DOCTOR OF PHILOSOPHY

Synthesis of an α-mycolic acid from Mycobacterium brumae containing a cis-alkene and an α-methyl-trans-alkene

Al-araj, Intisar Qahtan Mahmood

Award date:
2018

Awarding institution:
Bangor University

Synthesis of an α-mycolic acid from *Mycobacterium brumae* containing a *cis*-alkene and an α-methyl-*trans*-alkene

A thesis submitted to Bangor University

for the degree of Doctor of Philosophy

By

Intisar Qahtan Mahmood Al-araj

2018

Declaration and Consent

Details of the Work:

I hereby agree to deposit the following item in the digital repository maintained by Bangor University and/or in any other repository authorized for use by Bangor University.

Author Name: Intisar Qahtan Mahmood Al-araj

Title: Mrs.

Supervisors/Department: Professor Mark S. Baird & Dr. Juma'a R. Al-Dulayymi /Chemistry.

Funding body (if any):

Qualification/Degree obtained: PhD in Chemistry.

This item is a product of my own research endeavours and is covered by the agreement below in which the item is referred to as "the Work". It is identical in content to that deposited in the Library, subject to point 4 below.

Non-exclusive Rights:

Rights granted to the digital repository through this agreement are entirely non-exclusive. I am free to publish the Work in its present version or future versions elsewhere. I agree that Bangor University may electronically store, copy or translate the Work to any approved medium or format for the purpose of future preservation and accessibility. Bangor University is not under any obligation to reproduce or display the Work in the same formats or resolutions in which it was originally deposited.

Bangor University Digital Repository:

I understand that work deposited in the digital repository will be accessible to a wide variety of people and institutions, including automated agents and search engines via the World Wide Web.

I understand that once the Work is deposited, the item and its metadata may be incorporated into public access catalogues or services, national databases of electronic theses and dissertations such as the British Library's EThOS or any service provided by the National Library of Wales.

I understand that the Work may be made available via the National Library of Wales Online Electronic Theses Service under the declared terms and conditions of use (http://www.llgc.org.uk/index.php?id=4676). I agree that as part of this service the National Library of Wales may electronically store, copy or convert the Work to any approved

medium or format for the purpose of future preservation and accessibility. The National Library of Wales is not under any obligation to reproduce or display the Work in the same formats or resolutions in which it was originally deposited.

Statement 1:

This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree unless as agreed by the University for approved dual awards.

Signed ……..…. (candidate) Date ……..

Statement 2:

This thesis is the result of my own investigations, except where otherwise stated. Where correction services have been used, the extent and nature of the correction is clearly marked in a footnote(s).
All other sources are acknowledged by footnotes and/or a bibliography.

Signed …… (candidate) Date ……

Statement 3 (bar):

I hereby give consent for my thesis, if accepted, to be available for photocopying, for inter-library loans and for electronic repositories after expiry of a bar on access.

Signed…… (candidate) Date ……

Statement 4:

Choose **one** of the following options:

a) I agree to deposit an electronic copy of my thesis (the Work) in the Bangor University (BU) Institutional Digital Repository, the British Library ETHOS system, and/or in any other repository authorized for use by Bangor University and where necessary have gained the required permissions for the use of third party material.	
b) I agree to deposit an electronic copy of my thesis (the Work) in the Bangor University (BU) Institutional Digital Repository, the British Library ETHOS system, and/or in any other repository authorized for use by Bangor University when the approved **bar on access** has been lifted.	

c) I agree to submit my thesis (the Work) electronically via Bangor University's e-submission system, however I **opt-out** of the electronic deposit to the Bangor University (BU) Institutional Digital Repository, the British Library ETHOS system, and/or in any other repository authorized for use by Bangor University, due to lack of permissions for use of third party material.	

Options B should only be used if a bar on access has been approved by the University.

In addition to the above I also agree to the following:

1. That I am the author or have the authority of the author(s) to make this agreement and do hereby give Bangor University the right to make available the Work in the way described above.
2. That the electronic copy of the Work deposited in the digital repository and covered by this agreement, is identical in content to the paper copy of the Work deposited in the Bangor University Library, subject to point 4 below.
3. That I have exercised reasonable care to ensure that the Work is original and, to the best of my knowledge, does not breach any laws – including those relating to defamation, libel and copyright.
4. That I have, in instances where the intellectual property of other authors or copyright holders is included in the Work, and where appropriate, gained explicit permission for the inclusion of that material in the Work, and in the electronic form of the Work as accessed through the open access digital repository, *or* that I have identified and removed that material for which adequate and appropriate permission has not been obtained and which will be inaccessible via the digital repository.
5. That Bangor University does not hold any obligation to take legal action on behalf of the Depositor, or other rights holders, in the event of a breach of intellectual property rights, or any other right, in the material deposited.
6. That I will indemnify and keep indemnified Bangor University and the National Library of Wales from and against any loss, liability, claim or damage, including without limitation any related legal fees and court costs (on a full indemnity bases), related to any breach by myself of any term of this agreement.

Signature: …… Date: ……

Table of Contents

Acknowledgments

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

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

I would like to thank Dr. Alison Jones for her assistance with the mass spectrometry, and all her help throughout the project.

Many thanks to all my friends on the 10^{th} floor Dr. Deiniol Pritchard and Anahit Hovhannisyan, for all the help throughout the study.

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

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

List of abbreviations

AIDS	Acquired Immune Deficiency Syndrome
Aq	Aqueous
BCG	Bacillus Calmette-Guérin
Bn	Benzyl
br	Broad
CID-MS	Collision-induced dissociation mass spectrometry
cm^{-1}	Per centimetre
CoA	Coenzyme A
d	Doublet
DCM	Dichloromethane
dd	Double doublet
DEAD	Diethyl azodicarboxylate
DIBAL-H	Di-isobutylaluminium hydride
DMAP	4-Dimethylaminopyridine
DMF	*N*,*N*-Dimethylformamide
DMS	Dimethyl sulfide
dt	Doublet of triplet
d.w	Disttles water
EI	Electron Impact
eq	Equivalent
Ether	Diethyl ether
g	Gram
GMM	Glucose monomycolate
GroMM	Glycerol monomycolate
h	Hour
^{1}H	Proton
HIV	Human Immunodeficiency Virus
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
m	*meta*-
m	Multiplet
MAC	*Mycobacterium avium* complex
MALDI	Matrix-Assisted Laser Desorption/Ionization
MALDI-TOF	Matrix-Assisted Laser Desorption/Ionization-Time of Flight
MAM	Methyl Arabino-Mycolates
MCPA	*m*-Chloroperoxybenzoic acid
MDR-TB	Multidrug-resistance TB
MHz	Mega Hertz
mmol	Millimole
mol. equiv.	Molar equivalents
m.p	Melting point
MS	Mass spectrometry
M.tb	*Mycobacterium tuberculosis*
NBS	*N*-Bromosuccinimide
NMR	Nuclear Magnetic Resonance
NSI	Nano-Electrospray Ionization
PCC	Pyridinium chlorochromate
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

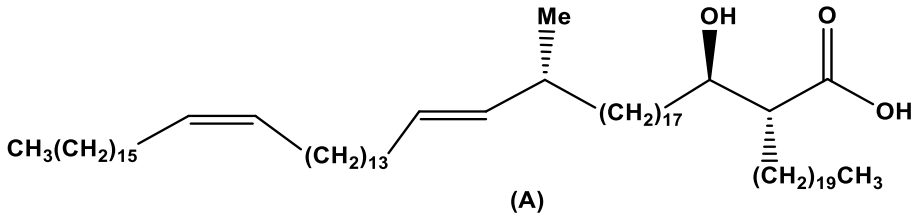
Sat	Saturated
NaHMDS	Sodium *bis*(trimethylsilyl)amide
t	Triplet
TB	Tuberculosis
TBAF	Tetra-*n*-butylammoniunfluoride
TBAH	Tetra-*n*-butylammoniunhydroxide
TBDMS	*Tert*-Butyldimethylsilyl
TDM	Trehalose dimycolate
THF	Tetrahydrofuran
THP	Tetrahydropyran
TLC	Thin-Layer-Chromatography
TMM	Trehalose monomycolate
TNF	Tumor necrosis factor
TsCl	*p*-Toluenesulfonyl chloride
v.br.	Very broad
WHO	World Health Organisation
XDR-TB	Extensively Drug Resistant tuberculosis

Abstract

Mycolic acids are high molecular weight α-alkyl-branched β -hydroxy long chain fatty acids (60-90 carbon atoms). Different species of Mycobacteria may produce different classes of mycolic acids including α-, methoxy and keto mycolic acids, each present as mixtures of homologues. These may also contain different functional groups such as ester, keto, methoxy, hydroxyl, and alkene groups. Mycolic acids and their trehalose esters are very important components of the mycobacterial cell wall. They 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 new methods for the detection of disease.

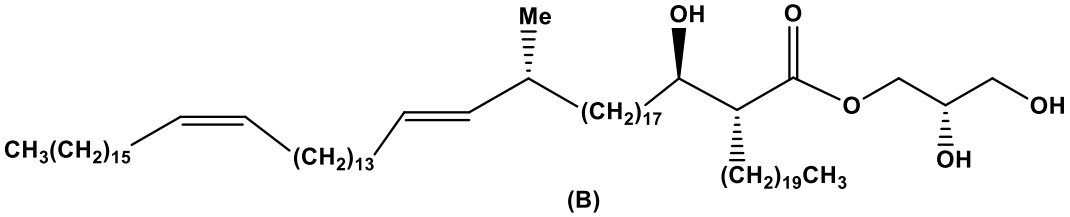
The first part of this project involved the development of a new method for the synthesis of the α-alkyl-β-hydroxy fragment of mycolic acids, which required fewer steps compared to previous syntheses and used *L*-malic acid as starting material.

The second part was to synthesise *cis*-alkene-α-methyl-*trans*-alkene mycolic acid **(A)**, which contains a double bond at the distal and proximal positions. This type of mycolic acid has been isolated from *Mycobacterium brumae*.

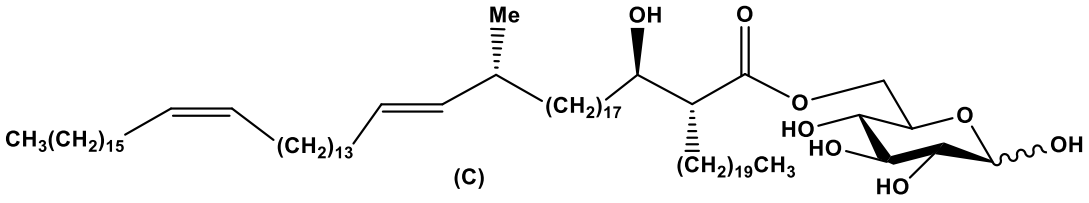


(A)

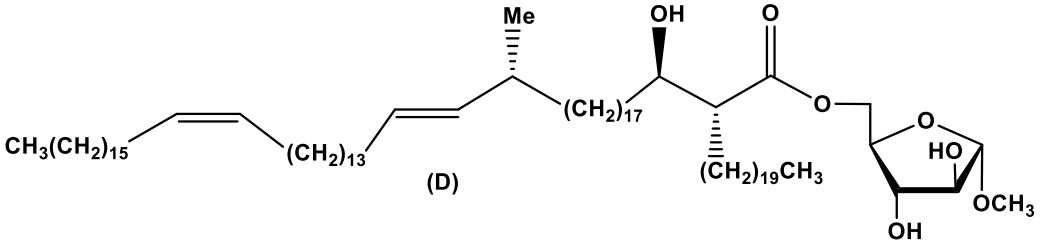
The third part of the project included the preparation of a number of sugar and glycerol esters of mycolic acid **(A)**. These included the GroMM **(B)**, GMM **(C),** the arabino-MA fragment from AG **(D)** and the trehalose esters TDM **(E)** and TMM **(F)**.



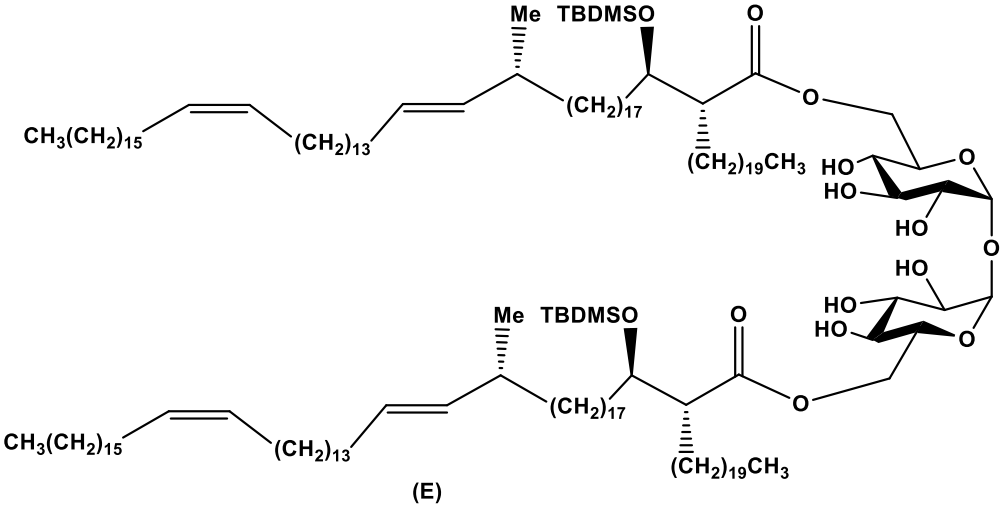
(B)


Me
OH
O
$CH_3(CH_2)_{15}$
$(CH_2)_{13}$
$(CH_2)_{17}$
$(CH_2)_{19}CH_3$
HO
HO
HO
OH

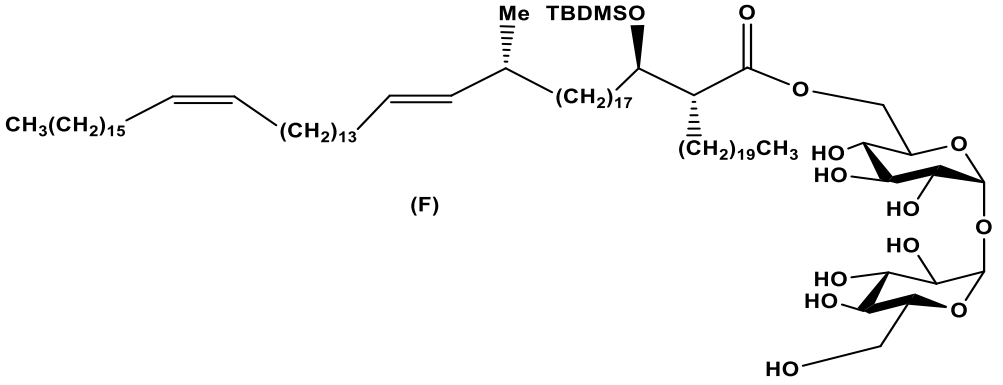
(C)


Me
OH
O
$CH_3(CH_2)_{15}$
$(CH_2)_{13}$
$(CH_2)_{17}$
$(CH_2)_{19}CH_3$
HO
OCH_3
OH

(D)

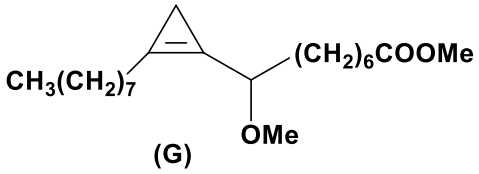

Me
TBDMSO
O
$CH_3(CH_2)_{15}$
$(CH_2)_{13}$
$(CH_2)_{17}$
$(CH_2)_{19}CH_3$
HO

(E)


Me
TBDMSO
O
$CH_3(CH_2)_{15}$
$(CH_2)_{13}$
$(CH_2)_{17}$
$(CH_2)_{19}CH_3$
HO

(F)

The final part of the project involved the synthesis of a different kind of fatty acid: the methyl esters of cyclopropene fatty acids, such as α-methoxy cyclopropane fatty acid (**G**).



(G)

These compounds have been tested and were shown to inhibit *Toxoplasma gondii* growth *in vitro,* with compound (**G**) being the most effective.

Chapter 1

1. Introduction

1.1 Tuberculosis (TB)

Tuberculosis is an infectious disease, which has an ancient history,[1,2] and is caused by *Mycobacterium tuberculosis* (*M.tb*). Although the lungs are the main place of infection in the body, other parts can be infected, such as the kidneys, lymph nodes and central nervous system.[3,4] The transmission of this disease usually occurs when a person breathes in air that contains species of *M. tuberculosis* from infected people, via coughing, sneezing, talking or spitting.[1] Nowadays it is believed that one third of the world's population is infected with TB, however only in 10% of the cases does TB become active and cause illness.[5] TB is known by many names, such as: 'The king of diseases' in India; 'The captain of all these men of death';[5] and 'phthisis' by the Greek physician, Hippocrates.[6] TB has been suggested to have originated in Africa as far back as 35,000 years ago.[2,7,8] Investigations have shown signs of death as a result of TB in Egyptian mummies, which are over 5,000 years old,[9] as well as in a 9000 year old human skeleton in the Eastern Mediterranean.[7] There is also evidence showing the presence of TB in China 2300 years ago and in India 3300 years ago.[10,11] It is believed that TB in the Americas was present before the arrival of European explorers, with a similarity to that found in Egypt.[12,13] Throughout the middle ages there is widespread archaeological evidence of TB.[13] TB was responsible for many deaths and it was not until 1819 when a group of French physicians, the most notable being Rene Theophile Hyacinthe Laennec who wrote *D'Ausculation Mediate,* outlined the physical signs of TB and its pathology.[13] During Laennec's era death rates in many major European and American cities had reached 800-1000 per 100,000 deaths per year.[13,14,15,16]

There were no significant advances in the knowledge of TB until 1882 when Robert Koch gave their famous presentation on the tubercle bacillus and on their work to postulate the link between a microbe and disease. More commonly known as the Koch postulates, they are still the standard explanation for how an infectious disease arises.[17,18] In 1905 Robert Koch, who was the winner of the Nobel Prize in Medicine and Physiology, first discovered a stain which enabled the visualisation of the *M. tuberculosis* bacilli and identified the causative agent of TB.[19,20] Koch isolated a substance from tubercle bacilli which rendered

the bacterium harmless. He named it tuberculin and in a demonstration, he injected himself with it.[21] Since Koch's work a reliable vaccine has never been developed because TB immunity differs from immunity to many other common microbial diseases.[18] Albert Calmette and Camille Guérin developed a vaccine from *Mycobacterium bovis (M. bovis)* called Bacille Calmette-Guérin (BCG).[22] Acceptance of the vaccine was slow as many people did not believe it to be safe, as it was based on live TB bacteria. The BCG vaccine was first used on humans in 1921 in France,[23] having agreed to share the responsibility for this first human experiment, Weill-Hallé and Calmette administered three oral doses of 2 milligrams of BCG to a new born baby, but it was not until after World War II that BCG received widespread acceptance in the United States, Great Britain and Germany.[24] The protection against disease by the vaccine does vary and overall is approximately 50%, for example, that against disseminated and meningeal disease in children is higher than that against pulmonary disease in adults.[25,26] In addition, it is not known how long and to what extent the protective effect lasts, although giving the vaccine to new-borns (who are mycobacterially naïve) increases its efficiency.[27] Although the reactivation of pulmonary TB cannot be protected against by routine vaccination, it does, however, reduce mortality and infection from TB in infants.[28,29] Thus the development of a more effective vaccine would help enormously in the fight against TB. A new vaccine would also assist against the scepticism surrounding the BCG vaccine, which has led to some countries not routinely vaccinating infants. Currently only 5% of deaths are prevented by the BCG vaccine,[30] a percentage which needs to be increased. One advantage of the BCG vaccine is that it also protects against Buruli ulcer, leprosy and helminth infection.[31] In addition, it reduces the risk of superficial bladder cancer progression, and it may help against atopic diseases such as asthma.[32] Recent studies show a 37% reduction in asthma in those who received a neonatal BCG vaccination.[33]

TB has surged in great epidemics and then receded, and epidemics have been recorded during the 18th and 19th centuries in Europe and North America.[15] During the early to mid-19th century there was a noticeable decline in TB cases in Europe and North America and the World Health Organization (WHO) has reported that TB infects one person every second, of whom 5-10% die as a direct result.[20] In 1993, the WHO announced TB as a major health problem and declared a global health emergency.[34] In 2007, there were an

estimated 9.27 million cases of TB,[35] as a comparison there were 9.24 million cases in 2006, 8.3 million cases in 2000 and 6.6 million cases in 1990. In 2009, 9.4 million new cases of TB were recorded, more than at any time in history.[36] A recent WHO report on Global TB Control, reports that from 2013-2015, new incidents of TB increased, with around 10.4 million new cases of TB being recorded worldwide in 2015 leading to 1.8 million deaths, with most being concentrated in six countries: South Africa, China, India, Nigeria, Indonesia and Pakistan.[37,38] **Figure (1)** shows the estimated number of new cases of TB per 100,000 population in 2016.[38]

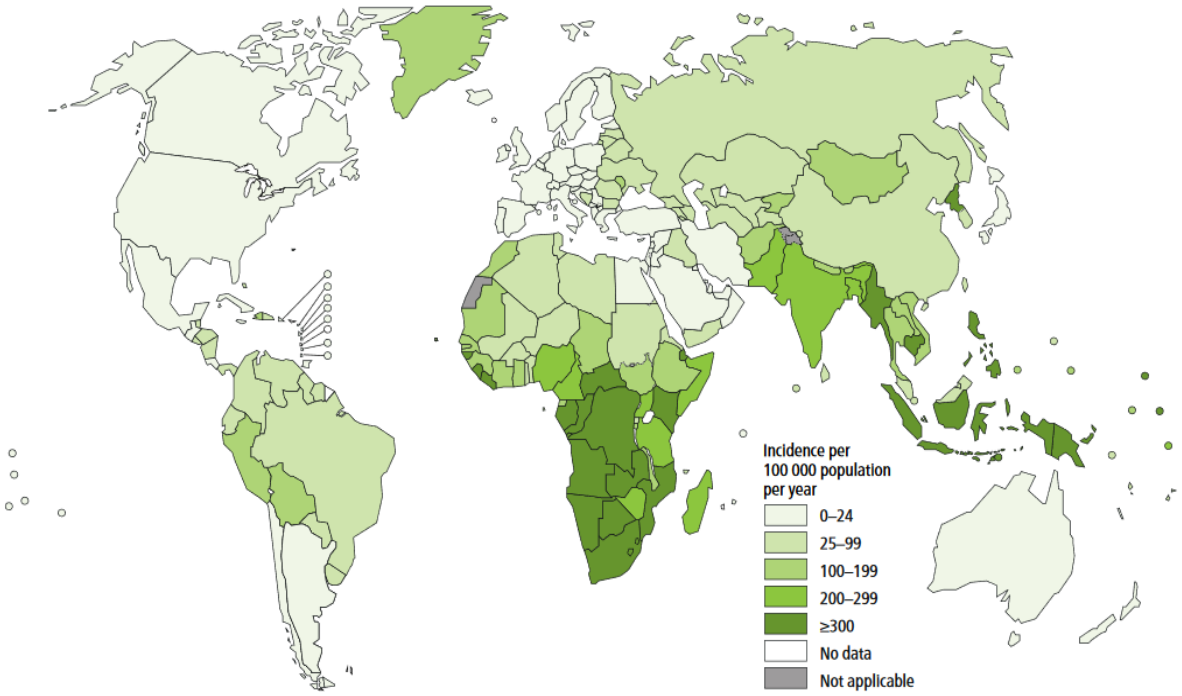


Figure (1): Map showing estimated TB incidence in 2016, for countries with at least 100 000 incident cases.[38]

Today TB is a major cause of preventable deaths by infection and is the leading cause of death in people who are infected with HIV/AIDS. People with HIV who are TB positive are more likely to move to active disease and are approximately 30 times as likely to die from TB compared to a person infected with TB alone,[39] because the HIV virus weakens the immune system, causing the TB bacteria to be more harmful. TB and HIV work in conjunction with each other and form a fatal combination, each increasing the progression rate of the other disease, with HIV infection being one of the major conditions that make people more

vulnerable to developing active TB. 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 the late diagnosis of TB patients co-infected with HIV result in the death of 48-85% of these cases, *i.e* one in three patients.[40] It is estimated that in 2010 alone there were 8.8 million new cases of TB, with an estimated 1.45 million deaths.[41] An estimated 1.1 million (13%) of the 8.6 million people who developed TB in 2012 were HIV-positive; about 75% of these cases were in the African Region and this is thought to be related to poor health services in these countries. About 82% of TB deaths among HIV-negative people occurred in the WHO African Region and the WHO South-East Asia Region in 2016; these regions accounted for 85% of the combined total of TB deaths in HIV-negative and HIV-positive people. India accounted for 33% of global TB deaths among HIV-negative people, and for 26% of the combined total TB deaths in HIV-negative and HIV-positive people (estimates of TB mortality rates per 100,000 population). **Figure (2)** shows the estimated worldwide distribution of TB patients co-infected with HIV. Statistics have shown a high incidence of HIV/AIDS-TB in Sub-Saharan Africa and other developing countries, where early and accurate diagnosis with effective therapy is still not easily accessible.[42,43,44,45] Invariably, the pathogenesis of the HIV/AIDS-TB pandemic presents an urgent challenge to global science.

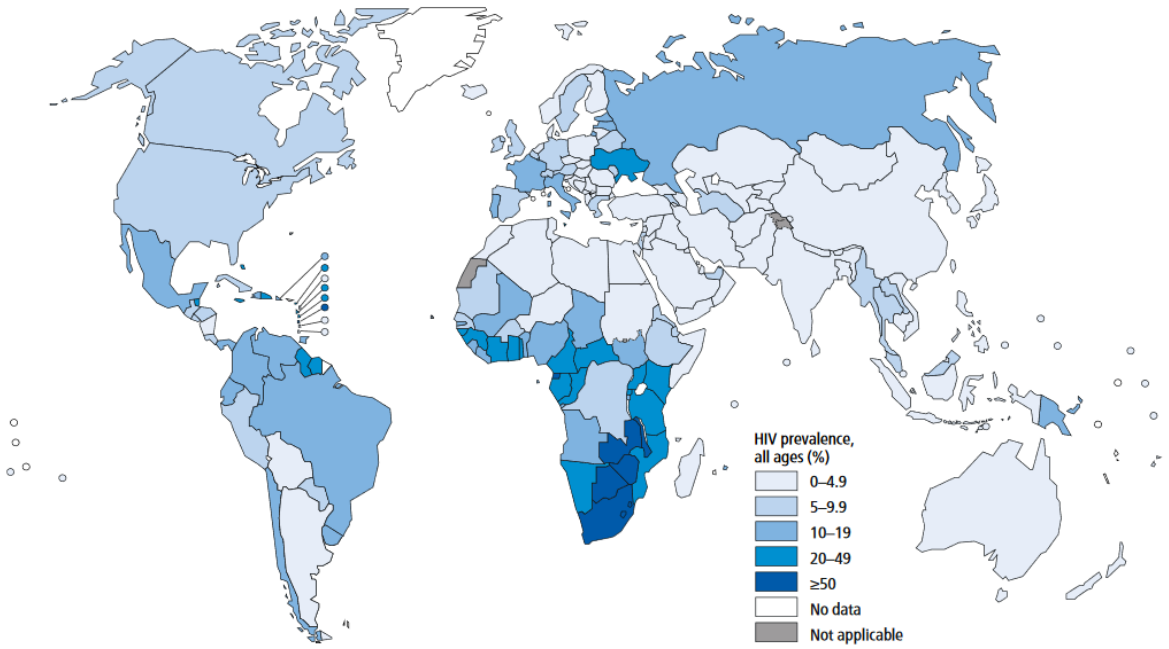


Figure (2): Map showing estimated HIV prevalence in new and relapse TB cases, 2016.[38]

Prevention and treatment are two aspects that must be harmonized in the battle against TB. The treatment of TB is hard and includes long courses of multiple antibiotics.[43,46] Antibiotics are usually part of the therapy in people who have no symptoms and whose germs are in an inactive state, and in this case are helpful in preventing the activation of infection. In the 1940s the first drug proven to be effective against TB was streptomycin **(1)** which was first isolated from *Streptomyces griseus*.[47] Another antibiotic, isoniazid **(2)** was introduced in the 1950s; subsequently, both drugs were used together in a multi-drug therapy.[48,49] The rifamycins (**3**) are a family of antibiotics obtained by fermentation and chemical modification. They were isolated in 1957 from a fermentation culture of *Streptomyces mediterranei*.[50] *para*-Aminosalicylic-acid (**4**),[51] is used to treat tuberculosis and is able to kill bacterial cells hiding in the intracellular compartment of macrophages. Ethambutol **(5)** is also used to kill the bacteria that cause tuberculosis by interfering with cell wall synthesis **(Figure 3)**.[52] These drugs are the recommended first-line treatments and are also used together to provide a multi-pronged sustained assault on the bacteria. The aim is to kill all the bacteria infecting the host, as a single cell may give rise to drug-resistant strains.

