were added. High diastereoselectivity was achieved for allylation due to the steric hindrance on the top face of the six-membered ring intermediate **(207)**, which allowed the allyl group to attach mainly from the bottom face producing the α-alkyl-β-hydroxy fragment in the (*R,R*)-configuration **(Scheme 44)**.[135]

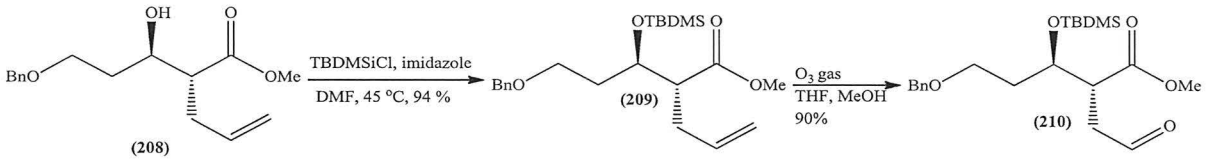


Scheme 44: The mechanism of the Fräter allylation

2.2.8.3 The chain extension of the α-allyl chain

In order to chain extend the allyl end to the required chain length in the structure of mycolic acids, protection of the β-hydroxy group and oxidative cleavage of the alkene were required **(Scheme 45)**.

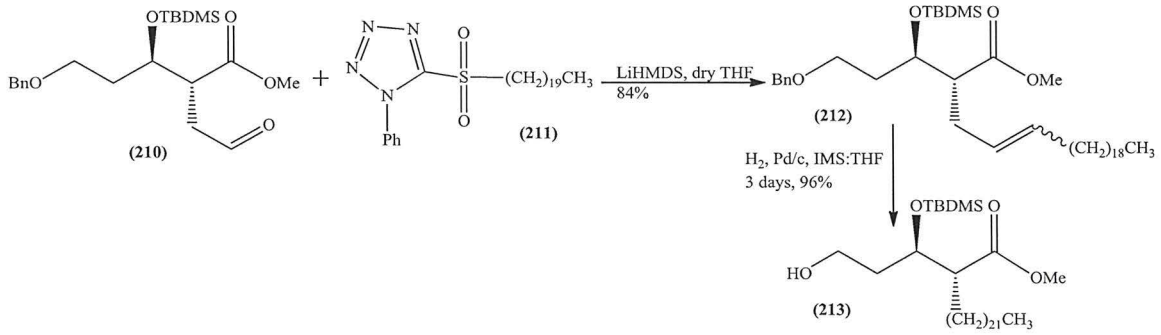
Firstly the β-hydroxy of the compound **(208)** was protected with the *tert*-butyldimethylsilyl (TBDMS) group to prevent undesired reactions in the subsequent steps. The β-hydroxy compound **(208)** was treated with imidazole and *tert*-butyldimethylsilyl chloride in DMF to give the protected conpound **(209)**.[217]



Scheme 45: The protection and oxidative cleavage of (**208**)

Secondly oxidative cleavage of the alkene (**209**) was required to obtain the aldehyde (**210**) for the chain extension. There are two common methods for alkene oxidative cleavage; either the use of OsO_4 and $NaIO_4$ in the presence of 2,6-lutidine, or ozonolysis. The former needs the use of very strong oxidizing agent such as OsO_4, which has a potential to cause safety issues to the user; also the difficulty of removing 2,6-lutidine has been always been a problem. For these reasons ozonolysis was chosen as a safer and easier method for alkene cleavage. Ozone gas was applied for 10 minutes to the alkene (**209**) in 1:1 THF/ methanol at –70 °C. The reaction was allowed to warm up and treated with acetic acid, water, followed by the addition of zinc metal, to give the aldehyde (**210**) in 90% yield.

The resulting aldehyde (**210**) was coupled with 20 carbon unit sulfone (**211**) in a modified Julia-Kocienski olefination reaction to give a mixture of *E*- and *Z*-alkenes (**212**) **(Scheme 46)**.



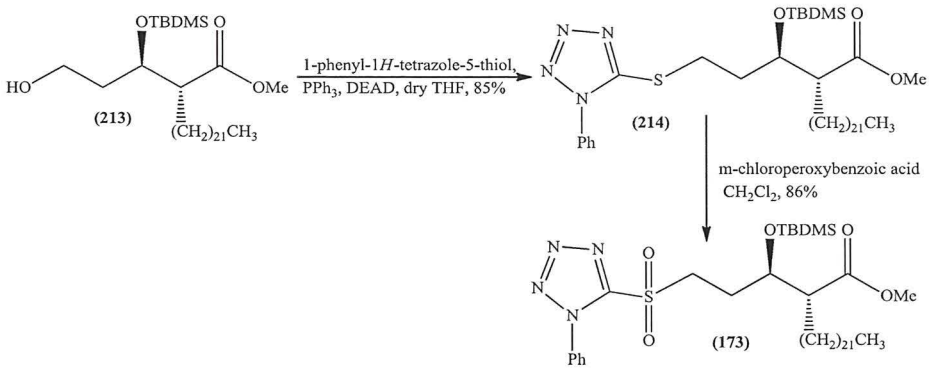
Scheme 46: The chain extension of the α-alkyl chain

The alkene (**212**) was hydrogenated and debenzylated in a one pot reaction under hydrogen gas for 3 days using Pd (10 %) on carbon as a catalyst to give the alcohol (**213**).

All of the above compounds **(Scheme 45, 46)** showed identical NMR data to those given in the literature, and the experimental procedures are given in the appendix.[135,149]

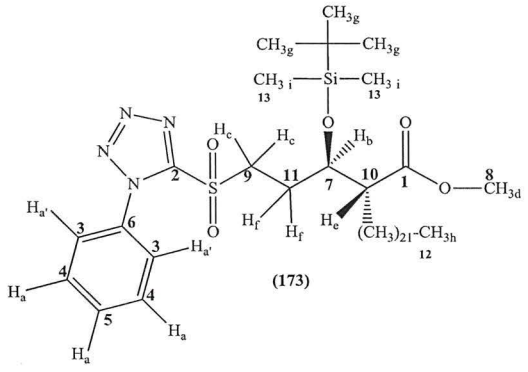
2.2.8.4 Synthesis of mycolic motif sulfone (173)

In order to link the mycolic motif with the meromycolate part, it was necessary to convert the mycolic motif alcohol **(213)** into sulphide **(214)** and then to the corresponding sulfone **(173)** **(Scheme 47)**.



Scheme 47: Synthesis of mycolic motif sulfone **(173)**

The proton NMR spectrum for compound **(214)** showed a multiplet at 7.59–7.52 ppm for the five aromatic protons, and the signals corresponding to the two protons next to the sulphide group shifted up-field and appeared as a two multiplets at 3.51–3.44 and 3.40–3.34 ppm. The NMR data of the sulfone **(173)** are summarised in **Table 1**.



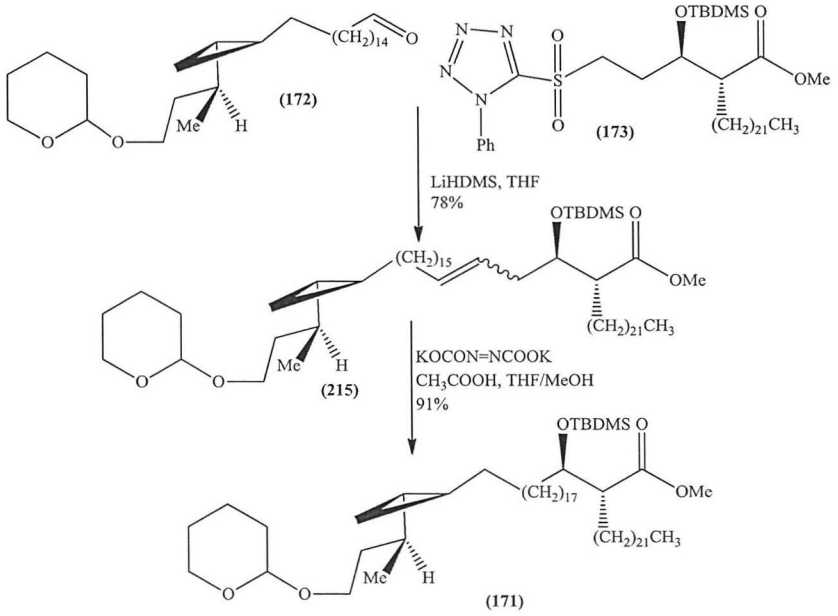
Proton	Ppm	Multip -licity	Integration	*J Hz*	Carbon	^{13}C NMR, ppm
$H_{a'}$	7.72–7.70	m	2	--	**C_1**	174.1
Ha	7.65–7.61	m	3	--	**C_2**	153.2
H_b	4.15	m	1	--	**C_3**	133.0
H_c	3.81	m	2	--	**C_4**	131.5
H_d	3.69	s	3	--	**C_5**	129.7

H_e	2.53	ddd	1	4.0, 7.4, 11.0	C_6	125.0
H_f	2.16-2.1	m	2	---	C_7	70.9
H_g	0.89	s	9	---	C_8	51.8
H_h	0.87	t	3	6.9	C_9	51.6
H_i	0.08	s	3		C_{10}	51.4
H_i	0.06	s	3		C_{11}	31.9
					C_{12}	14.1
					C_{13}	–4.5, –5.1

Table 1: The proton and carbon NMR data for mycolic motif sulfone **(173)**

2.2.9 The Julia reaction between the aldehyde (172) and sulfone (173)

The aldehyde **(172)** was coupled with the sulfone **(173)** through a modified Julia-Kocienski olefination reaction in dry tetrahydrofuran in the presence of lithium *bis*(trimethylsilyl) amide at –15 °C to give a mixture of *E*- and *Z*-alkenes **(215) (Scheme 48)**. This was followed by saturation of the double bond using dipotassium azodi carboxylate with acetic acid in MeOH/THF to give compound **(171)**.

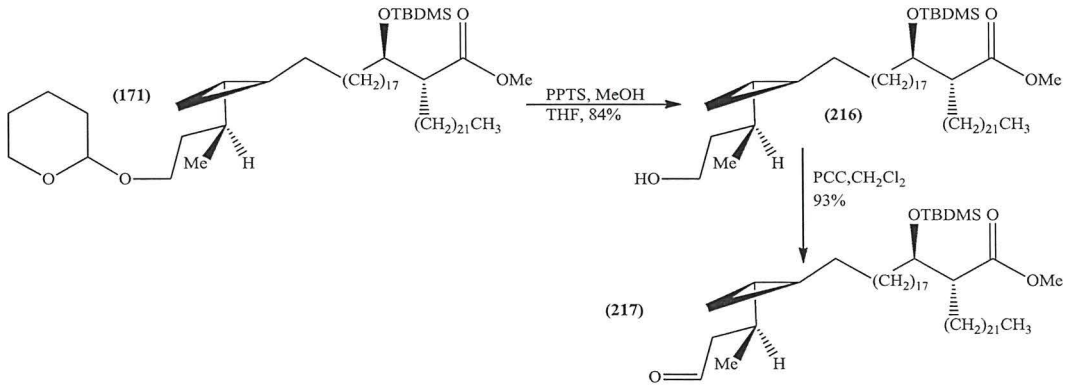


Scheme 48: The preparation of fragment **(171)**

The proton NMR for the compound **(171)** showed a broad multiplet at 4.58 ppm integrating for one proton corresponding to the acetal proton of the THP group. A broad multiplet occurring between 3.97–3.76 ppm integrated for three protons and included the β-postion proton and the two protons adjacent to the oxygen of the THP ring, while another multiplet was seen from 3.56–3.40 ppm integrating for two protons next to the THP protecting group. The α-position proton appeared as doublet of doublets of doublets at 2.53 ppm (*J* 3.7, 7.1, 10.8 Hz), and the methyl next to the cyclopropane as a doublet at 0.96 ppm (*J* 6.6 Hz). A complex multiplet appeared between 0.9–0.87 ppm integrating to the four protons corresponding to the proton of the chiral centre bearing the α-methyl next to cyclopropane and the terminal methyl group of the α-chain. The *tert*-butyl protons of the silyl group gave a singlet at 0.86 ppm, while the two methyl groups bound to silicon atom showed two sharp singlets at 0.05, and 0.02 ppm. The cyclopropane protons appeared as two multiplets at 0.57 ppm integrating for one proton, and at 0.27 ppm integrating for three protons. The carbon NMR spectrum showed the signals of the carbonyl carbon at 175.2 ppm, and signals at 99.0, 98.9 ppm for the carbons of the THP chiral centres. The β-carbon appeared at 73.2 ppm, while the α-carbon appeared at 51.5 ppm.

2.2.10 Deprotection and oxidation

The THP protecting group was removed from the compound **(171)** using pyridinium-*p*-toluenesulfonate (0.5 eq.) in 5:1 THF/methanol at 45 ° C to give the alcohol **(216)** **(Scheme 49)**. The disappearance of the signals corresponding to the THP protecting group in the proton and carbon NMR spectra confirmed the success of the reaction.



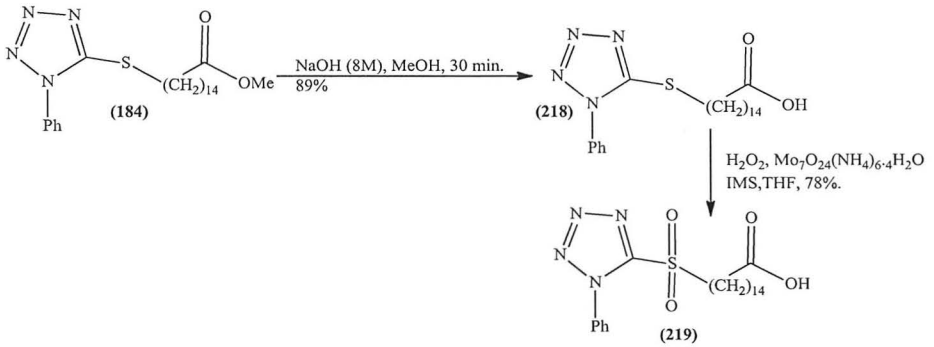
Scheme 49: Deprotection and oxidation of **(215)**

The oxidation of **(216)** using PCC gave **(217)** in 93 % yield **(Scheme 49)**. The proton NMR showed a triplet at 9.79 ppm (*J* 2.5 Hz) belonging to the aldehyde proton, and the

β-position proton appeared as a multiplet at 3.92 ppm. The carbon NMR showed a new signal for the aldehyde carbon atom at 202.9 ppm.

2.2.11 Synthesis of acid sulfone (219) for chain extension

The next stage required the coupling of the above aldehyde **(217)** with an acid sulfone **(219)** in a Julia-Kocienski olefination reaction to produce ω-carboxylic acid mono-ester (**170**). Firstly the acid sulfone **(219)** was prepared from sulphide **(184)**.



Scheme 50: Preparation of acid sulfone **(219)**

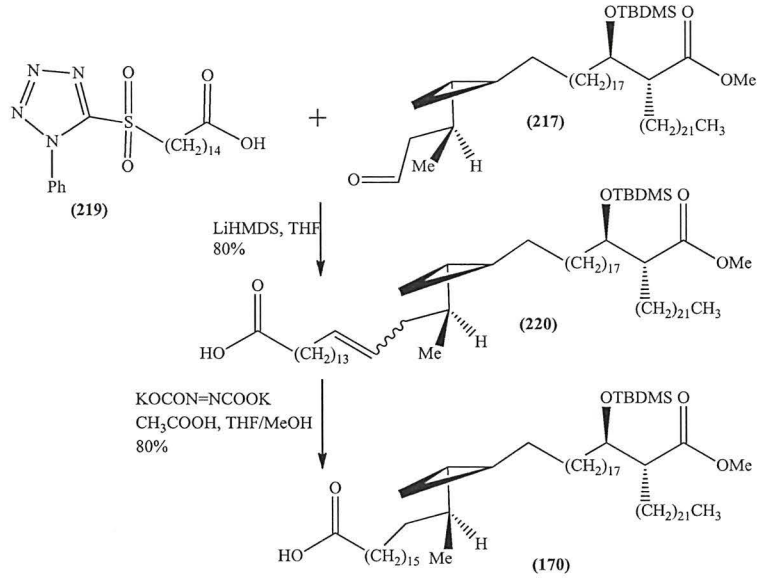
The sulphide **(184)** was hydrolysed using standard methods to give the acid sulphide **(218) (Scheme 50)**. The proton NMR spectrum showed the signals of the phenyl group as a multiplet at 7.74–7.59 ppm integrating to five protons, and a triplet at 3.40 ppm (*J* 7.4 Hz) corresponding to the two protons next to the sulphide group. Another triplet occurred at 2.36 ppm (*J* 7.5 Hz) corresponding to the other two protons next to the carbonyl group. Three pentets appeared at 1.82, 1.66 and 1.48 ppm with the same coupling constant (*J* 7.4 Hz) integrating to two protons each, while the remaining protons appeared as a multiplet at 1.30–1.26 ppm. The last step was the oxidation of the sulphide **(218)** by hydrogen peroxide with ammonium molybdate to prepare the target acid sulfone **(219)**.

2.2.12 Synthesis of ω-carboxylic acid mono-ester (170)

The chain extension of the aldehyde **(217)** to produce ω-carboxylic acid mono-ester **(170)** was one of the challenges in the synthesis of complete mycobacterial wax esters. Usually ester sulphones are used for this purpose, but in this case could not be used, because the presence of another methyl ester group on the right hand side of the compound **(217)** will produce a di-ester. The di-ester could not be hydrolysed selectively to the desired ω-dicarboxylic acid mono-ester **(170)**. After running numerous model

reactions, it was proved that the compound **(170)** could be obtained by the use of an acid sulphone **(219)**.

The aldehyde **(217)** was coupled with the acid sulphone **(219)** in a Julia-Kocienski reaction in the presence of strong base, lithium bis(trimethylsilyl) amide in dry THF at –15 °C, leading to a mixture of *E/Z*-alkenes **(220)**, which was then saturated using dipotassium azodicarboxylate and acid to give **(170)** **(Scheme 51)**.



Scheme 51: The preparation of ω-carboxylic acid mono-ester **(170)**

Parts of the expanded proton NMR spectrum of the ω-dicarboxylic acid mono-ester **(170)** are shown below in **Figure 29**. The disappearance of the signals for the alkene protons confirmed the hydrogenation. The β-position proton appeared as a doublet of triplets at 3.92 ppm (*J* 7.2, 4.6 Hz), and the methoxy group showed a sharp singlet at 3.66 ppm. The α-proton appeared as a doublet of doublets of doublets at 2.53 ppm (*J* 3.8, 7.2, 10.9 Hz), and a sharp triplet appeared at 2.36 ppm (*J* 7.5 Hz) integrating for two protons corresponding to those next to the carbonyl group. A multiplet was seen between 0.91–0.86 ppm including the protons of α-methyl next to cyclopropane and the terminal methyl group of the α-alkyl chain. The silyl protecting group protons appeared as a singlet at 0.88 ppm integrated for nine protons and another two singlets at 0.05 ppm and 0.02 ppm for the two methyl groups. The cyclopropane protons were seen as two multiplets at 0.73 and 0.51 ppm each integrating to one proton, and another multiplet at 0.26–0.07 ppm integrating to three protons. The carbon NMR showed two carbonyl carbon signals at 178.8, 175.2 ppm. The β-carbon appeared at 73.2 ppm, while the α-carbon appeared at 51.6 ppm.

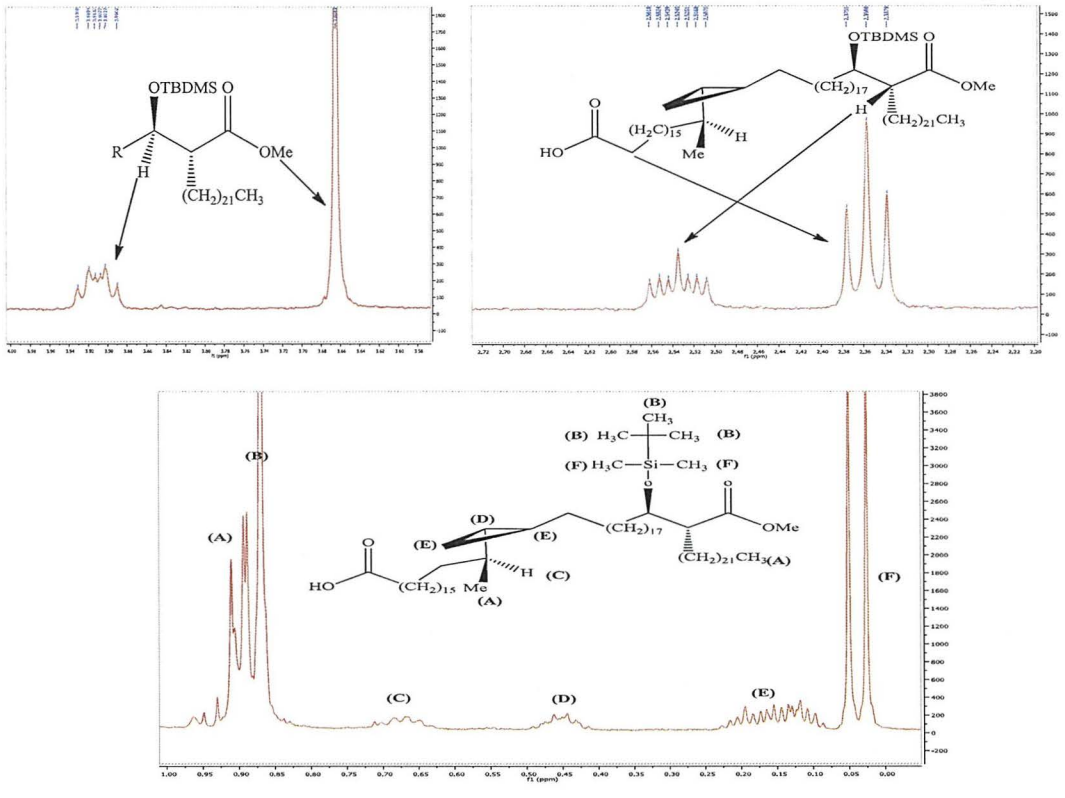
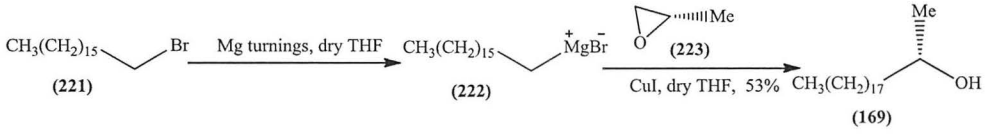


Figure 29: Expansion of the proton NMR of the ω-dicarboxylic acid mono-ester **(170)**

2.2.13 Synthesis of (*S*)-Eicosan-2-ol (169)

The ω-carboxylic acid mono-ester **(170)** required esterfication with (*S*)-eicosan-2-ol **(169)** to produce the complete wax ester. In order to synthesize (*S*)-eicosan-2-ol, firstly 1-bromoheptadecane **(221)** was added to magnesium turnings in dry THF with gentle reflux, and monitored by gas chromatography to produce heptadecylmagnesium bromide **(222)).** The freshly prepared Grignard reagent was added slowly to a stirred suspension of copper iodide at –30 °C, and then (*S*)-1-epoxypropane **(223)** was added dropwise to give the desired alcohol **(169) (Scheme 52)**.



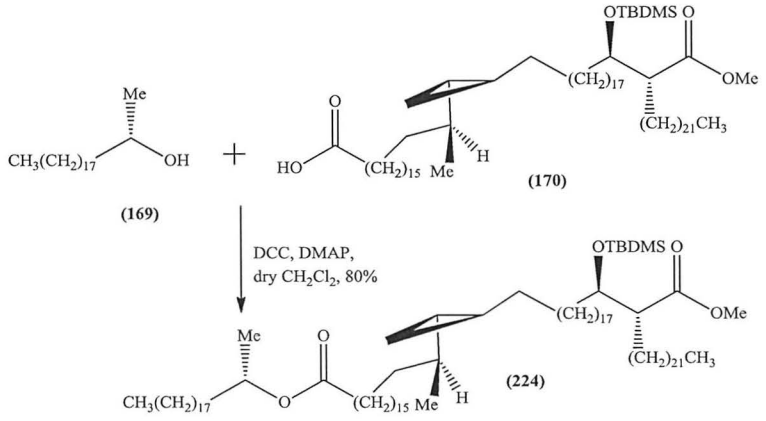
Scheme 52: the preparation of (*S*)-eicosan-2-ol **(169)** via a Grignard reaction

The proton NMR of the compound **(169)** showed the proton at the α-position as a sextet at 3.8 ppm (*J* 6.0 Hz), a doublet at 1.20 ppm (*J* 6.2 Hz) for the α-methyl, and the terminal methyl gave a triplet at 0.88 ppm (*J* 6.7 Hz). Carbon NMR also showed the α-position

carbon at 68.2 ppm, and the methyl group at 23.5 ppm. The specific rotation was compared with natural (*S*)-eicosan-2-ol; thus, the specific rotation of compound **(169)** ($[\alpha]_D$ = +3.8 (c= 1.01, ($CHCl_3$)) was very close to that of the natural compound, which gave $[\alpha]_D$ = +3.5 in $CHCl_3$).[101,102]

2.2.14 Steglich esterfication to produce complete wax ester (224)

The Steglich esterfication was applied to produce complete wax ester **(224)**. The ω-dicarboxylic acid mono-ester **(170),** (*S*)-eicosan-2-ol **(169)**, and 4-dimethyl-aminopyridine (DMAP) were dissolved in dry dichloromethane, followed by dropwise addition of dicyclohexylcarbodiimde (DCC). The reaction was stirred for 3 hrs at room temperature, giving the complete wax ester **(224)** in 80% yield **(Scheme 53)**.

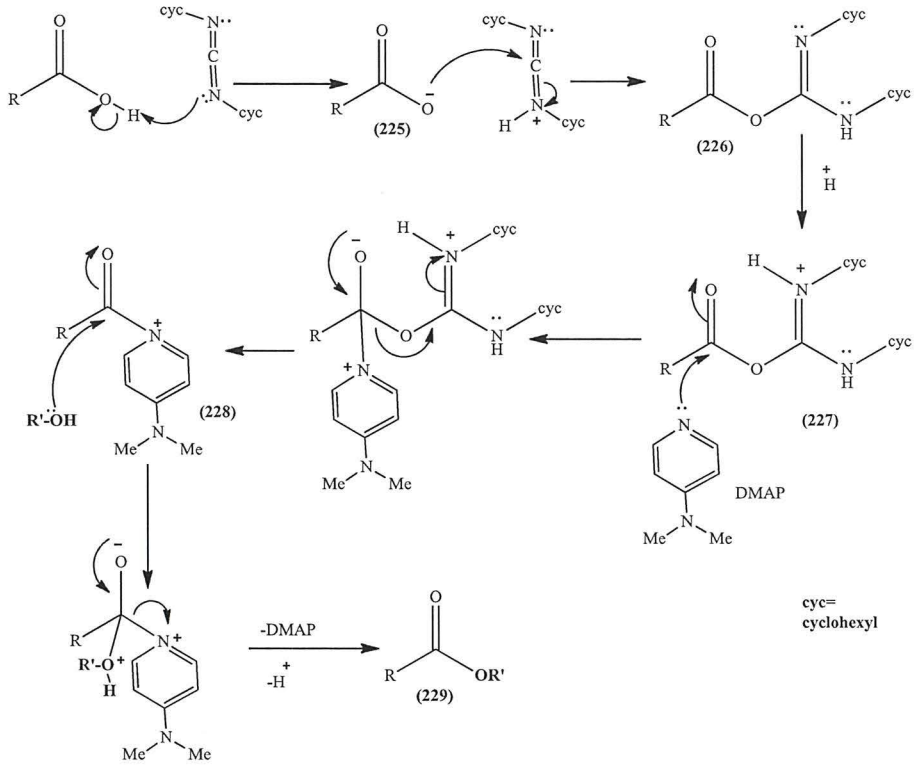


Scheme 53: preparation of complete wax ester **(224)** via Steglich esterfication

2.2.14.1 Overview on Steglich esterfication

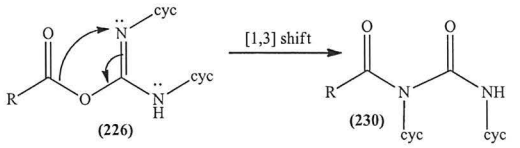
Steglich esterfication is a mild reaction for esterfication of carboxylic acids with alcohols using DCC as a coupling reagent and DMAP as a catalyst in dry dichloromethane; it was first reported by W. Steglich in 1978.[218] The Steglich esterfication has many advantages, as its conditions are very mild, it can be used for acid sensitive substrates, not producing water as a by-product, and it also can be used for sterically hindered substrates. The reaction takes place via several steps. Firstly DCC takes a proton from the carboxylic acids to form an active carboxylate anion **(225)**, which then forms *O*-acylisourea intermediate **(227)** after protonation of the **(226)**. At this stage, DMAP attacks the intermediate **(227)** to form a new reactive intermediate **(228)** after removal of dicyclohexylurea (DHU). Now the alcohol can react with the

intermediate **(228)** to form the desired ester **(229)** after the removal of DMAP and deprotonation **(Scheme 54a)**.



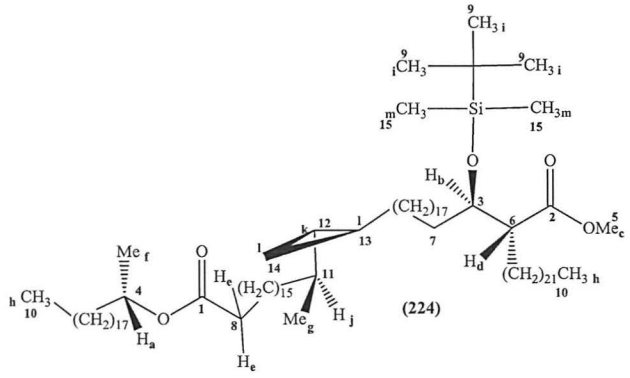
Scheme 54a: The mechanism of Steglich esterfication

DMAP is used as a catalyst; its function is to transform the *O*-acylisourea intermediate **(227)** rapidly in to the active amide intermediate **(228)**, which then reacts with the alcohol. In the absence of DMAP the reaction is slow, and allows for the possibility of an undesired [1,3] sigmatropic shift of the *O*-acylisourea intermediate **(226)** producing a new N-acyl urea intermediate **(230).** In this way, the intermediate **(230)** is unable to react with the alcohol to produce the ester **(Scheme 54b)**.



Scheme 54b: [1,3]-Sigmatropic shift of the O-acylisourea intermediate **(226)**

The HRMS (MALDI-TOF) of the protected wax ester **(224)** gave the correct mass at [M + Na^+]: 1418.3659 (calculated mass 1418.3649). The structure of the protected wax ester **(224)** was fully characterized by proton, and carbon NMR as shown in **(Table 2).**



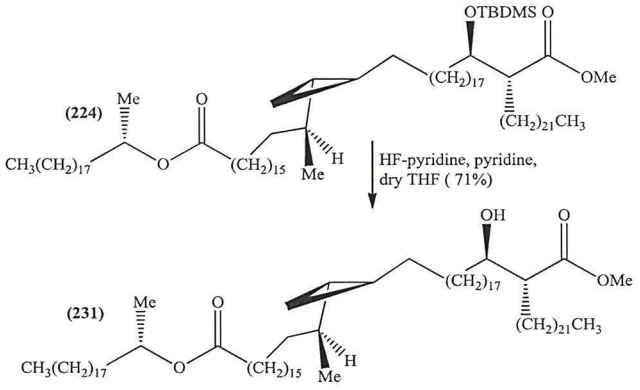
(224)

Proton	HNMR/ ppm	Multiplicity	Integration	*J*/ Hz	Carbon	^{13}C NMR/ ppm
H_a	4.91	sext	1	6.2	C_1	175.2
H_b	3.92	dt	1	7.0, 4.8	C_2	173.5
H_c	3.66	s	3	----	C_3	73.2
H_d	2.53	ddd	1	3.8,7.1,10.9	C_4	70.7
H_e	2.27	t	2	7.5	C_5	51.6
H_f	1.20	d	3	6.2	C_6	51.2
H_g	0.91	d	3	6.8	C_7	38.1
H_h	0.89	t	3	6.9	C_8	37.4
H_h	0.89	t	3	6.9	C_9	27.2–25.1
H_i	0.86	s	9	---	C_{10}	14.1
H_j	0.71–0.63	m	1	---	C_{11}	38.1
H_k	0.49–0.41	m	1	---	C_{12}	26.1
H_l	0.22–0.08	m	3	---	C_{13}	18.6
H_m	0.05	s	3		C14	10.5
H_m	0.02	s	3		C15	–4.4, –4.9

Table 2: The proton and carbon NMR data for complete wax ester **(224)**

2.2.15 Silyl deprotection

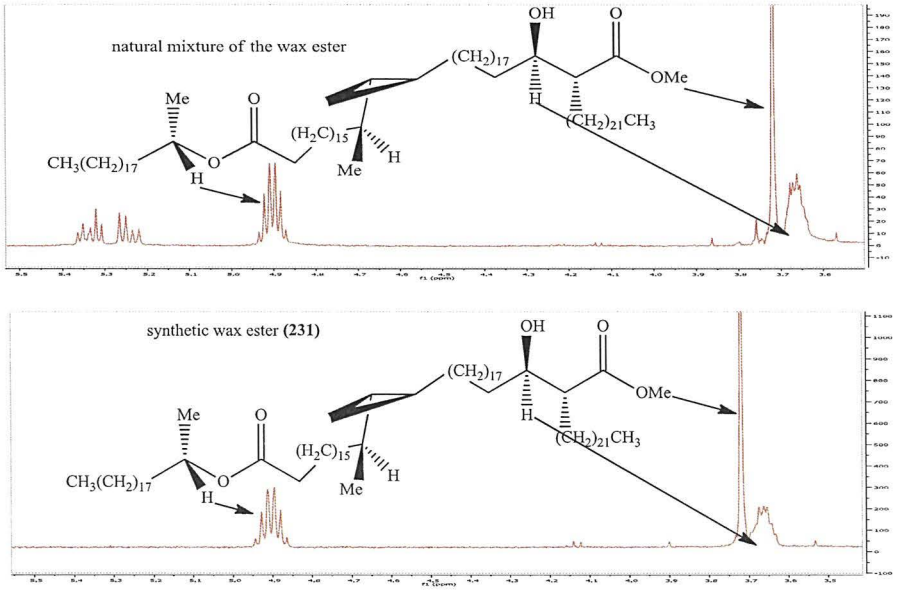
The silyl protecting group was removed from the methyl ester **(224)** using HF.pyridine complex, and pyridine in dry THF and stirring at 43 °C for 18 h, to give the β-hydroxy ester **(231) (Scheme 56)**.

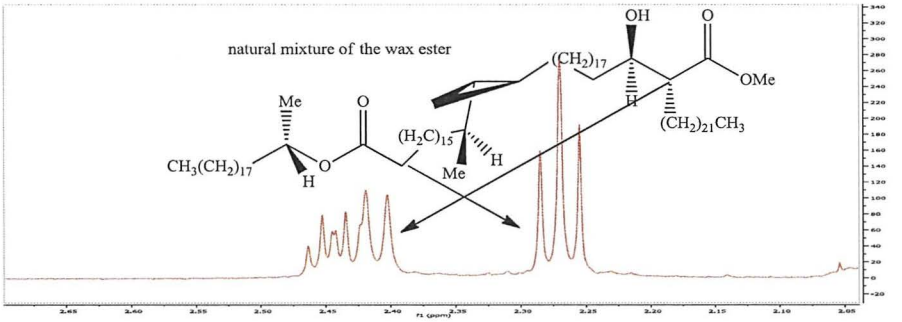


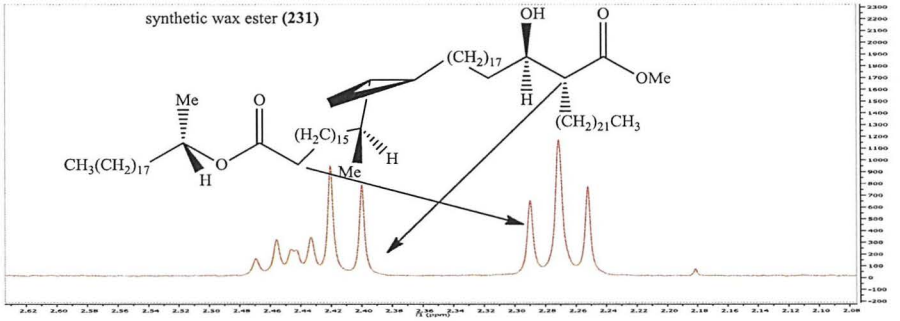
Scheme 55: Silyl deprotection to obtain β-hydroxy ester **(231)**

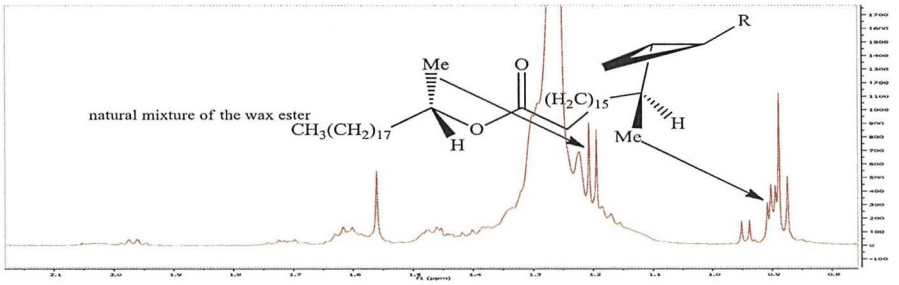
The HRMS (MALDI-TOF) also gave the expected mass ion.The success of the deprotection was confirmed by proton NMR, which showed the disappearance of the protons of *t*-butyl dimethyl silyl group and the remained signals appeared similar to the protected wax ester **(224)**.

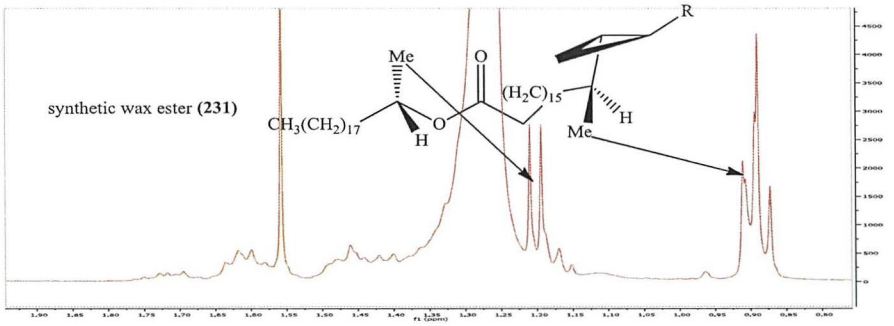
Figure 30 shows the proton and carbon NMR expansion of the synthetic wax ester methyl ester **(231)** and the natural mixture isolated from *M.avium* (thankfully provided by Dr.Alison Jones), which also contains *trans*-alkene wax ester.[219]

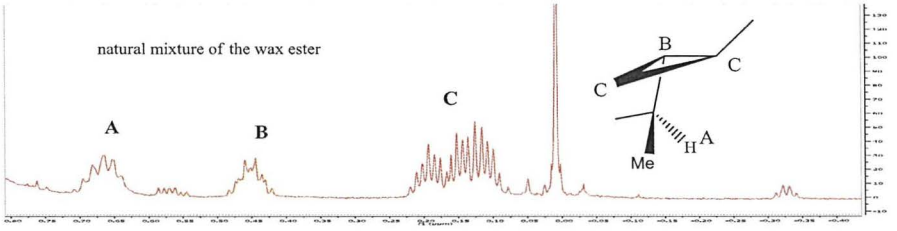


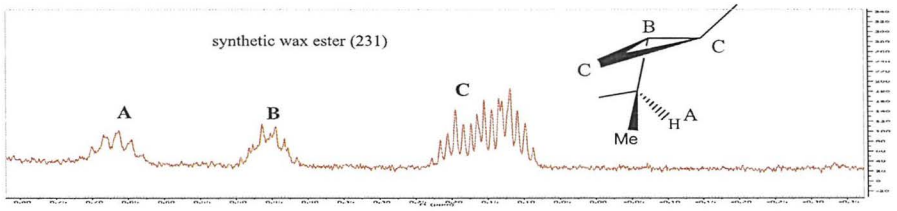

natural mixture of the wax ester
OH
O
(CH2)17
OMe
Me
O
(H2C)15
H
CH3(CH2)17
O
H
Me
(CH2)21CH3


synthetic wax ester (231)
OH
O
(CH2)17
OMe
Me
O
(H2C)15
H
CH3(CH2)17
O
H
Me
(CH2)21CH3


natural mixture of the wax ester
R
Me
O
(H2C)15
CH3(CH2)17
O
H
H
Me


synthetic wax ester (231)
R
Me
O
(H2C)15
CH3(CH2)17
O
H
H
Me


natural mixture of the wax ester
A
B
C
B
C
C
H
A
Me


synthetic wax ester (231)
A
B
C
B
C
C
H
A
Me

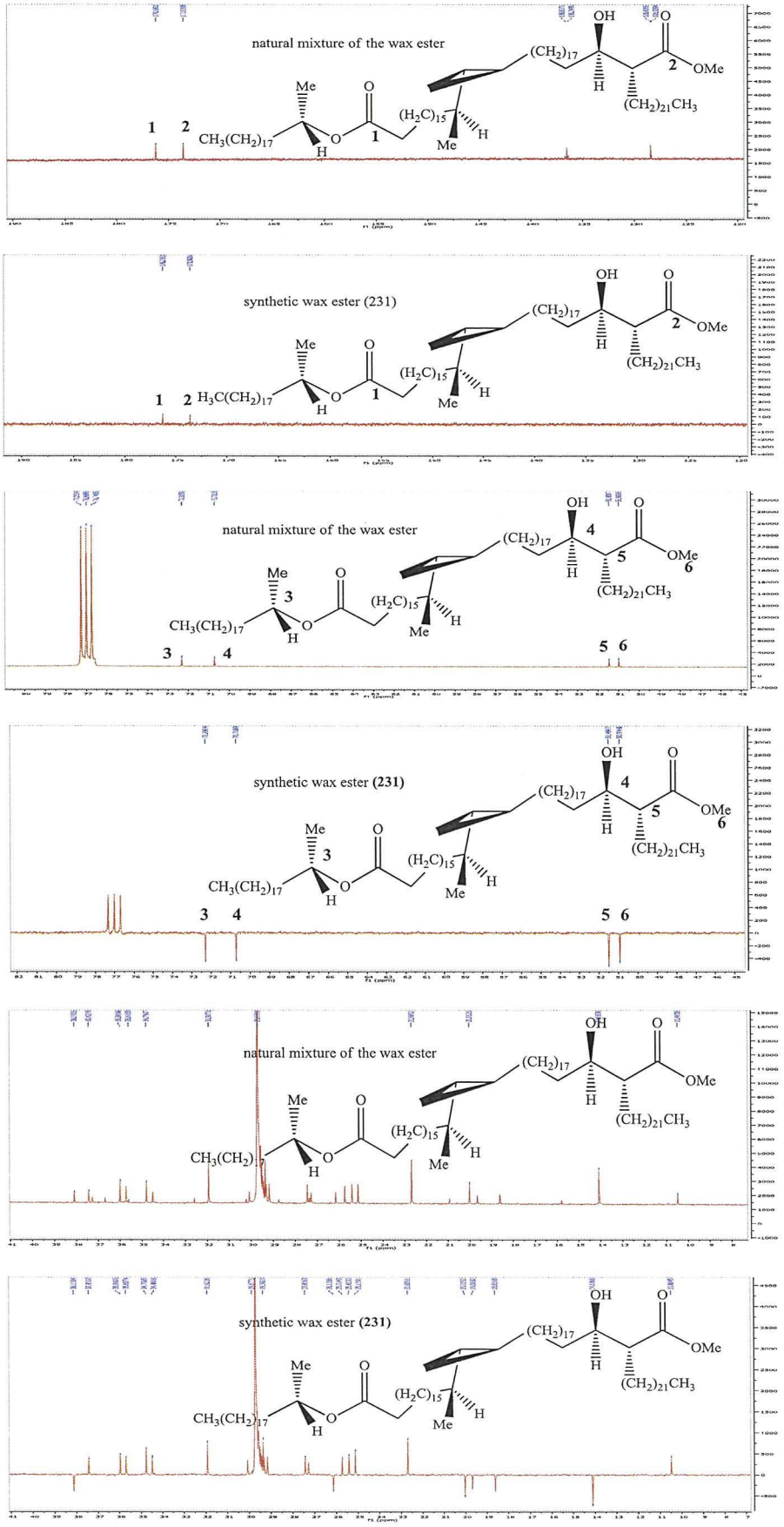


Figure 30: The proton and carbon NMR of the *M.avium* synthetic wax esters methyl ester **(231)** and natural mixture.

The crude *M.avium* sample containing (wax ester, α and keto-mycolic acids) was originally provided by Prof. Minikin, and was then separated by Dr. Alison Jones to give the above NMR spectra of the natural wax ester. The following procedure was carried out in order to separate the three components (wax ester, α and keto-mycolic acids).

(1) The crude *M.avium* sample was columned on silica gel giving three fractions including the methyl ester of α -mycolic acid, mixture of wax ester and keto-mycolic acid and methyl ester of ω -carboxymycolic acid.

(2) The fraction containing the mixture of wax ester and keto-mycolic acid was protected with TBDMS group, which was then reduced with $NaBH_4$ inorder to reduce the keto group.

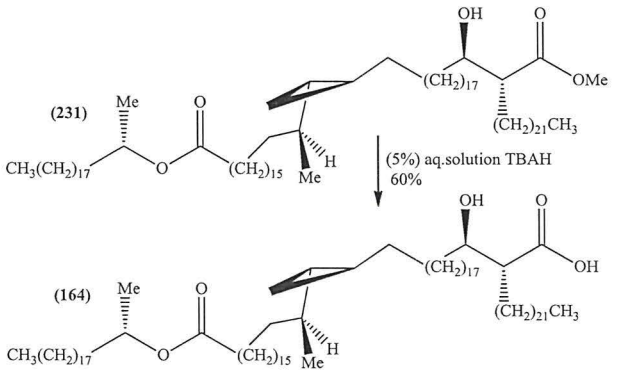
(3) The resulting alcohol and the protected wax ester were then separated by column chromatography.

(4) The TBDMS was removed on the protected wax ester using HF/pyridine complex producing the mixture homologous of the natural wax ester containing different chain lengths with some *trans*-alkene wax ester. This gave the above NMR spectrum thankfully provided by Dr.Alison Jones.

(5) The product obtained from step (4) was hydrolysed using tetrabutyl ammonium hydroxide solution (5%) to give the free wax ester. The NMR spectrum of the free wax ester will be shown later in this chapter.

2.2.16 Hydrolysis of (231)

The compound **(231)** required a selective reaction to hydrolyse the methyl ester. The difficulty was the possibility of the undesired hydrolysis of the eicosanol ester to give the ω-carboxymycolic acid. Finally, the hydrolysis of **(231)** was achieved using aqueous tetra-*n*-butyl ammonium hydroxide (5%, v/v) and heating at 100°C for 24 hrs., which afforded the free wax ester mycolic acid **(164) (Scheme 56)**.



Scheme 56: Hydrolysis of **(231)** to obtain the free wax ester

The MS (MALDI-TOF) confirmed that the synthetic free wax ester **(164) (Figure 31)** is identical to the major component of the *M.avium* natural mixture of the wax ester **(Figure 32)** (provided by Dr.Alison Jones).[219]

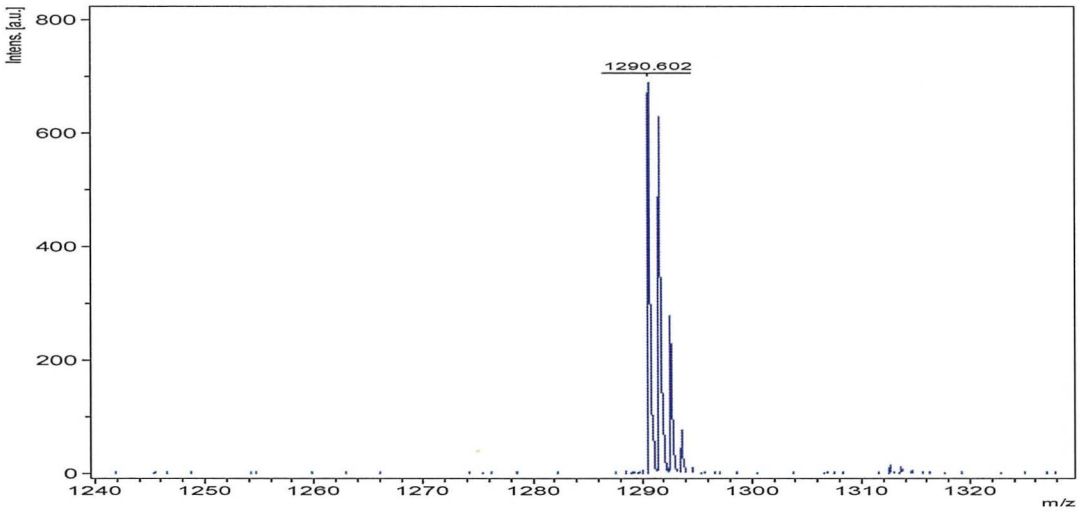


Figure 31: MALDI-MS of synthetic wax ester **(164)**

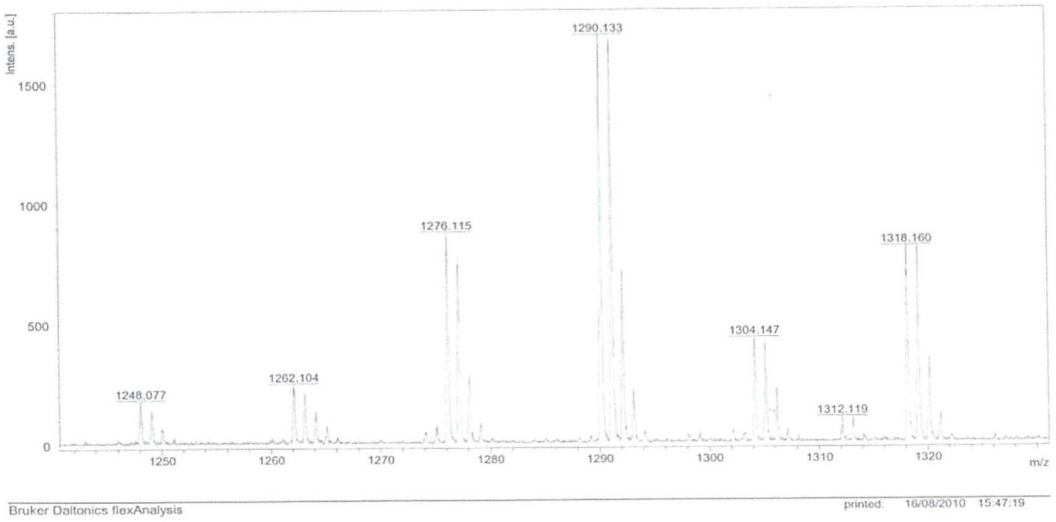
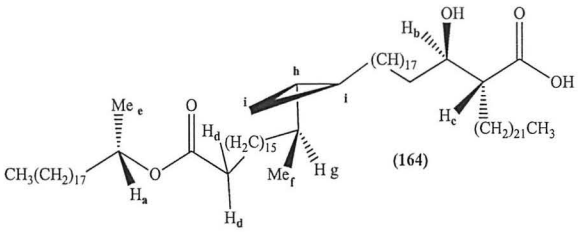


Figure 32: MALDI-MS of a mixture of natural wax ester.[219]

The proton NMR spectrum of compound **(164)** confirmed the disappearance of the singlet at 3.71 ppm belonging to the methyl ester. The selected proton NMR data are compared with those of the natural mixture of the wax ester **(Table 3)**.

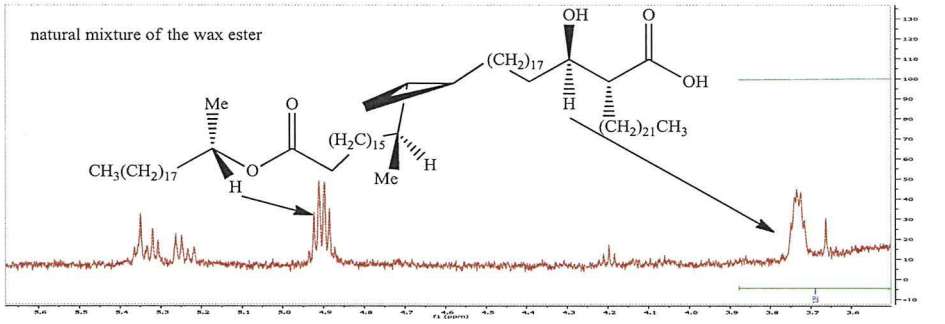


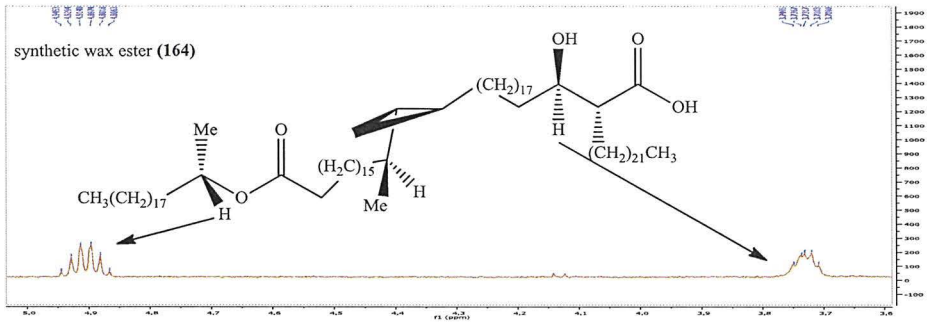
(164)

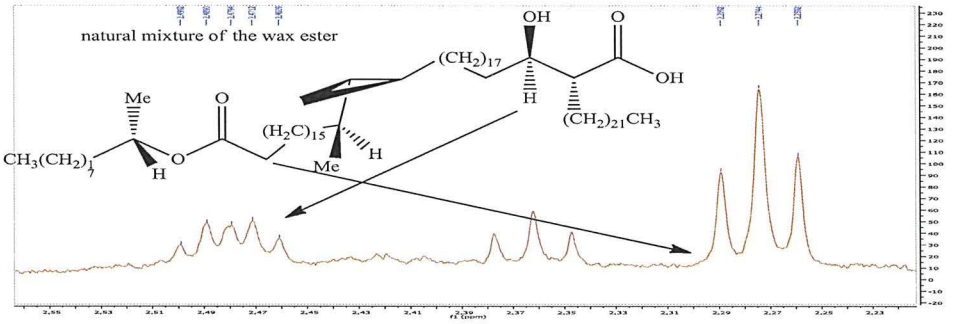
Synthetic wax ester **(164)**				Natural wax ester.[219]		
Proton	**ppm**	**Multiplicity**	**integration**	**ppm**	**Multiplicity**	**integration**
H_a	4.91	sext	1H (*J* 6.2 Hz)	4.91	sext	1H (*J* 6.2 Hz)
H_b	3.73	m	1H	3.73	m	1H
H_c	2.46	br.dt	1H (*J* 9.1, 5.3 Hz)	2.46	br.dt	1H (*J* 9.0, 5.2 Hz)
H_d	2.27	t	2H (*J* 7.5 Hz)	2.27	t	2H (*J* 7.5 Hz)
H_e	1.19	d	3H (*J* 6.2 Hz)	1.19	d	3H (*J* 6.2 Hz)
H_f	0.90	d	3H (*J* 6.8 Hz)	0.90	d	3H (*J* 6.8 Hz)
H_g	0.70–0.63	m	1H	0.69–0.63	m	1H
H_h	0.49–0.41	m	1H	0.48–0.42	m	1H
H_i	0.22–0.08	m	3H	0.22–0.09	m	3H

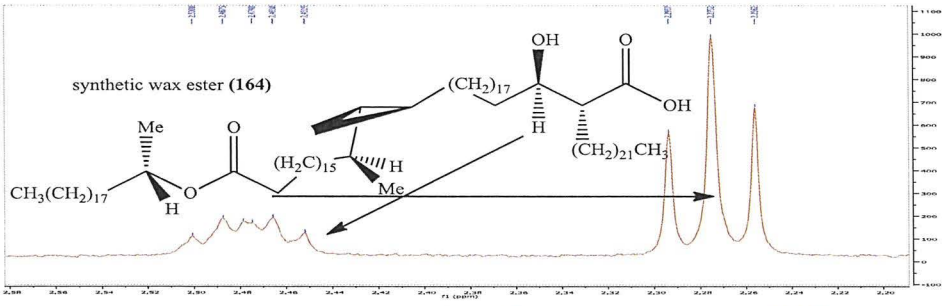
Table 3: The proton NMR data analysis of the free synthetic wax ester **(164)** and natural mixture of the wax ester

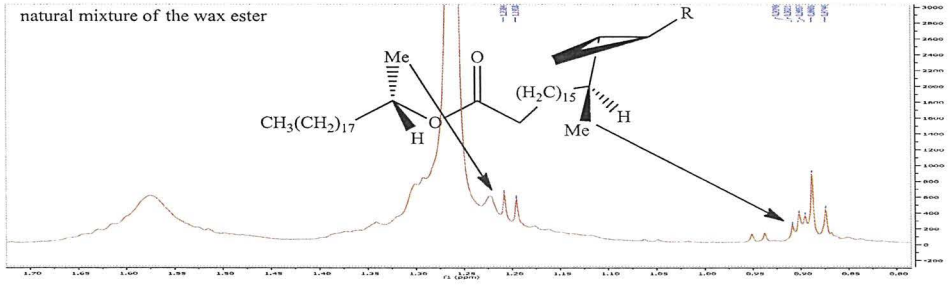
The proton NMR of the free synthetic wax ester (164) and a natural mixture of the wax ester











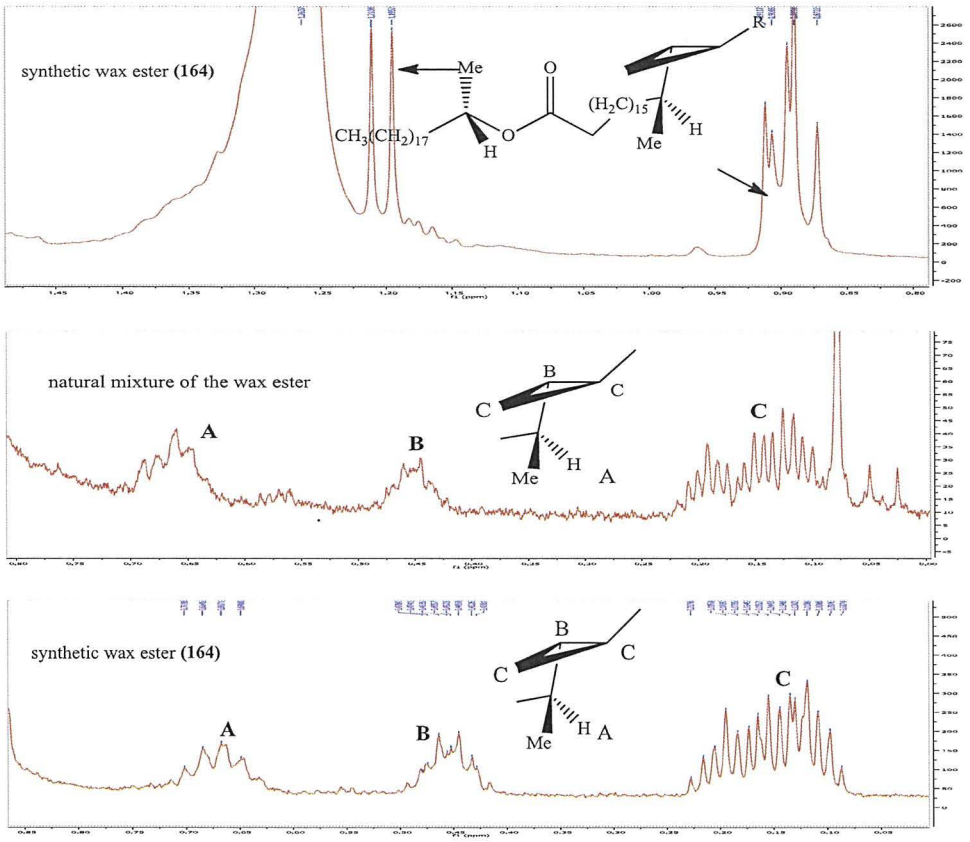
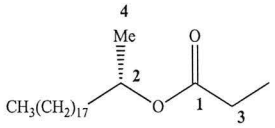


Figure 33: The expanded proton NMR of the free synthetic wax esters **(164)** and *M.avium* natural mixture of the wax ester. [219]

As can be seen in **(Figure 33)**, the proton NMR spectra showed the identical signals for the free synthetic wax ester **(164)** to those of the natural sample isolated from *M.avium*. The wax ester region in the carbon NMR from the natural *M.avium* wax ester was also reported by Minnikin *et al.* **(Table 4)**.[108]

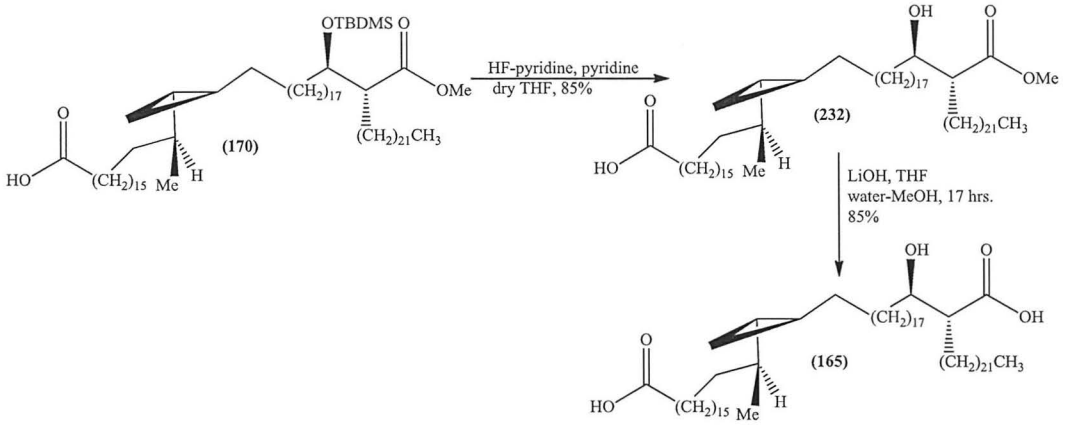


Carbon No.	Synthetic (ppm)	Natural (ppm).[108]
1	173.6	173.6
2	70.75	70.7
3	34.7	34.7
4	20.0	20.0

Table 4: Selected carbons chemical shifts for both of the synthetic **(164)** and natural wax ester.

2.2.17 Synthesis of ω-carboxymycolic acid (165)

Compound **(170)** was treated with HF.pyridine complex, pyridine in dry THF at 43 °C for 18 h in order to remove the silyl protecting group; this produced β-hydroxy acid-ester **(232)**. The proton NMR spectra of **(232)** was compared with the protected compound **(170)**, and showed the disappearance of the *tert*-butyl dimethylsilyl group **(Scheme 57)**.



Scheme 57: Synthesis of ω-carboxymycolic acid **(165)**

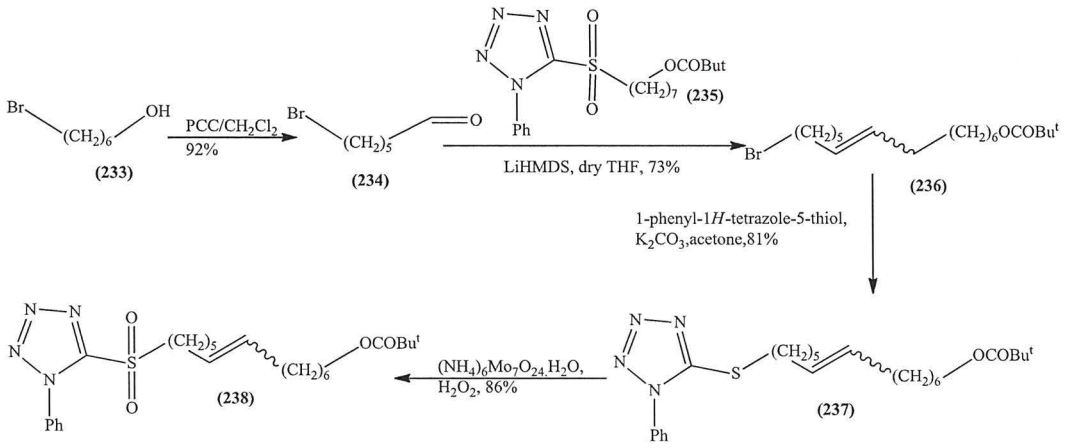
Finally compound **(232)** was hydrolysed to give the free ω-carboxymycolic acid **(165)** using lithium hydroxide in THF, methanol and water at 45 ° C for 18 h. The proton NMR spectrum showed the disapperance of the singlet at 3.66 ppm for the methyl ester, two multiplets at 3.74–3.70 ppm and 2.46 ppm for the α and β-position proton respectively. The two protons next to the carbonyl group appeared as a sharp triplet at 2.35 ppm (*J* 7.5 Hz). The cyclopropane protons appeared as two multiplets at 0.73–0.68 ppm and 0.50–0.44 ppm each integrated for one proton and another multiplet was seen at 0.24–0.10 ppm integrated for three protons. In addition, the carbon NMR showed two signals belonging to the carbonyl carbons at 180.1, 179.1 ppm. The β-carbon appeared at 72.2 ppm, while the α-carbon appeared at 50.9 ppm.

2.3 The synthesis of *M.gordonae* wax ester and corresponding ω-carboxymycolic acid

The *M.gordonae* wax ester **(166)** and its corresponding ω-carboxymycolic acid **(167)** were synthesized applying the same synthetic route described earlier for the *M.avium* wax ester. The only differences were the carbon chain lengths. Sixteen carbons to the right hand side of α-methyl cyclopropane and eighteen carbons the left hand side.[107]

2.3.1 Synthesis of sulfone (238) for chain extension

The intermediate sulfone **(238)** was prepared using established methods. The aldehyde **(234)** (prepared from 1-bromohexanol **(233)**), was coupled with sulphone **(235)** (provided by Dr. Al-dulayymi) through a modified Julia-Kocienski reaction in the presence of base lithium *bis*(trimethylsilyl)amide in dry THF to give the unsaturated bromo ester **(236)**. This requires hydrogenation of the alkene using Pd on carbon as a catalyst under an atmosphere of hydrogen, but this method usually leads to the simultaneous saturation of the double bond and the removal of some of the bromine, resulting in a terminal methyl group. Therefore, it was decided to avoid hydrogenation of the bromo-ester **(236)** at this stage, it being carried out subsequently after the second olefination reaction. The unsaturated bromo-ester **(236)** was therefore converted into the unsaturated sulphone **(238)** using the standard method described earlier **(Scheme 58)**.

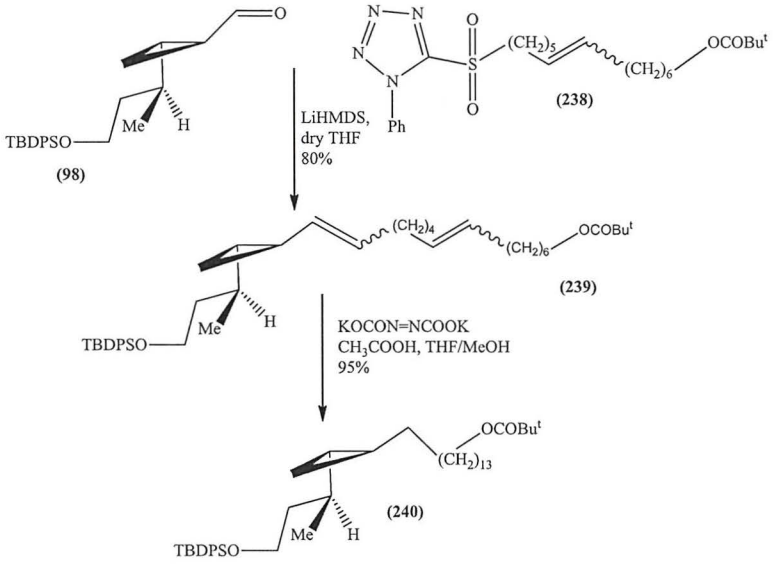


Scheme 58: Synthesis of the sulfone **(238)**

The proton NMR for the final sulphone **(238)** showed a multiplet at 7.71 ppm integrating to two protons, and another multiplet at 7.62 ppm integrating to three protons. The alkene protons gave a multiplet at 5.38 ppm for both the *(E/Z)* isomers. The two protons next to the butyl ester group appeared as a triplet at 4.04 ppm (*J* 6.5 Hz), while the two protons next to the sulphone group gave a multiplet at 3.73 ppm. The remaining protons appeared as a multiplet from 1.99–1.27 ppm, and the butyl ester protons gave a strong singlet at 1.21 ppm integrating for nine protons. The carbon NMR spectrum gave the expected signals, including a carbonyl carbon signal at 178.3 ppm with phenyl and alkene carbon signals occurring between 133.5–125 ppm.

2.3.2 Julia-Kocienski reaction between aldehyde (98) and sulphone (238)

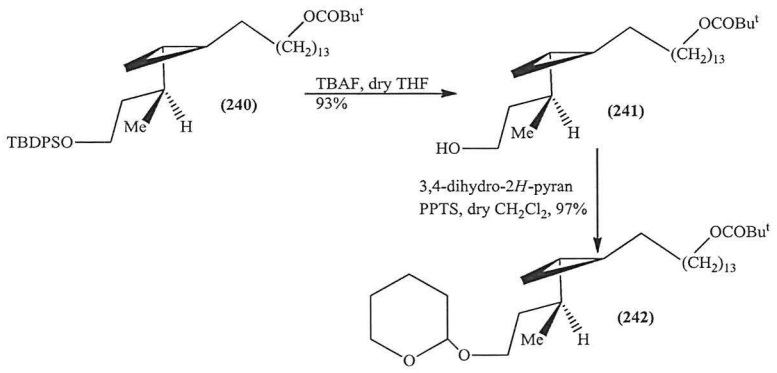
The previously prepared aldehyde **(98)** was coupled with the sulphone **(238)** again employing a Julia-Kocienski reaction which afforded the mixture of *E/Z*-alkenes **(239)**, which was then saturated using dipotassium azodicarboxylate and acid to give **(240)** **(Scheme 59)**.



Scheme 59: Chain extension of the *trans*-aldehyde (**98**) via Julia-Kocienski reaction

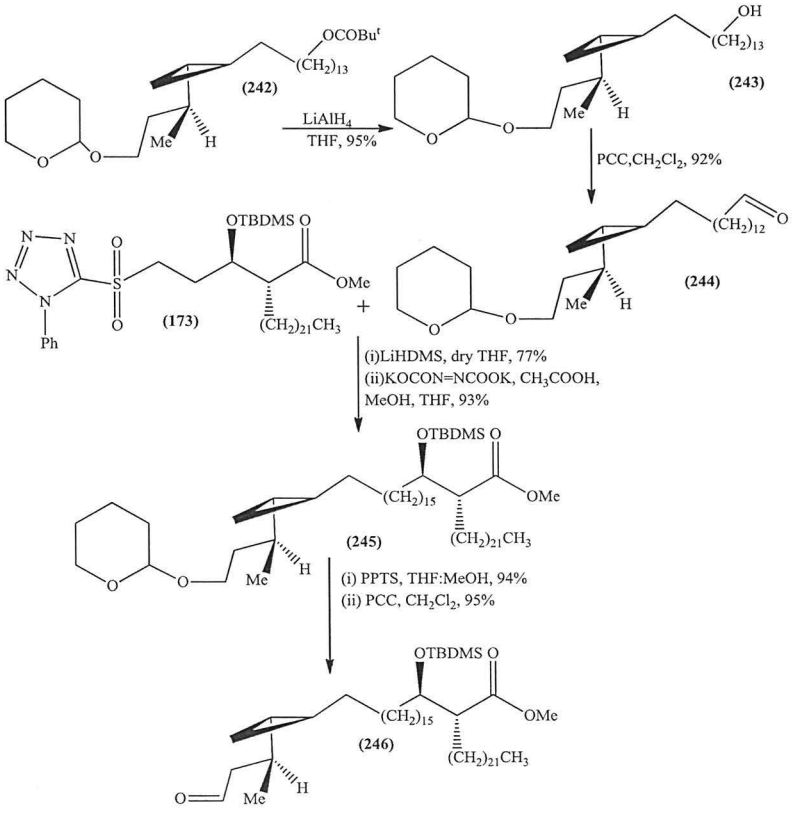
The proton NMR of the compound **(240)** confirmed the disappearance of the olefinic signals, and showed a broad multiplet at 7.68 ppm integrating to four protons and another multiplet at 7.42 ppm integrating for the other six protons corresponding to the protons of the diphenyl group. A sharp triplet was seen at 4.06 ppm (*J* 6.6 Hz) integrating for two protons adjacent to the *tert*-butyl ester, while the two protons next to the protecting group appeared as a multiplet at 3.78–3.68 ppm. The nine protons of the *tert*-butyl group of the ester and those bound to the silicon atom showed two singlets at 1.20 and 1.04 ppm respectively, while the α-methyl cyclopropane protons appeared as three multiplets at 0.88, 0.47 and 0.16 ppm.

Removal of the silyl protecting group from **(240)** was achieved using tetra-*n*-butyl ammonium fluoride (TBAF) in dry THF to give the alcohol **(241)** in 93% yield. The disappearance of the signals of the protecting group in the proton and carbon NMR spectrum confirmed the success of the reaction. The two protons next to the hydroxyl group appeared as a multiplet at 3.78–3.68 ppm in the proton NMR **(Scheme 60)**.



Scheme 60: Protecting group replacement on **(240)**

The alcohol (**241**) was treated with 3,4-dihydro-2*H*-pyran in dichloromethane in the presence of pyridinium-*p*-toluenesulfonate, stirring for 30 minutes to give the protected ester **(242) (Scheme 60)**. The ester **(242)** was reduced with lithium aluminium hydride to give **(243)**, which was then oxidized with PCC to give aldehyde **(244)**. Again, the Julia-Kocienski reaction was employed to couple the aldehyde **(244)** with a previously prepared mycolic motif sulfone **(173)** using lithium *bis*(trimethylsilyl)amide. This led to **(245)**, after saturation by dipotassium azodicarboxylate and acid **(Scheme 61)**.

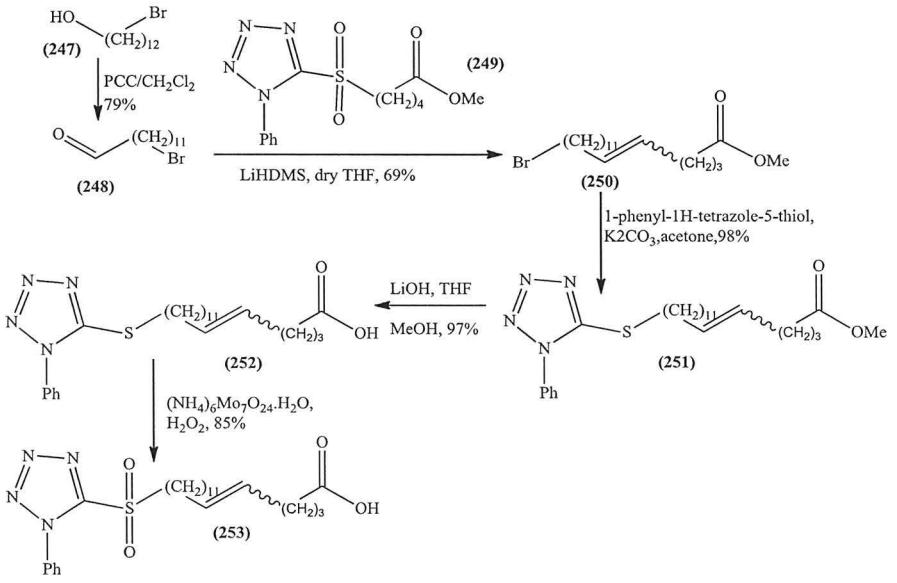


Scheme 61: Preparation of the aldehyde **(246)**

The THP protecting group was removed from compound **(245)** using pyridinium-*p*-toluenesulfonate (0.5 eq.) under the same conditions as described earlier, followed by oxidation of the alcohol with PCC to give the aldehyde **(246) (Scheme 61).** All the above compounds **(Scheme 61)** gave essentially identical NMR spectra to those of the intermediate compounds of the *M.avium* wax ester.

2.3.3 Synthesis of acid sulfone (253) for chain extension

The intermediate **(253)** was prepared in similar way to the intermediate **(238), (Scheme 58)**. The first step was the preparation of bromo-ester **(250)**, which was then converted into the sulphide **(251)**, following by hydrolysis to give **(252)**, which was then converted to the sulphone **(253)**, using the standard method described earlier **(Scheme 62)**.

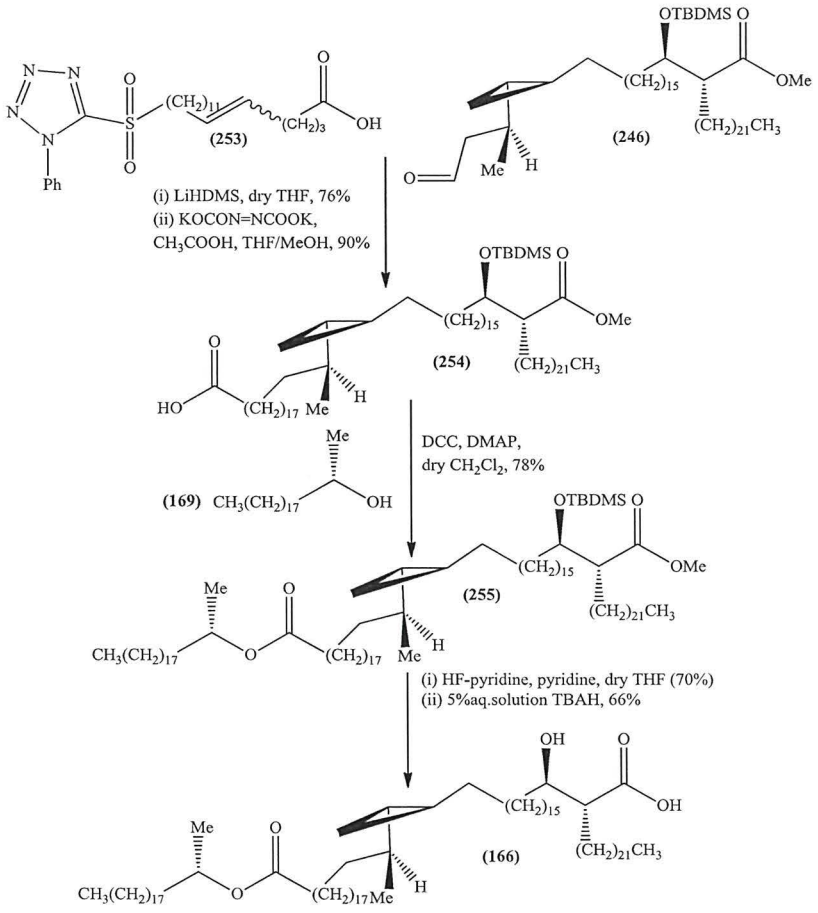


Scheme 62: Preparation of acid sulfone **(253)**

A close similarity in chemical shifts was seen between both intermediates **(253)** and **(238)** in NMR. The proton NMR for the final acid sulphone **(253)** showed a multiplet at 7.69 ppm integrating to two protons, and another multiplet at 7.62 ppm integrating to three protons. The alkene protons gave a multiplet at 5.38 ppm for both the *(E/Z)* isomers. The two protons next to the sulphone appeared as a triplet at 3.73 ppm (*J* 8 Hz), and the two protons next to the carbonyl group gave another triplet at 2.35 ppm (*J* 7.5 Hz). The carbon NMR spectrum gave the expected signals, including a quaternary carbon signal at 179.8 ppm, and signals for the phenyl and alkene carbons between 131.9–125.1 ppm.

2.3.4 Synthesis of complete wax ester (166)

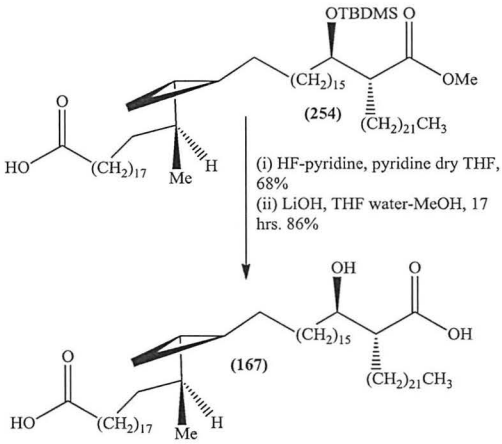
The complete wax ester **(166)** was prepared **(Scheme 63)** using the same reaction conditions described earlier for *M.avium* wax ester **(164)**, and these compounds showed essentially identical NMR spectra to those of the *M.avium* wax ester. The specific rotation of the compound **(166)** was found $[\alpha]_D = +7.1$ (c 0.55 ($CHCl_3$)); this compares closely to the value for *M.avium* wax ester **(164)**, $[\alpha]_D = +7.6$, (c 0.4 ($CHCl_3$)).



Scheme 63: Preparation of the complete wax ester **(166)**

2.3.5 Synthesis of ω-carboxymycolic acid (167)

The *M.gordonae* ω-carboxymycolic acid **(167)** was obtained under the same conditions described previously for the synthesis of the *M.avium* ω-carboxymycolic acid **(165)**, and showed identical NMR spectra **(Scheme 64)**. The specific rotation was found $[\alpha]_D = +5.9$ (c 0.56 $CHCl_3$); again, this is very similar to the value for the *M.avium* ω-carboxymycolic acid **(165)** $[\alpha]_D = +5.8$, (c 0.86 $CHCl_3$).



Scheme 64: Synthesis of ω-carboxymycolic acid **(167)**

2.3.6 Synthesis of non-natural wax ester (168)

The wax ester **(168)** was also prepared using the same reaction conditions described earlier for *M.avium* wax ester **(164)**, having nineteen carbons to the right hand side of α-methyl cyclopropane, sixteen carbons the left hand side, and 23 carbons in the α-alkyl chain instead of 21. The wax ester **(168)** and its intermediates showed essentially identical analysis data (NMR, specific rotation) to those of the *M.avium* wax ester **(164)**, and the experimental procedures are given in the appendix **(Figure 34)**.

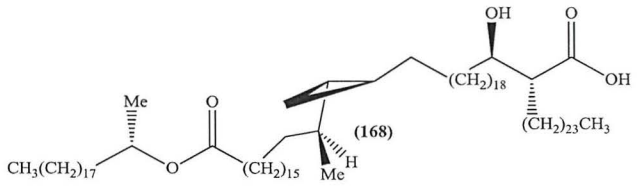


Figure 34: Synthesis of non-natural wax ester **(168)**

This allowed the effect of chain lengths on biological properties to be determined.

Chapter 3

3. Synthesis of the trehalose esters (TDM, TMM) of wax esters (164, 166, 168)

3.1 The aims

The aims of this chapter are to describe the first synthesis of the trehalose esters (TDM, TMM) of *M.avium* and *M.gordonae* wax esters **(164)** and **(166)** respectively. These compounds would be used as specific antigens to distinguish *M.avium* or *M.gordonae* from *M.tuberculosis* in serodiagnostic assays. In addition the synthesis of trehalose esters of the non-natural wax ester **(168)** was also applied to study their antigenicity.

3.1.1 Overview

Different classes of cord factors from different mycobacteria may show different biological activities. For example; natural TDM from *M.avium* shows different toxicity and granulomatogenic activity in mice in comparison with other mycobacteria such as *M. tuberculosis H37Rv, M. bovis BCG* and *M. kansasii*.[220] This behaviour is due to the presence of different mycolic acid and wax ester compounds in these species.

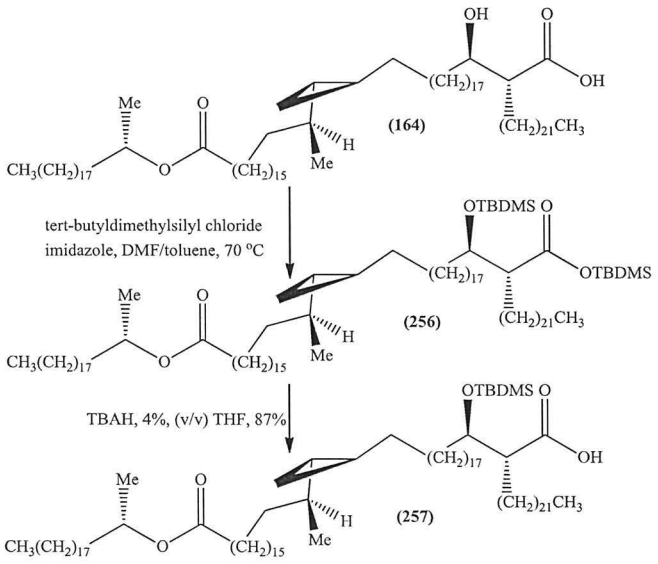
Yano *et al.*[192] have reported that TDM isolated from *M.tuberculosis* is antigenic to antibodies present in the serum of patients infected with tuberculosis. Antibodies in the serum of patients infected with TB responded less strongly to TDM isolated from *M.avium* complex (MAC), while antibodies in serum of MAC infected patients showed the opposite response pattern. Yano *et al.*[192] have also reported that the natural TDM isolated from *M.avium* is more antigenic than natural TDM from *M.tuberculosis* toward the sera of *M.avium* infected patients, and the opposite results were seen for the natural TDM from *M.tuberculosis*. In addition, specific subclasses of TDM have a role in antibody recognition, and the methoxy TDM is recognised more strongly than α- and keto TDMs by the antibodies of tuberculosis infected serum samples.[192] This suggests that the sera of patients infected with *M.avium* may recognize wax ester specifically, as it is found exclusively in *M.avium*. The interesting results found by Yano *et al.*, stimulated our interest in the synthesis of the trehalose esters of *M.avium* and *M.gordonae* wax esters.

3.2 Synthesis of the trehalose esters of *M.avium* wax ester (164)

The synthetic *M.avium* wax ester (prepared in Chapter 2) was esterified with trehalose to produce trehalose dimycolate (TDM) and trehalose monomycolate (TMM). Similar reaction conditions were employed to those described by Maza *et al.* **(Scheme 26),**[170] with some modifications which were deemed necessary in order to produce the desired wax ester TDM and TMM and to improve the yield of the reactions (these will be described in detail later in this chapter).

3.2.1 Protection of the β-position of wax ester

The β-hydroxy group of the wax ester was protected with the TBDMS protecting group using *tert*-butyldimethylsilylchloride, imidazole and DMAP and free wax ester **(164)** in a mixture of DMF/toluene at 70 ° C for 18 hours. The reaction usually leads to the protection of both the β-hydroxy group and the carboxylic acid to give **(256)**, which then requires the hydrolysis of the carboxylic acid in the next step to give the target compound **(257) (Scheme 65)**.

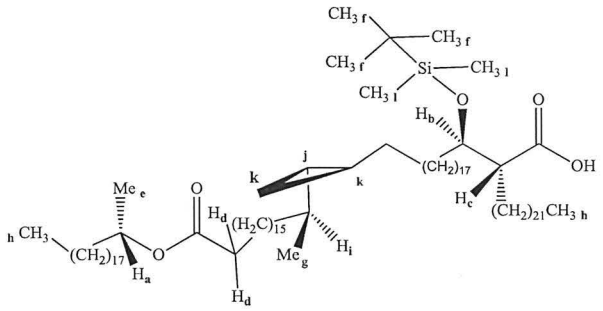


Scheme 65: Protection of the wax ester **(164)**

In order to hydrolyse the TBDMS ester on the acid group from compound **(256)**, the standard procedure includes stirring it in a mixture of THF, water and methanol in the presence of potassium carbonate at 45 ° C for 18 hours. This procedure could lead to the undesired hydrolysis of the wax ester to give dicarboxylic acid; for this reason a new procedure was required to obtain **(257)**. Finally, compound **(257)** was obtained successfully by dissolving the compound **(256)** in THF with a 4% (v/v) aqueous solution

of tetra-*n*-butyl ammonium hydroxide at room temperature for 15 minutes. This led to **(257)** in 87% yield without any undesired hydrolysis **(Scheme 65)**.

The proton NMR for the compound **(257)** showed the silyl protecting group protons as a nine proton singlet at 0.93 ppm and two singlets at 0.15 and 0.12 ppm for the methyl groups (the full proton NMR characterization is shown in Table 5). The carbon NMR also showed signals at –4.2 and –4.9 ppm for the methyl groups of the silyl protecting group.

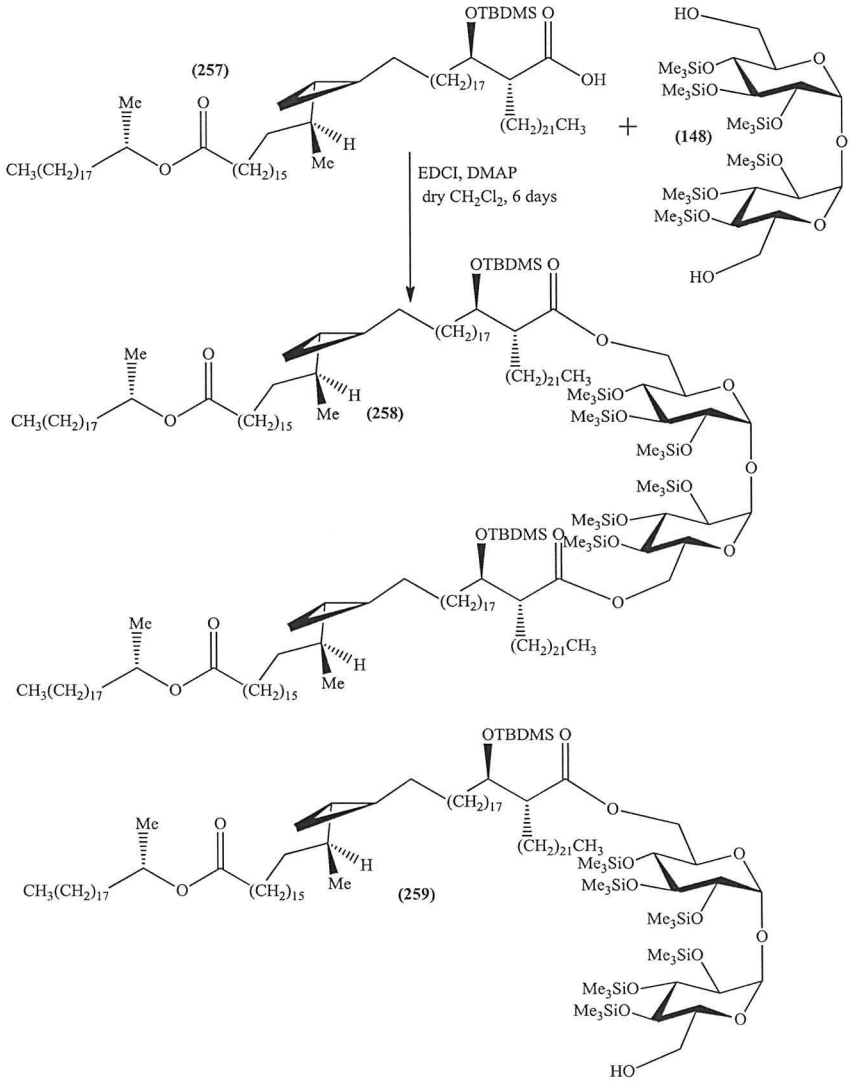


Proton	ppm	Multiplicity	Integration	*J*/Hz
H_a	4.92	sext	1	6.2
H_b	3.83	m	1	--
Hc	2.53	ddd	1	3.2, 5.6, 9.1
H_d	2.27	t	2	7.4
H_e	1.20	d	3	6.2
H_f	0.93	s	9	--
H_g	0.90	d	3	6.8
H_h	0.89	t	6	6.9
H_i	0.71–0.63	m	1	--
H_j	0.48–0.41	m	1	--
H_k	0.22–0.06	m	3	--
H_l	0.15	s	3	
H_l	0.12	s	3	

Table 5: Selected proton NMR data for the protected wax ester **(257)**

3.2.2 Coupling of protected wax ester to protected trehalose

The protected wax ester **(257)** was esterified with the protected trehalose **(148)** using coupling agents (EDCI and DMAP) in dry dichloromethane and stirring at room temperature for 6 days. The crude mixture was separated by column chromatography to give a first fraction TDM **(258)**, and a second fraction TMM **(259) (Scheme 66)**.



Scheme 66: Esterification of protected wax ester with protected trehalose

The MS (MALDI-TOF) for the TDM **(258)** found $[M+Na]^+$: 3524.01; the calculated value requires 3524.04. The TDM **(258)** was also fully characterized by proton NMR, which gave a sextet at 4.92 ppm (*J* 6.3 Hz) integrating for two proton corresponding to the proton adjacent to the wax ester group. The two acetal protons appeared as a doublet at 4.85 ppm (*J* 3.0 Hz), and the remaining trehalose protons, including the β-position protons of the wax ester, occurred at 4.38, 4.04–3.98, 4.0–3.3.96, 3.93, 3.52, 3.38 ppm.

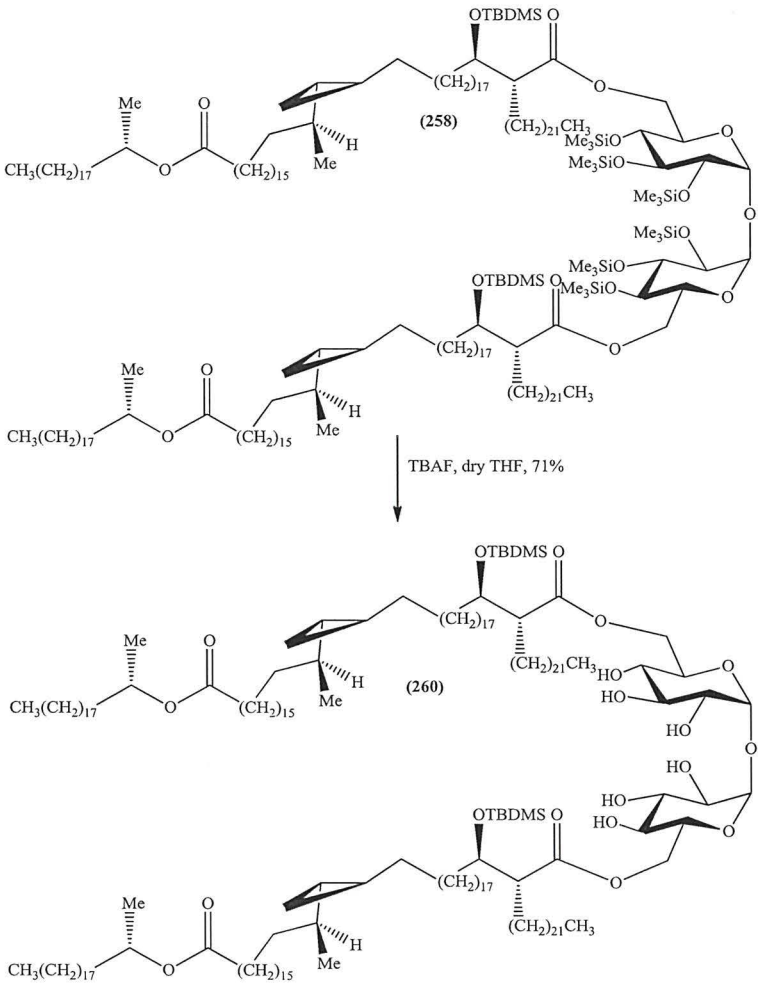
The α-position proton next to the alkyl chain of the wax ester appeared as a doublet of doublets of doublets at 2.55 ppm (*J* 3.50, 4.75, 10.1 Hz), and a sharp triplet appeared at 2.26 ppm (*J* 7.5 Hz) for the two protons of the methylene group adjacent to the carbonyl group. The α-methyl next to the ester group appeared as a doublet at 1.20 ppm (*J* 6.2 Hz), while the α-methyl next to cyclopropane ring showed another doublet at 0.90 ppm (*J* 6.8 Hz). The terminal methyl groups of the alkyl chains gave a broad triplet at 0.91–0.87 ppm (*J* 6.9 Hz), and the *tert*-butyl protons appeared as singlet at 0.88 ppm with an integration of eighteen protons. The cyclopropane protons were seen as two multiplets at 0.72–0.65 ppm and 0.48–0.41 ppm, each integrating to two protons, and a multiplet at 0.22–0.06 ppm integrating to six protons. The protons of the trimethylsilyl protecting groups on the trehalose showed three singlets at 0.16, 0.15, and 0.14 ppm each integrated for eighteen protons. The methyl groups of the TBDMS protecting group showed a singlet at 0.062 ppm integrated to twelve protons. The carbon NMR showed two carbonyl carbon signals at 173.8 and 173.6 ppm. The anomeric carbons signal appeared at 94.8 ppm, and the remaining trehalose carbon signals appeared between 73.5–70.7 ppm.

The MS (MALDI-TOF) of the TMM **(259)** found $[M+Na]^+$: 2160.58; the calculated value requires 2160.69. The proton NMR spectrum was more complicated than that of TDM **(258)** due to loss of the symmetry. The acetal protons appeared as two doublets at 4.91 ppm (*J* 3.0 Hz), 4.85 ppm (*J* 2.9 Hz), and the remaining sugar protons appeared between 4.36–3.38 ppm. The carbon NMR showed the anomeric carbon signals at 94.5 and 94.4 ppm. The remaining NMR data for the wax ester appeared similar to that of the TDM with only half of the integration as expected.

3.2.3 Trehalose deprotection of TDM (258)

The removal of the trimethylsilyl protecting groups on the trehalose core of the TDM **(258)** was achieved using tetra-*n*-butyl ammonium fluoride (TBAF) in dry THF. The reaction requires aqueous work up, which can lead to a significant decrease in the yield because of the high polarity of the product which is consequently difficult to completely extract. Therefore it was necessary to find an alternative method. It was decided to apply the residue directly to the silica column after the concentration in vacuum and without any work up, and this gave the target compound **(260)** successfully in 71 % yield (**Scheme 67**). The proton NMR spectrum confirmed the disappearance of the signals for

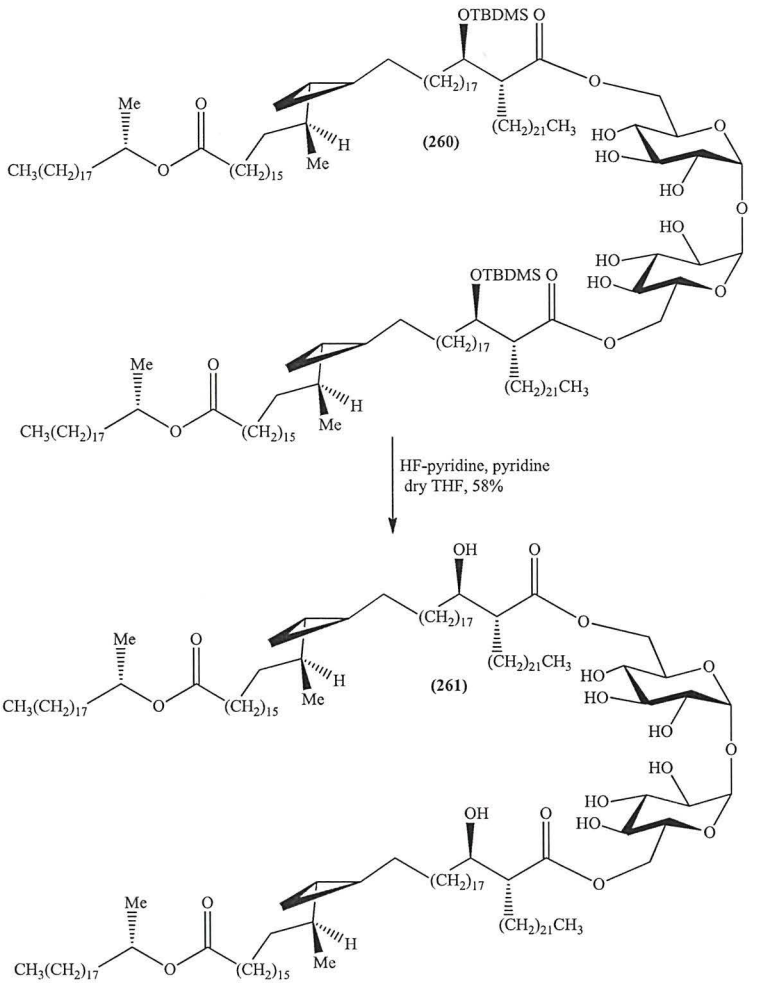
the trimethyl protecting groups at 0.16, 0.15, and 0.14 ppm, which means that the deprotection has been successful.



Scheme 67: Deprotection of the trehalose core of TDM **(258)**

3.2.4 β-Position deprotection of TDM (260)

The final step was the deprotection of the *tert*-butyldimethylsilyl group on the β- position of the wax ester using HF.pyridine complex and pyridine in dry THF at 43 °C for 17 hrs **(Scheme 68)**. The reaction mixture required aqueous work up with saturated solution of sodium hydrogen carbonate to neutralize the HF. It was observed that the aqueous work up led to the partial loss of the compound, probably via undesired hydrolysis of the wax ester part. In order to avoid the aqueous work up, the excess of HF was neutralized with triethylamine, and the excess of triethylamine was then removed under high vacuum. The residue was columned directly without aqueous work up, which gave the final target compound **(261)** in 58 % yield



Scheme 68: Synthesis of free wax ester TDM **(261)**

The HRMS (MALDI-TOF) of the TDM **(261)** gave $[M+Na]^+$: 2863.6340 **(Figure 35)**, the calculated value being 2863.6314. Figure 36 shows the MALDI-TOF MS of the natural mixture of *M.avium* TDM reported by Fujita *et al.*[160]

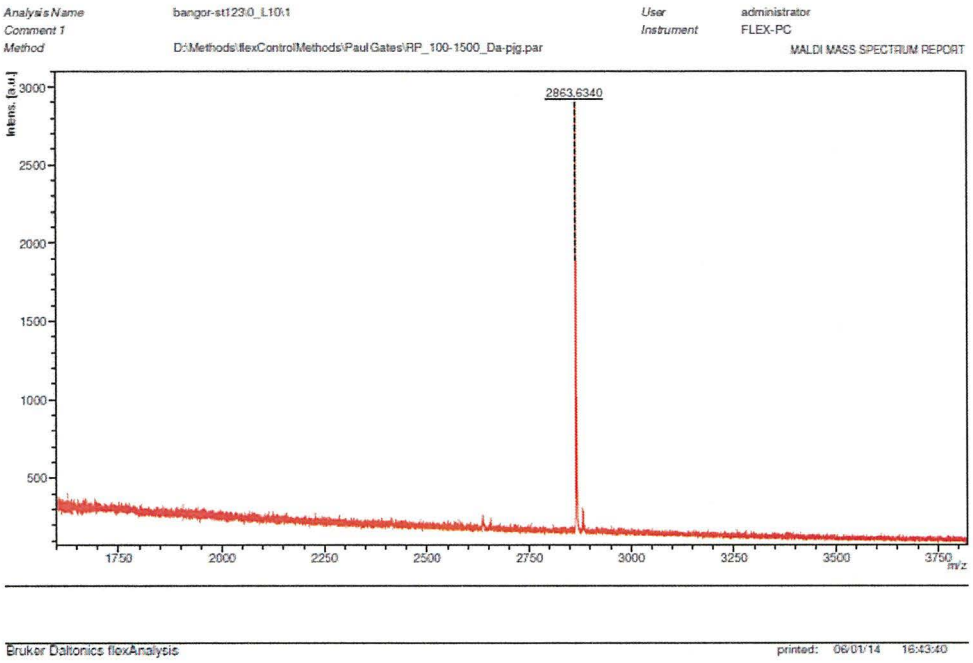


Figure 35: MALDI-MS of the synthetic *M.avium* wax ester TDM **(261)**

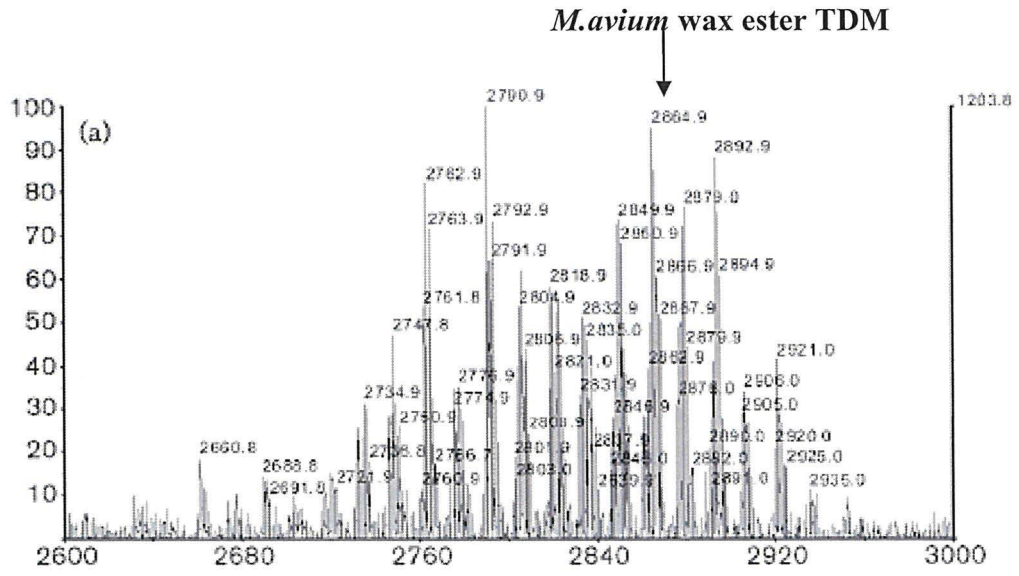


Figure 36: MALDI-MS of the natural mixture of *M.avium* TDM.[160]

The expanded proton NMR spectrum of the TDM **(261)** is shown in **(Figure 37)**.

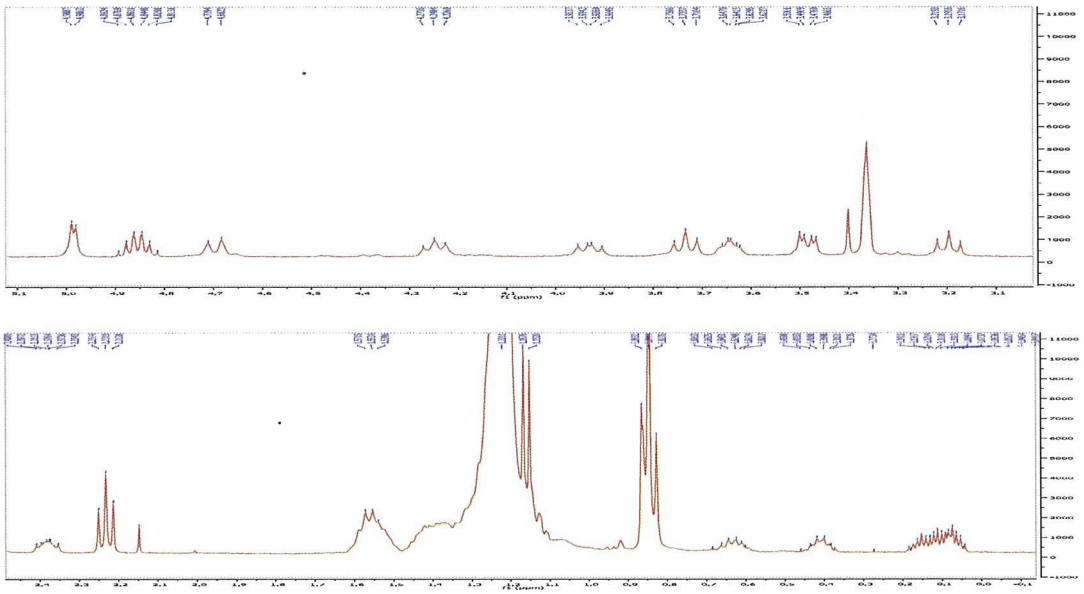
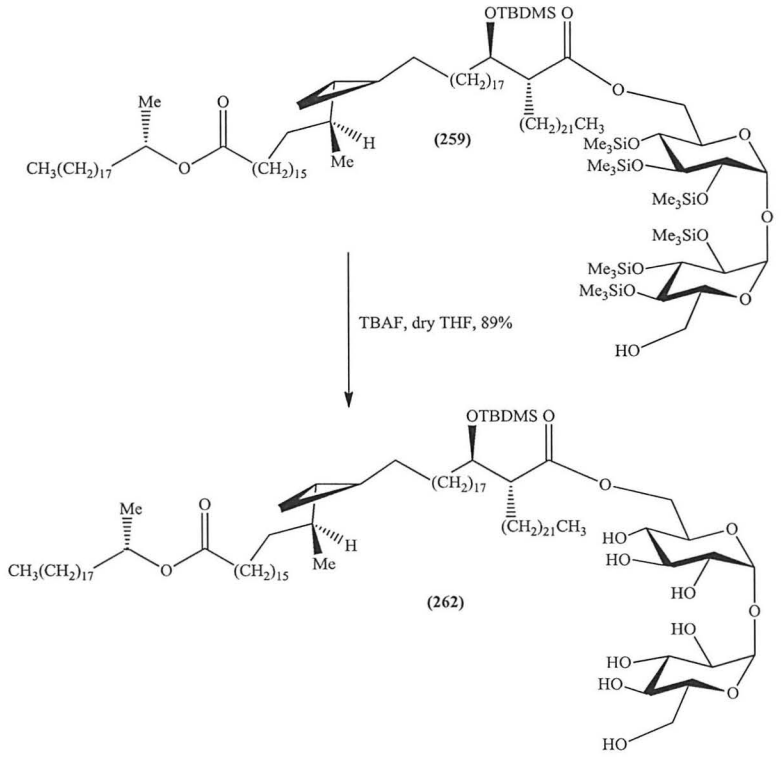


Figure 37: Expansion of the proton NMR of the TDM **(261)**

The proton NMR confirmed the loss of the signals for the *tert*-butyldimethylsilyl groups. In addition the final free TDM (**261**) was fully characterised by proton NMR which showed the acetal protons as a doublet at 4.98 ppm (*J* 3.4 Hz), and the chiral proton next to the ester group as a sextet at 4.87 ppm (*J* 6.4 Hz). The remaining sugar protons, including the two protons at the β-position appeared at 4.68, 4.24, 3.93, 3.73, 3.66–3.62, 3.49, 3.19 ppm, with an integration of two protons for each signal. The α-position proton next to the alkyl chain of the wax ester appeared as a multiplet at 2.4 ppm, and the two protons of the methylene next to the carbonyl group of the wax ester gave a sharp triplet at 2.23 ppm (*J* 7.4 Hz). The α-methyl next to the ester group appeared as a doublet at 1.16 ppm (*J* 6.2 Hz), and the α-methyl next to cyclopropane showed another doublet at 0.86 ppm (*J* 6.8 Hz). A broad triplet was seen at 0.86 ppm (*J* 6.9 Hz), integrated for twelve protons of the terminal methyl groups of the alkyl chains. The cyclopropane protons were seen as two multiplets at 0.66–0.59 ppm and 0.43–0.37 ppm, each integrating to two protons, and a multiplet at 0.18–0.04 ppm integrating to six protons. The carbon NMR also confirmed the disappearance of the *tert*-butyldimethyl silyl group signals, and showed two carbonyl carbon signals at 175.7 and 173.6 ppm. While the anomeric carbons signal appeared at 95.2 ppm.

3.2.5 Trehalose deprotection of TMM (259)

The removal of the trimethy silyl protecting groups on the trehalose core was achieved successfully using tetra-*n*-butyl ammonium fluoride (TBAF) in dry THF. The TBAF was added to a stirred solution of the TMM **(259)** in dry THF at 10 ° C, and then stirred for 30 minutes at room temperature. The residue was added directly to the silica column after the concentration in vacuum, and this gave the target compound **(262)** in 89 % yield (**Scheme 69**).

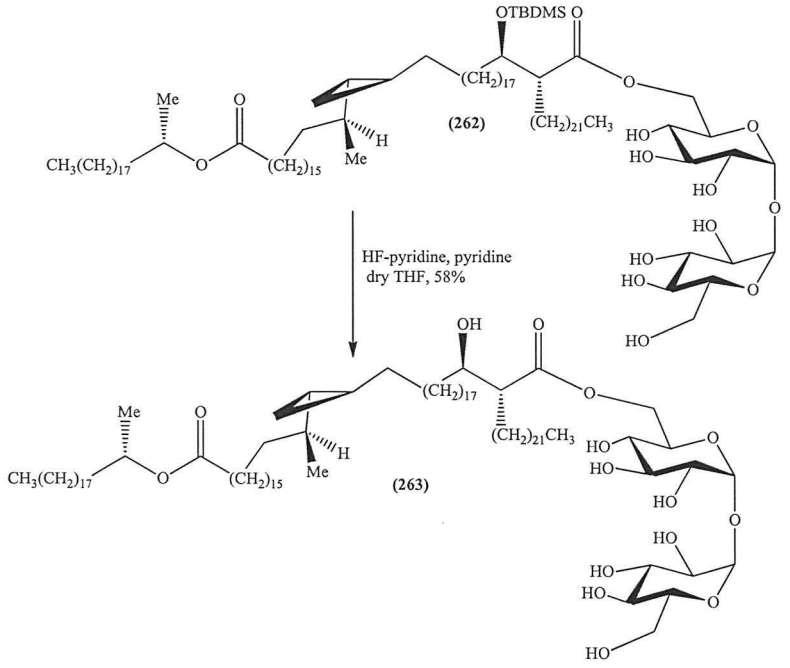


Scheme 69: Deprotection of the trehalose core of TMM **(259)**

The proton NMR spectrum confirmed the disappearance of the signals for the trimethyl protecting group at 0.17, 0.15, and 0.14 ppm, which means that the deprotection has been successful. The MS (MALDI-TOF) gave $[M+Na]^+$:1728.39; the calculated value requires 1728.45.

3.2.6 β-Position deprotection of TMM (262)

The deprotection of the *tert*-butyldimethylsilyl group on the β-position of the wax ester was achieved by using HF.pyridine complex and pyridine in dry THF and stirring at 43 °C for 17 hrs **(Scheme 70)**, and work up as above gave the final target compound **(263)** in 58 % yield.



Scheme 70: Synthesis of free wax ester TMM **(263)**

The MS (MALDI-TOF) of **(263)** found $[M+Na]^+$: 1614.00 **(Figure 38)**; the calculated value requires 1614.36. The MALDI MS of the natural mixture of *M.avium* TMM reported by Fujita *et al.* is shown in **Figure 39**.[161]

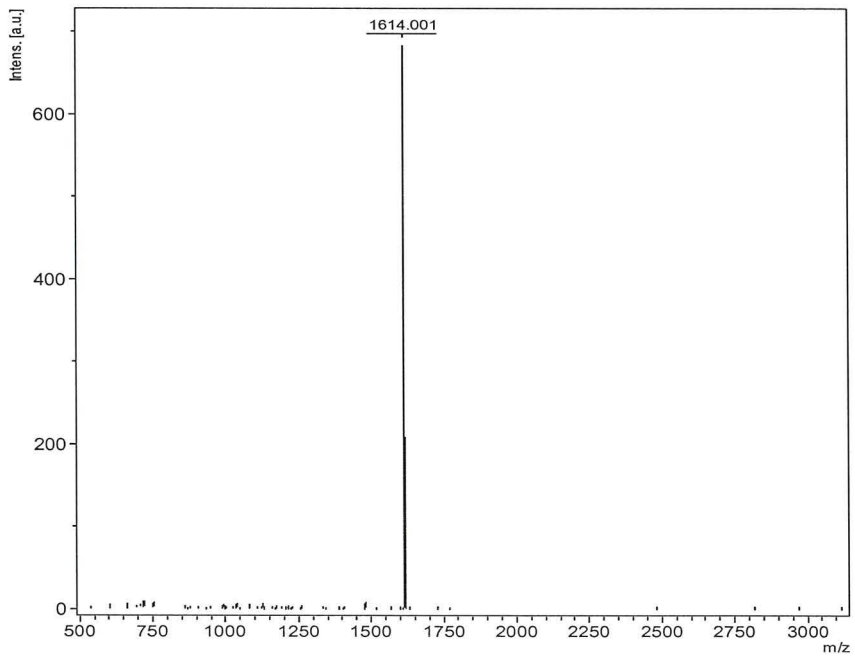


Figure 38: MALDI-MS of the synthetic *M.avium* wax ester TMM **(263)**

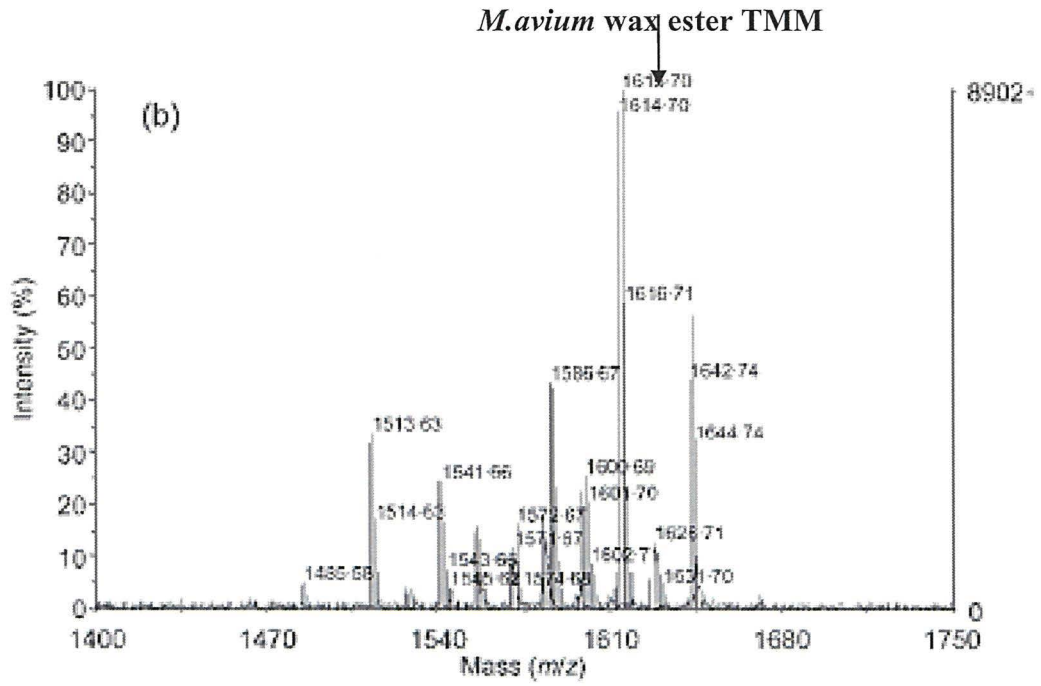


Figure 39: MALDI MS of the natural mixture of *M.avium* TMM.[161]

The expanded proton NMR spectrum of the TMM **(263)** is shown in **(Figure 40)**.

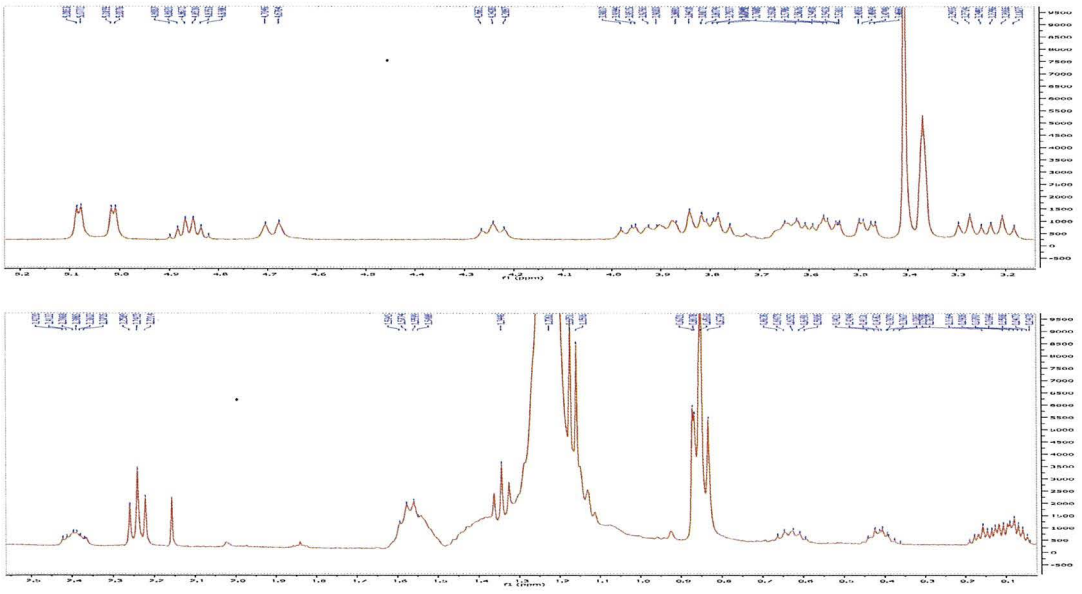


Figure 40: Expansion of the proton NMR of the TMM **(263)**

The disappearance of the signals of the *tert*-butyldimethyl silyl group confirmed the success of the reaction. The proton NMR showed two doublets at 5.08 ppm (*J* 3.2 Hz) and 5.02 ppm (*J* 3.3 Hz) corresponding to the acetal protons, and a sextet at 4.88 ppm (*J* 6.3 Hz) belonging to the chiral proton next to the ester group. The remaining sugar

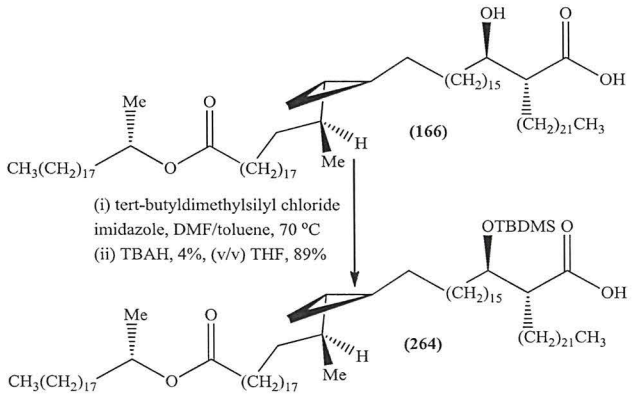
protons, including the β-position proton, appeared between 4.70–3.21ppm. The α-position proton next to the alkyl chain of the wax ester appeared as a multiplet at 2.38 ppm, and the two protons of the methylene adjacent to the carbonyl group of the wax ester gave a sharp triplet at 2.24 ppm (*J* 7.4 Hz). The α-methyl next to the ester group appeared as a doublet at 1.17 ppm (*J* 6.2 Hz), while the α-methyl next to the cyclopropane showed another doublet at 0.86 ppm (*J* 6.9 Hz). A broad triplet was seen at 0.86 ppm (*J* 6.9 Hz) integrated for six protons of the terminal methyl groups of the alkyl chains. The cyclopropane protons were seen as two multiplets at 0.66–0.59 ppm and 0.44–0.36 ppm, each integrating to one proton, and a multiplet at 0.18–0.04 ppm integrating to three protons. The carbon NMR also confirmed the disappearance of the *tert*-butyldimethyl silyl group signals.

3.3 Synthesis of the trehalose esters of *M.gordonae* wax ester (166)

The same method that was used to prepare *M. avium* trehalose esters **(261, 263)** was repeated to prepare *M. gordonae* trehalose esters **(267, 268)**.

3.3.1 Protection of the β-position of wax ester (166)

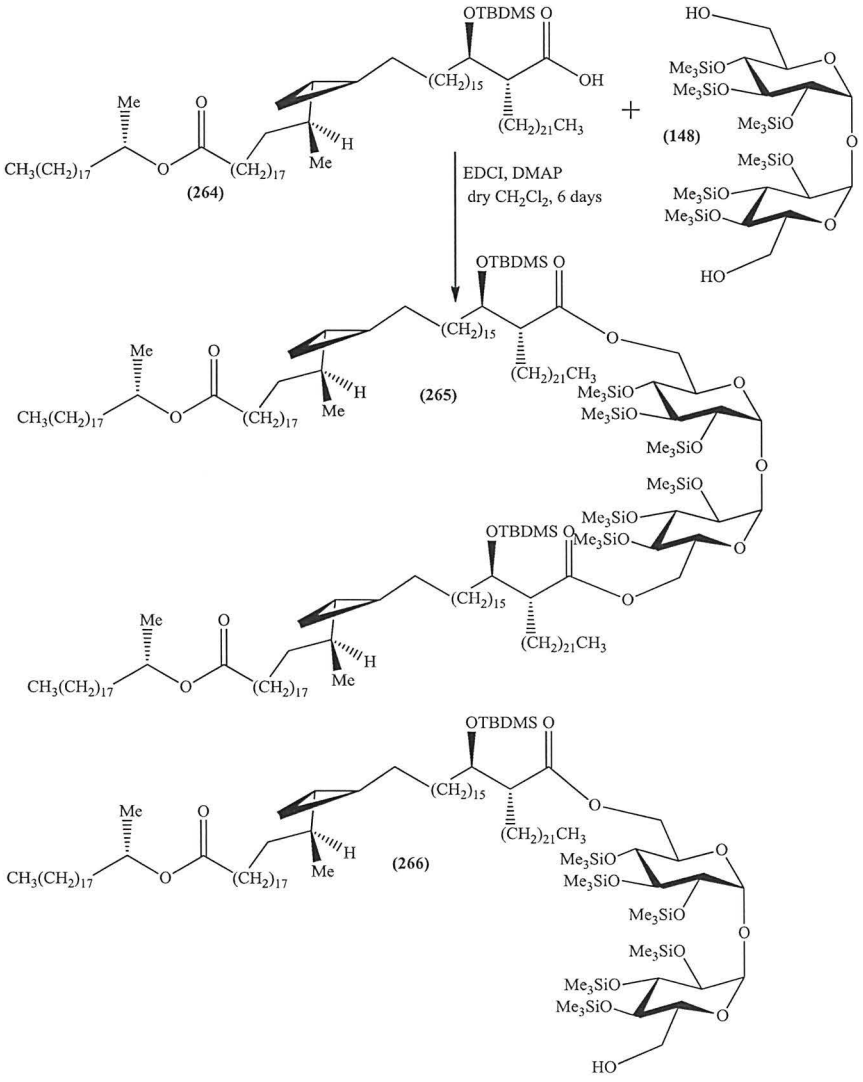
The β-hydroxy group of the wax ester **(166)** was protected with the TBDMS protecting group under the same conditions described for *M.avium* wax ester **(164) (Scheme 71)**. The compound **(264)** showed essentially identical NMR spectra to those of the *M.avium* protected wax ester.



Scheme 71: Protection of the free wax ester **(166)**

3.3.2 Coupling of protected wax ester (264) to protected trehalose

The protected wax ester **(264)** was coupled to protected trehalose **(148)** using EDCI and DMAP in dry dicholoromethane at room temperature for 6 days. This led to the TDM **(265)**, and TMM **(266)** after separation by column chromatography **(Scheme 72)**. The proton and carbon NMR data of the TDM **(265),** TMM **(266)** were essentially identical to that obtained for the *M. avium* TDM **(258)**, TMM **(259)**.

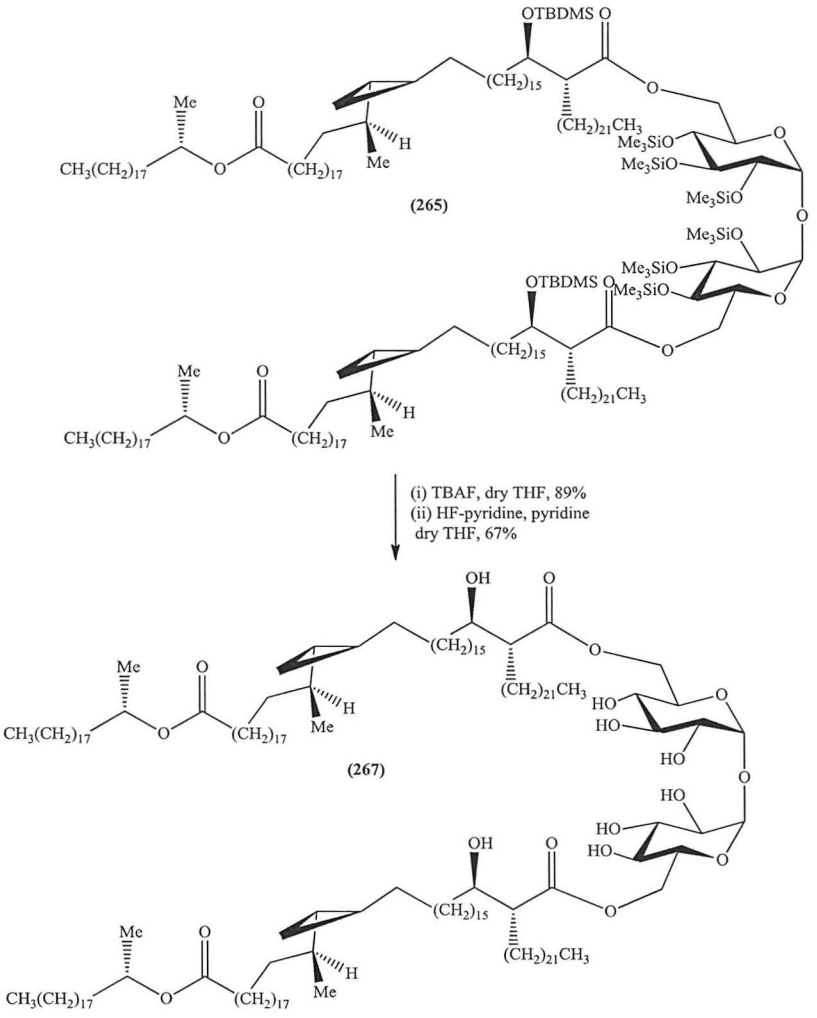


Scheme 72: Esterification of protected wax etser with protected trehalose

3.3.3 Deprotection of TDM (265)

The TDM was deproteced through two steps as described earlier. Firstly the trehalose was deprotected using TBAF in dry THF to give the desired product in 89 % yield. Finally, the deprotection of β-postion of the wax ester was carried out using HF-pyridine/pyridine in dry THF for 17 hrs, which gave the free TDM **(267)** in 67% yield

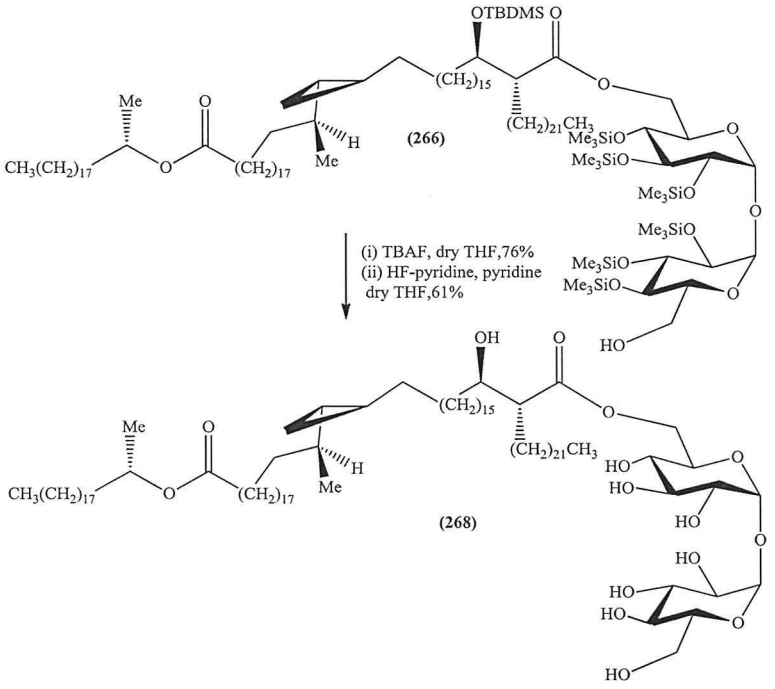
(Scheme 73). Both the proton and carbon NMR data were found to be identical to those of the *M.avium* TDM **(261).** The HRMS (MALDI-TOF) gave $[M+Na]^+$: 2863.6353, the calculated value requires: 2863.6314.



Scheme 73: Synthesis of free wax ester TDM **(267)**

3.3.4 Deprotection of TMM (266)

The silyl protecting groups of the trehalose and the wax ester were removed in two steps to give the free TMM **(268) (Scheme 74)** under the same reaction conditions described for *M.avium* TMM **(263)**. The TMM **(268)** showed identical NMR data to those of the *M.avium* TMM **(263).** The HRMS (MALDI-TOF) gave $[M+Na]^+$: 1614.3674; the calculated value requires 1614.3684.



Scheme 74: Synthesis of free wax ester TMM **(268)**

3.4 Synthesis of the trehalose esters of non-natural wax ester (168)

The same method that was used to prepare *M. avium* trehalose esters **(261, 263)** was applied to prepare trehalose esters (TDM, TMM) from the non-natural wax ester **(168)** **(Figure 41)**. Unfortunately, small quantity of the wax ester **(168)** was obtained to prepare both trehalose esters; owing to that, only a very small quantity of the TDM **(269)** was obtained, and no TMM was produced. The TDM **(269)** showed essentially identical NMR data to those of the *M.avium* TDM **(261).**

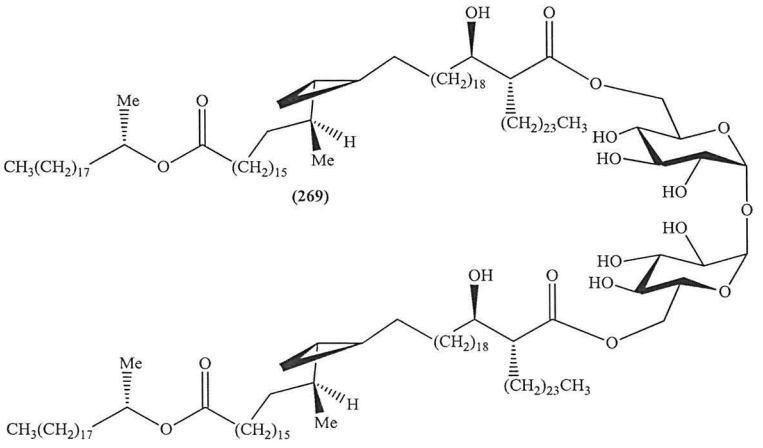


Figure 41: TDM **(269)** for the non-natural wax ester **(168)**

3.5 Biological activity results

ELISA assays and data analysis have been carried out by Dr. A. Jones for all of the synthetic wax esters and their corresponding trehalose esters. The ELISA experimental procedure is written at the end of the experimental **(Chapter 7)**. ELISA assays have been carried out on different serum samples including bovine TB, *M.avium paratuberculosis* (*Map*) and human TB serum samples (Discussed below).

3.5.1 Bovine TB serum samples from Veterinary Laboratories Agency (VLA)

20 tuberculin skin test positive samples (naturally infected) from cattle from farms with a confirmed history of bovine TB and 16 samples from non-vaccinated young cattle, which are expected to be TB negative, were tested using a range of wax ester compounds, from *M.avium* and *M.gordonae*, as antigens.

The graph below shows the average responses of the naturally infected and non-vaccinated samples to each of the synthetic antigens along with the responses to the natural wax ester and natural bovine TDM, for comparison. All samples were run at a 1 in 20 serum dilution and anti-bovine IgG (Fc specific) was used as the secondary antibody.

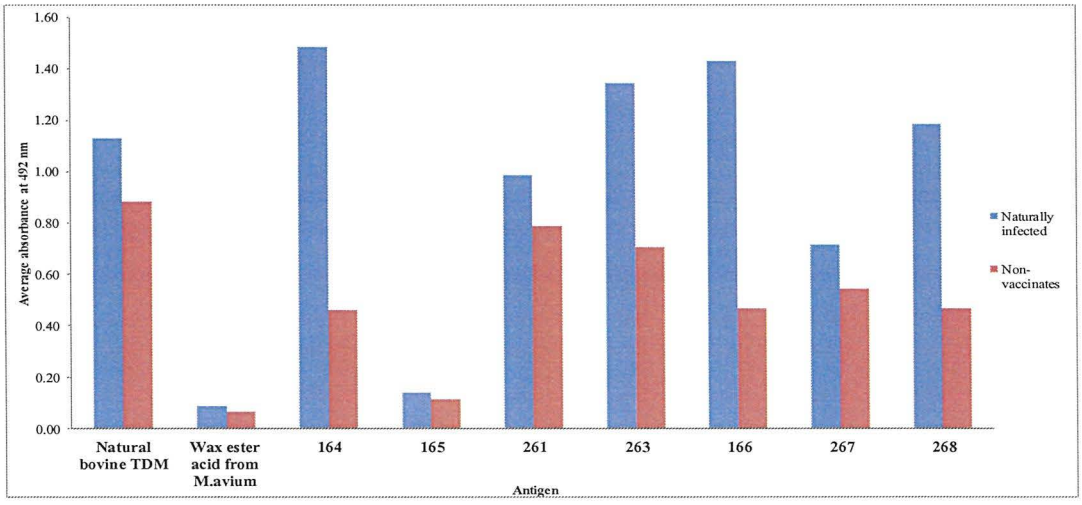


Figure (42): The average absorbance of VLA bovine TB serum samples to various wax esters antigens

As can be seen from the graph the *M.avium* and *M.gordonae* synthetic wax esters **(164)** and **(166)** show the best distinction between the 2 sets of samples. The corresponding TMMs **(263)** and **(268)** also show a good distinction, which is better than that of the corresponding TDMs **(261)** and **(263)**. The distinction observed for both the wax esters and the TMMs is also better than that for the natural TDM from *M.bovis*. The natural

wax ester and the diacid **(165)** show very little distinction between the 2 sets of samples. The absorbance values for all the samples with these antigens were however very low (<0.3, which is the same as the blank) this suggesting that there is no real response to these antigens.

Table 6 shows the sensitivity and specificity obtained for each of the above antigens to the samples from the VLA. Again a similar conclusion can be drawn with both the wax esters **(164)** and **(166)** giving the best values and showing a higher sensitivity and specificity than the corresponding TMMs and TDMs and also than the natural TDM from *M.bovis*.

	Natural bovine TDM	Natural *M.avium* wax ester	*M.avium* wax ester (164)	*M.avium* TDM (261)	*M.avium* TMM (263)	*M.avium* diacid (165)	*M.gordonae* wax ester (166)	*M.gordonae* TDM (267)	*M.gordonae* TMM (268)
Sensitivity (%)	63	30	75	60	65	20	75	65	60
Specificity (%)	56	94	75	69	63	94	75	81	69

Table 6: ELISA data of *M.avium* and *M.gordonae* antigens using VLA samples

The results discussed above, **(Figure 42)** and **(Table 6)**, indicate that the bovine serum samples from the VLA respond to the wax ester antigens. Moreover they also show a distinction between those infected with TB and those that are not infected. This was not expected as wax esters are found exclusively in *M.avium* and not in *M.tuberculosis*. A possible reason for these observations may be that in addition to being infected with TB the cattle are also infected with *M.avium*, as the *M.avium* status of the cattle is not known.

Another interesting observation from the above data is that the wax esters **(164)** and **(166)** show a higher response to and a better distinction between the serum samples than their corresponding TMMs and TDMs. This is in contrast to other synthetic antigens found in TB, where the free mycolic acids generally have a lower response than their corresponding TMMs and TDMs and also show a poorer distinction between the infected and uninfected samples (unpublished results within the MSB group).

3.5.2 M.avium paratuberculosis serum samples

In addition to the samples from the VLA, two samples from cattle infected with *M.avium paratuberculosis (Map)*, one naturally and one experimentally, from Brussels were also tested using the same set of antigens. A high response was observed with both samples to all the antigens, except the natural wax ester and synthetic diacid **(165)**. The response

of these samples to these 2 antigens was however higher than had been observed for the samples from the VLA.

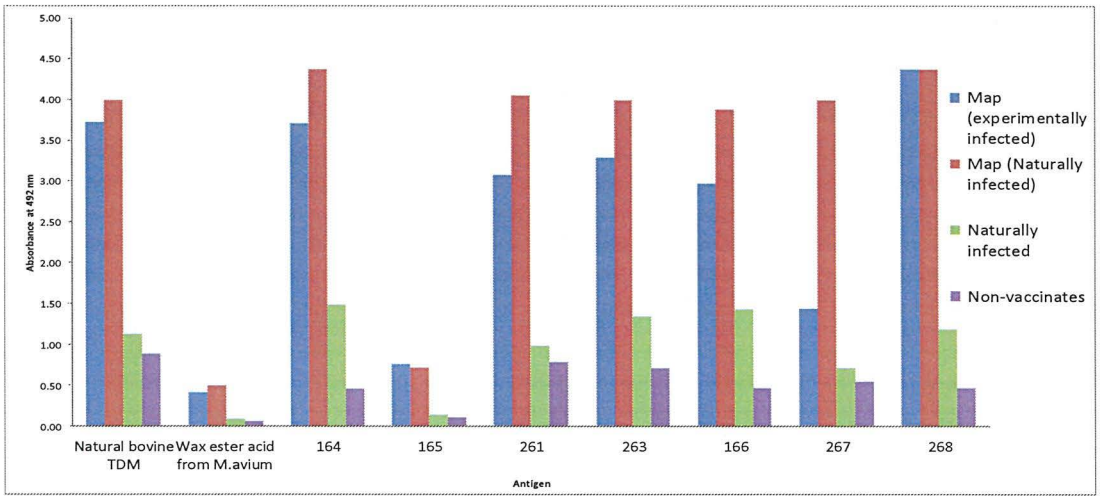


Figure (43): The average absorbance of 2 individual samples infected with *Map* and VLA bovine TB serum samples to various antigens.

The graph shows a comparison of the samples infected with *Map* and those infected with TB. As can be seen from the graph all the antigens show a higher response to the samples infected with *Map* compared to those infected with TB and those that are not infected. The values for the samples infected with *Map* are however for individual samples while those for the other two groups are the average for 20 and 16 samples respectively. A much larger set of samples infected with *Map* would therefore need to be tested in order to make a fair comparison between the different sets of samples and thus determine whether this pattern in the responses of the various antigens to the different sets is 'real'. An interesting observation from the above data is that although the values observed when using the natural wax ester and the diacid are generally low for all the samples tested, these two antigens only have a significant response to the samples infected with *Map*. This may suggest that these antigens would be suitable to distinguish between *Map* and TB. Again, in order to confirm whether this is the case a much larger set of samples infected with *Map* would need to be tested.

3.5.3 Human TB serum samples

A set of 64 serum samples from the WHO from patients from Gambia were tested using compounds **(261)** and **(263)** as antigens. Although all the patients had been hospitalised with TB related symptoms, only 9 were diagnosed as being TB+, with the other 55 being diagnosed as TB-.

The graph below shows the results obtained for TDM **(261)** and TMM **(263)** at a 1 in 80 serum dilution. The data for another antigen (AD132), run with serum samples at a 1 in 20 and 1 in 80 serum dilution, which had given the best results in a previous blind test (unpublished results within the MSB group) is also shown for comparison. Anti-human IgG (Fc specific) was used as secondary antibody in each case.

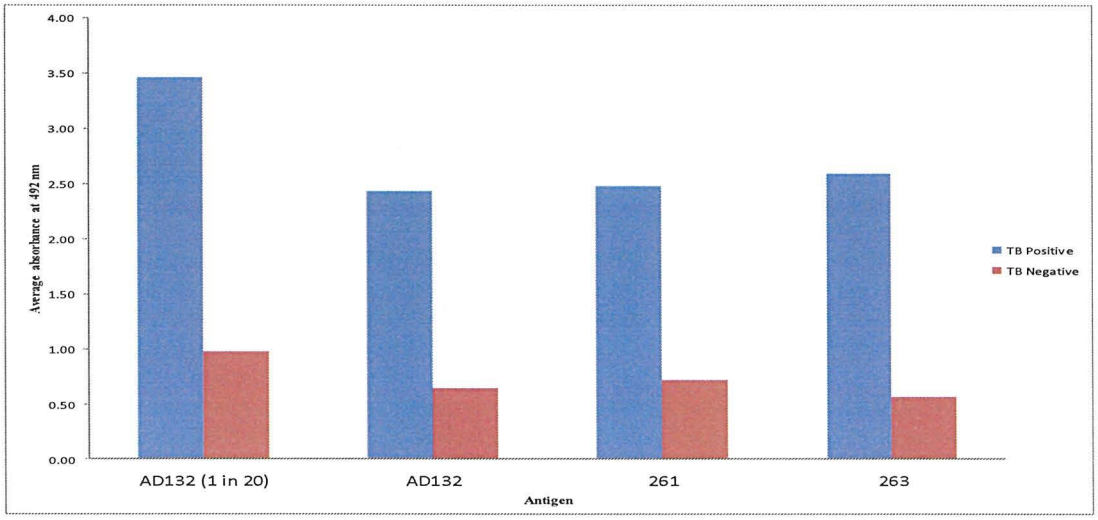


Figure (44): The average absorbance of 9 TB+ and 55 TB- human TB serum samples to various antigens

As can be seen from **(Figure 44)**, all the antigens show a good distinction between the TB+ and TB- serum samples. Although the values for the samples run at a 1 in 80 serum dilution are lower than those run at a 1 in 20 dilution they show a similar distinction to that obtained for AD132 at a 1 in 20 serum dilution.

The antigens also show good sensitivities and specificities, with the synthetic TDM **(261)** from *M.avium* giving the same values as those observed for AD132. The values for the TMM **(263)** were slightly lower with a sensitivity and specificity of 78 and 75 % respectively **(Table 7)**.

	AD132 (1 in 20)	**AD132 (1 in 80)**	**(261) (1 in 80)**	**(263) (1 in 80)**
Sensitivity (%)	89	89	89	78
Specificity (%)	80	78	78	75

Table 7: ELISA data of *M.avium* antigens

As was the case with the bovine serum samples infected with TB, the human samples from TB+ patients also show a response to the wax ester antigens. Again it is possible that these human serum samples may also be infected with *M.avium*, however the symptoms of TB and *M.avium* infection resembles each other.

Another set of serum samples from the WHO, from patients hospitalised with the symptoms of TB (17 found to be TB+ and 33 TB-), from various countries were tested using the non-natural wax ester TDM **(269)** as antigen. These were run at a 1 in 20 serum dilution using anti-human IgG (Fc specific) as the secondary antibody. Again the data for the best antigen AD132 is shown for comparison.

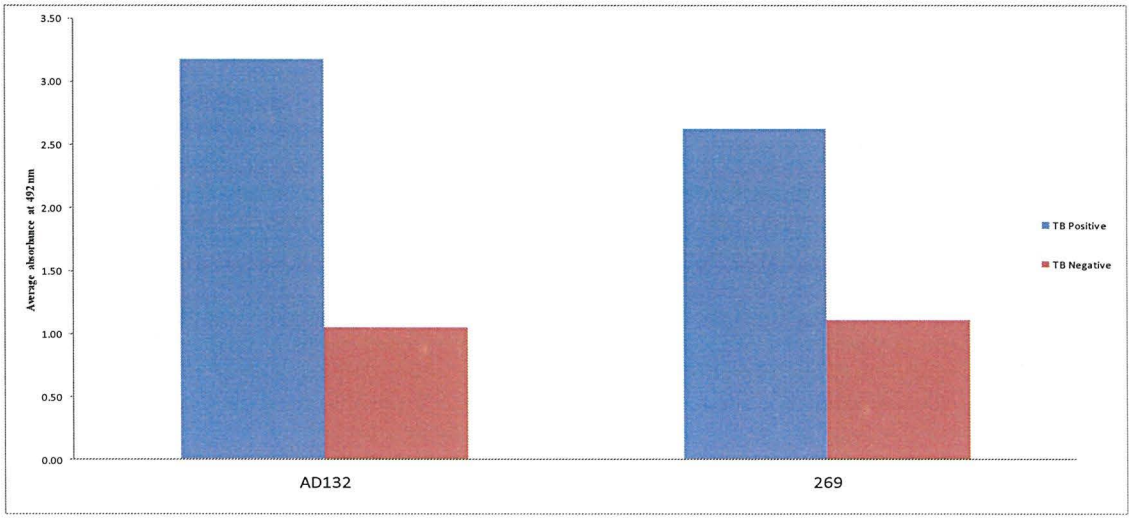


Figure (45): The average absorbance of TB+ and TB- human TB serum samples to various antigens

	AD132	**(269)**
Sensitivity (%)	100	86
Specificity (%)	76	63

Table 8: ELISA data of non-natural wax ester antigens

As can be seen from **(Figure 45)**, although the synthetic TDM **(269)** shows quite a good distinction between the TB+ and TB- serum samples it is not as good as AD132. This is also highlighted by the sensitivity and specificity values shown in **(Table 8)**, which are 86 and 63% respectively for the synthetic wax ester TDM **(269)** as compared to 100 and 76 % for AD132.

Chapter 4

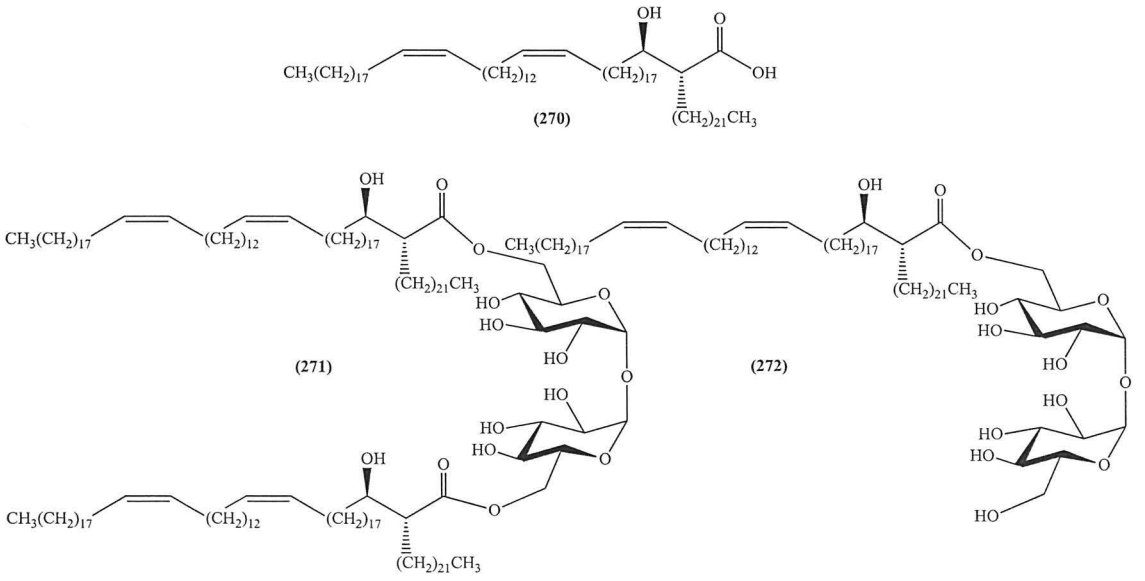
4. Synthesis of diene and alkene mycolic acids and their trehalose esters (TDM, TMM)

4.1 The aims

The aims of this chapter were the synthesis of diene and alkene mycolic acids and their trehalose esters, which then would be tested for their immunological properties (TNF-α cytokine stimulation), as well as the determination of their specificity and sensitivity in ELISA assays to detect mycobacterial infection.

This chapter consists of two parts. The first part was the synthesis of diene mycolic acid **(270)** and its trehalose esters **(271)** and **(272)**. The diene mycolic acid was synthesized using a modified method which gives a higher yield compared to the method described earlier by Muzael *et al.*[143]

The second part was the synthesis of alkene mycolic acid **(133)** and its trehalose esters **(273)** and **(274)**. The alkene mycolic acid is reported by Muzael *et al.*,[149] and its trehalose esters were attempted. In this work, firstly the alkene mycolic acid was synthesized using the same method, and then the syntheses of its trehalose esters were successfully completed **(Figure 46)**.



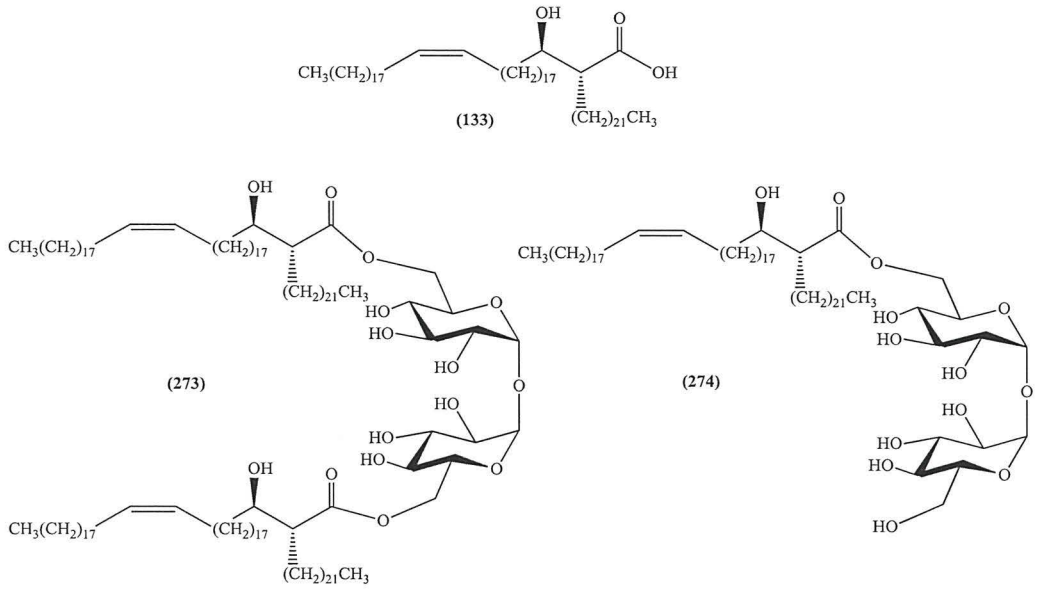
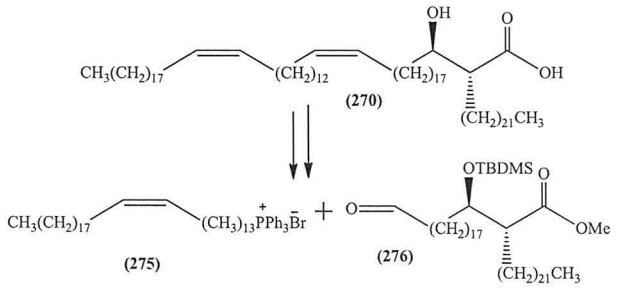


Figure 46: Synthesized mycolic acids and their trehalose esters discussed in this chapter

4.2 Synthesis of diene mycolic acid

4.2.1 Previous syntheses of diene mycolic acid (270)

Muzael *et al.*[143] was able to synthesize the diene mycolic acid (**270**) via Wittig coupling reaction between alkene phosphonium salt **(275)** and the mycolic motif aldehyde **(276)** using sodium *bis*(trimethylsilyl)amide in dry THF. This gave the desired product, but on a small scale and in low yield (32%) **(Scheme 75)**.

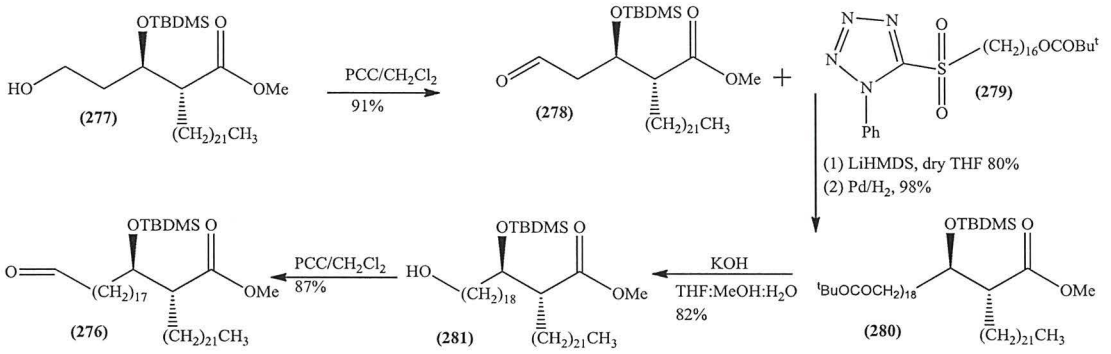


Scheme 75: Muzael *et al.*[143] method to prepare diene mycolic acid

The above method was repeated in several attempts in order to improve the yield of the reaction. These will be described below.

4.2.1.1 Synthesis of chain extended mycolic motif aldehyde (276)

Chain extended mycolic motif aldehyde **(276)** is the building block in the synthesis both of the alkene and diene mycolic acids. The route starts by oxidizing the known alcohol **(277)** using PCC in dichloromethane to give aldehyde **(278)**. The resulting aldehyde was coupled to the sulfone **(279)** (provided by Dr. Al Dulayymi) in a Julia-Kocienski olefination reaction using lithium *bis*(trimethylsilyl)amide, followed by hydrogenation to give **(280)**. The hydrolysis of the tertiary butyl ester of the compound **(280)** using KOH in a mixture of THF, MeOH, and H_2O led to the alcohol **(281)**, which was then oxidized using PCC to give the target aldehyde **(276)** **(Scheme 76)**.

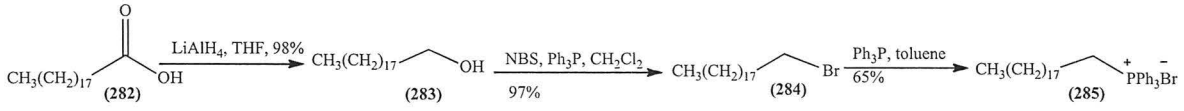


Scheme 76: The preparation of mycolic motif aldehyde **(258)**

All of the above compounds showed identical NMR spectra to those given in the literature, and the experimental procedures are given in the appendix.[149]

4.2.1.2 Synthesis of *Z*-alkene phosphonium salt (275)

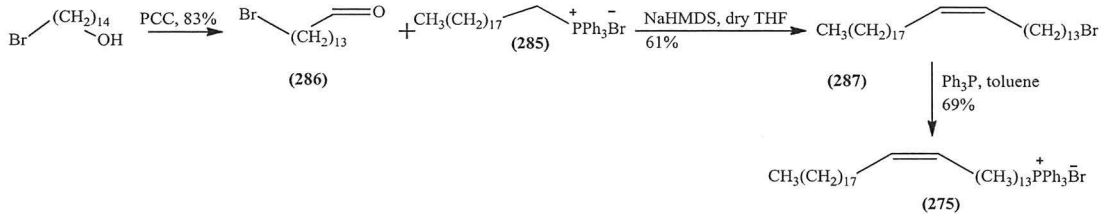
The synthesis of **(275)** firstly required the synthesis of alkyl phosphonium salt **(285)** in order to produce the desired *Z* alkene phosphonium salt **(275)**. Nonadecanoic acid **(282)** was reduced to alcohol **(283)** using lithium aluminium hydride in THF. This was converted into 1-bromononadecane **(284)** using *N*-bromosuccinimide with triphenylphosphine in dichloromethane. Finally the 1-bromononadecane **(284)** was refluxed with triphenyl phosphine in toluene for four days to give the phosphonium salt **(285)** **(Scheme 77)**. The resulting phosphonium salt **(285)** was purified by column chromatography eluting with petroleum ether and then dichloromethane /methanol (95:5), and then precipitated in dry ether.



Scheme 77: Synthesis of alkyl phosphonium salt **(285)**

The Z-alkene phosphonium salt **(275)** was prepared via the Wittig coupling reaction between the above alkyl phosphonium salt **(285)** and bromo aldehyde **(286)** using sodium *bis*(trimethylsilyl)amide in dry THF to give **(287)** **(Scheme 78)**.

The produced bromo alkene **(287)** was refluxed with triphenylphosphine in toluene to give the Z alkene phosphonium salt **(275)** **(Scheme 78)**.

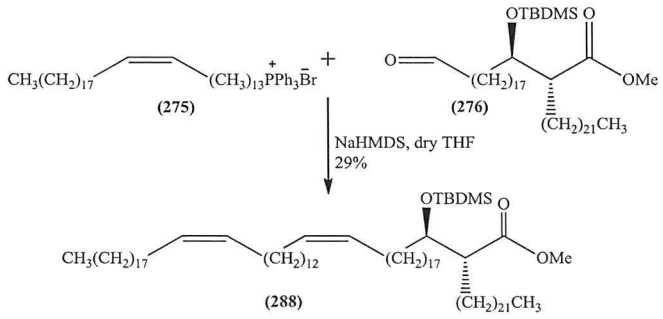


Scheme 78: Synthesis of *Z* alkene phosphonium salt **(275)**

All of the compounds in **Scheme (77)** and **(78)** showed identical NMR spectra to those in the literature,[143] and the experimental procedures are written in the appendix.