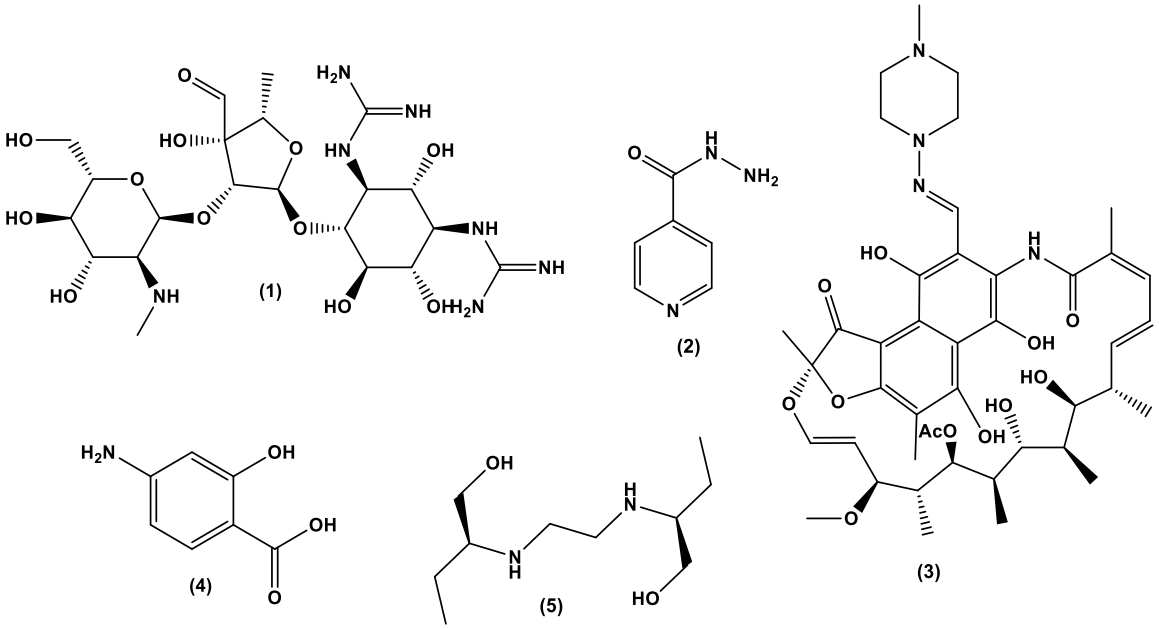


Figure (3): The structures of first-line anti-TB drugs.

The second-line drugs are for drug-resistant TB (see below) and for patients who cannot tolerate the first-line drugs; they are less effective, more toxic and require longer use than the first-line drugs.[53] Today, there are more than thirty different types of anti TB drugs being used, however most of them are analogues of the above products. According to the WHO, in 2007 the overall success rate for TB treatment was 70%.[35]

TB germs were found to be still growing in the sputum of a patient who had completed a course of streptomycin treatment, and a new deadly form of TB appeared in patients who had seemingly been treated with success.[54] This resistance has become more and more frequent and is most alarming as it is becoming apparent in the case of both first-line and second-line drugs.[55] This growing problem of antibiotic resistance, is coupled with the emergence of Multi–Drug Resistant Tuberculosis (MDR-TB) and Extensively-Drug Resistant Tuberculosis (XDR-TB).

MDR-TB caused by *M. tuberculosis* shows high-level resistance to both isoniazid and rifampicin with or without resistance to other drugs.[55,56] The probability of resistance is much higher for less effective antitubercular drugs.[57] MDR-TB can occur during the treatment for fully sensitive tuberculosis if a patient misses a dose, a patient doses not complete the course, or if the doctor administers the wrong treatment.[58]

XDR-TB is a form of TB caused by bacteria that are resistant to the most effective anti-TB drugs. XDR-TB is defined as TB that has developed resistance to the first line anti-TB drugs that define MDR-TB.[59] XDR-TB is associated with a much higher mortality rate than MDR-TB.[60]

1.2 Mycobacteria

Mycobacterium is a genus of actinobacteria, given its own family, which can cause fatal diseases in both humans and animals. Over 150 species are recognized in this genus and they can be divided into two classes: Tuberculous Mycobacteria such as *Mycobacterium tuberculosis, Mycobacterium bovis, Mycobacterium africanum, Mycobacterium microti, Mycobacterium caprae* and *M. canettii*;[61] and Non-tuberculous mycobacteria (NTM) which comprise over 90 species,[62] the most common ones associated with disease being *Mycobacterium abscessus*, *Mycobacterium avium* complex (MAC), *Mycobacterium brumae, Mycobacterium chelonae, Mycobacterium gordonae, Mycobacterium fortuitum* and *Mycobacterium smegmatis*.

1.3 Tuberculosis mycobacteria

The tuberculosis mycobacteria constitute a collection of *M. tuberculosis, M. bovis, M. microti* and *M. africanum,* collectively known as *M. tuberculosis* complex, which can all cause tuberculosis disease in both humans and animals.[63]

1.3.1 *Mycobacterium tuberculosis*

As indicated, TB is mainly caused in humans by certain members of the *Mycobacterium* genus. The most important of these, in terms of number of infections, is *M. tuberculosis* which can be seen in **Figure (4)** below.[1,64]

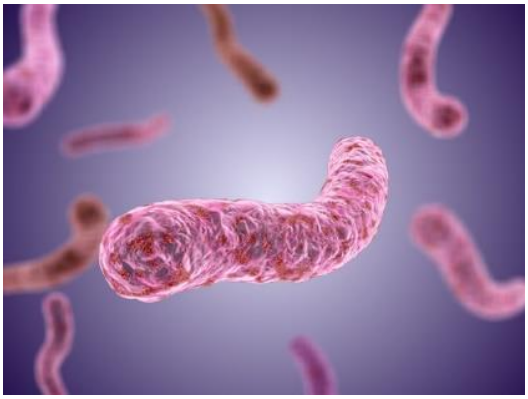


Figure (4): Scanning electron micrograph of *M. tb*.[65]

It is classified as an acid fast gram-positive bacterium; it is gram positive and is acid fast because it does not retain methyl violet stain well.[66] It is a small rod-shaped bacillus, approximately (1-4.4, 0.3-0.6 μm) in size and divides every 16 to 20 hours. This rate of division is slower than that of most other bacteria.[67,68]

1.3.2 Other relevant mycobacteria

M. tb is linked to other pathogenic mycobacteria, with some responsible for causing tuberculosis in other animal species.[69] Of the tuberculosis causing mycobacteria in mammals one of the more well-known is *M. bovis*, which is responsible for tuberculosis in cattle, which is of concern as there is evidence that *M. bovis* is transferable to humans via an infected cow's milk or via an aerosol.[70] *Mycobacterium africanum*, a heterogenous group of strains found in West Africa with similar symptoms to *M. tb*, has also been observed.[71,72] Other mycobacterial species causing disease in humans are *Mycobacterium leprae* which causes leprosy, *Mycobacterium ulcerans* which is responsible for the dangerous and potentially fatal Buruli ulcer, a skin and sometimes bone infection.[73,74] *Mycobacterium microti* is the causative agent behind tuberculosis in small mice and voles, but occurrence in humans is very limited.[75] Other pathogens include *Mycobacterium kansasii* which causes disease in HIV infected individuals, *Mycobacterium marinum* which causes disease in fish and skin infections in humans. Aronson first isolated this Mycobacterium in 1926 from a salt water fish. Tuberculosis is rarely caused by *M. marinum* in humans, however some skin infections can occur after contact with contaminated water sources.[76]

1.4 Non-tuberculous mycobacteria (NTM)

Non-tuberculosis mycobacteria, are small, rod shaped bacilli, also known as environmental mycobacteria, which enter the human body through environmental sources such as natural water, soils, foods, and water pipes of the distribution system. These kinds of bacteria are resistant to chlorine in water therefore they survive in the water pipes.[77] The species of NTM do not cause tuberculosis, but have the ability to cause other diseases in both humans and animals such as skin disease, disseminated disease, and pulmonary disease in HIV negative patients.[61] Unlike tuberculosis mycobacteria NTM are not transmitted directly from one person to another, with the organism being acquired exclusively from environmental sources.[78] It has been found that the rate of worldwide NTM infection is increasing significantly. In the UK, the rate of all NTM reported cases increased from 0.9/100,000 to 2.9/100,000 persons between the years 1996-2006.[79] 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.[80] Similar reports also showed the prevalence of pulmonary NTM infection in other countries.[81,82]

1.4.1 *Mycobacterium brumae*

Mycobacterium brumae (M. brumae) as seen in **Figure (5)** is a strain of non-tuberculosis mycobacteria (NTM). It is a new species of rapidly growing, environmental and non-photochromogenic mycobacteria. It is also gram-positive, nonmotile, strongly acid-fast and rod shaped (2.0-2.5 µm long and 0.3 to 0.5 µm wide). It was first identified having been isolated from water, soil, and human sputum samples in 1993 in Barcelona, Spain.[83]

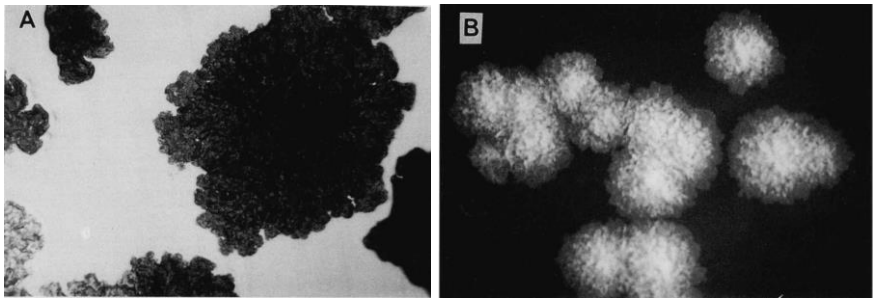


Figure (5): (A) Mature *M. brumae* colonies on micrograph (magnification x120). (B) No magnification.[83]

The inclusion of this organism in the genus *Mycobacterium* is based on its acid-alcohol fastness, DNA relatedness studies showed that the *M. brumae* strains studied form a single DNA hybridization group which is less than 30% related to 15 other species of the genus *Mycobacterium.* Thin-layer chromatographic analysis showed that only alpha-mycolates (see page 28 for mycolic acids) are present. Unlike *Mycobacterium fallax* and *Mycobacterium triviale* α-mycolates, *M. brumae* α-mycolates release only 22-carbon atom esters after pyrolysis.[83] One of the factors that has been related to the virulence of mycobacteria is their ability to form cords, with studies in this area suggesting that cords only occurred in virulent strains. A recent study suggested that this cording could be due to the fine structure of the α-mycolic acids, in particular the presence of a cyclopropane at the proximal position, in mycolic acids isolated from *Mycobacterium tuberculosis* and *M. bovis* BCG.[84] Luquin *et al*.[83], however, described the presence of cords in *M. brumae*, despite there being no cyclopropane α-mycolic acids present. They therefore investigated whether *M. brumae* formed true cords and by using SEM images showed this to be the case. This therefore suggests that the relationship between the presence of a cyclopropane in the α-mycolic acids and cording cannot be extended to all mycobacteria.[85]

The use of *M. brumae* as an efficient candidate for the treatment of bladder cancer (BC) has been investigated.[86,87] Although BCG has been shown to be an effective therapy, side effects, such as BCG infection, can occur, thus limiting its use. Much research has therefore been carried out in order to find alternative therapeutic agents. Yuksel *et al*.[86] found that *Mycobacterium phlei* showed antitumoral activity, and that fractions rich in cell wall proteins caused the activation of TNF-α and IL-12. They studied a further 88 mycobacterial strains and found that 12 additional strains, including *M. brumae* were candidates for superficial BC treatment. More recently Julián *et al*.[87] also investigated the use of *M. brumae* in inhibiting BC cell growth. They showed that *M. brumae* had improved effects, compared to BCG, in inhibiting low grade bladder cancer (BC) cell growth while similar effects against high grade cells were observed. In tumor-bearing mice, *M. brumae* treatment prolonged their survival, while posing a lower risk of infection, thus suggesting that *M. brumae* could be a potential candidate as a therapeutic agent for non-muscle-invasive BC. Julián *et al*.[88] also suggested that the antitumor effect could be improved by obtaining homogenous mycobacteria suspensions. Due to the high lipid content of the mycobacterial cell wall, they are likely to aggregate and form clumps when re-suspended in aqueous solutions, which could interfere with their activity. Different emulsions containing *M. brumae* were prepared and their efficacy studied, with that prepared using olive oil giving the best results. The olive oil-*M. brumae* emulsion triggered the highest cytokine production in infected tumour cell cultures and induced a robust immune response in tumour bearing mice. In a different study Valero-Guillén *et al*.[89] showed that trehalose dimycolates (TDM) known as 'cord factors' from *M. brumae* stimulate the secretion of some pro-inflammatory cytokines that are relevant in *tuberculosis*; they studied these cord factors and compared them to those from *M. tuberculosis*. The cord factors were strong inducers of TNF-α and IL-1β, with comparable or higher levels than the cord factor from *M. tuberculosis*, however the opposite was true for cytokines IL-6, IL-12p40 and IL-23. These results could are of interest when considering the adjuvanticity of these cord factors, however further investigation is required to determine whether mycobacteria such as *M. brumae* and its mycolic acids and cord factors could have a role as adjuvants (substances which enhance the body's immune response to an antigen).

1.4.2 *Mycobacterium smegmatis*

M. smegmatis is a fast-growing *Mycobacterium* which is typically 3.0 to 5.0 µm long with a bacillus shape belonging to the family of environmental NTM.[90] *M. smegmatis* is 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.[91] Lustgarten first discovered and isolated *M. smegmatis* in 1884, and found that it lives in aggregate layers of cells attached to each other in a community called a biofilm. It is found in water, soil and plants.[92] The study of biofilms in *M. smegmatis* as seen in **Figure (6)** can aid in understanding biofilm formation in other pathogenic mycobacteria like *M. tb*.[93] *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*.[94, 95]

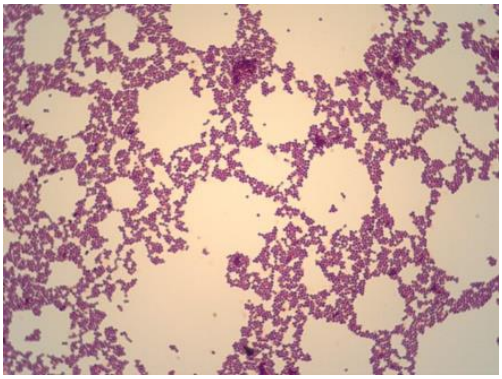


Figure (6): *M. smegmatis.*[96]

1.4.3 *Mycobacterium gordonae*

M. gordonae is another type of NTM which is classed as a slow growing NTM. *M. gordonae* is an acid and alcohol-fast bacillus belonging to group II of scotochromogenous mycobacteria.[97] It can be found and isolated from environmental sources such as soil, natural water, tap water, or even unpasteurized milk.[97] It was thought to be non-pathogenic and rarely cause disease to humans, but recent reports showed cases of significant disease caused by *M. gordonae* infection such as pulmonary disease, disseminated infection, and genitourinary disease.[98,99,100] *M. gordonae* infections, those which entail co-infection with HIV-negative patients is rarely a pathogen, but in HIV-positive patients with a low CD4 cell count,[101] it can cause significant disease and treatment is beneficial. Reports indicate that *M. gordonae* was

isolated from the blood of HIV infected patients and caused serious health complications.[102,103] Nevertheless, it is important to establish a timely diagnosis so that optimal treatment can be administered.[104] *M. gordonae* **(Figure 7)** is classed as the second most common non-tuberculosis Mycobacterium in the UK, with the rate of reported infections increasing 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.[79] Studies using molecular-based identification methods, such as PCR-restriction length polymorphism analysis (PRA), have concluded that *M. gordonae* is the most heterogenous member of the Mycobacterium genus studied to date.[105]

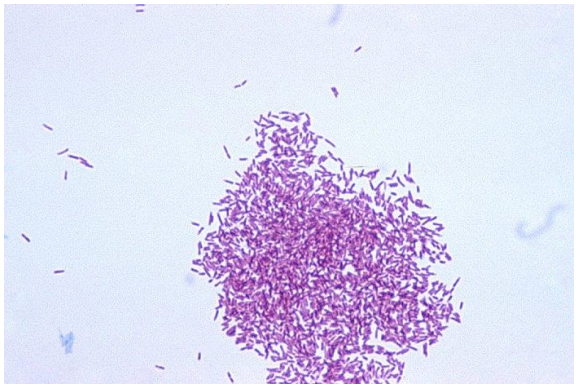


Figure (7): *M. gordonae* (coloration de Ziehl).[106]

1.4.4 *Mycobacterium avium* complex (MAC)

Mycobacterium avium complex (MAC), as seen in **Figure (8)** is the most frequently encountered NTM associated with both human and animal disease, classified as an acid-fast, slowly growing bacillus that may produce a yellow pigment in the absence of light (exposure to light often intensifies pigment production).[107]

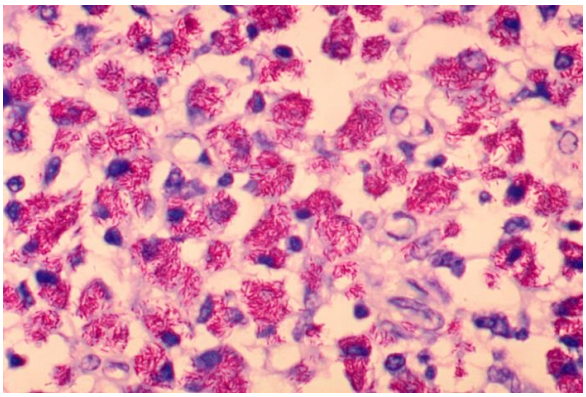


Figure (8): *Mycobacterium avium* complex (MAC).[108]

There are three subspecies of *M. avium* including *M. avium*, *M. avium* subsp *paratuberculosis* and *Mycobacterium intracellulare* collectively known as *M. avium* complex (MAC).[78,109,110] MAC is a serological (the scientific study or diagnostic examination of blood serum, especially with regard to the response of the immune system to pathogens or introduced substances) complex of 28 serovars (a subdivision of a species or subspecies distinguishable from other strains on the basis of antigenicity) of two species, *M. avium* and *M. intracellulare*, which sometimes has been extended to include three additional serovars of a third species, *Mycobacterium scrofulaceum.*[111] The distinction between *M. avium* and *M. intracellulare* is now well established, and Thorel *et al*.[112] have proposed three subspecies of *M. avium* on the basis of phenotypic properties and nucleic acid studies: *M. avium* subsp. *avium*, *M. avium* subsp. *paratuberculosis*, and *M. avium* subsp. *silvaticum.*[112] *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.[113,114] *M. avium paratuberculosis* (MAP) can cause Johne's disease in cattle and may have implications for human health.[115] Johne's disease is one of the most widespread and economically important disease of ruminants. Recently, MAP has been associated with different autoimmune diseases such as Crohn's disease,[116] type 1 diabetes (T1D) and multiple sclerosis in humans.[116] Bovine paratuberculosis and bovine TB are still a major concern in many countries and are responsible for heavy economic losses as a result of decreases in weight, milk production and fertility of the infected animals. Additional costs include increased culling rates, diagnostic testing, and control measures.[117]

1.4.5 *Mycobacterium kansasii*

M. kansasii is probably the easiest NTM pathogen to treat successfully, and it is the second most common NTM after MAC to cause disease in HIV infected individuals.[118] It causes both pulmonary and extra-pulmonary infections. Indeed, more information exists showing how effective antituberculosis drugs are for treating *M. kansasii* than all the other NTM infections. Also, because it is similar to *tb. M.*, mycobacterial isolates may be linked to lung disease which can be both aggressive and destructive **(Figure 9**).[119]

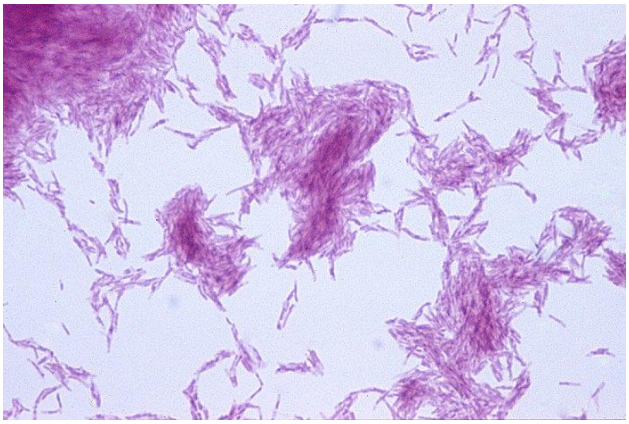


Figure (9): *Mycobacterium kansasii* (coloration de Ziehl).[119]

Infection by *M. kansasii* probably takes place via aerosols, although research has yet to confirm tap water as a major reservoir for *M. kansasii* causing human infection.[120] In fact, it can be found virtually anywhere in the environment; these bacteria live in water, soil, foods and a variety of animals. The organism has been isolated from tap water in some communities as cases of identified *M. kansasii* disease.[120,121,122]

1.5 The cell wall of *M. tuberculosis*

Significant to the pathogenesis of *M. tb* is the organisation and structure of the cell envelope. As a result it is important to have an understanding of these factors in the fight against TB and other related diseases.[123] The cell envelope in *M. tuberculosis* has an unusual structure, comprising a thick, multi-layered and extremely hydrophobic cell envelope, and this is important for the organism because it prevents the passage of antibiotics into the cell and protects it from the immune system of the host by allowing it to survive in macrophages, acting as a permeability barrier. The general structure of the mycobacterial cell envelope is now well understood **(Figure 10)** and it was proposed by Minnikin with its complex architecture of lipids, glycolipids, polysaccharides and proteins.[124,125] The cell wall of mycobacteria consists of three main organelles: the plasma membrane, the cell wall (inner layer) and the outer layer (capsule).

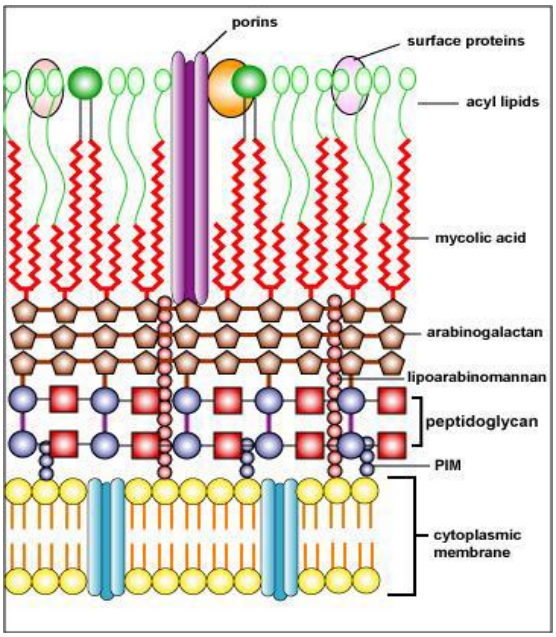


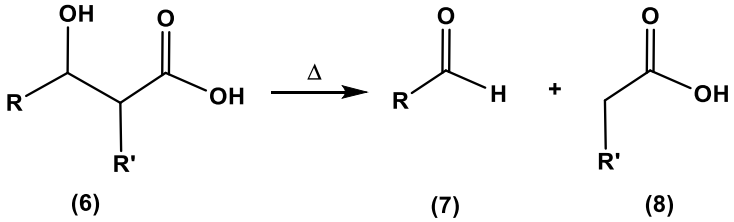
Figure (10): The cell wall of *M. tuberculosis.*[128]

The capsule-like layer consists of polysaccharides, proteins and also contains small amounts of lipids.[126] The composition of the plasma membrane remains similar to the lipid bilayer of other organisms. It is composed of phospholipid derivatives from phosphatidic acid (PIMs). The cell wall (inner layer) consists of peptidoglycan (PG), consisting of long polysaccharide chains that give shape and rigidity to bacteria. *N*-Glycolylmuramic acid is linked in β-(1,4) to *N*-acetyl glucosamine in alternating position and cross linked to a four chain amino acid L-alanine, disoglutamine, *meso*- diamino-pimmelic acid and D-alanine. The PG is linked to arabinogalactan (AG) by a phosphodiester bridge. The AG layer is composed of arabinose and galactose.[127] Mycolic acids, high molecular weight long chain fatty acids alkylated at the α-position and hydroxylated at the β-position,[128,129] are the main constituent of the cell wall; they occur along with a large number of different lipids, for example several different lipids with multi-methyl branched fatty acids.[128] The mycobacterial lipids, constituting up to 40% of the dry weight of the cell envelope, have been the subject of numerous studies in order to determine their structure, biosynthesis and role in the virulence of the mycobacteria.[130,131] MAs bound to trehalose are extractable lipids also found in the cell wall. These are known as 'cord factor' and are bio-medically important glycolipids. They are made up of a trehalose which is esterified at either one or both primary alcohol groups to form either a trehalose 6,6'–monomycolate (TMM) or trehalose 6,6'–dimycolate (TDM).[132,133] In biosynthesis,

TMM transfers newly synthesised mycolic acids to the cell wall. It remains unproven that this is the case for TDM.[132,134,135] Among the most interesting and potentially useful lipids found in *M. tuberculosis* are the mycolic acids, cord factors trehalose mono- and dimycolate (TMM, TDM), glucose monomycolates (GMM) and glycerol monomycolates (GroMM).

1.6 Mycolic acids

Mycolic acids are characteristic of mycobacteria and give unique properties to the cell wall structure, making it resistant to all of the host's defences and other chemotherapeutic agents which would otherwise damage or render unviable normal bacterial cells.[128,133,136] Anderson *et al.* characterised the first "hydroxyl acids of very high molecular weight" which contained complex mixtures with different compositions. They proposed the name "mycolic" acid and reported the presence of one carboxylic group, one hydroxyl group and possibly a methoxy group. It was also reported that pyrolysis of the mycolic acid at 300 °C under vacuum gave hexacosanoic acid.[137,138,139] However, pyrolysis at 300 °C also released a series of aldehydes (**7)** alongside the hexacosanoic acid (**8)**. This helped to determine the basic structure of mycolic acids as β–hydroxy fatty acids with a long alkyl chain at the α-position-[$RCH(OH)CH(CO_2H)R'$][140] **(Scheme 1)**.



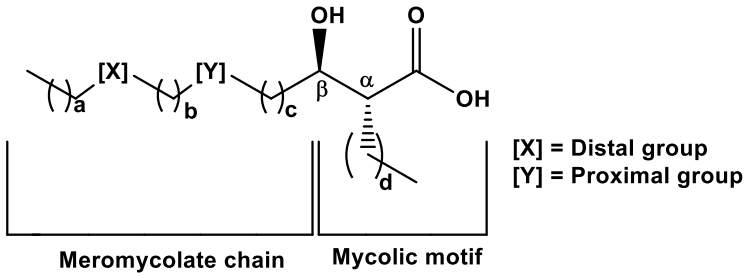
Scheme (1): Pyrolysis of a mycolic acid.

The first *M. tb* mycolic acid structures were published by Minnikin *et al.* in 1967[141,142,143,144] who used different analytical methods such as I.R. spectroscopy, proton NMR spectroscopy and mass spectrometry to show that mycolic acids have a cyclopropane ring in different sterochemistry, and also suggested the main structure of mycolic acids.[145,146]

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.

It has been noted that there maybe over 500 types of mycolic acids in *M. tuberculosis*, with closely related chemical structures.[147] They have been studied since their discovery and research continues because they are unique to mycobacteria and because they are important to their virulence. 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 is challenging. For this reason, highly developed analytical techniques were required in order to determine the correct structures of these series of mycolic acids. Recently a lot of research has been done on MAs using electron impact (EI) mass spectrometry, to determine chain lengths of the mycolates groups.[145,148,149,150]

Mycolic acids have two key moieties, the meromycolate chain, which is the main part of mycolic acids, and generally has two intra-chain functional groups at the distal and proximal positions labelled as [X] and [Y] **(Figure 11)**. The other part is the mycolic motif, which contains the hydroxyl group the and α-alkyl branched fatty acid, and is essentially similar all in mycolic acids, except for a slight difference in the chain length at the α-position.[151,152]



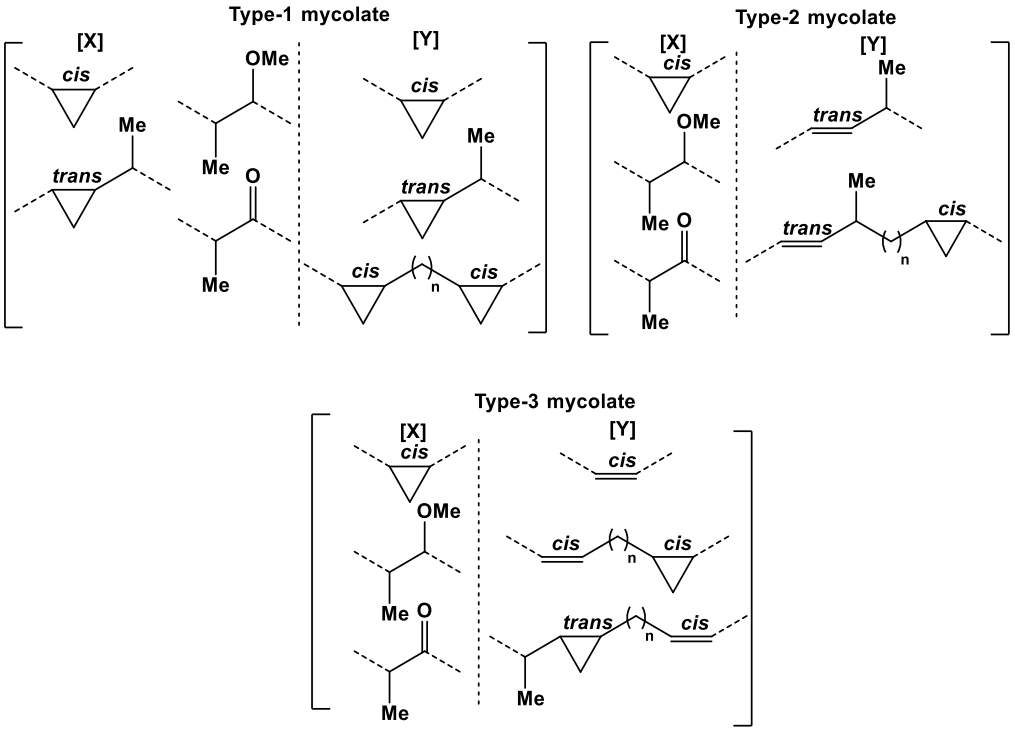


Figure (11): Generalized structures of major mycobacterial mycolic acids.[151,152]

The meromycolate part is different from one mycolic acid to another, and it can contain different groups at both the distal and proximal positions. As the functionality of these positions appears to play an important role in the mycobacterial cell envelope, the resultant mycolic acids have been classified by their functionality. These groups may be a cyclopropane ring, a methoxy group, a carbonyl group, a methyl group, oxirane ring or a double bond. The classification of mycolic acids depends on the groups present. Watanabe *et al*. suggested a comprehensive classification method.[152] There are three main types of MA which are present in *M. tuberculosis* the first one (**9)** where the proximal position, can be either a *cis* or *trans*-cyclopropane ring; the second **(10)** where the proximal position is a *trans* double bond; and finally **(11)** where the proximal position is a *cis* double bond. **Figure 12** shows these three different types.[151] It has been reported that when the double bond or the cyclopropane are in the *trans* configuration there is a methyl group on the adjacent distal carbon while in the *cis* configuration this is not present.[147,153]

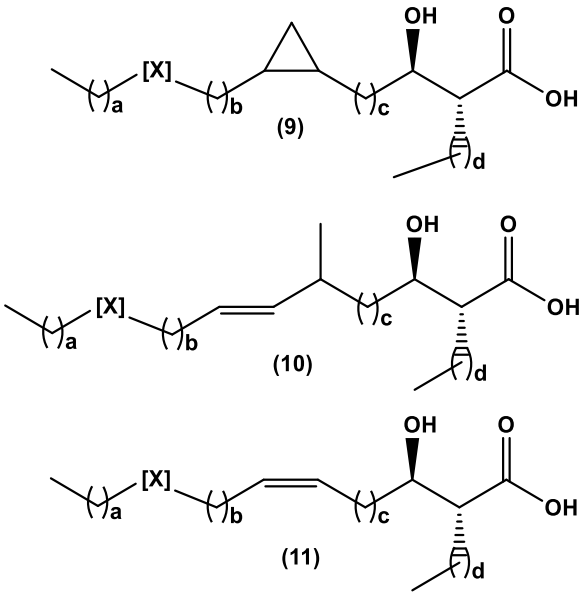


Figure (12): Types of MA structures according to Watanabe *et al*. classification.[151]

In addition, based on the nature of the functional groups present in the meromycolate chains, there are three main types of mycolic acid which are present in *M. tuberculosis*: α-mycolic acid (**12)** containing two cyclopropane rings usually in a *cis*-configuration **(Figure 13)**; methoxy-mycolic acid **(13)** in which the distal group contains a methoxy group **(Figure 14)** and keto-mycolic acid containing a carbonyl group with a *trans* cyclopropane **(14)** or a *cis* cyclopropane **(15) (Figure 15)**. Methoxy-mycolic acids and keto-mycolic acids have methyl branches next to the oxygenated functional group and natural mixtures have both *cis*-cyclopropane and α-methyl *trans*-cyclopropane rings.

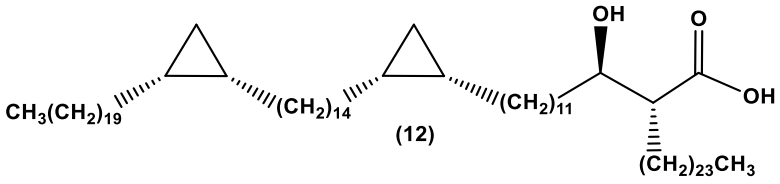


Figure (13): Structure of an α-MA.

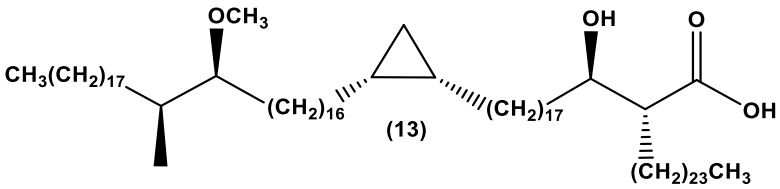


Figure (14): Methoxy-MA with a cyclopropane ring in the *cis*-configuration.

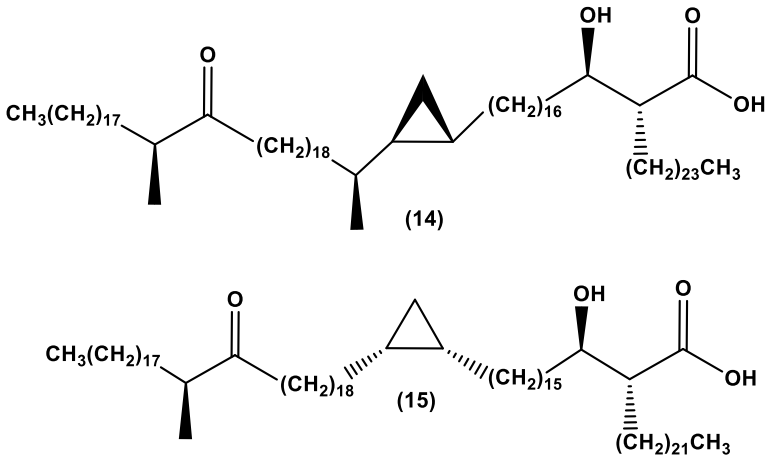
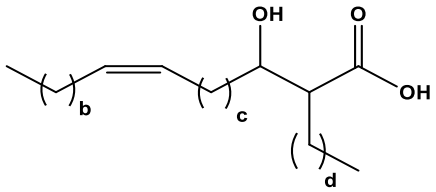


Figure (15): Keto-MAs with a cyclopropane ring in the *trans*-configuration (up) **(14)**; *cis*-configuration (down) **(15)**.

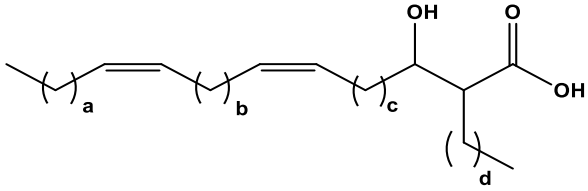
In addition, other mycobacteria contain completely different sets of mycolic acids with the other various functianal groups in the meromycolate chain as illustrated in **Figure (16)**. For example, *M. smegmatis* contains α'and α-mycolic acids **(16)** and **(17)** with either one or two double bonds, either in the *cis* or the *trans* configuration. The α'-mycolic acids are shorter than α-mycolic acids, containing 60 carbons instead of 80, and are widely distributed within mycobacteria although absent from *M. tuberculosis*. The MA of *Mycobacterim fortuitum* include an epoxide ring **(18)**.[154,155] Recently, other different oxygenated mycolic acids have been isolated from various mycobacteria; these include a ω-carboxy-MA **(19)**[156] from *Mycobacterim phlei* while a ω-methoxy-MA was found in *Mycobacterim alvei* **(20)**.[157] Wax ester **(21)** is another variant of MA, found in *M. aurum*.[158] Wax esters are the result of a Baeyer-Villiger type reaction on the keto group of the keto-mycolic acid.[159] MA (both major and minor) present in other mycobacteria, have different variations at the proximal and distal positions. Following a careful examination of all their mycolic-like fatty acids, small amounts of hydroxy MA **(22)** have been found in *M. bovis* (BCG) and also *M. tb*. It was found that a gene cluster isolated from *M. bovis* (BCG) conferred upon *M. smegmatis* the ability to synthesise hydroxy MA with cyclopropanated keto MAs.[148,160]

α'-mycolic acid



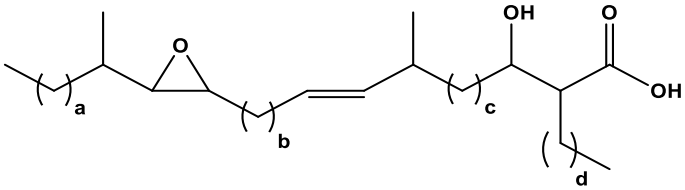
(16)

α–mycolic acid



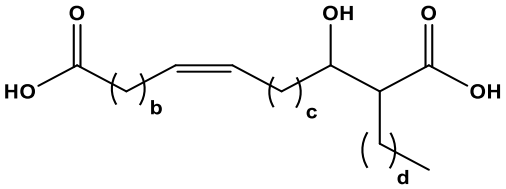
(17)

Epoxy-mycolic acid



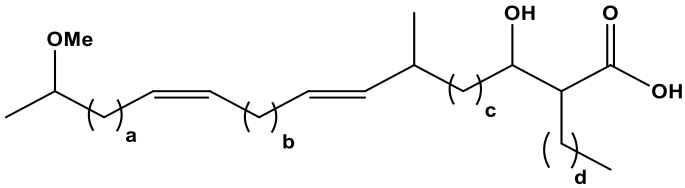
(18)

ω-Carboxy-mycolic acid



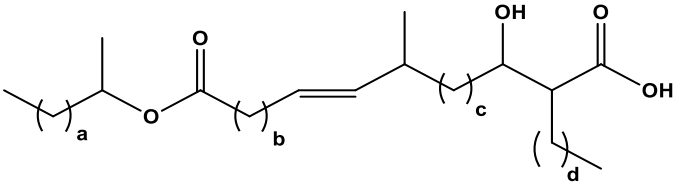
(19)

ω-1-Methoxy-mycolic acid



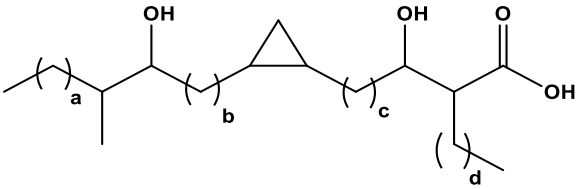
(20)

Ester-mycolic acid



(21)

Hydroxy-mycolic acid



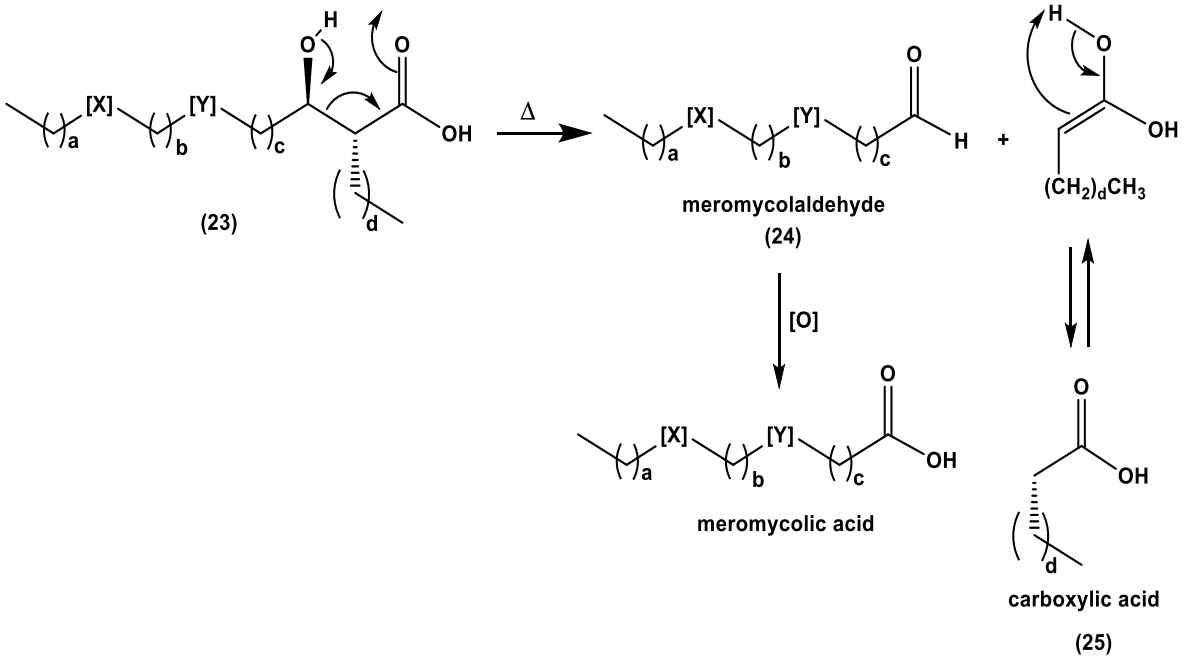
(22)

Figure (16): Some of the mycolic acids present in different mycobacteria.

1.7 The chain length of mycolic acids

The chain length represented in the different homologus of mycolic acids in all types of mycobacteria has been recognised.[147] In *M. tuberculosis* alone, a family of over 500 individual mycolic acids with closely related chemical structures have been documented,[147] while for *M. smegmatis* about 100 structural isomers are found in the mixture of α-mycolates.[161] In 2001, Watanabe *et al.*[151] was the first to analyse mycolic acids using MALDI-TOF mass spectrometry to provide a rapid and highly sensitive technique for the analysis of mycolic acids and other lipids. They studied the length of all the carbon chains of the various mycolates correlated with the growth rate of the mycobacterial strains of the main types of MA. It was found that pathogenic strains of mycobacteria such as *M. tuberculosis* and *M. ulcerans*, synthesised chains containing even numbers of carbons (C74-C82) of α-mycolic acids. Non-pathogenic strains produced chains with both odd and even numbers of carbons.[151] In fact, the main chains of oxygenated (methoxy and keto) mycolic acids were between four and six carbons longer than the corresponding α-MA in the slow-growing pathogenic species[162,163] whereas rapid growers provided oxygenated homologues possessing the same chain lengths as their α-mycolic acids.[67]

Watanabe *et al.* used MALDI mass spectrometry to study mycolic acids (both major and minor components) present in 19 strains of the *M. tuberculosis* complex.[151] Combining this new methodology with collision-induced dissociation (CID) mass spectrometry they succeeded in locating, precisely, the functional groups in the meromycolate moiety of different types of mycolic acids.[152] To determine the chain lengths (a, b and c) of meroaldehyde **(24)** and the saturated α-chain **(25)**, pyrolysis of MA **(23)** at 300 ˚C was carried out, followed by mass spectrometry. This gives information on the internal chain lengths as illustrated in **Scheme (2)**.[152] It was revealed that the mycolate chains vary between 60 and 90 carbon units and that the α-chain varies between 22 and 26 carbons in length.[147,164]



Scheme (2): Pyrolytic cleavage of a mycolic acid, followed by oxidation with Ag_2O to prepare meromycolic acids for analysis.[152,165]

The a-b-c values for the MAs were elucidated as shown in **Figures (17-19)**. Moreover, it was found that the methyl branch next to the *trans*-double bond was located nearest to the carboxylic end of the mycolic acids. In addition, they discovered a close structural relationship between the keto and the methoxy MAs, with their a-b-c values generally matching.[107]

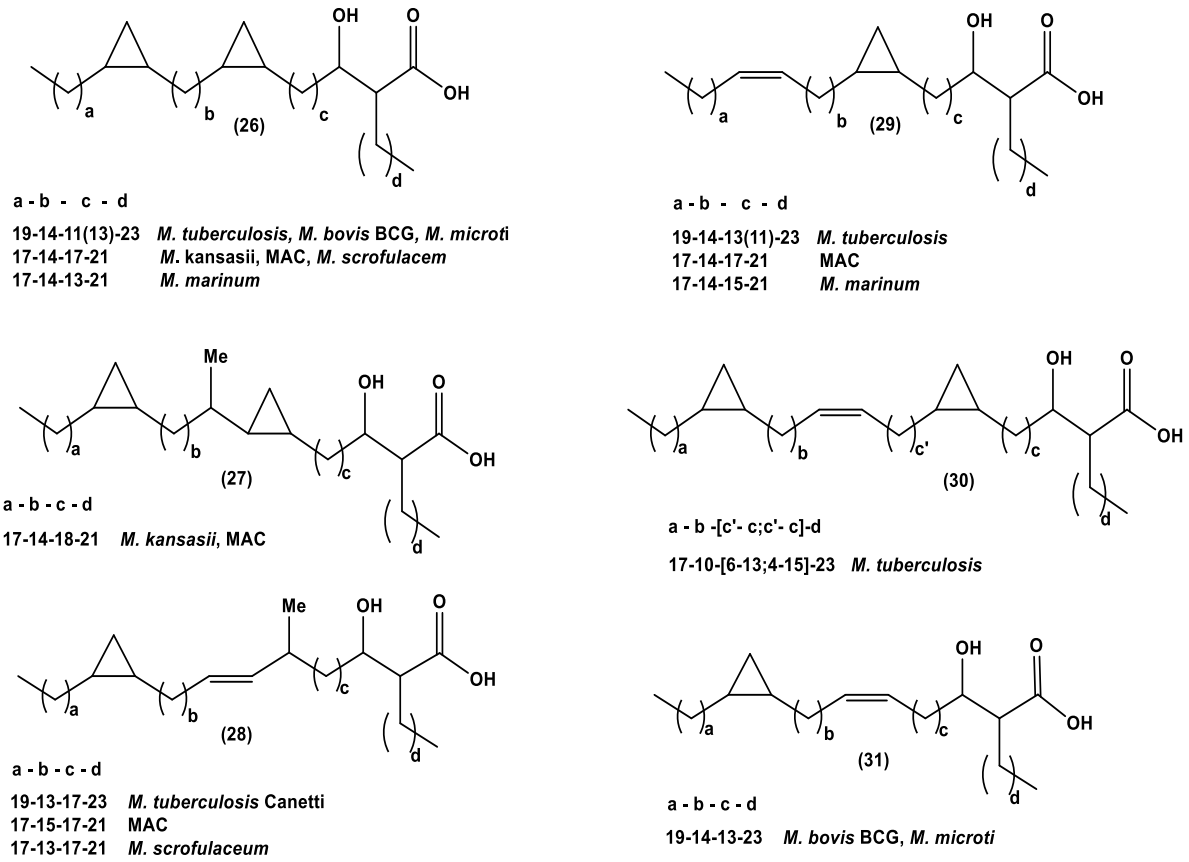


Figure (17): Structures and distribution of major and minor α-mycolic acids.[107]

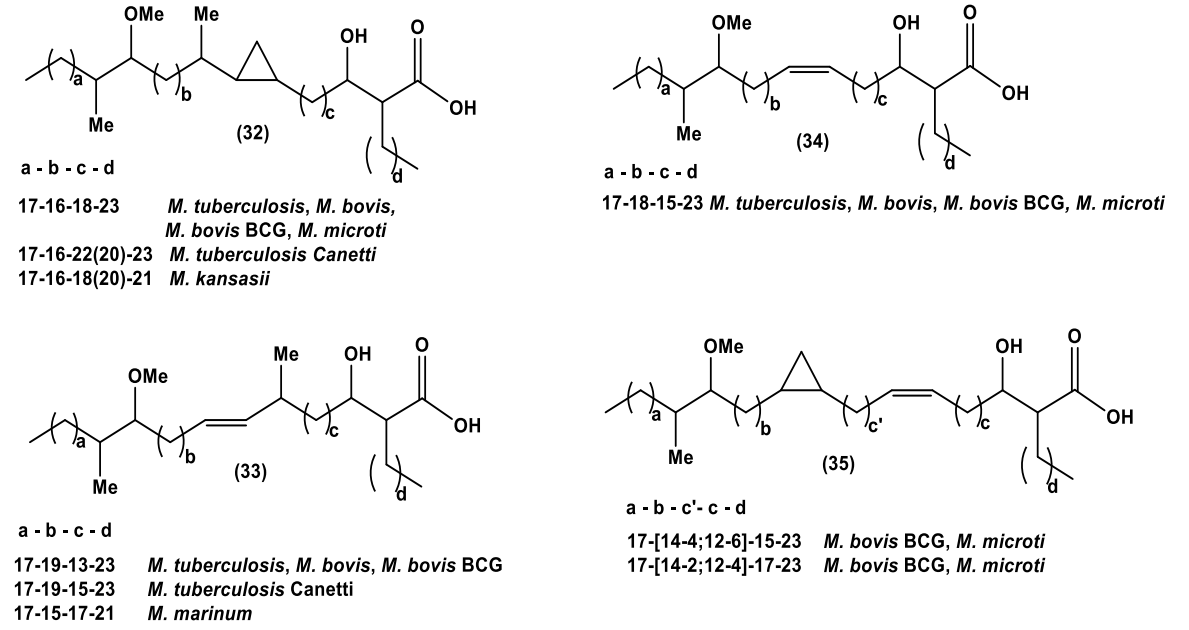
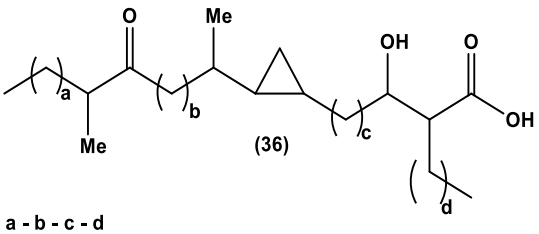
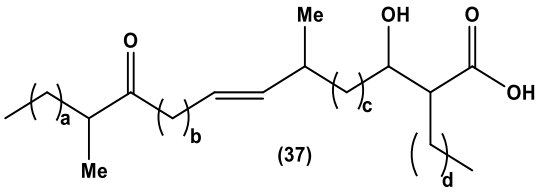


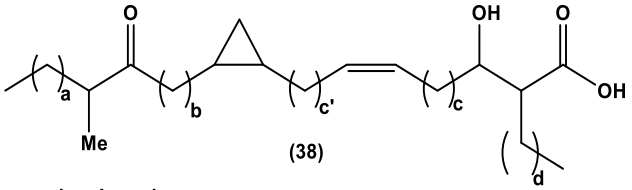
Figure (18): Structures and distribution of major and minor methoxy-mycolic acids.[152]



a - b - c - d	
17-18-16-23	*M. tuberculosis*, *M. bovis*, *M. bovis* BCG, *M. microti*
15-18-18-21	*M. kansasii*
17-16-18-21	MAC
15-16-18-21	*M. scrofulaceum*



a - b - c - d	
17-19-15(13)-23	*M. tuberculosis*, *M. bovis*, *M. bovis* BCG, *M. microti*
17-17-17-23	*M. tuberculosis* Canetti
17-17-17-21	MAC
15-17-17-21	*M. scrofulaceum*
17-15-17-21	*M. marinum*



a - b - c'- c - d	
17-[8-10;10-8;12-6;14-4]-15-23	*M. bovis* BCG

Figure (19): Structures and distribution of keto-mycolic acids.[152]

As previously mentioned, different species of mycobacteria may produce different classes of mycolic acids; thus *M. smegmatis* possesses different sets of α'- and α-mycolic acids with one or two double bonds, either in the *cis* or the *trans*-configuration. The α′-mycolic acids **(39)** contain one double bond in the *cis*-configuration The α-mycolic acids contains two double bonds either both in the *cis*- configuration **(40)**, or the one at the distal position in a *cis*-configuration and the one at the proximal position in a *trans*-configuration **(41)**.[161,166,167] Diene mycolic acids have also been found in *M. brumae* which contain two double bonds both in the *trans*- configuration **(42)**.[89] In addition diene methoxy mycolic acids have been isolated from *Mycobacterium alvei*; these include ω-1-methoxy-mycolic acids in two different configurations (**43, 44**).[156] The presence of epoxy mycolic acids was reported in *M. fortuitum* **(45-48) (Figure 20)**.[166,167]

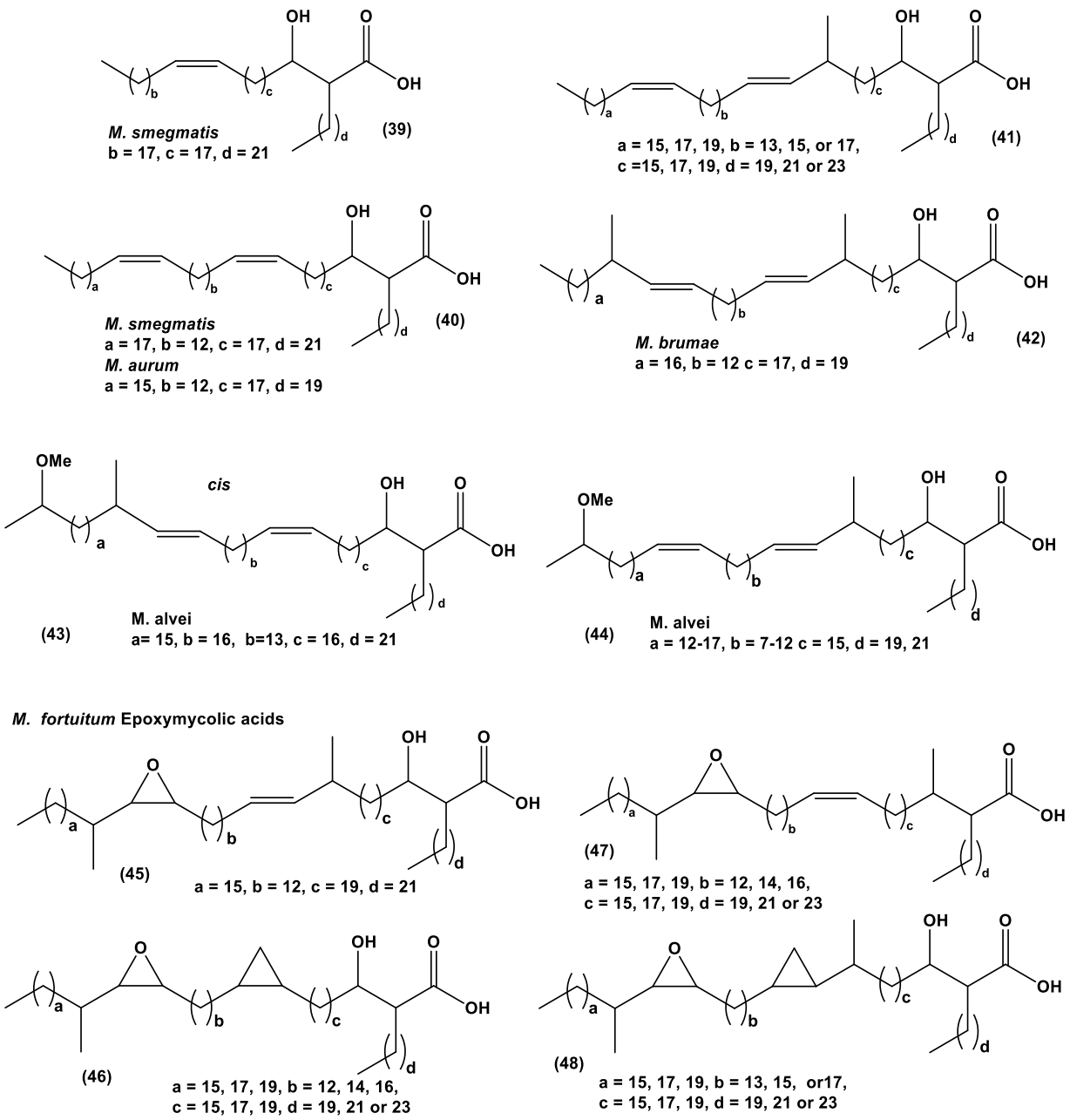


Figure (20): Mycolic acids from other mycobacteria.

1.8 Mycolic acid stereochemistry

Much work has been carried out in order to determine the absolute stereochemistries of various mycolic acids and to identify whether the specific structure has any effect on their biological properties.

The α- and β- fragment has been found to be in the *R,R*-configuration for all the mycolic acids examined, regardless of the groups in the meromycolate chain.[146,168] This was confirmed first for the corynomycolic acids **(Figure 21**).[169,170]

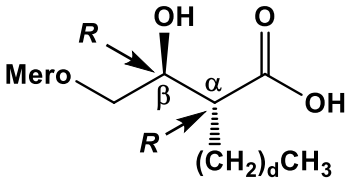


Figure (21): The (*R,R*)- configuration for all mycolic acids.

The relative configuration between the α-alkyl chain and the β-hydroxyl group has been proved to affect the film molecular packing in the cell wall of mycobacteria, as a result of hydrogen bond formation between the hydroxyl group and the carboxylic group; this is believed to have a stabilizing effect on the alignment between two long mycolic acid chains.[171,172,173,174] The configuration at these two chiral centres is thought to play a vital role in T cell recognition,[175] and the generation of an immune response by the host organism against pathogenic mycobacteria.[175,176]

The stereochemistry of the chiral centres in the meromycolate part is difficult to determine because it often contains several sets of chiral centres. Recent studies on different mycobacteria suggest that the methyl branch adjacent to the hydroxy (**49**), methoxy (**50**) and keto (**51**) groups at the distal position are in the *S*-configuration. The hydroxyl (**49**) and the methoxy (**50**) groups are also thought to be in the *S*-configuration **(Figure 22)**.[146,149,177] The wax ester fragment *S*-**(52)** derived from keto-mycolic acids, is believed to be via an enzymatic oxidation via a Baeyer-villiger type reaction.[178,179]

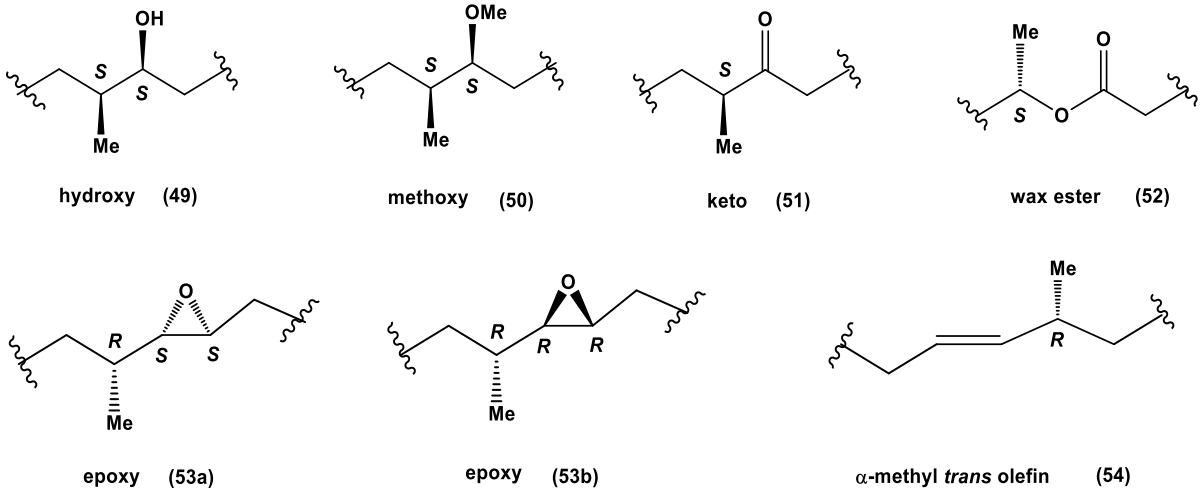


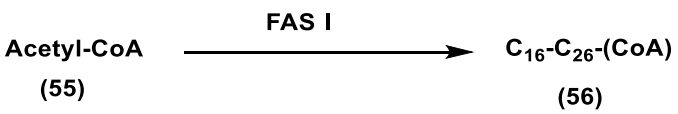
Figure (22): The strereochemistry of chiral centres of the mycolic acids.