4.2.1.3 Synthesis of complete diene mycolic acid

The previously prepared aldehyde **(276)** was coupled to the above *Z*-alkene phosphonium salt **(275)** by a Wittig reaction **(Scheme 79)**, which led to the diene mycolic acid **(288)** in 29 % yield.



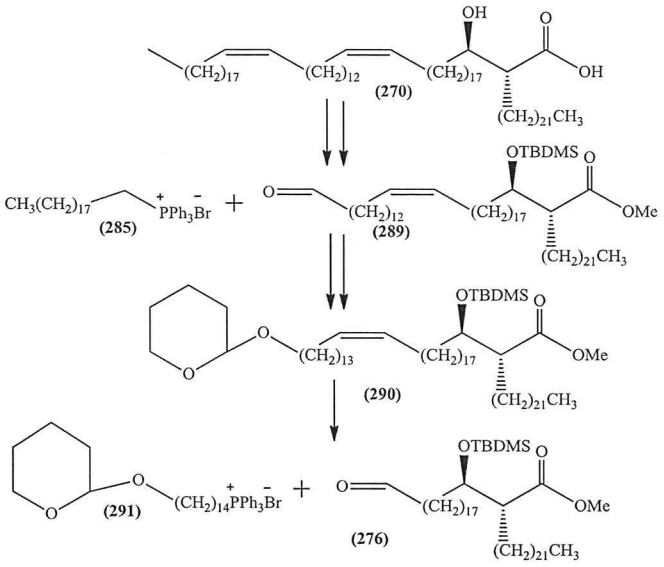
Scheme 79: Synthesis of complete diene mycolic acid using Muzael *et al.*[143] method.

4.2.2 Modified method to synthesize diene mycilic acid (270)

The ineffectiveness of the above method made it necessary to develop an alternative approach to diene mycolic acids on a larger scale in order for then to be used to synthesize trehalose esters.

The following route was employed, which led to the diene mycolic acid **(270)** in higher yield compared to the Muzael *et al.* method. The yield of the first Wittig reaction

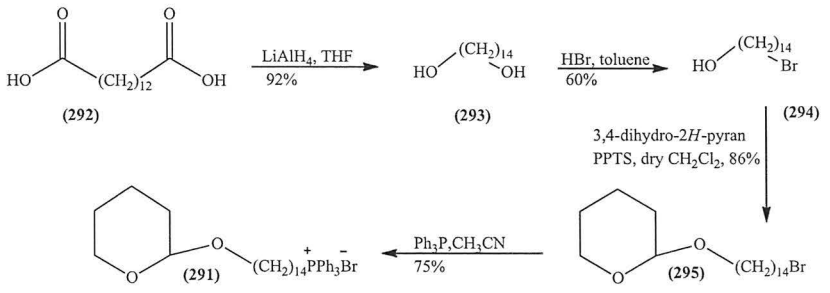
between **(276)** and **(291)** was 73% and for the second Wittig reaction between **(289)** and **(285)** was 59%. **(Scheme 80)**.



Scheme 80: The alternative approach to prepare diene mycolic acid

4.2.2.1 Synthesis of phosphonium salt (291)

The route to protected phosphoniunm salt **(291)** started by reducing 1,12-dodecanedicarboxylic acid **(292)** to the diol **(293)** using lithium aluminium hydride in dry THF **(Scheme 81)**. The diol **(293)** was characterized by proton NMR, which showed a triplet at 3.65 ppm (*J* 6.6 Hz) corresponding to the two protons next to the hydroxyl group, and the carbon NMR spectrum also confirmed the disappearance of the carbonyl groups. The diol **(293)** was converted to bromo alcohol **(294)** using hydrobromic acid in toluene. The proton NMR of compound **(294)** showed a triplet at 3.65 ppm (*J* 6.6 Hz) corresponding to the two protons next to the hydroxyl group, and another triplet appeared at 3.42 ppm (*J* 6.8 Hz) corresponding to the other two protons next to bromine. Two pentets appeared at 1.86 (*J* 6.9 Hz) and 1.57 ppm, (*J* 6.6 Hz), each integrating to two protons, while the remaining protons appeared as a multiplet between 1.48–1.27 ppm.

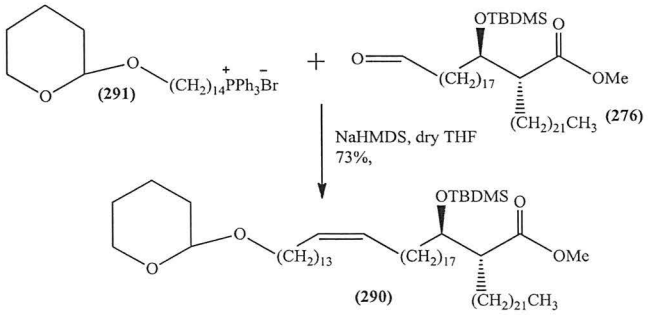


Scheme 81: The synthesis of phosphoniunm salt **(263)**

The hydroxyl group of the bromo-alcohol **(294)** was protected with dihydropyran in the presence of pyridinium-*p*-toluene–sulfonate in dichloromethane to give the bromo-compound **(295)** in 86% yield. The proton NMR showed signals for the THP group starting from broad multiplet at 4.57 ppm belonging to the acetal proton. A multiplet was seen at 3.85 ppm, and a doublet of triplets at 3.73 ppm (*J* 6.9, 9.5 Hz) each integrating to one proton belonging to the methylene next to the oxygen of the ring. A triplet appeared at 3.40 ppm (*J* 6.9 Hz) corresponding to the two protons next to bromine. The carbon NMR also showed THP signal at 98.8 ppm for the acetal carbon. The phosphonium salt **(291)** was obtained by refluxing the bromo-compound **(295)** and triphenylphosphine in dry acetonitrile at 70°C for 4 days **(Scheme 81)**. The pure phosphonium salt **(291)** was obtained in 75% yield by column chromatography, firstly eluting with petroleum ether and then dichloromethane/methanol (95:5), and was then precipitated in dry ether. It was found that this method of purifying the phosphonium salt is better than recrystallization alone, and can improve the yield of the subsequent Wittig reaction. The proton NMR of **(291)** showed the aromatic protons as a broad multiplet between 8.03–7.53 ppm integrated for fifteen protons, and the two protons next to the phosphorus appeared as a multiplet at 3.85 ppm. The existence of the THP protons was also confirmed, and showed similar signals to those given for the starting material **(295)**. The carbon NMR also showed the aromatic carbons between 135.1–117.8 ppm, and the THP acetal carbon at 98.9 ppm.

4.2.2.2 Insertion of the first double bond via a Wittig reaction

The aldehyde **(276)** was built up by first reacting with the phosphonium salt **(291)** and base in a Wittig reaction, giving the compound **(290)** in 73 % yield **(Scheme 82)**.

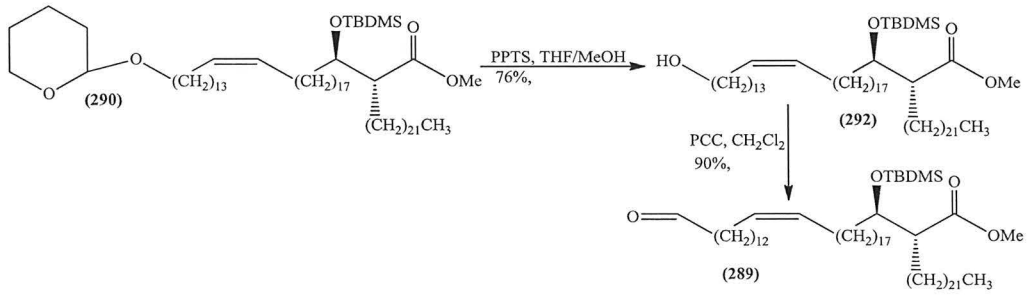


Scheme 82: The Insertion of the first double bond

The proton NMR of **(290)** showed a broad triplet at 5.35 ppm (*J* 4.7 Hz) corresponding to the two protons of the double bond. The signals of the THP group showed a broad multiplet at 4.58 ppm belonging to the acetal proton, while the remaining signals appeared between 3.93–3.39 ppm, including the β-position proton in the mycolic motif. The α-position proton appeared as a doublet of doublets of doublets at 2.55 ppm (*J* 3.8, 7.2, 10.9 Hz), and the protons next to the double bond showed a quartet at 2.02 (*J* 6.5 Hz). The terminal methyl group of the α-alkyl chain gave a triplet at 0.89 ppm (*J* 6.6 Hz). The silyl protecting group protons appeared as a singlet at 0.87 ppm integrating to nine protons and corresponding to those of the tertiary butyl substituent, and two singlets at 0.05 and 0.02 ppm for the two methyl groups bound to silicon. The carbon NMR showed the alkene carbons at 129.9 ppm, and the THP acetal carbon at 98.8 ppm. The β-position carbon appeared at 73.2 ppm, while the α-position carbon and the methoxy group gave peaks at 51.6, and 51.3 ppm respectively.

4.2.2.3 Deprotection and oxidation

The THP protecting group was removed by stirring compound **(290)** in a mixture of THF and MeOH in the presence of pyridinium-*p*-toluenesulfonate at 45 °C for six hours **(Scheme 83)**.



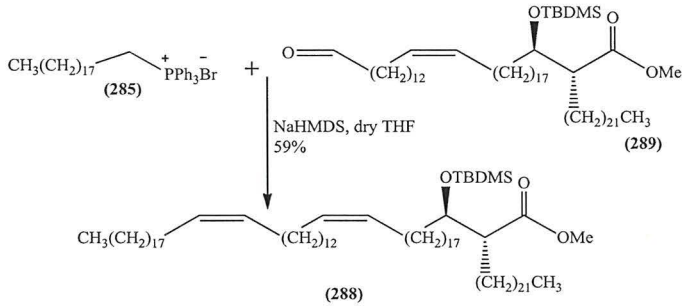
Scheme 83: Deprotection and oxidation of **(264)**

The oxidation of the alcohol **(292)** was carried out in CH_2Cl_2 solvent using PCC as oxidizing agent to give the aldehyde **(289)** in 90 % yield **(Scheme 83)**. The proton NMR showed a triplet at 9.77 ppm (*J* 1.8 Hz) corresponding to the aldehyde proton, and another triplet appeared at 5.35 ppm (*J* 4.7 Hz) corresponding to the alkene protons. The β-position proton appeared as a doublet of triplets at 3.9 ppm (*J* 7.0, 4.6 Hz), while the α-position proton gave a doublet of doublets of doublets at 2.53 ppm (*J* 3.7, 7.1, 10.9). The two protons next to the aldehyde functional group were seen as a doublet of triplets

at 2.43 ppm (*J* 1.8, 7.4). The carbon NMR showed a new signal for the aldehyde carbon atom at 202.9 ppm.

4.2.2.4 Insertion of the second double bond via a Wittig reaction

The Wittig reaction was carried out between the aldehyde **(289)** and the previously prepared phosphonium salt **(285)** **(Scheme 84)** under the same conditions as described earlier gave the complete diene mycolic acid **(288)** in 59% yield.



Scheme 84: Preparation of complete diene mycolic acid **(288)**

The proton NMR of **(288)** confirmed the success of the reaction, which showed the two *cis* double bonds protons as a broad triplet at 5.35 ppm (*J* 4.7 Hz) integrating to four protons **(Figure 47)**. The β-position proton appeared as a doublet of triplets at 3.91 ppm (*J* 7.0, 4.6 Hz), and the methoxy group as a singlet at 3.66 ppm. The α-position proton appeared as a doublet of doublets of doublets at 2.55 ppm (*J* 3.9, 7.2, 10.9 Hz), and the eight protons next to the two double bonds showed a quartet at 2.02 (*J* 6.7 Hz). The terminal methyl groups gave a triplet at 0.88 ppm (*J* 6.9 Hz). The silyl group protons appeared as singlet at 0.86 ppm integrated for nine protons of the tertiary butyl ester and two singlets at δ 0.05 and 0.02 for the methyl groups bound to silicon. The carbon NMR showed the carbonyl carbon at 175.2 ppm, and the alkene carbons at 129.9 ppm.

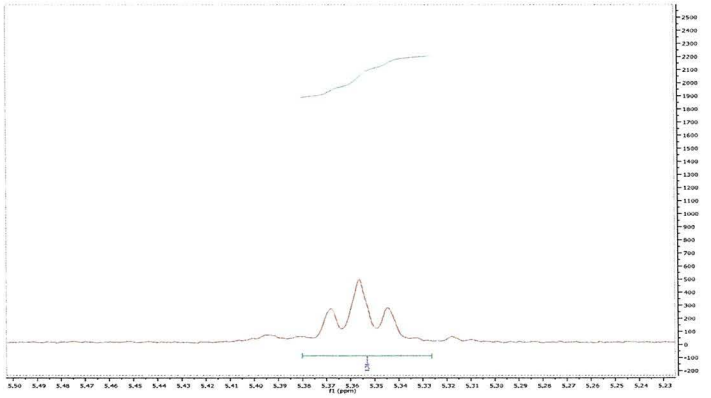
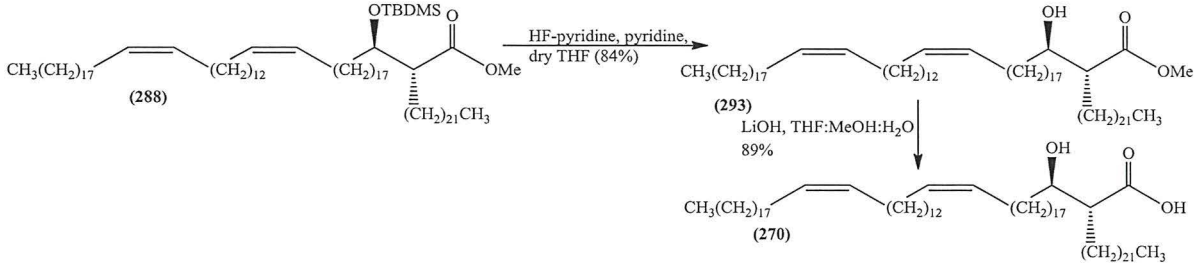


Figure 47: The proton NMR signal for the *cis* alkene protons of **(288)**

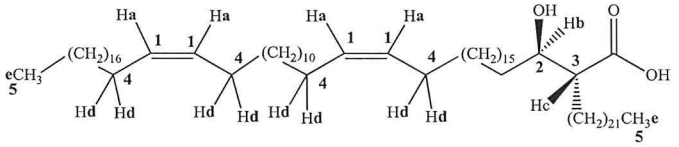
4.2.2.5 Deprotection and hydrolysis to produce free diene mycolic acid (270)

The *tert*-butyldimethylsilyl group was removed using HF-pyridine/pyridine in dry THF to give the methyl ester mycolic acid **(293) (Scheme 85)**.



Scheme 85: Deprotection and hydrolysis to produce the free diene mycolic acid **(270)**

Finally the methyl ester **(293)** was hydrolysed using lithium hydroxide in a mixture of THF: methanol: water at 40 °C for 18 hrs; this led to free diene mycolic acid **(270) (Scheme 85)**. Both the proton and carbon NMR data for the compound **(270)** were similar to those reported for the same compound prepared by the Muzael *et al.* method.[143] Selected proton and carbon NMR signals are shown in **(Table 9)**.

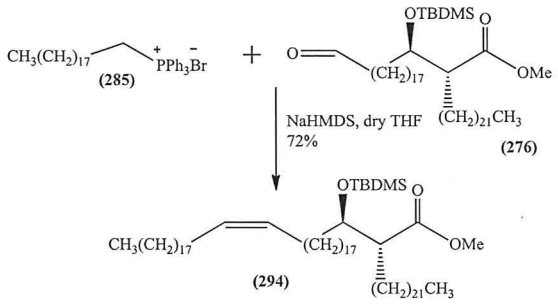


Proton	ppm	Multiplicity	Integration	*J* Hz	Carbon	(^{13}C NMR) ppm
Ha	5.35	br.t	4	4.7	**C1**	129.9
Hb	3.72	m	1	----	**C2**	72.1
Hc	2.47	br.dt	1	9.3, 5.3	**C3**	50.8
Hd	2.04	q	8	6.4	**C4**	32.6
He	0.89	t	6	6.6	**C5**	14.1

Table 9: Selected proton and carbon NMR data for diene mycolic acid **(270)**

4.3 Synthesis of alkene mycolic acid (294)

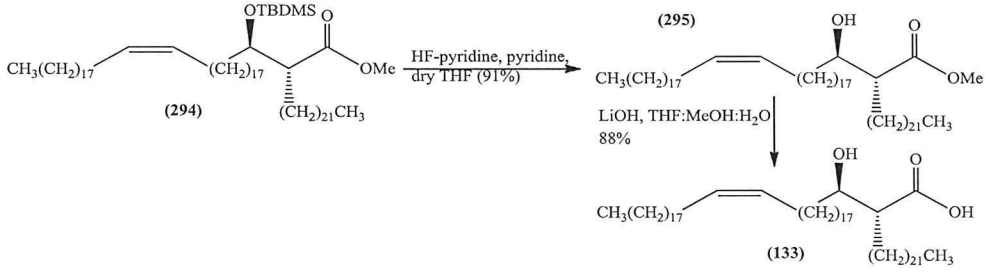
The alkene mycolic acid **(294)** was prepared using the method described by Muzael *et al.*[149] The aldehyde **(276)** was coupled to the phosphonium salt **(285)** in a Wittig reaction **(Scheme 86)** with sodium *bis*(trimethylsilyl)amide in THF at room temperature.



Scheme 86: Preparation of alkene mycolic acid **(294)**

4.3.1 Deprotection and hydrolysis to produce free alkene mycolic acid (133)

The protected mycolic acid **(294)** was treated with HF-pyridine/pyridine in dry THF to remove the *tert*-butyldimethylsilyl group to give the methyl ester mycolic acid **(295)**. Finally, the resulting compound was hydrolysed using lithium hydroxide in a mixture of THF: methanol: water at 40 °C for 18 hrs to give the free alkene mycolic acid **(133)** **(Scheme 87)**. This would be converted into the corresponding trehalose esters as described later in this chapter.



Scheme 87: Deprotection and hydrolysis to produce the free alkene mycolic acid **(133)**

The compounds produced in **Schemes (86)** and **(87)** showed identical NMR data to those given in the literature,[149] and the experimental procedures are in the appendix.

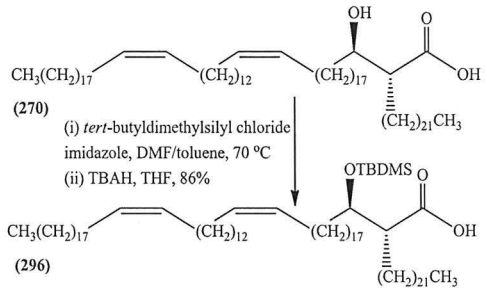
4.4 Synthesis of trehalose esters of diene and alkene mycolic acid

Another two sets of cord factors were prepared in this chapter using the method described in **Chapter 2**.

4.4.1 Synthesis of trehalose esters of diene mycolic acid

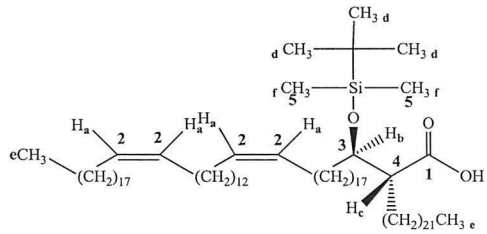
4.4.1.1 Protection of the β-position of the diene mycolic acid

The free diene mycolic acid **(270)** was protected by stirring it with *tert*-butyldimethylsilylchloride, imidazole and DMAP in a mixture of DMF/toluene at 70 °C for 18 hours **(Scheme 88)**. The work-up procedure described in Chapter 3 was used.



Scheme 88: Protection of the free diene mycolic acid **(270)**

The success of the reaction was confirmed by both proton and carbon NMR. Selected signals are shown in **Table 10**.

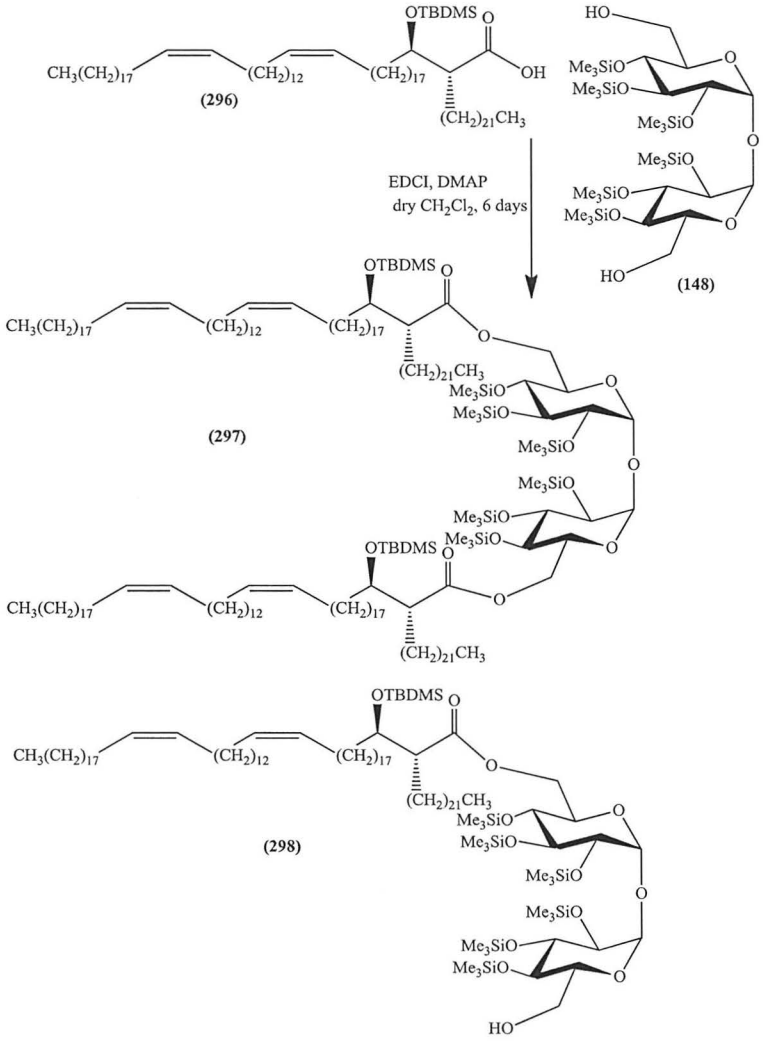


Proton	ppm	Multiplicity	Integration	*J* Hz	Carbon	^{13}C NMR/ppm
Ha	5.35	br.t	4	4.7	**C1**	175.2
Hb	3.85	m	1		**C2**	129.9
Hc	2.53	ddd	1	3.2, 5.6, 9.0	**C3**	73.7
Hd	0.92	s	9	--	**--**	---
He	0.87	t	6	6.9	**C4**	50.2
Hf	0.14, 0.13	s	6	--	**C5**	– 4.3, –4.90

Table 10: Selected proton and carbon NMR data for protected diene mycolic acid **(296)**

4.4.1.2 Coupling of protected diene mycolic acid to protected trehalose

The protected diene mycolic acid **(296)** was coupled with protected trehalose **(148)** using EDCI and DMAP **(Scheme 89)**. This gave TDM **(297)** and TMM **(298)** after separation by column chromatography.



Scheme 89: Esterification of protected diene mycolic acid with protected trehalose

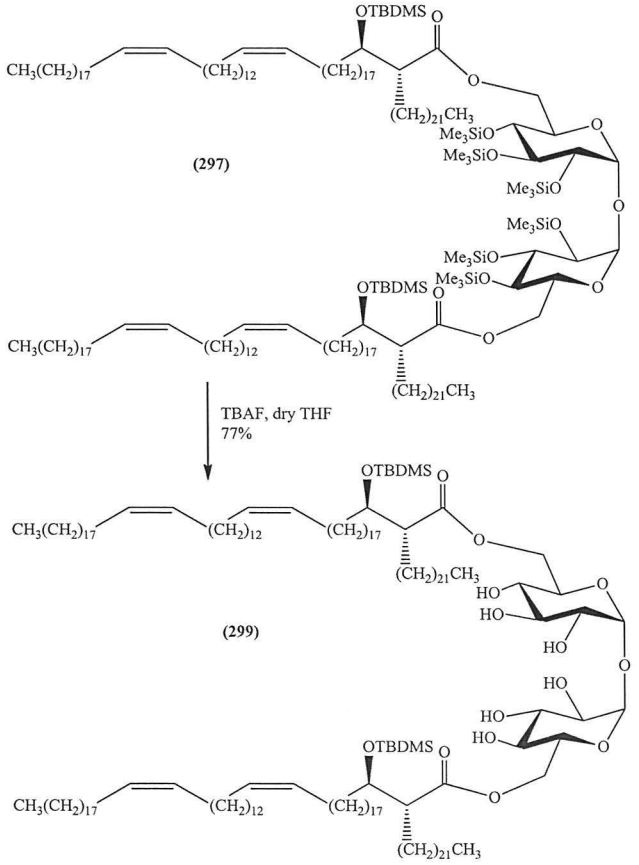
The MS (MALDI-TOF) for the TDM **(297)** gave the correct mass ion at $[M+Na]^+$: 3207.74; the calculated value: 3207.78. The TDM **(297)** was also fully characterized by proton NMR, which included a broad triplet at 5.35 ppm (*J* 4.7 Hz) for eight alkene protons. The two acetal protons of the trehalose appeared as a doublet at 4.85 ppm (*J* 3 Hz), and the remaining trehalose protons, including the β-position protons appeared at 4.37, 4.04–3.95, 3.79–3.72, 3.52, and 3.38 pm. The α-position proton of the mycolic acid appeared as a multiplet at 2.53 ppm, and a quartet appeared at 2.07 ppm (*J* 6.6 Hz), integrating for sixteen protons, corresponding to those next to the double bonds. The *tert*-butyl protons appeared as a singlet at 0.88 ppm, and the terminal methyl groups of

the alkyl chains appeared as a triplet at 0.84 ppm (*J* 6.8 Hz). The protons of the trimethylsilyl protecting groups on the trehalose gave three singlets at 0.16, 0.14, and 0.12 ppm each integrating for eighteen protons. The methyl groups of the TBDMS protecting group showed a singlet at 0.05 ppm integrating to twelve protons. The carbon NMR showed the carbonyl carbon signal at 173.8 ppm, and the alkene carbons at 129.9 ppm. The anomeric carbon signal appeared at 94.8 ppm, and the remaining trehalose carbon signals appeared between 75.5–70.7 ppm.

The MS (MALDI-TOF) for the TMM **(298)** gave the correct mass ion at $[M+Na]^+$: 2002.54; the calculated value 2002.56. The proton NMR spectrum of the TMM **(298)** was more complicated due to the loss of symmetry. The acetal protons appeared as two doublets at 4.92 ppm (*J* 3.1 Hz) and 4.85 ppm (*J* 3.0 Hz), and the remaining sugar protons appeared between 4.35–3.40 ppm. The remainder of the spectrum for the diene mycolic acid appeared similar to those of the TDM, with half of the integration as expected.

4.4.1.3 Trehalose deprotection of TDM (297)

The trimethylsilyl protecting groups of the trehalose of the TDM **(297)** were removed using TBAF in dry THF to give **(299)** in 77 % yield **(Scheme 90)**.

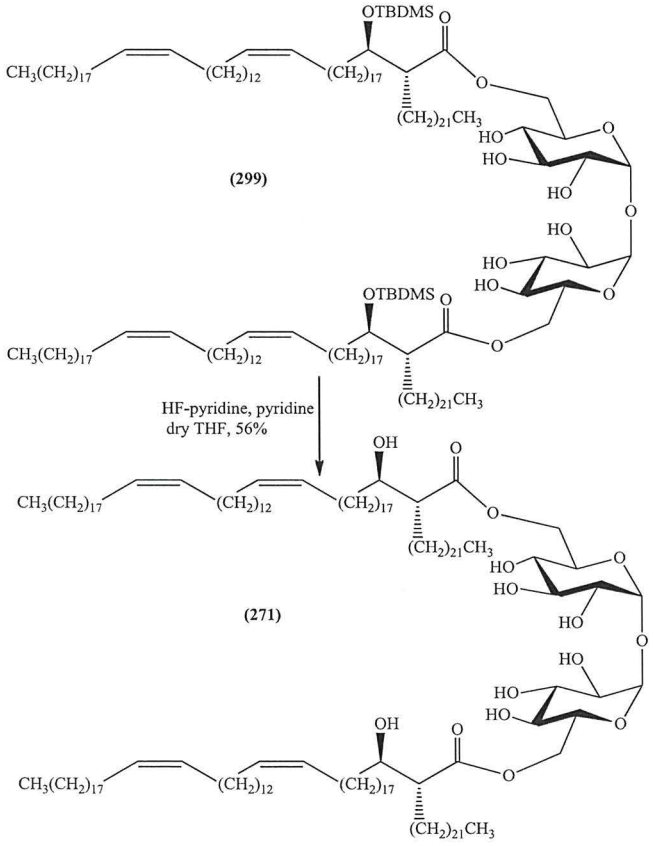


Scheme 90: Deprotection of the trehalose core of TDM **(297)**

The disappearance of the proton NMR signals of the trimethylsilyl groups at 0.16, 0.14, and 0.12 ppm confirmed the success of the deprotection reaction. The MS (MALDI-TOF) gave the correct mass, $[M+Na]^+$: 2775.57; the calculated value: 2775.54

4.4.1.4 β-Position deprotection of TDM (299)

Finally the *tert*-butyldimethylsilyl protecting group of the mycolic acid was removed by using HF.pyridine complex and pyridine to give the free TDM **(271)** in 56% yield (**Scheme 91**). The MS (MALDI-TOF) gave $[M+Na]^+$: 2547.37; the calculated value: 2547.37.



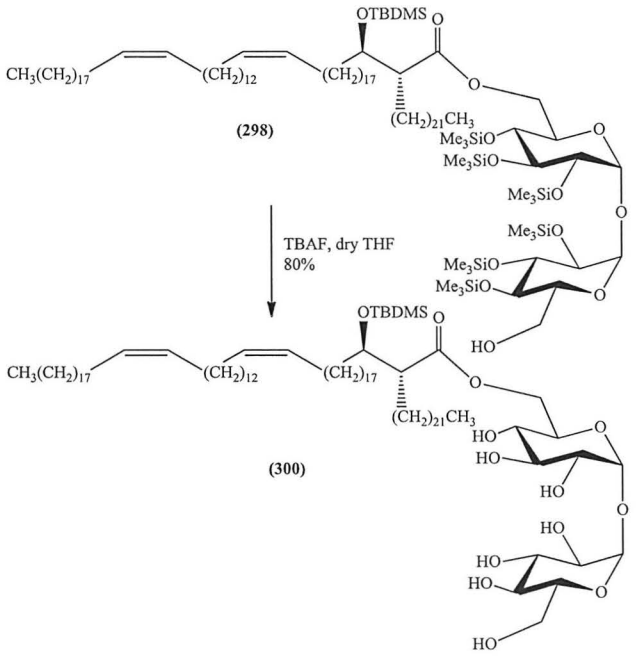
Scheme 91: Synthesis of free TDM **(271)**

The proton NMR spectrum of the free TDM (**271**) showed a broad triplet at 5.28 ppm (*J* 4.6 Hz), integrating for eight protons and corresponding to the alkene protons. The acetal protons gave a doublet at 4.96 ppm (*J* 3.2 Hz), and the remaining trehalose protons including the β-position protons resonated at 4.57, 4.14, 3.98, 3.69, 3.59, 3.47 and 3.19 ppm. The α-position proton of the mycolic acid appeared as a multiplet at 2.33 ppm, and the protons next to the double bonds appeared as a quartet at 1.95 ppm (*J* 6.5 Hz) integrating to sixteen protons. The carbon NMR showed the carbonyl carbon at 175.4,

and the alkene carbons at 129.7 ppm. The anomeric carbon signal appeared at 94.8 ppm, and the remaining trehalose carbon signals appeared between 72.5–71.1 ppm.

4.4.1.5 Trehalose deprotection of TMM (298)

Using the same conditions described earlier, the trimethylsilyl protecting groups of the TMM **(298)** were removed using TBAF in dry THF to give **(300)** in 80 % yield **(Scheme 92)**.

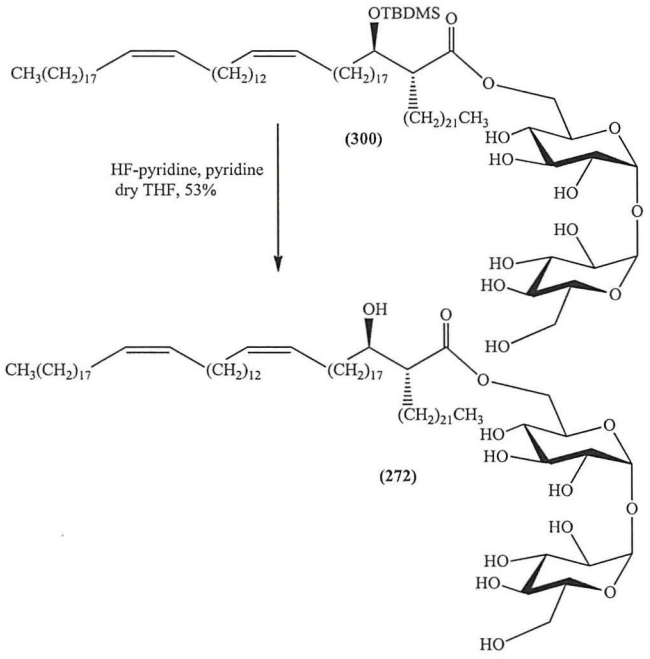


Scheme 92: Deprotection of the trehalose core of TMM **(298)**

The proton NMR showed no peak for the triemthylsily protons at 0.155, 0.14 and 0.12 ppm. The MS (MALDI-TOF) gave the correct mass $[M+Na]^+$: 1570.97, the calculated value: 1570.32.

4.4.1.6 β-Position deprotection of TMM (300)

Finally, the *tert*-butyldimethylsilyl protecting group at the β-postion of the mycolic acid was removed by using HF.pyridine complex and pyridine under the same conditions described earlier. This gave free TMM **(272) (Scheme 93)**. The HRMS (MALDI-TOF) now gave $[M+Na]^+$: 1456.2405; the calculated value: 1456.2383.



Scheme 93: Synthesis of free TMM **(272)**

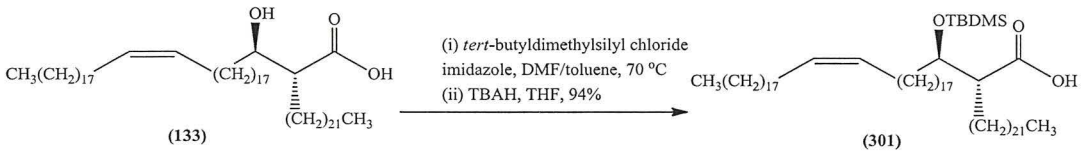
The disappearance of the *tert*-butyl signal at 0.83 ppm and the two methyl groups bonded to silicon at –0.02, –0.03 ppm, confirmed the success of the reaction. The olefinic signals appeared as a broad triplet at 5.30 ppm (*J* 4.7 Hz). The acetal protons appeared as doublets at 5.06 ppm (*J* 3.7 Hz) and 5.00 (*J* 3.5 Hz) respectively, and the remaining sugar protons, including the β-position proton appeared between 4.65–3.20 ppm. The remaining diene mycolic acid signals were similar to those of the corresponding TDM **(271)**, but with half of the proton integration. The carbon NMR showed the carbonyl carbon signals at 175.3 ppm, and the alkene carbons at 129.8 ppm. The anomeric carbons signal appeared at 94.2 and 94.15 ppm and the remaining trehalose carbon signals appeared between 73.1–70.3 ppm.

4.4.2 Synthesis trehalose esters of alkene mycolic acid

The same method and conditions were applied to prepare alkene mycolic acid TDM **(273)** and TMM **(274)**.

4.4.2.1 Protection of the β-position of alkene mycolic acid

The free alkene mycolic acid **(133)** was protected with the TBDMS group to give **(301)** (**Scheme 94)** under the same conditions described for diene mycolic acid.

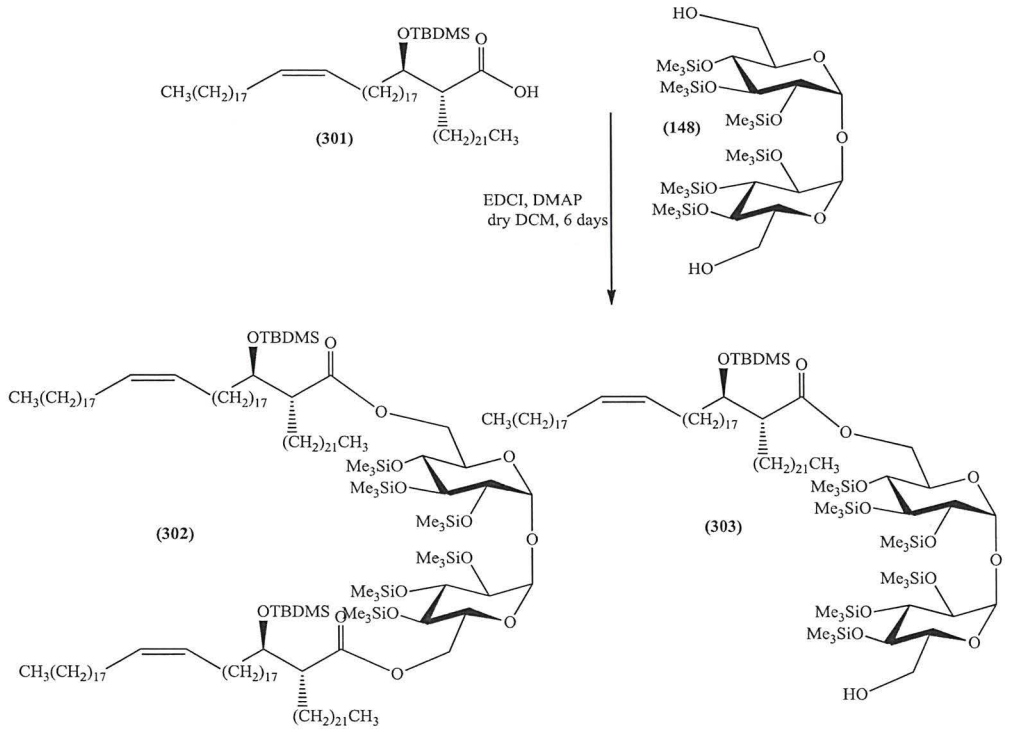


Scheme 94: protection of the free alkene mycolic acid **(133)**

The proton NMR spectrum of the compound **(301)** was essentially identical to that of protected diene mycolic acid **(296)**, except that the integration of both the alkene protons and of those protons adjacent to the double bond gave half the value compared to the diene mycolic acid **(296)**.

4.4.2.2 Coupling of the protected alkene mycolic acid to protected trehalose

The protected alkene mycolic acid **(301)** was coupled to the protected trehalose **(148)** using EDCI and DMAP at room temperature for 6 days **(Scheme 95)**. This gave protected TDM **(302)** and TMM **(303)** after column chromatography.

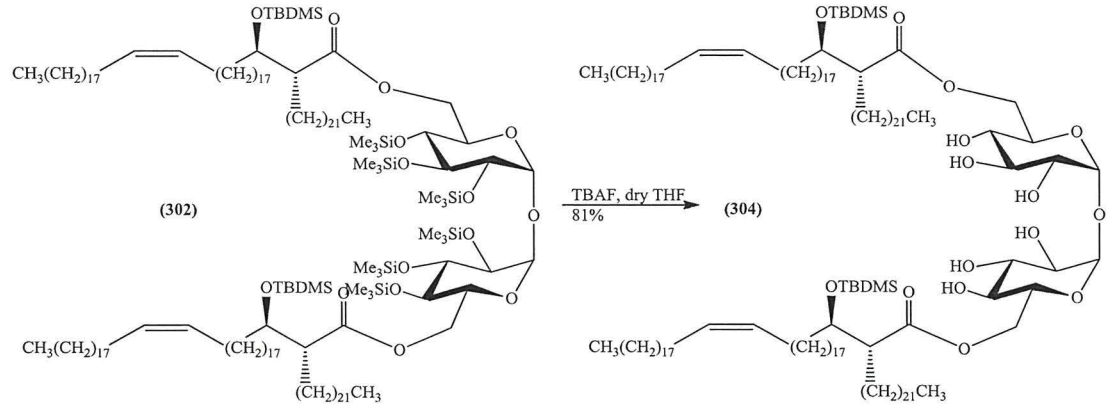


Scheme 95: Esterification of protected alkene mycolic acid with protected trehalose

The above TDM **(302)** and TMM **(303)** showed identical NMR spectra to those of the diene mycolic acid TDM **(297)** and TMM **(298)**, except that the integration of both the alkene protons and of those protons adjacent to the double bond were half as expected.

4.4.2.3 Trehalose deprotection of TDM (302)

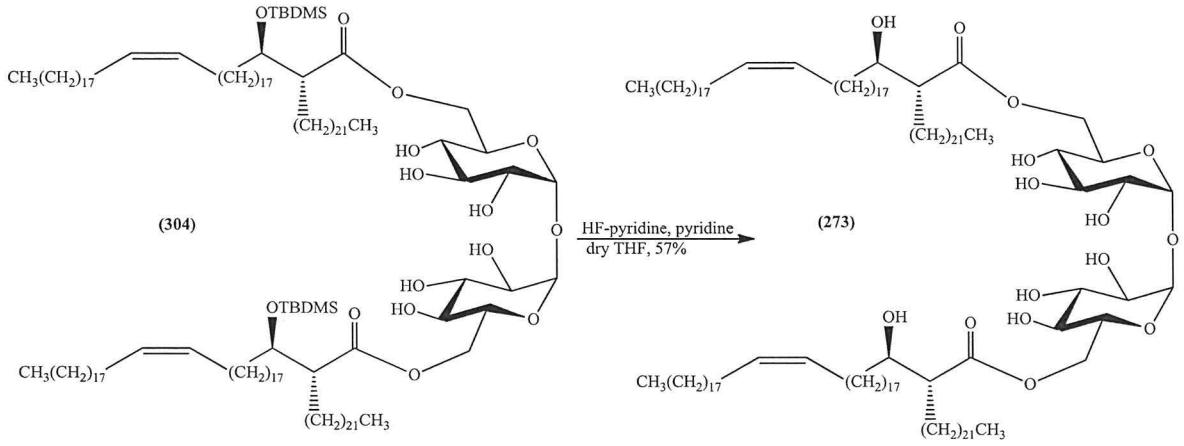
Using the same conditions as described earlier, the trimethylsilyl protecting groups on the trehalose of compound **(302)** were removed using TBAF in dry THF to give **(304)** in 81% yield **(Scheme 96)**. The proton NMR showed no peaks for the triemthylsilyl protons at 0.16, 0.14 and 0.13 ppm.



Scheme 96: Deprotection of the trehalose core of TDM **(302)**

4.4.2.4 β-Position deprotection of TDM (304)

The *tert*-butyldimethylsilyl protecting groups were removed from the TDM **(304)** using HF.pyridine complex and pyridine under the same condition described earlier. This gave free TDM **(273)** in 57% yield **(Scheme 97)**.



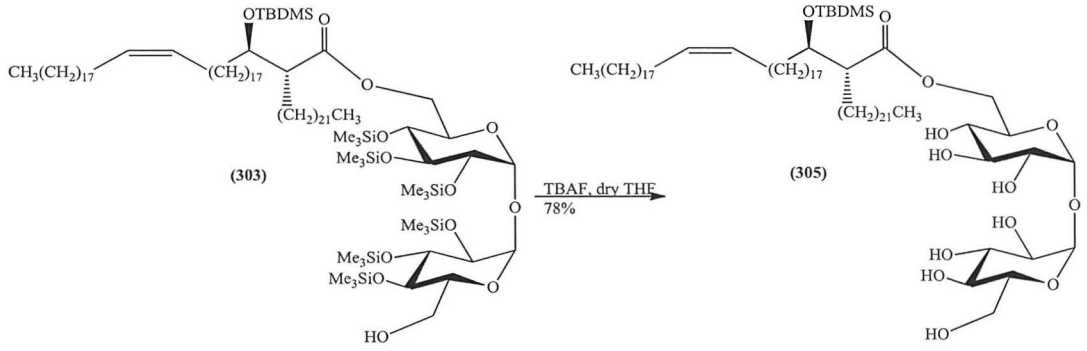
Scheme 97: Synthesis of free TDM **(273)**

The MS (MALDI-TOF) for the TDM **(273)** gave the correct mass ion at $[M+Na]^+$: 2158.61, the calculated value:2158.63.The disappearance of the *tert*-butyl signal at 0.85 ppm and the signals corresponding to the two methyl groups bonded to silicon at –0.015 and –0.013 ppm confirmed the success of the reaction. In addition the proton NMR showed the expected signals starting from a broad triplet at 5.31 ppm (*J* 4.7 Hz),

integrating for four protons and corresponding to the alkene protons. The acetal protons gave a doublet at 4.98 ppm (*J* 3.5 Hz), and the remaining trehalose protons, including the β-position protons resonated between 4.75–3.19 ppm. The α-position proton of the mycolic acid appeared as a multiplet at 2.38 ppm, and the eight protons next to the double bonds appeared as a quartet at 1.95 ppm (*J* 6.5 Hz). The terminal methyl groups gave a triplet at 0.84 (*J* 6.6 Hz). The carbon NMR showed the carbonyl carbon signals at 175.5 ppm, and the alkene carbons at 129.8 ppm. The anomeric carbon signal appeared at 94.9 ppm, and the remaining trehalose carbon signals appeared between 72.6–71.2 ppm.

4.4.2.5 Trehalose deprotection of TMM (303)

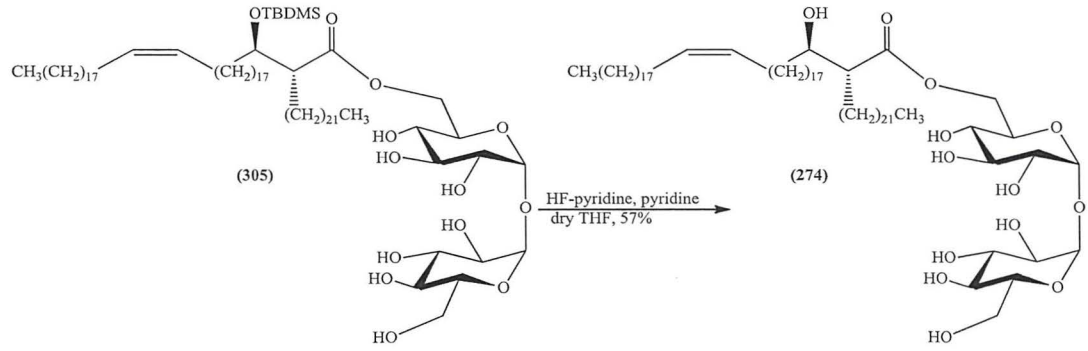
Again, TBAF in dry THF was used to deprotect the trehalose trimethyl groups under the same condition described earlier **(Scheme 98)**. The proton NMR showed no peak for the trimethylsilyl protons at 0.15, 0.14 and 0.13 ppm.



Scheme 98: Deprotection of the trehalose core of TMM **(303)**

4.4.2.6 β-Position deprotection of TMM (305)

The *tert*-butyldimethylsilyl protecting groups were removed from the TMM **(305)** using HF.pyridine complex and pyridine under the same conditions as described earlier. This gave free TMM **(274)** in 57% yield **(Scheme 99)**.



Scheme 99: Synthesis of free TMM **(274)**

The MS (MALDI-TOF) for the TDM **(274)** gave the correct mass ion at $[M+Na]^+$: 1262.61, the calculated value: 1262.03

The disappearance of the *tert*-butyl signal at 0.83 ppm and the signals corresponding to the two methyl groups bonded to silicon at 0.00 and –0.03 ppm confirmed the success of the reaction. The two olefinic protons appeared as a triplet at 5.25 ppm (*J* 4.8 Hz). The hemiacetal protons appeared as two doublets at 5.00 ppm (*J* 3.5 Hz) and 4.96 ppm (*J* 3.5 Hz), and the remaining sugar protons, including the β-position proton appeared between 4.53–3.13 ppm. The remaining alkene mycolic acid signals were similar to those of the TDM **(273)**, but with half the proton integration. The carbon NMR showed the carbonyl carbon signals at 175.2 ppm, and the alkene carbons at 129.8 ppm. The anomeric carbon signals appeared at 94.2 and 94.1 ppm.

4.5 Biological activity results

Generally, glycolipids possessing mycolic acids, and in particular TDM are able to induce the immune system, via the production of several chemokines (MCP-1, IL-8) and cytokines (TNF-α, IFN-γ, IL-4, IL-6, IL-10, IL-12) that play an important role in pathogen destruction.[221]

In this study, experiments have been conducted by a group in Brussels using synthetic TDMs and TMMs of diene mycolic acid **(271, 272)** and alkene mycolic acid **(273, 274)** to study their ability to stimulate the TNF-α cytokine.

Tumour necrosis factor (TNF) is a proinflammatory cytokine produced by host body immune system, particularly produced by activated macrophages, CD4+ lymphocytes, monocytes and Natural Killer Cells (NK cells) in response to an infection. In the past, its role was limited to cause necrosis of tumours,[222] however it has many roles in the immune response to infection involves granuloma formation to destruct pathogens, or protection against infections, such as TB.[223]

The below graphs shows the high stimulation of TNF-α cytokine in different time intervals using the above synthetic compounds.

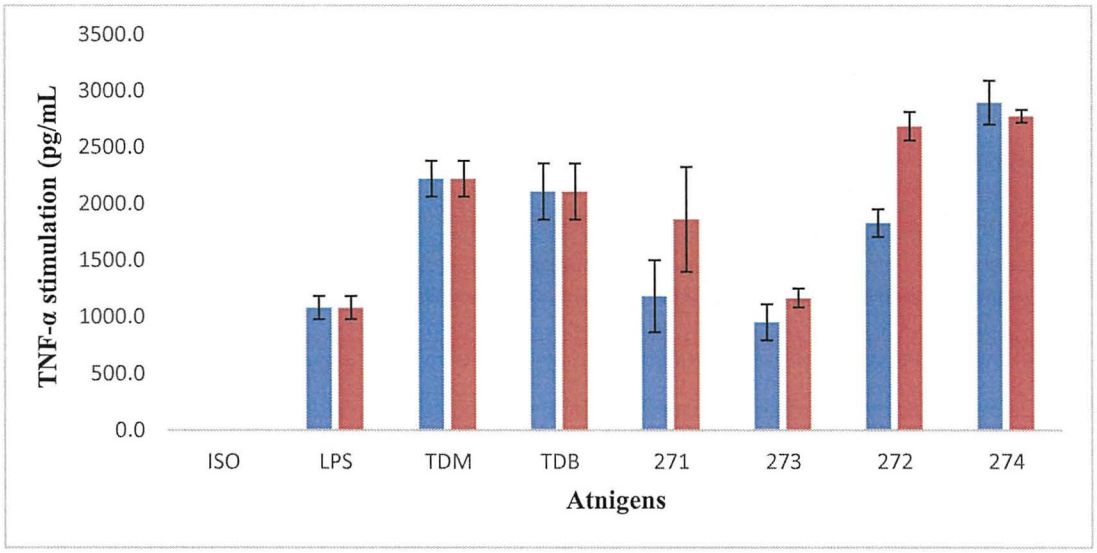


Figure 48: TNF-α stimulation after 6 hrs.

As shown in **(Figure 48)** the red and green bars shows the stimulation of TNF-α after 6 hrs. with the same experiment in two different times using bone marrow cells (DMDC). Interestingly all of the synthetic compounds **(271-274)** showed high stimulation of TNF-α, however the diene and alkene mycolic acid TMMs **(272, 274)** gave higher stimulation than either the natural TDM as the best compound to stimulate TNF-α.

In another study the same experiment was carried out, but the TNF-α stimulation was measured after 24 hrs. **(Figure 49)**.

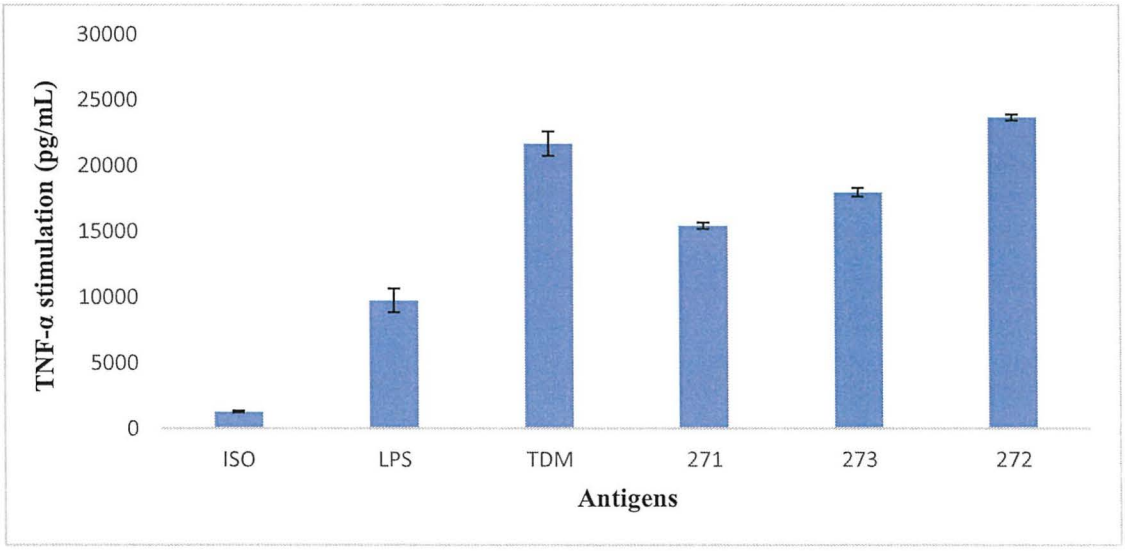


Figure 49: TNF-α stimulation after 24 hrs.

As can be seen in **(Figure 49)**, with the time increased form 6 hrs. to 24 hrs., enormous stimulation have been observed using the synthetic diene TMM **(272)** as the data not available for the alkene TMM **(274)**.

Chapter 5

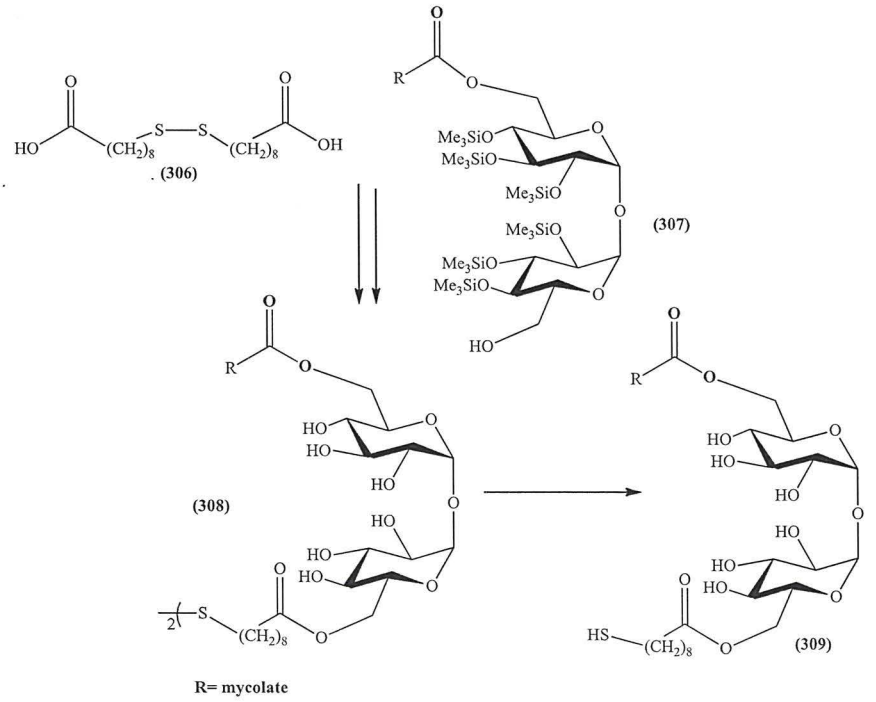
5. Synthesis of thiolated and disulfide cord factors

5.1 The aims

This chapter concerns the first synthesis of thiolated and disulfide cord factors. Both thiol and disulfide groups would be coated on a gold surface or gold nanoparticles, and their properties as antigens would be tested to detect TB antibodies.

Fast and accurate detection of TB is a fundamental factor toward the control of the disease, but to achieve this, highly specific antigens and an appropriate method for detection are required. It is believed that these gold-coated thiolated and disulfide cord factors could be used as specific synthetic antigens to detect TB in biosensors as a fast, accurate and multi-use method for the early detection of mycobacterial disease.

After running numerous model reactions, the first syntheses of thiolated and disulfide cord factors were achieved. The synthesis of the target compound **(308)** was achieved by esterifying both of the carboxylic terminals of disulfide diacid **(306)** with the primary alcohol position of synthetic TMM **(307)**, followed by the removal of the protecting groups from the trehalose and mycolic acid to give the free disulfide cord factor **(308)**. In addition, the disulfide cord factor **(308)** was cleaved to give the corresponding thiolated cord factor **(309)** using *DL*-dithiothreitol, "Cleland's reagent" **(Scheme 100)**.

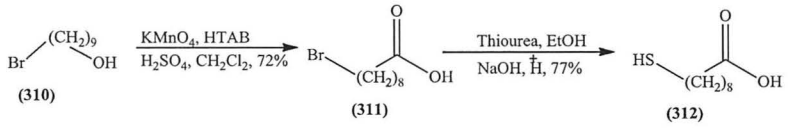


Scheme 100: General synthetic route to disulfide and thiolated cord factors

5.2 Synthesis of 9-mercaptononanoic acid (312)

The first step toward the synthesis of thiolated or disulfide cord factor was the synthesis of the thiol **(312)** as a starting material, which could be linked to trehalose esters in different forms to obtain either thiolated or disulfide cord factors.

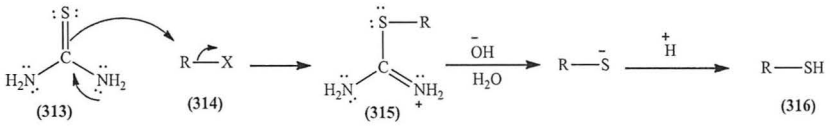
Firstly, a standard oxidation reaction was employed to oxidize 9-bromononan-1-ol **(310)** using potassium permanganate and hexadecyltrimethylammonium bromide (phase transfer catalyst) in the presence of sulphuric acid, to give 9-bromononanoic acid **(311)** **(Scheme 101)**. The proton NMR confirmed the success of the reaction, showing a triplet at 3.40 ppm (*J* 6.8 Hz) for the two protons next to the bromide, while the two protons next to the carbonyl group showed another triplet at 2.36 ppm (*J* 7.5 Hz). The carbon NMR spectrum showed the carbonyl signal at 180.1 ppm.



Scheme 101: The synthesis of 9-mercaptononanoic acid **(312)**

In order to convert the bromoacid **(311)** into the thiol **(312)** **(Scheme 101)**, it was firstly refluxed in ethanol with thiourea for 3 hrs, followed by addition of a 5M solution of sodium hydroxide and heating the mixture under reflux for another 2 hrs to cleave the thiolate and hydrolyse the ester to the acid. The proton NMR spectrum of the product **(312)** showed a significant difference to that of the starting material **(311)**, the two protons next to the thiol group appearing as a quartet at 2.53 ppm (*J* 7.4 Hz), while the two protons next to the bromide of the **(311)** was a triplet at 3.40 ppm (*J* 6.8 Hz).

The thiolation reaction occurs via an S_N2 mechanism, using thiourea **(313)** as a sulfur nucleophile generator, which attacks the alkyl halide **(314)** forming the isothiouronium salt **(315)**. Finally, base hydrolysis converts the isothiouronium salt in to the desired thiol **(316)** after acidification **(Scheme 102)**.



Scheme 102: The general mechanism of thiolation

5.3 Attempts to synthesize thiolated cord factors

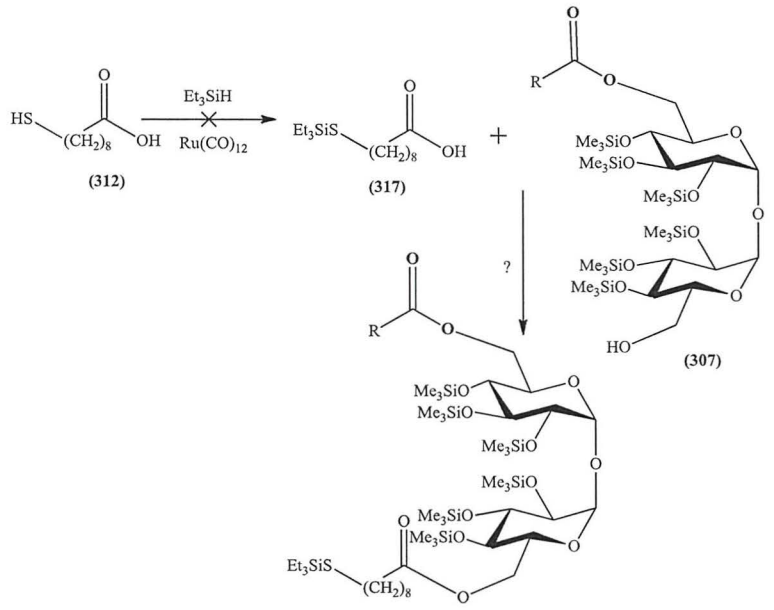
5.3 The first attempt

The first attempt to achieve thiolated cord factor, involved the production of silyl thioether **(317)** from the thiol **(312)** in order to couple it with the model trehalose ester **(307) (Scheme 103)**.

5.3.1 An attempt to prepare silyl thioether (317)

Silyl protecting groups are used to protect thiol groups as well as hydroxyl groups. The formation and cleavage of the silyl thioethers take place in a manner similar to those silyl ethers. The bond between sulfur and the silyl protecting group (S-Si) is weaker than the bond between the oxygen and silyl group (O-Si); this makes silyl protected thiols more susceptible to undesired hydrolysis. For this reason, introducing the silyl protecting group on thiols is limited to *in situ* protection of the thiol group or producing a more stable silylthioether by the use of sterically hindered silyl groups such as triethyl or triisopropyl silylthioether.[224,225] Recent reports have shown that organometallic complexes can catalyse the coupling of silane with thiols to produce silylthioethers. The most common catalysts are cyclopentadienyliron decacarbonyl dimer, and ruthenium complexes such as triruthenium dodecacarbonyl and [$RuCl(PPh_3)_2$(3-phenylindenyl)] complex.[226, 227, 228]

The preparation of silyl thioether compound **(317)** was attempted by refluxing triethyl silane with the thiol acid **(312)** in toluene in the presence of catalytic amounts of triruthenium dodecacarbonyl.[227] This did not lead to the desired product, and showed numerous spots on TLC. The proton NMR spectrum did not show clear signals for the triethyl group. It has been noted that this method is of use for the silyl protection of simple alkyl thiols only but not the thiol acid **(312)** due to the presence of the carboxyl functional group, which may undergo side reactions.



Scheme 103: The first attempt to achieve thiolated cord factor

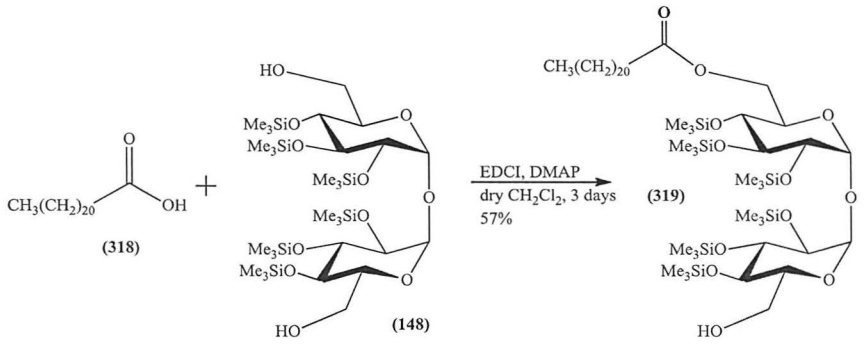
5.4 The second attempt

Another method to prepare thiolated compounds is from corresponding halides after reacting with a sulfur transfer reagent.

Throughout this work, the strategy entailed the synthesis of the model trehalose monobehenate (TMB) **(319)** first, and then reaction of the produced TMB with bromo acid **(311)** to give bromo-cord factor **(320)**. Finally, the conversion of **(320)** into the corresponding thiolated cord factor **(321)** using *bis*(tributyltin)sulphide as a sulfur transfer reagent was examined **(Scheme 104-106)**.

5.4.1 Synthesis of trehalose monobehenate (TMB) (319)

In order to produce the TMB **(319),** behenic acid **(299)** (1mol eq.) was reacted with protected trehalose (2 mol.eq) using EDCI and DMAP in dry dichloromethane and stirring the mixture for 3 days **(Scheme 104)**. This gave mainly TMB **(319)**.

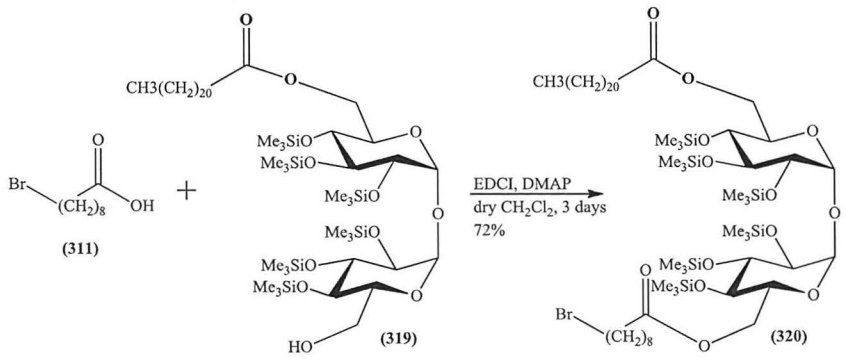


Scheme 104: Esterification of behenic acid with protected trehalose

Compound **(319)** was characterized using proton NMR, which showed the two acetal protons as a triplet at 4.92 ppm (*J* 3.1 Hz), and the remaining trehalose protons appearing between 4.31–3.44 pm. The two protons next to the carbonyl group appeared as two doublets of triplets at 2.43 ppm (*J* 16.0, 7.50 Hz) and 2.41 (*J* 15.3, 7.1 Hz) respectively, and the protons of the trimethyl sily group gave three singlets at 0.16, 0.14 and 0.13 ppm. The carbon NMR showed the carbonyl carbon at 173.8 ppm. The anomeric carbon signals appeared at 94.3 and 94.2 ppm, and the remaining trehalose carbon signals appeared between 73.3–70.7 ppm.

5.4.2 Synthesis of the model brominated cord factor (320)

The bromocord factor **(320)** was prepared by coupling TMB **(319)** with bromoacid **(311)** in the presence of the coupling agents EDCI and DMAP under the same reaction conditions as described to prepare TMB **(319)**.



Scheme 105: Esterification of TMB **(319)** with bromo acid **(311)**

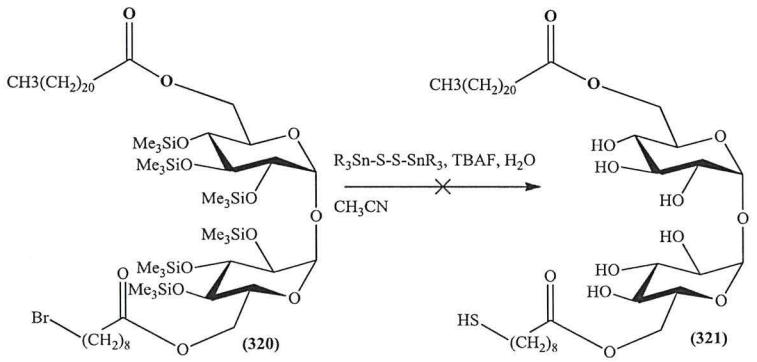
The proton NMR for the bromide **(320)** was found to be similar to those of the TMB **(319).** The significant difference was the appearance of a sharp triplet at 3.41 ppm (*J* 6.9 Hz) corresponding to the two protons next to the bromine. The protons next to the carbonyl groups gave a broad triplet at 2.35 ppm (*J* 6.9 Hz) integrating for four protons. The MS (MALDI-TOF) gave $[M+Na]^+$: 1338.01; the calculated value requires 1337.69.

5.4.3 An attempt to synthesize thiolated model cord factor (321)

Halide compounds can be converted into their corresponding thiols or thioethers by the use of sulfur generator reagents such as *bis*(tributyltin)sulphide or hexamethyl disilathiane $((CH_3)_3Si)_2S$ in the presence of tetrabutyl ammonium fluoride (TBAF).[229,230,231] It is likely that the sulphur generator reagent reacts with the free fluoride ion to produce a sulphur nucleophile, which then attacks the halide compound

via an S_N2 reaction to produce the thioether. It has been reported that in the presence of water the reaction goes toward the formation of thiol rather than thioether.[230]

An attempt was made to convert bromo cord factor **(320)** into the corresponding thiol **(321)** via a slow addition of **(320)** in dry acetonitrile to a mixture of *bis*(tributyltin) sulphide and TBAF in the presence of distilled water, using acetonitrile as a solvent **(Scheme 106)**.[230] From the TLC, which showed several spots close to each other, it was concluded that this method is not effective for the preparation of thiolated cord factors. The difficulties of the method might be related to the presence of the trimethyl silyl groups on the trehalose which are sensitive to TBAF. However, excess of TBAF was used in an attempt to produce the thiol group and remove the trimethyl silyl groups simultaneously, but this did not produce the desired product **(321).**



Scheme 106: An attempt to prepare thiolated cord factors **(321)**

5.5 Successful attempt to synthesize disulfide cord factors

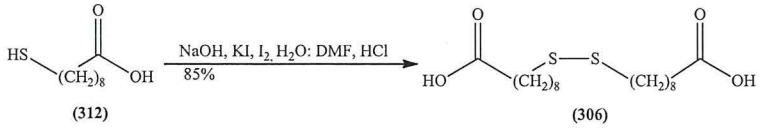
After running several unsuccessful model reactions to synthesize thiolated cord factors, it was decided to attempt the synthesis of disulfide cord factors instead of thiol cord factors. Disulfides could then be cleaved in the laboratory using *DL*-dithiothreitol, "Cleland's reagent". However, it has been proved that both of the disulfides and thiols can bind to the gold and that disulfides cleave to produce the corresponding thiol during the binding process on the gold surface.[199]

The method includes the esterification both its caboxylic terminals of disulfide **(306)** to the primary alcohol position of selected TMM as shown previously in **(Scheme 100)**.

In early attempts, two model reactions were carried out to obtain disulfide trehalose esters using simpler compounds similar to mycolic acids. This was to investigate the feasibility of the method to synthesize disulfide cord factors using complete mycolic acids. The success of these model reactions led to the first synthesis of disulfide cord factors of α-mycolic acid successfully.

5.6 Synthesis of diacid disulfide (306)

The thiol **(312)** was oxidized to give the disulfide **(306)** using a standard method **(Scheme 107)**.



(312) (306)

Scheme 107: The preparation of disulfide **(306)**

The disulfide **(306)** showed a significant difference to the thiol **(312)** in its proton NMR. The signal for the methylene group adjacent to the sulphur for the disulfide **(306)** appeared as a triplet at 2.69 ppm (*J* 7.3 Hz), while for the thiol, this methylene group was seen as a quartet at 2.53 ppm (*J* 7.4 Hz) **(Figure 50)**. The carbon NMR showed the signal for the carbon next to the sulphur for the disulfide **(306)** at 39.9 ppm, while the signal for the same carbon for the thiol **(312)** appeared at 34.0 ppm.

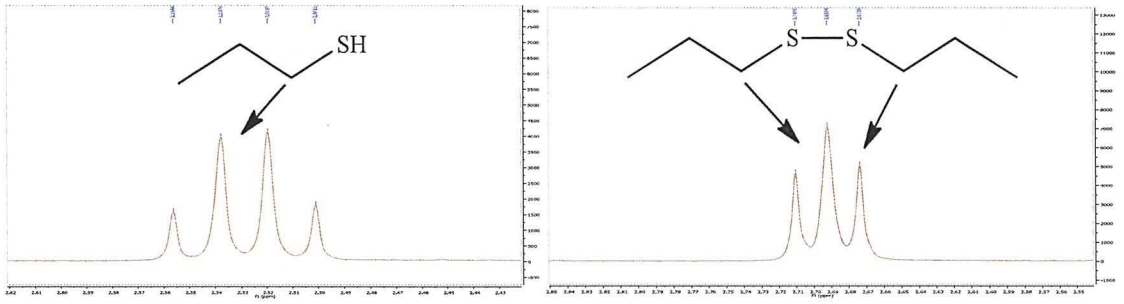
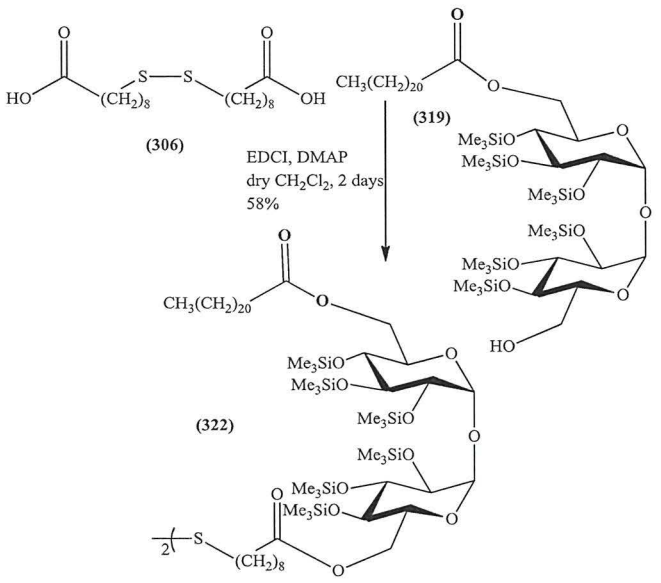


Figure 50: The Proton NMR signal for methylene group adjacent to the sulfur in thiol **(312)** and disulfide **(306)**

5.7 Synthesis of model disulfide cord factors

5.7.1 Synthesis of disulfide cord factor of trehalose mono behenate (300)

The disulfide **(306)** was esterified at both its caboxyl terminals with protected trehalose monobehenate **(319)** to give disulfide cord factor **(322)**. This was achieved by mixing the disulfide **(306)** (0.5 mol.eq.) with trehalose monobehenate **(319)** (1 mol.eq.) in the presence of EDCI and DMAP in dry dichloromethane and stirring the mixture for 2 days to give the target compound **(322) (Scheme 108)**.

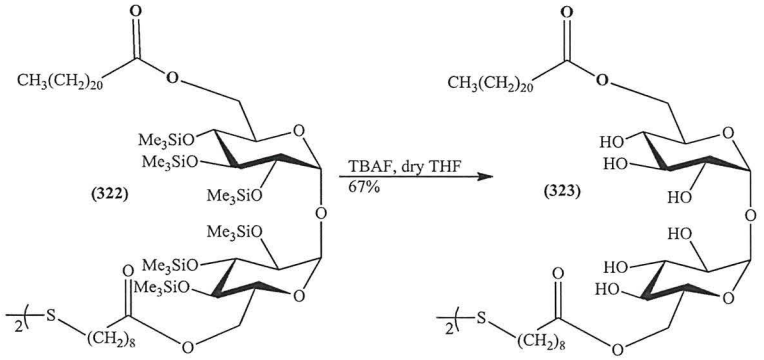


Scheme 108: Esterification of diacid disulfide **(306)** with trehalose monobehenate **(319)**

The MS (MALDI-TOF) for the disulfide cord factor **(322)** gave the correct mass ion at $[M+Na]^+$: 2559.52; the calculated value requires 2558.51. The success of the reaction was confirmed by the proton NMR spectrum, which showed the acetal protons as a doublet at 4.92 ppm (*J* 2.9 Hz) integrating for four protons, and the remaining trehalose protons appeared between 4.28–3.43 ppm. The methylene protons adjacent to the disulfide group gave a sharp triplet as expected at 2.67 ppm (*J* 7.3 Hz) integrated for four protons, and the protons next to the carbonyl groups appeared as a two doublets of triplets at 2.40 ppm (*J* 16.0, 7.3 Hz) and 2.37 (*J* 16.0, 7.4 Hz) each integrating for four protons. The trimethylsilyl protons gave three singlets at 0.16, 0.14 and 0.13 ppm. The carbon NMR showed the two carbonyl group signals at 173.6 and 173.5 ppm, and the carbon next to the disulfide group appeared at 38.4 ppm.

5.7.1.2 Deprotection of disulfide (322)

The removal of the trimethyl silyl groups from the trehalose was achieved to give **(323)** using TBAF in dry THF under the conditions described earlier **(Scheme 109)**.



Scheme 109: Deprotection of the trehalose core of disulfide **(322)**

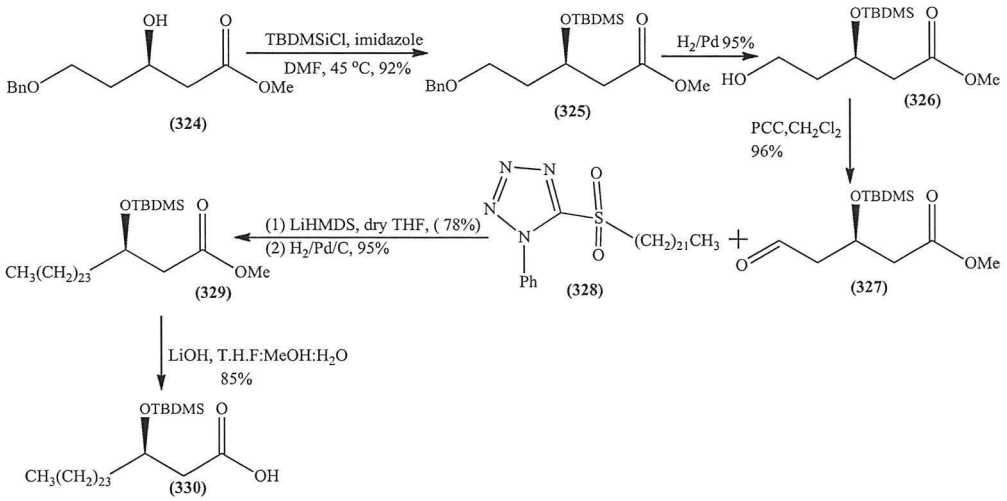
The disappearance of the signals corresponding to the trimethylsilyl protons at 0.16, 0.14 and 0.13 ppm in proton NMR showed that the reaction had been successful. The The MS (MALDI-TOF) found $[M+Na]^+$: 1694.68; the calculated value requires 1694.03.

5.7.2 The synthesis of the disulfide cord factor of a β-hydroxycarboxylic acid

Once the reaction conditions had been optimised, it was decided to synthesize β-hydroxy acid **(330)** and then to prepare its disulfide cord factor using the method described above. The reason for synthesizing the disulfide cord factor of a β-hydroxy carboxylic acid is that it contains a β-hydroxy position in common with the mycolic acids. This work was necessary in order to investigate whether there would be any effect on the disulfide group during the deprotection of the β-position in later stages.

5.7.2.1 Synthesis of β-hydroxycarboxylic acid (330)

Compound **(330)** was synthesized starting from **(324)** **(Scheme 110)**. Firstly, the protection of β-position of **(324)** with TBDMS protecting group was achieved using *tert*-butyldimethylsilyl chloride and imidazole in DMF for 18 hours at 45 °C to give the protected compound **(325)**. The proton NMR signals were similar to the starting material; however the product showed additional signals starting from a singlet at 0.9 ppm for the *tert*-butyl group and another two singlets at 0.06 and 0.04 ppm for the two methyl groups bound to silicon atom. Carbon NMR also gave two signals at –4.2 ppm and –4.9 ppm corresponding to the methyl carbons attached to the silicon atom.