Other reports identify *R*-stereochemistry for the three stereocentres of the α-methyl-*trans*-epoxy unit in related mycolic acids (**53a**, **53b**), and the methyl branch next to the *trans*-alkene unit (**54**) was found to be in *R*-configuration.[180] However the stereochemistry of the epoxide ring **(53a)** was recently reported to be in a *S*,*S*-configuration.[181]

1.9 Biosynthesis of mycolic acids

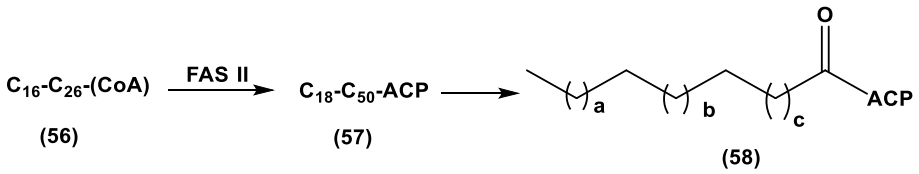
Mycolic acids play an essential role in the survival of TB bacteria, and it is important to find a new anti-mycobacterial drug because most of the drugs relate to the inhibition of mycolic acid biosynthesis.[182] The biosynthesis of mycolic acids has been investigated in great detail,[167,183] and although it is not fully understood, research has provided a number of different hypotheses.

There are three major steps in the biosynthesis of mycolic acids. Firstly, the mycobacterial FAS-I system (fatty acid synthase I) extends the acetyl group by two carbon units from acetyl-CoA **(55)** and also produces up to C_{16}-C_{26} chain length fatty acids **(56)** by elongating the short alkyl chain of mycolic acids **(Scheme 3)**.



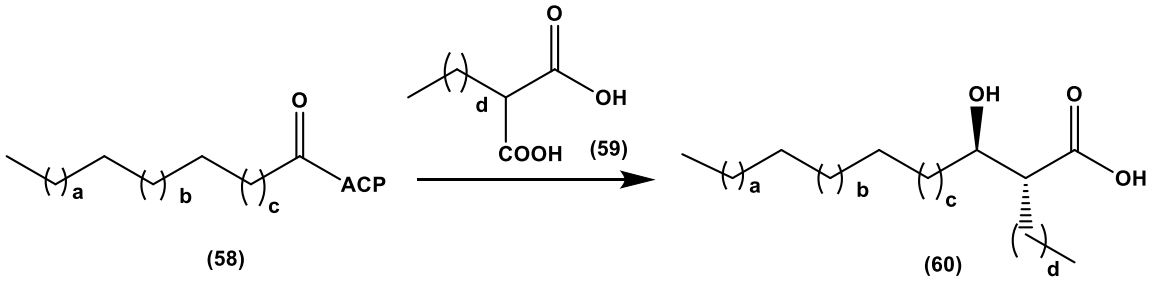
Scheme (3): FAS I system.

Secondly, the mycobacterial FAS II system is used for further chain extension, up to 50 carbon units in the synthesis of meroacids **(57)**, and modifications to form different functional groups **(58) (Scheme 4)**.[184,185] The available anti-tuberculosis drugs such as thiolactomycin, isoniazed, ethionamide and prothionamide are inhibitors of the enzymes involved in the FAS II system.[186,187]



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 (**58**) and alkyl dicarboxylic acid (**59**), with the occurrence of some more modifications like desaturation, cyclopropanation, methylation, elongation and formation of the other functional groups to give the mycolic acid **(60) (Scheme 5)**.[130]



Scheme (5): Condensation step.

There are numerous 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. The mechanism of the biosynthesis of methoxy and cyclopropane mycolic acids in *M. tuberculosis* was established by Yuan and Barry.[188]

It has been suggested that most major functionalities in the meromycolate are derived from a common intermediate. This intermediate is generated using *S*-adenosyl-*L*-methionine (SAM) **(61)**. Methylation of a *cis*-alkene (**62**), using SAM, gives the carbocation intermediate (**63**) **Figure (23)**. Labelled SAM has been used so that the methylation and any subsequent reaction can be monitored. This showed that the methyl group introduced occurs as a bridging methylene in *cis*- cyclopropanes, as methyl branches of *trans*-olefin and cyclopropanes, and the α-methyl branches of hydroxy, keto and methoxy mycolates. [161,177,189]

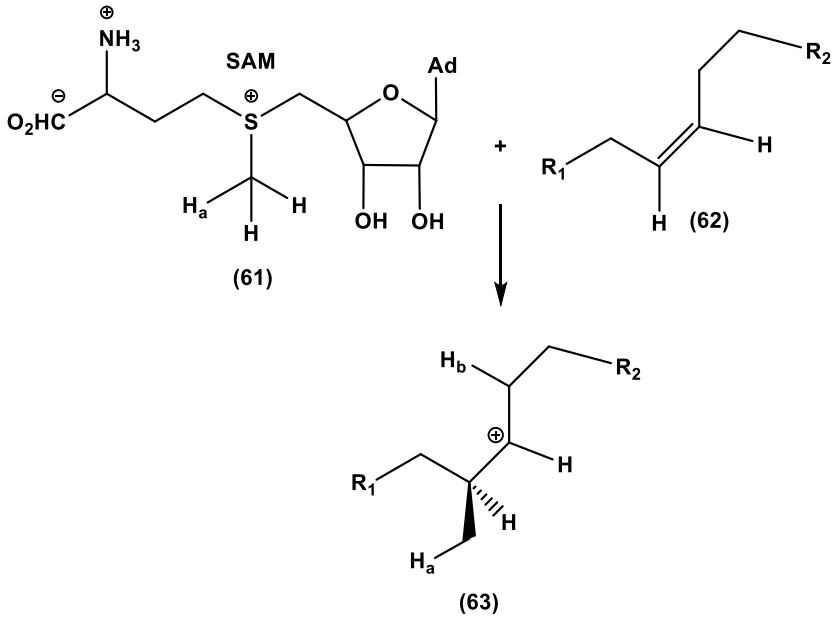


Figure (23): Formation of carbocation intermediate **(63)**.

The deprotonation of carbocation intermediate **(63)** could occur by two different processes. If the removal of H_a occurs this leads to a *cis*-cyclopropane ring **(64)**; conversely if deprotonation of H_b occurs then the *trans*-olefin **(65)** is formed. Additionally, methylation of the *trans*-double bond, involving SAM, gives the *trans*-cyclopropane mycolic acid **(66)**. If the carbocation intermediate **(63)** undergoes a hydration reaction, the hydroxymycolate **(67)** is formed, which is a precursor for the biosynthesis of the corresponding keto **(68)** and methoxy-mycolates **(69) (Figure 24)**.

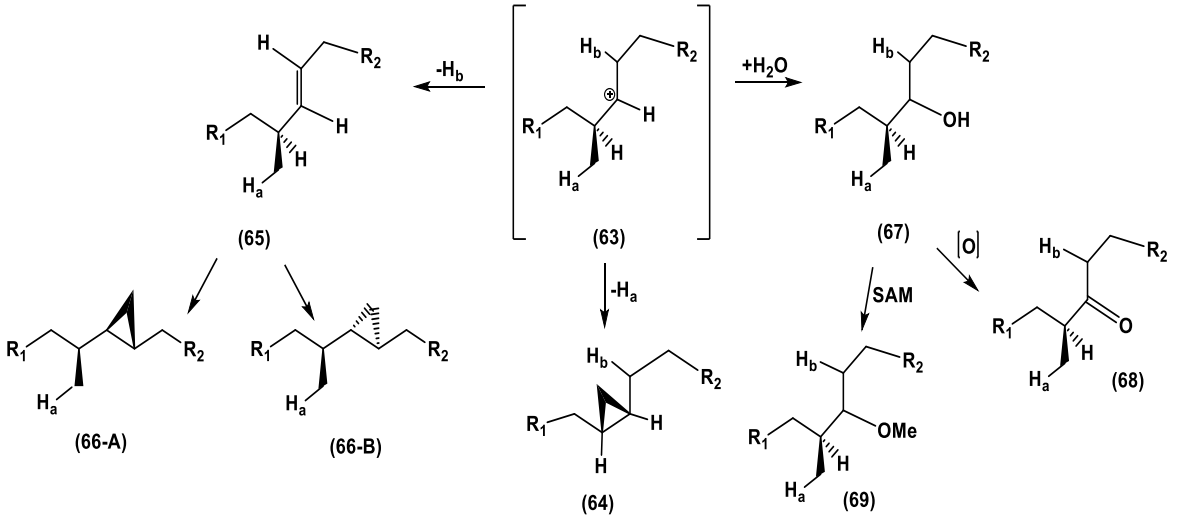


Figure (24): The insertion of the non-oxygenated functional groups in mycolic acids.

The gene in *M. tuberculosis* which is required for the biosynthesis of *trans*-cyclopropylmycolates,[190] confirms the hypothesized biosynthetic link between the different functionalities. The biosynthesis of mycolic acids is of great importance to understanding the impact of individual functionality on virulence, cell wall fluidity and permeability, and how the different mycolates are naturally produced enables one to determine how to manipulate the cell wall of the pathogen, and thus provides a tool with which to combat disease.[191]

1.10 Synthesis of mycolic acids

The synthesis of mycolic acids has been of interest to a variety of research groups, and the synthesis of single enantiomers is of the utmost importance in understanding the role they play 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. Moreover, one application of synthetic mycolic acids is in the detection of TB causing mycobacteria. The pattern of individual mycolic acids is exclusive to one Mycobacterium, therefore if the structure of these mycolic acids were to be known, an enantiomerically pure synthetic compound may be produced and compared to the natural mixture of mycolic acids obtained from the particular Mycobacterium infecting a patient.[192] In addition, most MAs include at least two chiral centres in the α- and β- positions relative to the carboxylic acid. The synthesis of a full MA usually involves the meromycolate moiety and mycolic motif being synthesized separately, prior to being coupled to each other to produce the complete mycolic acid.

1.10.1 Synthesis of the α-alkyl-β-hydroxy ester unit (mycolic motif)

The α-alkyl β-hydroxy fatty acid is present in the *R, R*-configuration and the mycolic motif unit is similar in all mycolic acids except for a slight variation in the length of the α-alkyl chain R'. There are many methods reported for the preparation of (2*R*,3*R*)-α-alkyl-β-hydroxy esters with different starting materials and catalysts **(Figure 25**).[146,168]

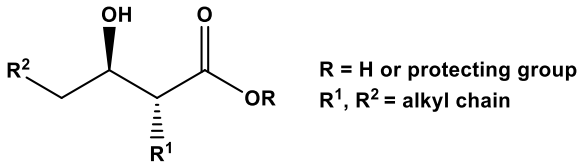
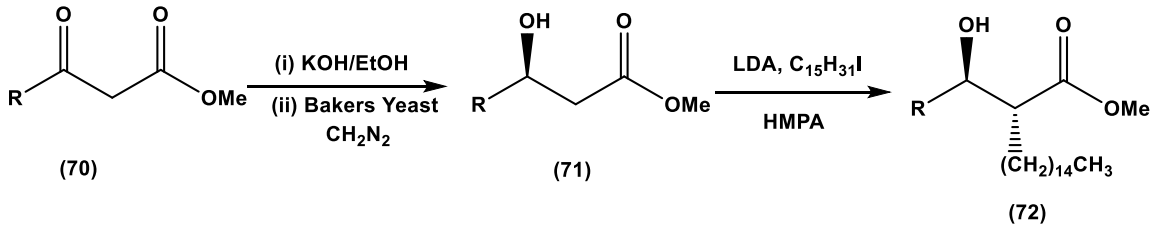


Figure (25): Structure of (*2R,3R*)-α-alkyl-β-hydroxy esters.

In 1952, Lederer *et al.*[193] first reported the synthesis of the mycolic motif via condensation between two fatty acid molecules. Further studies by the same group used a Claisen condensation to prepare corynomycolate analogues, but all the attempts produced a mixture of diasteromers[194,195] Kitano *et al.*[196,197] synthesised the first enantiomerically pure α-alkyl-β-hydroxy unit.

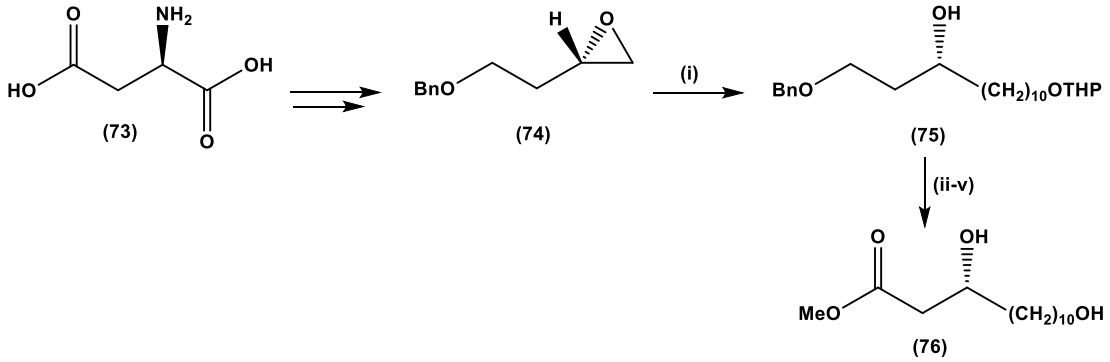
In 1987, Utaka *et al.*[198,199] used a stereo-selective chiral reduction of β-keto ester **(70)** to introduce the hydroxy group at the β-position **(71)** by a Baker's yeast type reduction. An alkyl chain was then directly introduced at the α-position by a Fräter reaction[200] to produce the α-alkyl-β-hydroxy carboxylate (**72**) with two chiral centres in the correct configuration and with an α-chain of the correct length **(Scheme 6)**.



Scheme (6): Method of Utaka *et al.*[198]

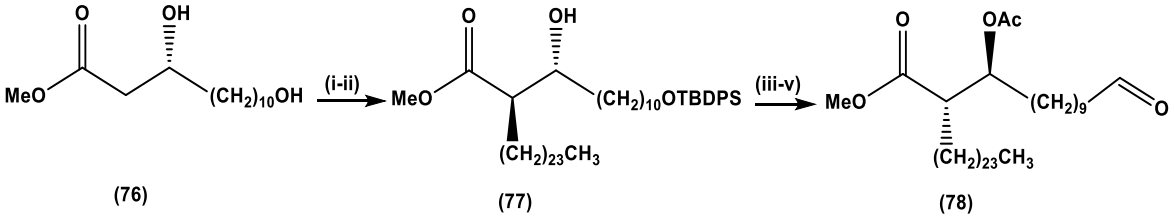
Subsequently, Al Dulayymi *et al.*[201] reported an improved approach to synthesize the α-alkyl-β-hydroxyl unit, originating from *R*-aspartic acid **(73)**. This was converted into epoxide **(74)** in 3 steps and was then ring opened with a Grignard reagent to give a single

enantiomer of the mono protected diol (**75**). This was transformed in four steps (protection, debenzylation, oxidation and esterfication) into the diol (**76**) **(Scheme 7**).



Scheme (7): Ring opening of epoxide **(74)** (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.

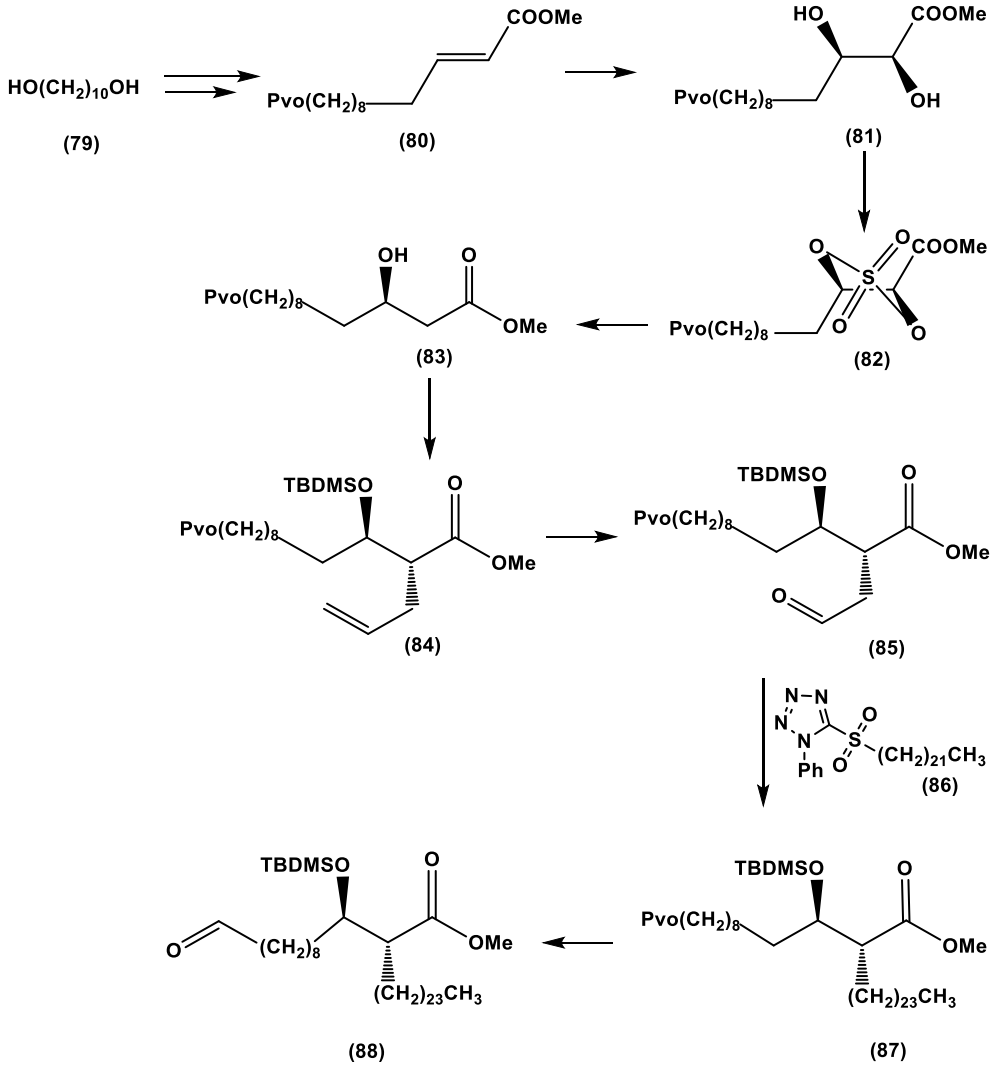
Protecting the diol **(76)** at the primary alcohol group as a silyl ether, followed by a Fräter alkylation with 1-iodotetracosane gave the α-alkyl-β-hydroxy ester **(77)**. Protection of the secondary alcohol of (**77**) as the acetate, deprotection of the primary alcohol and oxidation led to the mycolic motif aldehyde (**78**) **(Scheme 8)**.



Scheme (8): Reagents and conditions: (i) TBDPSCl, DMAP, Et_3N; (ii) LDA, $CH_3(CH_2)_{23}I$, HMPA; (iii) Ac_2O, pyridine; (iv) TBAF, (v) PCC.

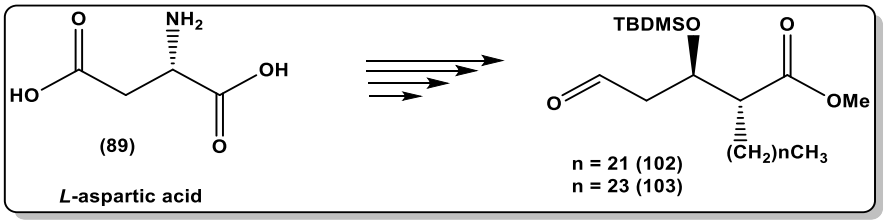
Baird *et al.*[202] also reported the preparation of the mycolic motif unit by an improved method using a simple diol as the starting material. 1,10-Decandiol **(79)** was first transformed in to the *E*-α, β-unsaturated ester **(80)** in four steps. After that a Sharpless asymmetric dihydroxylation was carried out to get the diol **(81)**, which was then transformed into a cyclic sulfate **(82)**. The sulfate was further reduced and hydrolysed to produce protected β-hydroxy ester (**83**) followed by a Fräter allylation at the α-position of the ester **(83)**. The β-hydroxy position was then protected with a TBDMS group to give the alkene **(84)** which was then oxidized with OsO_4 to give aldehyde **(85)**. A coupling reaction

was used to chain extend aldehyde **(85)**, with sulfone **(86)** via a Julia olefination followed by hydrogenation to give **(87)**. Finally, deprotection of compound **(87)** and subsequent oxidation gave the desired mycolic motif aldehyde **(88) (Scheme 9)**.



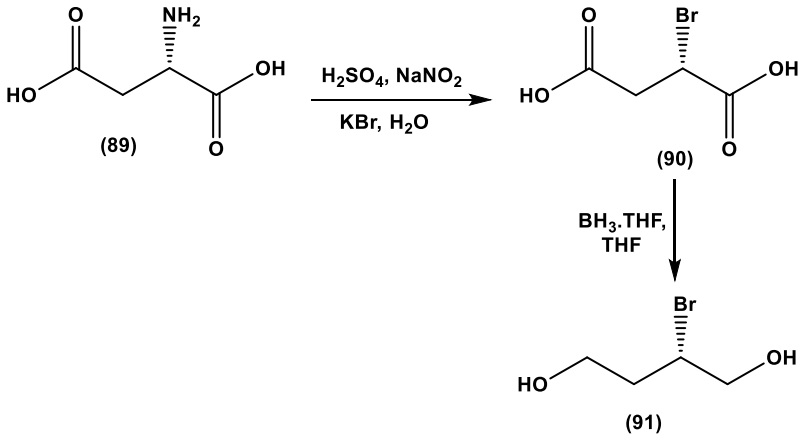
Scheme (9): Toschi *et. al*. synthesis of the mycolic motif.[202]

In a further development work described by Koza *et al.* to prepare the mycolic motif unit,[203] *L*-aspartic acid (**89**) was used as starting material instead of *R*-aspartic acid, for the preparation of the mycolic motif units (**102** and **103**) This was advantageous as *L*-aspartic acid is much cheaper than the *R*-aspartic acid **(Scheme 10).**



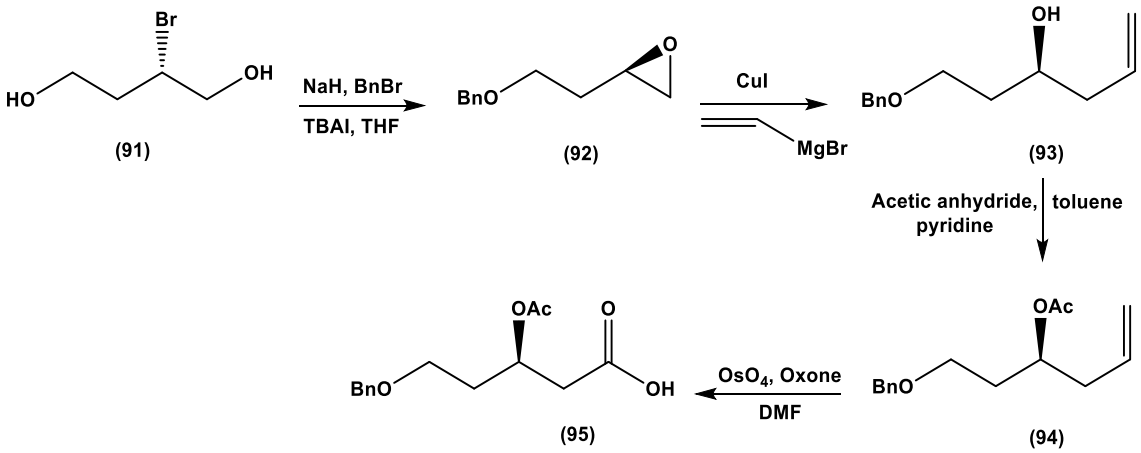
Scheme (10): The preparation of mycolic motif unit **(102** and **103)**.[203]

α-Bromosuccinic acid **(90)** was firstly synthesized from *L*-aspartic acid (α-amino acid) with retention of configuration by the use of $NaNO_2$ and potassium bromide in the presence of sulfuric acid. The concentration of sulfuric acid was critical in this reaction; if it was too high, the salt precipitated making the reaction impossible to complete. The subsequent step was conversion of the acid to the diol (**91**) using borane tetrahydrofuran complex solution in 1.0 M THF **(Scheme 11)**.[204] The borane was able to reduce the two carboxylic acids into diols, while leaving the bromine group unchanged because it is a mild reducing agent.



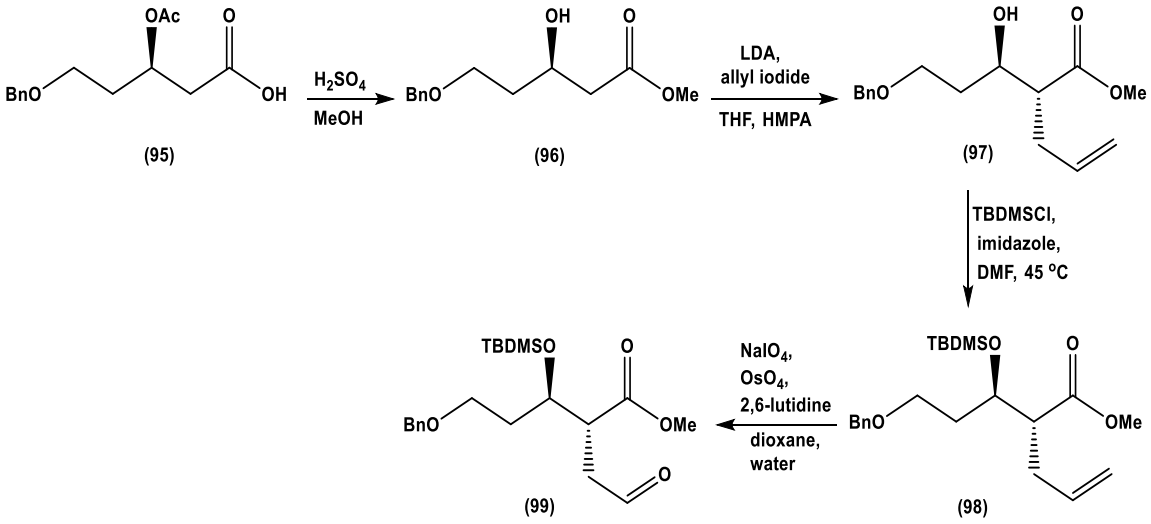
Scheme (11): Preparation of diol **(91)**.

The next step was cyclization to form the epoxide intermediate (**92**) with simultaneous protection of the other hydroxyl with a benzyl protecting group. The intermediate **(92)** was ring opened with a Grignard reagent to extend the chain by two carbon atoms **(93)**. After that the protection of the secondary alcohol with an acetyl group was carried out using acetic anhydride and pyridine as catalyst in dry toluene to form compound (**94**). Oxidative cleavage of the alkene (**94**) with OsO_4 and oxone in DMF gave the carboxylic acid **(95) (Scheme 12)**.[203]



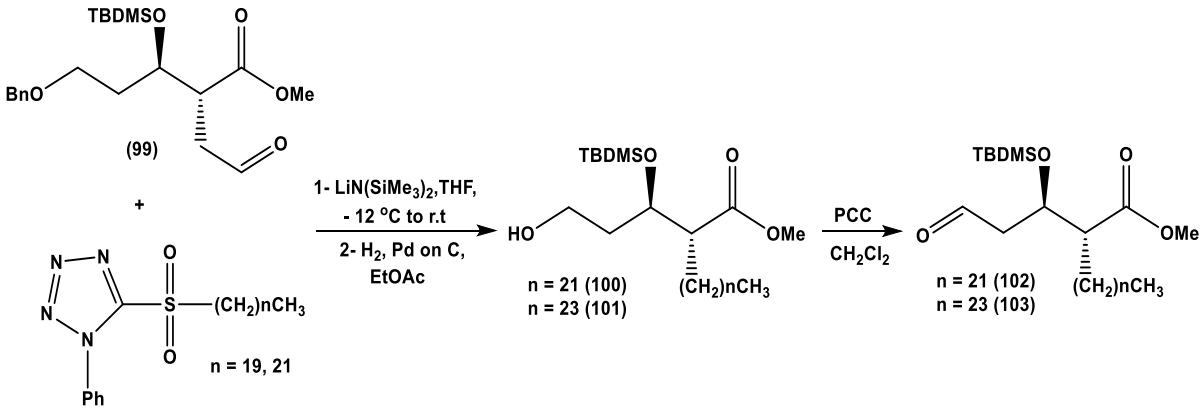
Scheme (12): Preparation of carboxylic acid **(95)**.[203]

In the next step both esterification of the carboxylic acid group and the deprotection of the secondary alcohol was achieved by refluxing the acid **(95)** in methanol/H_2SO_4 to form the desired ester **(96)** (methanol was used as reagent and solvent). After this, the insertion of the alkyl chain, using an allyl group at the α-position, with respect to the carboxylic acid group was achieved by a Fräter reaction to give alkene (**97**). The secondary alcohol was then protected as a *tert*-butyldimethylsilyl ether **(98)**. Subsequently, oxidative cleavage of olefin (**98**) with OsO_4–$NaIO_4$ and 2,6-lutidine in dioxane-water gave the aldehyde (**99**) **(Scheme 13)**.[203,205]



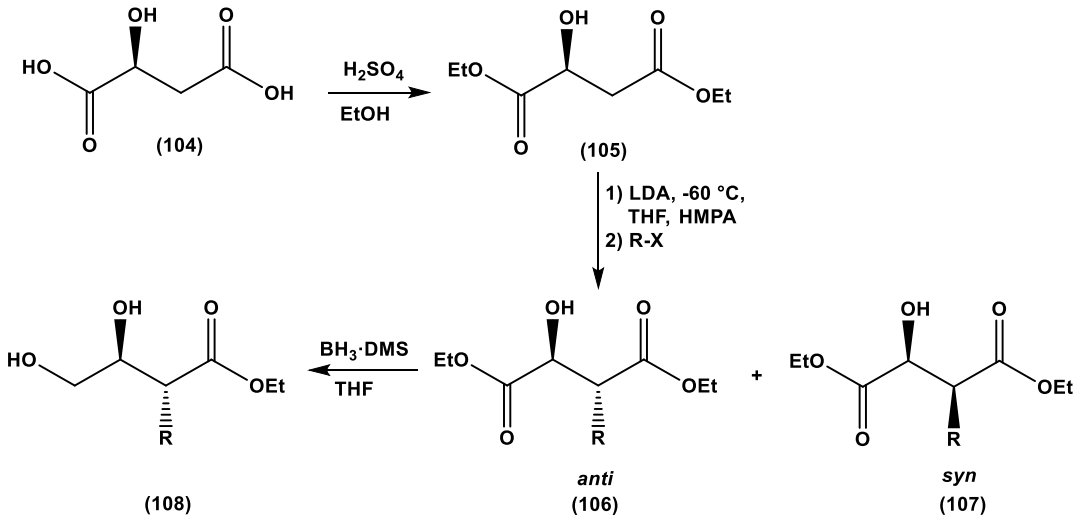
Scheme (13): Insertion of allyl group at the α-position.

The coupling reaction of the aldehyde compound **(99)** with a C-20 or C-22 sulfone via the modified Julia reaction, followed by hydrogenation of the resulting alkenes and debenzylation led to the formation of alcohols (**100**) and (**101**) respectively. The final step formed the mycolic motif unit (aldehyde compound) by oxidation of the alcohols (**100** and **101**) using pyridinium chlorochromate (PCC) in dichloromethane **(Scheme 14)**.



Scheme (14): Preparation of the mycolic motif with two different chain lengths.

Another route to prepare the β-hydroxy ester unit, starting from *L*-malic acid **(104)** was described by Khan *et al.*[206]

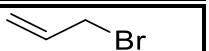
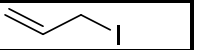
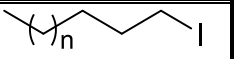
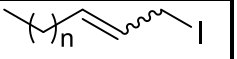
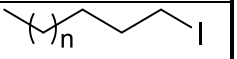
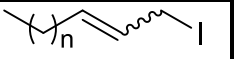
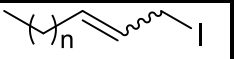
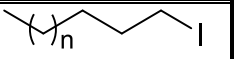
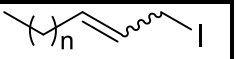
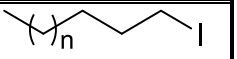


Scheme (15): The malic acid route for synthesis of the α-alkyl β-hydroxy unit.

Diethyl (*S*)-malate (**105**) was alkylated with allylic halides and their saturated counterparts in THF, using various conditions: A: LDA (2.5 equiv.), **(105)** (1 equiv.), R–X (1.5 equiv.); or B: addition of

HMPA (HMPA/THF 1:1), (**105)** (1 equiv.), R–X (1.5 equiv.) **(Table 1)**. Selective reduction of diester **(106)** by treatment with BH_3·DMS then gave diol **(108)**.[207]

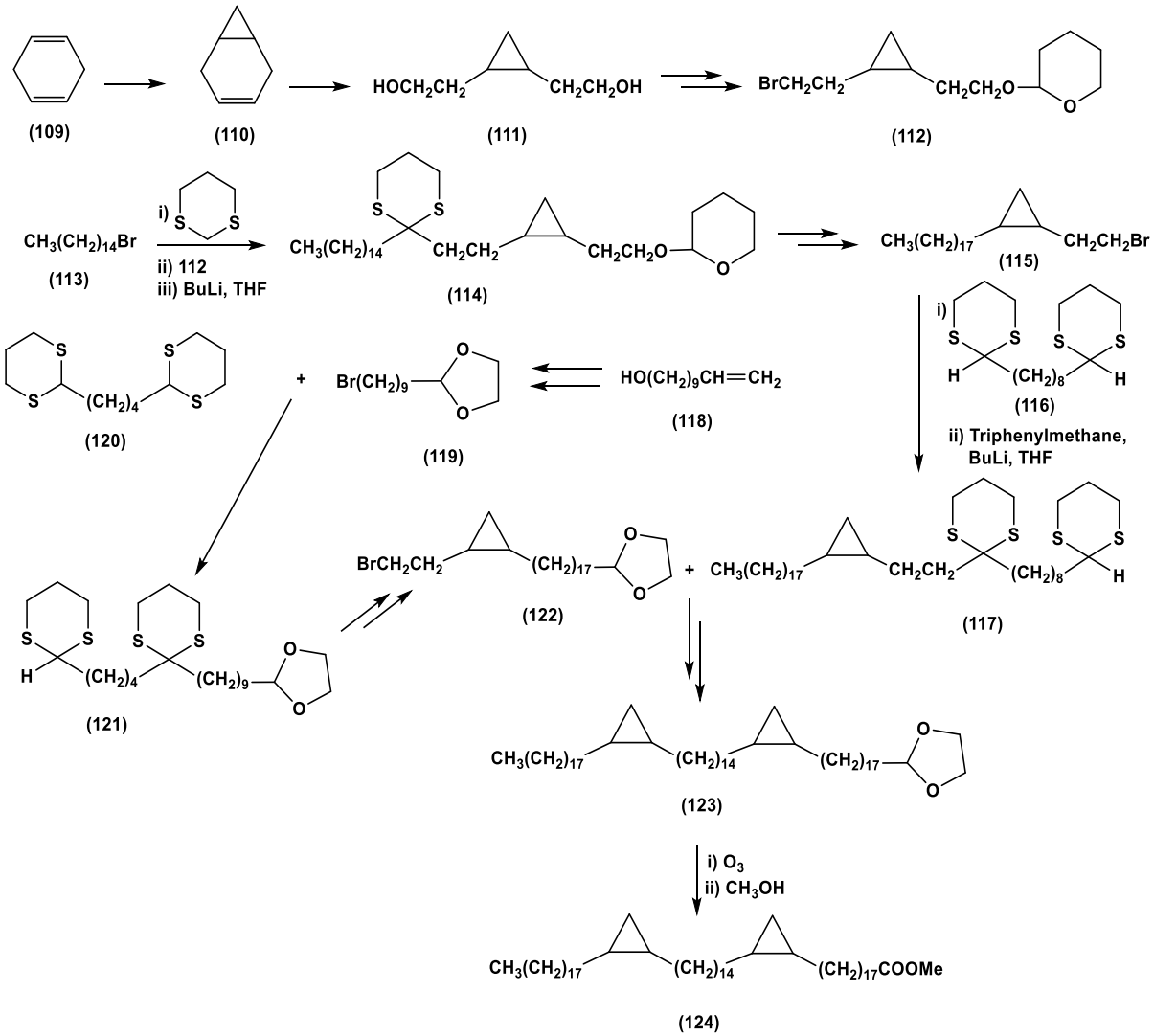
Table (1): The Fráter–Seebach alkylation using a variety of highly reactive long-chain alkyl halides.[206]

Entry	R-X	n	Conditions	Yield %	*Anti/syn*
1		-	A	57	14:1
2		-	A	52	9:1
3		3	A	37	7:1
4		3	A	55	9:1
5		10	A	15	9:1
6		10	A	7	7:1
7		10	B	35	7:1
8		10	A	51	10:1
9		10	B	45	9:1
10		16	A	5	6:1
11		16	A	49	9:1
12		18	A	8	6:1
13		18	A	42	9:1
14		20	A	41	9:1

1.10.2 Synthesis of the meromycolate unit

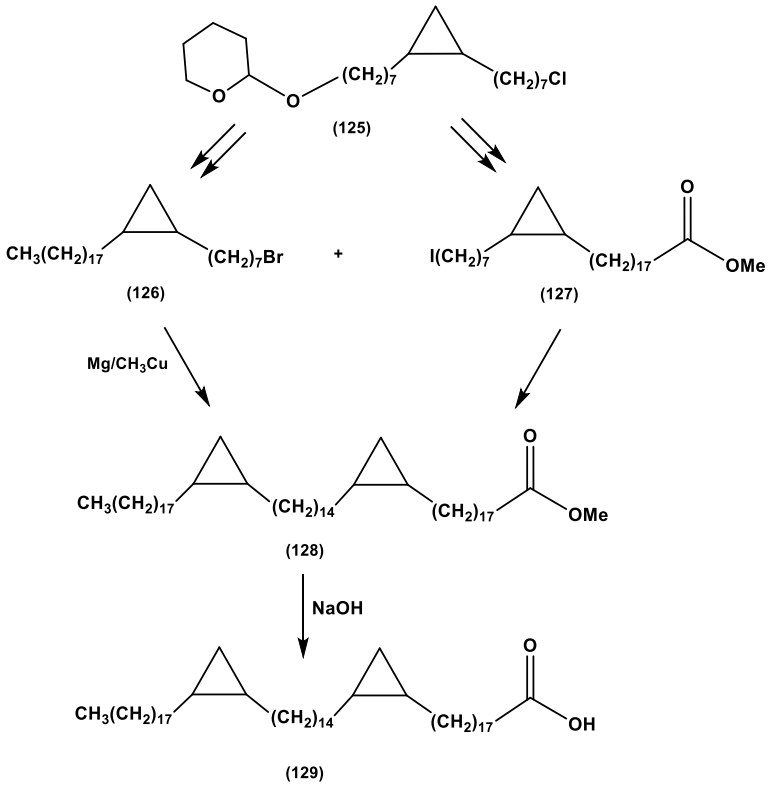
The second part of the mycolic acid, *i.e.* the meromycolate moiety was first synthesised in 1977 by Gensler *et al.*[208] who described the synthesis of a mixture of four stereoisomers of a meromycolic acid containing two *cis*-cyclopropanes. This method involved using 1,4-cyclohexadiene **(109)** as starting material to prepare norcarene **(110).** Ozonolysis of **(110)** followed by a reduction gave *cis*-1,2-cyclopropanediol **(111)**.[209] After numerous stages, the cyclopropane-containing bromide **(112)** was obtained. Cyclopropane **(115)** was then prepared by alkylation of pentadecyl bromide **(113)** with the cyclopropane-containing bromide **(112)**, followed by desulfurisation of compound **(114)** with Raney nickel.[210] Using the bisdithiane **(116)**, chain extension yielded **(117)**, one of two major components which

make up the meromycolate acid product. In a separate synthesis the second major component of the meromycolate was prepared. To begin with, ozonoylsis of 10-undecenol **(118)** was undertaken, followed by conversion of the resulting aldehyde into the acetal and bromination to yield **(119)**. Chain extension, by six carbons with the *bis*-dithiane (120), gave **(121)**, which was coupled to bromide **(112)**. Desulfurisation and deprotection of the tetrahydropyranyl group and bromination gave **(122)**, which was the second key intermediate. Coupling of the lithio derivative of the *bis*-dithiane intermediate **(117)** with intermediate **(122)** followed by desulfurization, gave the desired product **(123)**. Ozonolysis of this gave methyl meromycolate **(124)** which contained two *cis*-cyclopropane rings, **Scheme (16)**.



Scheme (16): The first synthetic meromycolic acid.

Subsequently, Gensler *et al.*[208] proposed another method involving the conversion of compound **(125)** to the alkyl bromide **(126)** and alkyl iodide **(127)** over several steps. The methyl meromycolate **(128)** was then prepared by combining the different portions using the Grignard reagent obtained from the alkyl bromide **(126)**, which formed a complex with the methyl copper, and the alkyl iodide **(127)**. The methyl meromycolate **(128)** was obtained as a mixture of four stereoisomeric di-*cis*-cyclopropanes. This mixture was then hydrolyzed to give meromycolic acid **(129) (Scheme 17)**.

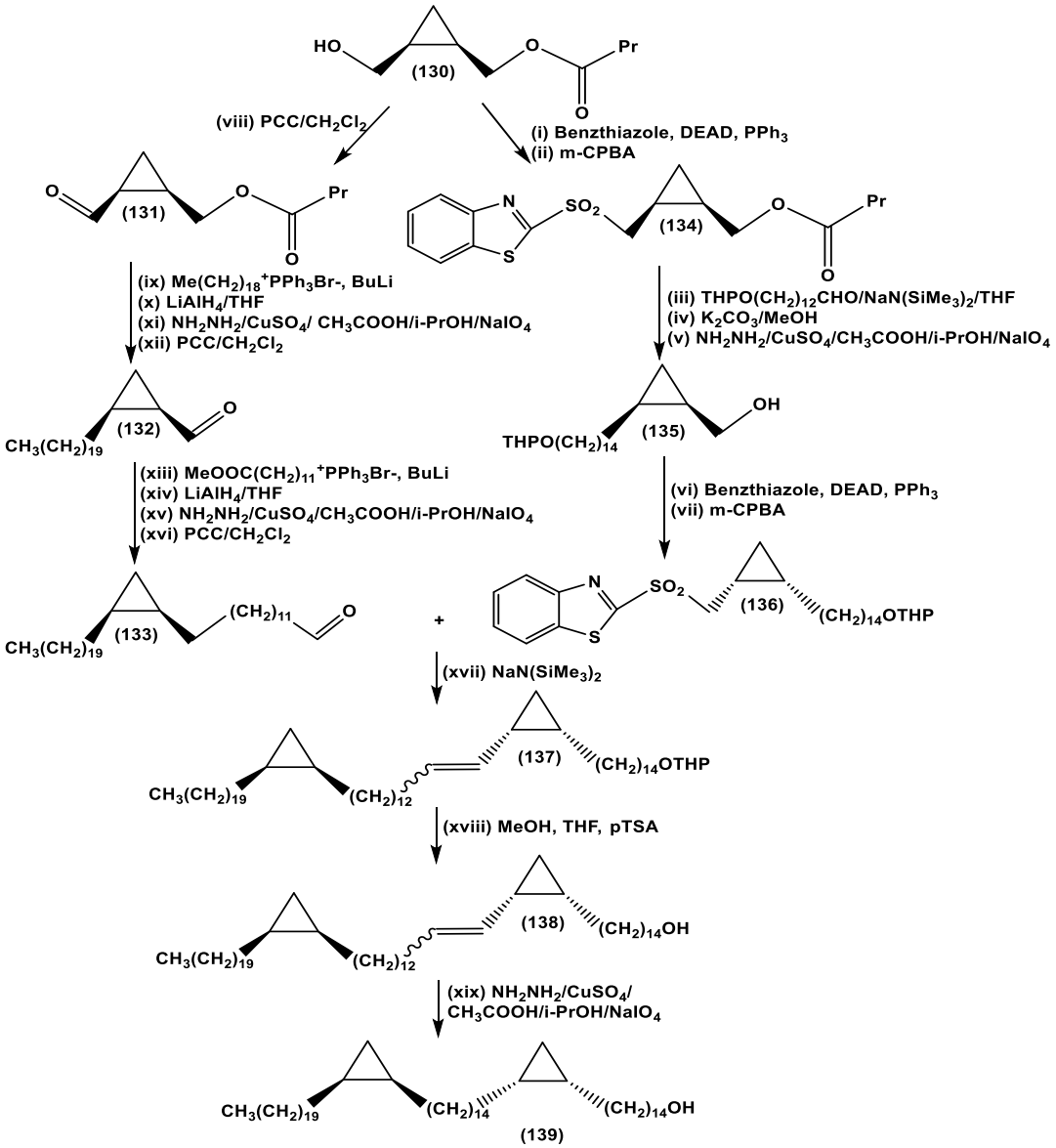


Scheme (17): Second Gensler *et.al.* approach.

This method is shorter and could be more easily scaled up, but suffers from low yield and does not control the stereochemistry.

The first enantiomerically pure meromycolic acid was prepared by Baird *et al.* in 2000, where single enantiomers of cyclopropane intermediates were coupled with no loss of stereochemistry.[211] The aldehyde **(131)** was prepared from the anhydride of cyclopropane-*cis*-1,2-dicarboxylic acid. A Wittig reaction of this with nonadecyltriphenylphosphonium bromide and *n*-butyl lithium, and reduction with lithium aluminium hydride, led to the alcohol as a mixture of *Z*- and *E*-isomers. Saturation of the alkene was achieved with di-imide, prepared *in situ* by reaction between hydrazine, sodium periodate

and acetic acid, this was followed by oxidation of the alcohol, which led to aldehyde **(131)**. Chain extension with a second Wittig reaction followed by saturation and oxidation to give the aldehyde **(133)**. An important feature of this approach is the coupling reaction, which was used to link the different units in several stages, securing the final desired stereochemistry. A Julia reaction of sulfone **(134)** with 13-tetrahydro-pyranyloxytridecanal yielded the protected alcohol **(135)**. This was converted into sulfone **(136)**. The Julia reaction between aldehyde **(133)** and sulfone **(136)** gave **(137)** as a mixture of *E*- and *Z*-alkenes. The enantiomerically pure alcohol **(139)** was obtained by deprotection of the alcohol group and saturation of the derived alkenes **(138)** with di-imide **(Scheme 18)**.

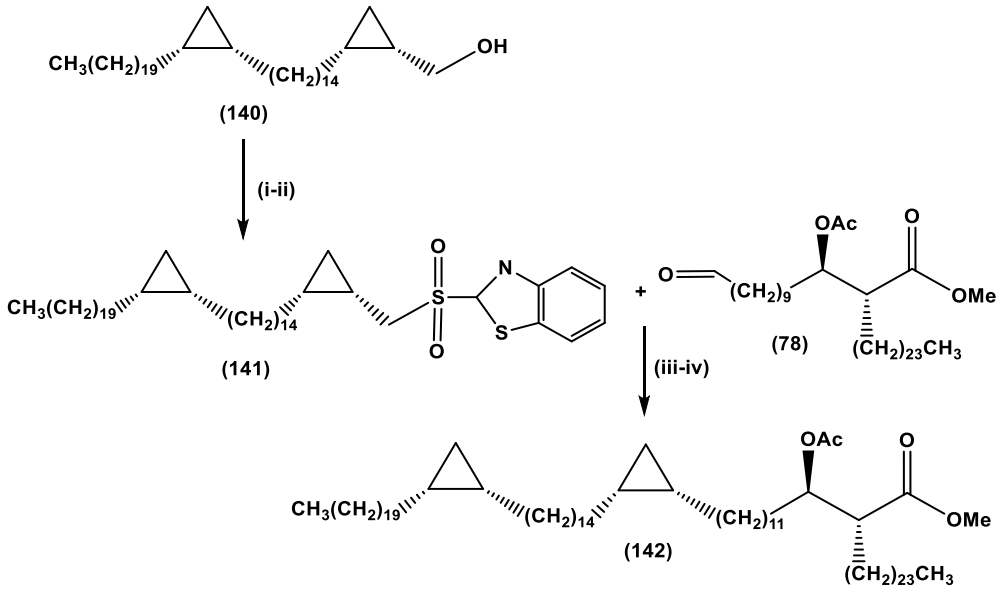


Scheme (18): The first synthesis of a single enantiomer of meromycolate.

A better overall yield (50%) and control of the stereochemistry are two important advantages of this method. This method can be applied to the synthesis of other meromycolates having varied functional groups and chain lengths and permits the preparation of different stereoisomers by modifying the sequence of reactions on the cyclopropyl groups, providing all the diastereoisomers of meromycolaldehydes.

1.11 Synthesis of complete mycolic acids

The full mycolic acid was prepared by linking the mycolic motif to the meromycolic acid. The single enantiomer of mero-mycolic acid **(140)** was converted into the sulfone **(141)**. Coupling of this to the mycolic motif aldehyde **(78)** via a Julia olefination, followed by hydrogenation of the resulting alkenes gave the full mycolic acid **(142) (Scheme 19)**.



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

Methoxy mycolic acids **(143–145)** of *M. tb* acids have also been synthesized using similar procedures. Also, the isolation of this type of molecule from *M. tb* has been achieved.[144,152]

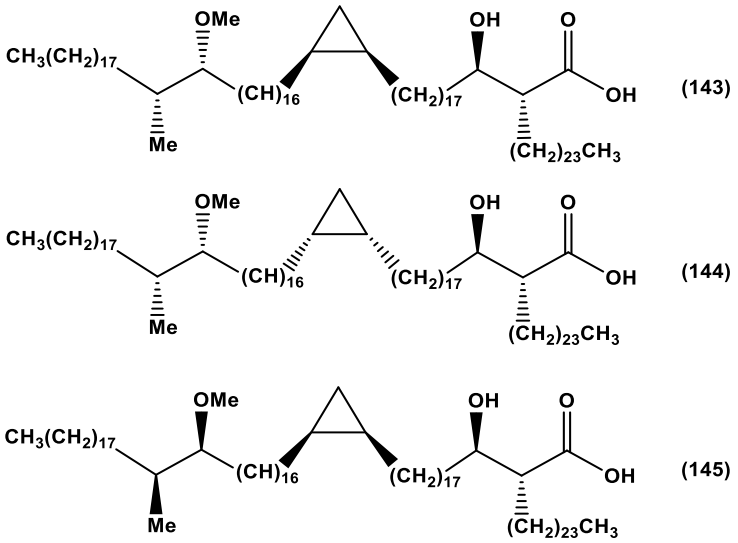


Figure (26): Synthetic methoxy-mycolic acids.

A set of hydroxy and keto-mycolic acids has also been prepared by the same group. The synthesis of ketomycolates which contain α-methyl-*trans* and *cis*-cyclopropane fragments **(146** and **147)** in order to produce a range of absolute stereochemistries and chain lengths was also undertaken **(Figure (27)**.[212,213]

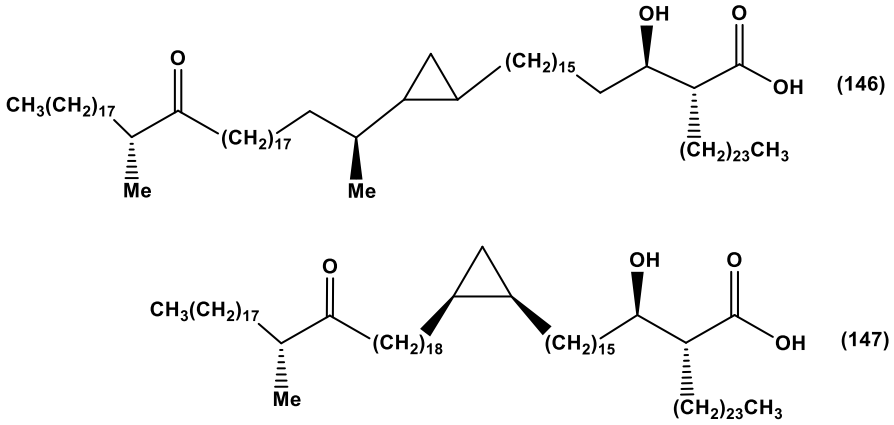


Figure (27): Synthetic keto-mycolic acids of *M. tb.*[212,213]

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 **(148)** and **(149)** containing an (*R*)-α-methyl-*trans*-alkene at the proximal position and the *cis*-alkene mycolic acid **(150) (Figure (28)**.[181,214] These have been found in *M. fortuitum* and *M. smegmatis.*

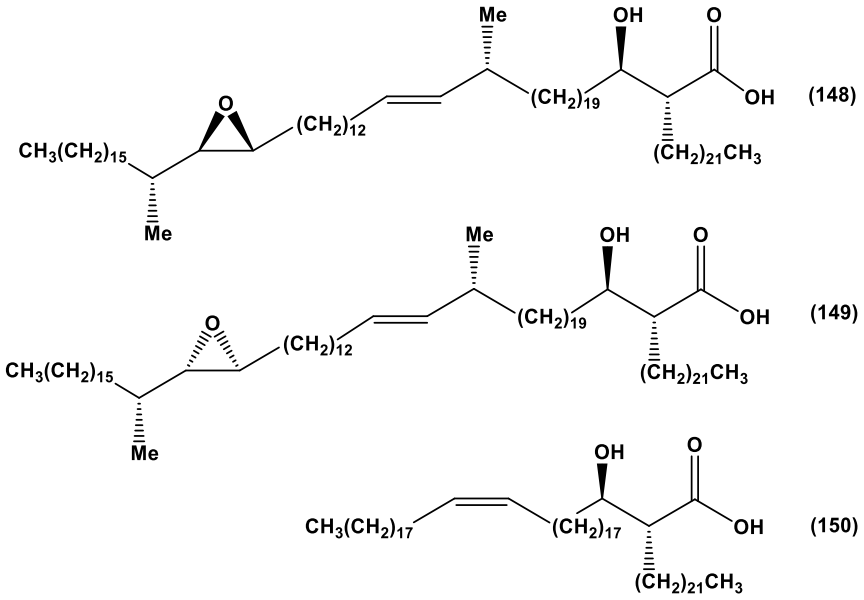


Figure (28): Synthesized epoxy and *cis* alkene mycolic acids.[181,214]

1.12 Derivatives of mycolic acids

Mycolic acids are found bound to the cell wall, as in penta-arabinose tetramycolate or as non-bound species such as sugar esters, usually trehalose di- or mono-mycolates (TDM and TMM), glucose monomycolate (GMM) or glycerol monomycolate (GroMM). These species are known to show interesting toxicological and immunological properties.[215,216]

1.12.1 Cord Factor

Cord factor is a complex glycolipid, trehalose dimycolate (TDM); trehalose is esterified at both primary alcohol positions with mycolic acids giving the 6,6'–dimycolate.[217,218,219] It is one of the most interesting and potentially valuable glycolipids found in the cell wall of *M. tuberculosis*. However, trehalose is also present in a wide variety of organisms including fungi, insects, invertebrates, and in plants.[220,221] Most bacteria use trehalose only as a general osmoprotectant or thermoprotectant.[222] There are two classes of cord factors, **(Figure 29)**; each of these derivatives consists of a non-reducing, disaccharide core, *i.e.* trehalose, esterified with one mycolic acid at the 6-position corresponding to trehalose monomycolate (TMM) **(151)** or esterified with two mycolic acids at the 6,6'-positions corresponding to TDM **(152)**.

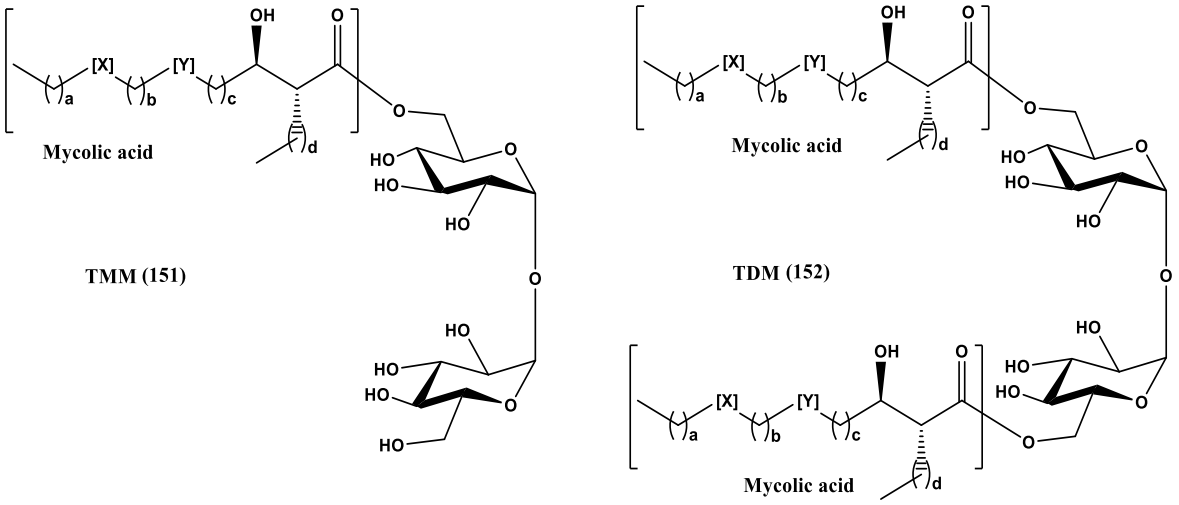


Figure (29): Structures of TMM and TDM.