Scheme 110: Synthesis of β-hydroxy carboxylic acid **(330)**

The compound **(325)** was debenzylated by stirring vigorously in a suspension of Pd (10%)/C in 1:1 IMS/THF under an atmosphere of hydrogen gas for 2 days to give the alcohol **(326) (Scheme 110)**. The proton NMR spectrum of alcohol (**326**) showed a multiplet at 4.38 ppm for the β-position proton, and the two protons next to the hydroxyl group gave another multiplet at 3.86 ppm. The two protons next to the β-position gave two doublets of doublets at 2.62 ppm (*J* 6.4, 14.9 Hz) and 2.54 ppm (*J* 6.6, 14.9 Hz) respectively. The carbon NMR spectrum showed signals for the β-position carbon at 68.2 ppm, while the carbon next to the hydroxyl group appeared at 59.6 ppm. Oxidation of the alcohol **(326)** with PCC in dichloromethane gave the aldehyde **(327)** in 96% yield. The proton NMR confirmed the success of the reaction, which showed the aldehyde proton as a triplet at 9.8 ppm (*J* 1.6 Hz). The carbon NMR gave a signal for the aldehyde carbon at 200.9 ppm.

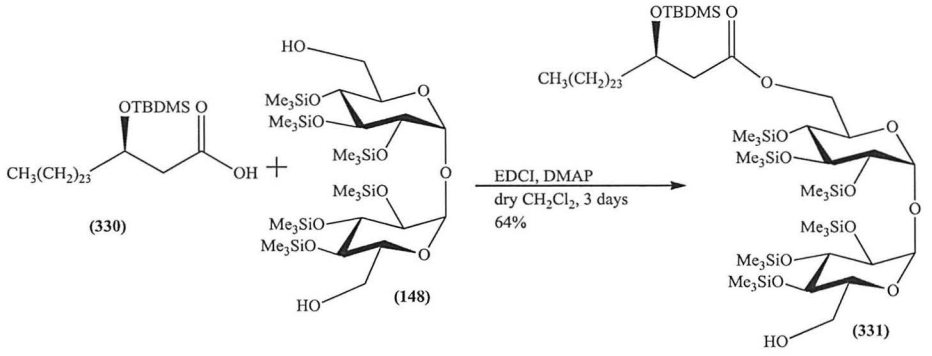
The aldehyde **(327)** was chain extended with a C22 carbon unit sulfone **(328)** in a modified Julia-Kocienski olefination reaction to give **(329)** after saturation **(Scheme 110)**. The proton NMR spectrum of (**329**) showed a multiplet at 4.14 ppm for the β-position proton and the two protons next to the β-position appeared as a doublet of doublets at 2.41ppm (*J* 5.1, 14.9 Hz). The terminal methyl group gave a triplet at 0.90 ppm (*J* 6.9 Hz). The carbon NMR spectrum showed a carbonyl signal at 172.4 ppm, and the signal for the β-position carbon at 69.5 ppm.

Finally, compound **(329)** was hydrolysed directly using lithium hydroxide mono hydrate to give **(330) (Scheme 110)**. The proton NMR of compound **(330)** showed no singlet at 3.67 ppm for the methyl ester, thereby determining the success of the reaction.

As shown in above Scheme **(110)**, it was possible to perform direct hydrolysis without prior removal of the silyl group. In mycolic acid synthesis, it is difficult to perform direct hydrolysis, it being first necessary to remove the silyl protecting groups, and then hydrolyse the methyl ester. The reason for this may be the steric hindrance effect of the long α-alkyl chain present in the α-position of mycolic acids.

5.7.2.2 Coupling of protected β-hydroxycarboxylic acid to protected trehalose

The β-hydroxy acid **(330)** was esterified with protected trehalose **(148)** using EDCI and DMAP in dry dichloromethane for 3 days **(Scheme 111)**. This gave mainly **(331).**

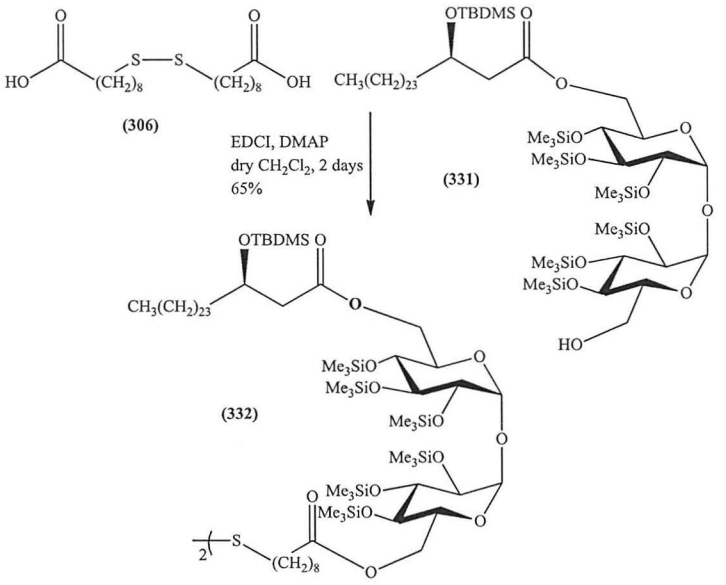


Scheme 111: Esterification of β-hydroxy acid with protected trehalose

The proton NMR for compound **(331)** showed two doublets at 4.92 (*J* 3.1 Hz) and 4.89 ppm (*J* 3.1 Hz) corresponding to the two acetal protons, and the remaining trehalose protons, including the β-position proton appeared between 4.29–3.44 ppm. The two protons next to the β-position gave two doublets of doublets at 2.53 ppm (*J* 6.8, 15.3 Hz) and 2.44 (*J* 6.0, 15.3 Hz) respectively. The *tert*-butyl group within the TBDMS group gave a singlet at 0.88 ppm integrating for nine protons. The trimethylsilyl protons gave three singlets at 0.16, 0.145 and 0.14 ppm each integrated for eighteen protons. The two methyl groups on silicon within the TBDMS group appeared as two singlets at 0.06 and 0.04 ppm. The carbon NMR showed the carbonyl carbon at 171.8 ppm. The anomeric carbon signals appeared at 94.5 and 94.3 ppm, and the remaining trehalose carbon signals occurred between 73.4–70.7 ppm. The β-position carbon gave a signal at 69.3 ppm, and the two methyl groups bonded to silicon appeared at –4.6 and –4.8 ppm.

5.7.2.3 Coupling of diacid disulfide (306) to TMM (331)

The diacid disulfide **(306)** was esterified at both its carboxyl terminals with the TMM **(331)** to give the disulfide **(332)** **(Scheme 112)**, using coupling reagents EDCI and DMAP under the same conditions as described earlier **(Scheme 108)**.

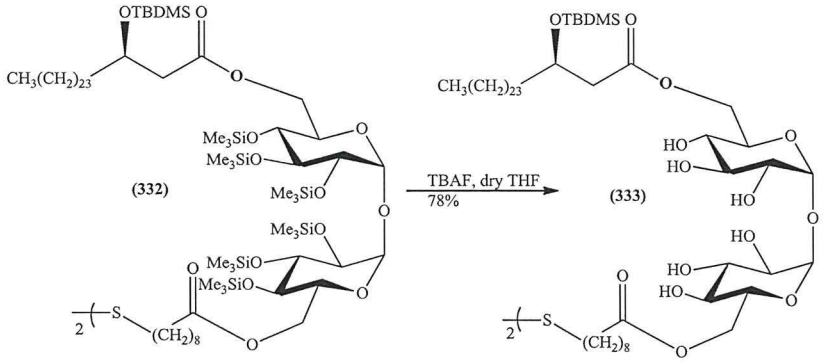


Scheme 112: Esterification of diacid disulfide **(306)** with TMM **(331)**

The MS (MALDI-TOF) for the disulfide cord factor **(332)** gave the correct mass ion at $[M+Na]^+$: 2958.77, the calculated value requires 2958.83. The proton NMR spectrum showed the acetal protons as two doublets at 4.92 ppm (*J* 3.0 Hz) and 4.9 ppm (*J* 3.0 Hz), each integrating for two protons, and signals for the remaining trehalose protons occurred between 4.29–3.44 ppm. The methylene groups adjacent to the disulfide gave a triplet as expected at 2.67 ppm (*J* 7.3 Hz) integrating for four protons, and the protons next to the β-position appeared as two doublets of doublets at 2.53 ppm (*J* 6.9, 15.3 Hz) and 2.43 ppm (*J* 5.9, 15.3 Hz) each integrating for two protons. The protons next to the carbonyl groups appeared as two doublets of triplets at 2.38 ppm (*J* 16.0, 7.4 Hz) and 2.34 ppm (*J* 15.9, 7.4 Hz) each integrating for two protons. The *tert*-butyl group within the TBDMS group gave singlet at 0.88 ppm integrating for eighteen protons. The trimethyl silyl protons gave three singlets at 0.16, 0.14 and 0.13 ppm. The two methyl groups bonded to the silicon atom within the TBDMS group appeared as singlets at 0.06 and 0.04 ppm. The carbon NMR showed the two carbonyl group signals at 173.8 and 171.6 ppm, and the carbon next to the disulfide group appeared at 42.5 ppm.

5.7.2.4 Trehalose deprotection of disulfide cord factor (332)

The removal of the trimethyl silyl groups from the trehalose was achieved to give **(333)** in 78% yield using TBAF in dry THF in under the same conditions described earlier **(Scheme 113)**.

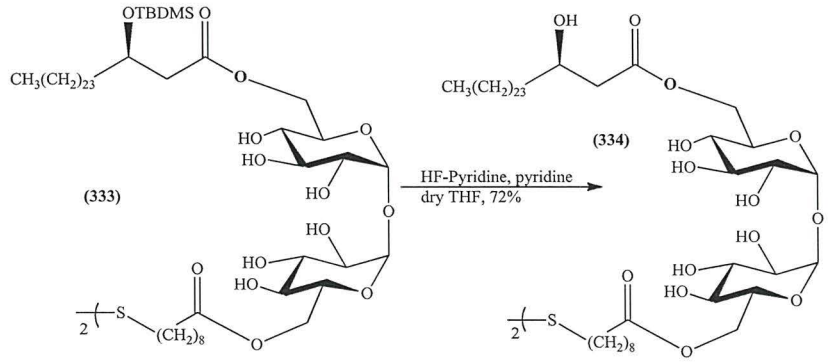


Scheme 113: Deprotection of the trehalose core of disulfide **(332)**

The disappearance of the signals corresponding to the triemethylsilyl protons at 0.16, 0.14 and 0.13 ppm in the proton NMR spectrum provided evidence that the reaction had been successful.

5.7.2.5 β-position deprotection of disulfide cord factor (333)

Finally, the *tert*-butyldimethylsilyl protecting groups of the β-position were removed using HF.pyridine complex and pyridine under the same conditions described earlier to give the free model disulfide cord factor **(334)** in 72 % yield **(Scheme 114)**.



Scheme 114: Synthesis of free disulfide **(334)**

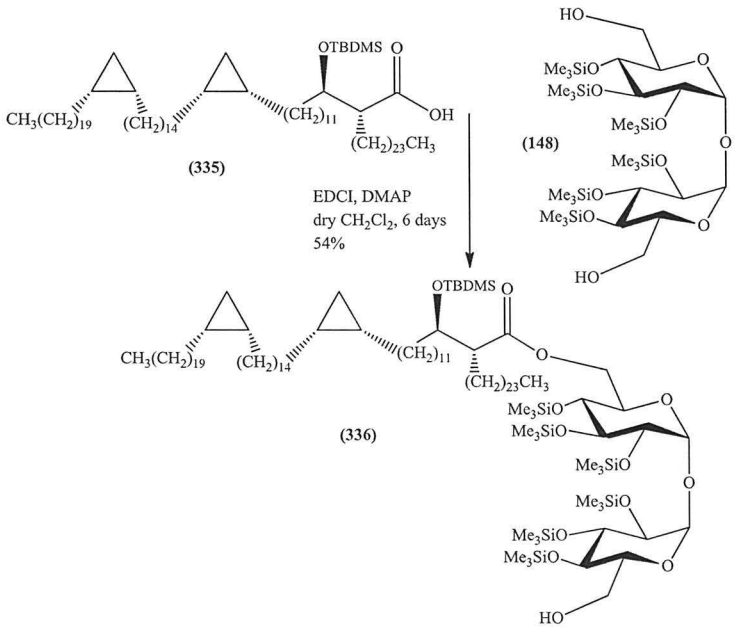
The MS (MALDI-TOF) for the compound **(334)** gave the correct mass ione $[M+Na]^+$: 1865.79, the calculated value requires 1866.18. The proton NMR spectrum showed the acetal protons as a triplet at 5.05 ppm (*J* 2.7 Hz), and the remaining trehalose protons appeared between 4.59–3.32 ppm. The methylene protons adjacent to the disulfide gave

a triplet at 2.64 (*J* 7.2 Hz) integrating for four protons, and the protons next to the β-position appeared as a doublet of doublets at 2.53 (*J* 8.1, 15.1 Hz) integrating for four protons. The protons next to the carbonyl groups appeared as a triplet at 2.30 ppm (*J* 7.6 Hz) integrating for four protons. The terminal methyl groups gave a triplet at 0.90 ppm (*J* 6.5 Hz). The carbon NMR showed the two carbonyl groups at 174.4 and 171.6 ppm, and the anomeric carbon at 93.7 ppm. The carbon atom next to the disulfide appeared at 41.6 ppm.

5.8 The first synthesis of a disulfide cord factor of a full α-mycolic acid

5.8.1 Coupling of protected α-mycolic acid to protected trehalose

The esterification between the protected α-mycolic acid **(335)** (1mol eq.) (Provided by Dr. Al-dulayymi) and the protected trehalose **(148)** (2 mol.eq) was carried out in the presence of EDCI and DMAP in dry dichloromethane, stirring the mixture for six days. This gave mainly TMM **(336) (Scheme 115)**.



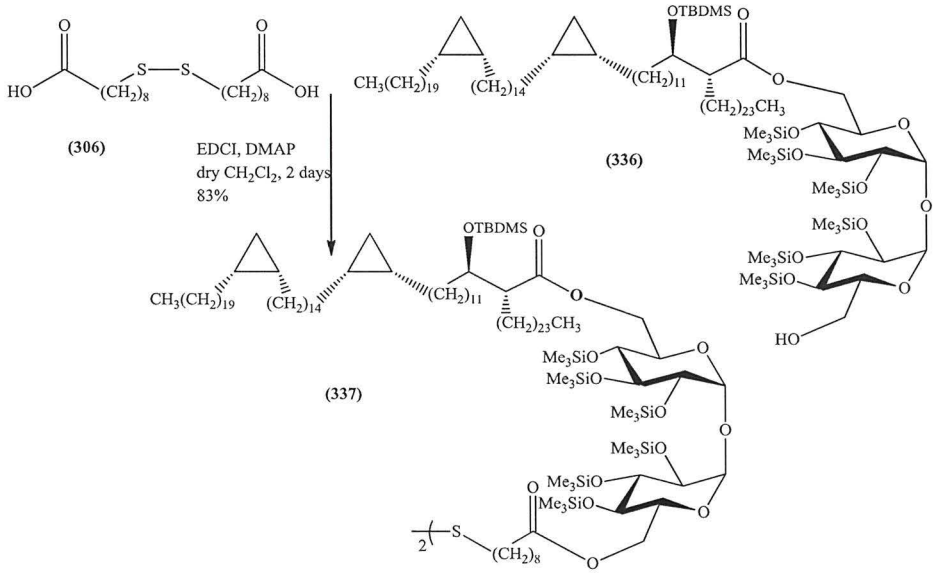
Scheme 115: Esterification of α-mycolic acid **(335)** with protected trehalose **(148)**

The proton NMR of the TMM **(336)** gave two doublets at 4.91 ppm (*J* 3.1 Hz) and 4.84 ppm (*J* 3.0 Hz), corresponding to the two acetal protons, and the remaining trehalose protons appeared between 4.35–3.40 ppm. The β-position proton of the mycolic acid gave a doublet of triplets at 3.84 ppm (*J* 3.5, 9.4 Hz), and the α-position proton as a doublet of doublets of doublets at 2.55 ppm (*J* 3.4, 5.5, 9.3, Hz). The terminal methyl group gave a triplet at 0.88 ppm (*J* 6.9 Hz) and the *tert*-butyl group gave a singlet at 0.88

ppm integrating for nine protons. The cyclopropane protons gave a multiplet at 0.67–0.64 ppm integrating for four protons, a doublet of triplets at 0.57 ppm (*J* 3.9, 7.8 Hz) for two protons and a quartet at –0.33 ppm (*J* 5.1 Hz) for another two protons. The trimethylsilyl protons gave six singlets at 0.17, 0.16, 0.156, 0.15, 0.14 and 0.12 ppm, each integrating for nine protons. The two methyl groups bonded to silicon in the TBDMS group appeared as singlets at 0.06 and 0.05 ppm. The carbon NMR showed the carbonyl signal at 174.1 ppm, and the anomeric carbons at 94.4 and 94.3 ppm.

5.8.2 Coupling of diacid disulfide (306) to α-TMM (336)

The disulfide **(306)** was esterified at both its carboxyl terminals with protected TMM **(336)** to give **(337)** using the coupling reagents EDCI and DMAP under the same conditions described earlier **(Scheme 116)**.



Scheme 116: Esterification of diacid disulfide **(306)** with TMM **(336)**

The MS (MALDI-TOF) of the disulfide **(337)** gave $[M+Na]^+$: 4380.83; the calculated value requires 4380.36. The expanded proton NMR signal is shown in (**Figure 51**), which gave two doublets at 4.92 ppm (*J* 3.0 Hz) and 4.86 ppm (*J* 3.0 Hz), corresponding to the two acetal protons, and the remaining trehalose protons, including the β-position proton of the mycolic acid, appeared at 4.36–3.39 ppm. The methylene protons adjacent to the disulfide group gave a sharp triplet at 2.67 ppm (*J* 7.3 Hz) integrating for four protons, and the α-position proton next to the α alkyl chain of the mycolic acid gave a doublet of doublets of doublets at 2.55 ppm (*J* 3.4, 5.0, 9.9, Hz). The protons next to the carbonyl groups of the disulfide part appeared as two doublets of triplets at 2.38 ppm (*J*

16.1, 7.5 Hz) and 2.34 ppm (*J* 16.0, 7.3 Hz) each integrating for two protons. The remaining signals of the compound **(337)** in the proton NMR spectrum showed a similar pattern to that given for the starting material TMM **(336).** The carbon NMR showed the carbonyl signals at 173.9 and 173.7 ppm, and the anomeric carbons at 94.7 and 94.6 ppm, with the carbon signal next to the disulfide group at 39.0 ppm.

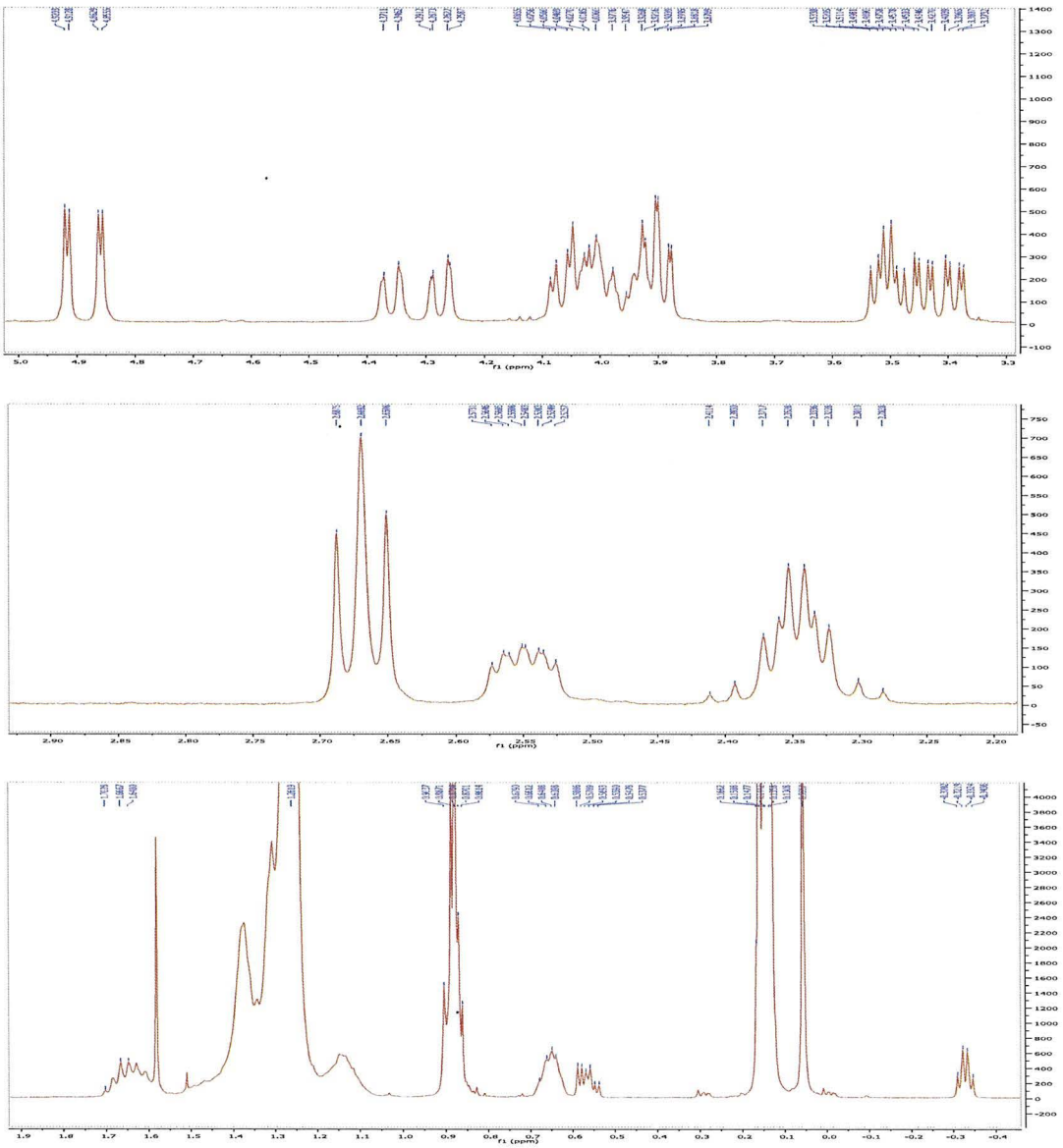
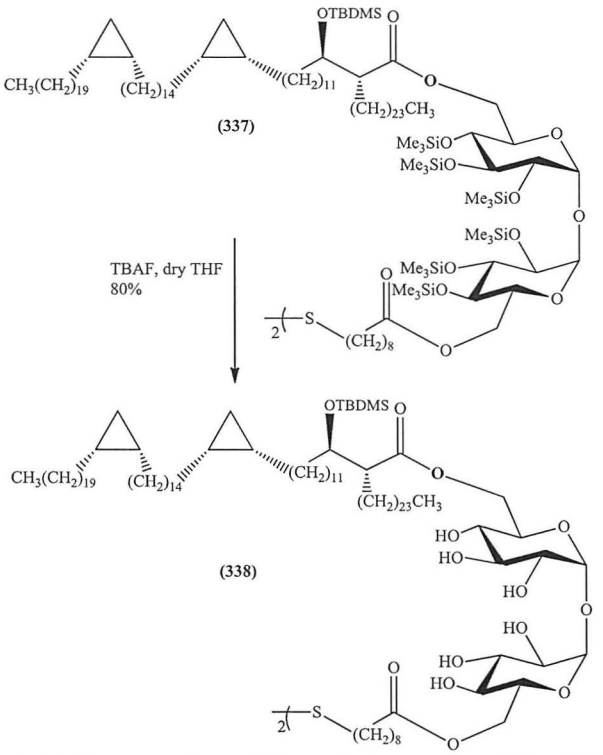


Figure 51: Proton NMR signals for the disulfide **(337)**

5.8.3 Trehalose deprotection of disulfide cord factor (337)

The removal of the trimethylsilyl groups from compound **(337)** was achieved to give **(338)** in 80% yield using TBAF in dry THF under the same conditions described earlier **(Scheme 117)**.

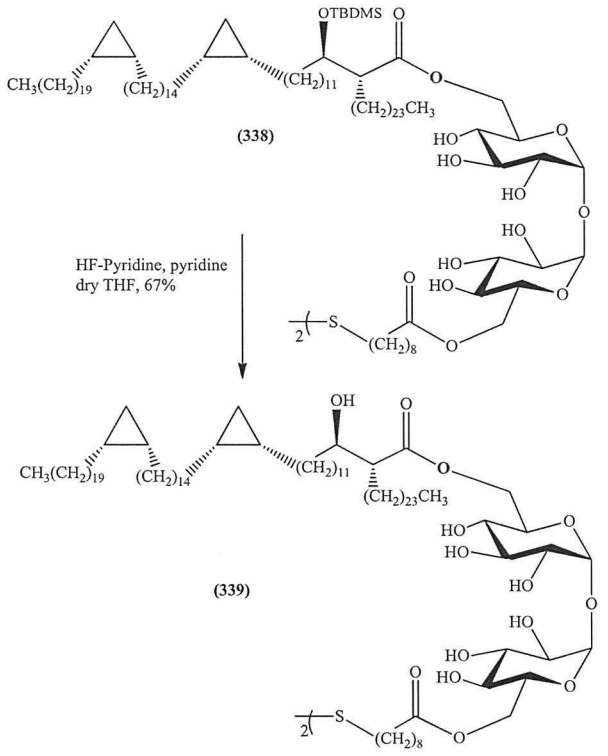


Scheme 117: Deprotection of the trehalose core of disulfide **(337)**

The disappearance of the trimethylsilyl protons in the proton NMR confirmed that the reaction had been successful. The MS (MALDI-TOF) gave $[M+Na]^+$: 3515.45; the calculated value is 3515.89.

5.8.4 β-position deprotection of disulfide cord factor (338)

The *tert*-butyldimethylsilyl protecting groups of the β-position of the α-mycolic acids were removed, using HF.pyridine complex and pyridine, to give the free disulfide cord factor **(339)** in 67% yield **(Scheme 118)**.



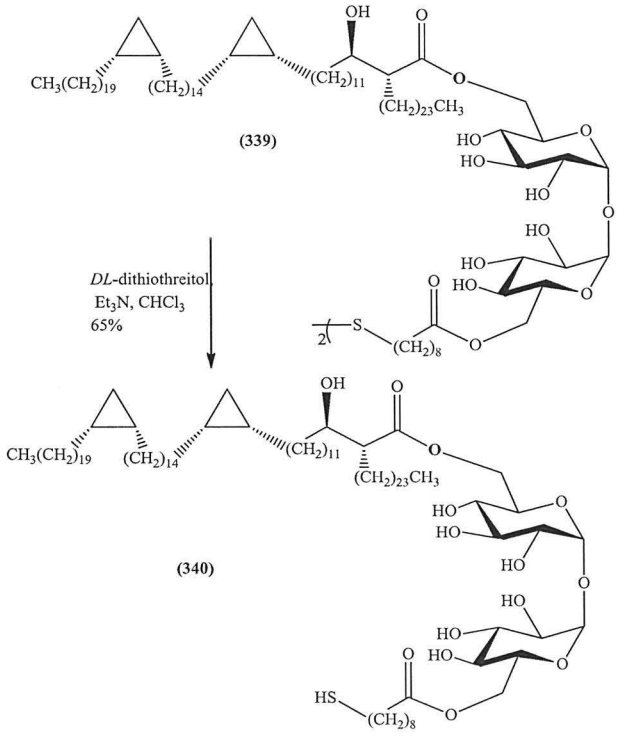
Scheme 118: Synthesis of free disulfide **(339)**

The HRMS (MALDI-TOF) for compound **(339)** gave the exact mass $[M+Na]^+$ value of 3287.7198, the calculated value requires 3287.7175 . The proton NMR spectrum showed the acetal protons as a triplet at 5.05 ppm (*J* 3.1 Hz), while the remaining trehalose protons and the β-position proton appeared between 4.64–3.24 pm. The methylene protons adjacent to the disulfide group gave a triplet at 2.64 (*J* 7.4 Hz) integrating for four protons, and the α-position proton gave a multiplet at 2.38 ppm integrating for two protons. The protons next to the carbonyl groups of the disulfide part appeared as a triplet at 2.31 ppm (*J* 7.3 Hz) integrating for four protons. The terminal methyl group gave a triplet at 0.83 ppm (*J* 6.6 Hz). The cyclopropane protons gave a multiplet at 0.64–0.60 ppm integrating for eight protons, a doublet of triplets at 0.51 ppm (*J* 3.8, 7.6 Hz) integrating for four protons and a quartet at –0.38 ppm (*J* 4.9 Hz) for another four protons. The carbon NMR showed the two carbonyl group signals at 175.4 and 174.3 ppm, and the anomeric carbons at 94.1 and 94.0 ppm. The carbon atom next to the disulfide group appeared at 39.3 ppm.

5.9 The cleavage of disulfide cord factor (339)

It has been proved that both the disulfides and thiols can bind to the gold surface.[199] The disulfides cleave to the thiol during the binding process on the gold surface. It was

decided to cleave the disulfide **(339)** in the laboratory to obtain the thiol **(340)** in order to compare their binding ability on the gold surface. *DL*-dithiothreitol, "Cleland's reagent", was added to a stirred solution of compound **(339)** in chloroform followed by the addition of one drop of triethylamine under a nitrogen atmosphere. The reaction flask was tightly covered by aluminium foil and allowed to stir at room temperature for 2 days. This gave the desired thiol **(340)** in 65% yield **(Scheme 119)**.



Scheme 119: Synthesis of free thiol **(340)**

The MS (MALDI-TOF) for the thiol **(340)** gave the correct mass ion $[M+Na]^+$ value of 1656.68, the calculated value requires 1656.36. The thiol **(340)** showed a similar NMR spectrum to the disulfide **(339)**, the significant difference being the chemical shift of the methylene signal adjacent to the sulfur group, which gave a triplet at 2.46 ppm (*J* 7.2 Hz) for the thiol **(340)**, while the same protons in the corresponding disulfide **(339)** gave a triplet at 2.64 ppm (*J* 7.4 Hz).

5.10 Cyclic voltammetry*

Cyclic voltammetry was used to confirm the coating of the synthesized disulfide and thiolated cord factors on the gold surface. Electron transfer from the electrode in the

* This work was carried out by Dr.J.Helliwell in the laboratory of Dr.C.D.Gwenin.

cyclic voltametry system reduces the gold coated thiols or disulfides, generating a potential current that could be observed to confirm the formation of gold-sulfur bond. Generally, in cyclic voltammetry three electrodes are used; a saturated calomel electrode as the reference electrode, a platinum electrode as the counter electrode and the working electrode is a gold slide coated with thiol or disulfide.

Gold electrodes were flame annealed and allowed to cool before being placed undisturbed for 48 hours in a solution of the selected thiol or disulfide compounds (1 mg/ml in chloroform) to form the thiol layers on the gold surface (gold coated slides). Prior to using the gold slides as the working electrode, it was washed several times with distilled water to wash away any unbound thiols on the surface. A special cell containing the electrolyte solution (0.1 M NaOH) was sealed by a rubber *o*-ring and linked to the working electrode in the bottom. The whole cell with the linked working electrode was put in the cyclic voltammetry system, and then degassed by passing nitrogen several times prior to use. The potential was then applied between the counter electrode and the working electrode for 15 minutes, and the current measured between the working electrode and the counter electrode **(Figure 52)**.

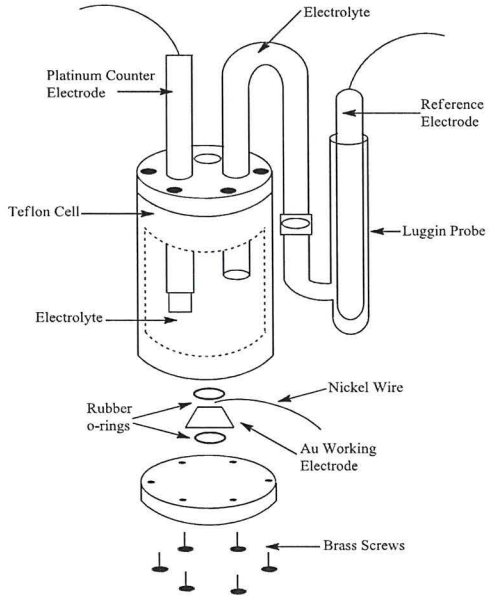


Figure 52: Schematic diagram of cyclic voltammetry system.[232]

In order to determine whether the thiol or disulfide is bound to the gold surfaces, the range of the potential is scanned twice, and then by using special software, the data is plotted as current vs. potential between 0 V to -1400 mV **(Figure 53)**.

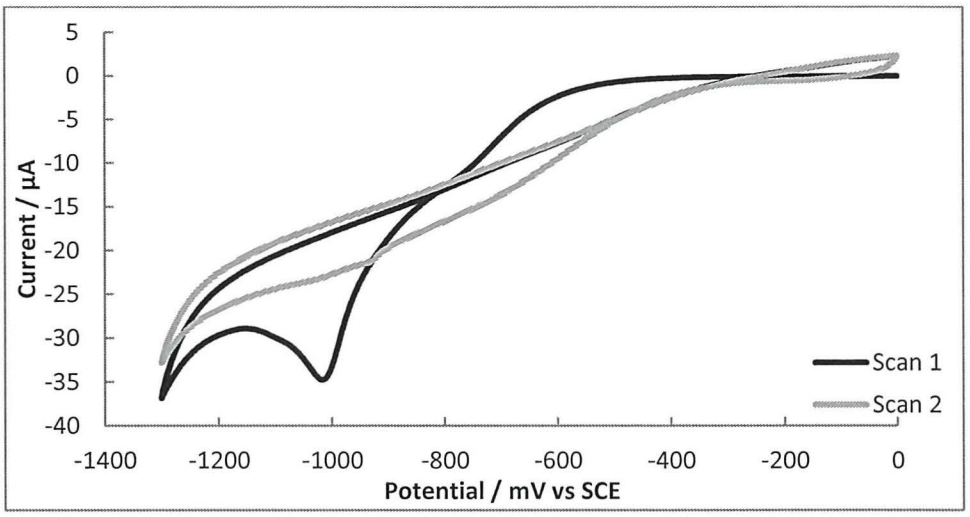


Figure 53: A voltammogram of gold electrode modified with disulfide **(339).**[232]

As can be seen in **Figure 53** the reduction peaks **(Scan 1, 2)** correspond to disulfide desorption from the gold surface. The disulfide desorption in scan 2 is smaller than scan 1, which means some of the disulfide is re-adsorbed on the gold surface (oxidation). The reduction peak for the disulfide **(339)** observed at 1010 mV confirmed that the disulfide **(339)** is bound to the gold **(Figure 53)**.

Reduction of the gold-sulfur bond occurred by the electron transfer from the electrode produces a current called "standard reduction potential" according to the following equation **(Scheme 120)**.

$$R(S\text{-}Au) + 1\,\bar{e} \longrightarrow Au + R(\bar{S})$$

Scheme 120: Reduction of the gold-sulfur

There is also small peak observed at 1300 mV in **(Figure 53)**, this corresponds to the electrolysis of water called "hydrogen evolution reaction" (HER). The reaction happens in acidic, neutral and basic medium, although the reaction is observed to occur in basic medium in cyclic voltammetry system at 1300 mV to reduce the water molecules to produce hydrogen, according to the following equation **(Scheme 121)**.

$$2H_2O + 2\bar{e} \longrightarrow H_2 + 2\bar{O}H$$

Scheme 121: Hydrogen evolution reaction

The process of HER occurs in two steps, firstly passing the current makes the protons from the solution migrate to the electrode, and then combine with the electrons from the electrode to form hydrogen atoms. Secondly, the atoms will then combine together in order to form hydrogen molecule.

5.11 Disulfide cord factor assay for detecting TB using Electrochemical Impedance Spectroscopy (EIS)

All of the assays and data analysis related to the disulphide compounds have been carried out by Dr.J.Helliwell according to the following procedure.[232]

Gold electrodes were flame annealed and allowed to cool before being placed in a solution of the compound (1 mg/ml in chloroform) for 48 hours at room temperature. The electrodes were removed from the solution and rinsed thoroughly with chloroform before being dried under nitrogen. They were then placed in a bovine serum albumin (BSA) solution (1%) for 1 hour before being thoroughly rinsed with pure water. The electrodes were then sealed in the electrochemical cell and redox probe (5 mM ferri/ferrocyanide in 100 mM phosphate buffered saline (PBS)) added and Electrochemical Impedance Spectroscopy (EIS) was performed at 230 mV from 60 kHz to 100 mHz. The electrodes were then removed from the cell and again thoroughly rinsed and dried before a TB+ serum was added (50 µl, 1:200 dilution) and incubated for 15 minutes. The serum was removed and thoroughly rinsed with pure water and the impedance re-measured. The electrode was again removed and thoroughly rinsed and dried before an IgG-HRP antibody was added (50 µl, 1:20,000) and incubated for a further 15 minutes before the Impedance was re-measured.

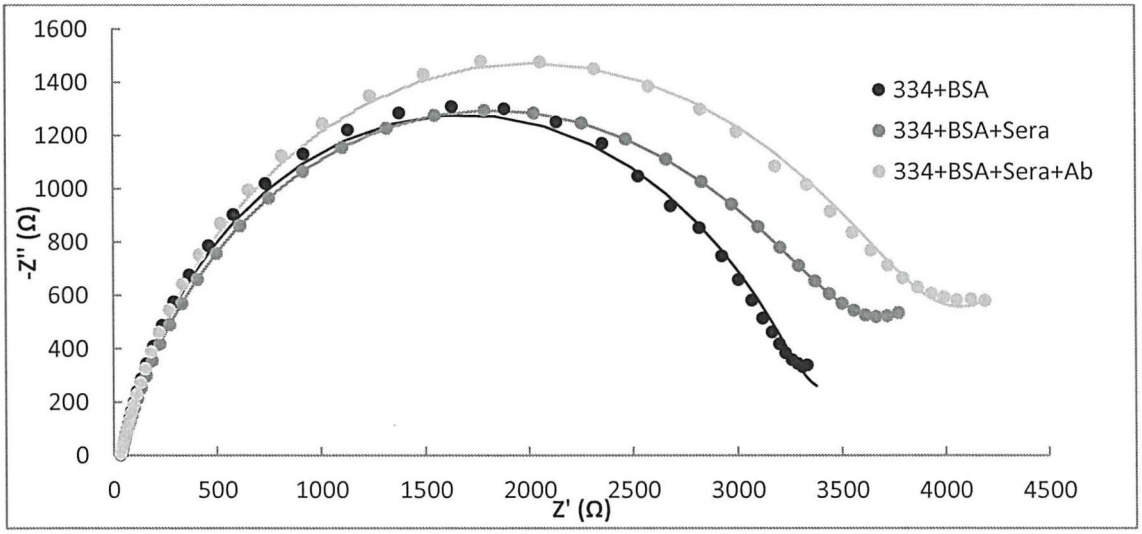


Figure 54a: EIS graph of the model disulphide **(334)** bound gold, followed by TB+ serum and then to IgG secondary antibodies

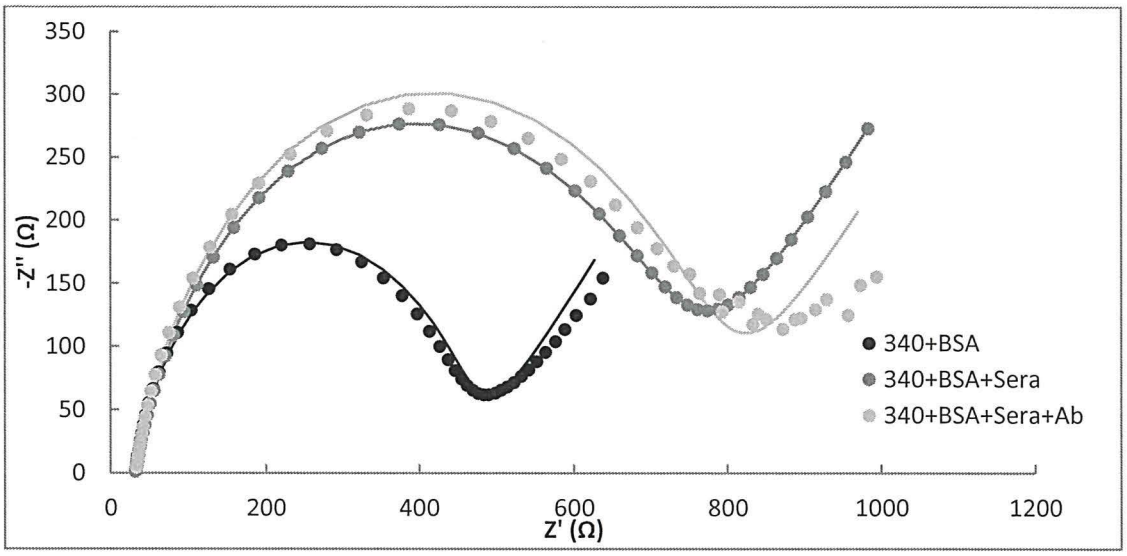


Figure 54b: EIS graph of the α-mycolic acid thiol **(340)** bound to gold, followed by TB+ serum and then to IgG secondary antibodies

As can be seen in graphs shown in **Figures 54a** and **54b,** the TB-positive sera bound strongly to the model disulphide **(334)** and the α-mycolic acid thiol **(340)**. However, it was observed that the secondary antibody (IgG-HRP) also bound to the disulphide **(334)**, but slightly bound with the thiol **(340)**. For future work these observations need further investigation to be clearly understood.

The binding status in EIS system is based on the increase in charge transfer resistance with the build-up of the layers, showing that the TB-positive serum and then the antibody bind to the mycolic acid layer. Charge transfer resistance is calculated from the diameter of the semi-circle of the graph. Ions in the redox probe can easily reach on to the gold electrode surface and become oxidised and reduced. On a bare electrode, their path is unblocked so they can easily reach the surface; however, when the layers build up on the surface as in the assays, their route becomes blocked and this causes the increase in charge transfer resistance that could be seen by the increase in the semi-circle. In a serum sample that is TB-negative, no binding would be expected to happen to the thiol compounds so there would be no change in the charge transfer resistance.

Further work needs to be carried out using different disulphide and thiol cord factors on a set of TB-positive and negative sera samples.

Chapter 6

Summary and Conclusions

Synthetic mycolic acids, wax esters 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.

The first part of this project involved the first synthesis of *M. avium* and *M. gordonae* wax esters **(164)** and **(166)**, and their corresponding ω-carboxymycolic acids **(165)** and **(167)**. The non-natural wax ester **(168)** was also prepared with longer chain length than the natural wax ester by introducing two additional carbons in α-alkyl chain and one additional carbon in the right hand side of cyclopropane. This was to study the effect of chain length on biological activities.

The first synthesis of trehalose esters (cord factors) of the above wax esters were achieved, which were then evaluated as specific antigens to distinguish *M. avium* from *M.tuberculosis* specifically in serodiagnostic assays (ELISA).

M. avium and *M.gordonae* belong to the family of Non-tuberculosis mycobacteria, and can cause diseases to both human and animals such as skin disease, disseminated disease, and pulmonary disease. HIV positive patients are particularly at risk of infection with Non-tuberculosis mycobacteria, especially *M.avium* infection.

M.avium is the causative agent of Crohn's disease in humans and Johne's disease (*paratuberculosis*) in animals. The specific detection of *M.avium* in humans and animals is a major issue in UK and elsewhere in the world and is an increasing problem in a number of other species. The issue is the difficulty entailed in distinguishing between *M.avium* and *M.tuberculosis* infection, since the symptoms of these two infections resemble each other closely. Wax esters are not found in tuberculosis mycobacteria, and therefore these could be used as specific antigens to distinguish *M. avium* or *M.gordonae* from *M.tuberculosis* in serodiagnostic assays (ELISA).

In order to synthesize the target wax esters **(164)**, **(166)** and **(168),** the four fragments (a, b, c, d) were linked together **(Figure 55)**. The building block is α-methyl-cyclopropane (fragment c). Elongation of both sides of the α-methyl cyclopropane unit was achieved by linking mycolic motif sulphone (fragment d) to the right hand side and

the acid sulphone (fragment b) to the left side via the use of the Julia-Kocineski olefination reaction (in a good yield). Hydrogenation of the produced alkenes produced the linked fragments (b, c, d) together. Finally esterfication with (*S*)-eicosan-2-ol (fragment a) was achieved successfully by means of the Steglich esterfication to give the complete structure of wax ester **(Figure 55)**.

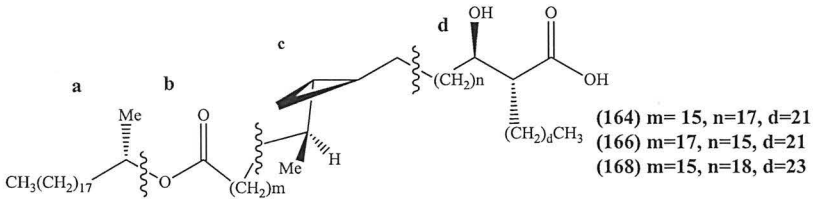


Figure 55: General structure of the complete wax ester

The first challenge in the synthesis of the complete mycobacterial wax esters was the production of the ω-dicarboxylic acid mono-ester (linking fragments b, c, d together); this was obtained successfully in high yield by the use of acid sulphone (fragment b) in a Julia-Kocineski olefination reaction.

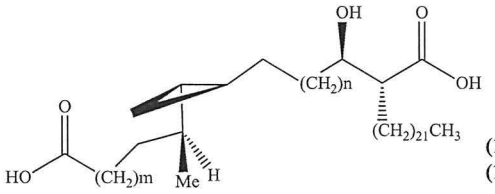
The second challenge was found in the coupling of (*S*)-eicosanol (fragment a) with the fragments (b, c, d) to produce the complete wax ester. However, it was found that applying the Steglich esterfication to link (S)-eicosanol with such a sterically hindered compound can give the desired esters successfully in very high yield (80%). In addition, it was found that the reaction is very straight forward with the conditions being very mild, not affecting the other functional groups.

The final hydrolysis reaction to produce free wax ester was the last and the most difficult challenge encountered in the synthesis of the wax esters. The difficulty lay in the possibility of the undesired hydrolysis of both of the sides of wax ester to give ω-carboxymycolic acids. Finally, the hydrolysis was achieved using a 5% aq. solution of tetrabutyl ammonium hydroxide and heating at 100 °C for 24 hrs, which gave the free wax ester in 66% yield.

The *M.avium* wax ester **(164)** and *M.gordonae* wax ester **(166)** were synthesized with the same chain length and stereochemistry to the natural wax ester isolated by Minnikin *et al.*[108] and Astola *et al.*[107] respectively. Moreover the wax esters **(164)** and **(166)** gave identical NMR spectrum to those reported for natural wax esters.

ω-carboxymycolic acids are also isolated from *M. avium* and *M.gordonae*. However, it is believed that ω -carboxymycolic acids are the result of the hydrolysis of wax esters during the process of extraction and other chemical treatment of the mycobacterial cell wall. It was also decided to synthesize ω-carboxymycolic acids starting from the

corresponding ω-dicarboxylic acid mono-ester, which was deprotected with HF-pyridine complex, and hydrolysed with lithium hydroxide to give ω-carboxymycolic acids **(165)** and **(167)** **(Figure 56)**.

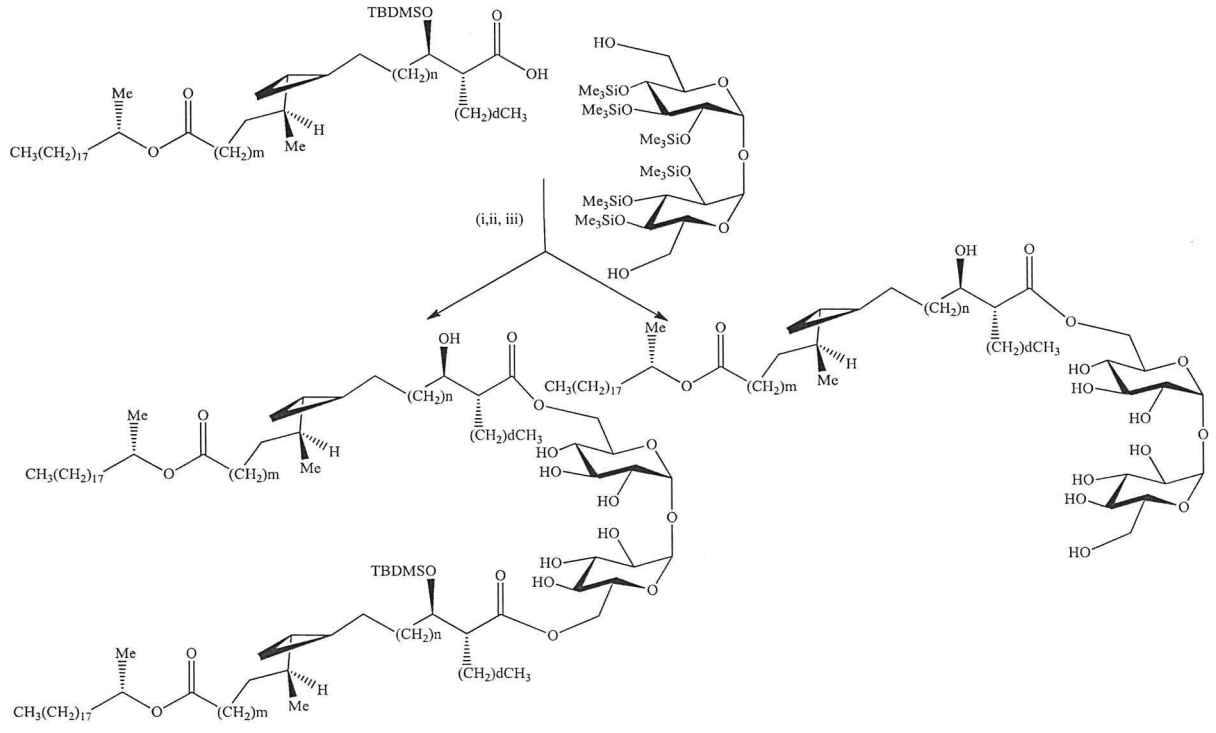


(165): m=15, n=17
(167): m=17, n=15

Figure 56: Synthesized ω-carboxymycolic acids

The successful synthesis of these wax esters **(164)**, **(166)** and **(168)** afforded the opportunity to synthesize their trehalose esters (TDM and TMM). The first step in the synthesis of the trehalose esters was the protection of the β-hydroxyl position present in the wax ester with a TBDMS group, and the secondary hydroxyl groups of the trehalose sugar with the trimethylsilyl protecting group.

The esterification reaction between the protected wax ester and trehalose 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 wax ester in two steps to give the free (TDM and TMM) **(Scheme 122)**.



Scheme 122: Synthesis of trehalose esters (TDM, TMM)

The silyl deprotection of the trehalose was achieved using TBAF in THF. At this stage, according to the old procedure, the reaction required aqueous work up with saturated sodium hydrogen carbonate solution, which leads to a decrease in the yield of the reaction and cause hydrolysis of the wax ester. Modification was developed which entails the addition of the residue of the TBAF reaction directly to the silica column without any work up. This resulted in a significant increase in the yield.

The TBDMS deprotection of the wax ester was achieved using HF. pyridine complex. Again in order to avoid the aqueous work up, the excess of the HF was neutralized with triethyl amine, and the residue was columned directly to give the final compound successfully. It was also found that using this alternative procedure of work up gave better yields than the old procedure, and could be applied as a standard method to work up the HF-pyridine reactions.

The hypothesis about the possibility of the use of wax ester compounds to distinguish between *M.avium* and *M.tuberculosis* was based on the results found by Yano *et al.*[193] using natural TDM. They found that natural TDM isolated from *M.avium* is more antigenic than natural TDM from *M.tuberculosis* toward the *M.avium* infected serum samples, and the opposite results were found using TB infected serum samples. This study suggested that the antibodies present in the serum samples may recognize mycolic acids present in the structure of TDMs. This means that the *M.avium* infected serum samples may recognize wax ester specifically, as it is found exclusively in *M.avium.* In order to confirmYano *et.al* results a set of *M.avium* infected serum samples are required. Unfortunately, until now only 2 *M.avium paratubeculosis (Map)* serum samples have been obtained for this purpose.

ELISA assays have been carried out using these 2 *Map* samples with another 2 sets of samples of naturally infected bovine TB and non-vaccinated serum samples in order to test the ability of the wax ester compounds to distinguish between *Map* and TB. The ELISA results showed that these 2 Map samples gave high responses to all of the wax ester antigens, while the other 2 sets of bovine TB samples also showed responses but were lower than the 2 *Map* samples. The responses of the natural *M.avium* wax ester and *M.avium* ω-carboxymycolic acid **(165)** were lower than the other wax ester compounds, but showed some distinction between the samples infected with *Map* and those infected with bovine TB. The above results are promising, but in order to make them more reliable, and clearly prove the ability of the synthetic wax esters to distinguish between TB and *M.avium,* larger sets of *M.avium* samples are needed.

Based on the above preliminary ELISA results obtained using the wax ester compounds, there are also other interesting findings that needs further investigation. It was found that free wax esters **(164)** and **(166)** gave high responses compared to their corresponding TDMs and TMMs, while other unpublished results within the MSB group showed that, the free mycolic acids gave significantly lower responses compared to their corresponding TDMs and TMMs.

In another study, ELISA assays were carried out on WHO human TB serum samples from Gambia using the *M.avium* wax ester TDM **(261)** and TMM **(263)**. As initial results, surprisingly the TB infected serum samples showed high responses to the TDM **(261)**, which gave the same sensitivity and specificity (89 and 78 % respectively) as that of the best synthetic keto-TDM antigen **(AD132),** while the values for the TMM **(263)** were slightly lower with a sensitivity and specificity of 78 and 75 % respectively being observed. For now, the explanation for this response might be the un-detected co-infection of these human TB serum samples with *M.avium,* as the symptoms of TB and M.*avium* infection resemble each other.

For future work, further ELISA assays are required using the synthetic *M.avium* wax ester compounds to confirm the detection of *M.avium* specifically, and also to understand the reason of responding TB serum samples to the synthetic wax esters. In addition, it could be interesting to apply non-natural wax ester compounds to find out whether the chain length has any effects on the antibody recognition.

The second aim of this project was the synthesis of the alkene and diene mycolic acids **(131)** and **(270)** found in *M. smegmatis* **(Figure 57)**, and then the synthesis of their corresponding trehalose esters. *M. smegmatis* is not pathogenic and belongs to the same genus as *M. tuberculosis.* Owing to this, the organism has been used for many research experiments as an alternative to the other pathogenic mycobacteria such as *M. tuberculosis* and *M. bovis.*

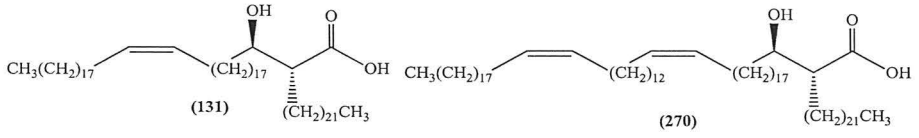


Figure 57: Synthetic alkene and diene mycolic acids

Synthetic alkene mycolic acid **(131)** has already reported by Muzael *et al.*[149] and due to this being an interesting biologically active compound, it was prepared on a large scale in this work in order to complete the synthesis of its trehalose esters.

Diene mycolic acid **(270)** was also been prepared by Muzael *et al.*[143] on small scale due to inefficiency of the method. This method was not promising for use in the production of trehalose esters, since the yield of the reaction was found to be very low (29%). Therefore, determining a more efficient method was our aim to obtain the diene mycolic acid on a large scale inorder to synthesize its cord factors.

Diene mycolic acid was prepared by a new method, which involved building up the mycolic motif aldehyde to give the final diene mycolic acid by insertion of two double bonds subsequently via a Wittig reaction, using the base sodium *bis*(trimethylsily)amide. The insertion of the first double bond was achieved successfully in 73% yield and the second double in 59% yield.

For future work, it would be possible to apply this method for the synthesis of triene mycolic acids, or any three functional group mycolic acids which have been reported from mycobacteria.

Following the successful synthesis of the alkene and diene mycolic acids, their trehalose esters (TDMs, TMMs) were prepared successfully via the method described previously. The synthetic TDMs and TMMs of diene mycolic acid **(271, 272)** and alkene mycolic acid **(273, 274)** were tested by a group in Brussels to study their ability to stimulate the TNF-α cytokine. Interestingly, high stimulation have been observed for all of the compounds **(271-273)**, particularly the diene and alkene mycolic acid TMMs **(272, 274)** showed higher stimulation than either the natural TDM as the best compound to stimulate TNF-α. These results are encouraging to further study the adjuvant activity of those synthetic TDMs and TMMs in order to use them as adjuvants against diseases.

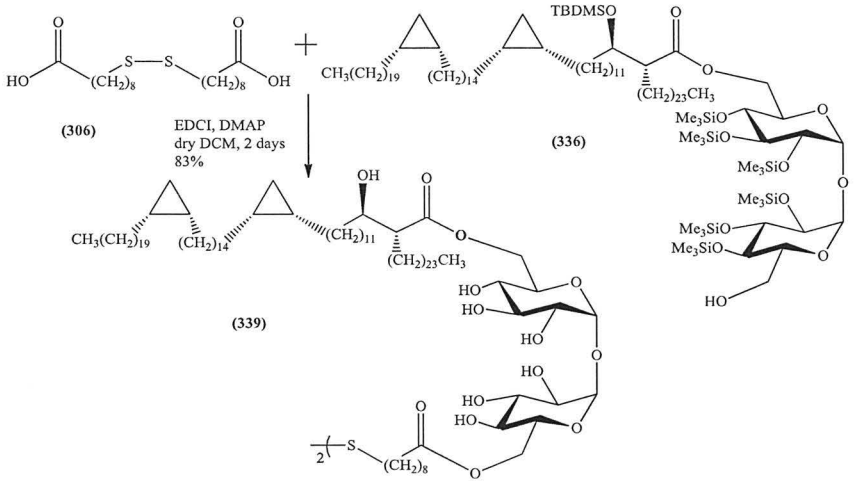
The final aim of this project was the synthesis of disulfide and thiolated cord factors in order to use them for TB detection.

The early detection of TB infection in humans is a particular problem in populations heavily compromised by HIV/AIDS. The life expectancy of a person with TB and HIV/AIDS is often shorter than the time required for a result to be obtained from current 'gold standard' testing methods. The disulfide and thiolated cord factors could be bound to gold nanoparticles in order to test their ability as antigens to detect TB antibodies. These compounds can be used in the development of biosensors as a fast, accurate and multiuse method for the early detection of mycobacterial disease *e.g.* TB.

The first strategy in this work was to decide the position of the thiol group within the cord factor. It was believed that linking the thiol group directly to the mycolic acid or

the trehalose might affect the antigenicity of the molecule. Therefore, it was decided to link the thiol group indirectly at the free primary hydroxyl position of the TMM.

This was achieved by esterifying the previously prepared diacid disulfide with the selected TMM to give the disulfide cord factor. Carrying out the synthesis involves silyl deprotection of the trehalose core and mycolic acid in two steps to give the free disulfide cord factor **(339)**.



Scheme 123: Synthesis of α-mycolic acid disulphide **(339)**

The structure of the disulfide was characterized by proton and carbon NMR, and the HRMS (MALDI-TOF) provided further evidence for the existence of the desired disulfide, which gave the correct mass ione $(M+Na)^+$: 3287.7198

The disulfide was also cleaved in the laboratory using *DL*-dithiothreitol "Cleland's reagent" to give the thiol **(340)** **(Figure 58)**. However, our results proved that the disulfide **(339)** bound to the gold surface in similar manner to thiol **(340)**. As the disulfides cleaves to produce thiol during the binding process on the gold surface.

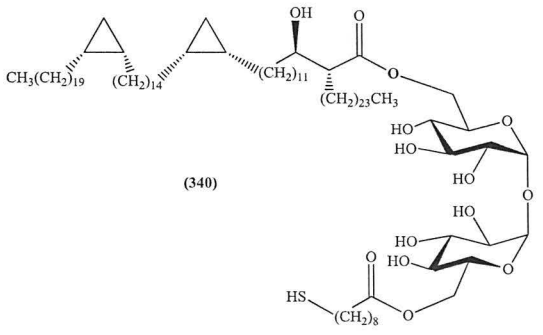


Figure 58: α-mycolic acid thiol **(340)**

The model disulphide compound **(334)** and α-mycolic acid thiol **(340)** were tested for their suitability in an assay for detecting TB in serum samples, using Electrochemical Impedance Spectroscopy (EIS). The results look promising with the compounds binding strongly to the TB antibodies present in a serum positive for TB.

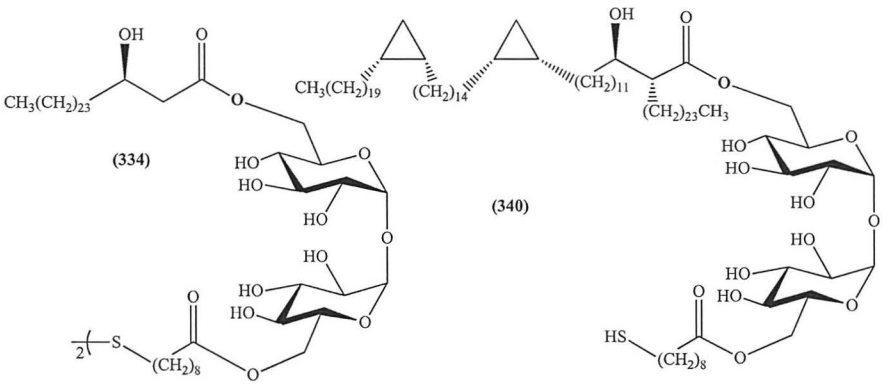


Figure 59: Synthetic compounds used as antigens to detect TB

For future, further work needs to be carried out using different disulphide and thiol cord factors on a range of TB-positive and negative sera samples to calculate the sensitivity and specificity. As the method to synthesize disulfides has been optimised, it is easy to prepare different mycolic acid disulfides in order to use them in such assays.

Chapter 7

7. Experimental

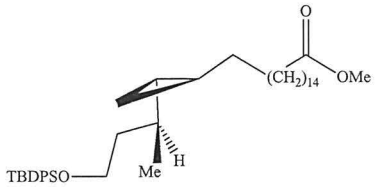
7. 1 General considerations

All chemicals and reagents were obtained from chemical companies (Sigma-Aldrich, Alfa Aesar, and Acros). All reactions were performed by using dried glassware in oven at (120 °C). THF was dried over sodium wire and benzophenone under nitrogen, whereas dichloromethane, diisopropylamine and HMPA were dried over calcium hydride. Nitrogen balloons and rubber septum were used to carry out reactions in inert conditions and liquid nitrogen with IMS was used to obtain low temperature, and the temperature was controlled by using digital thermometer. Anhydrous magnesium sulphate was used to dry organic solutions. Solid compounds were purified by re-crystallisation; column chromatography and distillation were used for purification and separation of oily compounds. Silica gel (Merck 7736) and silica gel plates (Merck 7736) used for column chromatography and the separated components were detected using potassium permanganate in water and phosphomolybdic acid solution in IMS.

GLC was performed using (Hewlett Packard 5890), using nitrogen as a carrier gas, and flame ionization detector (FID H_2/Air). All NMR samples were prepared in solution of deuterated chloroform and recorded on 400 MHz (Ultra Shield 400 plus Bruker). IR (infrared) spectra were performed on a Perkin-Elmer 1600 FTIR, and using KBr disc for the solid compounds. ($[\alpha]_D$) were recorded on a POLAR 2001 using chloroform. Accurate mass spectra for low molecular weight compounds (< 800) were obtained on micro-mass GCT time of flight mass spectrometer, using electron impact (EI). Accurate mass spectra for high molecular weight compounds (> 800) were have been carried out using MALDI-TOF mass using Bruker Daltonics flexAnalysis. Nominal mass spectra were obtained using MALDI-TOF mass spectra on a Bruker Reflex IV

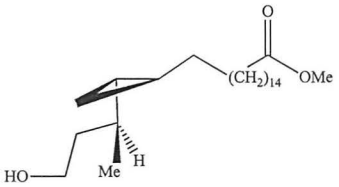
7.2 Experiments

Experiment 1: Methyl 16-((*1S,2R*)-2-((*S*)-4-((*tert*-butyldiphenylsilyl)oxy)butan-2-yl)cyclo propyl) hexadecanoate (195)



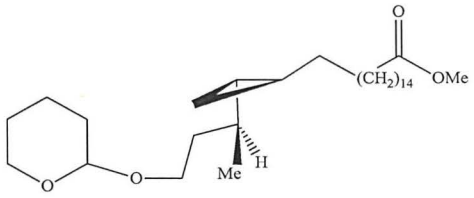
Lithium *bis*(trimethylsilyl) amide (17.8 mL, 18.9 mmol, 1.06 M) was added dropwise to a stirred solution of (*1S,2R*)-2-[(*S*)-3-(*tert*-butyldiphenylsilanyloxy)-1-methylpropyl] cyclopropane carbaldehyde **(98)** (4.01 g, 10.5 mmol) and 16-(1-phenyl-1*H*-tetrazole-5-sulfonyl)-pentadecanoic acid methyl ester **(185)** (5.86 g, 12.60 mmol) in dry THF (75 mL) under nitrogen at –15 ° C. The mixture was allowed to reach 0 °C, and stirred for 1 h at room temperature. When TLC showed all starting material had reacted, the reaction mixture was cooled to 0 °C and quenched with sat.aq. NH_4Cl (30 mL). The product was extracted with petrol/ethyl acetate (10:1, 3×50 mL), and the combined organic layers were dried over $MgSO_4$ and the solvent was evaporated to give a crude product. Column chromatography over silica gel, eluting with petrol/ethyl acetate (20:1) gave a mixture of *E/Z* alkenes **(186)** (5.60 g, 85%), [HRMS (EI^+): *m/z* 618.4518 $[(M)^+]$, calculated for $C_{40}H_{62}O_3Si$: 618.4468]. Dipotassium azodicarboxylate (5.03 g, 26.2 mmol) was added to a stirred solution of the above alkene (5.55 g, 8.98 mmol) in THF (75 mL) and methanol (5 mL) at 5 °C. A solution of glacial acetic acid (5 mL) in THF (5 mL) was added drop wise over a period of 72 hrs. The mixture was added portion wise into the solution of sat. aq. $NaHCO_3$ (250 mL), and the product was extracted with ethyl acetate (3 × 150 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was evaporated to give a thick oil. Column chromatography eluting with petrol/ethyl acetate (20:1) led to **(195)** (5.40 g, 96 %), $[\alpha]_D^{21}$ = +7.9 (*c* 0.96, $CHCl_3$) [HRMS (EI^+): *m/z* 620.4645 $[(M)^+]$, calculated for $C_{40}H_{64}O_3Si$: 620.4625]; δ_H: 7.68–7.66 (4H, m), 7.42–7.36 (6H, m), 3.76–3.71 (2H, m), 3.67 (3H, s), 2.30 (2H, t, *J* 7.5 Hz), 1.7–1.48 (2H, m), 1.43–1.26 (28H, br.m), 1.17–1.11 (1H, m), 1.05 (9H, s), 0.89–0.81 (3H, s), 0.42 (1H, m), 0.13 (3H, m); δ_C: 174.2, 135.9, 134.2, 129.6, 127.5, 62.4, 51.4, 40.2, 34.8, 34.4, 34.1, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 26.9, 25.9, 25.0, 19.9, 18.6, 10.6; ν_{max}/cm^{-1}: 2925, 2854, 1742, 1428, 1111, 701.

Experiment 2: Methyl 16-((*1S,2R*)-2-((S)-4-hydroxybutan-2-yl)cyclopropyl)hexadecanoate (196)



Tetra-*n*-butyl ammonium fluoride (10.2 mL, 10.2 mmol) was added to a stirred solution of methyl 16-((*1S,2R*)-2-*((S)*-4-((*tert*-butyl-diphenylsilyl)oxy)butan-2-yl)-cyclopropyl) hexadecanoate **(195)** (5.30 g, 8.54 mmol) in dry THF (70 mL) at 0 °C under nitrogen. The mixture was allowed to reach room temperature and stirred for 5 hrs. When TLC showed no starting material, the reaction was cooled to 5 °C and quenched with sat.aq. NH_4Cl (30 mL). The product was extracted with ethyl acetate (3 ×150 mL), then the combined organic layers were dried over $MgSO_4$ and the solvent was evaporated to give a crude product, which was purified by column chromatography eluting with petrol/ ethyl acetate (5:1) to give a colourless oil of **(196)** (3.12 g, 96 %), $[\alpha]_D^{21} = +14.1$ (*c* 0.93, $CHCl_3$), [HRMS (EI^+): *m/z* 382.3467 [$(M)^+$], calculated for $C_{24}H_{46}O_3$: 382.3447]; δ_H: 3.78–3.69 (2H, m), 3.67 (3H, s), 2.30 (2H, t, *J* 7.6 Hz), 1.76–1.67 (1H, m), 1.63–1.60 (2H, m), 1.59–1.52 (1H, m), 1.37–1.20 (26H, m), 1.18–1.11 (1H, m), 0.96 (3H, d, *J* 6.6 Hz), 0.90–0.81 (1H, m), 0.51–0.44 (1H, m), 0.25–0.13 (3H, m); δ_C: 174.8, 61.4, 51.4, 40.4, 35.0, 34.4, 34.1, 29.7, 29.7, 29.6, 29.5, 29.3, 29.2, 26.6, 25.9, 25.0, 19.9, 18.8, 10.6; ν_{max}/cm^{-1}: 3369, 2923, 2853, 1742, 1459, 1170.

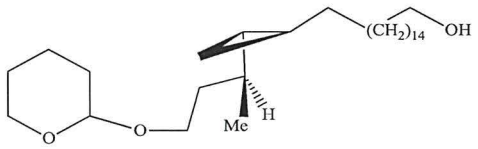
Experiment 3:Methyl16-((*1S,2R)*-2-*((2S)*-4-((tetrahydro-2*H*-pyran-2-yl)oxy) butan -2-yl)cyclopropyl)hexadecanoate (197)



Pyridinium-p-toluene–sulfonate (1.01 g, 4.01 mmol) was added to a stirred solution of methyl 16-((*1S,2R*)-2-((*S*)-4-hydroxybutan-2-yl)cyclopropyl)hexadecanoate (3.05 g, 7.98 mmol) and 3,4-dihydro-2*H*-pyran **(196)** (1.67 g, 20.96 mmol) in dry CH_2Cl_2 (40 mL) under nitrogen at room temperature and then stirred for 30 minutes. When TLC showed all starting material had reacted; the mixture was quenched with sat.aq. $NaHCO_3$ (20 mL). The product was extracted with dichloromethane (2 × 75 mL), dried over

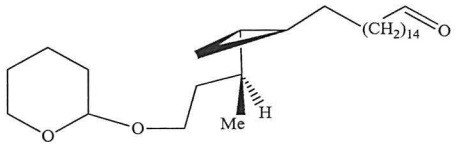
$MgSO_4$ and the solvent was evaporated to give the crude product, which was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give a colourless oil of **(197)** (3.67 g, 98%), $[\alpha]_D^{21} = +11.8$ (c 0.55, $CHCl_3$) [HRMS (EI^+): *m/z* 466.4011 [$(M)^+$], calculated for $C_{29}H_{54}O_4$: 466.4022]; δ_H: 4.57 (1H, br.m), 3.88-3.78 (2H, m), 3.68 (3H, s), 3.48 (2H, m), 2.31 (2H, t, *J* 7.5 Hz), 1.88–1.71 (1H, m), 1.69–1.64 (2H, m), 1.58–1.53 (7H, m), 1.37–1.20 (26H, m), 0.95 (3H, d, *J* 6.8 Hz), 0.90–0.80 (1H, m), 0.51–0.43 (1H, m), 0.18–0.14 (3H, m); δ_C: 174.4, 99.0, 98.9, 66.2, 65.9, 62.4, 51.4, 37.2, 37.1, 35.3, 35.2, 34.4, 34.1, 30.9, 29.8, 29.7, 29.6, 29.5, 29.3, 29.2, 26.0, 25.9, 25.5, 25.0, 19.82, 19.8, 18.64, 18.6, 10.6; ν_{max}/cm^{-1}: 2924, 2853, 1743, 1454.

Experiment 4: 16-((1*S*,2*R*)-2-((2*S*)-4-((tetrahydro-2*H*-pyran-2-yl)oxy)butan-2-yl) cyclopropyl)hexadecan-1-ol (198)



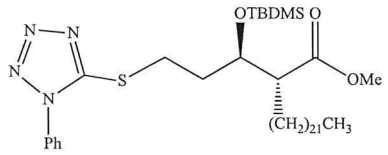
Methyl 16-((1*S*,2*R*)-2-((2*S*)-4-((tetrahydro-2*H*-pyran-2-yl)oxy)butan-2-yl)cyclopropyl) hexadecanoate **(197)** (3.63g, 7.78 mmol) in THF (10 mL) was added to a stirred suspension of $LiAlH_4$ (0.44 g, 11.7 mmol) in THF (60 mL) at –10 °C under nitrogen. The mixture was allowed to reach r.t. and then refluxed for 1 h. When TLC showed no starting material was left, it was quenched with sat. aq. sodium sulphate decahydrate at –10 °C until a white precipitate had formed. The mixture was stirred at r.t. for 30 min, filtered through a pad of celite and the solvent was evaporated to give crude product which was purified by column chromatography eluting with petrol/ethyl acetate (3:1) to give a colourless oil of **(198)** (3.35 g, 94%), $[\alpha]_D^{21} = +18.9$ (c 0.94, $CHCl_3$), [HRMS (EI^+): *m/z* 438.4084 [$(M)^+$], calculated for $C_{28}H_{54}O_3$: 438.4073]; δ_H: 4.57 (1H, br.m), 3.94–3.77 (2H, m), 3.64 (2H, t, *J* 6.6 Hz), 3.56–3.39 (2H, m), 1.92–1.64 (3H, m), 1.59–1.54 (6H, br.m), 1.34–1.26 (28H, br.m), 1.17 (1H, m), 0.96 (3H, d, *J* 6.9 Hz), 0.90–0.77 (1H, m), 0.58–0.40 (1H, m), 0.28–0.07 (3H, m); δ_C: 99.0, 98.9, 66.2, 65.9, 63.0, 62.4, 37.2, 37.1, 35.3, 35.2, 34.4, 32.8, 30.9, 30.8, 29.7, 29.65, 29.62, 29.60, 29.4, 26.0, 25.9, 25.8, 25.5, 19.82, 19.81, 19.8, 18.64, 18.6, 10.6; ν_{max}/cm^{-1}: 3392, 2923, 2852, 1468.

Experiment 5: 16-((1*S*,2*R*)-2-((2*S*)-4-((Tetrahydro-2*H*-pyran-2-yl)oxy)butan-2-yl)cyclopropyl)hexadecanal (172)



16-((1*S*,2*R*)-2-((2*S*)-4-((tetrahydro-2*H*-pyran-2-yl)oxy)butanyl)cyclopropyl)hexadecan -1-ol **(198)** (3.25 g, 7.41 mmol) in CH_2Cl_2 (10 mL) was added to stirred suspension of pyridinium chlorochromate (PCC) (3.99 g, 18.6 mmol) in CH_2Cl_2 (80 mL), and stirred for 2 hrs. The mixture was diluted with petrol/ ethyl acetate (10:1, 50 mL), and then filtered through a pad of silica gel and celite. The solvent was evaporated and the product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give a colourless oil of **(172)** (3.00 g, 93%), $[\alpha]_D^{21} = +5.23$ (*c* 1.01, $CHCl_3$), [HRMS (EI^+): *m/z* 436.3911 [$(M)^+$], calculated for $C_{28}H_{52}O_3$: 436.3916]; δ_H: 9.77 (1H, t, *J* 1.9 Hz), 4.61–4.53 (1H, br.m), 3.95–3.77 (2H, m), 3.56–3.40 (2H, m), 2.42 (2H, dt, *J* 1.9, 7.4 Hz), 1.80–1.75 (3H, m), 1.59–1.53 (7H, br.m), 1.34–1.26 (26H, br.m), 0.96 (3H, d, *J* 6.9 Hz), 0.91–0.78 (1H, m), 0.59–0.41 (1H, m), 0.29–0.10 (3H, m); δ_C: 203.0, 99.0, 98.9, 66.2, 65.9, 62.4, 43.9, 37.2, 37.0, 35.3, 35.2, 34.4, 30.9, 30.8, 29.7, 29.7, 29.65, 29.5, 29.4, 25.9, 25.5, 19.81, 19.8, 18.61, 18.6, 10.6; ν_{max}/cm^{-1}: 2923, 2852, 1728, 1455.

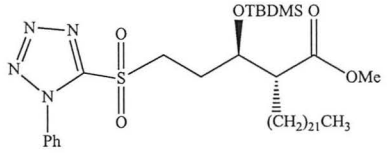
Experiment 6: Methyl (*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-3-((1-phenyl-1H-tetrazol-5-yl)thio)propyl)tetracosanoate (214)



Diethyl azodicarboxylate (DEAD) (2.38 g, 13.68 mmol) in dry THF (8 mL) was added to a stirred solution of Methyl (*R*)-2-((*R*)-3-(benzyloxy)-1-((*tert*-butyldimethylsilyl) oxy) propyl)tetracosanoate **(213)** (6.0 g, 10.50 mmol), Triphenylphosphine (3.58 g, 13.68 mmol) and 1-phenyl-1*H*-tetrazole-5-thiol (2.43 g, 13.86 mmol) in dry THF (70 mL) at 0 °C under nitrogen atmosphere. The reaction mixture was allowed to reach r.t. and then stirred for 3 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 (10:1) to give a thick oil of **(214)** (6.5g, 85%), $[\alpha]_D = -13.16$ (c 1.06 ($CHCl_3$), [HRMS (EI^+): *m/z* 673.4535 [$(M–Bu^t)^+$],

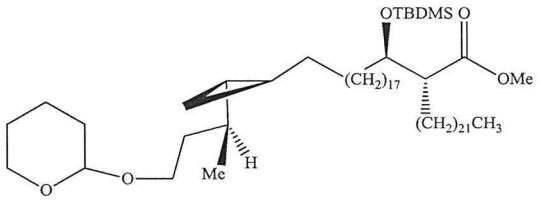
calculated for $C_{37}H_{65}N_4O_3SiS$: 673.4547]; δ_H: 7.59–7.52 (5H, m), 4.07 (1H, m), 3.67 (3H, s), 3.51–3.44 (1H, m), 3.40–3.34 (1H, m), 2.59 (1H, ddd, *J* 4.8, 6.9, 11.4 Hz), 2.16–2.09 (1H, m), 2.01–1.93 (1H, m), 1.59–1.21 (42H, br. m), 0.90–0.87 (12H, m, including a triplet at 0.87 ppm with *J* 6.9 Hz), 0.08 (3H, s), 0.06 (3H, s); δ_C: 174.4, 154.1, 130.1, 129.8, 123.8, 72.0, 51.5, 51.48, 33.1, 31.9, 29.7, 29.66, 29.6, 29.5, 29.4, 28.6, 27.9, 27.1, 25.7, 22.7, 14.1, –4.4, –4.9; ν_{max}/cm^{-1}: 2918, 2852, 1741, 1498, 1459, 1161.

Experiment 7: Methyl (*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-3-((1-phenyl-1H-tetrazol-5-yl)sulfonyl)propyl)tetracosanoate (173)



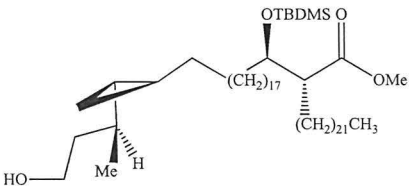
m-Chloroperoxybenzoic acid (7.87 g, 32.05 mmol) was added to a stirred solution of ester **(214)** (7.8 g, 10.68 mmol) in CH_2Cl_2 (50 mL), followed by addition of $NaHCO_3$ (3.59 g, 42.7 mmol). The mixture was stirred for 24 hrs at r.t. When TLC showed no starting material was left, it was poured to a sat.aq. solution of $NaHCO_3$ (150 mL), and stirred for 2 hrs. The product was extracted with CH_2Cl_2 (3 × 100 mL), and the combined organic layers were dried and evaporated to give a crude product which was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give a white solid of **(173)** (5.02 g, 86%), $[\alpha]_D^{21} = -14.45$ (c 1.12 ($CHCl_3$) [HRMS (EI^+): *m/z* 705.4431 [(M–Bu^t)$^+$], calculated for $C_{37}H_{65}N_4O_5SiS$: 705.4445]; δ_H: 7.72–7.7 (2H, m), 7.65–7.61 (3H, m), 4.15 (1H, m), 3.81 (2H, m), 3.69 (3H, s), 2.53 (1H, ddd, *J* 4.0, 7.4, 11.0 Hz), 2.16–2.09 (2H, m), 1.59–1.21 (42H, br. m), 0.90–0.87 (12H, m, including a triplet at 0.87 ppm with *J* 6.9 Hz), 0.08 (3H, s), 0.06 (3H, s); δ_C: 174.1, 153.2, 133, 131.5, 129.7, 125.0, 70.9, 51.8, 51.60, 51.4, 31.9, 29.7, 29.66, 29.6, 29.6, 29.5, 29.4, 29.3, 27.7, 27.4, 26.2, 25.7, 22.70, 14.1, –4.5, –5.1; ν_{max}/cm^{-1}: 2920, 2852, 1729, 1497, 1467, 1155.