Robert Koch, in 1884 was the first to discover that growing tubercle bacilli formed long strands or 'cords',[223]

The term 'cord factor' has been used since 1947; Middlebrook *et al.*[224] discovered that the formation of 'serpentine cords' was typical of contagious strains of *tubercle bacilli.* In 1950 Bloch for the first time isolated the glycolipid from the tubercle bacilli cells and he referred to it as a 'cord factor'. Bloch also reported the toxic behaviour of these cords when he extracted four different strains with petrol and they tested them on mice.[225]

Noll *et al.*[131] in 1956 proposed the structure of cord factor isolated from *M. tb* and subjected it to alkaline hydrolysis. This produced, a free mycolic acid and a non-reducing carbohydrate moiety, α,α-trehalose. This was confirmed in two ways: firstly the acid hydrolysis of the carbohydrate moiety produced *D*-glucose; and secondly acetylation to give crystalline α,α-trehalose octa-acetate. The position of the MA attached to the trehalose was clarified firstly by methylation and then by saponification of the TDM **(153)**; the result was hexa-methyltrehalose. Acid hydrolysis gave trimethylglucose **(154)**. This showed that the MA is attached to the trehalose sugar at the 6,6' positions respectively **(Figure 30**).[131]

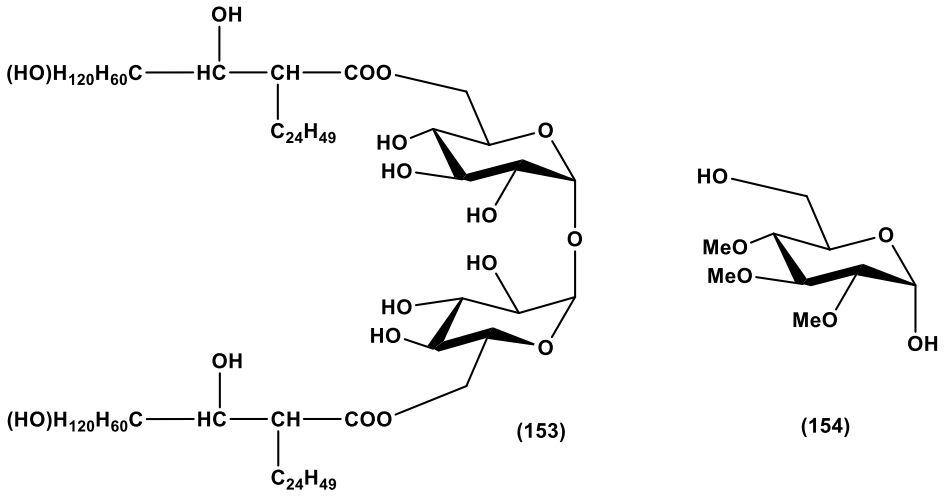


Figure (30): Proposed structure of trehalose 6,6-dimycolate by Noll *et al*.[131]

Ioneda *et al*. revealed the isolation of a glycolipid from *Corynebacterium diphtheria* and showed that it was a trehalose di-ester containing corynomycolic acid ($C_{32}H_{64}O_3$) and corynomycolenic acid ($C_{32}H_{62}O_3$) in equal proportions.[226,227] 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.[228] Using a strain of *M. tuberculosis brevanne,* Toubiana *et al.* in 1979 extracted and purified dimycolates of trehalose by C wax hydrolysis of the tubercle bacilli. They also isolated and characterised all types of mycolic acid (type α, β, γ) in a single strain and found molecular peak ions by mass spectrometry.[217]

Promé *et al.* isolated and identified mixtures of α-*D*-trehalose dimycolate and monomycolate from *M. phlei.* Two types of trehalose monomycolate (TMM) were found; the first one comprises an α-mycolic acid and the second one was the trehalose mono wax ester. Another three types of trehalose dimycolate (TDM) were also found, one containing two α-mycolic acids, one containing two wax esters, and one having one residue of each type.[229] In 1977, Takayama and Armstrong studied the H37Ra strain and identified a new disymmetrically substituted ester which was involved in mycolic acid transfer into the cell wall.[230] Fujita *et al.* used MALDI-TOF mass spectrometry in order to analyse the cell wall components of several types of mycobacteria. The structure of TMM and TDM were thus confirmed.[231] 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 spectrometric techniques. This led to a much clearer understanding of cord

factor composition.[231] Baird *et al*.[232] published the first synthesis of cord factors employing single synthetic enantiomers of mycolic acids in 2009 with the method used being easily modified to produce cord TDM or TMM from any synthetic mycolic acid. As with MAs, the synthesis of single enantiomers of TDM and TMM compounds allows the antigenic properties of each of these, and any combination thereof, to be investigated.

1.12.2 Glycerol monomycolate (GroMM)

GroMM is a mycolic acid esterified with glycerol at one of the primary hydroxyl groups **Figure (31)**. Glycerol mycolate-containing lipid species produced by mycobacteria, can stimulate innate immune cells and produce inflammatory cytokines.[216] It is found among different bacteria of the *Corynebacterium*, *Mycobacterium*, and *Nocardia* (CMN) group.[233] Andersen *et al.* specified GroMM *in vitro* as the most immunopotentiating compound among a number of different lipids isolated from the mycobacterial cell wall.[234]

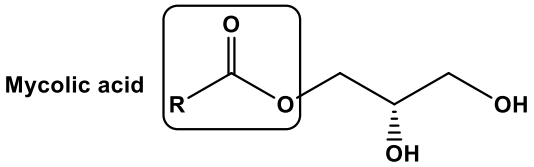


Figure (31): Example of GroMM.

Hattori *et al.* immunized guinea pigs with bacillus Calmette–Guérin (BCG) expressing high levels of GroMM and then monitored skin reactions at the site of inoculation with GroMM-containing liposomes. The host responses to GroMM produced by dormant mycobacteria contributed to their long-term survival in the host.[235] Similar to TDM and lipoarabinomannam (LAM), GroMM appears to directly stimulate innate immune cells to produce inflammatory cytokines.[233] Recent research pointed to a direct degradation pathway for TDM by means of enzymatic hydrolysis,[236] resulting in the accumulation of free mycolates that was associated with the increased influx of nutrients including glycerol.[237] The synthesis of GroMM using single synthetic mycolic acids will result in a rich knowledge of the nature of the cell wall of *M. tuberculosis*, and hence give a better understanding of their effects in the immune system.[234]

1.12.3 Glucose monomycolate (GMM)

GMM sugar ester mycolate, is a glycolipid consisting of a mycolic acid attached to the 6-position of glucose present in numerous bacterial species including *Mycobacterium*, *Rhodococcus* and *Nocardia*.[238,239] The structure of GMM from *M. tuberculosis* is shown in **Figure 32**. GMM is a mycobacterial antigen isolated from various species of mycobacteria, including *M. tuberculosis*.[238,239]

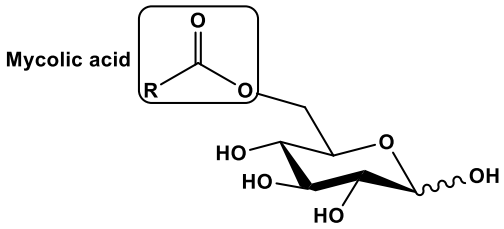


Figure (32): Example of GMM.

It has been proposed that TDM can be converted into GMM inside the host.[240] Environmental mycobacteria express TDM on the surface of their cell wall but fail to biosynthesize GMM because of the very limited availability of glucose.[241] Mycobacteria are unable to synthesise GMM outside of the host cell since a non-mycobacterial source of glucose is needed. Therefore, GMM is only produced by a pathogenic mycobacteria after infection of the host cell.[175] GMM can induce a memory T cell response.[239] GMM and other antigenic mycobacterial glycolipids are presented to T cells by the CD1 family of proteins and the antigen–protein complexes mediate the T cells response in the human host.[242] However, depending on the species, the meromycolic chain carries variable functionalities in the proximal and distal positions, which are characteristic of the species.[243] GMMs have the capacity to produce T cell proliferative responses in a number of species including humans, mice, guinea pigs, and cattle.[244] Nguyen *et al.* have described cell-mediated and humoral immune responses in cattle upon vaccination with GMM as the only antigen; as a result a T cell response was produced but no antibody responses, while the vaccine comprising a pure protein as the only antigen generated both T cell and antibody responses. However, in humans and cattle,[245] Nguyen *et al.* assumed that a conjugate of GMM with a protein may provide T cell help for B cells to produce antibodies against surface exposed glycolipids in mycobacteria.[244]

1.12.4 Synthesis of a fragment from arabinogalactan

The mycolyl-arabinogalactan complex (mAG) is the largest component structure in the cell wall of mycobacteria and is located outside the PG. mAG is essential for cell wall integrity and mycobacterial survival.[246] The arabinans and galactans of mycobacterial cell walls consist of D-arabinofuranose (D-Araf) and D-galactofuranose (D-Galf).[247] Synthetic arabino-mycolates induce the production of TNF-α in murine macrophage cell lines at an intensity similar to BCG-cell wall skeletons. However, the immunological activity of natural arabino-mycolates isolated from BCG has not been investigated; this is probably due to the complexity of the molecule.[248]

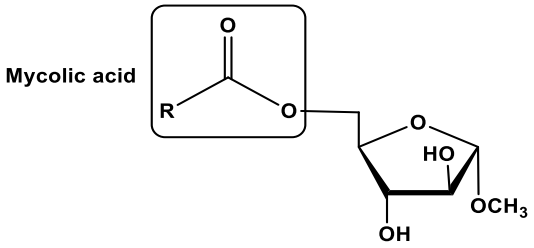


Figure (33): Example of fragment from arabinogalactan.

Isolated natural arabino-mycolates significantly induced TNF-α production and enhanced delayed type hypersensitivity (DTH) reactions against inactivated tumour cells and possess potent adjuvant immunostimulatory activity.[248,249] Intratumour injections of extracts of Re Mutant *Salmonella typhimurium* in combination with TDM or arabinose mycolate were highly effective in producing regression of tumours in guinea pigs.

1.12.5 Synthesis of TDM and TMM

Baird *et al.* reported the first unique synthesis of mycobacterial cord factors.[232] They were able to convert a methoxy mycolic acid and an α-mycolic acid into their corresponding TDM and TMM. According to the reported procedure,[250] both mycolic acids were protected at the β-hydroxy acid position with TBDMS, prior to the coupling with the trehalose, to avoid reactions of the alcohol group. The protected mycolic acid was coupled with protected trehalose to obtain a protected TDM and a protected TMM. The coupling was carried out by esterification, using EDCI and DMAP. Subsequently, deprotection was carried out in two stages. The first deprotection was done with TBAF, removing all the trimethylsilyl groups from the trehalose unit. Further deprotection using HF pyridine removed TBDMS protection from the β-hydroxy position of the acid to yield the desired cord factors **(see page 45)**.[232, 250]

Chapter 2

2. Results and Discussion

2.1 Project aims

This project consists of three main parts. The first objective was to reduce the number of steps currently involved in the synthesis of the mycolic motif, by developing an improved method for the synthesis of the α-alkyl, β-hydroxy fragment **(Figure 34)**. The method needs to be less expensive and involve fewer reaction steps. This fragment is a common unit in all mycolic acids and as such the development of an improved method for its synthesis would simplify the overall synthesis of all mycolic acids, as well as cord factors.

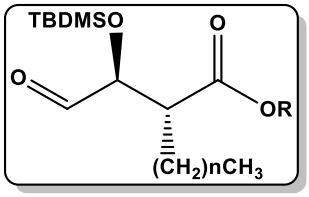


Figure (34): The α-alkyl-β-hydroxy fragment of mycolic acids (mycolic motif).

The second aim was to synthesise the specific *cis*-alkene-α-methyl-*trans*-alkene mycolic acid **(155)**, which contains a double bond at the distal and proximal positions. This type of mycolic acid has been isolated from *M. brumae.* Having prepared the MA **(155)**, the third part was the preparation of a number of mycolate esters, by esterification of the mycolic acid with different sugars. These include the GroMM **(156)**, GMM **(157)**, the arabino-MA fragment from AG **(158)**, TDM **(159)**, and TMM **(160) (Figure 35)**.

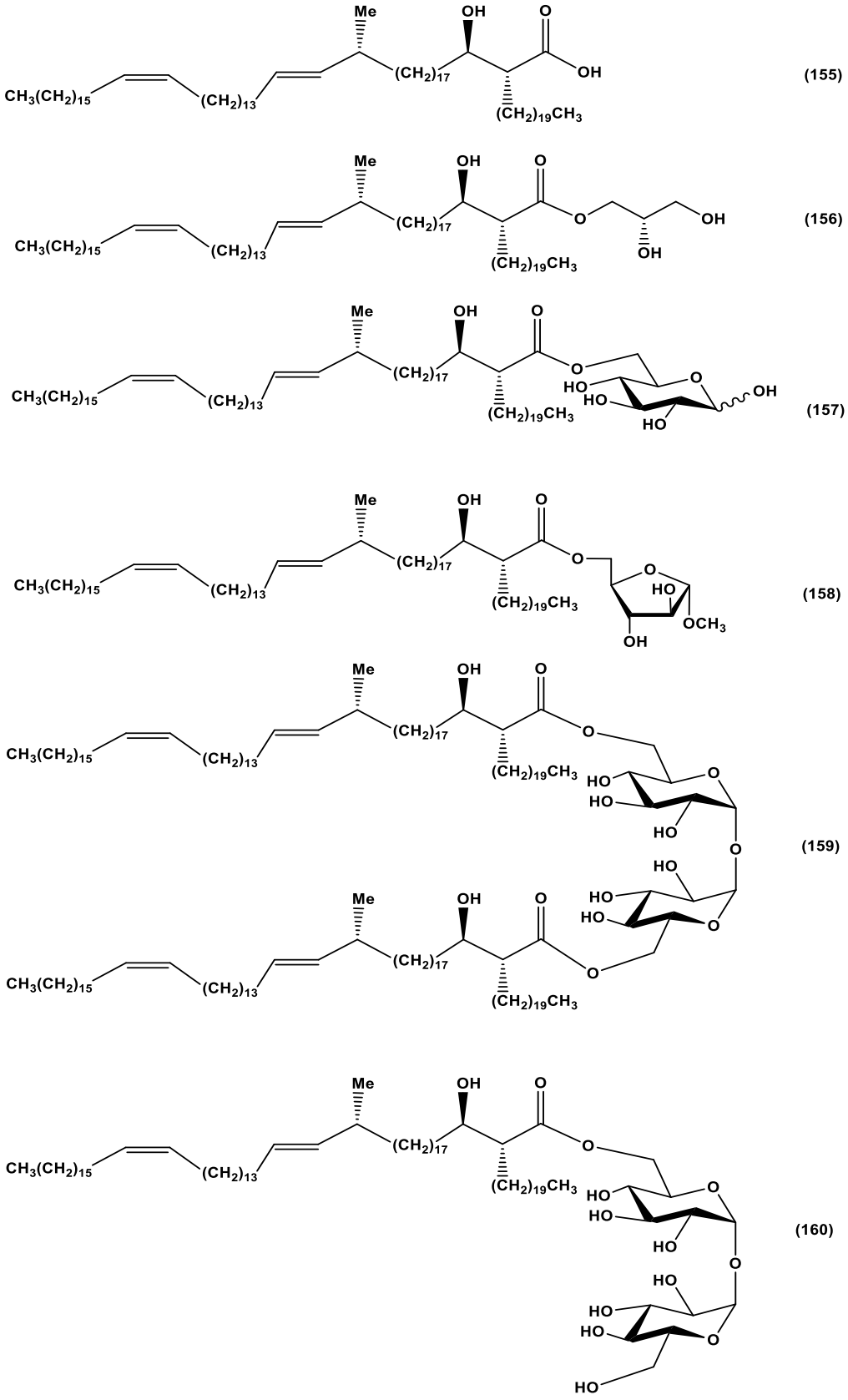


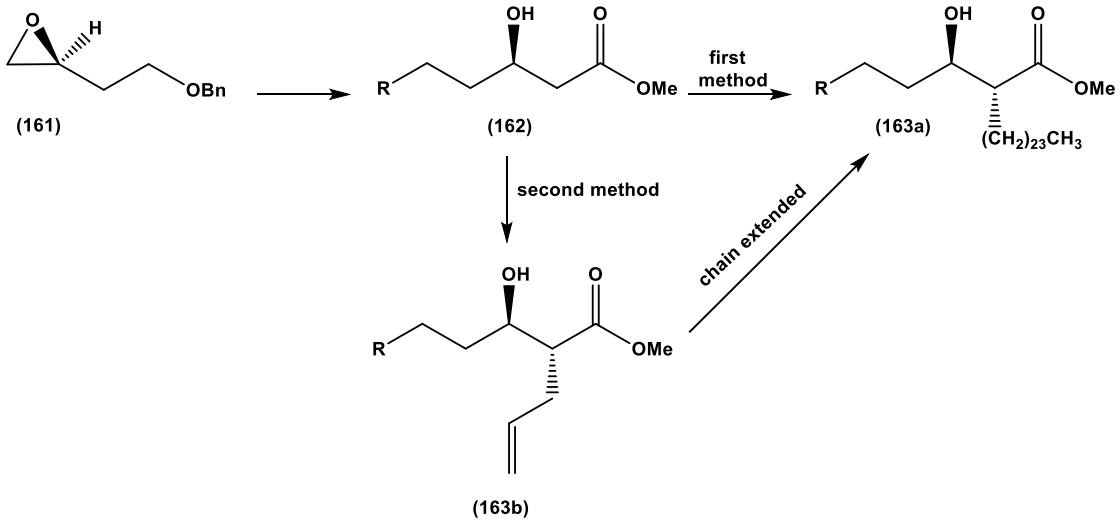
Figure (35): Target mycolic acid and its sugar and glycerol esters.

2.2 Preparation of the mycolic motif unit

The first target of this project was concerned with the development of a new method for the synthesis of the α-alkyl-β-hydroxy fragment of mycolic acids, which is also known as the mycolic motif unit. The preparation of this unit is almost certainly the most tedious part of mycolic acid synthesis and as such necessitates some improvement. Improving this synthetic method is also of major importance as the α-alkyl-β-hydroxy moiety is similar in all mycolic acids and their derivatives, such as cord factors.

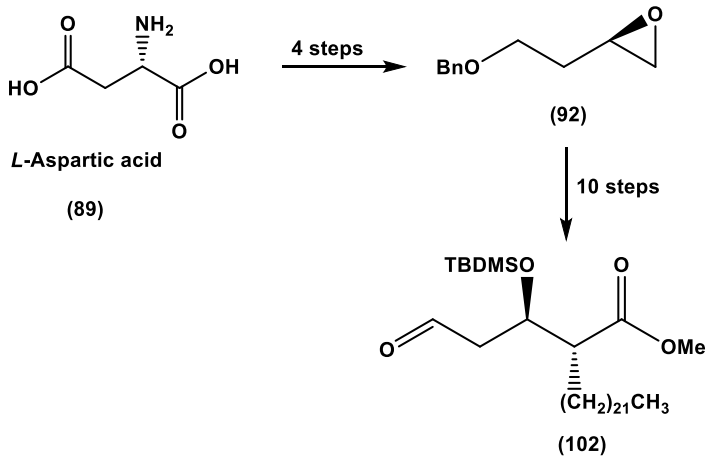
2.2.1 Previous work

Several methods for the preparation of the mycolic motif unit with *R,R* stereochemistry at the chiral α- and β- carbon atoms have been published.[202,251] The first includes a direct alkylation of a long chain at the α-position relative to the carboxylic group. In this method Al Dulayymi *et al.*[251] used (*S*)-(2-benzeneoxyethyl) oxirane **(161)** as a chiral intermediate prepared from commercially available *D*-aspartic acid based on a method developed by Frick *et al.*[252] In the second method, a short chain allylation was initially carried out, followed by further elongation of the chain using a modified Julia-Kocienski reaction in order to obtain the desired carbon chain length **(Scheme 20**). This short allyl chain was used because the insertion of the full alkyl group in one step has given variable results and low yield (10%).



Scheme (20): The two basic methods for the synthesis of the α-alkyl-β-hydroxy unit.

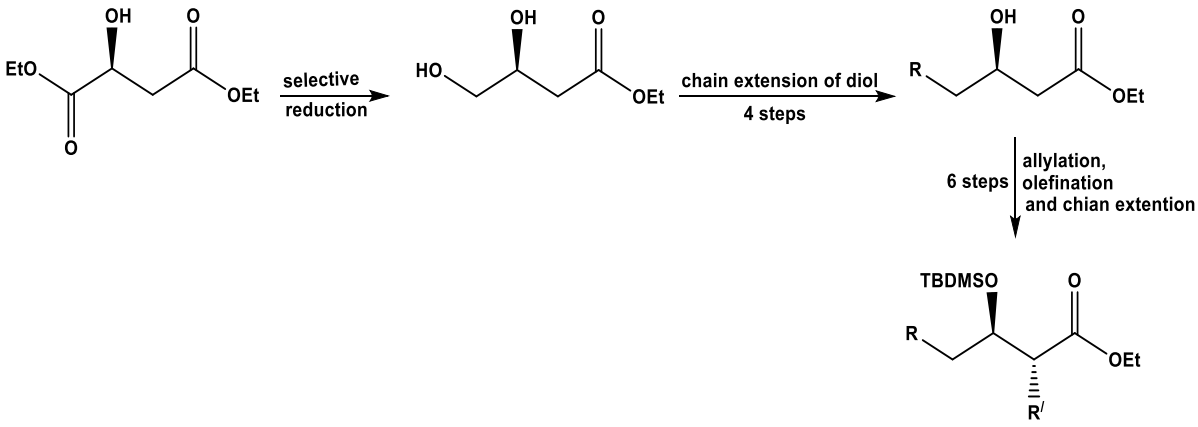
Another method described by Koza *et al.*[203] prepared the mycolic motif unit **(102)** starting from commercially available *L*-aspartic acid (**89**) for the synthesis of the (*R*)-(2-benzeneoxyethyl)oxirane intermediate **(92)** in four steps **Scheme (21**). A short chain Fräter allylation followed by a further Julia-Kocienski chain extension was then carried out at the *α*-position. They obtained the α-alkyl-β-hydroxy unit in 14 steps with an overall yield of 11%.



L-Aspartic acid
(89)

Scheme (21): Alternative starting materials for the synthesis of the α-alkyl-β-hydroxy unit.

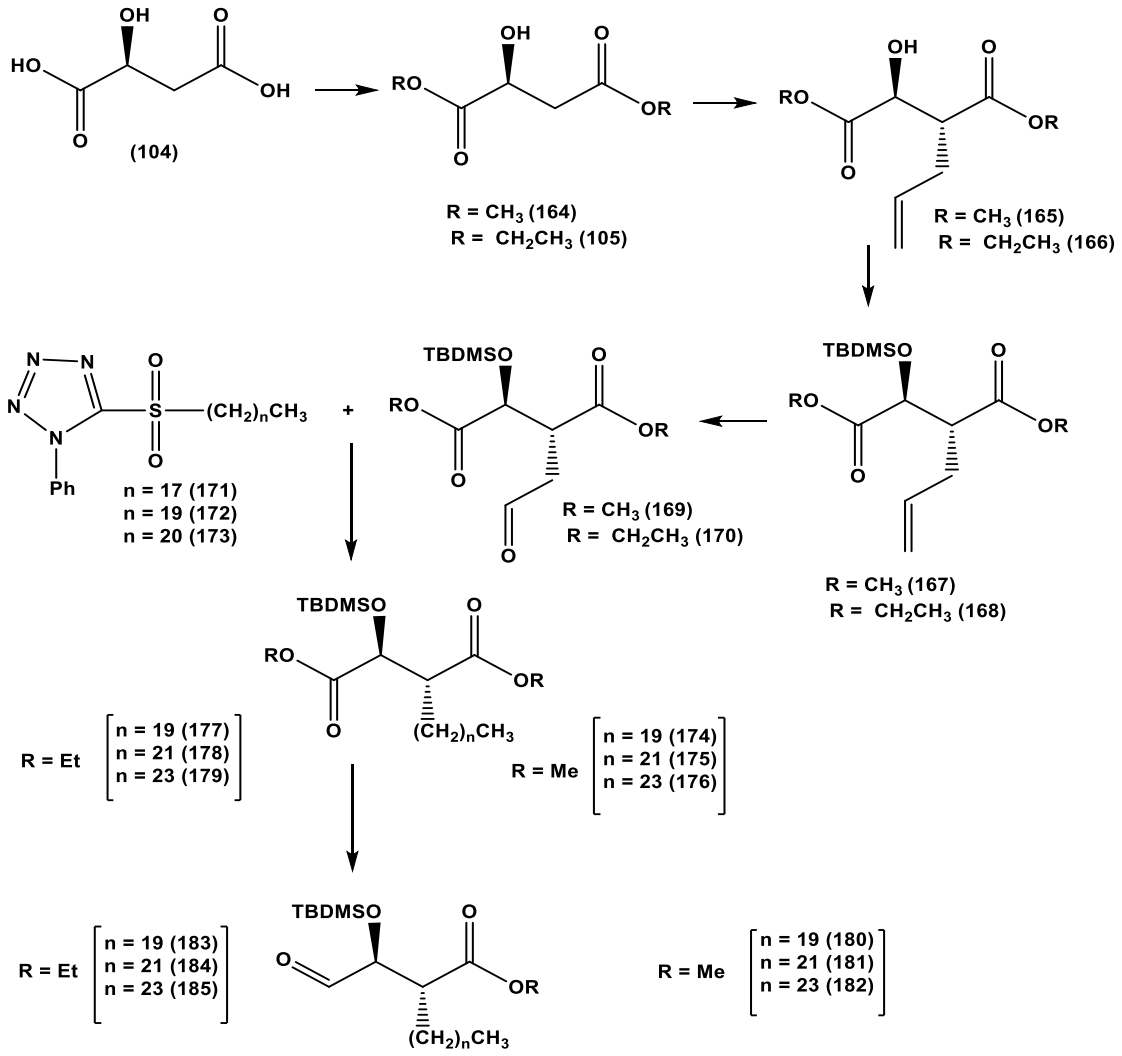
Another method for the synthesis of the α-alkyl-β-hydroxy fragment, starting from a dialkyl malate has also been investigated **(Scheme 22)**. A selective reduction of diethyl malate with borane dimethyl sulfide complex (BMS) and catalytic $NaBH_4$, was first carried out followed by chain elongation via a chiral epoxide intermediate. Alkylation at the α-position, which involved a Fräter allylation, was then carried out, followed by protection of the secondary alcohol, ozonoylsis, and Julia olefination respectively. The desired compound was then obtained, in the correct stereochemistry, after hydrogenation. This route was successfully completed in 10 steps with an overall yield of 10%.[253] The disadvantage of this approach is that it requires the use of expensive material such as silver oxide.



Scheme (22): Preparation of the α-alkyl-β-hydroxy unit from diethyl malate.

2.2.2 A new method for the synthesis of the mycolic motif unit

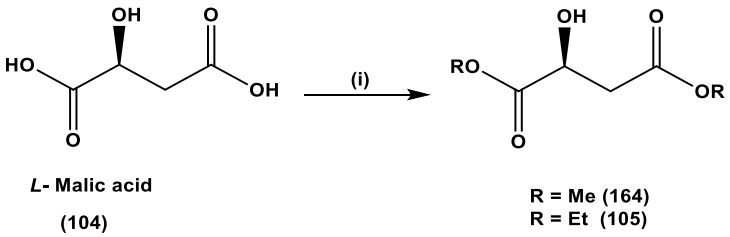
The work in this thesis involved developing an alternative, more efficient route starting from the commercially available and the cheaper starting material *L*-malic acid **(104)**, over fewer steps (6 steps), as shown in **Scheme (23)**. This sequence had been completed before for the series of ethyl esters,[254] but the key final step proved very difficult to repeat, and was optimised in this work using the methyl esters.



Scheme (23): The mycolic motif unit.

2.2.3 Preparation of dialkyl (*S*)-2-hydroxysuccinate

L-Malic acid **(104)** was esterified to the diesters **(164**, **105)** using MeOH or EtOH respectively in the presence of thionyl chloride at 0 ºC and stirring overnight at room temperature, and the crude products were purified by flash distillation in 98 and 99% yields respectively **(Scheme 24)**. The structures were confirmed by their NMR spectra which were the same as those reported in the literature.[255,256]



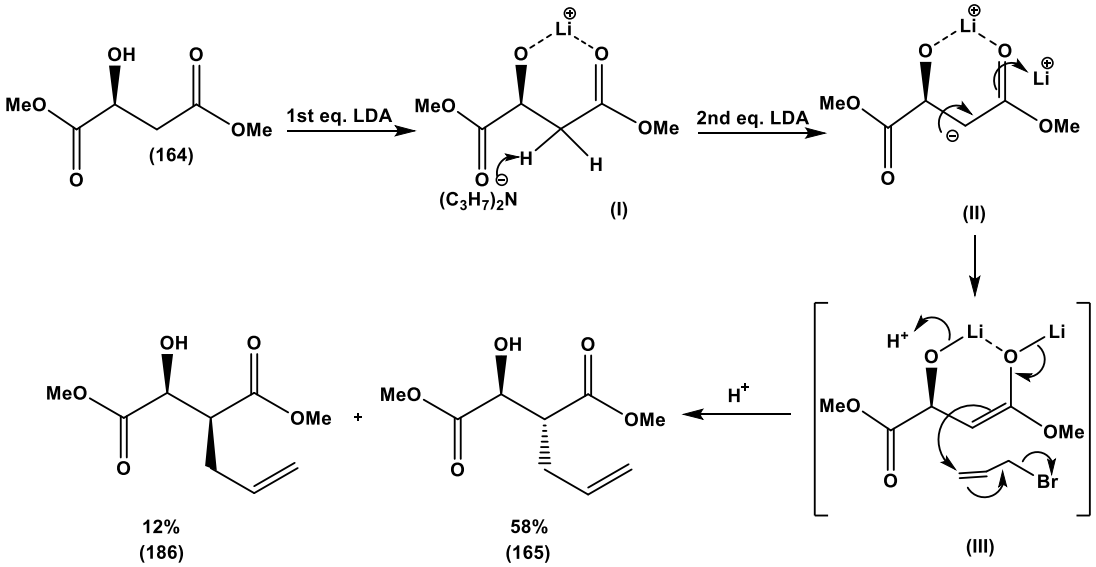
Scheme (24): Esterification of L-malic acid **(104)** into diesters **(164**, **105)**, reagents and conditions: (i) $SOCl_2$, ROH, 0 ºC.