Experiment 8: Methyl (2*R*)-2-((1*R*)-1-((*tert*-butyldimethylsilyl)oxy)-19-((1*S*,2*R*)-2-((2*S*)-4-((tetrahydro-2*H*-pyran-2-yl)oxy)butan-2-yl)cyclopropyl)nonadecyl)tetracosanoate (171)



The procedure used in Experiment **1** was repeated in order to couple ester **(173)** (5.97 g, 7.84 mmol) with aldehyde **(172)** (2.85 g, 6.53 mmol) using lithium *bis*(trimethylsilyl) amide (11.1 mL, 11.7 mmol, 1.06 M) in dry THF (50 mL) under nitrogen at -15 °C. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a mixture of *E/Z* alkenes (4.98 g, 78%), [MS (MALDI-TOF): *m/z* 995.94 $[(M+Na)^+]$, calculated for $C_{62}H_{120}NaO_5Si$: 995.88]. The procedure used in Experiment **1** was repeated to hydrogenate the above alkene (4.95 g, 5.09 mmol) using dipotassium azodicarboxylate (2.52 g, 13.2 mmol) and glacial acetic acid (5 mL) in THF (75 mL). The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a colourless oil of **(171)** (4.55 g, 91%), $[\alpha]_D^{21} = -8.6$ (*c* 0.91, $CHCl_3$) [HRMS (MALDI-TOF): *m/z* 997.8949 $[(M+Na)^+]$, calculated for $C_{62}H_{122}NaO_5Si$: 997.8954]; δ_H: 4.58 (1H, br.m), 3.97–3.76 (3H, m), 3.67 (3H, s), 3.56–3.40 (2H, m), 2.53 (1H, ddd, *J* 3.7, 7.1, 10.8 Hz), 1.75–1.64 (2H, m), 1.61–1.06 (84H, br.m), 0.96 (3H, d, *J* 6.6 Hz), 0.90–0.84 (4H, m, including a triplet at 0.87 ppm with *J* 6.9 Hz), 0.86 (9H, s), 0.57–0.40 (1H, m), 0.27–0.11 (3H, m), 0.05 (3H, s), 0.02 (3H, s); δ_C: 175.2, 99.0, 98.9, 73.2, 66.2, 65.9, 62.4, 51.5, 51.4, 41.3, 37.4, 37.2, 35.3, 35.2, 34.4, 32.8, 31.9, 30.8, 29.8, 29.7, 29.63, 29.60, 29.5, 29.4, 29.3, 27.9, 27.7, 27.6, 25.9, 25.7, 25.5, 23.7, 22.7, 22.6, 20.4, 19.8, 14.2, 10.0, –4.4, –4.9; ν_{max}/cm^{-1}: 2924, 2853, 1741, 1464.

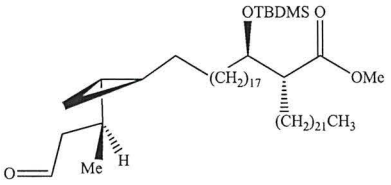
Experiment 9: Methyl (*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-19-((1*S*,2*R*)-2-((*S*)-4-hydroxybutan-2-yl)cyclopropyl)nonadecyl)tetracosanoate (216)



A solution of pyridinium-*p*-toluene sulfonate (0.58 g, 2.31 mmol) was added to a stirred solution of ester **(171)** (4.51 g, 4.62 mmol) in THF (50 mL), MeOH (10 mL) and stirred

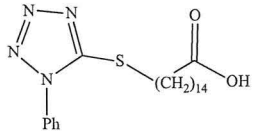
at 45 ° C for 6 hrs. Sat. aq. solution of sodium bicarbonate (20 mL) and water (20 mL) were added to the reaction mixture and the product was extracted with ethyl acetate (3 × 50 mL). The combined organic layers were dried and the solvent was evaporated to give the crude product, which was purified by column chromatography eluting with petrol/ethyl acetate (12:1) to give a white semi solid of **(216)** (3.45 g, 84%), $[\alpha]_D^{21}$ = +17.9 (c 1.09, $CHCl_3$); [HRMS (MALDI-TOF): *m/z* 913.8369 $[(M+Na)^+]$, calculated for $C_{57}H_{114}NaO_4Si$: 913.8379]; δ_H: 3.92 (1H, m), 3.80–3.68 (2H, m), 3.66 (3H, s), 2.53 (1H, ddd, *J* 3.8, 7.1, 10.9 Hz), 1.72 (1H, m), 1.54–1.26 (80H, br.m), 0.96 (3H, d, *J* 6.5 Hz), 0.91–0.84 (4H, m, including a triplet at 0.87 ppm with *J* 6.9 Hz), 0.86 (9H, s), 0.55–0.44 (1H, m), 0.29–0.13 (3H, m), 0.05 (3H, s), 0.03 (3H, s) ;δ_C: 175.2, 73.2, 61.4, 51.6, 51.2, 40.4, 35.0, 34.4, 33.7, 31.9, 31.6, 29.8, 29.7, 29.66, 29.6, 29.59, 29.5, 29.4, 29.1, 27.8, 27.7, 27.65, 27.5, 25.9, 25.8, 25.3, 23.7, 22.7, 21.0, 20.5, 19.8, 18.8, 14.0, 10.6, –4.4, –4.9; ν_{max}/cm^{-1}: 3584, 2924, 2853, 1740, 1463.

Experiment 10: Methyl (*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-19-((1*S*,2*R*)-2-((*S*)-4-oxobutan-2-yl)cyclopropyl)nonadecyl)tetracosanoate (217)



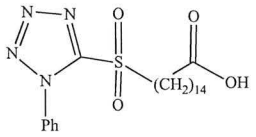
The procedure described in Experiment **5** was repeated to oxidize alcohol **(216)** (3.25 g, 3.65 mmol) using PCC (2.36 g, 10.9 mmol) in CH_2Cl_2 (120 mL). The product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give a colourless oil of **(217)** (3.0 g, 93%), $[\alpha]_D^{21}$ = +10.2 (c 0.91, $CHCl_3$) [MS (MALDI-TOF): *m/z* 912.24 $[(M+Na)^+]$, calculated for $C_{57}H_{112}NaO_4Si$: 911.82]; δ_H: 9.79 (1H, t, *J* 2.5 Hz), 3.92 (1H, m), 3.66 (3H, s), 2.50–2.48 (2H, m), 2.38 (1H, ddd, *J* 2.5, 7.7, 15.7 Hz), 1.54–1.26 (79H, br.m), 1.03 (3H, d, *J* 6.8 Hz), 0.90–0.80 (4H, m, including a triplet at 0.87 ppm with *J* 6.9 Hz), 0.86 (9H, s), 0.50–0.48 (1H, m), 0.38–0.19 (3H, m), 0.05 (3H, s), 0.02 (3H, s); δ_C: 202.9, 175.2, 73.2, 51.6, 51.5, 34.1, 33.9, 33.7, 31.9, 29.8, 29.7, 29.6, 29.5, 29.4, 27.8, 27.7, 27.7, 27.5, 25.9, 25.8, 25.3, 23.7, 22.7, 21.0, 20.5, 19.8, 18.8, 14.1, 11.4, –4.4, –4.9; ν_{max}/cm^{-1}: 2924, 2853, 1738, 1464.

Experiment 11: 15-((1-Phenyl-1*H*-tetrazol-5-yl)thio)pentadecanoic acid (218)



Aq.solution of sodium hydroxide (8M) 100 mL was added to a stirred solution of methyl 15-((1-phenyl-1H-tetrazol-5-yl)thio)pentadecanoate **(184)** (10.50 g, 93.75 mmol) in THF (100 mL), followed by addition of MeOH (15 mL). The reaction mixture was refluxed for 30 minutes. When TLC showed the hydrolysis was complete, the solvent was evaporated, and the residue was acidified with aq. solution of HCl (2M). The product was extracted with ethyl acetate (3 × 150 mL), and the combined organic layers were dried, and evaporated to give crude product. Purification of the product by re-crystalization in ethyl acetate gave a semi solid of **(218)** (9.03 g, 89%), [MS (MALDI-TOF): *m/z* 441.41 $[(M+Na)^+]$, calculated for $C_{22}H_{34}N_4O_2S$: 441.23]; δ_H: 7.74–7.59 (5H, m), 3.40 (2H, t, *J* 7.4 Hz), 2.36 (2H, t, *J* 7.5 Hz), 1.82 (2H, pent, *J* 7.4 Hz), 1.66–1.64 (2H, pent, *J* 7.4 Hz), 1.48–1.41 (2H, pent, *J* 7.4 Hz), 1.30–1.26 (18H, m); δ_C: 179.8, 153.5, 130.1, 129.8, 123.9, 33.7, 33.4, 29.6, 29.5, 29.49, 29.4, 29.3, 29.3, 29.1, 29.04, 28.6, 24.7; ν_{max}/cm^{-1}: 3436, 2918, 2849, 1725, 1594, 1496, 1463, 1413, 1381, 1172, 708.

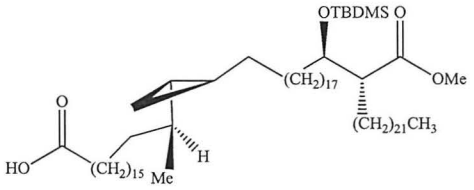
Experiment 12: 15-((1-Phenyl-1H-tetrazol-5-yl)sulfonyl)pentadecanoic acid (219)



A solution of ammonium molybdate (VI) tetrahydrate (7.60 g, 6.14 mmol) in 35 % H_2O_2 (15 mL) was added to a stirred solution of the sulfide **(218)** (5.01 g, 12.3 mmol) in THF (30 mL) and IMS (30 mL) at 10 °C, and then stirred at r.t for 2 hrs. A further solution of ammonium molybdate (VI) tetrahydrate (3.80 g, 3.07 mmol) in 35% H_2O_2 (8 mL) was added and the mixture was stirred at r.t. for 18 hrs. The mixture was poured into water (250 mL) and the product was extracted with ethyl acetate (3 × 100 mL). The combined organic layers were dried and the solvent was evaporated. The crude product was purified by recrystallization with MeOH/acetone (1:1) to give **(219)** (4.2 g, 78%), [MS (MALDI-TOF): *m/z* 472.96 $[(M+Na)^+]$, calculated for $C_{22}H_{34}N_4NaO_4S$: 473.21]; δ_H: 7.74–7.70 (2H, m), 7.66–7.61 (3H, m), 3.74 (2H, t, *J* 8.15 Hz), 2.36 (2H, t, *J* 7.5 Hz), 1.96 (2H, pent, *J* 7.4 Hz), 1.66 (2H, pent, *J* 7.4 Hz), 1.48 (2H, pent, *J* 7.4 Hz), 1.30–1.26 (18H, m); δ_C: 179.5, 153.1, 131.5, 129.7, 125.1, 56.1, 33.8, 29.5, 29.4, 29.4, 29.2, 29.17,

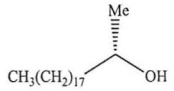
29.0, 28.9, 28.1, 24.7, 22.0; ν_{max}/cm^{-1}: 3467, 2921, 2851, 1694, 1597, 1466, 1359, 1152, 707.

Experiment 13: (*S*)-18-((1*R*,2*S*)-2-((19*R*,20*R*)-19-((*tert*-butyldimethylsilyl)oxy)-20-(methoxy carbonyl)dotetracontyl)cyclopropyl)nonadecanoic acid (170)



The procedure used in Experiment **1** was repeated in order to couple ester **(217)** (2.95 g, 3.32 mmol) with 15-((1-phenyl-1H-tetrazol-5-yl)sulfonyl)pentadecanoic acid **(219)** (1.79 g, 3.98 mmol) using lithium *bis*(trimethylsilyl)amide (9.40 mL, 9.97 mmol, 1.06 M) in dry THF (40 mL) under nitrogen at –15 °C. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a mixture of *E/Z* alkenes **(220)** (2.97 g, 80%), [MS (MALDI-TOF): *m/z* 1136.45 $[(M+Na)^+]$, calculated for $C_{72}H_{140}NaO_5Si$: 1136.03]. The procedure used in Experiment **1** was repeated to hydrogenate alkenes **(220)** (2.90 g, 2.60 mmol) using di-potassium azodicarboxylate (3.75 g, 19.5 mmol) and glacial acetic acid (3 mL) in THF (60 mL). The product was purified by chromatography eluting with petrol/ethyl acetate (10:1) to give a white semi solid of **(170)** (2.60 g, 80%), $[\alpha]_D^{21} = +4.1$ (c 0.48 ($CHCl_3$) [HRMS (MALDI-TOF): *m/z* 1138.0530 $[(M+Na)^+]$, calculated for $C_{72}H_{142}NaO_5Si$: 1138.0519]; δ_H: 3.92 (1H, dt, *J* 7.2, 4.6 Hz), 3.66 (3H, s), 2.53 (1H, ddd, *J* 3.8, 7.2, 10.9 Hz), 2.36 (2H, t, *J* 7.5 Hz), 1.64 (3H, m), 1.57–1.03 (105H, br.m), 0.90 (3H, d, *J* 6.7 Hz), 0.91–0.88 (3H, br.t, *J* 6.9 Hz), 0.87 (9H, s), 0.73–0.62 (1H, m), 0.51–0.40 (1H, m), 0.26–0.07 (3H, m), 0.05 (3H, s), 0.02 (3H, s); δ_C: 178.8, 175.2, 73.2, 51.6, 51.2, 38.1, 37.4, 33.9, 33.7, 32.7, 31.9, 29.7, 29.6, 29.4, 29.3, 27.6, 27.5, 27.3, 25.8, 25.7, 23.7, 22.7, 21.0, 19.3, 18.6, 14.1, 10.5, –4.4, –4.9; ν_{max}/cm^{-1}: 3583 (broad), 2924, 2853, 1743, 1711, 1464, 1241.

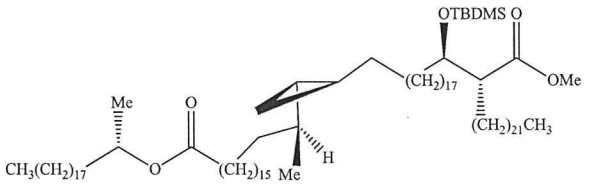
Experiment 14: (*S*)-Eicosan-2-ol (169)



A solution of bromoheptadecane **(221)** (5.01g, 15.6 mmol) in dry THF (5 mL) was added dropwise to a stirred suspension of magnesium turnings (2.26 g, 94.1 mmol) in dry THF (8 mL) with gentle warming. The reaction mixture refluxed for 1 hr. to complete the

formation of the Grignard reagent **(222)**. The Grignard reagent was added dropwise to a stirred suspension of cupper iodide (0.98 g, 5.17 mmol) in dry THF (40 mL) at –30 °C and stirred for 30 minutes. 1-Epoxy propane **(223)** (0.40 g, 6.89 mmol) in dry THF (5 mL) was added dropwise to the reaction mixture at -30 °C, and allowed to stir at room temperature for 16 hrs. The mixture was quenched with sat. aq. NH_4Cl and the product was extracted with ethyl acetate (3 × 100 mL). The combined organic layers were dried and evaporated to give the crude product, which was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give a white solid of (*S*)-eicosan- 2-ol **(169)** (1.10 g, 53%), $[\alpha]_D^{21}$ = +3.8 (*c* 1.01, $CHCl_3$) [HRMS (EI^+): *m/z* 297.3154 [$(M–H)^+$], calculated for $C_{20}H_{41}O$: 297.3157]; δ_H: 3.80 (1H, sext, *J* 6.0 Hz), 1.48 (2H, m), 1.38–1.25 (32H, br.m), 1.2 (3H, d, *J* 6.2 Hz), 0.88 (3H, t, *J* 6.7 Hz); δ_C: 68.2, 39.4, 31.9, 29.70, 29.66, 29.63, 29.61, 29.4, 25.8, 23.5, 22.5, 14.1; ν_{max}/cm^{-1}: 3386, 2924, 2853, 1464.

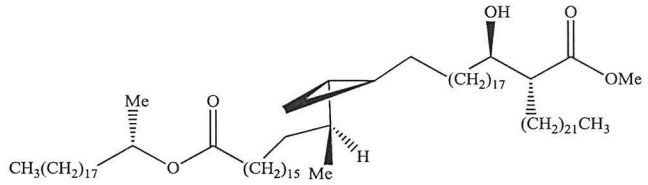
Experiment 15: Methyl (*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-19-((1*S*,2*R*)-2-((*S*)-19-((*S*)-icosan-2-yloxy)-19-oxononadecan-2-yl)cyclopropyl)nonadecyl)tetracosanoate (224)



Dicyclohexylcarbodiimide (DCC) (0.88 g, 4.30 mmol) in dry CH_2Cl_2 (7 mL) was added dropwise over 15 minutes to a stirred solution of (*S*)-18-((1*R*,2*S*)-2-((19*R*,20*R*)-19-((*tert*-Butyldimethylsilyl)oxy)-20-(methoxycarbonyl) dotetracontyl)cyclopropyl) nona decanoic acid **(170)** (2.41 g, 2.16 mmol), (*S*)-Eicosan-2-ol **(169)** (0.67 g, 2.22 mmol) and 4-Dimethyl aminopyridine (DMAP) (0.39 g, 3.22 mmol) in dry CH_2Cl_2 (15 mL) at r.t. The reaction mixture was stirred for 3 hrs. When TLC showed the reaction was complete, the mixture was filtered and the solvent was evaporated to give a crude product, which was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a thick colourless oil of **(224)** (2.4 g, 80%), $[\alpha]_D^{21}$ = +6.1 (*c* 0.9, $CHCl_3$) [HRMS (MALDI-TOF): *m/z* 1418.3659 [$(M+Na)^+$], calculated for $C_{92}H_{182}NaO_5Si$: 1418.3649]; δ_H: 4.91 (1H, sext, *J* 6.2 Hz), 3.92 (1H, dt, *J* 7.0, 4.8 Hz), 3.66 (3H, s), 2.53 (1H, ddd, *J* 3.8, 7.1, 10.9 Hz), 2.27 (2H, t, *J* 7.5 Hz), 1.54–1.26 (142 H, br.m), 1.20 (3H, d, *J* 6.2 Hz), 0.91 (3H, d, *J* 6.8 Hz), 0.90–0.84 (15H, m, including a singlet at 0.88 ppm),

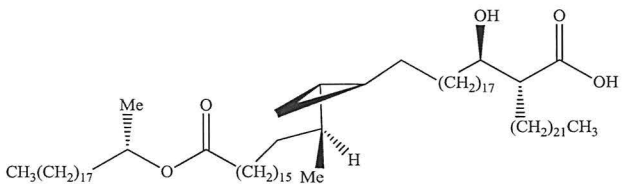
0.71–0.63 (1H, m), 0.49–0.41 (1H, m), 0.22–0.08 (3H, m), 0.05 (3H, s), 0.02 (3H, s); δ_C: 175.2, 173.5, 73.2, 70.7, 51.6, 51.2, 38.1, 37.4, 36.0, 34.8, 34.5, 33.7, 31.9, 30.1, 29.8, 29.8, 29.7, 29.7, 29.7, 29.60, 29.57, 29.52, 29.5, 29.4, 29.3, 29.2, 27.8, 27.5, 27.2, 26.1, 25.8, 25.4, 25.1, 23.8, 22.7, 20.0, 19.7, 18.6, 14.1, 10.5, –4.4, –4.9; ν_{max}/cm^{-1}: 2924, 2853, 1736, 1464, 1253, 1167, 836.

Experiment 16: Methyl (*R*)-2-((*R*)-1-hydroxy-19-((1*S*,2*R*)-2-((*S*)-19-((*S*)-icosan-2-yloxy)-19-oxononadecan-2-yl)cyclopropyl) nonadecyl) tetracosanoate (231)



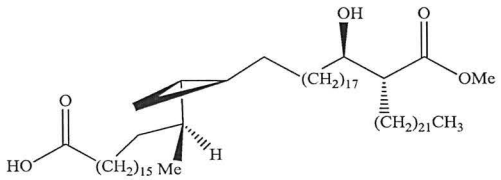
The methyl ester **(224)** (2.30 g, 1.79 mmol) was dissolved in dry THF (30 mL) in a dry polyethylene vial equipped with a rubber septum, followed by addition of pyridine (0.7 mL) at r.t under nitrogen. The reaction mixture was cooled to 10 °C, and then hydrogen fluoride-pyridine complex as ~70% (4.0 mL) was added dropwise. The mixture was stirred at 43 °C for 17 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 5:1 (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 (10:1) to give a semi-solid of **(231)** (1.50 g, 71%), $[\alpha]_D^{21} = +7.3$ (*c* 0.11, $CHCl_3$) [HRMS (MALDI-TOF): *m/z* 1304.2782 [$(M+Na)^+$], calculated for $C_{86}H_{168}NaO_5$: 1304.2784]; δ_H: 4.91 (1H, sext, *J* 6.2 Hz), 3.71 (3H, s), 3.66 (1H, m), 2.46 (1H, m), 2.27 (2H, t, *J* 7.5 Hz), 1.54–1.26 (143H, br.m), 1.20 (3H, d, *J* 6.2 Hz), 0.90 (3H, d, *J* 6.8 Hz), 0.89 (6H, br.t, *J* 6.9 Hz), 0.71–0.63 (1H, m), 0.49–0.41 (1H, m), 0.22–0.08 (3H, m); δ_C: 176.2, 173.6, 72.3, 70.7, 51.6, 50.9, 38.1, 37.4, 36.0, 34.8, 34.5, 33.7, 31.9, 30.1, 29.6, 29.5, 27.8, 27.5, 27.2, 26.1, 25.8, 25.4, 25.1, 23.8, 22.7, 20.1, 19.7, 18.6, 14.1, 10.5; ν_{max}/cm^{-1}: 3441, 2918, 2850, 1732, 1721, 1466, 1377, 1250, 1109, 721.

Experiment 17: (*R*)-2-((*R*)-1-Hydroxy-19-((1*S*,2*R*)-2-((*S*)-19-((*S*)-icosan-2-yloxy)-19-oxononadecan-2-yl)cyclopropyl)nonadecyl)tetracosanoic acid (164)



Methyl (*R*)-2-((*R*)-1-hydroxy-19-((1*S*,2*R*)-2-((*S*)-19-((*S*)-icosan-2-yloxy)-19-oxononadecan-2-yl)cyclopropyl)nonadecyl)tetracosanoate **(231)** (1.42 g, 1.11 mmol) was added to aq. solution of tetra-*n*-butyl ammonium hydroxide (64 mL, 5%) and refluxed at 100 °C for 24 hrs. The mixture was cooled to r.t. and the product was extracted with petroleum ether/ether 5:2 (3 × 75 mL). The combined organic layers were dried and evaporated to give a crude product, which was purified by column chromatography eluting with petrol/ethyl acetate (5:1) to give a white solid of **(164)** (0.84 g, 60%), $[\alpha]_D^{21}$ = +7.6 (*c* 0.4, $CHCl_3$), m.p. 55–56 °C, [HRMS (MALDI-TOF): *m/z* 1290.2617 $[(M+Na)^+]$, calculated for $C_{85}H_{166}NaO_5$: 1290.2627]; δ_H: 4.91 (1H, sext, *J* 6.2 Hz), 3.73 (1H, m), 2.46 (1H, br.dt, *J* 9.1, 5.3 Hz), 2.27 (2H, t, *J* 7.5 Hz), 1.81–0.95 (144H, br.m), 1.19 (3H, d, *J* 6.2 Hz), 0.90 (3H, d, *J* 6.8 Hz), 0.89 (6H, br.t, *J* 6.9 Hz), 0.70–0.63 (1H, m), 0.49–0.41 (1H, m), 0.22–0.08 (3H, m); δ_C: 179.6, 173.6, 72.1, 70.75, 50.8, 38.1, 37.4, 36.0, 35.5, 34.7, 34.5, 31.9, 30.1, 29.7, 29.52, 29.5, 29.43, 29.4, 29.3, 29.2, 27.3, 27.2, 26.1, 25.7, 25.4, 25.1, 22.7, 20.0, 19.7, 18.6, 14.1, 10.5; ν_{max}/cm^{-1}: 3432 (broad), 2918, 2850, 1721, 1712, 1470, 1377, 718.

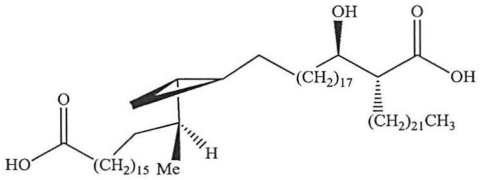
Experiment 18: (*S*)-18-((1*R*,2*S*)-2-((19*R*,20*R*)-19-Hydroxy-20-(methoxycarbonyl)-dotetra contyl)cyclopropyl)nonadecanoic acid (232)



The procedure used in Experiment **16** was repeated using (*S*)-18-((1*R*,2*S*)-2-((19*R*,20*R*)-19-((*tert*-butyldimethylsilyl)oxy)-20-(methoxycarbonyl)dotetracontyl)cyclopropyl)non-adecanoic acid **(170)** (0.10 g, 0.09 mmol), pyridine (0.1 mL), and hydrogen fluoride-pyridine complex as ~70% (0.4 mL) in dry THF (5 mL). The crude product was purified by chromatography eluting with petrol/ethyl acetate (4:1) to give a syrup of **(232)** (0.075 g, 85%), $[\alpha]_D^{21}$ = +7.8 (c 1.6 $CHCl_3$) [MS (MALDI-TOF): *m/z* 1024.68 $[(M+Na)^+]$, calculated for $C_{66}H_{128}NaO_5$: 1023.96]; δ_H: 3.71 (3H, s), 3.68–3.65 (1H, m), 2.46 (1H, dt, *J* 9.3, 5.4 Hz), 2.36 (2H, t, *J* 7.5 Hz), 1.74–1.62 (4H, m), 1.55–1.26 (106 H, m), 0.91–

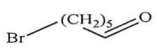
0.86 (6H, br.m), 0.69–0.62 (1H, m), 0.49–0.41 (1H, m), 0.22–0.08 (3H, m); δ_C: 178.6, 176.3, 72.3, 51.6, 51.0, 38.2, 37.4, 35.7, 34.5, 33.9, 31.9, 30.1, 29.77, 29.75, 29.74, 29.65, 29.6, 29.58, 29.54, 29.50, 29.47, 29.43, 29.4, 29.3, 29.1, 27.4, 27.2, 25.7, 24.7, 22.70, 21.1, 19.7, 18.6, 14.2, 10.5; ν_{max}/cm^{-1}: 2917, 2849, 1733, 1711, 1464, 1377, 719.

Experiment 19: (*R*)-2-((*R*)-19-((1*S*,2*R*)-2-((*S*)-18-Carboxyoctadecan-2-yl)cyclopropyl)-1-hydroxynonadecyl)tetracosanoic acid (165)



Lithium hydroxide monohydrate (0.044 g, 1.073 mmol) was added to a stirred solution of (*S*)-18-((1*R*,2*S*)-2-((19*R*,20*R*)-19-Hydroxy-20-(methoxycarbonyl) dotetra contyl) cyclopropyl) nonadecanoic acid **(232)** (0.071 g, 0.072 mmol) in THF (8 mL), water (1 mL) and MeOH (0.8 mL). The reaction mixture was heated at 45 °C for 16 hrs. When TLC showed no starting material was left, the mixture was diluted with petrol/ethylacetate (5:1, 4 mL), and then acidified with sat.aq.solution of $KHSO_4$. The product was extracted with petrol/ethyl acetate (5:1, 3 × 5 mL), and the combined organic layers were dried over $MgSO_4$, evaporated to give a crude product, which was purified by column chromatography eluting with petrol/ethyl acetate (1:3) to give a white solid of **(165)** (0.059 g, 85%); $[\alpha]_D^{21}$ = +5.8 (c 0.86, $CHCl_3$), m.p.77–78 °C, [HRMS (MALDI-TOF): *m/z* 1009.9520 $[(M+Na)^+]$, calculated for $C_{65}H_{126}NaO_5$: 1009.9497]; δ_H: 3.74–3.70 (1H, m), 2.53 (1H, m), 2.35 (2H, t, *J* 7.5 Hz), 1.77–1.62 (4H, m), 1.59–1.26 (107 H, m), 0.92–0.86 (6H, br. m), 0.73–0.68 (1H, m), 0.50–0.44 (1H, m), 0.24–0.10 (3H, m); δ_C: 180.1, 179.1, 72.2, 50.9, 38.1, 37.5, 35.6, 34.5, 34.0, 31.9, 30.8, 29.7, 29.6, 29.5, 29.4, 29.1, 27.4, 27.3, 26.2, 25.7, 24.7, 22.7, 19.7, 18.6, 14.0, 10.5; ν_{max}/cm^{-1}: 3585 (broad), 2916, 2849, 1735, 1464, 1377, 719.

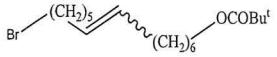
Experiment 20: 6-Bromohexanal (234)



The procedure described in Experiment **5** was repeated to oxidize bromohexan-1-ol **(233)** (3.80 g, 21.0 mmol) using PCC (11.30 g, 52.50 mmol) in CH_2Cl_2 (200 mL). The product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give a colourless oil of **(234)** (3.45 g, 92%); δ_H: 9.78 (1H, t, *J* 1.6 Hz), 3.42 (2H, t, *J*

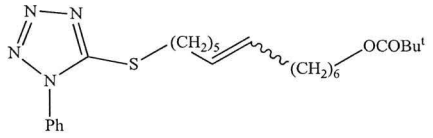
6.7 Hz), 2.47 (2H, dt, *J* 1.6, 7.2 Hz), 1.89 (2H, pent, *J* 6.9 Hz), 1.67 (2H, m), 1.49 (2H, m); δ_C: 202.2, 43.7, 33.4, 32.4, 27.7, 25.9; ν_{max}/cm^{-1}: 2939, 2863, 1708, 1412.

Experiment 21: (*E, Z*)-13-Bromotridec-7-en-1-yl pivalate (236)



The procedure used in Experiment **1** was repeated in order to couple 6-bromohexanal **(234)** (3.4 g, 18.9 mmol) with 7-((1-phenyl-1H-tetrazol-5-yl)sulfonyl)heptyl pivalate **(235)** (10.1g, 24.6 mmol) using lithium *bis*(trimethylsilyl) amide (34.9 mL, 37.1 mmol, 1.06 M) in dry THF (75 mL) under nitrogen at –15 °C. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (30:1) to give a colourless oil of **(236)** (4.95 g, 73%); [MS (MALDI-TOF): *m/z* 383.19 $[(M+Na)^+]$, calculated for $C_{18}H_{33}NaO_2Br$: 383.15]; δ_H: 5.38 (2H, m), 4.05 (2H, t, *J* 6.7 Hz), 3.42 (2H, t, *J* 6.7 Hz), 2.05 (2H, m), 1.89 (2H, m), 1.64 (4H, m), 1.39–1.34 (10H, m), 1.2 (9H, s); δ_C: 177.7, 130.6, 130.1, 129.84, 129.45, 64.4, 33.9, 33.9, 32.7, 32.4, 29.6, 29.4, 28.88, 28.85, 28.71, 28.70, 28.6, 27.8, 27.7, 27.2, 27.1, 27.0, 25.9, 25.8; ν_{max}/cm^{-1}: 2930, 2856, 1728, 1479, 1459,1155.

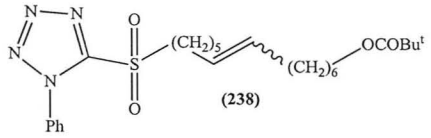
Experiment 22: (*E,Z*)-13-((1-phenyl-1H-tetrazol-5-yl)thio)tridec-7-en-1-yl pivalate (237)



1-Phenyl-1*H*-tetrazole-5-thiol (1.98 g, 11.10 mmol), (*E,Z*)-13-bromotridec-7-en-1-yl pivalate **(236)** (4.01 g, 11.10 mmol) and anhydrous potassium carbonate (3.06 g, 22.10 mmol) were dissolved in acetone (70 mL). The mixture was vigorously stirred for 18 hrs at r.t. When TLC showed the reaction was complete, the acetone was evaporated and then water (200 mL) was added. The product was extracted with CH_2Cl_2 (3 × 150 mL), 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 (8:1) to give a thick oil of **(237)** (4.16 g, 81%) [HRMS (EI^+): *m/z* 458.2732 $[(M)^+]$, calculated for $C_{25}H_{38}O_2N_4S$: 458.2715]; δ_H: 7.59–7.55 (5H, m), 5.38 (2H, m), 4.04 (2H, t, *J* 6.7 Hz) 3.39 (2H, t, *J* 7.4 Hz), 1.98 (2H, m), 1.82 (2H, m), 1.43–1.32 (14H, m), 1.19 (9H, s); δ_C: 177.7, 130.8, 130.6, 130.3, 130.1, 129.8, 129.3, 129.1, 123.9, 64.4, 33.4, 32.5,

32.4, 31.9, 29.6, 29.4, 28.9, 28.9, 28.71, 28.70, 28.6, 28.1, 27.2, 25.90, 25.7, 25.4; ν_{max}/cm^{-1}: 2928, 2855, 1726, 1597, 1500, 1479, 1459, 1387, 1284, 1157, 1155, 761, 694.

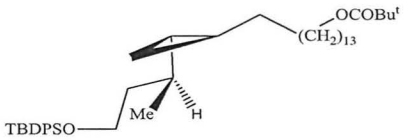
Experiment 23: (*E,Z*)-13-((1-Phenyl-1H-tetrazol-5-yl)sulfonyl)tridec-7-en-1-yl pivalate (238)



(238)

The procedure used in Experiment **12** was repeated using ammonium molybdate (VI) tetrahydrate (5.39 g, 4.36 mmol) in 35 % H_2O_2 (15 mL), (*E,Z*)-13-((1-phenyl-1H-tetrazol-5-yl)thio)tridec-7-en-1-yl pivalate **(237)** (4.00 g, 8.73 mmol) in THF (35 mL) and IMS (35 mL). The crude product was purified by column chromatography eluting with petrol/ethyl acetate (8:1) to give a thick oil of **(238)** (3.68 g, 86%) [HRMS (EI^+): *m/z* 490.2615 [$(M)^+$], calculated for $C_{25}H_{38}NaO_4N_4S$: 490.2614]; δ_H: 7.71 (2H, m), 7.62 (3H, m), 5.38 (2H, m), 4.04 (2H, t, *J* 6.5 Hz) 3.73 (2H, m), 1.99 (2H, m), 1.62 (6H, m), 1.35–1.27 (10H, m), 1.21 (9H, s); δ_C: 178.3, 133.5, 133.3, 133.0, 131.9, 131.5, 130.7, 130.6, 130.3, 130.1, 129.7, 129.2, 128.9, 128.5, 126.9, 125.2, 125, 64.4, 63.7, 63.6, 55.9, 55.4, 38.7, 34.1, 32.5, 32.4, 31.8, 30.8, 29.5, 29.3, 29.1, 28.9, 28.85, 28.8, 28.7, 28.5, 28.4, 28.3, 28.25, 28.2, 27.2, 25.9, 25.7, 25.4, 22.1, 21.3; ν_{max}/cm^{-1}: 2928, 2855, 1725, 1595, 1498, 1480, 1461, 1342, 1285, 1153, 763, 688.

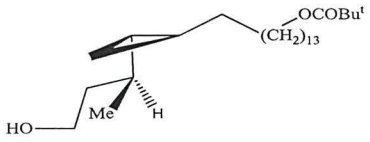
Experiment 24: 14-((1*S*,2*S*)-2-(4-((*tert*-Butyldiphenylsilyl)oxy)-2-methyl-butan-2-yl) cyclopropyl)tetradecyl pivalate (240)



The procedure used in Experiment **1** was repeated in order to couple (*1S,2R*)-2-[(*S*)-3-(*tert*-butyldiphenylsilanyloxy)-1-methylpropyl]cyclopropane carbaldehyde **(98)** (2.00 g, 5.26 mmol) with (*E,Z*)-13-((1-phenyl-1H-tetrazol-5-yl)sulfonyl)tridec-7-en-1-yl pivalate **(238)** (3.09g, 6.31 mmol) using lithium *bis*(trimethyl silyl) amide (8.93 mL, 9.47mmol, 1.06 M) in dry THF (30 mL) under nitrogen at –15 °C. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a mixture of *E/Z* alkenes **(239)** (2.70 g, 80 %) [HRMS (EI^+): *m/z* 587.3907 [$(M\text{-}Bu^t)^+$], calculated for $C_{38}H_{55}O_3Si$: 587.3920]. The procedure used in Experiment **1** was repeated

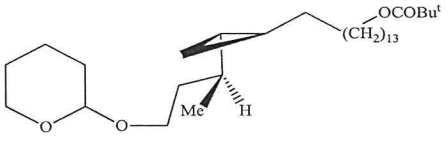
to hydrogenate the above alkenes **(239)** (2.68 g, 4.16 mmol) using dipotassium azodicarboxylate (2.50 g, 11.0 mmol) and glacial acetic acid (5 mL) in THF (60 mL). The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a colourless oil of **(240)** (2.57 g, 95%), $[\alpha]_D = +7.2$ (c 0.91($CHCl_3$) [HRMS (EI^+): *m/z* 591.4235 [$(M\text{-}Bu^t)^+$], calculated for $C_{38}H_{59}O_3$: 591.4233]; δ_H: 7.69–7.66 (4H, m), 7.42–7.38 (6H, m), 4.06 (2H, t, *J* 6.6 Hz), 3.78–3.68 (2H, m), 1.71–1.51 (6H, br.m), 1.38–1.26 (22H, br.m), 1.2 (9H, s), 1.18–1.11 (1H, m), 1.04 (9H, s), 0.88–0.84 (3H, br.s), 0.47–0.39 (1H, m), 0.16–0.08 (3H, m); δ_C: 135.9, 134.2, 129.5, 127.6, 64.5, 62.4, 40.3, 34.8, 34.4, 29.7, 29.7, 29.7, 29.6, 29.5, 29.2, 28.6, 27.7, 27.3, 27.2, 26.9, 25.9, 22.6, 21.3, 19.8, 19.2, 18.6, 10.6; ν_{max}/cm^{-1}: 2926, 2855, 1730, 1462, 1428, 1386, 1284, 1151, 1111, 701.

Experiment 25: 14-((1*S*,2*S*)-2-(4-Hydroxy-2-methylbutan-2-yl)cyclopropyl)tetradecyl pivalate (241)



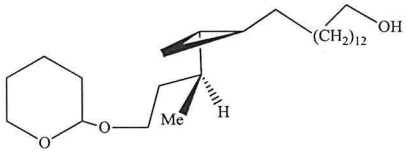
The same procedure used in Experiment **2** was repeated to deprotect 14-((1*S*,2*S*)-2-(4-((*tert*-butyldiphenylsilyl)oxy)-2-methylbutan-2-yl)cyclopropyl)tetradecylpivalate **(240)** (2.55 g, 3.93 mmol) using tetra-*n*-butyl ammonium fluoride (4.71 mL, 4.71 mmol) in dry THF (25 mL) at 0 °C under nitrogen. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (5:1) to give a colourless oil of **(241)** (1.50 g, 93 %), $[\alpha]_D^{21} = +15.71$ (c 2.62, $CHCl_3$); δ_H: 4.05 (2H, t, *J* 6.6 Hz), 3.78–3.68 (2H, m), 1.76–1.68 (1H, m), 1.62–1.50 (3H, br.m), 1.37–1.26 (24H, m), 1.20 (9H, s), 1.18–1.11 (1H, m), 0.96 (3H, d, *J* 6.6 Hz), 0.88–0.81 (1H, m), 0.50–0.45 (1H, m), 0.24–0.13 (3H, m); δ_C: 178.5, 64.4, 61.4, 40.4, 38.8, 35.0, 34.4, 29.7, 29.64, 29.62, 29.61, 29.6, 29.5, 28.6, 27.2, 26.6, 25.9, 19.9, 18.8, 10.6; ν_{max}/cm^{-1}: 3364, 2925, 2853, 1729, 1459, 1170.

Experiment 26: 14-((1*S*,2*R*)-2-((2*S*)-4-((tetrahydro-2*H*-pyran-2-yl)oxy)butan-2-yl) cyclopropyl)tetradecyl pivalate (242)



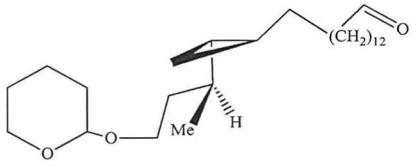
The procedure used in Experiment **3** was repeated using pyridinium-p-toluene–sulfonate (0.42 g, 1.67 mmol), 14-((1*S*,2*S*)-2-(4-hydroxy-2-methylbutan-2-yl)cyclo propyl) tetra decylpivalate **(241)** (1.40 g, 3.41 mmol) and 3,4-dihydro-2*H*-pyran (0.71 g, 8.44 mmol) in dry CH_2Cl_2 (20 mL) under nitrogen at r.t. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give a colourless oil of **(242)** (1.63 g, 97%), $[\alpha]_D^{21}$ = +13.1 (c 0.89, $CHCl_3$) [HRMS (EI^+): *m/z* 494.4357 $[(M)^+]$, calculated for $C_{31}H_{58}O_4$: 494.4335]; δ_H: 4.57 (1H, br.m), 4.05 (2H, t, *J* 6.6 Hz), 3.92–3.78 (2H, m), 3.53–3.42 (2H, m), 1.85–1.7 (4H, m), 1.62–1.50 (8H, m), 1.37–1.26 (22H, m), 1.2 (9H, s), 0.95 (3H, d, *J* 6.2 Hz), 0.88–0.81 (1H, m), 0.53–0.42 (1H, m), 0.27–0.13 (3H, m); δ_C: 178.7, 99.0, 98.9, 66.2, 65.9, 64.5, 62.4, 38.7, 37.5, 37.1, 34.4, 32.5, 30.9, 30.8, 29.7, 29.6, 29.4, 29.38. 29.32, 29.2, 26.6, 25.2, 19.8, 19.7, 18.6, 10.6; ν_{max}/cm^{-1}: 2925, 2853, 1731, 1597, 1459, 1170.

Experiment 27: 14-((1*S*,2*R*)-2-((2*S*)-4-((Tetrahydro-2*H*-pyran-2-yl)oxy)butan-2-yl) cyclopropyl)tetradecan-1-ol (243)



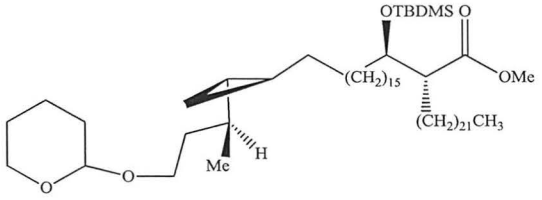
The procedure used in Experiment **4** was repeated to reduce 14-((1*S*,2*R*)-2-((2*S*)-4-((tetrahydro-2*H*-pyran-2-yl)oxy)butan-2-yl)cyclopropyl)tetradecylpivalate **(242)** (1.60 g, 3.23 mmol) using $LiAlH_4$ (0.18 g, 4.85 mmol) in THF (25mL) at -10 °C under nitrogen. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (3:1) to give a colourless oil of **(243)** (1.26 g, 95%), $[\alpha]_D^{21}$ = +19.83 (c 1.01, $CHCl_3$) [HRMS (EI^+): *m/z* 410.3753 $[(M)^+]$, calculated for $C_{26}H_{50}O_3$: 410.3760] ; δ_H: 4.57 (1H, br.m), 3.92–3.76 (2H, m), 3.64 (2H, t, *J* 6.6 Hz), 3.56–3.39 (2H, m), 1.89–1.66 (3H, m), 1.64–1.47 (7H, br.m), 1.34–1.26 (24H, br.m), 1.17 (1H, m), 0.96 (3H, d, *J* 6.7 Hz), 0.90–0.77 (1H, m), 0.53–0.41 (1H, m), 0.29–0.08 (3H, m); δ_C: 99.0, 98.9, 66.2, 65.9, 63.0, 62.4, 37.2, 37.1, 35.3, 35.2, 34.4, 32.8, 30.9, 30.8, 29.7, 29.69, 29.66, 29.64, 29.61, 29.4, 26.0, 25.9, 25.8, 25.5, 19.82, 19.81, 18.64, 18.6, 10.6; ν_{max}/cm^{-1}: 3386, 2924, 2852, 1463, 1034.

Experiment 28: 14-((1*S*,2*R*)-2-((2*S*)-4-((Tetrahydro-2*H*-pyran-2-yl)oxy)butan-2-yl) cyclopropyl)tetradecanal (244)



The procedure described in Experiment **5** was repeated to oxidize 14-((1*S*,2*R*)-2-((2*S*)-4-((tetrahydro-2*H*-pyran-2-yl)oxy)butan-2-yl)cyclopropyl) tetradecan-1-ol **(243)** (1.22 g, 2.97 mmol) in CH_2Cl_2 (10 mL) using PCC (1.92 g, 8.92 mmol) in CH_2Cl_2 (50 mL). The crude product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give a colourless oil of **(244)** (1.11 g, 92 %); $[\alpha]_D^{21}$ = +7.73 (*c* 1.13, $CHCl_3$) [HRMS (EI^+): *m/z* 407.3544 [$(M-H)^+$], calculated for $C_{26}H_{47}O_3$: 407.3525]; δ_H: 9.77 (1H, t, *J* 1.9 Hz), 4.61–4.53 (1H, br.m), 3.95–3.77 (2H, m), 3.56–3.40 (2H, m), 2.42 (2H, dt, *J* 1.9, 7.4 Hz), 1.80–1.75 (3H, m), 1.59–1.53 (7H, br.m), 1.34–1.26 (22H, br.m), 0.95 (3H, d, *J* 6.9 Hz), 0.91–0.78 (1H, m), 0.58–0.41 (1H, m), 0.25–0.10 (3H, m); δ_C: 203.0, 99.0, 98.9, 66.2, 65.9, 62.4, 43.2, 37.2, 37.1, 35.3, 35.2, 34.4, 30.9, 30.7, 29.7, 29.64, 29.62, 29.6, 29.4, 29.2, 25.9, 25.5, 19.84, 19.83, 18.63, 18.6, 10.6; ν_{max}/cm^{-1}: 2923, 2852, 1728, 1455.

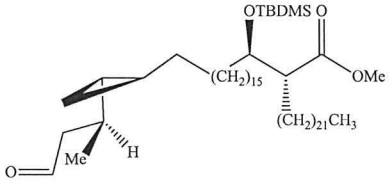
Experiment 29: Methyl (2*R*)-2-((1*R*)-1-((*tert*-butyldimethylsilyl)oxy)-17-((1*S*,2*R*)-2-((2*S*)-4((tetrahydro-2*H*-pyran-2-yl)oxy)butan-2-yl)cyclopropyl)-heptadecyl)-tetra cosanoate (245)



The procedure used in Experiment **1** was repeated in order to couple (*R*)-methyl 2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-3-((1-phenyl-1H-tetrazol-5-yl)sulfonyl)propyl) tetra-cosanoate **(173)** (2.39 g, 3.14 mmol) with 14-((1*S*,2*R*)-2-((2*S*)-4-((tetrahydro-2*H*-pyran-2-yl)oxy)butan-2-yl)cyclopropyl)tetra decanal **(244)** (1.07 g, 2.62 mmol) using lithium *bis*(trimethylsilyl)amide (4.45 mL, 4.71 mmol, 1.06 M) in dry THF (25 mL) under nitrogen at -15 °C. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a mixture of *E/Z* alkenes (1.91 g, 77%) [MS (MALDI-TOF): *m/z* 967.64 [$(M+Na)^+$], calculated for $C_{60}H_{116}NaO_5Si$: 967.84]. The

procedure used in Experiment **1** was repeated to hydrogenate the above alkene (1.88 g, 1.99 mmol) using dipotassium azodicarboxylate (2.52 g, 13.2 mmol) and glacial acetic acid (5 mL) in THF (50 mL). The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a colourless oil of **(245)** (1.74 g, 93%), $[\alpha]_D^{21} = -5.7$ (*c* 0.76, $CHCl_3$) [MS (MALDI-TOF): *m/z* 969.61 $[(M+Na)^+]$, calculated for $C_{60}H_{118}NaO_5Si$: 969.86], δ_H: 4.58 (1H, br.m), 3.97–3.76 (3H, m), 3.66 (3H, s), 3.56–3.40 (2H, m), 2.53 (1H , ddd, *J* 3.7, 7.1, 10.8 Hz), 1.75–1.64 (3H, m), 1.61–1.06 (80H, br.m), 0.96 (3H, d, *J* 6.3 Hz), 0.90–0.84 (4H, m, including a triplet at 0.87 ppm with *J* 6.9 Hz), 0.86 (9H, s), 0.57–0.40 (1H, m), 0.27–0.11 (3H, m), 0.05 (3H, s), 0.02 (3H, s); δ_C: 175.4, 99.1, 73.2, 66.2, 65.9, 62.3, 51.6, 51.4, 41.2, 37.4, 37.3, 37.1, 35.3, 35.2, 34.4, 32.8, 31.9, 30.8, 29.8, 29.7, 29.66, 29.60, 29.5, 29.4, 29.3, 27.9, 27.8, 27.6, 25.9, 25.7, 25.5, 23.7, 22.7, 22.6, 20.4, 19.8, 14.0, 10.0, –4.5, –4.9; ν_{max}/cm^{-1}: 2924, 2853, 1740, 1460.

Experiment 30: Methyl (*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-17-((1*S*,2*R*)-2-((*S*)-4-oxobutan-2-yl)cyclopropyl)heptadecyl)tetracosanoate (246)



(i) The procedure used in Experiment **9** was repeated to deprotect methyl (2*R*)-2-((1*R*)-1-((*tert*-butyldimethylsilyl)oxy)-17-((1*S*,2*R*)-2-((2*S*)-4-((tetrahydro-2*H*-pyran-2-yl) oxy) butan-2-yl)cyclopropyl)nonadecyl)tetracosanoate **(245)** (1.72 g, 1.81 mmol) using solution of pyridinium-*p*-toluene sulfonate (0.22 g, 0.90 mmol) in THF (30 mL), MeOH (7 mL) and stirred at 45 °C for 6 hrs. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (12:1) to give methyl (*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-17-((1*S*,2*R*)-2-((*S*)-4-hydroxybutan-2-yl)cyclo-propyl) heptadecyl)tetracosanoate (1.47 g, 94%), $[\alpha]_D^{21} = +11.2$ (*c* 1.03, $CHCl_3$), [MS (MALDI-TOF): *m/z* 885.99 $[(M+Na)^+]$, calculated for $C_{55}H_{110}NaO_4Si$: 885.80]; δ_H: 3.89 (1H, m), 3.80–3.68 (2H, m), 3.66 (3H, s), 2.53 (1H, ddd, *J* 3.8, 7.2, 10.9 Hz), 1.72 (1H, m), 1.54–1.26 (76H, br.m), 0.96 (3H, d, *J* 6.6 Hz), 0.91–0.86 (4H, m, including a triplet at 0.88 ppm with *J* 6.9 Hz), 0.86 (9H, s), 0.55–0.44 (1H, m), 0.29–0.13 (3H, m), 0.04 (3H, s), 0.02 (3H, s); δ_C: 175.2, 73.2, 61.7, 51.6, 51.3, 40.4, 35.1, 34.4, 33.6, 31.9, 31.6, 29.8, 29.7, 29.7, 29.65, 29.6, 29.59, 29.5, 29.4, 29.1, 27.8, 27.7, 27.65, 27.5, 25.9, 25.8, 25.3,

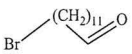
23.7, 22.7, 22.66, 21.0, 20.5, 19.8, 18.8, 14.1, 10.6, –4.4, –4.5; ν_{max}/cm^{-1}: 3583, 2924, 2853, 1741, 1464.

(ii) The procedure described in Experiment **5** was repeated to oxidize methyl (*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-17-((1*S*,2*R*)-2-((*S*)-4-hydroxybutan-2-yl)cyclopropyl)-heptadecyl)tetracosanoate (1.45 g, 1.68 mmol) using PCC (1.08 g, 5.04 mmol) in CH_2Cl_2 (60 mL). The crude product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give a colourless oil of methyl (*R*)-2-((*R*)-1-((*tert*-butyl-dimethylsilyl)oxy)-17-((1*S*,2*R*)-2-((*S*)-4-oxobutan-2-yl)cyclopropyl)hepta decyl) tetracosanoate **(246)** (1.37 g, 95%), $[\alpha]_D^{21} = +14.23$ (c 1.23, $CHCl_3$) [MS (MALDI-TOF): *m/z* 884.19 $[(M+Na)^+]$, calculated for $C_{55}H_{108}NaO_4Si$: 883.79]; δ_H: 9.78 (1H, t, *J* 2.5 Hz), 3.90 (1H, m), 3.66 (3H, s), 2.50–2.48 (2H, m), 2.39 (1H, ddd, *J* 2.5, 7.6, 15.7 Hz), 1.54–1.26 (75H, br.m), 1.03 (3H, d, *J* 6.8 Hz), 0.90–0.83 (4H, m, including a triplet at 0.87 ppm with *J* 6.9 Hz), 0.86 (9H, s), 0.50–0.48 (1H, m), 0.38–0.19 (3H, m), 0.05 (3H, s), 0.02 (3H, s); δ_C: 203, 175.2, 73.2, 51.7, 51.5, 34.1, 33.9, 33.8, 31.9, 29.8, 29.75, 29.7, 29.6, 29.5, 29.4, 27.8, 27.7, 27.7, 27.5, 25.9, 25.8, 25.3, 23.7, 22.7, 22.6, 21.0, 20.5, 19.8, 18.8, 14.3, 11.4, –4.5, –5.1; ν_{max}/cm^{-1}: 2924, 2853, 1732, 1464.

Experiment 31: 12-Bromododecan-1-ol (247)

Hydrobromic acid (20 mL, 48% aq.solution) was added to a stirred solution of Dodecane-1,12-diol (20.0 g, 99.0 mmol) in toluene (200 mL). The mixture was refluxed at 120 °C for 18hrs. The solvent was evaporated, and sat. aq. $NaHCO_3$ solution (200 mL) was added. The product was extracted with CH_2Cl_2 (2 × 100 mL) and the combined organic layers were dried and evaporated to give a crude product, which was purified by column chromatography eluting with petrol/ethyl acetate (5:1) to give 12-bromo dodecan-1-ol **(247)** (19.45 g, 74%); δ_H: 3.62 (2H, t, *J* 6.6 Hz), 3.39 (2H, t, *J* 6.9 Hz), 1.88 (2H, pent, *J* 6.9 Hz), 1.57 (2H, pent, *J* 6.6 Hz), 1.30 (16H, m); δ_C: 63.0, 60.4, 34.0, 32.8, 29.6, 29.4, 29.3, 28.7, 28.2, 25.7, 21.1; ν_{max}/cm^{-1}; 3296, 2918, 2850, 1462.

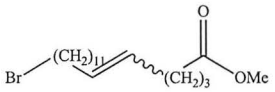
Experiment 32: 12-Bromododecanal (248)



The procedure described in Experiment **5** was repeated to oxidize 12-bromododecan-1-ol **(247)** (7.01 g, 26.40 mmol) using PCC (14.20 g, 66.00 mmol) in CH_2Cl_2 (500 mL).

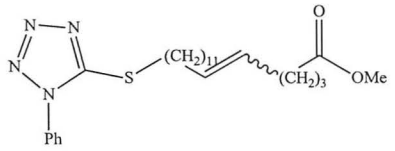
The crude product was purified by column chromatography eluting with petrol/ethyl acetate (7:1) to give a colourless oil of **(248)** (5.45 g, 79%); δ_H: 9.76 (1H, t, *J* 1.8 Hz), 3.40 (2H, t, *J* 6.9 Hz), 2.40 (2H, dt, *J* 1.8, 7.3 Hz), 1.89 (2H, pent, *J* 7.0 Hz), 1.62 (2H, m), 1.41 (2H, m), 1.27 (12H, m) ; δ_C: 203, 43.9, 34.1, 32.8, 29.5, 29.4, 29.3, 29.2, 28.7, 28.15, 22.0; ν_{max}/cm^{-1} 2919, 2852, 1705, 1412.

Experiment 33: Methyl (*E, Z*)-17-bromoheptadec-5-enoate (250)



The procedure used in Experiment **1** was repeated in order to couple 12-bromododecanal **(248)** (5.40 g, 20.50 mmol) with methyl 5-((1-phenyl-1H-tetrazol-5-yl)sulfonyl)penta noate **(249)** (7.98 g, 24.6 mmol) using lithium *bis*(trimethylsilyl)- amide (34.8 mL, 36.8 mmol, 1.06 M) in dry THF (100 mL) under nitrogen at –15 °C. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (25:1) to give a colourless oil of **(250)** (5.10 g, 69 %) [HRMS (EI^+): *m/z* 360.1662 [$(M)^+$], calculated for $C_{18}H_{33}O_2Br$: 360.1664]; δ_H: 5.39 (2H, m), 3.66 (3H, s), 3.41 (2H, t, *J* 6.9 Hz), 2.30 (2H, t, *J* 7.6 Hz), 2.05 (4H, m), 1.85 (2H, pent., *J* 7.0 Hz), 1.68 (2H, pent., *J* 7.6 Hz), 1.4 (2H, m), 1.39–1.34 (14H, m); δ_C: 177.7, 131.7, 131.2, 128.8, 128.3, 51.5, 34, 33.5, 33.4, 32.8, 32.6, 31.9, 29.7, 29.5, 29.4, 29.3, 29.2, 28.8, 28.2, 27.2, 26.5, 24.9, 24.7; ν_{max}/cm^{-1} 2926, 2854, 1741, 1459, 1436.

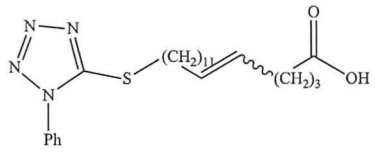
Experiment 34: Methyl (*E,Z*)-17-((1-phenyl-1H-tetrazol-5-yl)thio)heptadec-5-enoate (251)



The procedure used in Experiment **22** was repeated using 1-phenyl-1*H*-tetrazole-5-thiol (2.46 g, 13.8 mmol), (*E,Z*)-methyl17-bromoheptadec-5-enoate **(250)** (5.01 g, 13.80 mmol) and anhydrous potassium carbonate (3.82 g, 27.7 mmol) in acetone (70 mL). The crude product was purified by column chromatography eluting with petrol/ ethyl acetate (9:1) to give a thick colourless oil of **(251)** (6.23 g, 98 %) [HRMS (EI^+): *m/z* 458.2702 [$(M)^+$], calculated for $C_{25}H_{38}O_2N_4S$: 458.2715]; δ_H: 7.55 (5H, m), 5.38 (2H, m), 3.66 (3H, s), 3.39 (2H, t, *J* 7.3 Hz), 2.30 (2H, t, *J* 7.6 Hz), 2.05 (4H, m), 1.83 (2H, pent, *J* 7.3 Hz), 1.68 (2H, m), 1.43 (2H, m), 1.25 (14H, m); δ_C: 177.7, 154.3, 133.7, 131.7, 131.2,

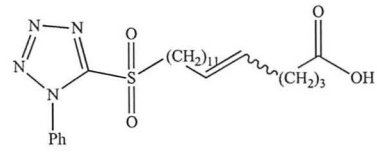
130, 129.8, 128.8, 128.3, 123.8, 51.5, 33.5, 33.4, 33.2, 32.6, 31.9, 29.7, 29.5, 29.4, 29.3, 29.2, 28.8, 28.4, 27.3, 26.5, 24.8, 24.7; ν_{max}/cm^{-1} 2924, 2853, 1719, 1498, 1460, 760.

Experiment 35: (*E,Z*)-17-((1-Phenyl-1H-tetrazol-5-yl)thio)heptadec-5-enoic acid (252)



The procedure used in Experiment **11** was repeated using aq.solution of sodium hydroxide (2.62 g, 65.5 mmol, 35 mL) with methyl (*E,Z*)-17-((1-phenyl-1H-tetrazol-5-yl)thio)heptadec-5-enoate **(251)** (3.75 g, 8.18 mmol) in THF (50 mL), and MeOH (6 mL). The crude product was purified by column chromatography eluting with petrol/ ethyl acetate (1:2) to give a thick colourless oil of **(252)** (3.55 g, 97%) [HRMS (EI^+): *m/z* 444.2553 [$(M)^+$], calculated for $C_{24}H_{36}O_2N_4S$: 444.2559]; δ_H: 7.55 (5H, m), 5.38 (2H, m), 3.39 (2H, t, *J* 7.3 Hz), 2.34 (2H, t, *J* 7.5 Hz), 2.05 (4H, m), 1.81 (2H, pent, *J* 7.2 Hz), 1.69 (2H, pent, *J* 7.4 Hz), 1.43 (2H, m), 1.25 (14H, m) δ_C: 153.5, 133.7, 131.9, 131.3, 130.1, 129.8, 128.7, 128.2, 123.9, 33.4, 33.3, 33.2, 32.5, 31.8, 29.7, 29.6, 29.4, 29.3, 29.2, 29.1, 28.7, 28.4, 27.2, 26.4, 24.6, 24.5, 20.5; ν_{max}/cm^{-1} 3417, 2924, 2852, 1708, 1597, 1499, 1460, 760.

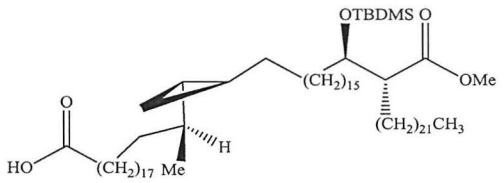
Experiment 36: (*E,Z*)-17-((1-Phenyl-1H-tetrazol-5-yl)sulfonyl)heptadec-5-enoic acid (253)



The procedure used in Experiment **12** was repeated using (*E,Z*)-17-((1-phenyl-1H-tetrazol-5-yl)thio)heptadec-5-enoic acid **(252)** (3.50 g, 7.88 mmol), ammonium molybdate (VI) tetrahydrate (4.87 g, 3.94 mmol) in 35 % H_2O_2 (10 mL) in THF (25 mL) and IMS (25 mL), and further solution of ammonium molybdate (VI) tetrahydrate (2.44 g, 1.97 mmol) in 35% H_2O_2 (5 mL) at 10 °C. The crude product was purified by column chromatography eluting with petrol/ ethyl acetate (3:2) to give a semi solid of **(253)** (3.18 g, 85%) [HRMS (EI^+): *m/z* 458.2356 [$(M–H_2O)^+$], calculated for $C_{24}H_{34}O_3N_4S$: 458.2352]; δ_H: 7.69 (2H, m), 7.62 (3H, m), 5.38 (2H, m), 3.73 (2H, t, *J* 8 Hz), 2.35 (2H, t, *J* 7.5 Hz), 1.96 (4H, m), 1.70 (2H, pent, *J* 7.5 Hz), 1.49 (2H, pent, *J* 7.6 Hz), 1.26

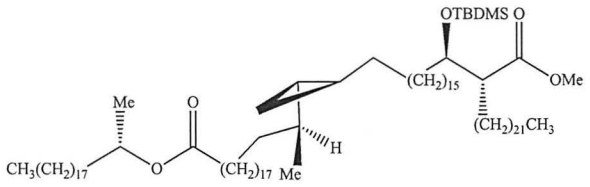
(16H, m); δ_C: 179.8, 153.5, 131.9, 131.5, 129.7, 128.7, 128.2, 125.1, 56, 33.4, 33.3, 33.2, 32.5, 31.8, 29.7, 29.6, 29.4, 29.3, 29.2, 29.1, 28.7, 28.4, 27.2, 26.4, 24.6, 24.5, 22.0; ν_{max}/cm^{-1} 2921, 2847, 1693, 1593, 1495, 1359, 1151, 759, 632.

Experiment 37: (*S*)-18-((1*R*,2*S*)-2-((19*R*,20*R*)-17-((*tert*-butyldimethylsilyl)oxy)-21-(methoxycarbonyl)dotetracontyl)cyclopropyl)nonadecanoic acid (254)



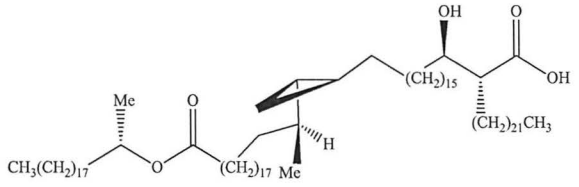
The procedure used in Experiment **1** was repeated in order to couple methyl (*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-17-((1*S*,2*R*)-2-((*S*)-4-oxobutan-2-yl)cyclopropyl)heptadecyl) tetracosanoate **(246)** (1.35 g, 1.56 mmol) with (*E,Z*)-17-((1-phenyl-1H-tetrazol-5-yl)sulfonyl)heptadec-5-enoic acid **(253)** (0.97 g, 2.04 mmol) using lithium *bis* (trimethylsilyl)amide (4.81 mL, 5.10 mmol, 1.06 M) in dry THF (40 mL) under nitrogen at –15 °C. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (12:1) to give a mixture of *E/Z* alkenes (1.32 g, 76 %) [MS (MALDI-TOF): *m/z* 1134.17 $[(M+Na)^+]$, calculated for $C_{72}H_{138}NaO_5Si$: 1134.02]. The procedure used in Experiment **1** was repeated to hydrogenate the above alkenes (1.30 g, 1.17 mmol) using dipotassium azodicarboxylate (2.52 g, 13.2 mmol), glacial acetic acid (5 mL) and methanol (5 mL) in THF (30 mL). The crude product was purified by was purified by column chromatography eluting with petrol/ethyl acetate (5:1) to give a white semi-solid of **(254)** (1.19 g, 90%), $[\alpha]_D$ = +2.4 (c 0.307 ($CHCl_3$) [MS (MALDI-TOF): *m/z* 1138.35 $[(M+Na)^+]$, calculated for $C_{72}H_{142}NaO_5Si$: 1138.05]; δ_H: 3.90 (1H, dt, *J* 7.2, 4.8 Hz), 3.66 (3H, s), 2.53 (1H, ddd, *J* 3.8, 7.2, 10.9 Hz), 2.35 (2H, t, *J* 7.5 Hz), 1.64 (2H, m), 1.57–1.03 (106H, br.m), 0.90 (3H, d, *J* 6.7 Hz), 0.91–0.88 (3H, br.t, *J* 6.9 Hz), 0.87 (9H, s), 0.73–0.62 (1H, m), 0.51–0.40 (1H, m), 0.26–0.07 (3H, m), 0.04 (3H, s), 0.02 (3H, s); δ_C: 179.5, 175.2, 73.2, 60.4, 51.6, 51.3, 38.1, 37.4, 34.2, 33.9, 33.7, 32.7, 31.9, 29.70, 29.60, 29.4, 29.3, 27.6, 27.5, 27.3, 25.8, 25.7, 23.7, 22.7, 21.0, 19.3, 18.6, 14.1, 10.4, –4.3, –4.9; ν_{max}/cm^{-1}: 3057, 2923, 2853, 1741, 1711, 1464, 1254.

Experiment 38: Methyl (*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-17-((1*S*,2*R*)-2-((*S*)-19-((*S*)-icosan-2-yloxy)-21-oxononadecan-2-yl)cyclopropyl)nonadecyl)tetracosanoate (255)



The procedure used in Experiment **15** was repeated in order to couple (*S*)-18-((1*R*,2*S*)-2-((19*R*,20*R*)-17-((*tert*-butyldimethylsilyl)oxy)-21-(methoxycarbonyl)dotetracontyl)-cyclopropyl)nonadecanoic acid **(254)** (1.16 g, 1.04 mmol), (*S*)-Eicosan-2-ol **(169)** (0.32 g, 1.09 mmol) using DMAP (0.19 g, 1.56 mmol) and DCC (0.42 g, 2.08 mmol) in dry CH_2Cl_2 (8 mL) under nitrogen at r.t. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a thick colourless oil of **(255)** (1.13 g, 78%), $[\alpha]_D^{21}$ = +6.26 (*c* 0.87, $CHCl_3$), [MS (MALDI-TOF): *m/z* 1418.79 [$(M+Na)^+$], calculated for $C_{92}H_{182}NaO_5Si$: 1418.36]; δ_H: 4.91 (1H, sext, *J* 6.2 Hz), 3.91 (1H, dt, *J* 7.1, 4.8 Hz), 3.66 (3H, s), 2.53 (1H, ddd, *J* 3.8, 7.1, 10.9 Hz), 2.26 (2H, t, *J* 7.5 Hz), 1.54–1.26 (142H, br.m), 1.20 (3H, d, *J* 6.2 Hz), 0.90 (3H, d, *J* 6.7 Hz), 0.90–0.84 (15H, m, including a singlet at 0.88 ppm), 0.71–0.63 (1H, m), 0.49–0.41 (1H, m), 0.22–0.08 (3H, m), 0.04 (3H, s), 0.02 (3H, s); δ_C: 175.2, 173.6, 73.2, 70.7, 51.6, 51.2, , 38.2, 37.4, 36.0, 34.8, 34.5, 33.7, 31.8, 30.1, 29.8, 29.8, 29.7, 29.7, 29.7, 29.60, 29.57, 29.52, 29.5, 29.4, 29.3, 29.2, 27.8, 27.5, 27.2, 26.2, 25.8, 25.4, 25.1, 23.8, 23.7, 22.7, 20.0, 19.7, 18.6, 14.1, 10.5, –4.4, –4.9; ν_{max}/cm^{-1}: 2924, 2853, 1738, 1464, 1253, 1164, 836.

Experiment 39: (*R*)-2-((*R*)-1-Hydroxy-17-((1*S*,2*R*)-2-((*S*)-19-((*S*)-icosan-2-yloxy)-21-oxononadecan-2-yl)cyclopropyl)nonadecyl)tetracosanoic acid (166)

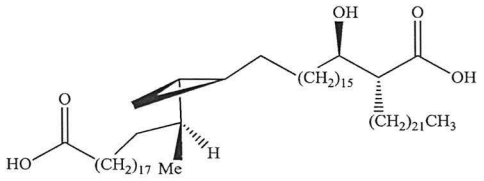


(i) The procedure used in Experiment **16** was repeated using methyl (*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-17-((1*S*,2*R*)-2-((*S*)-19-((*S*)-icosan-2-yloxy)-21-oxononadecan-2-yl)cyclopropyl)nonadecyl)tetracosanoate **(255)** (0.97 g, 0.69 mmol), pyridine (0.5 mL), and hydrogen fluoride-pyridine complex as ~70% (3.5 mL) in dry THF (25 mL). The crude product was purified by chromatography eluting with petrol/ ethyl acetate (10:1) to give a white semi-solid of methyl (*R*)-2-((*R*)-1-hydroxy-17-((1*S*,2*R*)-2-((*S*)-19-((*S*)-icosan-2-yloxy)-21-oxononadecan-2-yl)cyclopropyl)nonadecyl)tetracosanoate (0.62 g, 70%), $[\alpha]_D^{21}$ = +9.21 (*c* 0.21, $CHCl_3$) [MS (MALDI-TOF): *m/z* 1304.70

[(M+Na)$^+$], calculated for $C_{86}H_{168}NaO_5$: 1304.27]; δ_H: 4.91 (1H, sext, *J* 6.2 Hz), 3.71 (3H, s), 3.66 (1H, m), 2.53 (1H, m), 2.26 (2H, t, *J* 7.5 Hz), 1.55–1.25 (143H, br.m), 1.20 (3H, d, *J* 6.2 Hz), 0.90 (3H, d, *J* 6.8 Hz), 0.89 (6H, br.t, *J* 6.9 Hz), 0.71–0.63 (1H, m), 0.49–0.41 (1H, m), 0.22–0.08 (3H, m); δ_C: 176.3, 173.6, 72.3, 70.7, 51.6, 51.2, 38.1, 37.4, 36.0, 34.8, 34.5, 33.7, 31.9, 30.0, 29.6, 29.5, 27.8, 27.5, 27.2, 26.1, 25.8, 25.4, 25.1, 23.7, 22.70, 20.1, 19.7, 18.6, 14.1, 10.6; ν_{max}/cm^{-1}: 3437, 2921, 2851, 1732, 1722, 1467, 1376, 1250, 1108, 721.

(ii) The procedure used in Experiment **17** was repeated using methyl (*R*)-2-((*R*)-1-hydroxy-17-((1*S*,2*R*)-2-((*S*)-19-((*S*)-icosan-2-yloxy)-21-oxononadecan-2-yl)cycloprop yl)nonadecyl)tetracosanoate (0.6 g, 1.11 mmol) and aq.solution of tetrabutyl ammonium hydroxide (64 mL, 5%). The crude product was purified by column chromatography eluting with petrol / ethyl acetate (5:1) to give a white solid of **(166)** (0.39g, 66%), $[\alpha]_D^{21}$ = +7.1 (*c* 0.55, $CHCl_3$), m.p. 55–56 °C [HRMS (MALDI-TOF): *m/z* 1266.2643 [(M–H)$^+$], calculated for $C_{85}H_{165}O_5$: 1266.2642]; δ_H: 4.91 (1H, sext, *J* 6.2 Hz), 3.72 (1H, m), 2.46 (1H, br.dt, *J* 9.0, 5.3), 2.27 (2H, t, *J* 7.5 Hz), 1.81–0.95 (144 H, br.m), 1.20 (3H, d, *J* 6.2 Hz), 0.90 (3H, d, *J* 6.8 Hz), 0.89 (6H, br.t, *J* 6.9 Hz), 0.72–0.63 (1H, m), 0.50–0.41 (1H, m), 0.22–0.02 (3H, m); δ_C: 179.6, 173.5, 72.1, 70.8, 50.8, 38.1, 37.4, 36.0, 35.5, 34.7, 34.5, 31.8, 30.1, 29.8, 29.5, 29.5, 29.4, 29.4, 29.3, 29.2, 27.3, 27.3, 26.1, 25.7, 25.4, 25.1, 22.7, 20.0, 19.7, 18.6, 14.1, 10.5; ν_{max}/cm^{-1}: 3417, 2919, 2850, 1722, 1712, 1467, 1377, 717.

Experiment 40: (*R*)-2-((*R*)-17-((1*S*,2*R*)-2-((*S*)-20-Carboxyoctadecan-2-yl)cyclopropyl)-1-hydroxynonadecyl)tetracosanoic acid (167)

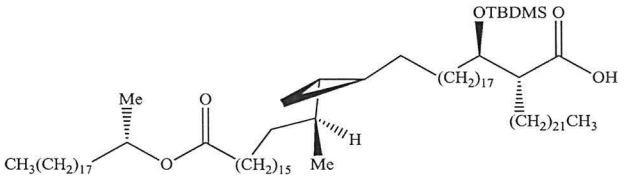


(i) The procedure used in Experiment **16** was repeated using *(S)*-18-((1*R*,2*S*)-2-((19*R*,20*R*)-17-((*tert*-butyldimethylsilyl)oxy)-21-(methoxycarbonyl)dotetracontyl)cyclopropyl)nonadecanoic acid **(254)** (0.062 g, 0.055 mmol), pyridine (0.1 mL), and hydrogen fluoride-pyridine complex as ~70% (0.4 mL) in dry THF (5 mL). The crude product was purified by chromatography eluting with petrol/ ethyl acetate (4:1) to give a syrup of (*S*)-20-((1*R*,2*S*)-2-((17*R*,18*R*)-17-hydroxy-18-(methoxycarbonyl) tetracontyl)cyclopropyl)nonadecanoicacid (0.038 g, 68 %), $[\alpha]_D^{21}$ = +8.9 (c 1.2 $CHCl_3$) . [MS

(MALDI-TOF): *m/z* 1024.36 [(M+Na)$^+$], calculated for $C_{66}H_{128}NaO_5$: 1023.96]; δ_H: 3.71 (3H, s), 3.67–3.65 (1H, m), 2.46 (1H, dt, *J* 9.3, 5.4 Hz), 2.34 (2H, t, *J* 7.5Hz), 1.74–1.62 (4H, m), 1.55–1.26 (106 H, m), 0.91–0.86 (6H, br.m), 0.69–0.62 (1H, m), 0.49–0.41 (1H, m), 0.22–0.08 (3H, m); δ_C: 178.6, 176.3, 72.3, 60.4, 51.6, 51.0, 38.2, 37.4, 35.7, 34.5, 33.9, 31.9, 30.1, 29.79, 29.75, 29.73, 29.66, 29.60, 29.58, 29.54, 29.50, 29.47, 29.43, 29.4, 27.3, 29.1, 27.4, 27.2, 25.7, 24.7, 22.7, 21.1, 19.7, 18.6, 14.2, 10.5; ν_{max}/cm^{-1}: 2921, 2850, 1732, 1711, 1467, 1377, 719

(ii) The procedure used in Experiment **19** was repeated to hydrolyse (*S*)-20-((1*R*,2*S*)-2-((17*R*,18*R*)-17-hydroxy-18-(methoxycarbonyl)tetracontyl)cyclopropyl)nonadecanoic acid (0.031 g, 0.031 mmol) using lithium hydroxide monohydrate (0.019 g, 0.465 mmol) in THF (4 mL), water (0.5 mL) and MeOH (0.4 mL). The crude product was purified by column chromatography eluting with petrol/ethyl acetate (1:3) to give a white solid of **(167)** (0.025 g, 86%), $[\alpha]_D^{21}$ = +5.9 (c 0.56 $CHCl_3$), m.p. 77–79 [MS (MALDI-TOF): *m/z* 1010.56 [(M+Na)$^+$], calculated for $C_{65}H_{126}NaO_5$: 1009.95]; δ_H: 3.74–3.70 (1H, m), 2.53 (1H, m), 2.35 (2H, t, *J* 7.5 Hz), 1.77–1.62 (4H, m), 1.59–1.26 (107 H, m), 0.91–0.86 (6H, br.m), 0.73–0.68 (1H, m), 0.50–0.44 (1H, m), 0.24–0.10 (3H, m); δ_C: 180.1, 179.1, 72.2, 51, 38.1, 37.5, 35.6, 34.5, 34.0, 31.9, 30.8, 29.7, 29.6, 29.5, 29.4, 29.1, 27.4, 27.3, 26.2, 25.7, 24.7, 22.7, 19.7, 18.6, 14.0, 10.5; ν_{max}/cm^{-1}: 2917, 2849, 1733, 1464, 1377, 719.

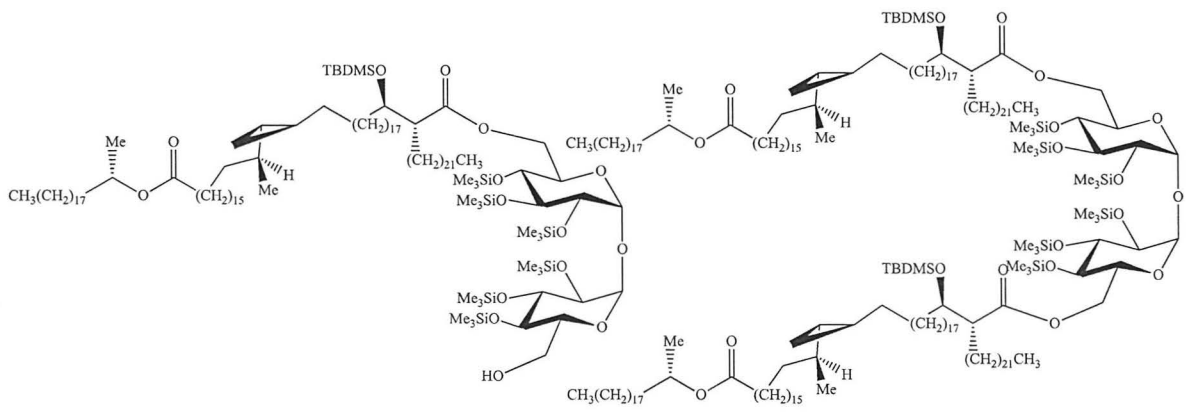
Experiment 41: (*R*)-2-((*R*)-1-((*tert*-Butyldimethylsilyl)oxy)-19-((1*S*,2*R*)-2-((*S*)-19-((*S*)-icosan-2-yloxy)-19-oxononadecan-2-yl)cyclopropyl)nonadecyl)tetracosanoic acid (257)



Imidazole (0.302 g, 4.507 mmol) was added to a stirred solution of (*R*)-2-((*R*)-1-hydroxy-19-((1*S*,2*R*)-2-((*S*)-19-((*S*)-icosan-2-yloxy)-19-oxononadecan-2-yl)cyclopropyl)nonadecyl) tetracosanoic acid **(164)** (0.560 g, 0.448 mmol) in dry DMF (3.5 mL) and dry toluene (5 mL) at r.t, followed by the addition of *tert*-butyl dimethyl silylchloride (0.675 g, 4.482 mmol) and 4-dimethylaminopyridine (0.055 g, 0.448 mmol). The reaction mixture was stirred at 70 °C for 18 hrs. When TLC showed no starting material was left, the solvent was removed under high vacuum and the residue was diluted with

petrol/ ethyl acetate (5:1, 30 mL) and water (20 mL). The organic layer was separated and the aqueous layer was re-extracted with petrol/ethyl acetate (5:1, 2 × 20 mL). The combined organic layers were dried and evaporated to give a residue. The residue was dissolved in THF (8 mL), and to this aq.solution of tetra-*n*-butyl ammonium hydroxide (4.5 mL, 4%) was added. The reaction mixture was stirred for 15 minutes at room temperature. When TLC showed no starting material was left, the product was extracted with petrol/ethyl acetate (2:1, 3 × 20 mL). The combined organic layers were dried and evaporated to give a crude product, which was purified by column chromatography eluting with petrol/ethyl acetate 20:1 to give a syrup of **(257)** (0.534g, 87%), $[\alpha]_D^{21}$ = +23.3 (*c* 0.49, $CHCl_3$) [MS (MALDI-TOF): *m/z* 1404.87 [$(M+Na)^+$], calculated for $C_{91}H_{180}NaO_5Si$: 1404.34]; δ_H: 4.92 (1H, sext, *J* 6.2 Hz), 3.83 (1H, m), 2.53 (1H, ddd, *J* 3.2, 5.6, 9.1 Hz), 2.27 (2H, t, *J* 7.4 Hz), 1.81–0.95 (140H, br.m), 1.20 (3H, d, *J* 6.2 Hz), 0.93 (9H, s), 0.91–0.87 (9H, m, including a triplet at 0.88 ppm with *J* 6.9 Hz), 0.71–0.63 (1H, m), 0.48–0.41 (1H, m), 0.15 (3H,s), 0.12 (3H, s), 0.22–0.06 (3H, m); δ_C: 179.7, 173.6, 73.7, 70.7, 50.1, 38.1, 37.4, 35.9, 35.5, 34.7, 34.4, 31.9, 30.09, 29.70, 29.51, 29.45, 29.42, 29.36, 29.30, 29.15, 27.32, 27.26, 26.1, 25.7, 25.4, 25.1, 22.7, 22.3, 20.0, 19.7, 18.6, 14.1, 14.0, 10.5, –4.2, –4.9; ν_{max}/cm^{-1}: 3432 (broad), 2918, 2850, 1721, 1712, 1470, 1372, 1254.

Experiment 42: 6,6'-*bis*-*O*-(*R*)-2-((*R*)-1-((*tert*-Butyldimethylsilyl)oxy)-19-((1*S*,2*R*)-2-((*S*)-19-((*S*)-icosan-2-yloxy)-19-oxononadecan-2-yl)cyclopropyl)nonadecyl) tetra cosanoic-2,3,4,2',3',4',-hexakis-*O*-(trimethylsiyl)α,α'-trehalose (258), 6-*O*-(*R*)-2-((*R*)-1-((*tert*-butyldimethyl silyl)oxy)-19-((1*S*,2*R*)-2-((*S*)-19-((*S*)-icosan-2-yloxy)-19-oxononadecan-2-yl) cyclopropyl) nonadecyl) tetra cosanoic-2,3,4,2',3',4',-hexakis-*O*-(trimethylsilyl)-α,α'-trehalose (259).



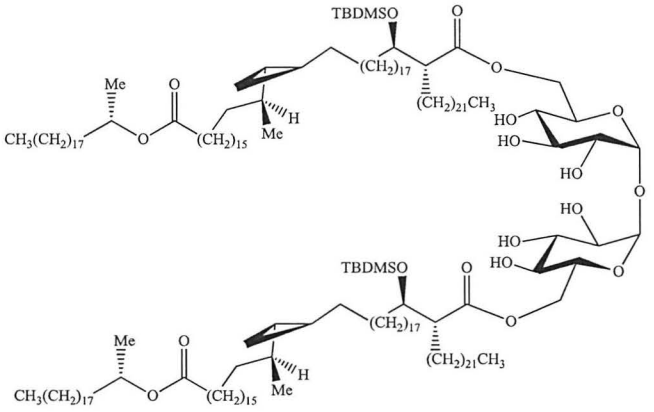
1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (0.242 g, 1.26

mmol) and 4-dimethylaminopyridine (DMAP) (0.154 g, 1.260 mmol) were added to a stirred solution of (*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-19-((1*S*,2*R*)-2-((*S*)-19-((*S*)-icosan-2-yloxy)-19-oxononadecan-2-yl)cyclopropyl)nonadecyl)tetracosanoic acid **(257)** (0.500 g, 0.360 mmol), 2,3,4,2',3',4'-hexaakis-O-(trimethylsilyl)-α,α'-trehalose **(148)** (0.141 g, 0.180 mmol) and powdered 4 A° molecular sieves in dry CH_2Cl_2 (4 mL) at r.t under nitrogen atmospher. The mixture was stirred for 6 days at r.t. When TLC showed no starting material was left, the solvent was evaporated to give a residue, which was purified by column chromatography on silica eluting with petroleum ether/ethyl acetate (25:1) to give the first fraction **(TDM) (258)** as a colourless oil (0.3238 g, 52%), $[\alpha]_D^{21}$ = +21.1 (*c* 1.17, $CHCl_3$) [MS (MALDI-TOF): *m/z* 3524.01 [$(M+Na)^+$], calculated for $C_{212}H_{426}NaO_{19}Si_8$: 3524.04]; δ_H: 4.92 (2H, sext, *J* 6.3 Hz), 4.85 (2H, d, *J* 3 Hz), 4.38 (2H, br, d, *J* 10.0 Hz), 4.04–3.98 (4H, m), 4.0–3.96 (2H, m) 3.93 (2H, m), 3.52 (2H, t, *J* 8.9 Hz), 3.38 (2H, dd, *J* 2.9, 9.3 Hz), 2.55 (2H, ddd, *J* 3.5, 4.75, 10.1 Hz), 2.26 (4H, t, *J* 7.5 Hz), 1.56–1.21 (284H, br.m), 1.20 (6H, d, *J* 6.2 Hz), 0.91–0.87 (18H, m, including a triplet at 0.88 ppm with *J* 6.9 Hz), 0.88 (18H, s), 0.72–0.65 (2H, m), 0.48–0.41 (2H, m), 0.22–0.06 (6H, m), 0.16 (18H, s), 0.15 (18H, s), 0.14 (18H, s), 0.062 (12H, s); δ_C: 173.8, 173.6, 94.8, 73.5, 73.4, 72 .8, 71.8, 70.7, 62.4, 60.4, 51.9, 41.3, 38.1, 37.4, 36.0, 34.5, 33.4, 31.9, 29.8, 29.50, 29.44, 29.42, 29.4, 29.3, 29.2, 27.3, 27.2, 26.2, 25.8, 25.4, 25.2, 22.7, 22.3, 20.0, 19.7, 18.6, 14.2, 14.1, 10.5, 1.1, 1.0, 0.1, –4.5, –4.7; ν_{max}/cm^{-1}: 2924, 2854, 1733, 1465, 1375, 1254, 1215, 836, 760.

The second fraction, **(TMM) (259)** as a colourless oil (0.114 g, 32%), $[\alpha]_D^{21}$ = +27.7 (*c* 0.77, $CHCl_3$) [MS (MALDI-TOF): *m/z* 2160.58 [$(M+Na)^+$], calculated for $C_{121}H_{248}NaO_{15}Si_7$: 2160.69]; δ_H: 4.92 (1H, sext, *J* 6.2 Hz), 4.91 (1H, d, *J* 3 Hz), 4.85 (1H, d, *J* 2.9 Hz), 4.36 (1H, dd, *J* 2, 11.6 Hz), 4.08 (1H, dd, *J* 4.0, 11.7 Hz), 4.04–3.98 (4H, m), 3.85 (1H, dt, *J* 3.2, 9.5 Hz), 3.69 (2H, m), 3.5 (2H, dt, *J* 4.6, 9.1 Hz), 3.41 (1H, dd, *J* 3.0, 9.1 Hz), 3.38 (1H, dd, *J* 2.90, 9.0 Hz), 2.55 (1H, ddd, *J* 3.4, 5.44, 10.3 Hz), 2.27 (2H, t, *J* 7.4 Hz), 1.73 (1H, br. m), 1.68–1.21 (143H, br.m), 1.20 (3H, d, *J* 6.2 Hz), 0.91–0.87 (9H, m, including a triplet at 0.88 ppm with *J* 6.9 Hz), 0.88 (9H, s), 0.70–0.40 (1H, m), 0.48–0.41 (1H, m), 0.22–0.06 (3H, m), 0.17 (18H, s), 0.15 (18H, s), 0.14 (18H, s), 0.060 (3H, s), 0.052 (3H, s); δ_C: 174.5, 173.6, 94.5, 94.4, 73.4, 73.3, 72.9, 72.8, 72.7, 72.0, 71.4, 70.7, 62.4, 61.7, 51.8, 38.1, 37.4, 36.0, 34.8, 34.4, 34.12, 33.4, 31.9, 30.1, 29.72, 29.70, 29.68, 29.65, 29.63, 29.55, 29.50, 29.45, 29.36, 28.10, 27.27, 26.29, 26.13, 25.81, 25.40, 25.12, 22.62, 22.60, 22.3, 20.0, 19.7, 18.7, 14.10, 14.0, 10.5, 1.05,

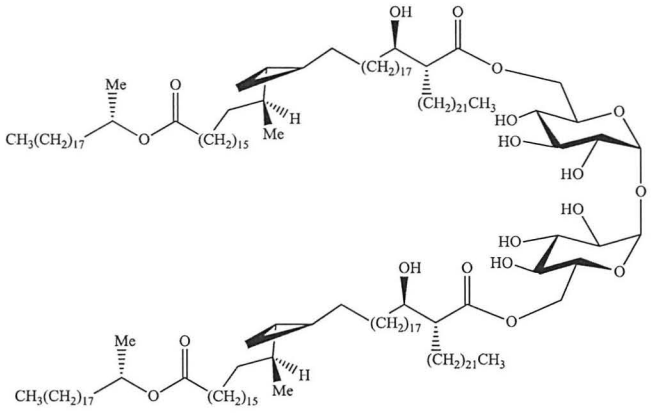
1.004, 1.0, 0.8, 0.2, 0.03, –4.5, –4.7; ν_{max}/cm^{-1}: 3448, 2923, 2853, 1733, 1465, 1375, 1253, 1215, 836, 760.

Experiment 43: 6,6-*bis*-*O*-(*R*)-2-((*R*)-1-((*tert*-Butyldimethylsilyl)oxy)-19-((1*S*,2*R*)-2-((*S*)-19-((*S*)-icosan-2-yloxy)-19-oxononadecan-2-yl)cyclopropyl)nonadecyl)tetracosanoic α,α'-trehalose (260)



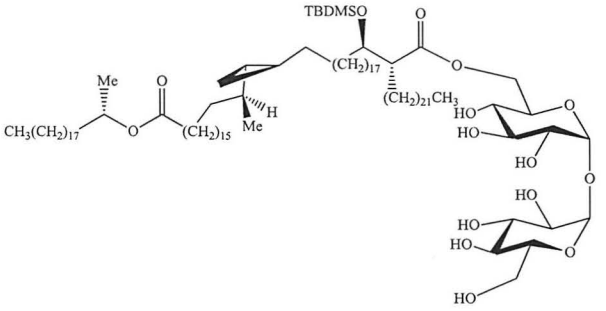
Tetra-*n*-butylammonium fluoride (0.287 mL, 0.287 mmol, 1M) was added to a stirred solution of 6,6'-*bis*-*O*-(*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-19-((1*S*,2*R*)-2-((*S*)-19-((*S*)-icosan-2-yl-oxy)-19-oxononadecan-2-yl)cyclopropyl)nonadecyl)tetracosanoic-2,3,4,2',3',4',-hexakis-*O*-(trimethylsiyl)-α,α'-trehalose **(258)** (0.305 g, 0.087 mmol) in dry THF (25 mL) at 5 °C under nitrogen. The mixture was allowed to reach r.t and stirred for 30 minutes. When TLC showed no starting material, the solvent was evaporated to give a residue, which was purified by column chromatography eluting with $CHCl_3$/MeOH (10:1) to give a syrup of **(260)** (0.188 g, 71 %), $[\alpha]_D^{21}$ = +25.5 (*c* 0.13, $CHCl_3$) [MS (MALDI-TOF): *m/z* 3091.83 $[(M+Na)^+]$, calculated for $C_{194}H_{378}NaO_{19}Si_2$: 3091.80]; δ_H ($CDCl_3$ + few drops of CD_3OD): 5.05 (2H, d, *J* 3.2 Hz), 4.92 (2H, sext, *J* 6.3 Hz), 4.37 (2H, br.dd, *J* 3.5, 11.3 Hz), 4.21 (2H, br. d, *J* 11.1 Hz), 3.93–3.85 (2H, m), 3.77 (2H, t, *J* 9 Hz), 3.51 (2H, dd, *J* 3.4, 9.4 Hz), 3.36 (2H, m), 3.30 (2H, t, *J* 9.4 Hz), 2.55 (2H, ddd, *J* 3.5, 4.7, 10.0 Hz), 2.22 (4H, t, *J* 7.4 Hz),1.61–1.24 (284 H, br.m), 1.18 (6H, d, *J* 6.3 Hz), 0.90–0.86 (18H, m, including a triplet at 0.88 ppm with *J* 6.9 Hz), 0.88 (18H, s), 0.68–0.58 (2H, m), 0.49–0.38 (2H, m), 0.20–0.06 (6H, m), 0.02 (6H, s), 0.007 (6H, s); δ_C: 175.2, 173.8, 93.5, 73.2, 72.8, 70.8, 70.2, 70.1, 67.8, 62.9, 51.6, 38.0, 37.3, 34.7, 34.4, 33.8, 31.95, 29.7, 29.61, 29.48, 29.42, 29.36, 29.30, 29.2, 26.3, 25.8, 25.4, 25.1, 22.7, 22.4, 20.0, 19.7, 18.6, 14.0, 10.4, –4.6, –5.0; ν_{max}/cm^{-1}: 3421, 2922, 2853, 1732, 1728, 1465, 1375, 1253, 836, 721.

Experiment 44: 6,6-*bis*-*O*-(*R*)-2-((*R*)-1-Hydroxy-19-((1*S*,2*R*)-2-((*S*)-19-((*S*)-icosan-2-yloxy)-19-oxononadecan-2-yl)cyclopropyl)nonadecyl)tetracosanoate-α,α'-trehalose (261)



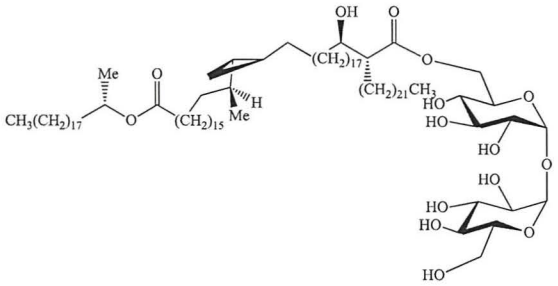
6,6-*bis*-*O*-(*R*)-2-((*R*)-1-((*tert*-Butyldimethylsilyl)oxy)-19-((1*S*,2*R*)-2-((*S*)-19-((*S*)-icosa-n-2-yloxy)-19-oxononadecan-2-yl)cyclopropyl)nonadecyl)tetracosanoic-α,α'-trehalose **(260)** (0.180 g, 0.058 mmol) was dissolved in dry THF (20 mL) in a small dry polyethylene vial equipped with a rubber septum, followed by addition of pyridine (0.1 mL) at r.t under nitrogen. The reaction mixture cooled to 10 °C, and then hydrogen fluoride-pyridine complex as ~70% (1.350 mL) was added dropwise. The mixture was stirred at 43 °C for 17 hrs. When TLC showed no starting material was left, the excess of the HF was neutralized with triethyl amine (2 mL), and the solvent was evaporated. The crude product was purified by column chromatography eluting with $CHCl_3$/MeOH (10:1) to give a syrup of **(261)** (0.095 g, 58 %); $[\alpha]_D^{21}$ = +31.3 (*c* 1.01, $CHCl_3$) [HRMS (MALDI-TOF): *m/z* 2863.6340 [$(M+Na)^+$], calculated for $C_{182}H_{350}NaO_{19}$: 2863.6314]; δ_H ($CDCl_3$ + few drops of CD_3OD): 4.98 (2H, d, *J* 3.4 Hz), 4.87 (2H, sext, *J* 6.4 Hz), 4.68 (2H, br. d, *J* 10.8 Hz), 4.24 (2H, t, *J* 9.3 Hz), 3.93 (2H, dd, *J* 3.8, 10.9 Hz), 3.73 (2H, t, *J* 9.2 Hz), 3.66–3.62 (2H, m), 3.49 (2H, dd, *J* 3.7, 9.8 Hz), 3.19 (2H, t, *J* 9.5 Hz), 2.40 (2H, br.m), 2.23 (4H, t, *J* 7.4 Hz), 1.58–1.53 (8H, m), 1.52–1.22 (280 H, br.m), 1.16 (6H, d, *J* 6.2 Hz), 0.86 (6H, d, *J* 6.8 Hz), 0.86–0.82 (12H, br.t, *J* 6.9 Hz), 0.66–0.59 (2H, m), 0.43–0.37 (2H, m), 0.18–0.04 (6H, m); δ_C: 175.7, 173.6, 95.2, 72.5, 71.1, 70.9, 70.7, 69.8, 64.7, 52.1, 38.0, 37.3, 34.7, 34.4, 33.8, 31.95, 29.7, 29.61, 29.48, 29.42, 29.4, 29.30, 29.2, 29.0, 27.2, 26.0, 25.3, 25.0, 22.7, 22.6, 19.9, 19.5, 18.5, 14.0, 10.4; ν_{max}/cm^{-1}: 3357, 2919, 2850, 1731, 1467, 1375, 759, 721.

Experiment 45: 6-*O*-(*R*)-2-((*R*)-1-((*tert*-Butyldimethylsilyl)oxy)-19-((1*S*,2*R*)-2-((*S*)-19-((*S*)-icosan-2-yloxy)-19-oxononadecan-2-yl)cyclopropyl)nonadecyl)tetracosanoic -α,α'-trehalose (262)



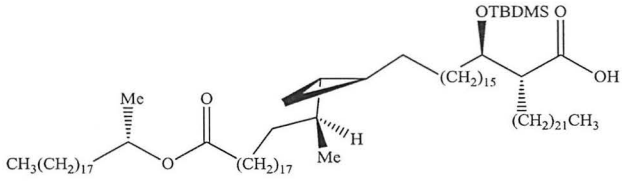
The same procedure used in Experiment **43** was repeated to deprotect 6-*O*-(*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-19-((1*S*,2*R*)-2-((*S*)-19-((*S*)-icosan-2-yloxy)-19-oxo nona decan-2-yl)cyclopropyl)nonadecyl)tetracosanoic-2,3,4,2',3',4',-hexakis-*O*-(trimethyl silyl)-α,α'-trehalose **(259)** (0.103 g, 0.048 mmol) using tetra-*n*-butylammonium fluoride (0.144 mL, 0.144 mmol, 1M) in dry THF (13 mL) at 5 °C under nitrogen. The residue was purified by column chromatography on silica eluting with $CHCl_3$/MeOH (5:1) to give a semi-solid of **(262)** (0.073 g, 89%), $[\alpha]_D^{21} = +17.1$ (*c* 0.78, $CHCl_3$) [MS (MALDI-TOF): *m/z* 1728.39 [$(M+Na)^+$], calculated for $C_{103}H_{200}NaO_{15}Si$: 1728.45]; δ_H ($CDCl_3$ + few drops of CD_3OD): 5.05 (2H, d, *J* 3.4 Hz), 4.85 (1H, sext, *J* 6.2 Hz), 4.32–4.22 (2H, m), 3.92 (1H, br.d, *J* 9.6 Hz), 3.86–3.78 (4H, m), 3.67 (1H, dd, *J* 5.5, 11.5 Hz), 3.5 (2H, dd, *J* 3.4, 9.7 Hz), 3.37–3.25 (3H, m), 2.52 (1H, ddd, *J* 3.4, 5.44, 10.3 Hz), 2.22 (2H, t, *J* 7.5 Hz), 1.58–1.50 (4H, m), 1.49–1.21 (138H, br.m), 1.16 (3H, d, *J* 6.3 Hz), 0.90–0.86 (9H, m, including a triplet at 0.88 ppm with *J* 6.9 Hz), 0.89 (9H, s), 0.66–0.57 (1H, m), 0.44–0.36 (1H, m), 0.10–0.03 (3H, m), 0.015 (3H, s), 0.0068 (3H, s); δ_C: 175.1, 173.9, 93.5, 93.4, 73.2, 73.0, 72.6, 72.1, 71.5, 70.9, 70.7, 70.2, 69.9, 67.9, 62.4, 61.7, 51.6, 38.0, 37.3, 35.8, 34.7, 33.5, 31.8, 30.0, 29.72, 29.70, 29.60, 29.50, 29.40, 29.39, 29.25, 29.20, 29.0, 27.6, 26.90, 26.1, 25.6, 25.3, 25.0, 24.2, 22.6, 22.60, 19.8, 19.6, 18.5, 14.6, 10.4, –4.6, –5.0; ν_{max}/cm^{-1}: 3428, 2924, 2852, 1732, 1465, 1375, 1251, 835, 759.

Experiment 46: 6-*O*-(*R*)-2-{(2-((*R*)-1-Hydroxy-19-((1*S*,2*R*)-2-((*S*)-19-((*S*)-icosan-2-yloxy)-19-oxononadecan-2-yl)cyclopropyl)nonadecyl)tetracosanoate-α,α'-trehalose (263)



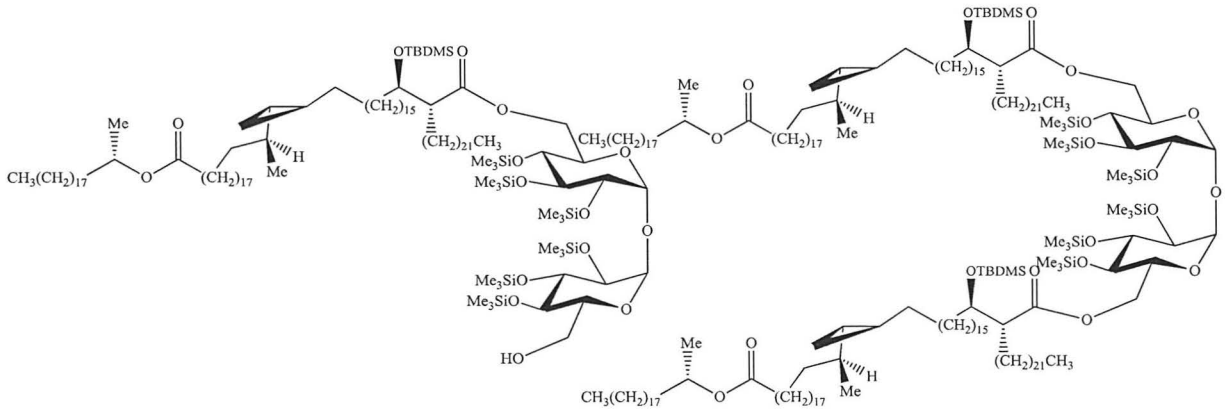
The procedure used in Experiment **44** was repeated to deprotect 6-*O*-(*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-19-((1*S*,2*R*)-2-((*S*)-19-((*S*)-icosan-2-yloxy)-19-oxono-nadecan -2-yl)cyclopropyl)nonadecyl) tetracosanoic -α,α'-trehalose **(262)** (0.065 g, 0.038 mmol) using pyridine (0.06 mL), and hydrogen fluoride-pyridine complex as ~70% (0.45 mL) in dry THF (7 mL). The product was purified by column chromatography eluting with $CHCl_3$/MeOH (5:1) to give a white solid of **(263)** (0.035 g, 58%), $[\alpha]_D^{21}$ = +25.5 (*c* 1.12, $CHCl_3$), m.p. 116–117 °C, [HRMS (MALDI-TOF): *m/z* 1614.3698 $[(M+Na)^+]$, calculated for $C_{97}H_{187}NaO_{15}$: 1614.3684]; δ_H ($CDCl_3$ + few drops of CD_3OD): 5.08 (1H, d, *J* 3.2 Hz), 5.02 (1H, d, *J* 3.3 Hz), 4.88 (1H, sext, *J* 6.3 Hz), 4.70 (1H, d, *J* 11.3 Hz), 4.24 (1H, t, *J* 9.6 Hz), 3.99–3.90 (2H, m), 3.88–3.78 (3H, m), 3.63–3.54 (3H, m), 3.5 (1H, dd, *J* 3.1, 9.8 Hz), 3.28 (1H, t, *J* 9.3 Hz), 3.21 (1H, t, *J* 9.4 Hz), 2.38 (1H, m), 2.24 (2H, t, *J* 7.4 Hz), 1.58–1.50 (4H, m), 1.49–1.21 (140 H, m), 1.17 (3H, d, *J* 6.2 Hz), 0.86 (3H, d, *J* 6.9 Hz), 0.86–0.83 (6H, br.t, *J* 6.9 Hz), 0.66–0.59 (1H, m), 0.44–0.36 (1H, m), 0.18–0.04 (3H, m); δ_C: 175.4, 173.9, 94.32, 94.3, 74.4, 73.3, 72.5, 71.4, 71.3, 71.2, 70.7, 64.0, 62.9, 52.4, 38.0, 37.2, 35.7, 34.5, 34.3, 33.4, 31.7, 30.1, 29.9, 29.50, 29.3, 29.2, 29.1, 28.10, 27.27, 25.9, 25.2, 25.1, 24.9, 22.6, 19.8, 19.5, 18.7, 14.0, 10.3; ν_{max}/cm^{-1}: 3357, 2919, 2851, 1730, 1467, 1375, 759, 721.

Experiment 47: (*R*)-2-((*R*)-1-((*tert*-Butyldimethylsilyl)oxy)-17-((1*S*,2*R*)-2-((*S*)-19-((*S*)-eicosan-2-yloxy)-21-oxononadecan-2-yl)cyclopropyl)nonadecyl)tetracosanoic acid (264)



The procedure used in Experiment **41** was repeated using (*R*)-2-((*R*)-1-hydroxy-17-((1*S*,2*R*)-2-((*S*)-19-((*S*)-icosan-2-yloxy)-21-oxononadecan-2-yl)cyclopropyl)nonadecyl)tetracosanoic acid **(166)** (0.380 g, 0.299 mmol), imidazole (0.203 g, 2.999 mmol), *tert*-butyldimethylsilylchloride (0.451 g, 2.999 mmol) and 4-dimethyl aminopyridine (0.036g, 0.299 mmol) in dry DMF (2.3 mL) and dry toluene (3.4 mL). The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a syrup of **(264)** (0.374 g, 89%), $[\alpha]_D^{21} = +23.3$ (*c* 0.42, $CHCl_3$) [MS (MALDI-TOF): *m/z* 1404.99 [$(M+Na)^+$], calculated for $C_{91}H_{180}NaO_5Si$: 1404.34]; δ_H: 4.91 (1H, sext, *J* 6.2 Hz), 3.84 (1H, m), 2.53 (1H, ddd, *J* 3.3, 5.6, 9.1 Hz), 2.27 (2H, t, *J* 7.4 Hz), 1.81–0.95 (140H, br.m), 1.20 (3H, d, *J* 6.2 Hz), 0.93 (9H, s), 0.91–0.87 (9H, m, including a triplet at 0.88 ppm with *J* 6.9 Hz), 0.70–0.60 (1H, m), 0.48–0.41(1H, m), 0.14 (3H, s), 0.13 (3H, s), 0.22–0.06 (3H, m); δ_C: 179.7, 173.6, 73.8, 70.7, 50.1, 38.0, 37.4, 35.9, 35.5, 34.8, 34.5, 31.9, 30.1, 29.70, 29.5, 29.4, 29.4, 29.36, 29.32, 29.11, 27.3, 27.29, 26.18, 25.8, 25.4, 25.1, 22.7, 22.4, 20.1, 19.7, 18.6, 14.1, 14.0, 10.5, –4.2, –4.9; ν_{max}/cm^{-1}: 3439, 2921, 2851, 1722, 1712, 1470.

Experiment 48: 6,6'-*bis*-*O*-(*R*)-2-((*R*)-1-((*tert*-Butyldimethylsilyl)oxy)-17-((1*S*,2*R*)-2-((*S*)-19-((*S*)-icosan-2-yloxy)-21-oxononadecan-2-yl)cyclopropyl)nonadecyl)tetracosanoic-2,3,4,2',3',4',-hexakis-*O*-(trimethylsilyl)-α,α'-trehalose (265), 6-*O*-(*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-17-((1*S*,2*R*)-2-((*S*)-19-((*S*)-icosan-2-yloxy)-21-oxononadecan-2-yl) cyclopropyl)nonadecyl)tetracosanoic-2,3,4,2',3',4'-hexakis-*O*-(trimethylsilyl)-α,α'-trehalose (266)

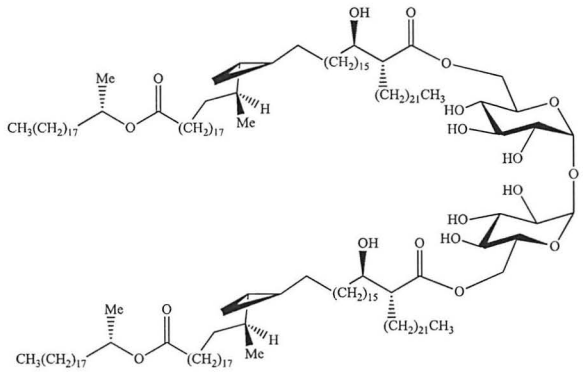


The procedure used in Experiment **42** was repeated using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (0.181 g, 0.940 mmol) and 4-dimethyl-aminopyridine (0.115 g, 0.94 mmol) (*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-17-

((1*S*,2*R*)-2-((*S*)-19-((*S*)-icosan-2-yloxy)-21-oxononadecan-2 yl) cyclopropyl) nonadecy l) tetracosanoic acid **(264)** (0.370 g, 0.360 mmol), and 2,3,4,2',3',4'-hexaakis-O-(trimethylsilyl)-α,α'-trehalose **(148)** (0.100 g, 0.130 mmol) and powdered 4 A° molecular sieves in dry dichloromethane (2.3 mL). The residue was purified and separated by column chromatography eluting with petroleum ether/ethyl acetate (25:1) to give a first fraction, **(TDM) (265)** as a colourless oil (0.106 g, 24%), $[\alpha]_D^{21} = +16.7$ (*c* 1.07 $CHCl_3$) [MS (MALDI-TOF): *m/z* 3524.06 $[(M+Na)^+]$, calculated for $C_{212}H_{426}NaO_{19}Si_8$: 3524.04]; δ_H: 4.92 (2H, sext, *J* 6.3 Hz), 4.86 (2H, d, *J* 3.0 Hz), 4.38 (2H, br, d, *J* 9.9 Hz), 4.04–3.98 (4H, m), 4.0–3.96 (2H, m) 3.93 (2H, m), 3.53 (2H, t, *J* 9 Hz), 3.38 (2H, dd, *J* 3, 9.3 Hz), 2.55 (2H, ddd, *J* 3.5, 4.7, 10.1 Hz), 2.26 (4H, t, *J* 7.5 Hz), 1.56–1.21 (284H, br.m), 1.20 (6H, d, *J* 6.2 Hz), 0.90–0.86 (18H, m, including a triplet at 0.88 ppm with *J* 6.9 Hz), 0.88 (18H, s), 0.72–0.65 (2H, m), 0.48–0.41 (2H, m), 0.22–0.06 (6H, m), 0.16 (18H, s), 0.15 (18H, s), 0.14 (18H, s), 0.062 (12H, s); δ_C: 173.8, 172.6, 94.8, 73.5, 73.4, 72 .8, 71.8, 70.7, 62.4, 60.4, 51.9, 41.3, 38.0, 37.4, 36.0, 34.5, 33.4, 32.0, 29.8, 29.6, 29.44, 29.41, 29.4, 29.33, 29.2, 27.4, 27.1, 26.2, 25.8, 25.40, 25.1, 22.7, 22.3, 20.1, 19.7, 18.5, 14.2, 14.1, 11.4, 10.5, 1.1, 1.0, 0.15, –4.5, –4.8; ν_{max}/cm^{-1}: 2924, 2853, 1733, 1465, 1375, 1252, 1215, 842, 760.

The second fraction **(TMM) (266)** as a colourless oil (0.193 g, 70%), $[\alpha]_D^{21} = +23.5$ (*c* 0.63 $CHCl_3$) [MS (MALDI-TOF): *m/z* 2160.93 $[(M+Na)^+]$, calculated for $C_{121}H_{248}NaO_{15}Si_7$: 2160.69]; δ_H: 4.91 (1H, d, *J* 3.0 Hz), 4.91 (1H, sext, *J* 6.2 Hz), 4.85 (1H, d, *J* 3.0 Hz), 4.36 (1H, dd, *J* 2.0, 11.6 Hz), 4.08 (1H, dd, *J* 4.0, 11.6 Hz), 4.04–3.98 (4H, m), 3.85 (1H, dt, *J* 3.2, 9.5 Hz), 3.69 (2H, m), 3.5 (2H, dt, *J* 4.6, 9.1 Hz), 3.41 (1H, dd, *J* 3.1, 9.3 Hz), 3.38 (1H, dd, *J* 2.8, 9.1 Hz), 2.55 (1H, ddd, *J* 3.3, 5.4, 10.3 Hz), 2.27 (2H, t, *J* 7.4 Hz), 1.73 (1H, br.m), 1.68–1.21 (143H, br.m), 1.20 (3H, d, *J* 6.2 Hz), 0.90–0.86 (9H, m, including a triplet at 0.88 ppm with *J* 6.9 Hz), 0.88 (9H, s), 0.48–0.41 (1H, m), 0.22–0.06 (3H, m), 0.17 (18H, s), 0.15 (18H, s), 0.14 (18H, s), 0.060 (3H, s), 0.052 (3H, s); δ_C: 174.5, 173.6, 94.50, 94.4, 73.5, 73.3, 72.9, 72.8, 72.7, 72.1, 71.4, 70.8, 62.4, 61.7, 51.8, 38.0, 37.4, 36.0, 34.7, 34.4, 34.12, 33.4, 31.9, 30.1, 29.77, 29.74, 29.7, 29.67, 29.61, 29.53, 29.51, 29.49, 29.35, 28.13, 27.3, 26.3, 26.13, 25.80, 25.45, 25.1, 22.64, 22.60, 22.3, 20.0, 19.7, 18.8, 14.0, 10.5, 1.1, 1.009, 1.004, 1.0, 0.8, 0.2, 0.03, –4.5, –4.8; ν_{max}/cm^{-1}: 3391, 2923, 2852, 1734, 1466, 1377, 1251, 1215, 843, 760.

Experiment 49: 6,6-*bis*-*O*-(*R*)-2-((*R*)-1-Hydroxy-17-((1*S*,2*R*)-2-((*S*)-19-((*S*)-icosan-2-yloxy)-21-oxononadecan-2-yl)cyclopropyl)nonadecyl)tetracosanoate-α,α'-trehalose (267)

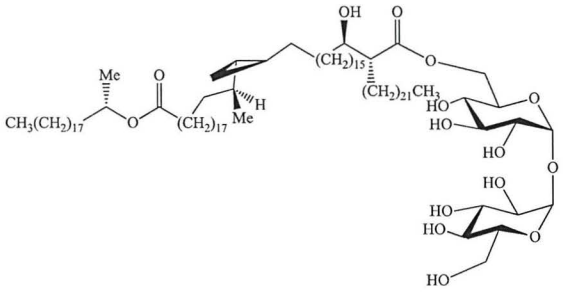


(i) The same procedure used in Experiment **43** was repeated to deprotect 6,6'-*bis*-*O*-(*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-17-((1*S*,2*R*)-2-((*S*)-19-((*S*)-icosan-2-yloxy)-21-oxononadecan-2-yl)cyclopropyl)nonadecyl)tetracosanoic-2,3,4,2',3',4'-hexakis-*O*-(trimethylsiyl)α,α'-trehalose **(265)** (0.0854 g, 0.0242 mmol) using tetra-*n*-butyl ammonium fluoride (0.0726 mL, 0.0726 mmol, 1M) in dry THF (10 mL) at 5 °C under nitrogen. The residue was purified by column chromatography on silica eluting with $CHCl_3$/MeOH (10:1) to give a syrup of 6,6-*bis*-*O*-(*R*)-2-((*R*)-1-((*tert*-butyl dimethyl silyl)oxy)-17-((1*S*,2*R*)-2-((*S*)-19-((*S*)-icosan-2-yloxy)-21-oxononadecan-2-yl) cyclopro-pyl)nonadecyl)tetracosanoic α,α'-trehalose **(267)** (0.0660 g, 89 %), $[\alpha]_D^{21}$ = +19.13 (c 0.19, $CHCl_3$) [MS (MALDI-TOF): *m/z* 3091.79 $[(M+Na)^+]$, calculated for $C_{194}H_{378}NaO_{19}Si_2$: 3091.80]; δ_H ($CDCl_3$ + few drops of CD_3OD): 5.04 (2H, d, *J* 3.4 Hz), 4.92 (2H, sext, *J* 6.2 Hz), 4.37 (2H, br. dd, *J* 3.4, 11.5 Hz), 4.21 (2H, br. d, *J* 11.4 Hz), 3.93–3.85 (2H, m), 3.77 (2H, t, *J* 9.2 Hz), 3.51 (2H, dd, *J* 3.4, 9.4 Hz), 3.36 (2H, m), 3.30 (2H, t, *J* 9.4 Hz), 2.55 (2H, m), 2.22 (4H, t, *J* 7.4 Hz), 1.61–1.24 (284 H, br.m), 1.18 (6H, d, *J* 6.3 Hz), 0.90–0.86 (18H, m, including a triplet at 0.88 ppm with *J* 6.9 Hz), 0.88 (18H, s), 0.68–0.58 (2H, m), 0.49–0.38 (2H, m), 0.20–0.06 (6H, m), 0.02 (6H, s), 0.007 (6H, s); δ_C: 175.2, 174.0, 93.6, 73.2, 72.8, 71.23, 70.8, 70.2, 70.1, 69.8, 67.8, 62.9, 51.6, 38.0, 37.3, 34.7, 34.4, 33.8, 31.95, 29.7, 29.63, 29.46, 29.40, 29.37, 29.30, 29.1, 26.1, 25.8, 25.41, 25.2, 22.8, 22.4, 20.0, 19.8, 18.6, 18.4, 14.0, 10.4, –4.6, –4.9; ν_{max}/cm^{-1}: 3391, 2923, 2853, 1732, 1728, 1466, 1375, 1250, 760.

(ii) The procedure used in Experiment **44** was repeated to deprotect 6,6-bis-O-(*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-17-((1*S*,2*R*)-2-((*S*)-19-((*S*)-icosan-2-yloxy)-21-oxo-nonadecan-2-yl)cyclopropyl)nonadecyl)tetracosanoic-α,α'-trehalose (0.058 g, 0.019 mmol) using pyridine (0.08 mL) and hydrogen fluoride-pyridine complex as

~70% (0.8 mL) in dry THF (8 mL). The crude product was purified by column chromatography eluting with $CHCl_3$/MeOH (10:1) to give a syrup of **(267)** (0.036 g, 67 %), $[\alpha]_D^{21}$ = +34.2 (c 1.32, $CHCl_3$), [HRMS (MALDI-TOF): *m/z* 2863.6353 $[(M+Na)^+]$, calculated for $C_{182}H_{350}NaO_{19}$: 2863.6314]; δ_H ($CDCl_3$ + few drops of CD_3OD): 4.98 (2H, d, *J* 3.4 Hz), 4.87 (2H, sext, *J* 6.2 Hz), 4.68 (2H, br. d, *J* 10.9 Hz), 4.24 (2H, t, *J* 8.8 Hz), 3.93 (2H, m), 3.73 (2H, t, *J* 9.2 Hz), 3.66-3.62 (2H, m), 3.49 (2H, dd, *J* 3.4, 9.7 Hz), 3.19 (2H, t, *J* 9.5 Hz), 2.40 (2H, br.m), 2.23 (4H, t, *J* 7.5 Hz), 1.58–1.53 (8H, m), 1.52–1.22 (280 H, m), 1.16 (6H, d, *J* 6.2 Hz), 0.86 (6H, d, *J* 6.8 Hz), 0.86–0.82 (12H, br.t, *J* 6.9 Hz), 0.66–0.59 (2H, m), 0.43–0.38 (2H, m), 0.18–0.05 (6H, m); δ_C: 175.5, 174.1, 94.9, 72.5, 71.8, 71.3, 71.0, 70.6, 69.8, 64.7, 52.1, 38.0, 37.3, 34.7, 34.4, 33.8, 31.9, 29.7, 29.6, 29.5, 29.4, 29.4, 29.30, 29.2, 29.0, 27.3, 26.0, 25.3, 25.0, 22.7, 22.6, 19.8, 19.5, 18.5, 14.0, 10.5; ν_{max}/cm^{-1}: 3371, 2918, 2850, 1732, 1467, 758, 723.

Experiment 50: 6-O-(*R*)-2-{(2-((*R*)-1-Hydroxy-17-((1*S*,2*R*)-2-((*S*)-19-((*S*)-icosan-2-yloxy)-21-oxononadecan-2-yl)cyclopropyl)nonadecyl)tetracosanoate-α,α'-trehalose (268)

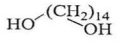


(i) The same procedure used in Experiment **43** was repeated to deprotect 6-*O*-(*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-17-((1*S*,2*R*)-2-((*S*)-19-((*S*)-icosan-2-yloxy)-21-oxononadecan-2-yl)cyclopropyl)nonadecyl)tetracosanoic-2,3,4,2',3',4'-hexakis-*O*-(trimethyl silyl)-α,α'-trehalose **(266)** (0.1680 g, 0.0786 mmol) using tetrabutylammonium fluoride (0.2358 mL, 0.2358 mmol, 1M) in dry THF (10 mL) at 5 °C under nitrogen. The residue was purified by column chromatography on silica eluting with $CHCl_3$/MeOH (5:1) to give a semi-solid of 6-*O*-(*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-17-((1*S*,2*R*)-2-((*S*)-19-((*S*)-icosan-2-yloxy)-21-oxononadecan-2-yl) cyclopropyl)nonadecyl)tetracosa noic-α,α'-trehalose **(268)** (0.1018 g, 76%), $[\alpha]_D^{21}$= +17.7 (c 0.6, $CHCl_3$) [MS (MALDI-TOF): *m/z* 1728.47 $[(M+Na)^+]$, calculated for $C_{103}H_{200}NaO_{15}Si$: 1728.45]; δ_H ($CDCl_3$ + few drops of CD_3OD): 5.05 (2H, d, *J* 3.4 Hz), 4.85 (1H, sext, *J* 6.2 Hz), 4.32–4.22 (2H, m), 3.92 (1H, br.d, *J* 9.6 Hz), 3.86–3.78 (4H, m), 3.67 (1H, m), 3.5 (2H, dd, *J* 3.4, 9.8

Hz), 3.37–3.25 (3H, m), 2.52 (1H, ddd, *J* 3.4, 5.4, 10.2 Hz), 2.22 (2H, t, *J* 7.5 Hz), 1.58–1.50 (4H, m), 1.49–1.21 (138H, m), 1.16 (3H, d, *J* 6.2 Hz), 0.90–0.86 (9H, m, including a triplet at 0.88 ppm with *J* 6.9 Hz), 0.88 (9H, s), 0.66–0.57 (1H, m), 0.44–0.35 (1H, m), 0.11–0.03 (3H, m), 0.014 (3H, s), 0.0067 (3H, s); δ_C: 175.2, 173.9, 93.5, 93.4, 73.2, 73.0, 72.6, 72.0, 71.5, 71, 70.7, 70.2, 70, 67.9, 62.3, 61.7, 51.6, 37.9, 37.2, 35.8, 34.7, 33.5, 31.8, 30.1, 29.73, 29.70, 29.60, 29.50, 29.40, 29.37, 29.24, 29.20, 29.1, 27.6, 26.9, 26.1, 25.6, 25.3, 25.1, 24.2, 22.6, 22.5, 19.8, 19.6, 18.5, 14.1, 14.0, 10.4, –4.6, –4.9; ν_{max}/cm^{-1}: 3391, 2921, 2852, 1733, 1466, 1375, 1250, 835, 759.

(ii) The procedure in Experiment **44** was repeated to deprotect 6-O-(*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-17-((1*S*,2*R*)-2-((*S*)-19-((*S*)-icosan-2-yloxy)-21-oxononadecan-2-yl)cyclopropyl)nonadecyl)tetracosanoic-α,α'-trehalose (0.087 g, 0.051 mmol) using pyridine (0.05 mL) and hydrogen fluoride-pyridine complex as ~70% (0.3 mL) in dry THF (5 mL). The crude product was purified by column chromatography eluting with $CHCl_3$/MeOH (5:1) to give a white solid of **(268)** (0.050 g, 61%), $[\alpha]_D^{21}$ = +32.85 (*c* 1.3, $CHCl_3$), m.p. 115–117 °C, [HRMS (MALDI-TOF): *m/z* 1614.3674 $[(M+Na)^+]$, calculated for $C_{97}H_{187}NaO_{15}$: 1614.3684]; δ_H ($CDCl_3$ + few drops of CD_3OD): 5.07 (1H, d, *J* 3.1 Hz), 5.02 (1H, d, *J* 3.2 Hz), 4.88 (1H, sext, *J* 6.2 Hz), 4.69 (1H, d, *J* 11.3 Hz), 4.22 (1H, t, *J* 9.1 Hz), 3.99–3.90 (2H, m), 3.88–3.78 (3H, m), 3.63–3.54 (3H, m), 3.5 (1H, dd, *J* 2.9, 10.0 Hz), 3.28 (1H, t, *J* 9.4 Hz), 3.21 (1H, t, *J* 9.3 Hz), 2.38 (1H, m), 2.23 (2H, t, *J* 7.5 Hz), 1.58–1.50 (4H, m), 1.49–1.21 (140H, br.m), 1.17 (3H, d, *J* 6.2 Hz), 0.86 (3H, d, *J* 6.8 Hz), 0.86–0.83 (6H, br.t, *J* 6.9 Hz), 0.67–0.59 (1H, m), 0.44–0.37 (1H, m), 0.18–0.04 (3H, m); δ_C: 175.5, 173.9, 94.3, 94.2, 74.5, 73.3, 72.5, 71.5, 71.3, 71.2, 70.6, 63.9, 62.9, 52.4, 38.0, 37.2, 35.7, 34.5, 34.4, 33.4, 31.7, 30.0, 29.9, 29.6, 29.3, 29.2, 29.1, 28.10, 27.3, 25.9, 25.2, 25.0, 24.9, 22.5, 19.8, 18.8, 18.4, 14.0, 10.3; ν_{max}/cm^{-1}: 3369, 2922, 2852, 1732, 1466, 1373, 759.

Experiment 51: 1, 14-Tetradecandiol (293)

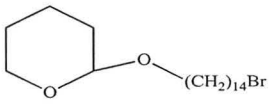


The procedure used in Experiment **4** was repeated to reduce 1,14-tetradecanoic acid **(292)** (16 g, 62 mmol) using $LiAlH_4$ (11.75 g, 310.00 mmol) in THF (200 mL) at 0 °C under nitrogen. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (1:1) to give a white solid of **(293)** (13.2 g, 92%);[143] δ_H: 3.65 (4H, t, *J* 6.6 Hz), 1.58 (4H, m), 1.27 (20H, m); δ_C: 63.13, 32.82, 29.61, 29.60, 29.57, 29.42, 25.73; ν_{max}/cm^{-1}: 3350, 2923, 2852, 1461, 1376, 1051.

Experiment 52: 14-Bromotetradecan-1-ol (294)

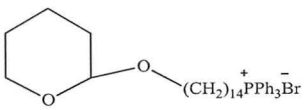
The procedure used in Experiment **31** was repeated using hydrobromic acid (20 mL, 48% in water) and 1,14-teradecandiol **(293)** (10.0 g, 43.4 mmol) in toluene (180 mL). The crude product was purified by column chromatography eluting with petrol/ethyl acetate (5:1) to give a white solid of **(294)** (7.4 g, 60 %); [143] δ_H: 3.65 (2H, t, *J* 6.6 Hz), 3.42 (2H, t, *J* 6.8 Hz), 1.86 (2H, pent, *J* 6.9 Hz), 1.57 (2H, pent, *J* 6.6 Hz), 1.48–1.27 (20H, m); δ_C: 63.1, 34.1, 32.85, 32.81, 29.61, 29.58, 29.54, 29.44, 29.42, 29.41, 28.8, 28.2, 25.7; ν_{max}/cm^{-1}: 3294, 2922, 2849, 1607, 1462, 1376, 1202, 1122.

Experiment 53: 2-((14-Bromotetradecyl)oxy)tetrahydro-2*H*-pyran (295)



The procedure used in Experiment **3** was repeated using pyridinium-p-toluene-sulfonate (0.98 g, 3.92 mmol), 14-bromo-tetradecan-1-ol **(294)** (2.30 g, 7.84 mmol) and 3,4-dihydro-2*H*-pyran (1.65 g, 19.6 mmol) in dry CH_2Cl_2 (30 mL) under nitrogen at r.t. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give a colourless oil of **(295)** (2.55 g, 86 %), [HRMS (EI^+): *m/z* 375.1894 $[(M)^+]$, calculated for $C_{19}H_{36}O_2Br$: 375.1899]; δ_H: 4.57 (1H, m), 3.85 (1H, m), 3.73 (1H, dt, *J* 6.9, 9.5 Hz), 3.54 (1H, m), 3.40 (2H, t, *J* 6.9 Hz), 3.39 (1H, dt, *J* 6.8, 9.6 Hz), 1.91 (2H, pent, *J* 6.9 Hz), 1.57 (6H, m), 1.48–1.27 (22H, m); δ_C: 98.8, 67.7, 62.3, 34.0, 32.8, 30.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 28.8, 28.2, 26.2, 25.5, 19.7; ν_{max}/cm^{-1}: 2925, 2854, 1465, 1135, 1121, 1034.

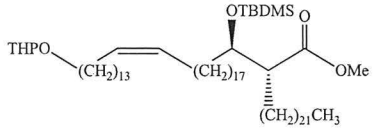
Experiment 54: (14-((Tetrahydro-2H-pyran-2-yl)oxy)tetradecyl)triphenyl-phosphornium bromide (291)



2-((14-bromotetradecyl)oxy)tetrahydro-2*H*-pyran **(295)** (1.90 g, 5.03 mmol) was added to a stirred solution of triphenylphosphine (1.98 g, 7.55 mmol) in dry acetonitrile (15 mL) and heated under refluxe for 4 days at 70 °C. The solvent was evaporated and the residue was purified by column chromatography firstly eluting with petroleum ether and then with dichloromethane/methanol (95:5), which was later precipitated in ether to give

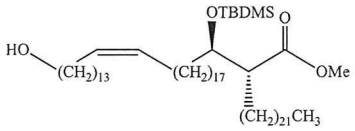
a white powder of **(291)** (2.41 g, 75%), δ_H: 8.03–7.53 (15H, m), 4.57 (1H, m), 3.85 (3H, m), 3.73 (1H, dt, *J* 6.9, 9.6 Hz), 3.49 (1H, m), 3.39 (1H, dt, *J* 6.7, 9.6 Hz), 1.88 (2H, m), 1.57 (14H, m), 1.48–1.27 (14H, m); δ_C: 135.1, 135.0, 133.6, 133.5, 130.6, 130.4, 118.6, 117.8, 98.9, 67.7, 62.4, 46.4, 34.0, 30.8, 30.5, 30.3, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 28.8, 28.2, 26.2, 25.7, 25.4, 22.6, 22.5, 22.4, 19.7; ν_{max}/cm^{-1}: 2920, 2851, 1473, 1115, 1027.

Experiment 55: Methyl (2*R*,3*R*,*Z*)-3-((*tert*-butyldimethylsilyl)oxy)-2-docosyl-35-((tetrahydro-2*H*-pyran-2-yl)oxy)pentatriacont-21-enoate (290)



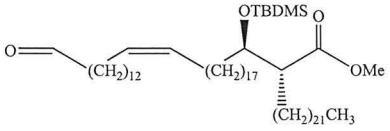
Sodium *bis*(trimethylsilyl)amide (4.16 mL, 4.16 mmol, 1.0M in THF) was added to a stirred solution of (14-((tetrahydro-2*H*-pyran-2-yl)oxy)tetradecyl) triphenyl phosphonium bromide **(291)** (1.78 g, 2.78 mmol) in dry THF (8 mL) at room temperature under nitrogen atmosphere. The reaction mixture was stirred for 30 min and then (*R*)-2-[(*R*)-1-*tert*-butyldimethylsilanyloxy)-19-oxononadecyl] tetracosanoic acid methyl ester **(276)** (1.10 g, 1.38 mmol) in dry THF (4 mL) was added. The reaction mixture was stirred for 2 hours at room temperature, and then quenched with sat. aq. NH_4Cl (10 mL). The product was extracted with petrol/ethyl acetate (20:1, 3×30 mL), the combined organic layers were dried and the solvent was evaporated to give the crude product, which was purified by chromatography eluting with petrol/ethyl acetate (40:1) to give a thick colourless oil of **(290)** (1.08 g, 73%), $[\alpha]_D^{21} = -9.3$ (*c* 0.9, $CHCl_3$) [MS (MALDI-TOF): *m/z* 1095.71 $[(M+Na)^+]$, calculated for $C_{69}H_{136}NaO_5Si$: 1096.00]; δ_H: 5.35 (2H, br.t, *J* 4.7 Hz), 4.58 (1H, m), 3.93 (2H, m), 3.73 (1H, dt, *J* 6.9, 9.5 Hz), 3.66 (3H, s), 3.49 (1H, m), 3.39 (1H, dt, *J* 6.7, 9.6 Hz), 2.53 (1H, ddd, *J* 3.8, 7.2, 10.9 Hz), 2.02 (4H, br.q, *J* 6.5 Hz), 1.6–1.2 (102H, br.m), 0.89 (3H, t, *J* 6.6 Hz), 0.87 (9H, s), 0.05 (3H, s), 0.02 (3H, s); δ_C: 175.6, 129.9, 98.8, 73.2, 67.7, 62.3, 60.1, 51.6, 51.3, 33.7, 32.6, 30.8, 29.9, 29.8, 29.7, 29.6, 29.4, 29.3, 29.2, 29.1, 27.7, 27.5, 27.3, 26.4, 25.6, 25.4, 23.3, 22.7, 19.7, 18.0, 14.1, –4.4, –4.9; ν_{max}/cm^{-1}: 2923, 2852, 1742, 1464, 1437, 1361, 1255, 1122, 836, 775.

Experiment 56: Methyl (2*R*,3*R*,*Z*)3-((*tert*-butyldimethylsilyl)oxy)-2-docosyl-35-hydroxy pentatriacont-21-enoate (292)



The procedure used in Experiment **9** was repeated to deprotect methyl (2*R*,3*R*,*Z*)-3-((*tert*-butyldimethylsilyl)oxy)-2-docosyl-35-((tetrahydro-2*H*-pyran-2-yl)oxy) pentatri acont-21-enoate **(290)** (1.040 g, 0.960 mmol) using a solution of pyridinium-*p*-toluene sulfonate (0.12 g, 0.48 mmol) in THF (20 mL), MeOH (8 mL). The crude product was purified by column chromatography eluting with petrol/ethyl acetate (12:1) to give a semi-solid of **(292)** (0.728 g, 76%), $[\alpha]_D^{21} = -2.3$ (*c* 0.82, $CHCl_3$) [MS (MALDI-TOF): *m/z* 1011.78 [$(M+Na)^+$], calculated for $C_{64}H_{128}NaO_4Si$: 1011.94]; δ_H: 5.35 (2H, br.t, *J* 4.7 Hz), 3.9 (1H, dt, *J* 7.0, 4.7 Hz), 3.66 (3H, s), 3.64 (2H, t, *J* 6.6 Hz), 2.53 (1H, ddd , *J* 3.8, 7.1, 10.9 Hz), 2.02 (4H, br.q, *J* 6.6 Hz), 1.60–1.20 (96 H, br.m), 0.89 (3H, t, *J* 6.6 Hz), 0.87 (9H, s), 0.05 (3H, s), 0.02 (3H, s); δ_C: 175.2, 129.9, 73.2, 63.1, 51.6, 51.2, 33.7, 32.8, 32.6, 31.9, 29.9, 29.8, 29.7, 29.6, 29.4, 29.3, 29.2, 29.1, 27.8, 27.5, 27.2, 26.4, 25.7, 23.7, 14.2, –4.4, –4.9; ν_{max}/cm^{-1}: 3337, 2924, 2853, 1741, 1465, 1437, 1361, 1179, 1120, 836, 775, 720, 695.

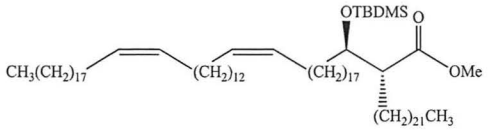
Experiment 57: Methyl (2*R*,3*R*,*Z*)-3-((*tert*-butyldimethylsilyl)oxy)-2-docosyl-35-oxopenta triacont-21-enoate (289)



The procedure described in Experiment **5** was repeated to oxidize methyl (2*R*,3*R*,Z)-3-((*tert*-butyldimethylsilyl)oxy)-2-docosyl-35-hydroxypentatriacont-21-enoate **(292)** (0.70 g, 0.70 mmol) using PCC (0.45 g, 2.12 mmol) in dichloromethane (10 mL). The crude product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give a thick colourless oil of **(289)** (0.63 g, 90 %), $[\alpha]_D^{21} = +1.3$ (*c* 0.5, $CHCl_3$) [MS (MALDI-TOF): *m/z* 1009.74 [$(M+Na)^+$], calculated for $C_{64}H_{126}NaO_4Si$: 1009.93]; δ_H: 9.77 (1H, t, *J* 1.8 Hz), 5.35 (2H, br.t, *J* 4.7 Hz), 3.90 (1H, dt, *J* 7.0, 4.7 Hz), 3.66 (3H, s), 2.53 (1H, ddd *J* 3.7, 7.0, 10.9 Hz), 2.43 (2H, dt, *J* 1.8, 7.4), 2.02 (4H, br.q, *J* 6.6 Hz), 1.6–1.2 (94H, br.m), 0.89 (3H, t, *J* 6.6 Hz), 0.87 (9H, s), 0.05 (3H, s), 0.02 (3H, s); δ_C: 202.9, 175.2, 129.9, 129.8, 73.2, 51.6, 51.2, 43.9, 33.7, 31.9, 29.9, 29.8, 29.7, 29.6,

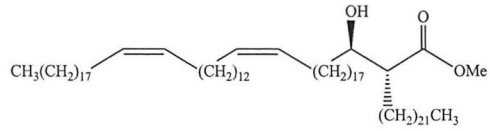
29.4, 29.3, 29.2, 29.1, 27.8, 27.5, 27.2, 25.7, 23.7, 22.7, 14.2, –4.4, –4.9; ν_{max}/cm^{-1}: 2924, 2853, 1739, 1464, 1255, 1120, 836, 759, 720.

Experiment 58: *(20Z,34Z)*-(2*R*,3*R*)-3-(*tert*-Butyldimethylsilanyloxy)-nonatriacont a- 20,34-dienoic-2-tetracosanoic acid methyl ester (288)



The procedure used in Experiment **55** was repeated to couple nonadecyltriphenyl phosphonium bromide **(285)** (0.740 g, 1.190 mmol) and methyl (*2R,3R,Z*)-3-((*tert*-butyldimethylsilyl)oxy)-2-docosyl-35-oxopentatriacont-21-enoate **(289)** (0.59 g, 0.59 mmol) using sodium bis(trimethylsilyl)amide (1.79 mL, 1.79 mmol, 1.0 M in THF) in dry THF (12 mL). The crude was purified by column chromatography eluting with petrol/ethyl acetate (40:1) to give a thick colourless oil of **(288)** (0.438 g, 59 %), $[\alpha]_D^{21}$= +1.1 (*c* 0.98, $CHCl_3$) [MS (MALDI-TOF): *m/z* 1260.35 $[(M+Na)^+]$, calculated for $C_{83}H_{163}NaO_3Si$: 1260.23]; δ_H: 5.35 (4H, br.t, *J* 4.7 Hz), 3.91 (1H, dt, *J* 7.0, 4.6 Hz), 3.66 (3H, s), 2.53 (1H, ddd, *J* 3.9, 7.2, 10.9 Hz), 2.02 (8H, q, *J* 6.7 Hz), 1.26 (126H, br.m), 0.88 (6H, t, *J* 6.6 Hz), 0.86 (9H, s), 0.05 (3H, s), 0.02 (3H, s); δ_C: 175.2, 129.9, 73.2, 51.6, 51.3, 33.7, 32.6, 31.9, 29.8, 29.77, 29.70, 29.6, 29.4, 29.36, 29.3, 29.2, 27.82, 27.5, 27.20, 25.8, 23.7, 22.7, 14.1, –4.4, –4.9; ν_{max}/cm^{-1}: 2923, 2853, 1741, 1653, 1465, 1370, 1253, 1166, 1082, 836, 774, 724.

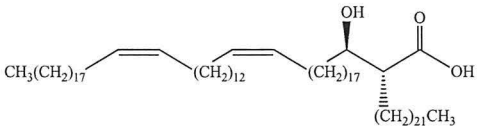
Experiment 59: (21*Z*,35*Z*)-(2*R*,3*R*)-3-(Hydroxy)-hentetraconta-21,35-dienoic-2 tetracosanoic acid methyl ester (293)



The procedure used in Experiment **16** was repeated to deprotect (20*Z,* 34*Z*)-(2*R,* 3*R*)-3-(*tert*-Butyldimethylsilanyloxy)-nonatriaconta-20,34-dienoic-2-tetracosanoicacid methy -l ester **(288)** (0.420 g, 0.320 mmol) using pyridine (0.3 mL) and hydrogen fluoride-pyridine complex as ~70% (2 mL) in dry THF (20 mL). The crude product was purified by chromatography eluting with petrol/ ethyl acetate (10:1) to give a semi-solid of **(293)** (0.320 g, 84 %) [MS (MALDI-TOF): *m/z* 1145.93 $[(M+Na)^+]$, calculated for $C_{77}H_{150}NaO_3$: 1146.14]; δ_H: 5.35 (4H, br.t, *J* 4.7 Hz), 3.71 (3H, s), 3.66 (1H, m), 2.44

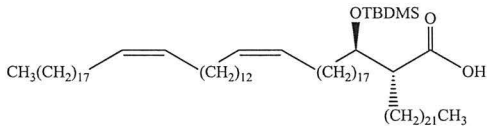
(1H, br.dt, *J* 9.0, 5.3 Hz), 2.06 (8H, q, *J* 6.7 Hz), 1.67–1.26 (126H, br.m), 0.88 (6H, t, *J* 6.6 Hz); δ_C: 176.3, 129.9, 72.3, 51.6, 50.9, 35.7, 32.6, 31.9, 29.8, 29.77, 29.70, 29.6, 29.4, 29.36, 29.32, 29.17, 27.5, 27.2, 25.7, 22.7, 14.2; ν_{max}/cm^{-1}: 3418, 2917, 2849, 1717, 1683, 1471, 1377, 1253, 1166, 1082, 836, 718.

Experiment 60: (21*Z*,35*Z*)-(2*R*,3*R*)-3-(Hydroxy)-hentetraconta-21,35-dienoic-2 tetracosanoic acid (270)



The procedure used in Experiment **19** was repeated using lithium hydroxide monohydrate (0.089 g, 2.130 mmol) and (21*Z*,35*Z*)-(2*R*,3*R*)-3-(hydroxy)-hentetraconta-21,35-dienoic-2-tetracosanoic acid methyl ester **(293)** (0.320 g, 0.284 mmol) in THF (12 mL), methanol (1.2 mL) and water (1.8 mL). The crude product was purified by column chromatography eluting with petrol/ethyl acetate (7:2) to give a white solid of **(270)** (0.282, 89 %) [MS (MALDI-TOF): *m/z* 1132.10 $[(M+Na)^+]$, calculated for $C_{76}H_{148}NaO_3$: 1132.13]; δ_H: 5.35 (4H, br.t, *J* 4.7 Hz), 3.72 (1H, m), 2.47 (1H, br.dt, *J* 9.3, 5.3 Hz), 2.04 (8H, q, *J* 6.4 Hz), 1.76–1.70 (1H, m), 1.65–1.59 (1H, m), 1.54–1.48 (4H, m), 1.26 (120H, br.m), 0.89 (6H, t, *J* 6.6 Hz); δ_C: 179.2, 129.9, 72.1, 50.8, 35.5, 32.6, 31.9, 29.8, 29.72, 29.67, 29.62, 29.6, 29.58, 29.5, 29.4, 29.35, 29.32, 29.23, 29.2, 27.3, 27.2, 25.8, 22.7, 14.1; ν_{max}/cm^{-1}: 3275, 2918, 2850, 1707, 1684, 1466, 1377, 719.

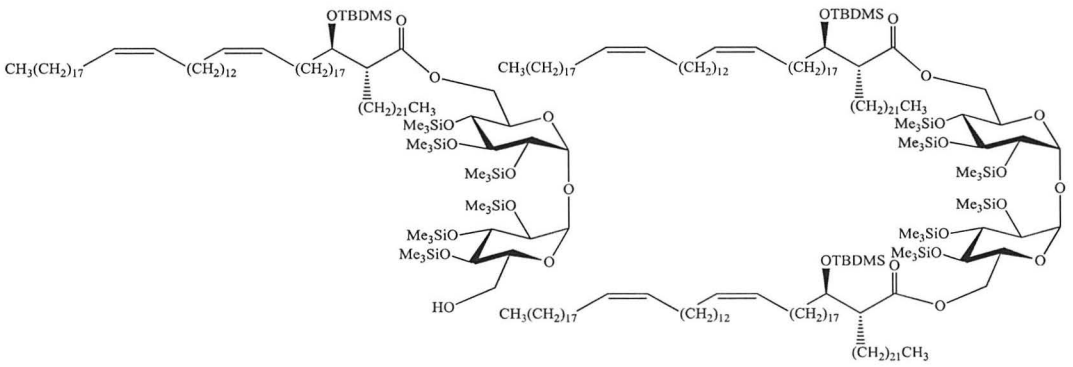
Experiment 61: 20*Z*,34*Z*)-(2*R*,3*R*)-3-(*tert*-Butyldimethylsilanyloxy)-nonatriaconta-20,34-dienoic-2-tetracosanoic acid (296)



The procedure used in Experiment **41** was repeated using (21*Z*,35*Z*)-(2*R*,3*R*)-3-(hydroxy)-hentetraconta-21,35-dienoic-2-tetracosanoic acid **(270)** (0.306 g, 0.275 mmol), imidazole (0.187 g, 2.750 mmol), *tert*-butyl-dimethylsilylchloride (0.415 g, 2.75 mmol) and 4-dimethylaminopyridine (0.033 g, 0.275 mmol) in dry DMF (2.0 mL) and dry toluene (3.0 mL). The residue was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give a thick colourless oil of **(296)** (0.292 g, 86 %) [MS (MALDI-TOF): *m/z* 1246.18 $[(M+Na)^+]$, calculated for $C_{82}H_{162}NaO_3Si$: 1246.21]; δ_H:

5.35 (4H, br.t, *J* 4.7 Hz), 3.85 (1H, m), 2.53 (1H, ddd, *J* 3.2, 5.6, 9.0 Hz), 2.02 (8H, q, *J* 6.7 Hz), 1.72–1.64 (2H, m), 1.58–1.52 (4H, m), 1.23 (120H, br.m), 0.92 (9H, s), 0.87 (6H, t, *J* 6.9 Hz), 0.14 (3H, br s), 0.13 (3H, br s); δ_C: 175.2, 129.9, 73.7, 50.2, 35.6, 31.9, 29.8, 29.7, 29.67, 29.65, 29.6, 29.58, 29.5, 29.46, 29.4, 29.36, 29.33, 29.32, 27.5, 27.4, 25.9, 25.8, 24.9, 22.7, 22.6, 14.1, – 4.3, –4.90; ν_{max}/cm^{-1}: 3433, 2923, 2852, 1709, 1467, 1255, 1070, 836, 775, 720.

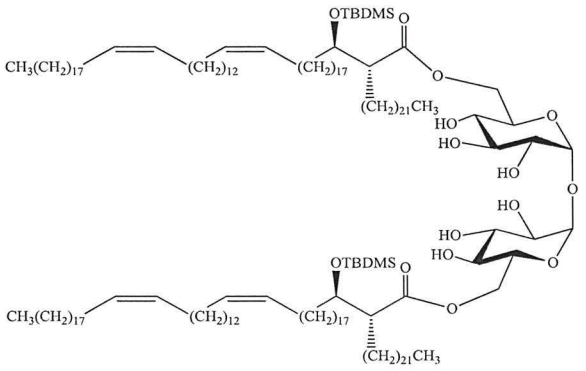
Experiment 62: 6,6'-*bis*-*O*-(20*Z*,34*Z*)-(2*R*,3*R*)-3-(*tert*-Butyldimethylsilanyloxy)-nonatriaconta- 20,34-dienoic-2- tetracosanoic-2,3,4,2',3',4',-hexakis-*O*-(trimethyl siyl) α,α'-trehalose (297), 6-*O*-(20*Z*,34*Z*)-(2*R*,3*R*)-3-(*tert*-Butyldimethylsilanyloxy)-nonatriaconta-20,34-dienoic-2-tetracosanoic-2,3,4,2',3',4',-hexakis-*O*-(trimethyl silyl)-α,α'-trehalose (298).



The procedure used in Experiment **42** was repeated using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (0.207 g, 1.084 mmol) and 4-dimethyl-l aminopyridine (0.134 g, 1.084 mmol), (20*Z*,34*Z*)-(2*R*,3*R*)-3-(*tert*-butyldimethylsilanyl-oxy)-nonatriaconta-20,34-dienoic-2-tetracosanoic acid **(296)** (0.280 g, 0.240 mmol), 2,3,4,2',3',4'-hexaakis-*O*-(trimethylsilyl)-α,α'-trehalose **(148)** (0.120 g, 0.154 mmol) and powdered 4 A° molecular sieves in dry dichloromethane (2.5 mL). The residue was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a first fraction **(TDM)** as a colourless oil **(297)** (0.181 g, 37 %), $[\alpha]_D^{21}$ = +31.3 (*c* 0.46, $CHCl_3$) [MS (MALDI-TOF): *m/z* 3207.74 [$(M+Na)^+$], calculated for $C_{194}H_{390}NaO_{15}Si_8$: 3207.78]; δ_H: 5.35 (8H, br.t, *J* 4.7 Hz), 4.85 (2H, d, *J* 3.0 Hz), 4.37 (2H, br d, *J* 10.1 Hz), 3.95 (4H, m), 3.79–3.72 (4H, m), 3.53 (2H, t, *J* 8.7 Hz), 3.38 (2H, dd, *J* 2.9, 9.3 Hz), 2.53 (2H, m), 2.07 (16H, q, *J* 6.6 Hz), 1.45–1.26 (256H, br.m), 0.88 (18H, s), 0.84 (12H, t, *J* 6.8 Hz), 0.16 (18H, s), 0.14 (18H, s), 0.12 (18H, s), 0.05 (12H, br.s); δ_C: 173.8, 129.9, 94.8, 75.5, 73.4, 72.8, 71.8, 70.7, 62.4, 51.8, 33.4, 32.6, 29.9, 29.8, 29.7, 29.6, 29.57,

29.5, 29.4, 29.3, 29.1, 28.1, 27.6, 26.2, 25.9, 25.8, 25.2, 22.7, 14.0, 1.1, 0.8, 0.01, –4.6, –4.7; ν_{max}/cm^{-1}: 2925, 2854, 1744, 1607, 1466, 1452, 1403, 1251, 1076, 1050, 825, 686. The second fraction **(TMM)** as a colourless oil **(298)** (0.192 g, 63 %),$[\alpha]_D^{21}$ = +43.3 (*c* 0.42, $CHCl_3$) [MS (MALDI-TOF): *m/z* 2002.54 $[(M+Na)^+]$, calculated for $C_{112}H_{230}NaO_{13}Si_7$: 2002.56]; δ_H: 5.36 (4H, br.t, *J* 4.7 Hz), 4.92 (1H, d, *J* 3.1 Hz), 4.85 (1H, d, *J* 3.0 Hz), 4.35 (1H, dd, *J* 2.1, 11.8 Hz), 4.08 (1H, dd, *J* 4.0, 11.8 Hz), 3.99 (1H, m), 3.96–3.92 (1H, m), 3.89 (2H, m), 3.84 (1H, dt, *J* 3.2, 9.5 Hz), 3.72–3.65 (2H, m), 3.48 (2H, dt, *J* 4.9, 8.9 Hz), 3.43 (1H, dd, *J* 3.1, 9.3 Hz), 3.40 (1H, dd, *J* 2.9, 9.3 Hz), 2.55 (1H, m), 2.07 (8H, br.q, *J* 6.6 Hz), 1.26 (126H, br.m), 0.89 (9H, s), 0.88 (6H, t, *J* 6.6 Hz), 0.17 (9H, s), 0.159 (9H, s), 0.155 (18H, s), 0.14 (9H, s), 0.12 (9H, s), 0.052 (3H, s), 0.05 (3H, s); δ_C: 174.1, 129.9, 94.5, 94.4, 73.4, 73.3, 72.9, 72.8, 72.7, 72.6, 72.0, 70.4, 62.4, 61.1, 51.8, 33.4, 32.6, 29.9, 29.8, 29.7, 29.69, 29.56, 29.36, 29.31, 29.05, 28.1, 27.6, 27.2, 26.4, 25.8, 24.8, 22.7, 22.6, 14.1, 1.05, 0.9, 0.8, 0.17, 0.03, –4.5, –4.7; ν_{max}/cm^{-1}: 3368, 2918, 2851, 1722, 1607, 1493, 1453, 1403, 1255, 1147, 1104, 1004, 870, 760, 720.

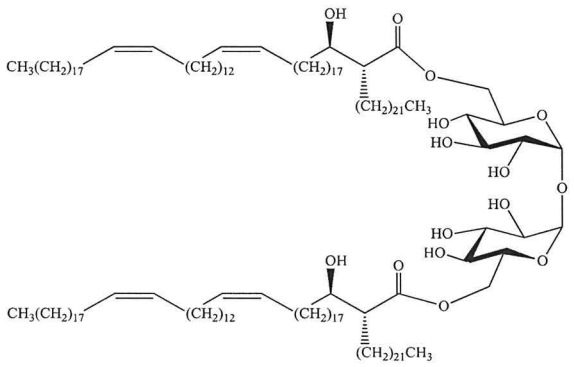
Experiment 63: 6,6-*bis*-*O*-(20*Z*,34*Z*)-(2*R*,3*R*)-3-(*tert*-Butyldimethylsilanyloxy)nona triaconta-20,34-dienoic-2-tetracosanoic α,α'-trehalose (299)



The same procedure used in Experiment **43** was repeated to deprotect 6,6'-*bis*-*O*-(20*Z*, 34*Z*)-(2*R*,3*R*)-3-(*tert*-Butyldimethylsilanyloxy)-nonatriaconta-20,34-dienoic-2-tetracos -anoic-2,3,4,2',3',4',-hexakis-*O*-(trimethylsiyl)α,α'-trehalose **(297)** (0.125 g, 0.039 mmol) using tetra-n-butylammonium fluoride (0.117 mL, 0.117 mmol, 1M) in dry THF (8 mL) at 5 °C under a nitrogen atmosphere. The residue was purified by column chromatography eluting with $CHCl_3$/MeOH (10:1) to give **(299)** as a thick colourless oil (0.083 mg, 77 %), $[\alpha]_D^{21}$ = +22.1 (*c* 0.69, $CHCl_3$) [MS (MALDI-TOF): *m/z* 2775.57 $[(M+Na)^+]$, calculated for $C_{176}H_{342}NaO_{15}Si_2$: 2775.54]; δ_H ($CDCl_3$ + few drops of

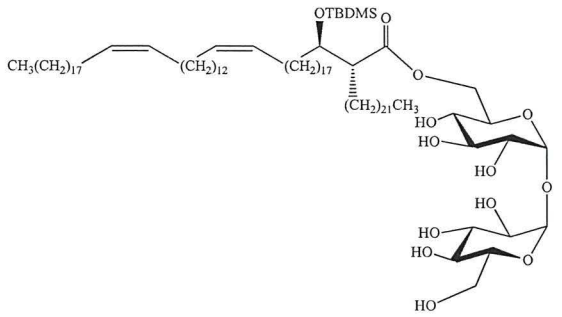
CD_3OD): 5.28 (8H, br.t, *J* 4.6 Hz), 5.03 (2H, d, *J* 3.4 Hz), 4.29 (2H, dd, *J* 5.3, 10.8 Hz), 4.19 (2H, d, *J* 10.5 Hz), 3.92–3.83 (4H, m), 3.74 (2H, t, *J* 9.3 Hz), 3.43 (2H, dd, *J* 3.5, 9.8 Hz), 3.38 (2H, m), 2.50 (2H, ddd, *J* 4.0, 6.2, 10.4 Hz), 1.95 (16H, q, *J* 6.5 Hz), 1.19 (260H, br.m), 0.85 (18H, s), 0.82 (12H, t, *J* 6.8 Hz), –0.014 (6H, s), –0.013 (6H, s); δ_C: 175.2, 129.8, 93.4, 73.1, 72.2, 71.6, 70.2, 69.8, 62.9, 51.6, 33.5, 32.4, 31.7, 29.8, 29.5, 29.4, 29.32, 29.2, 29.15, 27.9, 27.8, 25.7, 25.4, 25.1, 22.5, 13.9, –4.9, –5.1; ν_{max}/cm^{-1}: 3400, 2922, 2852, 1733, 1464, 1076, 836.

Experiment 64: 6,6-*bis*-*O*-(20*Z*,34*Z*)-(2*R*,3*R*)-3-(Hydroxy)-nonatriaconta-20,34-dienoic-2-tetracosanoic α,α'-trehalose (271)



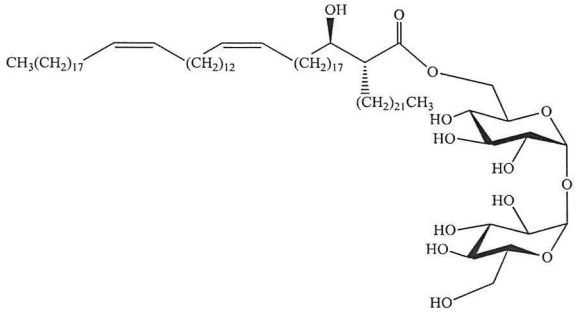
The procedure used in Experiment **44** was repeated to deprotect 6,6-*bis*-*O*-(20*Z*,34*Z*)-(2*R*,3*R*)-3-(*tert*-Butyldimethylsilanyloxy)-nonatriaconta-20,34-dienoic-2-tetracosanoic α,α'-trehalose **(299)** (0.070 g, 0.025 mmol) using of pyridine (0.080 mL) and hydrogen fluoride-pyridine complex as ~70% (0.7 mL) in dry THF (7 mL). The crude product which was purified by chromatography eluting with $CHCl_3$/MeOH (8:1) to give a semi-solid of **(271)** (0.036 g, 56 %), $[\alpha]_D^{21} = +27.3$ (*c* 0.52, $CHCl_3$) [MS (MALDI-TOF): *m/z* 2547.37 [$(M+Na)^+$], calculated for $C_{164}H_{314}NaO_{15}$: 2547.37]; δ_H ($CDCl_3$ + few drops of CD_3OD): 5.28 (8H, br.t, *J* 4.6 Hz), 4.96 (2H, d, *J* 3.2 Hz), 4.57 (2H, d, *J* 11.7 Hz), 4.14 (2H, t, *J* 9.3 Hz), 3.98 (2H, dd, *J* 7.2, 11.4 Hz), 3.69 (2H, t, *J* 9.4 Hz), 3.59 (2H, m), 3.47 (2H, m), 3.19 (2H, t, *J* 9.4), 2.33 (2H, m), 1.95 (16H, q, *J* 6.5 Hz), 1.15 (262H, br.m), 0.80 (12H, t, *J* 6.6 Hz); δ_C: 175.4, 129.8, 94.9, 72.5, 72.4, 71.2, 71.1, 69.8, 64.3, 52.3, 34.6, 32.4, 31.9, 30.0, 29.9, 29.6, 29.50, 29.45, 29.35, 29.30, 29.25, 29.20, 28.6, 27.2, 27.1, 25.2, 22.5, 13.9; ν_{max}/cm^{-1}: 3370, 2917, 2849, 1722, 1607, 1467, 1376, 1078, 760, 720.