2.2.4 Inserting an α-alkyl chain in the mycolic motif unit

There are a number of methods for the preparation of the *α*-alkyl-*β*-hydroxy unit with the correct stereochemistry at both the α- and β-carbon atoms. The widely utilized asymmetric alkylation method based on the Fräter alkylation is highly diastereoselective[160,200] and was believed to be the best route for the stereocontrolled insertion of the allyl chain at the α-position of the β-hydroxy ester.

2.2.4.1 Preparation of diesters (2*R*,3*S*)-2-allyl-3-hydroxysuccinate

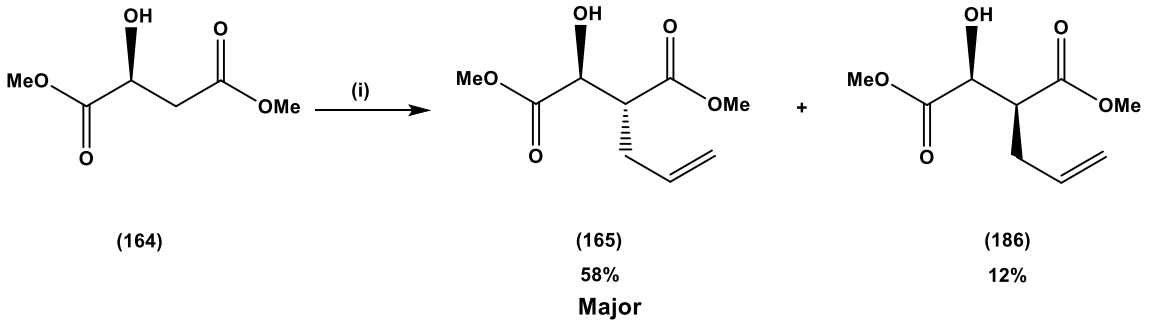
The Fräter alkylation was used for the stereoselective installation of the required allyl chain at the α-position of the β-hydroxy diester **(164)**. The allyl chain was attached from the bottom face due to the steric hinderance encountered on the top face from the six-membered ring chelate, intermediate (III). This gave the *anti*–alkylated product (2*R*,3*S*)-hydroxy ester **(165)** in 58% yield **Scheme (25**).[202]



Scheme (25): Mechanism of the Fräter alkylation.

The allylation was carried out using LDA generated in situ from MeLi and di-isopropylamine in dry THF. After 30 minutes, it was cooled to – 61°C and then reacted with dimethyl (*S*)-2-hydroxysuccinate **(164)**. In order to ensure the formation of the intermediates **(I)** and **(II)** the mixture was stirred at – 45 to – 10 °C for 2 hours, then cooled to – 62 °C followed by the addition of allyl bromide. A further quantity of allyl bromide was added to the mixture which was then stirred at the same temperature for 60 minutes.

The reaction was slowly allowed to reach 0 °C and then worked up with sat. NH_4Cl solution. The product was obtained as a mixture of distereoisomers **(165)** and **(186)** respectively which were separated by column chromatography to give the required product **Scheme (26)**.

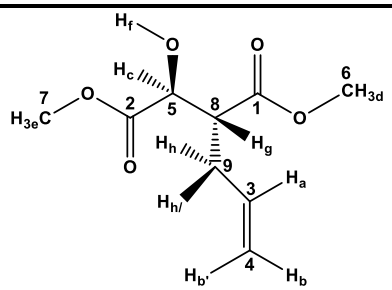


(164) (165) 58% Major (186) 12%

Scheme (26): The insertion of the α-allyl chain, reagents and conditions: (i) LDA, – 78 °C, allylbromide, dry THF.

The allylation was confirmed by the ^1H NMR spectrum which showed a double double doublet at δ 2.99 (*J* 3.2, 6.6, 8.7 Hz) for the proton at the α-position **Table (2)**. The two protons adjacent to the alkene group were also revealed as multiplets at δ 2.50 – 2.40 and a doublet of triplets at δ 2.62 (*J* 6.3, 14.1 Hz) and two singlets corresponding to the methyl groups appeared at δ 3.80 and δ 3.69. For the alkene signals, a multiplet corresponding to one C=C proton adjacent to the $-CH_2-$ group appeared at δ 5.88 – 5.73 and the two other terminal C=C protons were revealed as double doublet and doublet at δ 5.17 and 5.12 respectively. In addition, there was a peak at δ 3.17 for the hydroxyl proton and another one at δ 4.31 for the proton at the β-position. The ^{13}C NMR spectrum revealed two carbonyl carbon signals at δ 173.8 and 172.4, two olefinic signals at δ 134.6 and 117.8, and the signal at δ 70.0 was due to for the carbon adjacent to the hydroxyl group. Then two methoxy signals at δ 51.8 and 52.5, and at δ 48.0 for the α-carbon and a CH_2 signal at δ 31.9. In the IR spectrum there were significant peaks for the hydroxyl, alkene and carbonyl functional groups at ν_{max} 3468, 1642 and 1741 cm^{-1} respectively. The specific rotation was recorded as $[\alpha]_D^{23} = +11$ (c 1.5, $CHCl_3$).

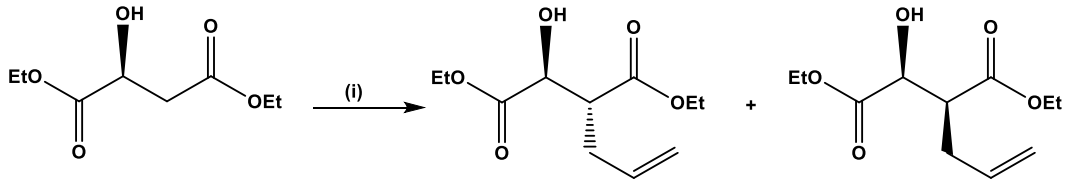
Table (2): Selected ^{1}H and ^{13}C NMR signals for compound **(165)**.



Proton	Integration	Multiplicity	*δ/ppm*	*J/* Hz	Carbon	*δ/ppm*
H_a	1	m	5.88 – 5.73	-----	C_1	173.8
H_b	1	dd	5.17	1.4, 17.1	C_2	172.4
H_b'	1	d	5.12	10.2	C_3	134.6
H_c	1	dd	4.31	3.2, 7.1	C_4	117.8
H_d	3	s	3.80	-----	C_5	70.0
H_e	3	s	3.69	-----	C_6	52.5
H_f	1	br.d	3.17	7.1	C_7	51.8
H_g	1	ddd	2.99	3.2, 6.6, 8.7	C_8	48.0
H_h	1	dt	2.62	6.3,14.1	C_9	31.9
H_h/	1	m	2.5			

In order to discover the effect of the HMPA on the yield, the reaction was carried out once using HMPA and gave the required product in 59% yield, compared to 58% yield, without HMPA. This is therefore advantageous compared to previous methods as it can be carried out without the use of HMPA, which is considered to be a carcinogenic compound, without having any significant effect on the yield of the required product.

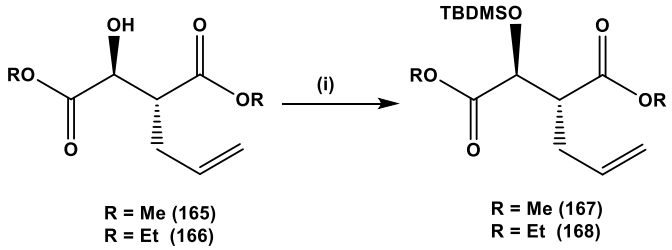
This allylation reaction was also repeated under the same conditions described above using butyl lithium, instead of methyl lithium, and diethyl (*S*)-2-hydroxysuccinate (**105 R = Et)** to give (**166**) in 53% yield **Scheme (27)** with a specific rotation of $[\alpha]_D^{23}$ = + 13.4 (c 1.79, $CHCl_3$). (*lit.*[206] $[\alpha]_D^{20}$ = + 15.1 (c 1.4, $CHCl_3$)).



Scheme (27): The insertion of the α-allyl chain: (i) LDA, – 78 °C, allylbromide, dry THF.

2.2.5 Protection of the secondary alcohol

In order to complete the chain extension at the α-position the secondary alcohol of (2*R*, 3*S*)-2-allyl-3-hydroxysuccinate compounds **(165)** and **(166)** were protected with a *tert*-butyldimethylsilyl group to give products **(167)** and **(168)** in 86% yield **Scheme (28)**. The protecting group protons for dimethyl (*2R,3S*)-2-allyl-3-((*tert*-butyldimethylsilyl) oxy) succinate **(167)** appeared in the ^{1}H NMR spectrum as a singlet at δ 0.88 for the *tert*-butyl group and two singlets at δ 0.07 and 0.05 for the two methyl groups. The two methyl groups are not equivalent because of the nearby chiral centre. The specific rotation of product **(167)** was $[\alpha]_D^{22} = -49$ (c 1.5, $CHCl_3$) and that of compound **(168)** was $[\alpha]_D^{22} = -44$ (c 0.81, $CHCl_3$).

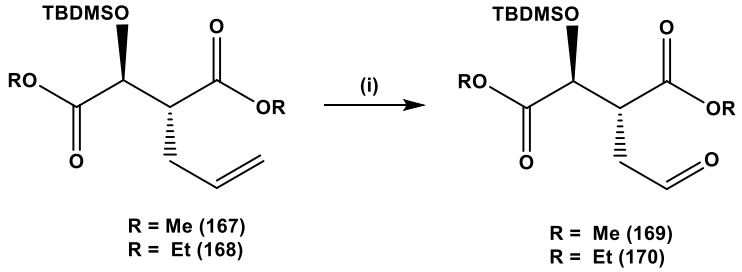


Scheme (28): Protection of the secondary alcohol, reagents and conditions: (i) imidazole, TBDMSCl, DMF, 45 ºC, 86%.

2.2.6 Oxidation of dialkyl (2*R*, 3*S*)-2-allyl-3-((*tert*-butyldimethylsilyl) oxy)-succinate to aldehyde

The olefins **(167)** and **(168)** were oxidised to the aldehydes **(169)** and **(170)** with ozone and oxygen in dichloromethane at –78 °C for 30 minutes, then the mixture was allowed to reach room temperature and triphenylphosphine was added. The aldehydes **(169)** and **(170)** were obtained in yields of 93% and 84% respectively **Scheme (29)**. Their formation was confirmed by their ^{1}H and ^{13}C NMR spectra, which showed signals corresponding to the

aldehyde proton, which appeared as a singlet at δ 9.81 and its carbon resonated at δ 199.4 for compound **(169)** and at δ 9.81 and δ 199.8 for compound **(170)**. The specific rotation of aldehydes **(169)** and **(170)** were $[\alpha]_D^{23} = -38$ (c 0.72, $CHCl_3$) and -41 (c 2.2, $CHCl_3$) respectively.



Scheme (29): Oxidation of the olefin to the aldehyde, reagents and conditions (i): O_3/O_2, DCM, – 78 ºC, PPh_3.

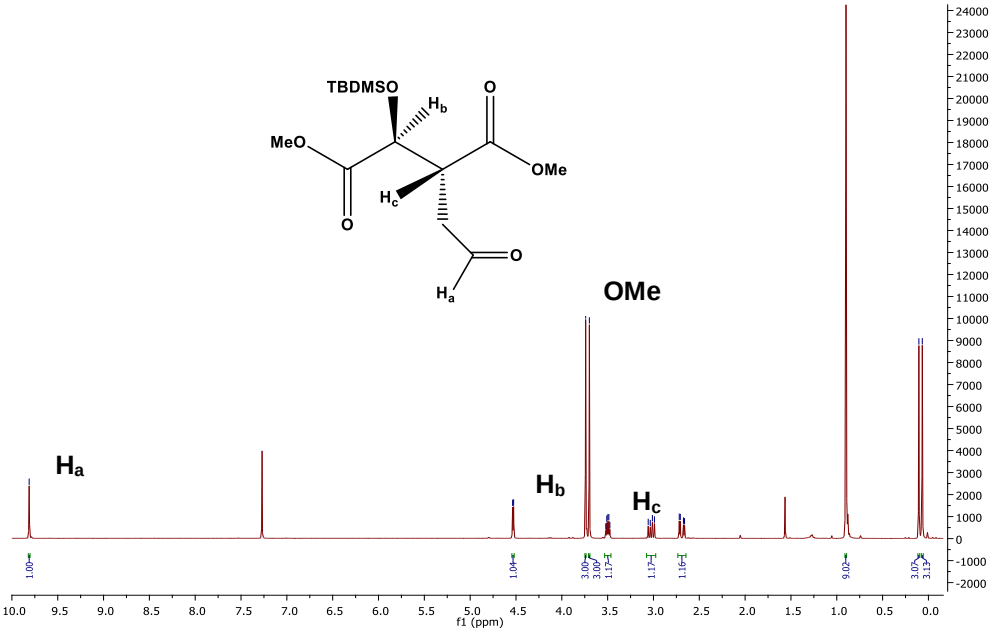


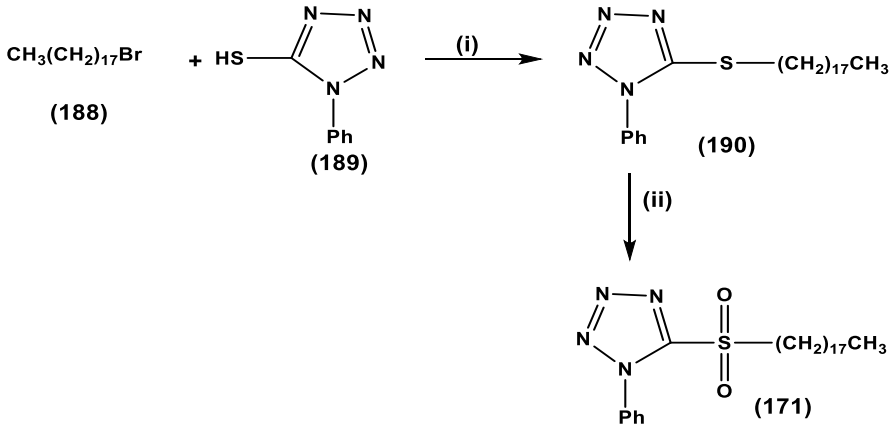
Figure (36): ^{1}H NMR spectrum of dimethyl (*2S,3R*)-2-((*tert*-butyldimethylsilyl)oxy)-3-(2oxoethyl)succinate **(169)**.

2.2.7 Preparation of sulfone C18

2.2.7.1 Preparation of 5-(octadecylsulfonyl)-1-phenyl-1*H*-tetrazole (171)

In order to extend the carbon chain at the α-position, the aldehyde prepared in **Scheme (30)** needed to be coupled with sulfone reagents of appropriate carbon chain lengths; For this purpose the modified Julia reaction was employed, which will be discussed in more detail in section **2.2.8**. The first sulfone prepared was the C-18 chain 5-(octadecylsulfonyl)-1-phenyl-1*H*-tetrazole **(171)**.

In order to prepare this, 5-(octadecylthio)-1-phenyl-1*H*-tetrazole **(190)** firstly needed to be synthesised, and this was achieved by reacting 1-phenyl-1*H*-tetrazole-5-thiol **(189)** with 1-bromooctadecane **(188)** using K_2CO_3 and acetone in a yield of 89%.



Scheme (30): Preparation of the sulfone **(171)**, reagents and conditions: (i) K_2CO_3, 1-phenyl-1*H*-tetrazole-5-thiol in acetone and THF, reflux 3 hours, 89%; (ii) H_2O_2, $(NH_4)_6Mo_7O_{24}.4H_2O$, THF, IMS, 97%.

The oxidation of the sulfide with hydrogen peroxide in the presence of ammonium molybdate (VI) tetrahydrate in THF and IMS gave the target sulfone **(171) Scheme (30)**.[212] The ^{1}H NMR spectrum of the sulfone **(171)** included a multiplet at δ 7.77 – 7.57 for the five aromatic protons, and a complex triplet at δ 3.79 – 3.69 (*J* 7.9 Hz) for the two protons adjacent to the sulfone **Figure (37**), which confirmed the success of the reaction.

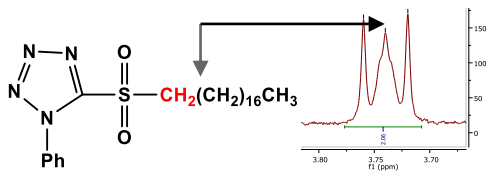


Figure (37): Signal in the ^{1}H NMR spectrum of the sulfone **(171)** corresponding to the CH_2 next to the sulfonyl group.

This signal displayed the typical pattern for an AA'BB' system, where the two substituents on the C–C bond mean that A and A' and B and B', respectively, are not magnetically equivalent **Figure (38**).

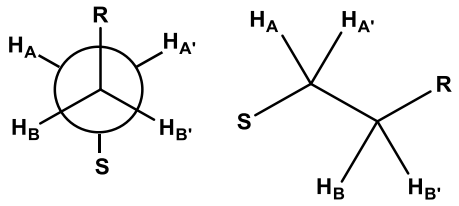
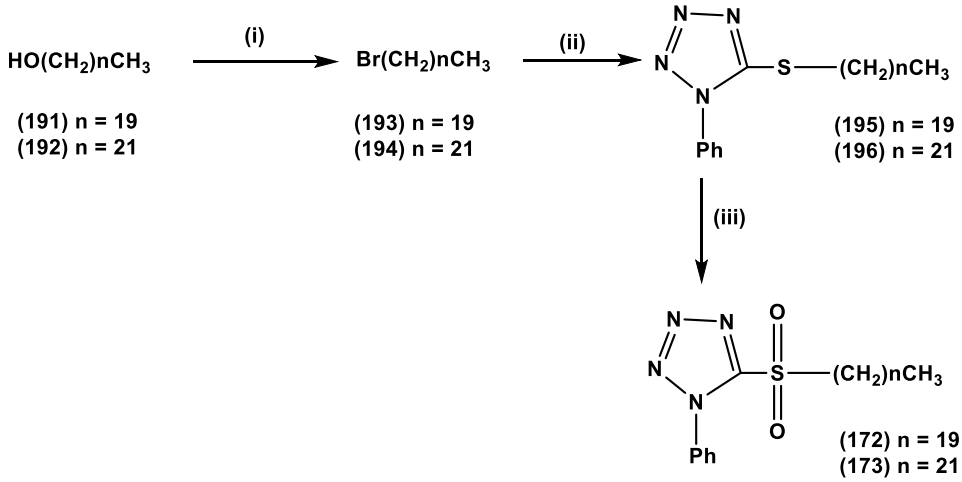


Figure (38): The protons ($H_{A\,A'}$ and $H_{\,BB'}$) adjacent to a sulfonyl group.

The ^{13}C NMR showed a signal at δ 56.0 for the (-CH_2SO_2-) group this again confirming that the sulfone had been formed. This system was seen throughout this work and was a good indication to confirm the success of the reaction.

2.2.7.2 Preparation of sulfone (C-20 and C-22) for the chain extension

Two other sulfones with a C-20 **(172)** and C-22 **(173)** chain were also prepared. Alcohol **(191** and **192)** were brominated using *N*-bromosuccinimide and triphenylphosphine in dichloromethane followed by reaction with 1-phenyl-1*H*-tetrazole-5-thiol to give the sulfide compounds **(195)** and **(196)**. The subsequent oxidation of these sulfides with *meta*-chloroperbenzoic acid was achieved by using dichloromethane and $NaHCO_3$ at 0 ^oC, and this gave the sulfones **(172)** and **(173)** in yields of 96%, **Scheme (31**).



Scheme (31): Preparation of sulfones (C-20 and C-22), reagents and conditions: (i) *N*-bromosuccinimide, Ph_3P, DCM, (ii**)** 1-phenyl-1*H*-tetrazole-5-thiol, K_2CO_3, acetone and THF, (iii) m-CPBA, $NaHCO_3$, DCM.

The ^{1}H NMR spectrum of compound **(195)** showed a multiplet at δ 7.65 – 7.48 for the five aromatic protons, a triplet at δ 3.40 (*J* 7.4 Hz) for the (-CH_2S-) and a triplet at δ 0.89 (*J* 6.8 Hz) for the terminal methyl group. The ^{13}C NMR spectrum showed a signal at δ 154.5 for the tetrazole ring carbon, four signals for the aromatic carbons at δ 133.8 – 123.9 and signals at δ 33.4 for the long chain carbon bonded to the sulfur atom (-CH_2S-) and at δ 14.1 for the terminal methyl group. The ^{1}H NMR and ^{13}C NMR spectra of compound **(196)** were similar to those of compound **(195)**, mentioned above.

The ^{1}H NMR spectrum of the sulfone (**172**) showed a multiplet at δ 7.73 – 7.57 for the five aromatic protons and a distorted triplet at δ 3.74 (*J* 7.9 Hz) for the two protons adjacent to the sulfonyl group. Again, the data for sulfone **(173)** was similar to that of **(172)**. For the oxidation of sulfides **(195)** and **(196)** to sulfones **(172)** and **(173)** m-CPBA, DCM and $NaHCO_3$ were used, rather than using the same conditions as were used for the oxidation of **(190)** to **(171)** (see **Scheme 30**). Both sets of reagents can be used for the oxidation, with those chosen depending on the solubility of the sulfide compound.

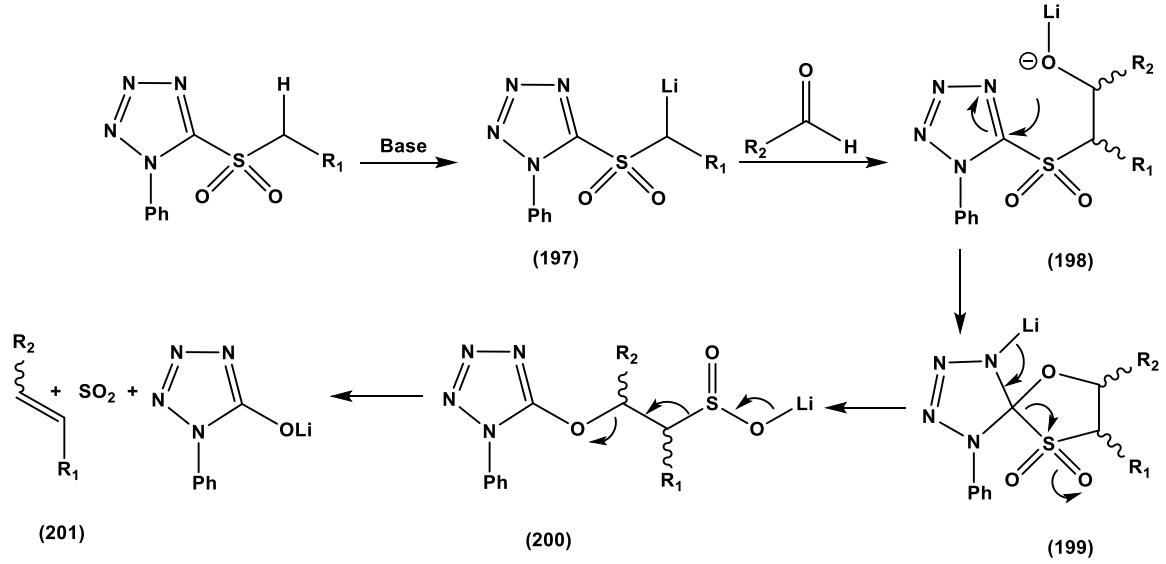
2.2.8 Extension of the chain

2.2.8.1 An overview of the Julia-Kocienski olefination reaction

As mentioned earlier chain extension at the α-position can be carried out using a modified Julia reaction to couple the aldehyde prepared in section **2.2.6** with sulfone reagents of appropriate carbon chain lengths. This reaction was first described by Marc Julia, and work carried out by Kocienski *et al.*[257,258] to modify this method led to it becoming an important C – C bond forming reaction in organic synthesis.[259] For this reason it became known as the Julia-Kocienski reaction. The reaction was then further modified by Sylvestre Julia,[260] who replaced the use of phenylsulfones with a new kind of heteroarylsulfones, and since then it has been named the modified Julia-Kocienski olefination reaction. This type of chain extension is used frequently throughout this study.

It involves the reaction of metallated sulfone **(197)** with an aldehyde to form a β-alkoxysulfone intermediate **(198)**. Due to the instability of the newly formed intermediate, it undergoes a Smiles rearrangement via the intermediate **(199)** with the transfer of the negative charge from

sulfur to oxygen. Finally, elimination of sulfur dioxide and lithium 1-phenyl-1*H*-tetrazolone from **(200)** gives a mixture of the two alkene isomers **(201) (Scheme 32**).



Scheme (32): Mechanism of the modified Julia-Kocienski reaction.

Four heterocyclic activators have been identified for the modified Julia olefination, which provide useful levels of stereoselectivity in certain scenarios: benzothiazol-2-yl (**BT**), pyridin-2-yl (**PYR**), 1-phenyl-1*H*-tetrazol-5-yl (**PT**) and 1-*tert*-butyl-1*H*-tetrazol-5-yl (**TBT**) **(Figure 39)**.

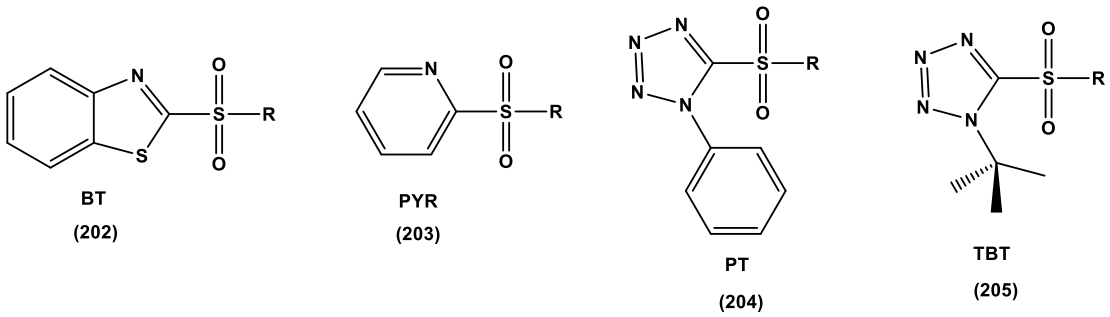
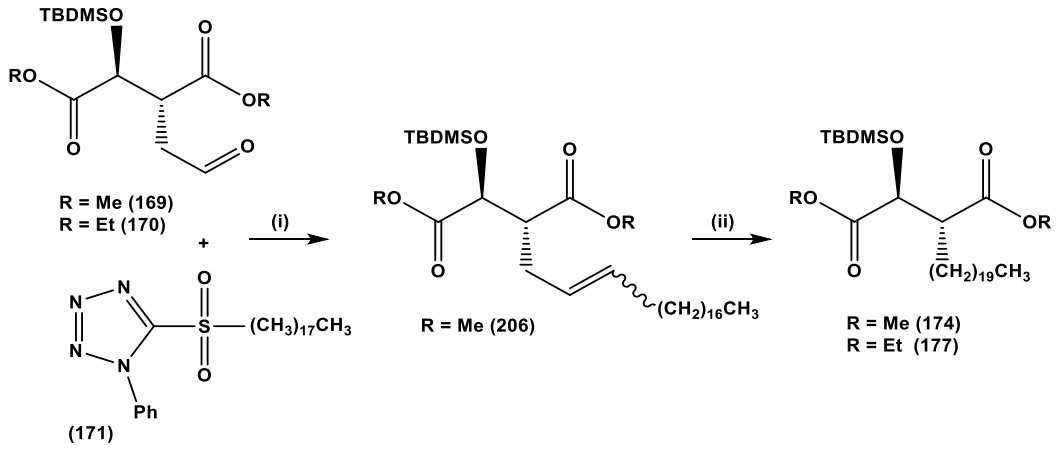


Figure (39): Four heterocyclic sulfones for the modified Julia olefination.

1-Phenyl-1*H*-tetrazol-5-yl sulfonyl **(204)** was chosen in this work, because it was easy to prepare from commercially available materials and it has not shown any problems with self-condensation.[261,262] Julia coupling reactions are also the best method for the preparation of the long chain alkenes in the work described here. Furthermore, the by-product of the Julia coupling reaction is easily removed compared to the by-products in olefinations such as the Wittig reaction; this produces triphenyl phosphonium oxide as a by-product, which is difficult to remove.

2.2.8.2 The chain extension of diesters (2*S*,3*R*)-2-((*tert*-butyldimethylsilyl)oxy)-3-(2-oxoethyl)succinate compounds (169 and 170)

Having the sulfone units **(171)**, **(172)** and **(173)** in hand, they could now be coupled to the previously prepared aldehydes (**169** and **170**) in a modified Julia olefination reaction. The reaction with the C-18 sulfone **(171)** was firstly carried out and the reaction was started by addition of a non-nucleophilic strong base, *i.e.* lithium bis(trimethylsilyl) amide at – 20 ºC. Subsequently, the mixture was allowed to reach room temperature and stirred for 3 hours to complete the reaction. The reaction appeared to be very straightforward, giving the desired alkenes in 76% and 77% yields respectively. The products were a mixture of *E*- and *Z*-stereoisomers in a 2:1 ratio **Scheme (33)**; The ^{1}H NMR spectrum of the product **(206)** showed the protons of the C–C double bond at δ 5.53 – 5.39 and δ 5.36 – 5.24, while the signal for the aldehyde proton had disappeared. The ^{13}C NMR spectrum also confirmed the success of the reaction due to the signals at δ 129.9 and 128.6 corresponding to the alkene carbons.