Experiment 65: 6-*O*-(20*Z*,34*Z*)-(2*R*,3*R*)-3-(*tert*-Butyldimethylsilanyloxy)-nonatriaconta-20,34-dienoic-2-tetracosanoic α,α'-trehalose (300)



The same procedure in Experiment **43** was repeated to deprotect 6-*O*-(20*Z*, 34*Z*)-(2*R*, 3*R*)-3-(*tert*-Butyldimethylsilanyloxy)-nonatriaconta-20,34-dienoic-2-tetracosanoic-2,3, 4,2',3',4',-hexakis-O-(trimethylsilyl)-α,α'-trehalose **(298)** (0.105 g, 0.053 mmol) using tetra-*n*-butylammonium fluoride (0.159 mL, 0.159 mmol, 1M) in dry THF (12 mL) at 5 °C under a nitrogen atmosphere. The residue was purified by column chromatography eluting with $CHCl_3$/MeOH (8.5:1.5) to give a syrup of **(300)** (0.065 mg, 80 %), $[\alpha]_D^{21}$ = +19.3 (*c* 0.72, $CHCl_3$) [MS (MALDI-TOF): *m/z* 1570.97 $[(M+Na)^+]$, calculated for $C_{94}H_{182}NaO_{13}Si$: 1570.32]; δ_H ($CDCl_3$ + few drops of CD_3OD): 5.28 (4H, br.t, *J* 4.6 Hz), 5.04 (2H, d, *J* 3.3 Hz), 4.30 (1H, dd, *J* 4.7, 12.6 Hz), 4.25 (1H, dd, *J* 4.5, 11.8 Hz), 3.95–3.86 (2H, m), 3.70 (2H, m), 3.64 (2H, m), 3.46 (1H, t, *J* 3.8 Hz), 3.43 (1H, t, *J* 3.7 Hz), 3.2 (3H, m), 2.52–2.49 (1H, m), 1.96 (8H, q, *J* 6.6 Hz), 1.24–1.20 (134H, br.m), 0.84 (6H, t, *J* 6.4 Hz, 0,83 (9H, s), –0.02 (3H, s), –0.03 (3H, s); δ_C: 174.9, 129.6, 93.5, 93.4, 73, 72.9, 72.7, 72.0, 71.5, 71.4, 70.5, 70, 69.9, 62.6, 61.6, 51.5, 33.5, 32.3, 31.6, 29.7, 29.60, 29.50, 29.27, 29.2, 27.1, 26.7, 25.6, 22.3, 13.6, –4.9, –5.3; ν_{max}/cm^{-1}: 3374, 2922, 2852, 1722, 1607, 1467, 1377, 1075, 839, 760, 720.

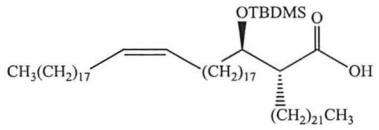
Experiment 66: 6-*O*-(20*Z*,34*Z*)-(2*R*, 3*R*)-3-(hydroxy)-nonatriaconta-20,34-dienoic -2- tetracosanoic α,α'-trehalose (272)



The procedure used in Experiment **44** was repeated to deprotect 6-*O*-(*20Z, 34Z*)-(*2R, 3R*)-3-(*tert*-butyldimethylsilanyloxy)-nonatriaconta-20,34-dienoic-2-tetracosanoic α,α'

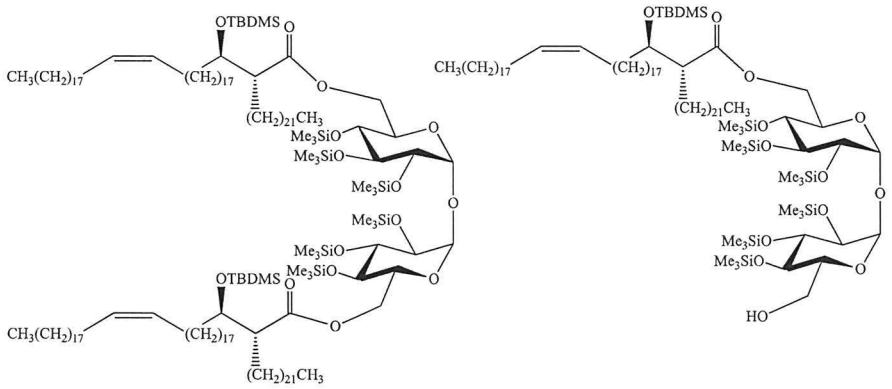
-trehalose **(300)** (0.055 g, 0.035 mmol) using pyridine (0.080 mL) and hydrogen fluoride-pyridine complex as ~70% (0.60 mL) in dry THF (7 mL). The crude product was purified by chromatography eluting with CHCl3/MeOH (5:1) to give a semi-solid of **(272)** (0.027, 53%), $[\alpha]_D^{21}$ = +26.3 (*c* 0.81, $CHCl_3$), [HRMS (MALDI-TOF): *m/z* 1456.2405 [$(M+Na)^+$], calculated for $C_{88}H_{168}NaO_{13}$: 1456.2383]; δ_H ($CDCl_3$ + few drops of CD_3OD): 5.30 (4H, br.t, *J* 4.7 Hz), 5.06 (1H, d, *J* 3.7 Hz), 5.00 (1H, d, *J* 3.5 Hz), 4.65 (1H, d, *J* 10.5 Hz), 4.19 (1H, t, *J* 8.3 Hz), 3.97 (1H, dd, *J* 8.0, 11.8), 3.7 (4H, m), 3.6 (2H, m), 3.52 (1H, dd, *J* 3.3, 10.0 Hz), 3.46 (1H, dd, *J* 3.3, 9.7 Hz), 3.26 (1H, t, *J* 9.3 Hz), 3.2 (1H, t, *J* 9.4 Hz), 2.32 (1H, ddd, *J* 4.5, 6.5, 8.7), 1.96 (8H, q, *J* 5.5 Hz), 1.44–1.20 (136H, br.m), 0.84 (6H, t, *J* 6.6 Hz); δ_C: 175.4, 129.7, 94.2, 94.15, 73.1, 73, 72.8, 72.2, 71.8, 71.5, 70.6, 70.3, 64.9, 62.3, 53.3, 33.9, 31.7, 29.9, 29.7, 29.65, 29.6, 29.27, 29.21, 27.10, 25.6, 25.5, 23.2, 14.1; ν_{max}/cm^{-1}: 3391, 2917, 2850, 1717, 1617, 1467, 1075, 839, 801, 720.

Experiment 67: (2*R*,*Z*)-2-Docosyl-3-(*R*)-3-(*tert*-butyldimethylsilanyloxy)-21-enoic acid (301)



The procedure used in Experiment **41** was repeated using (2*R*,3*R*,*Z*)-2-docosyl-3-hydroxytetracont-21-enoic acid **(133)** (0.348 g, 0.381 mmol), imidazole (0.259 g, 3.810 mmol), *tert*-butyl-dimethylsilylchloride (0.570 g, 3.810 mmol) and 4-dimethylamino-pyridine (0.046 g, 0.389 mmol) in dry DMF (2.3 mL) and dry toluene (3.44 mL). The residue was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give colourless oil of **(301)** (0.372 g, 94%) [MS (MALDI-TOF): *m/z* 1052.91 [$(M+Na)^+$], calculated for $C_{68}H_{136}NaO_3Si$: 1052.01]; δ_H: 5.35 (2H, br.t, *J* 4.7 Hz), 3.85 (1H, m), 2.53 (1H, ddd, *J* 3.75, 5.4, 9.2 Hz), 2.02 (4H, q, *J* 6.6 Hz), 1.72–1.64 (2H, m), 1.58–1.52 (4H, m), 1.23 (100H, br.m), 0.92 (9H, s), 0.87 (6H, t, *J* 3.4 Hz), 0.13 (3H, br.m), 0.12 (3H, br s); δ_C: 129.9, 73.6, 50.2, 35.4, 31.9, 29.8, 29.7, 29.65, 29.62, 29.6, 29.55, 29.5, 29.4, 29.37, 29.33, 29.30, 27.5, 27.2, 25.7, 25.6, 22.7, 22.6, 14.1, – 4.3, – 4.9; ν_{max}/cm^{-1}: 3413, 2919, 2851, 1709, 1638, 1467, 1254, 1070, 835, 760, 720.

Experiment 68: 6,6'-*bis*-*O*-(2*R*,*Z*)-2-Docosyl-3-(*R*)-3-(*tert*-butyldimethylsilanyloxy 21-enoic -2,3,4,2',3',4',-hexakis-*O*-(trimethylsiyl)α,α'-trehalose (302), 6-*O*-(2*R*,*Z*)-2-Docosyl-3-(*R*)-3-(*tert*-butyldimethylsilanyloxy)-21-enoic-2,3,4,2',3',4',-hexakis-*O*-(trimethylsilyl)-α,α'-trehalose (303).

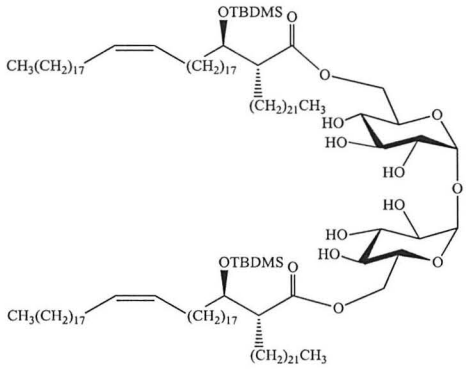


The procedure used in Experiment **42** was repeated using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (0.320 g, 1.680 mmol), 4-dimethylamino-pyridine (0.206 g, 1.680 mmol), (2*R*,*Z*)-2-docosyl-3-(*R*)-3-(*tert*-butyldimethylsilanyl-oxy)-21-enoic acid **(301)** (0.372 g, 0.361 mmol) and 2,3,4,2',3',4'-hexaakis-O-(trimethylsilyl)-α,α'-trehalose **(148)** (0.187 g, 0.240 mmol) and powdered 4 A° molecular sieves in dry dichloromethane (3 mL). The residue was purified and separated by column chromatography eluting with petrol/ethyl acetate (20:1) to give the first fraction **(TDM) (302)** as a colourless oil (0.134 g, 21%) [MS (MALDI-TOF): *m/z* 2821.74 [$(M+Na)^+$], calculated for $C_{166}H_{338}NaO_{15}Si_8$: 2819.37]; δ_H: 5.35 (4H, br.t, *J* 4.7 Hz), 4.84 (2H, d, *J* 3.0 Hz), 4.37 (2H, br.d, *J* 9.9 Hz), 3.95 (4H, m), 3.79–3.72 (4H, m), 3.56 (2H, t, *J* 8.7 Hz), 3.39 (2H, dd, *J* 2.7, 9.1 Hz), 2.53 (2H, m), 1.86 (8H, q, *J* 6.3 Hz), 1.26 (210H, br.m), 0.88 (18H, s), 0.89 (12H, t, *J* 6.7 Hz), 0.16 (18H, s), 0.14 (18H, s), 0.13 (18H, s), 0.05 (12H, s); δ_C: 173.8, 129.9, 94.8, 73.5, 73.4, 72.8, 71.8, 70.7, 62.4, 51.9, 33.4, 31.9, 29.9, 29.8, 29.7, 29.6, 29.5, 29.3, 29.3, 29.0, 28.1, 27.6, 27.2, 26.2, 25.9, 25.8, 25.2, 22.7, 14.1, 1.1, 0.9, 0.1, –4.5, –4.6; ν_{max}/cm^{-1}: 2924, 2853, 1744, 1607, 1493, 1466, 1403, 1251, 1077, 1052, 838.

The second fraction was **(TMM) (303)** as a colourless oil (0.123 g, 29%) [MS (MALDI-TOF): *m/z* 1809.93 [$(M+Na)^+$], calculated for $C_{98}H_{204}NaO_{13}Si_7$: 1808.35]; δ_H: 5.35 (2H, br.t, *J* 4.7 Hz), 4.91 (1H, d, *J* 3.0 Hz), 4.85 (1H, d, *J* 3.0 Hz), 4.36 (1H, dd, *J* 2.2, 11.8 Hz), 4.08 (1H, dd, *J* 4.1, 11.7 Hz), 3.99 (1H, m), 3.96–3.92 (1H, m), 3.89 (2H, m), 3.84 (1H, m), 3.72–3.65 (2H, m), 3.48 (2H, dt, *J* 4.9, 9.0 Hz), 3.43 (1H, dd, *J* 3.1, 9.3 Hz), 3.40 (1H, dd, *J* 2.85, 9.3 Hz), 2.55 (1H, ddd, *J* 3.1, 5.35, 9.2 Hz), 2.07 (4H, br.q, *J* 6.6

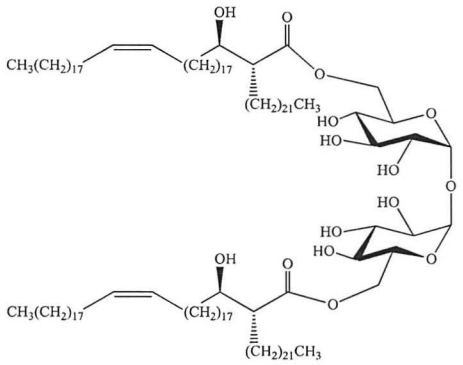
Hz), 1.26 (108H, br.m), 0.88 (6H, t, *J* 6 Hz), 0.87 (9H, s), 0.17 (9H, s), 0.159 (18H, s), 0.155 (9H, 232 s), 0.14 (9H, s), 0.12 (9H, s), 0.052 (3H, s), 0.05 (3H, s); δ_C: 173.2, 129.9, 94.5, 94.3, 72.9, 72.8, 72.7, 72.0, 71.4, 70.7, 62.5, 61.6, 51.8, 33.4, 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 29.1, 28.1, 27.7, 27.2, 26.4, 25.8, 22.7, 22.6, 20.4, 14.1, 1.05, 1.00, 0.9, 0.8, 0.1, 0.0, –4.5, –4.7; ν_{max}/cm^{-1}: 3368, 2918, 2851, 1722, 1607, 1467, 1377, 1251, 1076, 1050, 1006, 874, 760, 720.

Experiment 69: 6,6-*bis*-*O*-(2*R*,*Z*)-2-Docosyl-3-(*R*)-3-(*tert*-butyldimethylsilanyl-oxy)-21-enoic α,α'-trehalose (304)



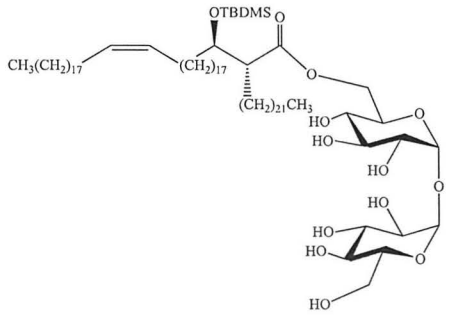
The same procedure used in Experiment **43** was repeated to deprotect 6,6'-*bis*-*O*-(2*R*, *Z*)-2-docosyl-3-(*R*)-3-(*tert*-butyldimethylsilanyloxy)-21-enoic-2,3,4,2',3',4',-hexakis-*O*-(trimethylsilyl)-α,α'-trehalose **(302)** (0.126 g, 0.045 mmol) using tetra-*n*-butylammonium fluoride (0.135 mL, 0.135 mmol, 1M) in dry THF (12 mL) at 5 °C under nitrogen. The residue was purified by column chromatography eluting with $CHCl_3$/MeOH (10:1) to give a thick colourless oil of **(304)** (0.085 mg, 81 %) [MS (MALDI-TOF): *m/z* 2387.35 $[(M+Na)^+]$, calculated for $C_{148}H_{288}NaO_{15}Si_2$: 2387.13]; δ_H ($CDCl_3$ + few drops of CD_3OD): 5.28 (4H, br.t, *J* 4.7 Hz), 5.02 (2H, d, *J* 3.5 Hz), 4.27 (2H, dd, *J* 4.2, 12.1 Hz), 4.20 (2H, d, *J* 10.5 Hz), 3.92–3.83 (4H, m), 3.74 (2H, t, *J* 9.3 Hz), 3.43 (2H, dd, *J* 3.5, 9.7 Hz), 3.38 (2H, m), 2.49 (2H, ddd, *J* 4.0, 6.2, 10.4 Hz), 1.95 (8H, q, *J* 6.5 Hz), 1.20 (222H, br.m), 0.85 (18H, s), 0.82 (12H, t, *J* 6.8 Hz), –0.015 (6H, s), –0.013 (6H, s); δ_C: 175.3, 129.8, 93.5, 73.3, 73.2, 71.7, 70.3, 69.9, 67.5, 62.9, 51.6, 33.5, 32.5, 29.8, 29.48, 29.4, 29.30, 29.22, 29.18, 27.6, 27.1, 25.7, 25.4, 25.1, 22.5, 14.1, –4.6, –5.0; ν_{max}/cm^{-1}: 3401, 2923, 2852, 1735, 1607, 1464, 1377, 1253, 1076, 836.

Experiment 70: 6,6-*bis*-*O*-(2*R*,*Z*)-2-Docosyl-3-(*R*)-3-(hydroxy)-21-enoic-α,α' trehalose (273)



The procedure used in Experiment **44** was repeated to deprotect 6,6-*bis*-*O*-(2*R*, *Z*)-2-docosyl-3-(*R*)-3-(*tert*-butyldimethylsilanyloxy)-21-enoic-α,α'-trehalose **(304)** (0.075 g, 0.032 mmol) using pyridine (0.080 mL) and hydrogen fluoride-pyridine complex as ~70% (0.7 mL) in dry THF (8 mL). The crude product was purified by chromatography eluting with $CHCl_3$/MeOH (5:1) to give a syrup of **(273)** (0.038 g, 57 %) [MS (MALDI-TOF): *m/z* 2158.61 [$(M+Na)^+$], calculated for $C_{136}H_{262}NaO_{15}$: 2158.63]; δ_H ($CDCl_3$ + few drops of CD_3OD): 5.31 (4H, br.t, *J* 4.7 Hz), 4.98 (2H, d, *J* 3.5 Hz), 4.75 (2H, d, *J* 11.2 Hz), 4.30 (2H, t, *J* 8.8 Hz), 3.89 (2H, dd, *J* 8.4, 11.7 Hz), 3.75 (2H, t, *J* 9.3 Hz), 3.66 (2H, m), 3.50 (2H, dd, *J* 3.4, 9.8 Hz), 3.19 (2H, t, *J* 9.6 Hz), 2.38 (2H, m), 1.95 (8H, q, *J* 6.5 Hz), 1.15 (218H, br.m), 0.84 (12H, t, *J* 6.6 Hz); δ_C: 175.5, 129.8, 94.9, 72.6, 72.3, 71.3, 71.2, 69.8, 64.4, 52.3, 34.7, 31.9, 30.0, 29.9, 29.57, 29.50, 29.45, 29.35, 29.30, 29.23, 29.20, 28.6, 27.2, 27.0, 25.2, 22.6, 14.0; ν_{max}/cm^{-1}: 3370, 2917, 2850, 1733, 1466, 1376, 1078, 760, 720.

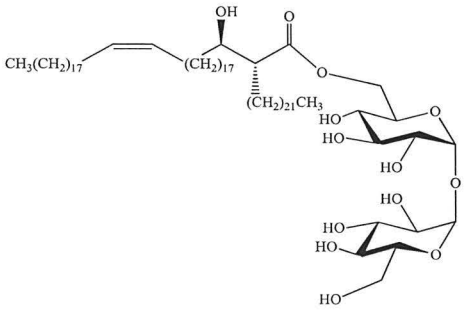
Experiment 71: 6-*O*-(2*R*,*Z*)-2-Docosyl-3-(*R*)-3-(*tert*-butyldimethylsilanyloxy)-21-enoic-α,α'-trehalose (305)



The same procedure used in Experiment **43** was repeated to deprotect 6-*O*-(2*R*,*Z*)-2-docosyl-3-(*R*)-3-(*tert*-butyldimethylsilanyloxy)-21-enoic-α,α'-trehalose **(303)** (0.113 g, 0.072 mmol) using tetra-*n*-butylammonium fluoride (0.189 mL, 0.189 mmol, 1M) in dry

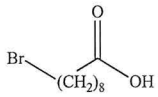
THF (12 mL) at 5 °C under a nitrogen atmosphere. The residue was purified by column chromatography eluting with $CHCl_3$/MeOH (8.5:1.5) to give a thick colourless oil of **(305)** (0.073 mg, 78 %) [MS (MALDI-TOF): *m/z* 1377.18 [$(M+Na)^+$], calculated for $C_{80}H_{156}NaO_{13}Si$: 1376.12]; δ_H ($CDCl_3$ + few drops of CD_3OD): 5.30 (2H, br.t, *J* 4.7 Hz), 5.04 (2H, d, *J* 3.8 Hz), 4.30 (1H, d, *J* 4.1 Hz), 4.25 (1H, dd, *J* 3.8, 11.7 Hz), 3.95–3.86 (2H, m), 3.70 (2H, m), 3.64 (2H, m), 3.47 (1H, t, *J* 3.8 Hz), 3.43 (1H, t, *J* 3.2 Hz), 3.2 (3H, m), 2.52–2.49 (1H, m), 1.96 (4H, q, *J* 5.6 Hz), 1.24– 1.20 (114 H, br.m), 0.84 (6H, t, *J* 6.4 Hz), 0.83 (9H, s), 0.00 (3H, s), –0.03 (3H, s); δ_C: 175.3, 129.8, 93.6, 93.5, 73.1, 73.0, 72.7, 72.2, 71.6, 70.2, 70.0, 62.7, 62, 51.6, 33.5, 31.8, 29.7, 29.6, 29.5, 29.3, 29.2, 27.1, 25.5, 22.6, 14.0, –5.0, –5.6; ν_{max}/cm^{-1}: 3368, 2918, 2851, 1722, 1607, 1467, 1075, 839.

Experiment 72: 6-*O*-(2*R*,*Z*)-2-Docosyl-3-(*R*)-3-(hydroxy)-21-enoic-α,α'-trehalose (274)



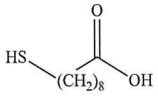
The procedure used in Experiment **44** was repeated to deprotect 6-*O*-(*2R,Z*)-2-docosyl-3-(*R*)-3-(*tert*-butyldimethylsilanyloxy)-21-enoic-α,α'-trehalose **(305)** (0.068 g, 0.046 mmol) using pyridine (0.080 mL) and hydrogen fluoride-pyridine complex as ~70% (0.5 mL) in dry THF (8 mL). The crude product was purified by chromatography eluting with chloroform/methanol (5:1) to give a semi-solid of **(274)** (0.036, 57%), $[\alpha]_D^{21} = +15.9$ (*c* 0.67, $CHCl_3$) [MS (MALDI-TOF): *m/z* 1262.61 [$(M+Na)^+$], calculated for $C_{74}H_{142}NaO_{13}$: 1262.03]; δ_H ($CDCl_3$ + few drops of CD_3OD): 5.25 (2H, br.t, *J* 4.8 Hz), 5.0 (1H, d, *J* 3.5 Hz), 4.96 (1H, d, *J* 3.5 Hz), 4.53 (1H, d, *J* 10.9 Hz), 4.12 (1H, m), 3.98 (1H, dd, *J* 7.1,11.7), 3.7 (4H, m), 3.56 (2H, dd, *J* 5.8, 11.9 Hz), 3.43 (1H, dd, *J* 3.6, 9.8 Hz), 3.40 (1H, dd, *J* 3.2, 9.8 Hz), 3.25–3.13 (2H, m), 2.32 (1H, m), 1.96 (4H, q, *J* 5.5 Hz), 1.44–1.20 (110H, br.m), 0.84 (6H, t, *J* 6.8 Hz); δ_C: 175.2, 129.8, 94.2, 94.1, 73.1, 73.0, 72.9, 72.3, 71.8, 71.4, 70.2, 70.0, 64.7, 62, 52.7, 33.5, 31.8, 29.67, 29.60, 29.50, 29.3, 29.2, 27.10, 25.7, 25.5, 22.6, 14.0; ν_{max}/cm^{-1}: 3368, 2917, 2850, 1717, 1611, 1467, 1075, 720.

Experiment 73: 9-Bromononanoic acid (311)



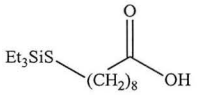
Potassium permanganate (17.8 g, 113 mmol) was added in portions to a stirred solution of 9-bromononan-1-ol **(310)** (12.6 g, 56.5 mmol), water (250 mL), acetic acid (6 mL), hexadecyltrimethylammonium bromide (1.60 g) and sulphuric acid 1 M (30 mL) in CH_2Cl_2 (250 mL) at 0 °C. The reaction mixture was stirred at room temperature for 4 hrs. When TLC showed no starting material was left, it was quenched with powdered sodium metabisulphite until a white solution had formed. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (2 × 100 mL). The combined organic layers were dried, and the solvent was evaporated to give crude product, which was purified by column chromatography eluting with petrol/ethyl acetate (6:1) to give a semi-solid of **(311)** (9.65 g, 72 %); δ_H: 3.40 (2H, t, *J* 6.8 Hz), 2.36 (2H, t, *J* 7.5 Hz), 1.85 (2H, pent, *J* 7.2 Hz), 1.60 (2H, m), 1.44 (2H, m), 1.25 (6H, br.s), δ_C: 180.1, 34.1, 33.9, 32.8, 29.0, 28.9, 28.1, 27.9, 24.6; ν_{max}/cm^{-1} 2800–3200 (broad), 2926, 2855, 1709, 1464, 935, 724 .

Experiment 74: 9-Mercaptononanoic acid (312)



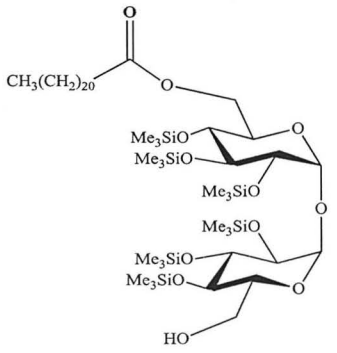
Thiourea (2.41 g, 31.6 mmol) was added to a stirred solution of 9-bromononanoic acid **(311)** (5.00 g, 21.0 mmol) in ethanol (70 mL) and heated under reflux for 3 hrs. When TLC showed no starting material was left, the solvent was evaporated and 5 M NaOH (130 mL) was added. The solution was heated under reflux for another 2 hours, it was then cooled and acidified to PH 2 with 10 % aq. solution of HCl. The product was extracted with ether (3 × 100 mL), and the combined organic layers were dried over $MgSO_4$ and evaporated to give a crude product, which was purified by column chromatography eluting with petrol/ ethyl acetate (3:1) to give a semi-solid of **(312)** (3.10 g, 77 %) [HRMS (EI^+): *m/z* 190.1044 [$(M)^+$], calculated for $C_9H_{18}O_2S$: 190.1028]; δ_H: 2.53 (2H, q, *J* 7.4 Hz), 2.36 (2H, t, *J* 7.5 Hz), 1.65 (4H, m), 1.25 (8H, br.m), δ_C: 180.1, 34.0, 33.9, 29.9, 28.9, 28.8, 28.2, 24.6; ν_{max}/cm^{-1} 2700–3300 (broad), 2921, 2851, 1701, 1465, 1411, 931.

Experiment 75: 9-((Triethylsilyl)thio)nonanoic acid (317)



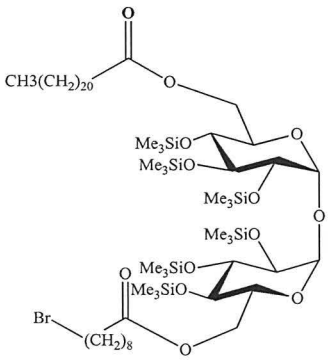
9-Mercaptononanoic acid **(312)** (0.1844 g, 0.970 mmol) was dissolved in toluene (4 mL), followed by addition of rutheniumdodecacarbonyl (0.0062 g, 0.0097 mmol) and refluxed at 80 °C for 4 hours. The solvent was evaporated and the crude residue was columned on silica eluting with petrol/ethyl acetetae (20:1), but no product was obtained and gave complicated NMR.

Experiment 76: 6-*O*-Docosanoic-2,3,4,2',3',4',-hexakis-*O*-(trimethylsilyl)-α,α'-trehalose (319)



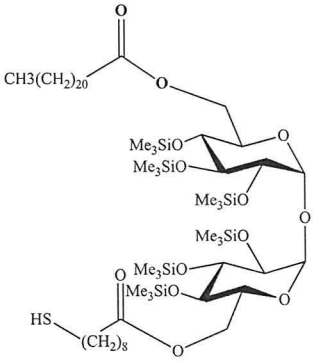
1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (1.48 g, 7.77 mmol) and 4-dimethylaminopyridine (0.94 g, 7.77 mmol) were added to a stirred solution of behenic acid **(318)** (0.66 g, 1.93 mmol), 2,3,4,2',3',4'-hexaakis-*O*-(trimethylsilyl)-α,α'-trehalose **(148)** (3.00 g, 3.8 mmol) and powdered 4 A° molecular sieves in dry CH_2Cl_2 (20 mL) at r.t under nitrogen. The mixture was stirred for 3 days at r.t. When TLC showed no starting material was left, the solvent was evaporated to give a residue, which was purified by column chromatography eluting with petroleum ether/ethyl acetate (15:1) to give a thick oil of (**319**) (1.20 g, 57%), δ_H: 4.92 (2H, t, *J* 3.1 Hz), 4.31 (1H, dd, *J* 2.0, 11.9 Hz), 4.07 (1H, dd, *J* 4.5, 11.8), 4.02 (1H, m), 3.93 (2H, dt, *J* 4.2, 8.9 Hz), 3.85 (2H, dt, *J* 3.3, 9.4 Hz), 3.7 (1H, m), 3.48 (2H, dd, *J* 3.1, 9.0 Hz), 3.44 (2H, m), 2.43 (1H, dt, *J* 16.0, 7.5 Hz), 2.41 (1H, dt, *J* 16.0, 7.1 Hz), 1.26 (38H, m), 0.89 (3H, t, *J* 6.9 Hz), 0.16 (18H, s), 0.145 (18H, s), 0.138 (18H, s); δ_C: 173.8, 94.3, 94.2, 73.3, 73.2, 72.8, 72.6, 72.4, 71.8, 71.2, 70.7, 63.2, 61.4, 34.4, 31.9, 29.8, 29.50, 29.44, 29.42, 29.4, 29.3, 24.7, 22.5, 14.0, 0.7, 0.01,–0.05 ; ν_{max}/cm^{-1}: 3432, 2925, 2854, 1742, 1251, 1215, 842, 748.

Experiment 77: 6-*O*-Docosanoic-6'-*O*-(9-bromononanoic)-2,3,4,2',3',4',-hexakis-*O*-(trimethylsilyl)-α,α'-trehalose (320)



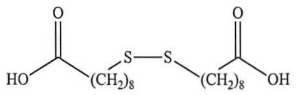
The procedure used in Experiment **76** was repeated to couple 6-*O*-docosanoic-2,3,4,2',3',4',-hexakis-*O*-(trimethylsilyl-α,α'-trehalose **(319)** (0.1000 g, 0.0911 mmol) with 9-bromononanoic acid **(311)** (0.0259 g, 0.1093 mmol) using 1-(3-Dimethyl aminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (0.0349 g, 0.1822 mmol) and 4-dimethylaminopyridine (0.0222 g, 0.1822 mmol) and powdered 4 A° molecular sieves in dry CH_2Cl_2 (1 mL) at r.t under nitrogen. The residue was purified by column chromatography eluting with petroleum ether/ethyl acetate (15:1) to give a thick oil of **(320)** (0.0860 g, 72%) [MS (MALDI-TOF): *m/z* 1338.01 $[(M+Na)^+]$, calculated for $C_{61}H_{127}BrNaO_{13}Si_6$: 1337.69]; δ_H: 4.93 (2H, d, *J* 3.0 Hz), 4.29 (2H, d, *J* 11.7 Hz), 4.07 (2H, dd, *J* 4.0, 11.9), 4.01 (2H, d, *J* 11.0 Hz), 3.93 (2H, t, *J* 8.9 Hz), 3.48–3.42 (4H, m), 3.41 (2H, t, *J* 6.9 Hz), 2.35 (4H, br.t, *J* 6.9 Hz), 1.86 (2H, q, *J* 7.6 Hz), 1.32 (4H, m), 1.26 (48H, m), 0.89 (3H, t, *J* 6.7 Hz), 0.16 (18H, s), 0.145 (18H, s), 0.138 (18H, s); δ_C: 173.6, 173.5, 94.2, 73.3, 73.1, 72 .8, 72.6, 71.8, 70.2, 70.7, 63.2, 63.1, 33.9, 33.7, 32.6, 31.8, 29.7, 29.50, 29.47, 29.42, 29.4, 29.30, 24.7, 24.5, 22.5, 14.9, 0.7, 0.58, 0.01,–0.05; ν_{max}/cm^{-1}: 2925, 2854, 1742, 1457, 1251, 1212, 966, 862, 748, 683.

Experiment 78: 6-*O*-Docosanoic-6'-*O*-(9-mercaptononanoic)-2,3,4,2',3',4',-hexakis-*O*-(trimethylsilyl)-α,α'-trehalose (321)



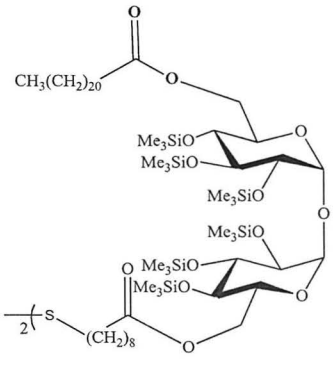
6-*O*-Docosanoic-6'-*O*-(9bromononanoic)-2,3,4,2',3',4',-hexakis-*O*-(trimethylsilyl)-α,α'-trehalose **(320)** (0.025 g, 0.019 mmol) in dry acetonitrile (1 mL) was added over 1 hr to a stirred solution of *bis*(tributyltin)sulphide (0.083 g, 0.012 mmol), TBAF (0.114 mL, 0.114 mmol) and distilled water (0.2 mL) in dry acetonitrile (2 mL) at 20 °C. The reaction mixture was stirred for 20 hrs at room temperature. TLC contained several spots close to each other, no purification was performed.

Experiment 79: 9,9'-Disulfanediyldinonanoic acid (306)



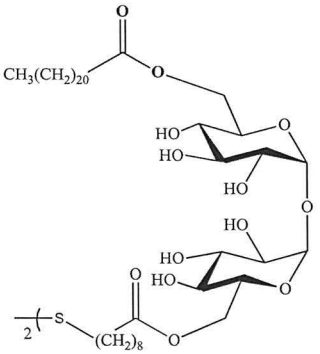
9-Mercaptononanoic acid **(312)** (0.25 g, 1.30 mmol) was added to a stirred solution of NaOH (0.057 g, 1.44 mmol), and KI (0.07 g, 0.42 mmol) in H_2O: DMF (8 mL, 1:1). Iodine crystals (0.083 g, 0.65 mmol) was added in portions until the yellow colour persisted, and then sodium sulphite powder was added until the yellow colour has completely disappeared. The reaction mixture was acidified with aq. solution of HCl (1 N), and the product was extracted with $CHCl_3$ (3 × 100 mL). The combined organic layers were dried over $MgSO_4$ and evaporated to give a crude product, which was purified by column chromatography eluting with petrol/ ethyl acetate (1:2) to give **(306)** (0.21 g, 85 %) [MS (MALDI-TOF): *m/z* 401.11 $[(M+Na)^+]$, calculated for $C_{18}H_{34}NaO_4S_2$: 401.17]; δ_H: 2.69 (4H, t, *J* 7.3 Hz), 2.36 (4H, t, *J* 7.3 Hz), 1.67 (8H, m), 1.33 (16H, br.m); δ_C: 179.9, 39.9, 33.9, 29.2, 29.1, 28.9, 28.8, 28.2, 24.6; ν_{max}/cm^{-1} : 2800-3038 (broad), 2921, 2851, 1699, 1470, 1411, 1310, 944, 721.

Experiment 80: 6-*bis*-*O*-Docosanoic-2,3,4,2',3',4',-hexakis-*O*-(trimethylsilyl)-α,α'-trehalose-10,11-dithio-eicosane-1,20-dioate (322)



1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (0.020 g, 0.109mmol) and 4-dimethylaminopyridine (0.013 g, 0.109 mmol) were added to a stirred solution of 6-O-docosanoic-2,3,4,2',3',4',-hexakis-O-(trimethylsilyl)-α,α'-trehalose **(319)** (0.060 g, 0.054 mmol) , 9,9'-disulfane diyldinonanoic acid **(306)** (0.010 g, 0.027 mmol) and powdered 4 A° molecular sieves in dry CH_2Cl_2 (1 mL) at r.t under nitrogen. The mixture was stirred at r.t for 2 days. When TLC showed no starting material was left, the solvent was evaporated to give a residue, which was purified by column chromatography on silica eluting with petroleum ether/ethyl acetate (15:1) to give a colourless thick oil of **(322)** (0.041 g, 58%) [MS (MALDI-TOF): *m/z* 2559.52 $[(M+Na)^+]$, calculated for $C_{122}H_{254}NaO_{26}S_2Si_{12}$: 2558.51]; δ_H: 4.92 (4H, d, *J* 2.9 Hz), 4.28 (4H, d, *J* 11.7 Hz), 4.06 (4H, dd, *J* 4.3, 11.8), 4.01 (4H, m), 3.93 (4H, t, *J* 8.9 Hz), 3.50 (4H, t, *J* 9.0 Hz), 3.43 (4H, dd, *J* 3.2, 9.3), 2.67 (4H, t, *J* 7.3 Hz), 2.43 (4H, dt, *J* 16.0, 7.3 Hz), 2.41 (4H, dt, *J* 16.0, 7.4 Hz), 1.67 (16H, m), 1.26 (96H, br.m), 0.88 (6H, t, *J* 6.7 Hz), 0.16 (36H, s), 0.145 (36H, s), 0.138 (36H, s); δ_C: 173.6, 173.5, 94.2, 73.3, 72.4, 71.7, 70.5, 70.4, 63.2, 63.1, 38.4, 34.0, 33.9, 31.7, 29.5, 29.50, 29.44, 29.42, 29.4, 29.30, 24.6, 24.5, 22.5, 13.9, 0.9, 0.0,–0.3; ν_{max}/cm^{-1}: 2925, 2854, 1738, 1457, 1251, 1167, 1112, 1100, 1009, 844, 735.

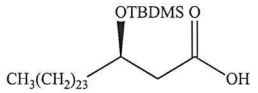
Experiment 81: 6-*bis*-*O*-Docosanoic-α,α'-trehalose-10,11-dithio-eicosane-1,20-dioate (323)



The procedure used in Experiment **43** was repeated to deprotect *bis*-6-*O*-docosanoic-2,3,4,2',3',4',-hexakis-*O*-(trimethylsilyl)-α,α'-trehalose-10,11-dithio-eicosane-1,20 dioate **(322)** (0.0251 g, 0.0098) using tetra-*n*-butylammonium fluoride (0.0588 mL, 0.0588 mmol, 1M) in dry THF (3 mL) at 5 °C under nitrogen. The residue was purified by column chromatography on silica eluting with $CHCl_3$/MeOH (10:1) to give colourless thick oil of **(323)** (0.0110, 67%) [MS (MALDI-TOF): *m/z* 1694.68 $[(M+Na)^+]$, calculated for $C_{86}H_{158}NaO_{26}S_2$: 1694.03]; δ_H ($CDCl_3$ + few drops of

CD_3OD): 5.07 (4H, d, *J* 3.4 Hz), 4.28 (8H, m), 3.94 (8H, m), 3.51 (4H, dd, *J* 2.7, 9.8), 3.34 (4H, m), 2.66 (4H, t, *J* 7.3 Hz), 2.34 (8H, m), 1.63 (16H, m), 1.26 (96H, br.m), 0.84 (6H, t, *J* 6.8 Hz), δ_C: 174.5, 93.4, 72.8, 71.5, 70.2, 69.9, 63.2, 63.1, 39.2, 34.0, 33.9, 31.7, 29.54, 29.50, 29.44, 29.42, 29.4, 29.30, 28.2, 24.7, 24.6, 22.5, 13.9; ν_{max}/cm^{-1}: 3369, 2920, 2851, 1730, 1465, 1377.

Experiment 82: *(R)*-3-((*tert*-Butyldimethylsilyl)oxy)heptacosanoic acid (330)



(i) *tert*-Butyldimethylchlorosilane (3.28 g, 21.8 mmol) was added to a stirred solution of imidazole (2.84 g, 41.7 mmol) and (*R*)-methyl-5-benzyloxy-3-hydroxy-pentanoate **(324)** (4.00 g, 16.8 mmol) in dry DMF (25 mL) at r.t. The reaction mixture was stirred at 45 °C for 18 hrs. When TLC showed the reaction was complete, the DMF was removed under high vacuum and then quenched with water (200 mL). The product was extracted with CH_2Cl_2 (3 × 150 mL), and the combined organic layers were dried over $MgSO_4$ and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give a colourless oil of methyl *(R)*-5-(benzyloxy)-3-((*tert*-butyldimethylsilyl)oxy)penta noate **(325)** (5.45 g, 92 %), $[\alpha]_D^{22}$ = -14.5 (*c*1.48, $CHCl_3$); δ_H: 7.38–7.27 (5H, m), 4.52 (2H, s), 4.33 (1H, m), 3.66 (3H, s), 3.65 (2H, t, *J* 6.3 Hz), 2.5 (2H, d, *J* 6.3 Hz), 1.86 (2H, m), 0.9 (9H, br.s), 0.06 (3H, s), 0.04 (3H, s); δ_C: 172.1, 138.6, 138.56, 128.5, 127.7, 127.5, 73.1, 67.1, 66.3, 51.5, 42.9, 27.4, 25.8, –4.2, –4.9; ν_{max}/cm^{-1}: 2953, 2929, 2857,1741, 1437, 1438, 1254, 1168, 1098, 836, 776, 697.

(ii) Palladium 10% on carbon (1.0 g) was added to a stirred solution of methyl *(R)*-5-(benzyloxy)-3-((*tert*-butyldimethylsilyl)oxy)pentanoate **(325)** (5.40 g, 15.3 mmol) in IMS/ THF 1:1 (100 mL), and the solution was stirred under hydrogen gas for 2 days. When TLC showed no starting material was left, the mixture was filtered on a pad of celite and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (5:1) to give colourless thick oil of methyl *(R)*-3-((*tert*-butyldimethylsilyl)oxy)-5-hydroxypent-anoate **(326)** (3.80 g, 95 %), $[\alpha]_D^{22}$ = – 2.98 (*c* 1.24, $CHCl_3$); δ_H: 4.38 (1H, m), 3.86 (2H, m), 3.68 (3H, s), 2.62 (1H, dd, *J* 6.4, 14.9 Hz), 2.54 (1H, dd, *J* 6.56, 14.9 Hz), 1.91–1.83 (1H, m), 1.78–1.69 (2H, m), 0.89 (9H, br.s), 0.11 (3H, s), 0.07 (3H, s); δ_C: 171.8, 68.2, 59.6, 51.6, 42.1, 40.1,

38.8, 25.7,–4.8, –4.9; ν_{max}/cm^{-1}: 3447, 2954, 2857, 1741, 1473, 1438, 1256, 1163, 1090, 837, 777.

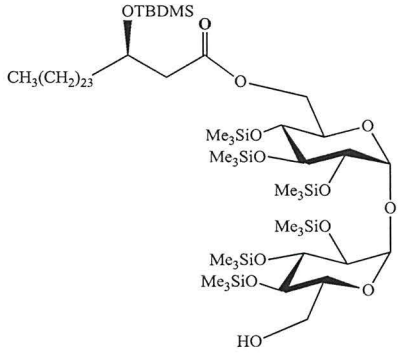
(iii) The procedure in Experiment **5** was repeated to oxidize methyl *(R)*-3-((*tert*-butyldimethylsilyl)oxy)-5-hydroxypentanoate **(326)** (1.30 g, 4.96 mmol) using pyridiniumchlorochromate (PCC) (2.67 g, 2.5 eq, 12.4 mmol) in CH_2Cl_2 (300 mL). The crude product was purified by chromatography petrol/ethyl acetate (5:1) to give a colourless oil of methyl *(R)*-3-((*tert*-butyldimethylsilyl)oxy)-5-oxopentanoate **(327)** (1.25 g, 96%), $[\alpha]_D^{22} = -22.5$ (*c* 2.62, $CHCl_3$); δ_H: 9.80 (1H, t, *J* 1.6 Hz), 4.64 (1H, m), 3.69 (3H, s), 2.67 (2H, m), 2.54 (2H, dd, *J* 3.9, 6.1 Hz), 0.86 (9H, s), 0.081 (3H, s), 0.08 (3H, s); δ_C: 200.9, 65.0, 51.7, 50.9, 42.4, 25.6,–4.8; ν_{max}/cm^{-1}: 2955, 2858, 1738, 1473, 1438, 1256, 1167, 837, 778.

(iv) The procedure used in Experiment **1** was repeated in order to couple *(R)*-methyl 3-((*tert*-butyldimethylsilyl)oxy)-5-oxopentanoate **(327)** (0.60 g, 2.30 mmol) with 5-(docosane-1-sulfonyl)-1-phenyl-1H-tetrazole **(328)** (1.43 g, 2.76 mmol) using lithium *bis*(trimethylsilyl)amide (3.91 mL, 4.15 mmol, 1.06 M) in dry THF (15 mL) under nitrogen at -15 °C. The crude product was purified by column chromatography eluting with petrol/ ethyl acetate (20:1) to give a mixture of (*E,Z*) alkenes. This was hydrogenated using palladium on carbon 10% (0.80 g) as a catalyst under hydrogen atmosphere. Column chromatography eluting with petrol/ ethyl acetate (20:1) gave a semi-solid of methyl *(R)*-3-((*tert*-butyldimethylsilyl)oxy)heptacosanoate **(329)** (0.89 g, 94.5 %), $[\alpha]_D^{21} = -12.98$ (*c* 1.24, $CHCl_3$), [HRMS (EI^+): *m/z* 497.4395 [$(M–Bu^t)^+$], calculated for $C_{30}H_{61}O_3Si$: 497.4390]; δ_H: 4.14 (1H, m), 3.67 (3H, s), 2.41 (2H, dd, *J* 5.1, 14.9 Hz), 1.47 (2H, m), 1.26 (44H, s), 0.90 (3H, t, *J* 6.9 Hz), 0.89 (9H, s), 0.06 (3H, s), 0.04 (3H, s); δ_C: 172.4, 69.5, 51.5, 42.5, 37.6, 31.9, 29.7, 29.5, 25.8, 25.0, 22.7, 14.1, –4.5, –4.85. ν_{max}/cm^{-1}: 2955, 2851, 1736, 1463, 1080.

(vi) The procedure used in Experiment **19** was repeated using (*R*)-methyl-3-((*tert*-butyl-dimethylsilyl)oxy)heptacosanoate **(329)** (0.80 g, 1.44 mmol) and lithium hydroxide monohydrate (0.89 g, 21.6 mmol, 15 mol eq.) in THF (12 mL), water (2 mL), MeOH (1.5 mL). The crude product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give a semi-solid of **(330)** (0.67 g, 85.6%), $[\alpha]_D^{21} = -3.23$ (*C* 0.5, $CHCl_3$) [Found $(M+Na)^+$: 563.20, $C_{33}H_{68}NaO_3Si$ requires: 563.42]; δ_H: 4.09 (1H, m), 2.53 (1H, dd, *J* 5.3, 15.3 Hz), 2.41 (1H, dd, *J* 5.4, 15.4 Hz), 1.54–1.50 (4H, m), 1.26

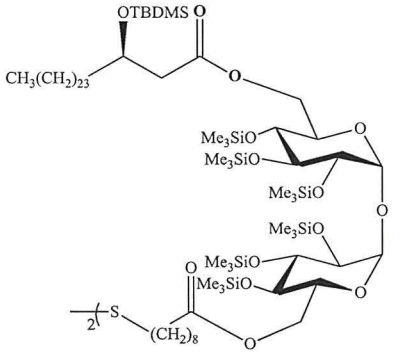
(42H, br.m), 0.91 (3H, t, *J* 6.9 Hz), 0.89 (9H, s), 0.12 (3H, s), 0.11(3H, s); δ_C: 69.6, 41.4, 37.0, 31.9, 29.71, 29.63, 29.55, 29.52, 29.37, 25.7, 25.3, 22.7, 14.1, –4.5, –4.9; ν_{max}/cm^{-1}:3749, 2917, 2851, 1708, 1464, 1253, 827.

Experiment 83: 6-*O*-(*R*)-3-((*tert*-butyldimethylsilyl)oxy)heptacosanoic-2,3,4,2',3', 4',-hexakis-*O*- (trimethylsilyl)-α,α'-trehalose (331)



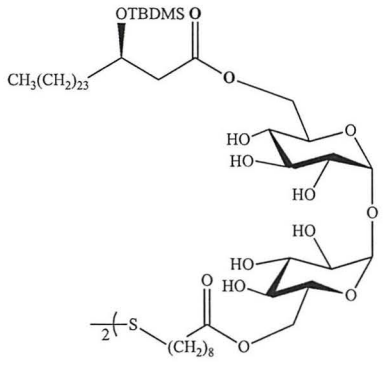
The procedure in Experiment **76** was repeated using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (0.222 g, 1.161 mmol), 4-dimethylamino-pyridine (DMAP) (0.141 g, 1.161 mmol), (*R*)-3-((*tert*-butyldimethylsilyl)oxy)hepta-cosanoic acid **(330)** (0.156 g, 0.290 mmol), 2,3,4,2',3',4'-hexaakis-O-(trimethyl silyl)-α,α'-trehalose **(148)** (0.450 g, 0.580 mmol) and powdered 4 A° molecular sieves in dry dichloromethane (3 mL). Column chromatography on silica eluting with petrol/ethyl acetate (15:1) gave a colourless oil of **(331)** (0.240 g, 64%), [MS (MALDI-TOF): *m/z* 1320.33 [$(M+Na)^+$], calculated for $C_{63}H_{136}NaO_{13}Si_7$: 1319.82]; δ_H: 4.92 (1H, d, *J* 3.1 Hz), 4.89 (1H, d, *J* 3.1 Hz), 4.29 (1H, dd, *J* 2.0, 11.7 Hz), 4.07 (1H, m), 4.02 (2H, m), 3.91 (2H, dt, *J* 2.6, 9 Hz), 3.86 (1H, dt, *J* 3.3, 9.4 Hz), 3.7 (2H, m), 3.45 (2H, m), 3.44 (2H, m), 2.53 (1H, dd, *J* 6.8, 15.3 Hz), 2.44 (1H, dd, *J* 6.0, 15.3 Hz), 1.5 (2H, m), 1.26 (46H, br.m), 0.90–0.87 (12H, m, including a singlet at 0.86 ppm), 0.16 (18H, s), 0.15 (18H, s), 0.14 (18H, s), 0.06 (3H, s), 0.04 (3H, s); δ_C: 171.8, 94.5, 94.3, 73.4, 73.3, 72.9, 72.8, 72.6, 71.9, 71.4, 70.7, 69.3, 63.4, 61.6, 60.3, 42.5, 36.4, 31.9, 31.5, 29.8, 29.50, 29.44, 29.42, 29.4, 29.30, 24.7, 22.5, 14.0, 0.7, 0.01,–0.05, –4.6, –4.8 ; ν_{max}/cm^{-1}: 2926, 2855, 1741, 1463, 1251, 1213, 1111, 1009, 842, 747.

Experiment 84: 6-*Bis*-*O*-(*R*)-3-((*tert*-Butyldimethylsilyl)oxy)heptacosanoic-2,3,4, 2',3',4',-hexakis-*O*-(trimethylsilyl)-α,α'-trehalose-10,11-dithio-eicosane-1,20-dioate (332)



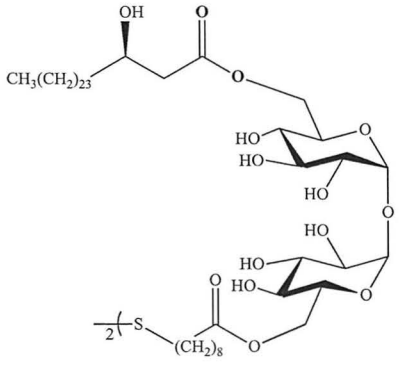
1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (0.059 g, 0.308 mmol) and 4-dimethylaminopyridine (0.037 g, 0.308 mmol) were added to a stirred solution of 6-*O*-(*R*)-3-((*tert*-butyldimethylsilyl)oxy)heptacosanoic-2,3,4,2',3',4',-hexa kis-*O*-(trimethylsilyl)-α,α'-trehalose **(331)** (0.201 g, 0.154 mmol), 9,9'-disulfane-diyl dinonanoic acid **(306)** (0.029 g, 0.077 mmol) and powdered 4 A° molecular sieves in dry CH_2Cl_2 (3 mL) at r.t under nitrogen. The mixture was stirred at r.t for 3 days. When TLC showed no starting material was left, the solvent was evaporated under reduced pressure to give a residue, which was purified by column chromatography on silica eluting with petroleum ether/ethyl acetate (15:1) to give colourless thick oil of **(332)** (0.145, 65%) [MS (MALDI-TOF): *m/z* 2958.77 $[(M+Na)^+]$, calculated for $C_{144}H_{302}NaO_{28}S_2Si_{14}$: 2958.83]; δ_H: 4.92 (2H, d, *J* 3.0 Hz), 4.90 (2H, d, *J* 3.0 Hz), 4.29 (2H, dd, *J* 2.0, 5.1 Hz), 4.26 (2H, dd, *J* 1.8, 4.9 Hz), 4.15 (2H, m), 4.07 (2H, t, *J* 4.1 Hz), 4.05 (2H, t, *J* 4.0 Hz), 4.03 (4H, m), 3.93 (4H, dt, *J* 1.5, 9.2 Hz), 3.48 (4H, dd, *J* 5.9, 13.9 Hz), 3.44 (4H, dt, *J* 3.4, 9.8 Hz), 2.67 (4H, t, *J* 7.3 Hz), 2.53 (2H, dd, *J* 6.9, 15.3 Hz), 2.43 (2H, dd, *J* 5.9, 15.3 Hz), 2.38 (2H, dt, *J* 16.0, 7.4 Hz), 2.34 (2H, dt, *J* 15.9, 7.4 Hz), 1.67 (8H, m), 1.26 (110H, m), 0.90–0.87 (24H, m, including a singlet at 0.86 ppm), 0.16 (36H, s), 0.15 (36H, s), 0.14 (36H, s), 0.06 (6H, s), 0.03 (6H, s); δ_C: 173.8, 171.6, 94.5, 94.4, 73.5, 73.4, 72.6, 71.9, 70.7, 69.3, 63.4, 63.2, 42.0, 39.3, 39.0, 34, 33.9, 31.7, 29.5, 29.50, 29.44, 29.42, 29.4, 29.30, 24.6, 24.5, 22.5, 13.9, 1.05, 1.04, 0.88, 0.18, 0.15, –4.6, –4.8; ν_{max}/cm^{-1}: 2924, 2853, 1743, 1464, 1251, 1215, 844, 748.

Experiment 85: 6-*Bis*-*O*-(*R*)-3-((*tert*-Butyldimethylsilyl)oxy)heptacosanoic-α,α'-trehalose-10,11-dithio-eicosane-1,20-dioate (333)



The same procedure used in Experiment **43** was repeated to deprotect 6-*bis*-O-(*R*)-3-((*tert*-butyldimethylsilyl)oxy)heptacosanoic-2,3,4,2',3',4',-hexakis-O-(trimethylsilyl)-α,α'-trehalose-10,11-dithio-eicosane-1,20-dioate **(332)** (0.112 g, 0.038 mmol) using tetra-*n*-butylammonium fluoride (0.228 mL, 0.228 mmol, 1M) in dry THF (12 mL) at 5 °C under nitrogen. The residue was purified by column chromatography on silica eluting with $CHCl_3$/MeOH (5:1) to give a syrup of **(333)**; (0.061, 78%), [MS (MALDI-TOF): *m/z* 2094.61 $[(M+Na)^+]$, calculated for $C_{108}H_{206}NaO_{28}S_2Si_2$: 2094.35]; δ_H ($CDCl_3$ + few drops of CD_3OD): 5.04 (4H, t, *J* 4 Hz), 4.29 (8H, m), 4.05 (2H, m), 3.98 (4H, m), 3.88 (4H, m), 3.5 (4H, m), 3.32 (4H, m), 2.65 (4H, t, *J* 7.3 Hz), 2.53 (4H, m), 2.35 (4H, t, *J* 7.5 Hz), 1.61 (8H, m), 1.26 (108H, m), 0.90–0.87 (24H, m, including a singlet at 0.86 ppm), 0.06 (6H, s), 0.03 (6H, s); δ_C: 174.3, 172.4, 93.3, 72.8, 71.5, 70.1, 69.9, 69.8, 69.3, 63.1, 42.3, 39.2, 37.4, 33.9, 31.7, 29.6, 29.5, 29.4, 29.45, 29.4, 29.30, 24.9, 24.8, 23.7, 22.5, 13.9, –4.8, –5.0; ν_{max}/cm^{-1}: 3340, 2923, 2853, 1738, 1457, 1252, 834.

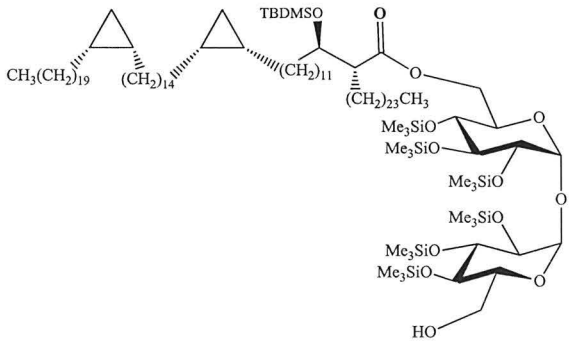
Experiment 86: 6-*Bis*-*O*-(*R*)-3-(Hydroxy)heptacosanoic-α,α'-trehalose-10,11-dithio-eicosane-1,20-dioate (334)



The procedure used in Experiment **44** was repeated to deprotect ester **(333)** (0.040 g, 0.024 mmol) using pyridine (0.08 mL) and hydrogen fluoride-pyridine complex as ~70% (0.50 mL) in dry THF (6 mL). The crude product was purified by column

chromatography eluting with $CHCl_3$/MeOH 4:1 to give a syrup of **(334)**; (0.026, 72%), [MS (MALDI-TOF): *m/z* 1865.79 [$(M+Na)^+$], calculated for $C_{96}H_{178}NaO_{28}S_2$: 1866.18]; ; δ_H ($CDCl_3$ + few drops of CD_3OD): 5.05 (4H, t, *J* 2.7 Hz), 4.59 (2H, d, *J* 10.7 Hz), 4.27 (4H, dd, *J* 8.1, 12.8 Hz), 4.25 (4H, m), 3.98 (8H, m), 3.55 (4H, m), 3.32 (4H, m), 2.64 (4H, t, *J* 7.2 Hz), 2.53 (4H, dd, *J* 8.1, 15.1 Hz), 2.30 (4H, t, *J* 7.6 Hz), 1.61 (8H, m), 1.26 (116H, m), 0.90-0.87 (6H, t, *J* 6.5 Hz); δ_C: 174.4, 173.8, 93.7, 72.9, 72.5, 71.6, 71.2, 70.5, 70.1, 70, 69.9, 68.1, 63.9, 63.1, 41.6, 39.3, 36.9, 33.9, 31.8, 29.6, 29.53, 29.44, 29.42, 29.38, 29.32, 28.8, 28.4, 28.2, 25.4, 24.6, 24.5, 22.5, 13.9; ν_{max}/cm^{-1}: 3369, 2922, 2852, 1723, 1465, 1377, 1251, 1147, 734.

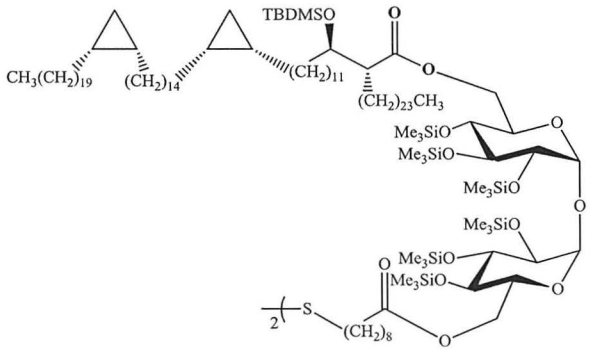
Experiment 87: 6-*O*-(*R*)-2-((*R*)-1-(*tert*-Butyldimethylsilanyloxy)-12-{(1*R*,2*S*)-2-[14-((1*R*,2*S*)-2-eicosylcyclopropyl)tetradecyl]cyclopropyl}dodecyl)hexacosanoic-2,3,4,2',3',4'-hexakis-*O*-(trimethylsilyl)-α,α'-trehalose (336)



The procedure used in Experiment **42** was repeated using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (0.094 g, 0.490 mmol), 4-dimethyl amino pyridine (DMAP) (0.059 g, 0.490 mmol), (*R*)-2-((*R*)-1-(*tert*-butyldimethyl- silanyloxy)-12-{(1*S*,2*R*)-2-[14-((1*S*,2*R*)-2-eicosylcyclopropyl)tetradecyl]cyclopropyl}- dodecyl)-hexacosanoic acid **(335)** (0.153 g, 0.122 mmol), 2,3,4,2',3',4'-hexaakis-*O*-(tri methylsilyl)-α,α'-trehalose **(148)** (0.190 g, 0.245 mmol) and powdered 4 A° molecular sieves in dry CH_2Cl_2 (3 mL). The residue was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a colourless oil of **(336)**, (0.132, 54%) [MS (MALDI-TOF): *m/z* 2031.66 [$(M+Na)^+$], calculated for $C_{114}H_{234}NaO_{13}Si_7$: 2032.66]; δ_H: 4.91 (1H, d, *J* 3.1 Hz), 4.84 (1H, d, *J* 3.0 Hz), 4.35 (1H, dd, *J* 2.1, 11.8 Hz), 4.08 (1H, dd, *J* 4.1, 11.8 Hz), 3.99 (1H, dt, *J* 2.2, 6.0 Hz), 3.96–3.94 (1H, m), 3.91 (2H, dt, *J* 2.2, 6.6 Hz), 3.84 (1H, dt, *J* 3.5, 9.4 Hz), 3.72–3.67 (2H, m), 3.49 (2H, dt, *J* 5.6, 9.2 Hz), 3.43 (1H, dd, *J* 3.1, 9.4 Hz), 3.4 (1H, dd, *J* 3.2, 9.4 Hz), 2.55 (1H, ddd, *J* 3.4, 5.5, 9.3, Hz), 1.62–160 (2H, m), 1.38–1.14 (134H, v br.s), 0.88 (6H, t, *J* 6.9 Hz),

0.88 (9H, s), 0.67–0.64 (4H, m), 0.57 (2H, dt, *J* 3.9, 7.8 Hz), 0.17 (9H, s), 0.16 (9H, s), 0.16 (9H, s), 0.158 (9H, s), 0.152 (9H, s), 0.14 (9H, s), 0.12 (9H, s), 0.06 (3H, s), 0.05(3H, s), –0.33 (2H, q, J 5.1 Hz); δ_C: 174.1, 94.4, 94.3, 73.5, 73.4, 72.8, 72.7, 72.5, 71.8, 71.6, 70.7, 62.5, 61.8, 51.8, 33.5, 31.9, 30.3, 29.9, 29.8, 29.75, 29.7, 29.6, 29.56, 29.3, 29.2, 29, 28.8, 28.0, 27.6, 26.4, 25.8, 24.8, 22.6, 15.6, 14.1, 10.9, 1.1, 1.0, 0.9, 0.86, 0.2, 0.0, –4.5, –4.7; ν_{max}/cm^{-1}: 3073, 2924, 2852, 1742, 1467, 1376, 1251, 1172, 1063, 1001, 872, 746, 710.

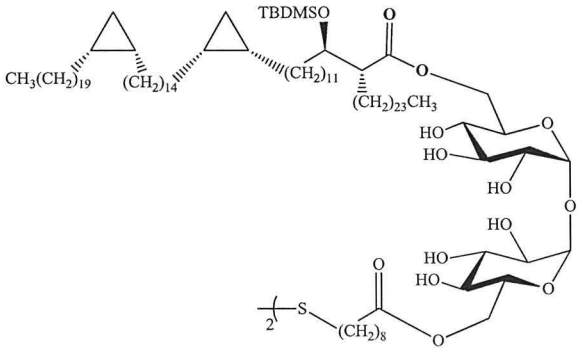
Experiment 88: 6-*bis*-*O*-(*R*)-2-((*R*)-1-(*tert*-Butyldimethylsilanyloxy)-12-{(1*R*,2*S*)-2-[14-((1*R*,2*S*)-2-eicosylcyclopropyl)-tetradecyl]-cyclopropyl}dodecyl)hexacosanoic-2,3,4,2',3',4'-hexakis-O-(trimethylsilyl)-α,α'-trehalose-10,11-dithio-eicosane-1,20-dioate (337)



The procedure used in Experiment **80** was repeated using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (0.0219 g, 0.1152 mmol), 4-dimethyl-aminopyridine (0.0140 g, 0.1152 mmol), 6-O-(*R*)-2-((*R*)-1-(*tert*-butyldimethyl-silanyloxy)-12-{(1*R*,2*S*)-2-[14-((1*R*,2*S*)-2-eicosylcyclopropyl)tetradecyl]cycloproPyl} dodecyl)hexacosanoic-2,3,4,2',3',4'-hexakis-O-(trimethylsilyl)-α,α'-trehalose **(336)**, (0.1153 g, 0.0576 mmol), 9,9'-disulfanediyldinonanoic acid **(306)** (0.0108 g, 0.0286 mmol) and powdered 4 A° molecular sieves in dry CH_2Cl_2 (2.5 mL). The residue was purified by column chromatography on silica eluting with petroleum ether/ethyl acetate (15:1) to give colourless thick oil of **(337)**; (0.1030, 83 %) [MS (MALDI-TOF): *m/z* 4380.83 $[(M+Na)^+]$, calculated for $C_{246}H_{498}NaO_{28}S_2Si_{14}$: 4380.36]; δ_H: 4.92 (2H, d, *J* 3.0 Hz), 4.86 (2H, d, *J* 3.0 Hz), 4.36 (2H, dd, *J* 1.6, 10.5 Hz), 4.27 (2H, dd, *J* 1.5, 11.5 Hz), 4.07 (2H, dd, *J* 3.8, 11.6 Hz), 4.05–4.02 (6H, m), 3.97 (2H, m), 3.95 (2H, m), 3.89 (2H, dd, *J* 1.9, 9.0 Hz), 3.5 (4H, dt, *J* 5.3, 9.0 Hz), 3.44 (2H, dd, *J* 3.0, 9.3 Hz), 3.39 (2H, dd, *J* 3.0, 9.3 Hz), 2.67 (4H, t, *J* 7.3 Hz), 2.55 (2H, ddd, *J* 3.4, 5.0, 9.9 Hz), 2.38 (2H, dt, *J* 16.1, 7.4 Hz), 2.34 (2H, dt, *J* 16.0, 7.3 Hz),1.62–160 (8H, m), 1.38–1.14 (296H, v br.m),

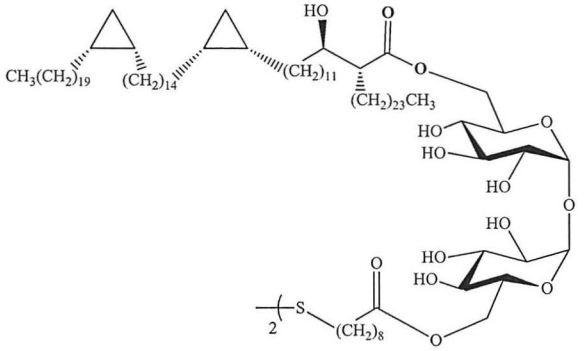
0.88 (12H, t, *J* 6.9 Hz), 0.88 (18H, s), 0.67–0.65 (8H, m), 0.57 (4H, dt, *J* 3.9, 7.8 Hz), 0.17 (18H, s), 0.16 (18H, s), 0.16 (18H, s), 0.15 (18H, s), 0.14 (18H, s), 0.12 (18H, s), 0.06 (6H, s) , 0.05 (6H, s), –0.34 (4H, q, *J* 5.1 Hz); δ_C: 173.9, 173.7, 94.7, 94.6, 73.6, 73.4, 73.3, 72.8, 72.6, 71.8, 70.7, 70.6, 63.3, 62.4, 51.8, 39, 34.1, 33.4, 31.9, 29.8, 29.75, 29.65, 29.6, 29.58, 29.2, 29.1, 29, 28.7, 28.5, 28.1, 26.3, 25.8, 25.1, 24.8, 22.7, 15.8, 14.1, 10.9, 1.08, 1.06, 0.9, 0.88, 0.2, 0.1, –4.5, –4.7; ν_{max}/cm^{-1}: 2924, 2853, 1743, 1464, 1362, 1251, 1165, 1112, 1077, 1010, 964, 843, 749.

Experiment 89: 6-*bis*-*O*-(*R*)-2-((*R*)-1-(*tert*-Butyldimethylsilanyloxy)-12-{(1*R*,2*S*)-2-[14-((1*R*,2*S*)-2-eicosylcyclopropyl)-tetradecyl]cyclopropyl}dodecyl)-hexacosanoic-α,α'-trehalose-10,11-dithio-eicosane-1,20-dioate (338)



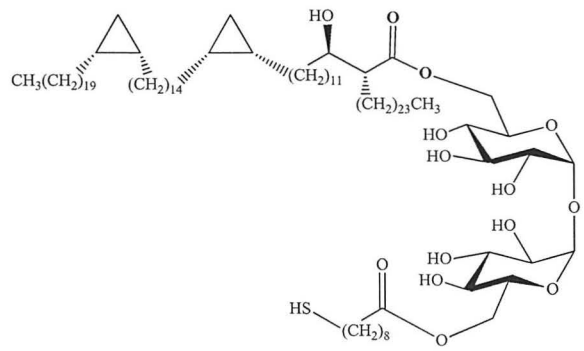
The same procedure used in Experiment **43** was repeated to deprotect ester **(337)** (0.090 g, 0.020 mmol, 1M) using tetra-*n*-butylammonium fluoride (0.123 mL, 0.123 mmol, 1M) in dry THF (8 mL) at 5 °C under nitrogen. The residue was purified by column chromatography on silica eluting with $CHCl_3$/MeOH (5:1) to give a syrup of **(338)** (0.058 g, 80%) [MS (MALDI-TOF): *m/z* 3515.45 $[(M+Na)^+]$, calculated for $C_{210}H_{402}NaO_{28}S_2Si_2$: 3515.89]; δ_H ($CDCl_3$ + few drops of CD_3OD): 5.05 (2H, d, *J* 3.3 Hz), 5.03 (2H, d, *J* 3.1 Hz), 4.55 (4H, m), 4.37 (4H, m), 3.94 (4H, t, *J* 10.8 Hz), 3.85 (4H, m), 3.7 (2H, m), 3.5 (2H, dd, *J* 3.7, 9.9 Hz), 3.46 (2H, dd, *J* 3.5, 10.0 Hz), 3.37 (2H, m), 3.30 (2H, dd, *J* 3.0, 10.0 Hz), 2.64 (4H, t, *J* 7.4 Hz), 2.51 (2H, ddd, *J* 3.6, 6.4, 10.2, Hz), 2.31 (4H, t, *J* 7.4 Hz), 1.64–160 (4H, m), 1.57–152 (4H, m), 1.38–1.14 (284H, v br s), 0.88 (12H, t, *J* 6.9 Hz) , 0.88 (18H ,s), 0.65–0.60 (8H, m), 0.52 (4H, dt, *J* 4.0, 8.0 Hz),0.00 (6H, s) ,– 0.01(6H, s), –0.34 (4H, q, *J* 5.0 Hz); δ_C: 175.1, 174.4, 93.7, 93.4, 73.2, 72.9, 72.8, 71.5, 71.4, 70.2, 70.1, 70.0, 69.9, 63.1, 62.7, 51.6, 39.1, 33.9, 33.1, 31.8, 29.78, 29.75, 29.64, 29.61, 29.6, 29.2, 29.1, 29, 28.6, 28.3, 28.2, 27.6, 26.9, 25.6, 24.6, 24.1, 22.5, 15.6, 13.9, 10.7, –4.7, –5.1; ν_{max}/cm^{-1}: 3367, 2921, 2852, 1738, 1459, 1251, 1169, 1071, 984, 844.

Experiment 90: 6-*bis*--*O*-(*R*)-2-((*R*)-1-(Hydroxy)-12-{(1*R*,2*S*)-2-[14-((1*R*,2*S*)-2-eicosylcyclopropyl)-tetradecyl]-cyclopropyl}dodecyl)hexacosanoic-α,α'-trehalose-10,11-dithio-eicosane-1,20-dioate (339)



The procedure used in Experiment **44** was repeated to deprotect **(338)** (0.045 g, 0.012 mmol) using pyridine (0.080 mL) and hydrogen fluoride-pyridine complex as ~70% (0.50 mL) in dry THF (6 mL). The crude product was purified by column chromatography eluting with $CHCl_3$/MeOH (4:1) to give a syrup of **(339)** (0.028 g, 67%), [HRMS (MALDI-TOF): *m/z* 3287.7198 $[(M+Na)^+]$, calculated for $C_{198}H_{374}NaO_{28}S_2$: 3287.7175]; δ_H ($CDCl_3$ + few drops of CD_3OD): 5.02 (4H, t, *J* 3.1 Hz), 4.64 (2H, br.d, *J* 11.9 Hz), 4.33 (2H, br.d, *J* 12.0 Hz), 4.18 (4H, m), 4.11–4.02 (4H, m), 4.01 (2H, m), 3.81 (4H, dd, *J* 8.8, 14.9 Hz), 3.65 (2H, m), 3.94 (4H, br.d, *J* 10.0 Hz), 3.24 (2H, t, *J* 9.3 Hz), 2.64 (4H, t, *J* 7.4 Hz), 2.38 (2H, m), 2.31 (4H, t, *J* 7.3 Hz), 1.64–160 (4H, m), 1.57–152 (4H, m), 1.38–1.14 (284H, br.s), 0.83 (12H, t, *J* 6.6 Hz), 0.64–0.60 (8H, m), 0.51 (4H, dt, *J* 3.8, 7.6 Hz), –0.38 (4H, q, *J* 4.9 Hz); δ_C: 175.4, 174.3, 94.1, 94.0, 72.7, 72.4, 71.3, 70.8, 70.3, 70.1, 70.0, 69.9, 64.0, 63.1, 52.3, 39.3, 34.6, 33.9, 31.8, 29.5, 30.1, 30.0, 29.8, 29.7, 29.6, 29.5, 29.2, 29.1, 29, 28.6, 28.2, 27.1, 25.1, 24.6, 22.5, 15.6, 13.9, 10.7; ν_{max}/cm^{-1}: 3367, 2922, 2851, 1732, 1464, 1377, 719.

Experiment 91: 6-*O*--(*R*)-2-((*R*)-1-(Hydroxy)-12-{(1*R*,2*S*)-2-[14-((1*R*,2*S*)-2-eicosyl cyclopropyl)-tetradecyl]-cyclopropyl}-dodecyl)-hexacosanoic-6'-*O*-(9-mercaptono nanoic-α,α'-trehalose (340)



DL-Dithiothreitol (0.1500 g) was added to a stirred solution of **(339)** (0.0100 g, 0.0031 mmol) in chloroform (1.5 mL) followed by the addition of one drop of triethylamine under nitrogen. The flask was covered with aluminium foil, and stirred at room temperature for 48 hours. The solvent was evaporated and the crude product was purified by column chromatography eluting with $CHCl_3$/MeOH (4:1) to give a semi-solid of **(340)** (0.0065 g, 65%) [MS (MALDI-TOF): *m/z* 1656.68 [$(M+Na)^+$], calculated for $C_{99}H_{188}NaO_{14}S$: 1656.36]; δ_H ($CDCl_3$ + few drops of CD_3OD): 5.02 (2H, t, *J* 3.4 Hz), 4.56 (1H, br.d, *J* 11.3 Hz), 4.26 (1H, m), 4.08 (2H, m), 3.98 (1H, m), 3.91 (1H, m), 3.76 (2H, br.t, *J* 9.1 Hz), 3.68–3.62 (2H, m), 3.54 (1H, m), 3.47 (1H, m), 3.24 (1H, t, *J* 8.5 Hz), 2.46 (2H, t, *J* 7.2 Hz), 2.38 (1H, m), 2.29 (2H, t, *J* 7.6 Hz), 1.56–153 (4H, m), 1.38–1.14 (148H, v br s), 0.83 (6H, t, *J* 6.7 Hz), 0.64–0.60 (4H, m), 0.51 (2H, dt, *J* 3.8, 7.6 Hz), –0.38 (2H, q, *J* 4.8 Hz).

ELISA method

ELISA were carried out on 96-well flat-bottomed polystyrene micro-plates. Antigens were dissolved in hexane to give an antigen solution of concentration 15 μg/ml. 50 μl of this solution was added to each well, and the solvent was left to evaporate at room temperature. Control wells were coated with hexane (50 μl/well) only. Blocking was done by adding 400 μl of buffer (pH = 7.4) to each well, and the plates were incubated at 25 °C for 30 minutes. The buffer was aspirated and any excess buffer was flicked out until the plates were dry. Serum (1in 20 dilution in buffer)(50 μl / well) was added and incubated at 25 °C for 1 hour. The plates were washed with 400 μl buffer 3 times using an automatic washer, and any excess buffer was flicked out onto a paper towel until dry. Secondary antibody (diluted to the appropriate concentration) (50 μl / well) was added, and incubated at 25 °C for 30 minutes. The plates were again washed 3 times with 400 μl casein/PBS buffer using an automatic washer, and any excess buffer was again flicked out. OPD substrate (50 μl/ well) (*o*-phenylenediamine (1 mg/ml) and H_2O_2 (0.8 mg/ml) in 0.1 M citrate buffer) was then added, and the plates were incubated for a further 30 minutes at 25 °C. The colour reaction was terminated by adding 2.5 M H_2SO_4 (50 μl / well), and the absorbance was read at 492nm (This was carried out by Dr.A.Jones).

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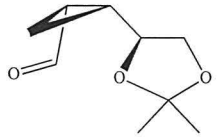
Appendix

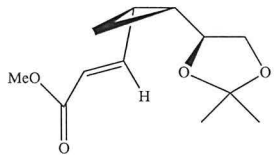
The appendix contains experiments reported before but repeated for this work, plus experiments with some minor variations of those reported in the full experimental section.

1: [(1*S*,2*R*)-2-(*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl]cyclopropanecarbaldehyde (175)



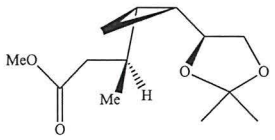
[(1*S*,2*R*)-2-(*S*)-2,2-dimethyl[1,3]dioxolan-4-ylcyclopropylmethanol **(174)** (15 g, 86 mmol) was added to a stirred suspension of pyredinium chlorochromate (43.2 g, 200 mmol) in CH_2Cl_2 (500 mL). The mixture was stirred for 2 h, when TLC showed all the starting material had reacted, then petrol/ethylacetate (10:1) 300 mL was added and filtered through pad of silica and celite and washed with 200 mL petrol/ethylacetate (10:1). The filtrate was evaporated to give a pale oil; this was purified by column chromatography eluting with petrol/ethyl acetate (1:1) to give a colourless oil of **(175)** (13 g, 88%).[115]

2: (*E*)-3-[(1*R*,2*R*)-2-(*S*)-2,2-dimethyl[1,3]dioxolan-4yl)cyclopropyl]acrylicacid methyl ester (176)



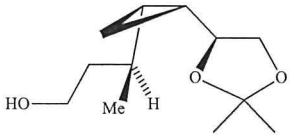
Methyl(triphenyl phosphoranylidene)acetate (26.6 g, 88 mmol) was added in portions to a stirred solution of **(175)** (12.6 g, 37 mol) in toluene (300 mL) at 10 °C. The mixture was stirred at room temperature for 16 h. The solvent was evaporated and the residue refluxed with petrol/ethyl acetate (1:1, 150 mL) for 30 min. The precipitate was filtered and washed with ethyl acetate (100 mL). The solvent was evaporated and the residue was purified by column chromatography petrol/ethyl acetate (5:2) to give a colourless oil of **(176)** (13.6 g, 83 %).[115]

3: (*S*)-3-[(1*R*,2*R*)-2-(*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl) cyclopropyl] butyric acid methyl ester (99)



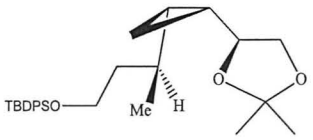
Methyl magnesium bromide (59.2 mL, 176 mmol) was added dropwise to a stirred suspension of copper bromide (12.74 g, 88 mmol) in dry THF (200 mL) under nitrogen at -40 °C. The mixture was stirred for 30 min, then **(176)** (13.4 g, 58 mmol) in dry THF (50 mL) was added dropwise under nitrogen at -30 ^{0}C. The reaction mixture was allowed to stir at -5 °C over 2 h when TLC showed no starting material was left, then cooled to -30 °C and quenched with sat. aq. NH_4Cl (15 mL). The product was extracted with ethyl acetate (3 × 100 mL) and the combined organic layers were washed with brine (30 mL), then dried over $MgSO_4$ and evaporated to give yellow oil which was purified and separated by column chromatography eluting with petrol/ethyl acetate (5:2) to give a pale yellow oil of **(99)** (9.1 g, 64 %).[115]

4: (*S*)-3-[(*1R,2R*)-2-(S)-2,2-dimethyl-[1,3]dioxolan-4-yl) cyclopropyl] butan-1-ol (177)



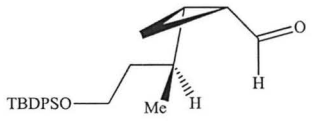
(*S*)-3-[(*1R,2R*)-2-(*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)cyclopropyl]butyric acid methyl ester **(99)** (9 g, 37 mmol) in dry THF (25 mL) was added dropwise to a stirred suspension of lithium aluminium hydride (2.11 g, 55 mmol) in dry THF (80 mL) under nitrogen at 10 °C. The mixture was refluxed for 1 h when TLC showed no starting material was left, then cooled to 0 °C and quenched carefully with saturated aqueous sodium sulphate dehydrate (20 mL) until a white precipitate formed. The precipitate was filtered through a pad of celite and washed with THF (150 mL), then evaporated to give crude product, which was purified by chromatography (petrol/ethyl acetate 1:1) giving **(177)** as a colourless oil (7.15 g, 90%).[115]

5: {(*S*)-3-[(1*R*,2*R*)-2-(*S*)-2,2dimethyl-[1,3]dioxolan-4-yl)-cyclopropyl] -butoxy}-2,2-dimethyl-1,1-diphenyl-ethyl)silane (178)



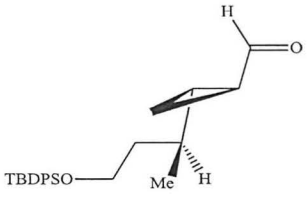
The alcohol (**177**) (7.05 g, 30 mmol) in DMF (25 mL) and imidazole (3.36 g, 42 mmol) under nitrogen at 0 °C. The mixture was stirred for 16 h when TLC showed all the starting material had reacted. DMF was removed by distillation (under high vacuum), then the reaction was quenched with water (15mL) and the product was extracted with CH_2Cl_2 (100 mL). The aqueous layer was re-extracted with CH_2Cl_2 (3 × 20 mL), and then the combined organic layers were evaporated to give a crude oil, which was purified by column chromatography eluting with petrol/ether (5:1) to give a colourless oil of **(178)** (13.85 g, 93%).[115]

6: *Cis*-(*1R,2R*)-2-[(*S*)-3-(2,2-Dimethyl-1,1-diphenylpropylsilanoxy)-1-methyl prop yl] cyclopropane carbaldehyde (100)



Periodic acid (17.30 g, 76.05 mmol) was added to stirred solution of (**178**) (13.75 g, 30.42 mmol) in dry ether (200 mL) at r.t under nitrogen. The mixture was stirred at r.t for 16 h. When TLC showed no starting material, then filtered through a pad of celite and washed with ethyl acetate. The solvent was evaporated to yield an oil which was purified by column chromatography eluting with petrol/ethyl acetate (15:1) giving **(100)** (10.4 g, 90%).[115]

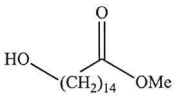
7: *Trans*-(1*S*,2*R*)-2-[(*S*)-3-(2,2-Dimethyl-1,1-diphenylethylsilanoxy)-1-methyl prop-yl]cyclopropane carbaldehyde (101)



Sodium methoxide (1.75 g, 32.5 mmol) was added to a stirred solution of **(100)** (10.32 g, 27 mmol) in methanol (300 mL), and refluxed for 67 h. The mixture was cooled to room temperature and quenched with sat. aq. NH_4Cl (50 mL). The product was extracted

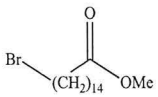
with CH_2Cl_2 (3 × 50 mL), dried and evaporated to give a colourless oil which separated and purified by column chromatography eluting with petrol/ethyl acetate (15:1) led to a colourless oil of **(101)** (7.1 g, 68%).[115]

8: Methyl 15-hydroxypentadecanoate (182)



Sodium (4.75 g, 206.5 mmol) was added to MeOH (250 mL) at 0 °C with stirring. The mixture was allowed to warm up to r.t until all of the sodium was consumed. Pentadecanolide **(181)** (20 g, 83.3 mmol) was added, and the reaction mixture was heated to 80 °C and stirred for 2 hrs. The reaction was quenched with aq.soln. of HCl (250 mL, 1N) and water (200 mL) was added. The product was extracted with ethyl acetate (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 MeOH (150 mL) and H_2SO_4 (2mL) was added. The solution was refluxed for 1 hr until no acid was left, cooled to r.t. and methanol was evaporated. The product was dissolved with ethyl acetate (200 mL), washed with sat. aq. $NaHCO_3$ (150 mL), and the product was extracted. The solvent was evaporated and the crude product was purified by recrystalization using petroleum ether to give **(182)** (20.4 g, 93%); δ_H: 3.67 (3H, s), 3.66 (2H, t, *J* 6.6 Hz), 2.31 (2H, t, *J* 7.55 Hz), 1.64–1.54 (4H, m), 1.36–1.25 (20H, m); δ_C: 174.4, 63.1, 51.5, 34.1, 32.8, 29.6, 29.6, 29.5, 29.4, 29.25, 29.15, 25.7, 25; ν_{max}/cm^{-1}: 3309, 2920, 2851, 1743, 1463.

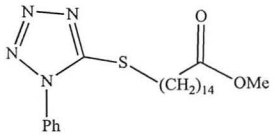
9: Methyl 15-bromopentadecanoate (183)



Triphenyl phosphine (24.31 g, 92.6 mmol) was added to a stirred solution of methyl 15-hydroxypentadecanoate **(182)** (20 g, 72.3 mmol) in CH_2Cl_2 (350 mL) followed by adition of $NaHCO_3$ (0.5 g). The mixture was cooled to 0 °C and N-bromosuccinamide (17.01 g, 95.5 mmol) was added in portions and stirred at r.t for 1 h. A sat.aq solution of sodium bisulphate (150 mL) was added and the product was extracted with CH_2Cl_2 (2 ×200mL). The combined organic layers were dried, and the solvent was evaporated to give a residue, which was then refluxed in petrol/ethyl acetate (20:1, 100 mL) for 30 min. The triphenylphosphonium oxide was filtered on a pad of celite and washed

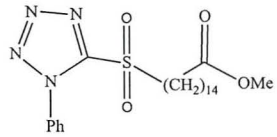
petrol/ethyl acetate (20:1, 100 mL). The solvent was evaporated and the crude product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give **(183)** (21.8 g, 88%); δ_H: 3.67 (3H, s), 3.41 (2H, t, *J* 6.6 Hz), 2.31 (2H, t, *J* 7.55 Hz), 1.9–1.82(2H, quintet, *J* 7 Hz), 1.64–1.60 (2H, m), 1.44–1.39 (2H, m), 1.30–1.26 (18H, m);δ_C: 174.3, 63.1, 51.4, 34.1, 34.1, 32.8, 29.6, 29.5, 29.4, 29.2, 29.15, 28.7, 28.2, 25; ν_{max}/cm^{-1}: 2919, 2851, 1739, 1463.

10: Methyl 15-((1-phenyl-1H-tetrazol-5-yl)thio)pentadecanoate (184)



1-Phenyl-1*H*-tetrazole-5-thiol (11.49 g, 64.4 mmol) was added to a stirred solution of methyl 15-bromopentadecanoate **(183)** (21.60 g, 64.4 mmol) and anhydrous potassium carbonate (17.82 g, 128.9 mmol) in acetone (300 mL) and refluxed for 1 hr. The solvent was evaporated , and the crude product was purified by recrystalization with MeOH / Acetone (1:1) to give a white solid of **(184)** (23.6 g, 84%); δ_H: 7.74–7.59 (5H, m), 3.67 (3H, s), 3.40 (2H, t, *J* 7.4 Hz), 2.31 (2H, t, *J* 7.55 Hz), 1.84 (2H, quintet, *J* 7.4 Hz), 1.64–1.62 (2H, quintet, *J* 7.4 Hz), 1.48–1.41 (2H, quintet, *J* 7.4 Hz), 1.30–1.26 (18H, m);δ_C: 174.4, 133.8, 130.1, 129.8, 123.9, 51.5, 34.1, 33.4, 29.62, 29.61, 29.59, 29.55, 29.45, 29.26, 29.16, 29.08, 29.04, 28.7, 25; ν_{max}/cm^{-1}: 2916, 2851, 1742, 1499, 1471.

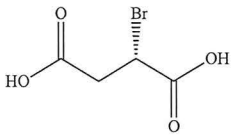
11: Methyl 15-((1-phenyl-1H-tetrazol-5-yl)sulfonyl)pentadecanoate (185)



The procedure used in Experiment **12** was repeated using (methyl 15-((1-phenyl-1H-tetrazol-5-yl)thio)pentadecanoate **(184)** (5.50 g, 12.75 mmol), ammonium molybdate (VI) tetrahydrate (7.85 g, 6.35 mmol) in 35 % H_2O_2 (10 mL) in THF (50 mL) and IMS (30 mL), and further solution of ammonium molybdate (VI) tetrahydrate (7.85 g, 6.35 mmol) in 35% H_2O_2 (10 mL) at 10 °C. The crude product was purified by column chromatography eluting with petrol/ ethyl acetate (1:1) to give a white solid of **(185)** (4.6 g, 78%); δ_H: 7.71–7.69 (2H, m), 7.62–7.58 (3H, m), 3.73 (2H, t, 7.8), 3.66 (3H, s), 2.32 (2H, t, *J* 7.5 Hz), 1.98–1.95 (2H, m), 1.64–1.6 (2H, m), 1.54–1.46 (2H, m), 1.35–1.26 (18H, m); δ_C: 174.3, 153.5, 133.1, 131.4, 129.7, 125.1, 56.0, 51.4, 34.1, 29.57,

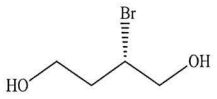
29.55, 29.53, 29.44, 29.3, 29.2, 29.1, 28.9, 28.1, 25.0, 21.9; ν_{max}/cm^{-1}: 2923, 2858, 1730, 1497, 1468, 1340, 1255, 1200, 1156.

12 : (*S*)-(-)Bromosuccinic acid (200)



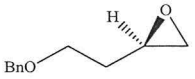
(L)-aspartic acid **(199)** (50.03g, 380 mmol), and KBr (201.6 g, 1690 mol) were dissolved in sulphuric acid (2.5M, 1L). The solution was cooled to -5 °C, and a solution of $NaNO_2$ (46.68 g, 680 mol) in 90 mL of water added dropwise through a dropping funnel between -5 to 0 °C with in a period of 1 hr. The resulting diazonium salt solution was stirred for 2 hrs at -5 °C, 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 **(200)** (76%). This was used directly into next step without purification.[214]

13: (*S*)-2-Bromo-1,4-butandiol (201)



Borane tetrahydrofurane (BH_3.THF) (800 mL, 1M, 0.8 mol) was added drop wise over a period of 1 hour to a stirred solution of (*S*)-(-)Bromosuccinic acid (52.46 g, 260 mmol) in dry THF (400 mL) at 0 °C. The reaction mixture was stirred for 5 hours 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 × 100mL). 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 **(201)** (40 g, 88%).[214]

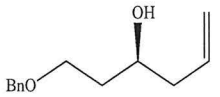
14: (*R*)-(2-Benzyloxyethyl)oxirane (202)



A solution of (*S*)-2-Bromo-1,4-butandiol **(201)** (40 g, 237.4 mmol) in dry THF (50mL) was added to a stirred suspension of sodium hydride (30g,1250 mmol) in dry THF (400 mL) at -10 °C under nitrogen. The soulution was stirred at -10 °C for 30 minutes, and

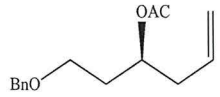
then benzyl bromide (30.8mL) and tetrabutylammonium iodide (TBAI) (8.0 g, 22 mmol) were added. The reaction mixture was allowed to reach room temperature and stirred for 18 hrs. It was then quenched with sa.aq. NH_4Cl (200 mL). The product was extracted with ethyl acetate (3 × 200 mL), then the combined 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 **(202)**: (78%).[214]

15: (*S*)-1-Benzyloxy-hex-5-en-3-ol (203)



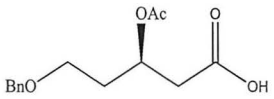
Vinylmagnesium bromide (353.8 mL, 353.8 mmol, 1M in THF) was added to a stirred suspension of copper iodide (10.11 g, 53.07 mmol) in dry THF (300 mL) at – 75 °C, and then the reaction mixture was stirred for 30 min at – 40 °C. It was re-cooled again to – 75 °C and a solution of the (*R*)-(2-benzyloxyethyl)oxirane **(202)** (31.51 g, 176.94 mmol) in dry THF (100 mL) was added and stirred at – 40 °C to – 20 °C for 2 hrs then allowed to reach room temperature. The reaction mixture was cooled to –20 °C, and then quenched with sat.aq. NH_4Cl (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/ethylacetate (5:1) to give a yellow oil of **(303)** (81%).[135]

16: Acetic acid (*S*)-1-(2-benzyloxy-ethyl)-but-3-enyl ester (204)



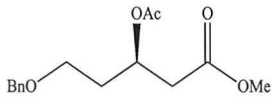
Acetic anhydride (109.5 mL) was added to stirred solution of (*S*)-1-Benzyloxy-hex-5-en-3-ol **(203)** (61.3 g, 297.3 mmol) in dry toluene (300 mL) at r.t, and followed by addition of anhydrous pyridine (35 mL). The mixture was stirred for 18 hrs at r.t under nitrogen. When TLC showed no starting material was left, the solvent was evaporated and the crude product was columned eluting with petrol/ethyl acetate (5:1) to give a colourless oil of **(204)** (88%).[135]

17: (*R*)-3-Acetoxy-5-benzyloxy-pentanoic acid (205)



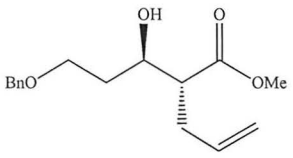
Osmium tetroxide (OsO_4 2.5 % in 2-methyl-2-propanol, 9 mL, 0.72 mmol) was added to a stirred solution of acetic acid (*S*)-1-(2-benzyloxy-ethyl)-but-3-enyl ester **(204)** (17.8 g, 71.77 mmol) and oxone salt (176.5 g, 287.1 mmol) in dry DMF (300 mL) at 10 °C under nitrogen atmospher. The reaction mixture was allowed to reach 32 °C 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 **(205)**: (73 %).[135]

18: (*R*)-5-Benzyloxy-3-hydroxy-pentanoic acid methyl ester (206)



Conc. H_2SO_4 (5mL) was added to a stirred solution of (*R*)-3-acetoxy-5-benzyloxy-pentanoic acid **(205)** (29.5 g, 110.9 mmol) in MeOH (400 mL), after reflux for 3 hrs. When TLC confirmed the completion of the reaction, the solvent was evaporated and the reaction mixture was neutralized with sat. aq. $NaHCO_3$ (200 mL), and then extracted with ethyl acetate (400mL). The aq. layer was re-extracted with ethyl acetate (2 × 150 mL) and the combined organic layers were dried and evaporated. The crude product was purified by column chromatography eluting with petrol / ethyl acetate (3:2) to give a pale yellow oil of **(206)** (88%).[135]

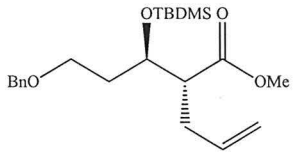
19: (*R*)-Methyl 2-((*R*)-3-(benzyloxy)-1-hydroxypropyl)pent-4-enoate (208)



Diisopropylamine (5.31 g, 7.4mL, 52.5 mmol) was dissolved in dry THF (30 mL) and cooled to – 20 °C. BuLi (22 mL, 52.5 mmol, 2.5 M) was added between – 20 °C to -10 °C and allowed to reach + 16 °C, then re-cooled to – 55 °C and (*R*)-5-benzyloxy-3-hydroxy-pentanoic acid methyl ester **(206)** (5 g, 21 mmol) in dry THF (15 mL) was added and the mixture was stirred between – 55 °C to – 50 °C for 1.5 hr. The reaction

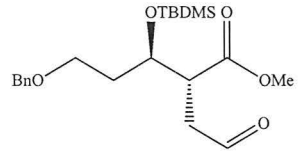
mixture was allowed to warm up to – 25 °C over 30 minutes, it was re-cooled to – 65 °C and allyl iodide (5.3 g, 2.9 mL, 31.5 mmol) and HMPA (7.3 mL, 41.9 mmol) in dry THF (12 mL) were added keeping the temperature between – 65 °C to – 55 °C. The reaction mixture was then stirred between - 50 °C to – 45 °C for 2hrs and then allowed to reach - 30 °C to stirr for 30 min. When TLC showed no starting material was left, the reaction mixture was quenched with sat. aq. NH_4Cl (50 mL) and extracted with ethyl acetate (3 × 100 mL). The combined organic layers were dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol / ethyl acetate (2:1) to give a pale yellow oil of **(208)** (4.52 g, 73 %), $[\alpha]_D^{22}$ = - 7.09 (*c* 1.03, $CHCl_3$); *litt* $[\alpha]_D$= -6.9 (c 1.09 ($CHCl_3$).[135]

20: (*R*)-Methyl 2-((*R*)-3-(benzyloxy)-1-((*tert* butyl dimethylsilyl)oxy) propyl)pent-4-enoate (209)



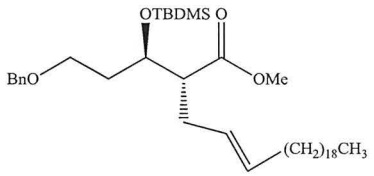
Tert-butyldimethylchlorosilane (4.813 g, 31.9 mmol) was added to a stirred solution of (*R*)-methyl 2-((*R*)-3-(benzyloxy)-1-hydroxypropyl)pent-4-enoate **(208)** (6.83 g, 24.5 mmol) and imidazole (4.17 g, 61.32 mmol) in DMF (50 mL), and the mixture was stirred for 16 h at 45 ^{0}C. When TLC showed all the starting material had reacted, DMF was removed by distillation (under high vacuum), then the reaction was quenched with water (15mL) and the product was extracted with CH_2Cl_2 (100 mL). The aqueous layer was re-extracted with CH_2Cl_2 (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 **(209)** (9.1 g, 94 %).[135]

21: (2*R*,3*R*)-Methyl-5-(benzyloxy)-3-((*tert*-butyldimethylsilyl)oxy)-2-(2-oxoethyl) pentanoate (210)



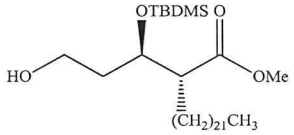
Ozone gas was applied for 10 minutes on a stirred solution of (*R*)-methyl 2-((*R*)-3-(benzyloxy)-1-((*tert* butyl dimethylsilyl)oxy) propyl)pent-4-enoate **(209)** (10 g, 25.5 mmol) in dry THF (100 mL), and MeOH (100 mL) at – 70 °C. The reaction mixture was allowed to warm up to – 34 °C, and ozone gas was removed. Acetic acid (25mL) and water (1mL) were added, followed by addition of zinc metal (2 g). The reaction mixture was allowed to reach r.t., and then sat.aq. $NaHCO_3$ (200 mL) and water (200 mL) were added. The product was extracted with petrol/ethyl acetate (1:1, 3 × 100 mL), and the combined organic layers were dried and the solvent was evaporated to give the crude product, which was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give a colourless oil of **(210)** (9 g, 90%).[135]

22: (*R,E*)-methyl 2-((*R*)-3-(benzyloxy)-1-((*tert*-butyldimethyl silyl)oxy) propyl) tetracos-4-enoate (212)



The procedure used in Experiment **1** was repeated in order to couple 5-(icosylsulfonyl)-1-phenyl-1H-tetrazole (6.68 g, 13.6 mmol) with (2*R*,3*R*)-methyl 5-(benzyloxy)-3-((*tert*-butyldimethylsilyl)oxy)-2-(2-oxoethyl)pentanoate (4.5 g, 11.36 mmol) using lithium *bis*(trimethylsilyl)amide (19.2 mL, 21 mmol, 1.06 M) in dry THF (100 mL) under nitrogen at -15 °C. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a colourless oil of **(212)** (84%).[143]

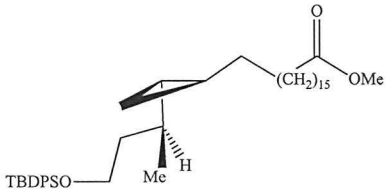
23: (*R*)-methyl 2-((*R*)-3-(benzyloxy)-1-((*tert*-butyldimethylsilyl)oxy)propyl) tetracosanoate (213)



Palladium 10% on carbon (1.8 g) was added to a stirred solution of the (*R,E*)-methyl 2-((*R*)-3-(benzyloxy)-1-((*tert*-butyldimethylsilyl)oxy)propyl) tetracos-4-enoate **(212)** (7.2 g, 10.6 mmol) in IMS: THF 1:1 (100 mL). The mixture was stirred for 3 days under hydrogen atmosphere. The solution was filtered through a pad of celite and the solvent

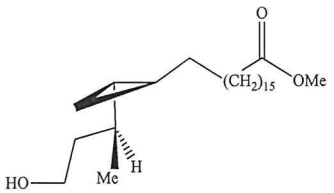
was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give a semi-solid of **(213)** (6.04 g, 96%).[143]

24: Methyl 17-((1S,2R)-2-((S)-4-((tert-butyldiphenylsilyl)oxy)butan-2-yl)cyclopropyl)heptadecanoate



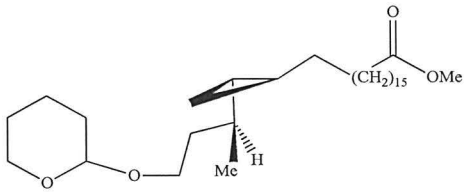
The procedure used in Experiment **1** was repeated in order to couple (1*S*,2*R*)-2-[(*S*)-3-(*tert*-butyl diphenylsilanyloxy)-1-methylpropyl]cyclopropane carbaldehyde **(98)** (3.55 g, 9.34 mmol) with 16-(1-phenyl-1*H*-tetrazole-5-sulfonyl)-hexadecanoic acid methyl ester (5.28 g, 11.05 mmol) using lithium *bis*(trimethylsilyl) amide (15.6 mL, 16.57 mmol, 1.06 M) in dry THF (15 mL) under nitrogen at –15 °C. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a mixture of (*E/Z*) alkenes (4.40 g, 75%); [HRMS (EI^+): *m/z* 577.4034 [$(M–Bu^t)^+$], calculated for $C_{37}H_{57}O_3Si$: 577.4077]. The procedure used in Experiment **1** was repeated to hydrogenate the above alkene (4.39 g, 2.16 mmol using dipotassium azodicarboxylate (2.5 g, 13 mmol) and glacial acetic acid (3 mL) in THF (15 mL) and methanol (5 mL) at 5 °C. The product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give a colourless oil of the title compound (4.10 g, 93 %); $[\alpha]_D = +7.3$ (c 0.91($CHCl_3$) [MS (MALDI-TOF): *m/z* 657.24 [$(M+Na)^+$], calculated for $C_{41}H_{66}NaO_3Si$: 657.46]; δ_H: 7.68–7.66 (4H, m), 7.44–7.36 (6H, m), 3.75–3.70 (2H, m), 3.67 (3H, s), 2.31 (2H, t, *J* 7.55 Hz), 1.7–1.48 (6H, m), 1.43–1.26 (28H, br.m), 1.17–1.11 (1H, m), 1.05 (9H, s), 0.89 (3H, s), 0.42 (1H, m), 0.13 (3H, m); δ_C:135.8, 134.2, 129.5, 127.5, 62.4, 51.4, 40.2, 34.7, 34.4, 34.1, 29.7, 29.7, 29.6, 29.5, 29.3, 29.2, 26.9, 25.9, 24.9, 19.8, 18.6, 10.6 ; ν_{max}/cm^{-1}: 2925, 2854, 1743, 1428, 1111.

25:Methyl17-((1S,2S)-2-(4-hydroxy-2-methylbutan-2-yl)cyclopropyl)heptadecan-oate



The same procedure used in Experiment **2** was repeated to deprotect methyl 17-((1S,2R)-2-((S)-4-((*tert*-butyl-diphenylsilyl)oxy)butan-2-yl)cycl-opropyl)heptadecan oate (4.00 g, 6.45 mmol) using tetra-*n*-butyl ammonium fluoride (8.38 mL, 8.38 mol) in dry THF (100 mL) at 0 °C under nitrogen. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (5:1) to give a colourless oil of the title product; (2.45 g, 98 %), $[\alpha]_D^{21}$ = +15.2 (c1.03, $CHCl_3$) [HRMS (EI^+): *m/z* 396.3607 $[(M)^+]$, calculated for $C_{25}H_{48}NaO_3$: 396.3607]; δ_H: 3.78–3.69 (2H, m), 3.67 (3H, s), 2.30 (2H, t, *J* 7.56 Hz), 1.77–1.70 (1H, m), 1.66–1.60 (2H, m), 1.59–1.52 (1H, m), 1.37–1.20 (28H, m), 1.17–1.11 (1H, m), 0.96 (3H, d, *J* 6.6 Hz), 0.90–0.81 (1H, m), 0.51–0.44 (1H, m), 0.26–0.14 (3H, m); δ_C: 61.4, 51.4, 40.4, 34.9, 34.4, 34.1, 29.7, 29.6, 29.6, 29.5, 29.3, 29.2, 26.6, 25.9, 24.9, 19.9, 18.8, 10.6; ν_{max}/cm^{-1}: 3338, 2918, 2851, 1739, 1468, 1169, 756.

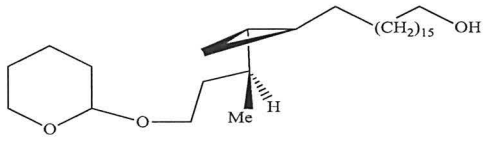
26: Methyl17-((1S,2R)-2-((2S)-4-((tetrahydro-2H-pyran-2-yl)oxy)butan-2-yl) cycl-opropyl)heptadecanoate



The procedure used in Experiment **3** was repeated using pyridinium-p-toluene–sulfonate (0.78 g, 3.10 mmol), methyl 17-((1S,2S)-2-(4-hydroxy-2-methyl butan-2-yl) cyclopro pyl)heptadecanoate (2.40 g, 6.28 mmol) and 3,4-dihydro-2*H*-pyran (1.32 g, 15.6 mmol) in dry CH_2Cl_2 (40 mL) under nitrogen at r.t. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give a colourless oil of the title product; (2.50 g, 86%), $[\alpha]_D^{21}$ = +19.3 (*c* 0.75, $CHCl_3$) [HRMS (EI^+): *m/z* 480.4168 $[(M)^+]$, calculated for $C_{30}H_{56}O_4$: 480.4179]; δ_H: 4.58 (1H, br.m), 3.88–3.79 (2H, m), 3.67 (3H, s), 3.51(2H, m), 2.31 (2H, t, *J* 7.56 Hz), 1.87–1.71 (1H, m), 1.69–1.64 (2H, m), 1.58–1.54 (7H, m), 1.37–1.20 (27H, m), 0.96 (3H, d, *J* 6.8 Hz), 0.90–0.80 (1H, m), 0.51–

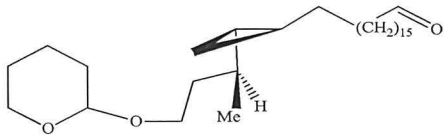
0.43 (1H, m), 0.27–0.11 (3H, m); δ_C: 99.0, 98.9, 66.2, 65.9, 62.4, 51.4, 37.2, 37.1, 35.3, 35.2, 34.4, 34.1, 30.9, 29.7, 29.66, 29.62, 29.61, 29.4, 29.2, 29.1, 26.0, 25.0, 25.5, 24.9, 19.83, 19.8, 18.7, 18.6, 10.5; ν_{max}/cm^{-1}: 2924, 2853, 1743, 1454.

27:16-((1S,2R)-2-((2S)-4-((Tetrahydro-2H-pyran-2-yl)oxy)butan-2-yl)cyclopropyl) hexadecan-1-ol



The procedure used in Experiment **4** was repeated to reduce methyl-17-((1S,2R) -2-((2S)-4-((tetrahydro-2H-pyran-2-yl)oxy)butan-2-yl)cyclopro-pyl)heptadecanoate (2.40 g , 5 mmol) using $LiAlH_4$ (0.28 g, 7.5 mmol) in THF (40mL) at 0 °C under nitrogen. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (3:1) to give to give a colourless oil of the title product; (2.20 g, 97%); $[\alpha]_D^{21}$ = +22.2 (*c* 1.04, $CHCl_3$); [HRMS (EI^+): *m/z* 452.4226 [$(M)^+$], calculated for $C_{29}H_{56}O_3$: 452.4229]; δ_H: 4.58 (1H, br.m), 3.91–3.83 (2H, m), 3.66 (2H, t, *J* 6.6 Hz), 3.52–3.41 (2H, m), 1.87–1.75 (3H, m), 1.59–1.54 (7H, br.m), 1.34–1.26 (30H, br.m,), 1.17 (1H, m), 0.96 (3H, d, *J* 6.6 Hz), 0.89–0.8 (1H, m), 0.55–0.43 (1H, m), 0.25–0.13 (3H, m); δ_C: 99.0, 98.9, 66.2, 65.9, 63.0, 62.4, 37.2, 37.1, 35.36, 35.2, 34.4, 32.8, 30.86, 30.82, 29.7, 29.6, 29.62, 29.61, 29.4, 26.0, 25.9, 25.7, 25.5, 19.82, 19.81, 19.8, 18.64, 18.60, 10.6; ν_{max}/cm^{-1}: 3438, 2921, 2849, 1490.

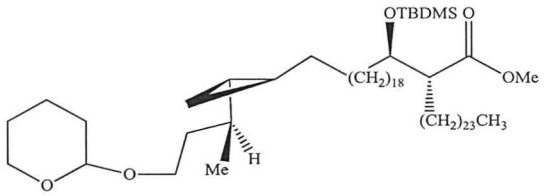
28:17-{(1*S*,2*R*)-2-((*S*)-1-Methyl-3-(tetrahydro-pyran-2-yloxy)-propyl]cyclopropyl} -heptadecanal



The procedure described in Experiment **5** was repeated to oxidize 16-((1S,2R)-2-((2S) -4-((tetrahydro-2H-pyran-2-yl)oxy)butan-2-yl)cyclopropyl) hexa decane 1-ol (2.12 g, 4.6 mmol) using PCC (3.01 g, 14 mmol) in CH_2Cl_2 (50 mL), and stirring for 2 hrs. The product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give a colourless oil of the title product; (2.05 g, 95%), $[\alpha]_D^{21}$ = +5.9 (*c* 1.04, $CHCl_3$); [MS (MALDI-TOF): *m/z* 473.59 [$(M+Na)^+$], calculated for $C_{29}H_{54}NaO_3$: 473.39]; δ_H: 9.77 (1H, t, *J* 1.9 Hz), 4.58 (1H, br.m), 3.91–3.79 (2H, m), 3.54–3.43 (2H, m), 2.42 (2H,

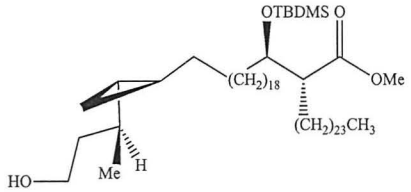
dt, *J* 1.8, 7.4 Hz), 1.80–1.75 (3H, m), 1.59–1.53 (7H, br.m), 1.34–1.26 (28H, br.m), 0.96 (3H, d, *J* 6.8 Hz), 0.89–0.80 (1H, m), 0.55–0.43 (1H, m), 0.25–0.14 (3H, m); δ_C: 202.1, 99.0, 98.9, 66.2, 65.9, 62.4, 43.5, 37.2, 37.0, 35.3, 35.2, 34.4, 30.9, 30.8, 29.7, 29.65, 29.61, 29.6, 29.4, 26.2, 25.9, 25.5, 19.8, 19.7, 18.62, 18.6, 10.6; ν_{max}/cm^{-1}: 2924, 2853, 1729, 1455.

29: (2*R*)-Methyl 2-((1*R*)-1-((*tert*-butyldimethylsilyl)oxy)-20-((*1S,2R*)-2-((2*S*)-4-((tetrahydro-2*H*-pyran-2-yl)oxy)butan-2-yl)cyclopropyl)icosyl)hexacosa noate (9):



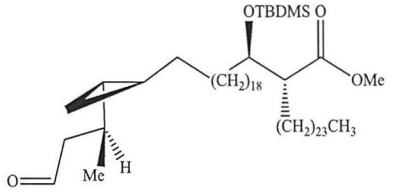
The same procedure used to prepare the compound **(1)** was repeated to couple (R)-methyl 2-((*R*)-1-((*tert*-butyldimethylsilyl) oxy)-3-((1-phenyl-1H-tetrazol-5-yl) sulfonyl)propyl) hexacosanoate (4.14 g, 5.33 mmol) with 17-{(1*S*,2*R*)-2-((*S*)-1-Methyl-3-(tetrahydro-pyran-2-yloxy)-propyl]-cyclopropyl}-heptadecanal (2.00 g, 4.44 mmol) using lithium *bis*(trimethyl silyl)amide (7.5 mL, 8 mmol, 1.06 M) to give a mixture of (*E/Z*) alkenes; (3.20 g, 71 %), [MS (MALDI-TOF): *m/z* 1037.93 $[(M+Na)^+]$, calculated for $C_{65}H_{126}NaO_5Si$: 1037.92]. The procedure used in Experiment **1** was repeated to hydrogenate the above alkene (3.15 g, 3.10 mmol) using dipotassium azodicarboxylate (2.52 g, 13.2 mmol) and glacial acetic acid (5 mL) in THF (50 mL) and methanol (5 mL) at 5 °C. The product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a colourless oil of the title product (3.01 g, 95%), $[\alpha]_D^{21} = -6.9$ (*c* 1.04, $CHCl_3$) δ_H: 4.58 (1H, br.m), 3.96–3.74 (3H, m), 3.66 (3H, s), 3.56–3.43 (2H, m), 2.53 (1H ,ddd, *J* 3.9, 7.0, 10.7 Hz), 1.75–1.64 (2H, m), 1.60–1.03 (90H, br.m), 0.96 (3H, d, *J* 6.6 Hz), 0.90–0.84 (4H, m, including a triplet 0.87 ppm with *J* 6.9 Hz), 0.86 (9H, s), 0.57–0.39 (1H, m), 0.27–0.11 (3H, m), 0.055 (3H, s), 0.03 (3H, s) δ_C: 175.2, 99.1, 98.9, 73.2, 66.2, 65.8, 62.4, 51.9, 51.5, 41.0, 37.4, 37.19, 37.1, 35.3, 35.2, 34.1, 32.8, 3.7, 31.9, 30.8, 29.8, 29.7, 29.63, 29.60, 29.5, 29.4, 29.3, 27.9, 27.7, 27.6, 25.9, 25.7, 25.5, 22.7, 22.6, 20.4, 19.8, 14.4, 10.7, –4.4, –4.8; ν_{max}/cm^{-1}:, 2924, 2853, 1741, 1464.

30: (*R*)-Methyl 2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-20-((*1S,2R*)-2-((*S*)-4-hydroxy butan-2-yl)cyclopropyl)icosyl)hexacosanoate



The procedure used in Experiment **9** was repeated to deprotect (2*R*)-methyl 2-((1*R*)-1-((*tert*-butyldimethylsilyl)oxy)-20-((1*S*,2*R*)-2-((2*S*)-4-((tetrahydro-2*H*-pyran-2-yl)oxy) butan-2-yl)cyclopropyl)icosyl)hexacosanoate (3.00 g, 2.94 mmol) using a solution of pyridinium-*p*-toluene sulfonate (0.36 g, 1.47 mmol) in THF (20 mL), methanol (5mL) and stirred at 45 °C for 6 hrs. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (12:1) to give a semi-solid of the title product (2.00 g, 79%), $[\alpha]_D^{21} = +18.3$ (*c* 2.56, $CHCl_3$) [MS (MALDI-TOF): *m/z* 955.55 $[(M+Na)^+]$, calculated for $C_{60}H_{120}NaO_4Si$: 955.88]; δ_H: 3.92 (1H, m), 3.73 (2H, m), 3.66 (3H, s), 2.56 (1H, ddd, *J* 3.7, 7.1, 10.7 Hz), 1.72 (1H, m), 1.54 (4H, m), 1.26 (82H, br.m), 0.96 (3H, d, *J* 6.42 Hz), 0.91–0.86 (4H, m, including a triplet 0.87 with *J* 6.9 Hz), 0.86 (9H, s), 0.52–0.45 (1H, m), 0.23–0.13 (3H, m), 0.05 (3H, s), 0.03 (3H, s); δ_C: 175.2, 73.2, 61.4, 60.4, 51.5, 51.3, 40.4, 37.4, 34.9, 34.4, 33.7, 32.8, 31.9, 31.6, 29.8, 29.7, 29.7, 29.64, 29.61, 29.59, 29.45, 29.37, 29.06, 27.83, 27.76, 27.65, 27.52, 25.9, 25.8, 25.28, 23.6, 22.7, 22.66, 21.0, 20.5, 19.8, 18.7, 17.8, 14.1, 10.5, –4.4, –4.92; ν_{max}/cm^{-1}: 3467, 2921, 2852, 1740, 1493, 1454, 1035.

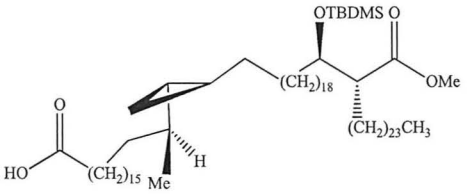
31: (*R*)-methyl 2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-20-((1*S*,2*R*)-2-((*S*)-4-oxo butan-2-yl)cyclopropyl)icosyl)hexacosanoate



The procedure described in Experiment **5** was repeated to oxidize (*R*)-methyl 2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-20-((1*S*,2*R*)-2-((*S*)-4-hydroxybutan-2-yl)cyclopropyl) icosyl)hexacosanoate (1.90 g, 2.04 mmol) using PCC (1.32 g, 6.1 mmol) in CH_2Cl_2 (100 mL). The crude product was purified by column chromatography eluting with petrol/ ethyl acetate (10:1) to give a colourless oil of the title product (1.76, 93%), $[\alpha]_D^{21} = +11.6$ (c 1.06, $CHCl_3$) [MS (MALDI-TOF): *m/z* 953.69 $[(M+Na)^+]$, calculated for $C_{60}H_{118}NaO_4Si$: 953.86]; δ_H: 9.79 (1H, t, *J* 2.5 Hz), 3.90 (1H, m), 3.66 (3H, s), 2.53–2.48 (2H, m), 2.38 (1H, ddd, *J* 2.5, 7.9, 15.7 Hz), 1.54–1.26 (85H, br.m), 1.03 (3H, d, *J*

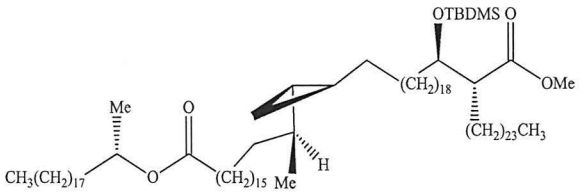
6.8 Hz), 0.91–0.86 (4H, m, including a triplet 0.87 ppm with *J* 6.9 Hz), 0.86 (9H, s), 0.50–0.48(1H, m), 0.38–0.19(3H, m), 0.05 (3H, s), 0.02 (3H, s); δ_C: 202.9, 73.2, 51.6, 51.4, 34.0, 33.9, 33.7, 31.9, 29.8, 29.7, 29.6, 29.5, 29.4, 27.8, 27.7, 27.6, 27.5, 25.9, 25.8, 25.3, 23.7, 22.7, 22.66, 21.0, 20.4, 19.8, 18.8, 14.1, 11.4, –4.4, –4.9; ν_{max}/cm^{-1}: 2924, 2853, 1738, 1464.

32: (*S*)-18-((1*R*,2*S*)-2-((20*R*,21*R*)-20-((*tert*-butyldimethylsilyl)oxy)-21 (methoxy carbonyl)pentatetracontyl)cyclopropyl)nonadecanoic acid



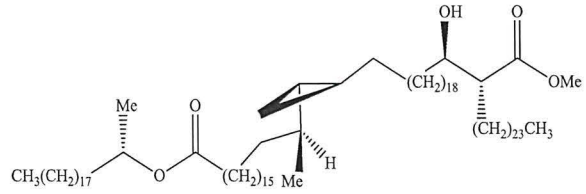
The same procedure used in experiment **1** was repeated to couple (R)-methyl 2-((R)-1-((*tert*-butyldimethylsilyl)oxy)-20-((*1S,2R*)-2-((S)-4-oxobutan-2-yl)cyclopropyl)icosyl) hexacosanoate (1.70 g, 1.82 mmol with the acid sulphone **(219)** (1.06 g, 2.3 mmol) to give to give a mixture of (*E/Z*) alkenes (1.55 g, 73%), [MS (MALDI-TOF): *m/z* 1177.86 $[(M+Na)^+]$, calculated for $C_{75}H_{146}NaO_5Si$: 1178.08. The procedure used in Experiment **1** was repeated to hydrogenate the above alkene (1.50 g, 1.29 mmol) using dipotassium azodicarboxylate (3.75 g, 19.5 mmol) and glacial acetic acid (3 mL) in THF (50 mL) and methanol (5 mL) at 5 °C. The product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give a white semi-solid of the title product (1.43 g, 95%); $[\alpha]_D$ = +2.8 (c 0.32 ($CHCl_3$)); [MS (MALDI-TOF): *m/z* 1180.87 $[(M+Na)^+]$, calculated for $C_{75}H_{148}NaO_5Si$: 1181.04]; δ_H: 3.92 (1H, dt, *J* 7.1, 4.8 Hz), 3.66 (3H, s), 2.53 (1H, ddd, *J* 3.8, 7.2, 10.9 Hz), 2.35 (2H, t, *J* 7.5 Hz), 1.64 (3H, m), 1.56–1.03 (105H, br.m), 0.91 (3H, d, *J* 6.7 Hz), 0.88 (3H, br.t, *J* 6.9 Hz), 0.86 (9H, s), 0.73–0.62 (1H, m), 0.51–0.40 (1H, m), 0.26–0.06 (3H, m), 0.05 (3H, s), 0.02 (3H, s); δ_C: 178.8, 175.2, 73.2, 68.1, 60.4, 51.5, 51.2, 38.7, 38.1, 37.4, 33.9, 33.7, 32.7, 31.9, 29.7, 29.60, 29.4, 29.2, 27.6, 27.4, 27.2, 25.7, 25.7, 23.7, 22.6, 21.0, 19.3, 18.6, 14.1, 10.5, –4.3, –4.9; ν_{max}/cm^{-1}: 2924, 2853, 1743, 1711, 1464.

33: (*R*)-methyl 2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-20-((*1S,2R*)-2-((*S*)-19-((*S*)-icosan-2-yloxy)-19-oxononadecan-2yl)cyclopropyl)icosyl) hexacosano ate



The procedure used in Experiment **15** was repeated in order to couple (S)-18-((1R,2S)-2-((20*R*,21*R*)-20-((*tert*-butyldimethylsilyl)oxy)-21(methoxycarbonyl)pentatetracontyl) cyclopropyl)nonadecanoic (1.40 g, 1.20 mmol) with (*S*)-eicosan-2-ol **(169)** (0.39 g, 1.3 mmol) using DMAP (0.13 g, 1 mmol) and DCC (0.37 g, 1.80 mmol) in dry CH_2Cl_2 (4 mL). The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a thick colourless oil of the title product (1.25 g, 72%); $[\alpha]_D^{21} = +6.6$ (c 1.7, $CHCl_3$); [MS (MALDI-TOF): *m/z* 1460.03 $[(M+Na)^+]$, calculated for $C_{95}H_{188}NaO_5Si$: 1460.41]; δ_H: 4.91 (1H, sext, *J* 6.2 Hz), 3.92 (1H, dt, *J* 7.0, 4.8 Hz), 3.66 (3H, s), 2.53 (1H, ddd, *J* 3.8, 7.1, 10.4 Hz), 2.26 (2H, t, *J* 7.5 Hz), 1.54–1.26 (148H, br.m), 1.20(3H, d, *J* 6.2 Hz), 0.91 (3H, d, *J* 6.8 Hz), 0.91 (3H, d, *J* 6.7Hz), 0.90–0.84 (15H, m, including a singlet at 0.88 ppm), 0.71–0.63(1H, m), 0.49–0.41(1H, m), 0.22–0.04(3H, m), 0.05 (3H, s), 0.02 (3H, s); δ_C: 175.1, 173.6, 73.2, 70.7, 51.6, 51.2, 38.1, 37.4, 35.9, 34.8, 34.5, 33.7, 31.9, 30.1, 29.87, 29.84, 29.75, 29.7, 29.67, 29.63, 29.54, 29.52, 29.46, 29.39, 29.33, 29.2, 27.8, 27.5, 27.2, 26.1, 25.8, 25.4, 25.1, 23.8, 23.7, 22.7, 20.0, 19.6, 18.6, 10.4, –4.4, –4.9; ν_{max}/cm^{-1}: 2924, 2853, 1738, 1464, 1253, 1166, 836.

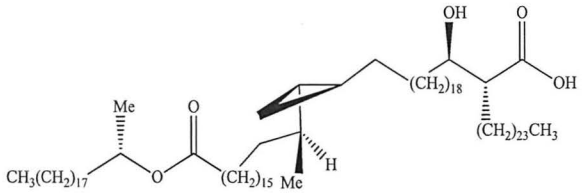
34: (*R*)-methyl 2-((*R*)-1-hydroxy-20-((1S,2R)-2-((S)-19-((S)-icosan-2-yl oxy)-19-oxononadecan-2-yl)cyclopropyl)icosyl)hexacosanoate.



The procedure used in Experiment **16** was repeated using (*R*)-methyl 2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-20-((1*S*,2*R*)-2-((*S*)-19-((*S*)-icosan-2-yloxy)-19-oxononadecan -2-yl)cyclopropyl)icosyl) hexacosanoate (1.20 g, 0.08 mmol), pyridine (0.7 mL) and hydrogen fluoride-pyridine complex as ~70% (4.2 mL). The residue was purified by chromatography eluting with petrol/ethyl acetate (10:1) to give a white semi-solid of the title product (0.75 g, 68%); $[\alpha]_D^{21} = +6.9$ (*c* 1.5, $CHCl_3$); [MS (MALDI-TOF): *m/z* 1345.83 $[(M+Na)^+]$, calculated for $C_{89}H_{174}NaO_5$: 1346.32]; δ_H: 4.91 (1H, sext, *J* 6.2 Hz), 3.71 (3H, s), 3.66 (1H, m), 2.47 (1H, m), 2.26 (2H, t, *J* 7.5 Hz), 1.54–1.26 (149H, br.m),

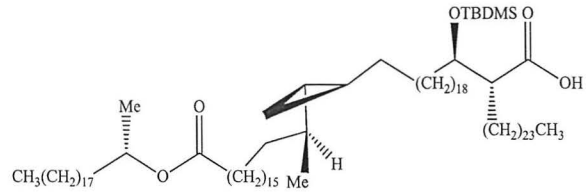
1.20 (3H, d, *J* 6.2 Hz), 0.90 (3H, d, *J* 6.8 Hz), 0.89 (6H, br.t, *J* 6.9 Hz), 0.71–0.63(1H, m), 0.49–0.41 (1H, m), 0.22–0.08 (3H, m); δ_C: 176.2, 173.5, 72.3, 70.7, 51.5, 51.2, 38.1, 37.4, 36.0, 34.7, 34.5, 33.7, 31.9, 30.1, 29.6, 29.5, 27.8, 27.5, 27.2, 26.1, 25.8, 25.4, 25.1, 23.8, 23.7, 22.7, 20.0, 19.7, 18.6, 14.1, 10.5; ν_{max}/cm^{-1}: 3441, 2918, 2850, 1734, 1722, 1466, 1377,1250, 1109, 720.

35: (*R*)-2-((*R*)-1-hydroxy-20-((1*S*,2*R*)-2-((*S*)-19-((S)-icosan-2-yloxy)-19-oxonona decan-2-yl)cyclopropyl)icosyl)hexacosanoic acid (168)



The procedure used in Experiment **17** was repeated to hydrolyse (*R*)-methyl-2-((*R*)-1-hydroxy-20-((1*S*,2*R*)-2-((*S*)-19-((*S*)-icosan-2-yloxy)-19-oxonona decan-2-yl)cyclopro pyl)icosyl)hexacosanoate (0.70 g, 0.52 mmol) using aq. solution of tetrabutyl ammonium hydroxide (28 mL, 5%). The crude product was purified by column chromatography eluting with petrol/ethyl acetate (5:1) to give a white solid of **(168)** (0.36 g, 51%), $[\alpha]_D^{21} = +8.7$ (*c* 0.5, $CHCl_3$); m.p. 56–57 °C [MS (MALDI-TOF): *m/z* 1332.07 $[(M+Na)^+]$, calculated for $C_{88}H_{172}NaO_5$: 1332.31]; δ_H: 4.91 (1H, sext, *J* 6.4 Hz), 3.72 (1H, m), 2.46 (1 H, br.dt, *J* 9.0, 5.3), 2.27 (2H, t, *J* 7.5), 1.81–0.95 (150H, br.m), 1.20 (3H, d, *J* 6.2 Hz), 0.90 (3H, d, *J* 6.8 Hz), 0.89 (6H, br.t, *J* 6.9 Hz), 0.72–0.63(1H, m), 0.50–0.41(1H, m), 0.22–0.02 (3H, m); δ_C: 179.6, 173.5, 72.1, 70.8, 50.8, 38.1, 37.4, 35.9, 35.5, 34.8, 34.4, 31.9, 30.1, 29.7, 29.58, 29.5, 29.45, 29.3, 29.2, 27.3, 27.2, 26.1, 25.7, 25.40, 25.1, 22.7, 20.1, 19.7, 18.6, 14.1, 10.5; ν_{max}/cm^{-1}: 3432, 2918, 2850, 1721, 1712, 1470, 1377, 718.

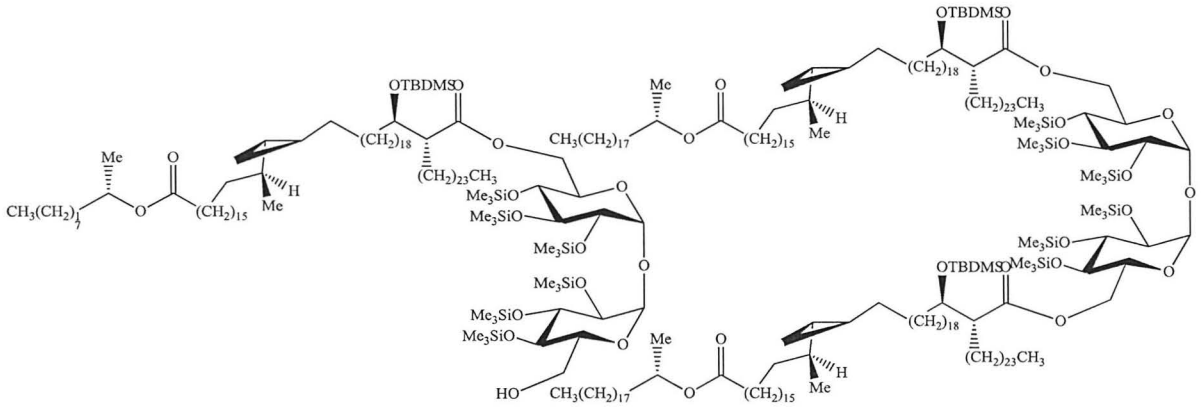
36: (*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-20-((1*S*,2*R*)-2-((*S*)-19-((*S*)-icosan-2-yloxy)-19-oxononadecan-2-yl)cyclopropyl)icosyl)hexacosanoic acid



The procedure used in Experiment **41** was repeated using (*R*)-2-((*R*)-1-hydroxy-20-((1*S*,2*R*)-2-((*S*)-19-((*S*)-icosan-2-yloxy)-19-oxononadecan-2-yl)cyclopropyl)icosyl)

hexacosanoic acid (0.26 g, 0.198 mmol), Imidazole (0.135 g, 1.9 mmol), *tert*-butyl dimethylsilylchloride (0.298 g, 1.9 mmol) and 4-dimethylaminopyridine (0.0242 g, 0.1 mmol) in dry DMF (2 mL) and dry toluene (3.5 mL). The crude product was purified by column chromatography eluting with petrol/ethyl acetate 20:1 to give a syrup of the title product; (0.26 g, 94%); $[\alpha]_D^{21} = +19.1$ (*c* 0.5, $CHCl_3$); [MS (MALDI-TOF): *m/z* 1446.41 $[(M+Na)^+]$, calculated for $C_{94}H_{186}NaO_5Si$: 1446.39]; δ_H: 4.92 (1H, sext, *J* 6.2 Hz), 3.83 (1H, m), 2.53 (1H, ddd, *J* 3.2, 5.6, 9.1 Hz), 2.27 (2H, t, *J* 7.4), 1.81–0.95 (140H, br.m), 1.20 (3H, d, *J* 6.2 Hz), 0.93 (9H, s), 0.91–0.87 (9H, m, including a triplet at 0.88 ppm with *J* 6.9 Hz), 0.70–0.40 (1H, m), 0.48–0.41 (1H, m), 0.15 (3H,s), 0.12 (3H, s), 0.22–0.06 (3H, m); δ_C: 179.6, 173.6, 73.7, 70.7, 50.1, 38.1, 37.4, 36.0, 35.5, 34.7, 34.4, 31.9, 30.1, 29.7, 29.5, 29.5, 29.4, 29.30, 29.15, 27.3, 27.2, 26.1, 25.8, 25.4, 25.1, 22.7, 22.3, 19.9, 19.7, 18.6, 14.1, 10.4, –4.2, –4.9; ν_{max}/cm^{-1}: 3432, 2918, 2850, 1721, 1712, 1470.

37: 6,6'-bis-O-(*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-20-((1*S*,2*R*)-2-((*S*)-19-((*S*)-icosan-2-yloxy)-19-oxononadecan-2-yl)cyclopropyl)icocyl) hexacosa noic-2,3,4,2',3',4',-hexakis-*O*-(trimethylsiyl)α,α'-trehalose (TDM), 6-*O*-(*R*)-2-((*R*)-1-((*tert*-butyldimethyl silyl)oxy)-20-((1*S*,2*R*)-2-((*S*)-19-((*S*)-icosan-2-yloxy)-19-oxo nonadecan-2-yl)cyclopropyl)icocyl)hexacosanoic-2,3,4,2',3',4',-hexakis-O-(trime thylsilyl)-α,α'-trehalose (TMM).

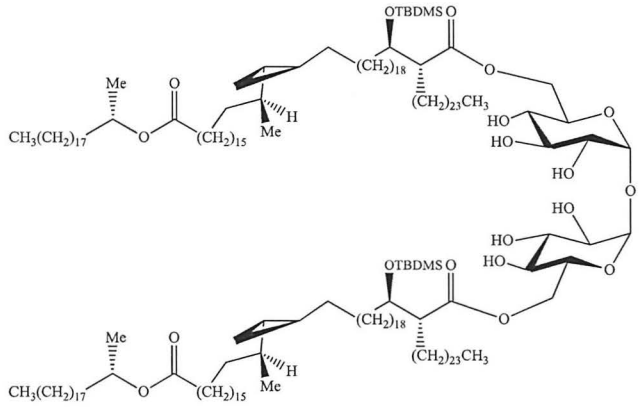


The procedure used in Experiment **42** was repeated using 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (0.107 g, 0.550 mmol), 4-dimethyl amino pyridine (DMAP) (0.068 mg, 0.55 mmol), (*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-20-((1*S*,2*R*)-2-((*S*)-19-((*S*)-icosan-2-yloxy)-19-oxononadecan-2-yl)cyclopropyl) icosyl) hexacosanoic acid (0.250 g, 0.204 mmol) and protected trehalose **(148)** (0.062 g, 0.080 mmol). The crude product was purified by column chromatography on silica eluting

with petroleum ether/ethyl acetate (25:1) to give a first fraction **TDM** as colourless oil (0.142 g); $[\alpha]_D^{21}$ = +23.2 (c 1.0, $CHCl_3$) [MS (MALDI-TOF): *m/z* 3608.18 $[(M+Na)^+]$, calculated for $C_{218}H_{438}NaO_{19}Si_8$: 3608.13]; δ_H: 4.92 (2H, sext, *J* 6.8 Hz), 4.85 (2H, d, *J* 2.9 Hz), 4.37 (2H, br, d, *J* 10.0 Hz), 4.04–3.98 (4H, m), 4.0–3.96 (2H, m) 3.93 (2H, m), 3.52 (2H, t, *J* 8.9 Hz), 3.38 (2H, dd, *J* 2.9, 9.3 Hz), 2.55 (2H, ddd, *J* 3.5, 4.75, 10.1 Hz), 2.27 (4H, t, *J* 7.5 Hz), 1.56–1.21 (296H, m), 1.20 (6H, d, *J* 6.2 Hz), 0.90–0.86 (18H, m, including a triplet at 0.88 ppm with *J* 6.9 Hz), 0.88 (18H, s), 0.70–0.40 (2H, m), 0.48–0.41(2H, m), 0.22–0.06 (6H, m), 0.16 (18H, s), 0.15 (18H, s), 0.14 (18H, s), 0.062 (12H, s); δ_C: 172.8, 172.6, 94.8, 73.5, 73.4, 72.8, 71.8, 70.7, 62.4, 51.8, 38.1, 37.4, 35.9, 34.5, 33.4, 31.9, 30.1, 29.6, 29.5, 29.5, 29.4, 29.37, 29.30, 29.15, 27.3, 27.26, 26.1, 25.7, 25.4, 25.1, 22.7, 22.3, 20.0, 19.7, 18.6, 14.1, 10.5, 1.0, 0.97, 0.94, 0.84, 0.15, 0.04, –4.5, –4.6; ν_{max}/cm^{-1}: 2918, 2850, 1731, 1470, 1375, 1254, 1215, 836, 760.

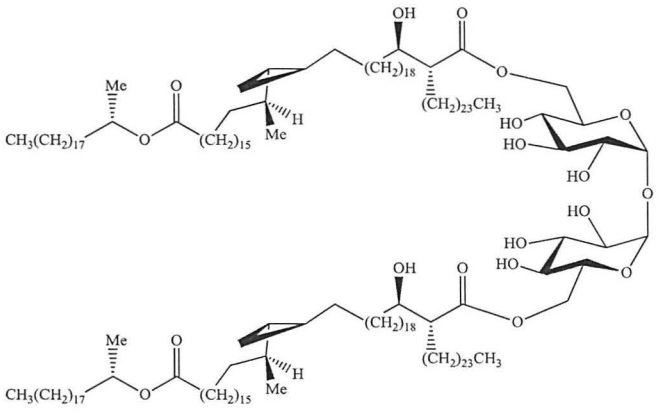
second fraction **TMM** as a colourless oil (0.068 g); $[\alpha]_D^{21}$ = +32.1 (*c* 0.9, $CHCl_3$) [MS (MALDI-TOF): *m/z* 2203.70 $[(M+Na)^+]$, calculated for $C_{124}H_{255}NaO_{15}Si_7$: 2202.73]; δ_H: 4.92 (2H, d, *J* 3.1 Hz), 4.91 (1H, sext, *J* 6.2 Hz), 4.85 (1H, d, *J* 3.0 Hz), 4.35 (1H, dd, *J* 2.1, 11.8 Hz), 4.08 (1H, dd, *J* 4.0, 11.8 Hz), 4.04–3.98 (4H, m), 3.85 (1H, dt, *J* 3.2, 9.5 Hz), 3.7 (2H, m), 3.49 (1H, dt, *J* 4.6, 9.1 Hz), 3.41 (1H, dd, *J* 3.1, 9.3 Hz), 3.38 (1H, dd, *J* 2.8, 9.1 Hz), 2.55 (1H, m), 2.27 (2H, t, *J* 7.5 Hz), 1.73 (1H, br. m), 1.68–1.21 (149H, br.m), 1.20 (3H, d, *J* 6.2 Hz), 0.90–0.86 (9H, m, including a triplet at 0.88 ppm with *J* 6.9 Hz), 0.88 (9H, s), 0.70–0.40 (1H, m), 0.48–0.41(1H, m), 0.22–0.06 (3H, m), 0.17 (18H, s), 0.15 (18H, s), 0.14 (18H, s), 0.060 (3H, s), 0.052 (3H, s); δ_C: 174.0, 173.6, 94.5, 94.4, 73.4, 73.3, 72.9, 72.8, 72.74, 71.9, 71.4, 70.7, 62.4, 61.7, 51.7, 38.1, 37.4, 35.9, 34.7, 34.5, 34.1, 33.4, 31.9, 30.0, 29.72, 29.70, 29.68, 29.65, 29.61, 29.52, 29.50, 29.45, 29.36, 28.1, 27.3, 26.2, 26.1, 25.8, 25.4, 25.1, 22.62, 22.60, 22.3, 20.0, 19.7, 18.6, 14.1, 14.0, 10.4, 1.2, 1.0, 0.97, 0.9, 0.8, 0.1, 0.0, –4.5, –4.5; ν_{max}/cm^{-1}: 3432, 2918, 2850, 1732, 1470, 1375, 1251, 836, 760.

38: 6,6-*bis*-*O*-(*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-20-((1*S*,2*R*)-2-((*S*)-19-((*S*)-icosan-2-yloxy)-19-oxononadecan-2-yl)cyclopropyl)icoecyl) hexacosa noic α,α'-trehalose



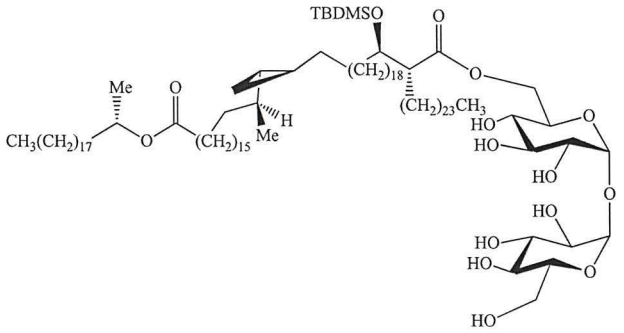
The same procedure used in Experiment **43** was repeated to deprotect the **TDM** (0.125 g, 0.034 mmol) using tetrabutylammonium fluoride (0.100 mL, 0.100 mmol, 1M) in dry THF (7 mL). The residue was purified by column chromatography on silica eluting with $CHCl_3$/MeOH 10:1 to give a syrup of the title product (0.060 g, 55 %); $[\alpha]_D^{21} = +22.3$ (*c* 0.42, $CHCl_3$) [MS (MALDI-TOF): *m/z* 3175.53 $[(M+Na)^+]$, calculated for $C_{200}H_{390}NaO_{19}Si_2$: 3175.89]; δ_H ($CDCl_3$ + few drops of CD_3OD): 5.05 (2H, d, *J* 2.7 Hz), 4.92 (2H, sext, *J* 6.4 Hz), 4.37 (2H, br, d, *J* 8.3 Hz), 4.23 (2H, br, d, *J* 10.8 Hz), 3.95–3.89 (4H, m), 3.51 (2H, t, *J* 8.8 Hz), 3.34 (2H, t, *J* 9.0 Hz), 3.30 (2H, t, *J* 9.4 Hz), 2.55 (2H, br.m), 2.27 (4H, t, *J* 7.5 Hz),1.61–1.24 (298H, m), 1.18 (6H, d, *J* 6.3 Hz), 0.90–0.86 (18H, m, including a triplet at 0.88 ppm with *J* 6.9 Hz), 0.88 (18H, s), 0.72–0.61 (2H, m), 0.49–0.38 (2H, m), 0.20–0.06 (6H, m), 0.03 (6H, s), 0.01 (6H, s); δ_C: 175.2, 173.2, 93.3, 73.2, 72.8, 71.6, 70.8, 70.3, 70.0, 62.4, 51.6, 38.0, 37.4, 35.9, 34.5, 33.4, 31.9, 30.0, 29.7, 29.5, 29.48, 29.4, 29.34, 29.30, 29.15, 26.1, 25.7, 25.4, 25.1, 22.9, 22.3, 20.1, 19.6, 18.6, 14.0, 10.4, –4.5, –4.8; ν_{max}/cm^{-1}: 3432, 2918, 2850, 1731, 1721, 1470, 1372, 1250.

39: 6,6-*bis*-*O*-(*R*)-2-((*R*)-1-hydroxy-20-((1*S*,2*R*)-2-((*S*)-19-((*S*)-icosan-2-yloxy)-19-oxononadecan-2-yl)cyclopropyl)icocyl)hexacosanoate-α,α'-trehalose (269)



The procedure used in Experiment **44** was repeated to deprotect the **TDM** (0.050 g, 0.016 mmol) using pyridine (0.1 mL) and hydrogen fluoride-pyridine complex as ~70% (0.35 mL). The residue was purified by chromatography eluting with $CHCl_3$/MeOH (10:1) to give a syrup of **(269)** (0.006 mg, 11%); $[\alpha]_D^{21}$ = +30.1 (*c* 0.3, $CHCl_3$) [MS (MALDI-TOF): *m/z* 2947.18 [$(M+Na)^+$], calculated for $C_{188}H_{362}NaO_{19}$: 2947.72]; δ_H ($CDCl_3$ + few drops of CD_3OD): 4.99 (2H, d, *J* 3.2 Hz), 4.87 (2H, sext, *J* 6.4 Hz), 4.68 (2H, m), 4.27 (2H, br.t, *J* 8.7 Hz), 3.93 (2H, br, t, *J* 10.6 Hz), 3.79–3.73 (2H, m), 3.68–3.62 (2H, m), 3.51 (2H, d, *J* 8.4 Hz), 3.22 (2H, t, *J* 8.2 Hz), 2.40 (2H, br.m), 2.23 (4H, t, *J* 7.5 Hz), 1.61–1.24 (294H, br.m), 1.17 (6H, d, *J* 6.3 Hz), 0.87 (6H, d, *J* 6.8 Hz), 0.87–0.83 (12H, br.t, *J* 6.9 Hz), 0.67–0.62 (2H, m), 0.46–0.38 (2H, m), 0.19–0.03 (6H, m); δ_C: 175.2, 173.2, 95.3, 73.2, 72.8, 71.7, 70.8, 70.3, 70.0, 62.4, 51.6, 38.0, 37.3, 35.9, 34.5, 33.4, 31.9, 30.1, 29.7, 29.5, 29.5, 29.4, 29.36, 29.31, 29.18, 26.1, 25.8, 25.4, 25.1, 22.7, 22.3, 20.0, 19.7, 18.6, 14.0, 10.4, –4.5, –4.9; ν_{max}/cm^{-1}: 3432, 2918, 2850, 1734, 1470, 1372.

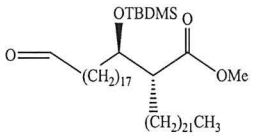
40:6-*O*-(*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-20-((1*S*,2*R*)-2-((*S*)-19-((*S*)-icosan-2-yloxy)-19-oxononadecan-2-yl)cyclopropyl)icocyl)hexacosanoic -α,α'-trehalose.



The procedure used in Experiment **43** was repeated to deprotect the **TMM** (0.060 g, 0.027 mmol) using tetrabutylammonium fluoride (0.082 mL, 0.082 mmol, 1M) in dry THF (5 mL) at 5 °C under nitrogen. The residue was purified by column chromatography on silica eluting with $CHCl_3$/MeOH (85:15) to give a semi-solid of the title product (0.026 g, 54 %); $[\alpha]_D^{21}$ = +20.2 (*c* 0.88, $CHCl_3$) [MS (MALDI-TOF): *m/z* 1770.52 [$(M+Na)^+$], calculated for $C_{106}H_{206}NaO_{15}Si$: 1770.50]; δ_H ($CDCl_3$ + few drops of CD_3OD): 5.07 (2H, d, *J* 3.2 Hz), 4.86 (1H, sext, *J* 6.1 Hz), 4.33–4.23 (2H, br.m), 3.94–3.87 (4H, br.m), 3.81 (1H, d, *J* 9.8 Hz), 3.7 (1H, m), 3.51 (2H, d, *J* 8.9 Hz), 3.38–3.35 (2H, br.m), 3.34 (1H, d, *J* 9.6 Hz), 2.55 (1H, m), 2.27 (2H, t, *J* 7.5 Hz), 1.59–1.22 (148H, m), 1.17 (3H, d, *J* 6.3 Hz), 0.90–0.86 (9H, m, including a triplet at 0.88 ppm with *J* 6.9

Hz), 0.86 (9H, s), 0.66–0.60 (1H, m), 0.44–0.32 (1H, m), 0.20–0.06 (3H, m), 0.011 (3H, s), 0.010 (3H, s); δ_C: 175.1, 173.8, 93.4, 93.3, 73.2, 73.2, 72.9, 72 .7, 72.5, 72.1, 71.6, 70.8, 70.7, 69.9, 62.7, 62.0, 51.6, 38.0, 37.3, 35.8, 34.7, 34.4, 31.8, 29.7, 29.7, 29.6, 29.55, 29.51, 29.4, 29.37, 28.1, 27.3, 26.2, 26.1, 25.8, 25.4, 25.1, 22.66, 22.60, 22.3, 20.0, 19.7, 18.5, 17.9, 14.0, 10.4, –4.6, –5.0; ν_{max}/cm^{-1}: 3432, 2918, 2850, 1733, 1470, 1374, 1249, 836, 760.

41: (*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-19-oxononadecyl]-tetra-cosanoic acid methyl ester (276)



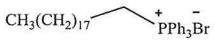
(i) The procedure used in Experiment **1** was repeated in order to couple (*R*)-2-[(*R*)-1-(*tert*-butyl-dimethyl-silanyloxy)-3-oxo-propyl]-tetracosanoic acid methyl ester (2.72 g, 6.91 mmol) and 2,2-dimethyl-propionic acid and 16-(1-phenyl- 1H-tetrazole-5-sulfonyl)-hexadecyl ester (1.67 g, 3.12 mmol) using lithium *bis*(trimethylsilyl)amide (4.35 mL, 4.68 mmol) in dry THF (75 mL) under nitrogen at -15 °C. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give the pure product (3.55 g, 80 %).[149] Palladium 10 % on carbon (0.50 g) was added to a stirred solution of (*E*/*Z*)-(*R*)-2-[(*R*)- 1-(*tert*-butyldimethylsilanyloxy)-3-benzyloxy-propyl]-tetracos-4-enoic acid methyl ester (3.45 g, 3.92 mmol) in IMS (60 mL) and (60 mL) THF. The reaction mixture was stirred under hydrogen for 1 hour. The solution was filtered on a pad of celite and the solvent was evaporated to give a colourless oil of (*R*)–2-[(*R*)–1-(*tert*-Butyldimethylsilanyloxy)-19-(2,2-dimethyl propio nyloxy)-nonadecyl]-tetracosanoic acid methyl ester **(280)** (3.40 g, 98 %).[149]

(ii) Water (2 mL), MeOH (20 mL) were added to a stirred solution of (*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilanyloxy)-19-(2,2-dimethylpropionyloxy)-nonadecyl]-tetra-cosanoic acid methyl ester **(280)** (3.40 g, 3.87 mmol) in THF (25 mL), followed by addition of KOH (3.25 g, 58.08 mmol, 15 mol eq.). The reaction mixture was refluxed for 45 minutes. When TLC showed no starting material was left, the mixture was quenched with water 25 mL, and then extracted with ethyl acetate (3 × 75 mL). The combined organic layers were dried, and the solvent was evaporated to give crude product, which was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give

a colourless oil of (*R*)-2-[(*R*)-1-(*tert*-Butyl-dimethyl-silanyloxy)-19 hydroxynonadecyl] -tetracosanoic acid methyl ester **(281)** (2.50 g, 82%).[149]

(iii) The procedure described in Experiment **5** was repeated to oxidize (*R*)-2-[1-(*tert*-Butyldimethylsilanyloxy)-19-hydroxynonadecyl]-tetracosanoic acid methyl ester (0.7 g, 0.88 mmol) using PCC (0.46 g, 2.22 mmol) in CH_2Cl_2 (70 mL). The crude product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give a colourless oil of **(276)** (0.61 g, 87 %).[149]

42: Nonadecyltriphenylphosphonium bromide (285)

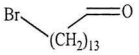


(i) the procedure used in Experiment **4** was repeated to reduce methyl nonadecanoate **(282)** (14.0 g, 44.8 mmol) using lithium aluminium hydride (2.55 g, 67.3 mmol) in THF (180 mL) at 0 °C. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) gave a white solid of nonadecan-1-ol **(283)** (12.6 g, 98 %).[143]

(ii) The procedure used in Appendix **9** was repeated to brominate nonadecan-1-ol **(283)** (12.6 g, 57.6 mmol) using *N*-Bromosuccinimide (10.2 g, 76.7 mmol) and triphenyl phosphine (14.5 g, 55.4 mmol) in CH_2Cl_2 (300 mL) at -10 °C. The crude product was purified by column chromatography eluting with petrol to give a white solid of 1-bromo-nonadecane **(284)** (14.9 g, 97 %).[143]

(iii) The procedure used in Experiment **54** was repeated using 1-bromononadecane **(284)** (14.84 g, 46.57 mmol) using triphenylphosphine (11.24 g, 46.57 mmol) in toluene (200 mL). The residue was purified by column chromatography firstly eluting with petroleum ether and then CH_2Cl_2/MeOH (95:5) to give a white solid of **(285)** (17.15 g, 65%).[143]

43: 14-Bromo-tetradecanal (286)



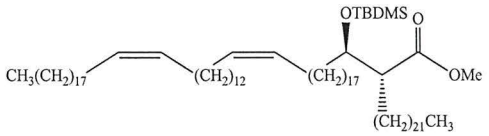
The procedure used in Experiment **5** was repeated to oxidize 14-Bromotetradecan-1-ol (5 g, 17.12 mmol) using PCC (12.86 g, 51.11 mmol) in CH_2Cl_2 (250 mL). The crude product was purified by column chromatography eluting with petrol/ethyl actate (5:1) to give a colourless oil of **(286)** (4.1 g, 83%).[143]

44: ((*Z*)-Dotriacont-14-enyl)-triphenylphosphonium bromide (275)

(i) Sodium *bis*(trimethylsilyl)amide (12.8 mL, 12.8 mmol, 1.0M in THF) was added to a stirred solution of nonadecyltriphenylphosphonium bromide **(285)** (4.35 g, 7.14 mmol) in (70 mL) dry THF at room temperature under nitrogen atmosphere. The mixture was stirred for 30 min then 14-bromo-tetradecanal **(286)** (1.65 g, 5.49 mmol) in dry THF (20 mL) was added, and the stirring was continued for 3 h at r.t. The reaction was quenched with sat. aq. NH_4Cl (10 mL) and the product was extracted with 20:1 petrol: ethyl acetate (3 × 50 mL). The combined organic layers were dried and the solvent was evaporated. The product was purified by column chromatography eluting with petrol/ethyl acetate (40:1) to give a white solid of *(Z)*-1-Bromo-tritriacont-14-ene **(287)** (1.88 g, 61 %).[143]

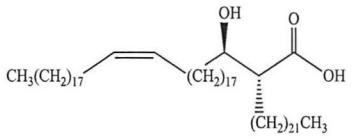
(ii) The procedure used in Experiment **54** was repeated using (*Z*)-1-Bromo-dotriacont-14-ene **(287)** (2.56 g, 4.72 mmol) and triphenylphosphine (1.85 g, 7.08 mmol) in toluene (30 mL). The residue was purified by column chromatography firstly with petrol and then with CH_2Cl_2/MeOH (9:1) to give a white solid of **(275)** (2.60 g, 69 %).[143]

45: (*20Z, 34Z*)-(*2R, 3R*)-3-(*tert*-Butyldimethylsilanyloxy)-nonatriaconta- 20,34-dienoic -2- tetracosanoic acid methyl ester (288)



Sodium *bis*(trimethylsilyl)amide, (0.46 mL, 0.46 mmol, 1.0 M in THF) was added to a solution of ((*Z*)-dotriacont-14-enyl)-triphenylphosphonium bromide **(275)** (0.205 g, 0.252 mmol) in (5 mL) of dry THF at room temperature under a nitrogen atmosphere. The mixture was stirred for 30 min and then 3-(*tert*-butyldimethylsilanyloxy)-21-hydroxy-heneicosanoic acid methyl ester **(276)** (0.145 g, 0.180 mmol) in dry THF (2 mL) was added at room temperature. The mixture was stirred for 18 hours, and then the reaction quenched with sat. aq. NH4Cl (10 mL). The product was extracted with petrol/ethyl acetate (10:1, 3 × 30 mL), and the combined organic layers dried and the solvent was evaporated to give the crude product, which was purified by column chromatography eluting with petrol/ethyl acetate (40:1) to give **(288)** (0.065 g, 29 %).[143]

46: (*2R, 3R, Z*)-2-Docosyl-3-hydroxytetracont-21-enoic acid (133)



(i) The procedure used in Appendix **45** was repeated to couple nonadecyltriphenyl phosphonium bromide **(285)** (0.90 g, 1.46 mmol) with (*R*)-2-[(*R*)-1-*tert*-butyl dimethyl silanyloxy)-19-oxononadecyl] tetracosanoic acid methyl ester **(276)** (0.58 g, 0.73 mmol) using sodium *bis*(trimethylsilyl)amide (2.19 mL, 2.19 mmol, 1.0M in THF) in dry THF (3 mL). The product was purified by chromatography eluting with petrol/ethyl acetate (40:1) to give syrup of (*Z*)-(*R*)-3-(*tert*-Butyldimethyl silanyloxy)-octatriacont-20-enoic -2- tetracosa noic acid methyl ester **(294)** (0.561 g, 72 %).[149]

(ii) The procedure used in Experiment **16** was repeated to deprotect (*Z*)-(*R*)-3-(*tert*-butyldimethylsilanyloxy)-octatriacont-20-enoic-2-tetracosanoic acid methyl ester **(294)** (0.54 g, 0.50 mmol) using pyridine and hydrogen fluoride-pyridine complex as ~70% (1.5 mL) (0.3 mL) in dry THF (15 mL). The crude product was purified by chromatography eluting with petrol/ ethyl acetate (10:1) to give a semi-solid of methyl (2*R*, 3*R*, *Z*)-2-docosyl-3-hydroxytetracont-21-enoate **(295)** (0.42 g, 91 %), [MS (MALDI-TOF): *m/z* 952.6 [$(M+Na)^+$], calculated for $C_{63}H_{124}NaO_3$: 951.9].[149]

(iii) The procedure used in Experiment **19** was repeated using methyl ester **(295)** (0.404 g, 0.434 mmol) and lithium hydroxide monohydrate (0.30 g, 5.24 mmol) in THF (13 mL), MeOH (1.4 mL) and water (2.2 mL). The crude product was purified by column chromatography eluting with petrol/ethyl acetate (7:2) to give a white solid of **(133)** (0.35 g, 88 %).[149]