Scheme (33): Chain extension of the aldehydes (**169** and **170**) by a Julia-Kocienski reaction, reagents and conditions: (i) $LiN(SiMe_3)_2$, dryTHF, – 20 ˚C, (ii) H_2, Pd on carbon, THF and IMS.

The alkenes were then saturated by hydrogenation using palladium (10%) on carbon as a catalyst and hydrogen gas in IMS and THF (1:1) as shown in **Scheme (33)**. The hydrogenation sometimes proceeded in a straightforward way, however sometimes it took a long time and in some cases, it was even necessary to repeat the hydrogenation. The products **(174)** and **(177)** gave NMR spectra similar to the olefin compounds but without the signals for the alkene protons, which proved that the hydrogenation had been completed. The ^{1}H NMR spectrum for compound **(174)** showed a doublet at δ 4.34 (*J* 7.2

Hz) corresponding to the proton at the β-position, and two singlets at δ 3.73 and δ 3.67 for the two (OCH_3) groups in the compound. The proton at the α-position appeared as a double double doublet at δ 2.83 (*J* 4.6, 7.2, 10.2 Hz), while the triplet at δ 0.88 (*J* 6.4 Hz) belonged to the terminal methyl group. The ^{13}C NMR spectrum also confirmed the success of the reaction due to the disappearance of the signal corresponding to the C=C at δ 129.9 and 128.6 **Figure (40)**.

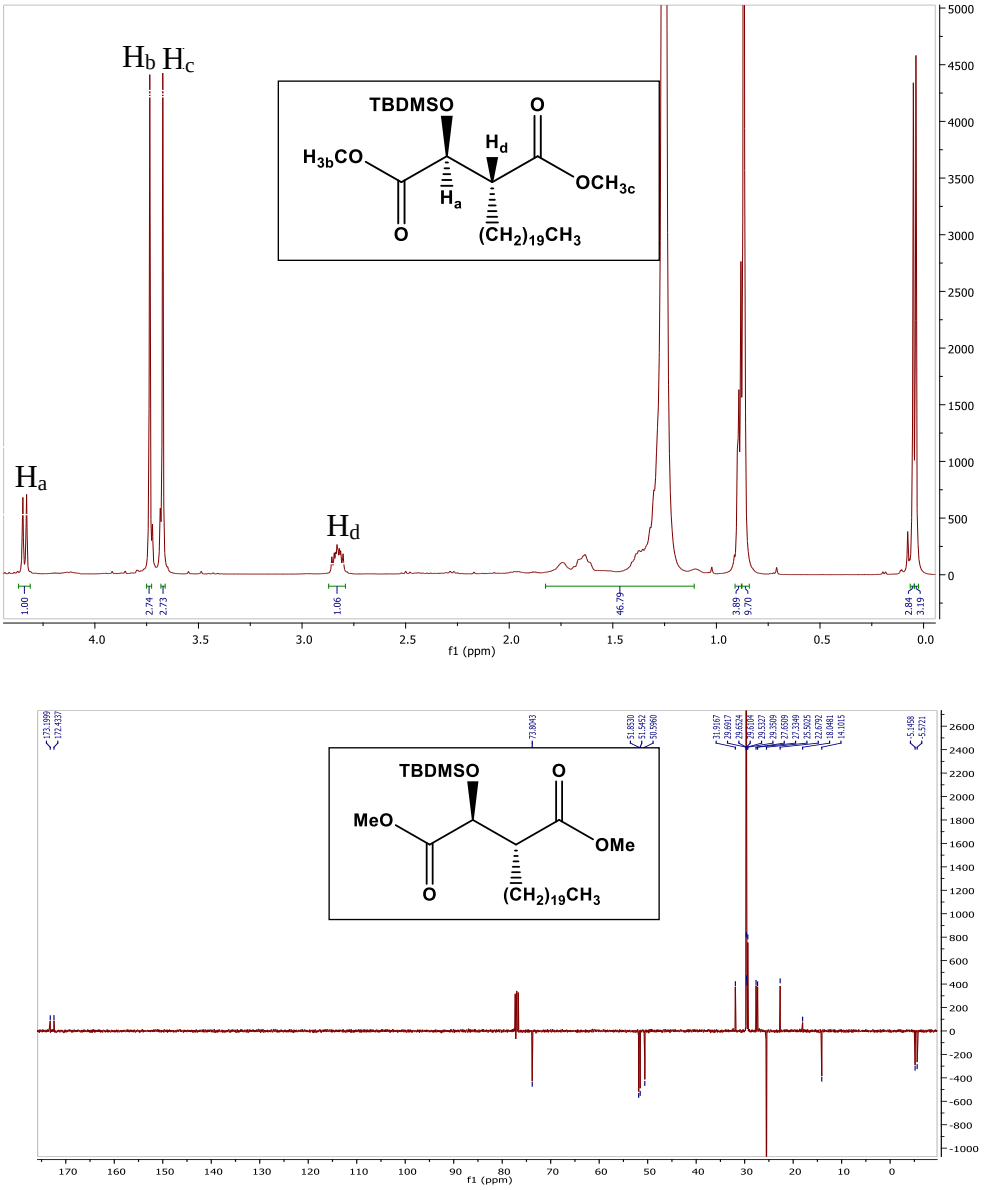
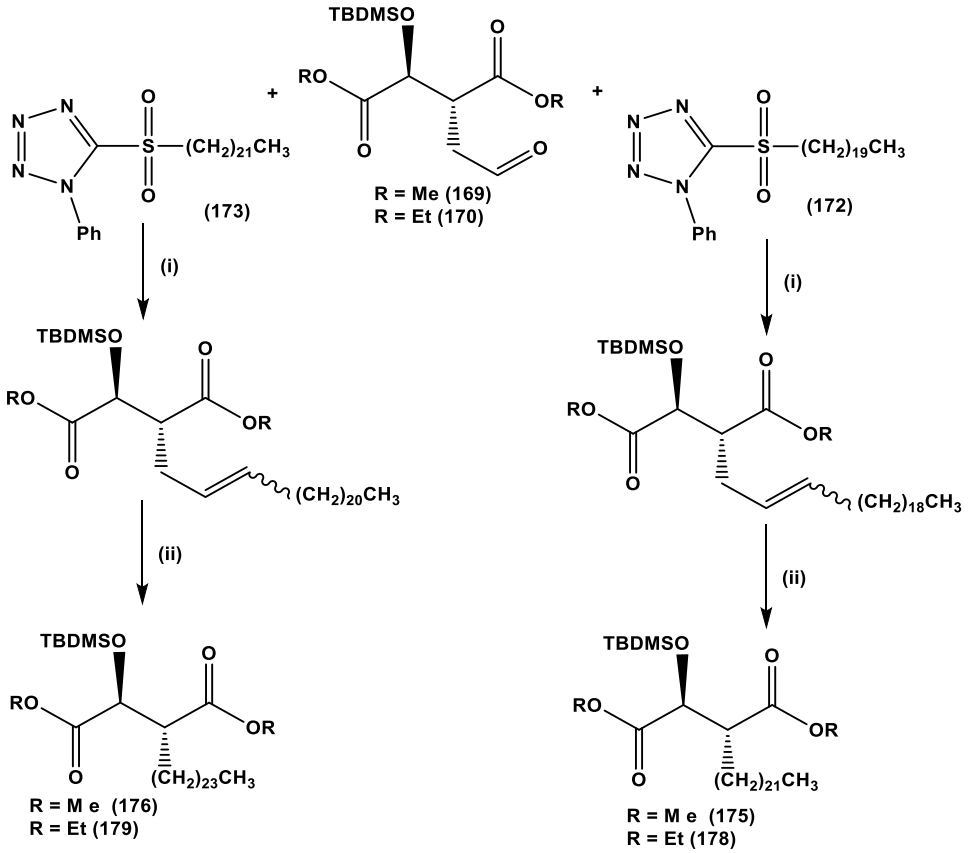


Figure (40): Selected signals in the ^{1}H and ^{13}C NMR spectrum of **(174)**.

Similar observations were also made in the NMR spectra of compound **(177)**. The specific rotation of compound **(174)** was $[\alpha]_D^{22} = -27$ (c 0.69, $CHCl_3$), while that of compound **(177)** was $[\alpha]_D^{22} = -24$ (c 0.91, $CHCl_3$).

This modified Julia-Kocienski olefination was also applied for the coupling of aldehydes **(169** and **170)** with the **C-20** and **C-22** sulfones, with the NMR spectra of the products similar to those described for compound **(174)**, thus again confirming the success of the reaction **(Scheme 34)**.



Scheme (34): Julia-Kocienski copling reaction to form **(175**, **178)** and **(176, 179)**, reagents and conditions: (i) $LiN(SiMe_3)_2$, dryTHF, – 20 ˚C; (ii) H_2, Pd on carbon, THFand IMS.

2.2.9 Selective reduction of the diesters

The key step for the preparation of the mycolic motif unit from the diester compounds, is the selective reduction of the ester (**1**) **(Figure 41**). The selective reduction of such systems without the α-chain has been reported in the literature.[263,264] Moreover the selective reduction of the di-ethyl ester (**179**) has been investigated by M. Sahb.[254] In this earlier work a range of conditions were used to reduce (179) and it was observed that at -78 °C no reaction occurred, while between -78 °C and -55 °C some of the required aldehyde was formed but there was also unreacted starting material. Using various equivalents of the reagents diisobutylaluminium hydride (DIBAL-H) and magnesium bromide diethyl etherate ($MgBr_2.Et_2O$) at a higher temperature of -55°C to 0°C gave different combinations of products, with 1.5 equivalents of $MgBr_2.Et_2O$ and 2.2 equivalents of DIBAL-H giving the best result (no starting material and 33 % of the required aldehyde, however 45% of the alcohol was also obtained). Because the presence of an ethyl ester caused problems later in the synthesis of complete mycolic acids, and to try to improve the yield, similar conditions to those described by Sahb were therefore attempted here, a number of times, for the selective reduction of diester **(176)**. However, at -55 °C no reaction occurred. This is likely to be due to the poor solubility of the reagents at this temperature; the formation of a solid was observed when the reagents were added. A range of alternative reaction conditions were therefore investigated **(see Table 3)**, with the best yield and selectivity being achieved when using DIBAL–H (1.5 equivalents) and $MgBr_2.Et_2O$ (1.7 equivalents) between -38 and -34 °C. This gave an improved yield (78%), compared to previous studies, of the desired aldehyde **(182)**, with only 10% of the alcohol **(182a)** (entry 4), **(see Table 3)**.

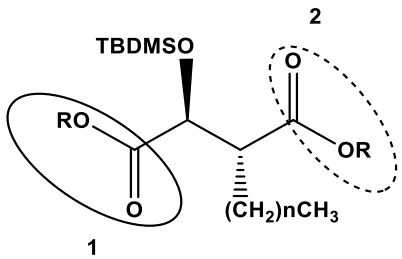
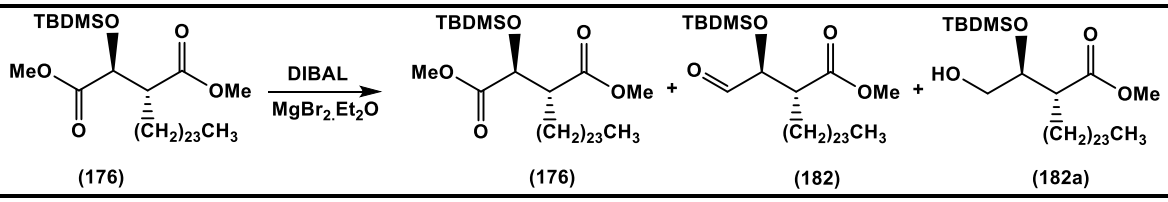


Figure (41): Selective reduction of the diester.

Table (3): Conditions used for the selective reduction of diester compound **(176)**, with entry 4 giving the best result.

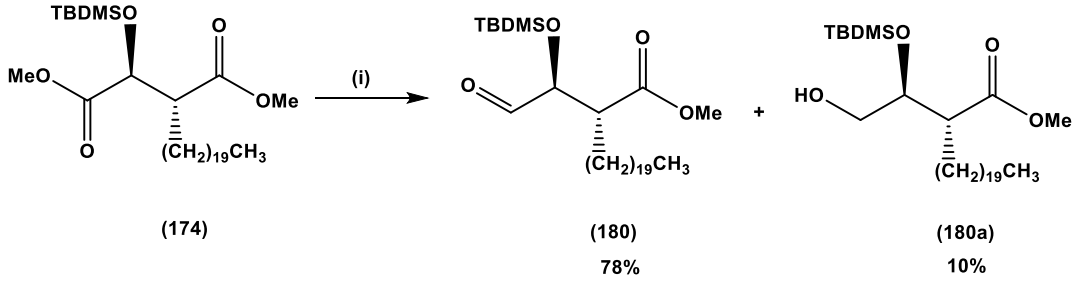


Entry	Temperature	$MgBr_2.Et_2O$ eq.	DIBAL eq.	Product	Yield %
1	– 55 °C	1.5	1.2	176	No reaction
2	– 45 to – 40 °C	1.5	1.3	176 and 182	73, 15
3	– 40 to – 33 °C	1.5	1.5	176, 182, 182a	62, 20, 6
4	**– 38 to – 34 °C**	**1.5**	**1.7**	**182, 182a**	**78, 10**
5	– 36 to – 30 °C	1.5	2.5	182a	70

The above conditions were also found to work well with the ethyl ester compounds.

2.2.9.1 Selective reduction of diesters (2*S*,3*R*)-2-((*tert*-butyldimethylsilyl)oxy)-3-tetracosylsuccinate

Having identified the optimal conditions for the selective reduction, the reduction of compound **(174)** with a shorter α-chain, could be carried out. A solution of magnesium bromide diethyl etherate (1.5 eq) and compound **(174)** in dichloromethene was stirred at room temperature for *ca*.one hour, and then the mixture was cooled to – 38 °C. Diisobutylaluminum hydride (1.7 eq) was added dropwise over 5 minutes, and the mixture was stirred for 1 hour at – 38 to – 34 °C before being quenched with methanol and saturated aqueous sodium sulfate. The crude product was purified by column chromatography to give **(180)** and **(180a) (Scheme 35)**.



Scheme (35): Selective reduction of diesters **(174)**, reagents and conditions: (i) DIBAL (1.7 eq), $MgBr_2.Et_2O$ (1.5eq), dry DCM, – 38 °C.

The structure of product **(180)** was verified by the ^{1}H NMR spectrum, which gave a doublet at δ 9.63 (*J* 1.9 Hz) corresponding to the aldehyde proton, a doublet of doublets at δ 4.07 (*J* 1.9, 5.6 Hz) for the proton at the β position, a doublet of triplets at δ 2.80 (*J* 5.7, 8.8) for the proton at the α-position and a singlet at δ 3.71 for the one ester group. The ^{13}C NMR spectrum gave a signal at δ 203.1 ppm for the aldehyde group **(Figure 42)**.

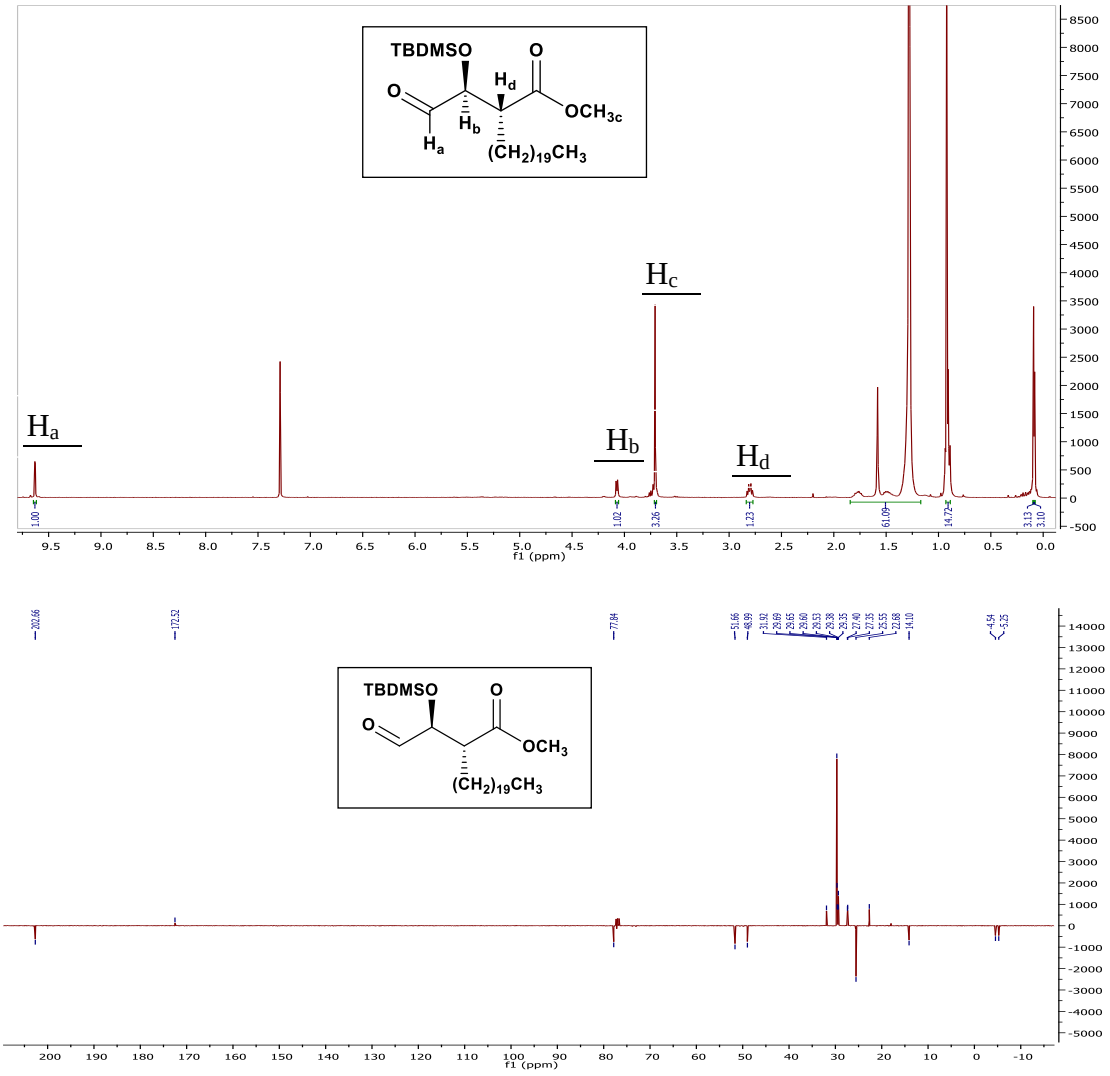
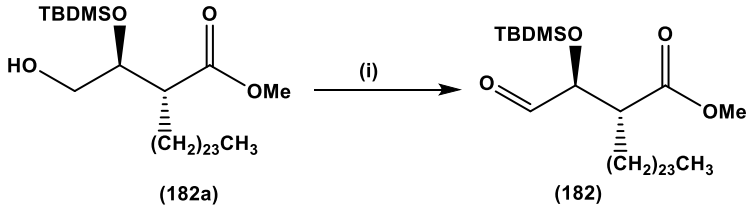


Figure (42): The ^{1}H and ^{13}C NMR spectra for compound **(180)**.

The second fraction that was obtained with aldehyde **(180)** was the alcohol **(180a)**. The ^{1}H NMR spectrum of **(180a)** showed a doublet of triplets at δ 3.91 (*J* 3.6, 7.4 Hz) for the proton at the β-position, a br. doublet at δ 3.60 (*J* 3.6 Hz) for the CH_2 adjacent to the primary alcohol and a multiplet at δ 2.76 – 2.68 for the α-proton. The ^{13}C NMR spectrum

showed signals at δ 173.9 for the carbonyl carbon of the ester, at δ 49.1 for the carbon at the α-position, and at δ 73.8 for the carbon at the β-position.

The alcohols could easily be oxidised to the required aldehydes using PCC in dichloromethane, as shown in **Scheme (36)** below for the oxidation of the longer chain alcohol **(182a)** to the aldehyde **(182)**.



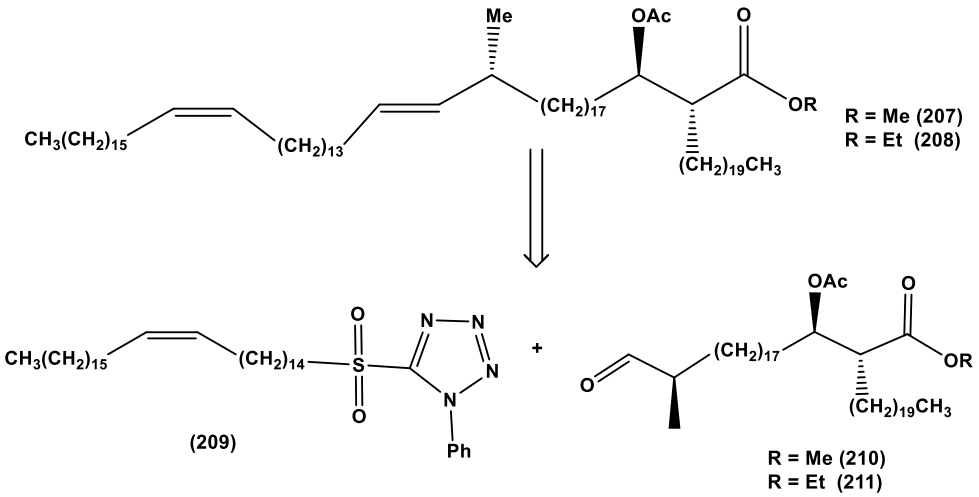
Scheme (36): Oxidation of alcohol **(182a)** to aldehyde **(182)**, reagents and conditions: (i) PCC, DCM, 87%.

2.2.10 Summary

Following the methods described above the mycolic motif was successfully prepared in an overall yield of 27% in only 6 steps. This is a significant improvement compared to previous methods starting from *L*-aspartic acid (14 steps, 10% yield)[203] and *L*-malic acid (10% yield over 10 steps).[253] Moreover, the method described here eliminates the use of HMPA. As the mycolic motif is a common unit in all MAs this method can be incorporated into the synthesis of all MAs and will reduce the cost and amount of time required in the full MA synthesis.

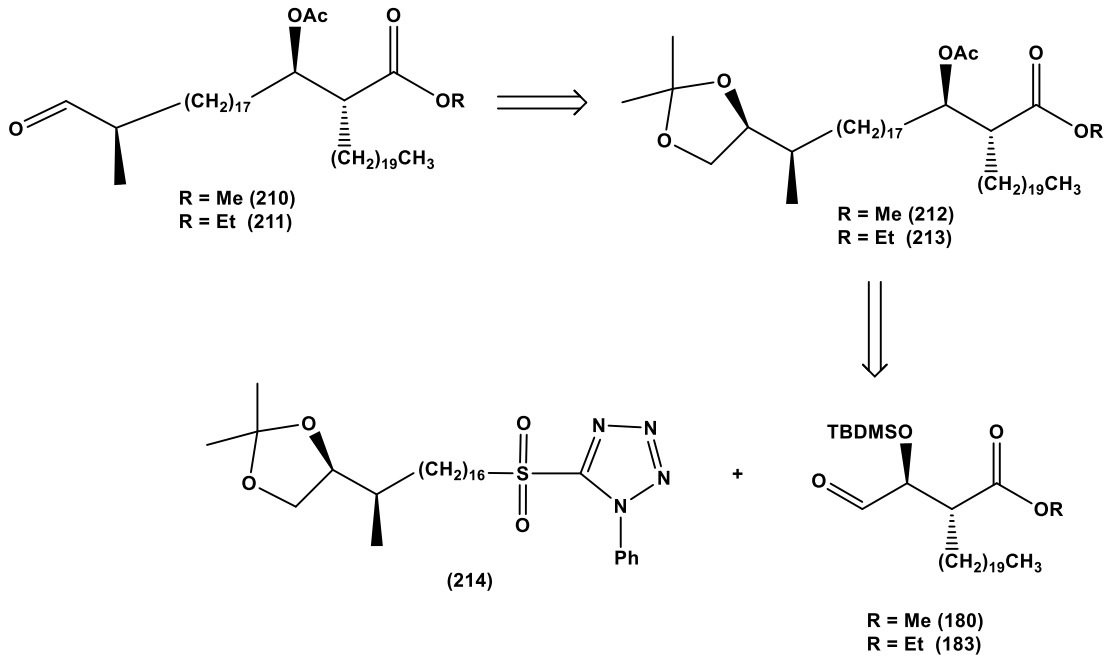
2.3 Synthesis of a *cis*-alkene-α-methyl-*trans*-alkene mycolic acid

Mycolic acid **(155)** contains double bonds at both the distal and proximal positions: a *cis* double bond at the distal position and an α-methyl *trans* alkene at the proximal position. This type of mycolic acid has been isolated from the environmental Mycobacterium, *M. brumae* (see page 9). By preparing a single synthetic enantiomer of this type of mycolic acid it can be compared to the natural material and also, along with it's related sugar esters, could be used in biological assays for the development of new methods to detect infection by this Mycobacterium and to distinguish it from other mycobacterial infections. *M. brumae* has also been shown to have potential to inhibit bladder cancer cell growth,[87] therefore synthesis of mycolic acids present in this Mycobacterium could also lead to further developments in this area. The synthetic route to prepare **(155)** will be discussed in detail in this section and the retrosynthesis is summarised below **(Schemes 37**–**40)**. The protected mycolic acids **(207** and **208)** can be broken down into 2 main fragments, that containing the distal position of the meromycolate moiety **(209)**, and the intermediate **(210)** and **(211) (Scheme 37)**.



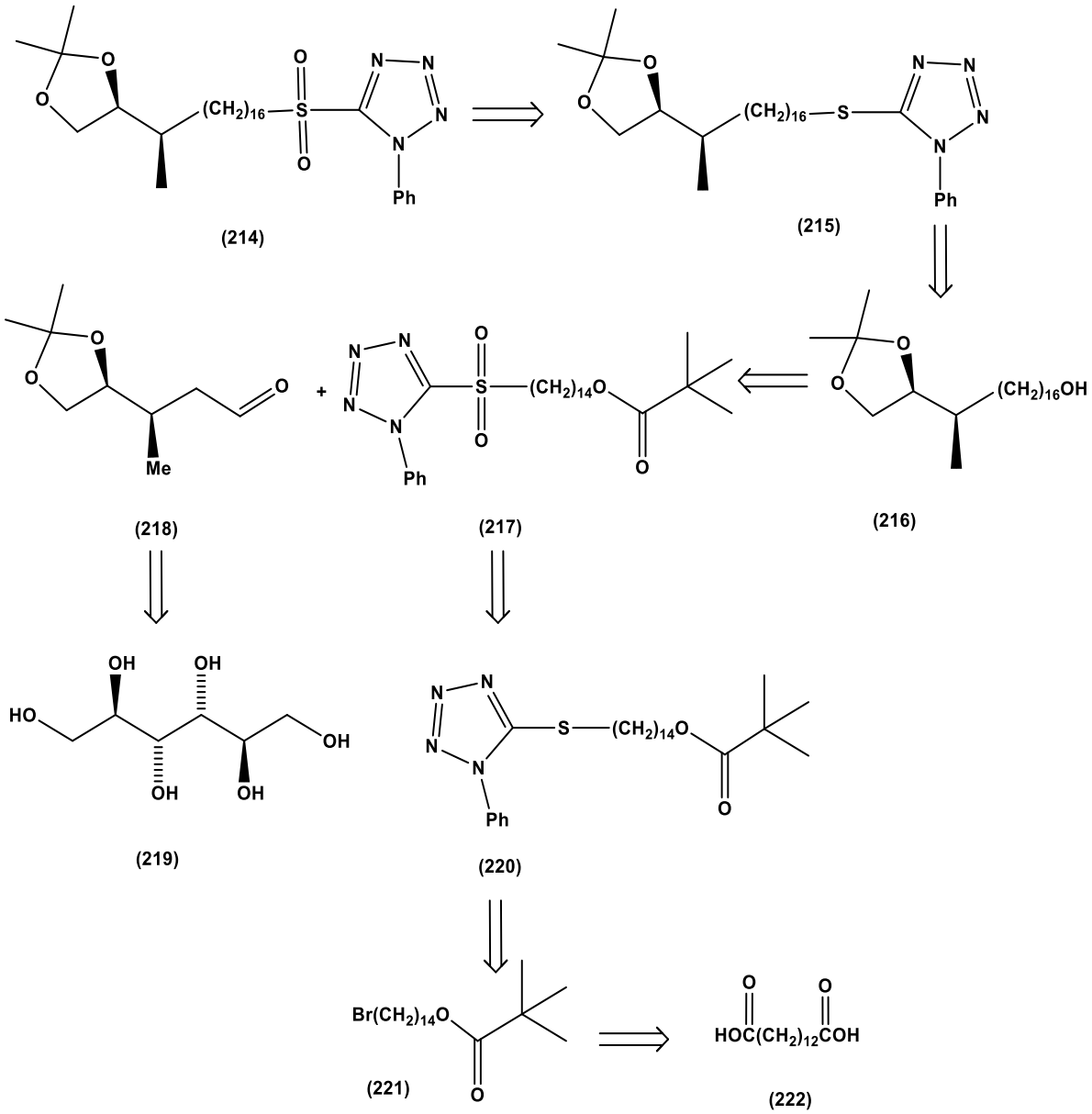
Scheme (37): The fragment containing the distal position of the meromycolate moiety (**209**) and the intermediates **(210** and **211)**.

The intermediates **(210 and 211)** can then be broken down into the mycolic motif **(180)** and **(183)**, the synthesis of which has already been described, and the proximal position **(212)** and **(213) (Scheme 38)**.



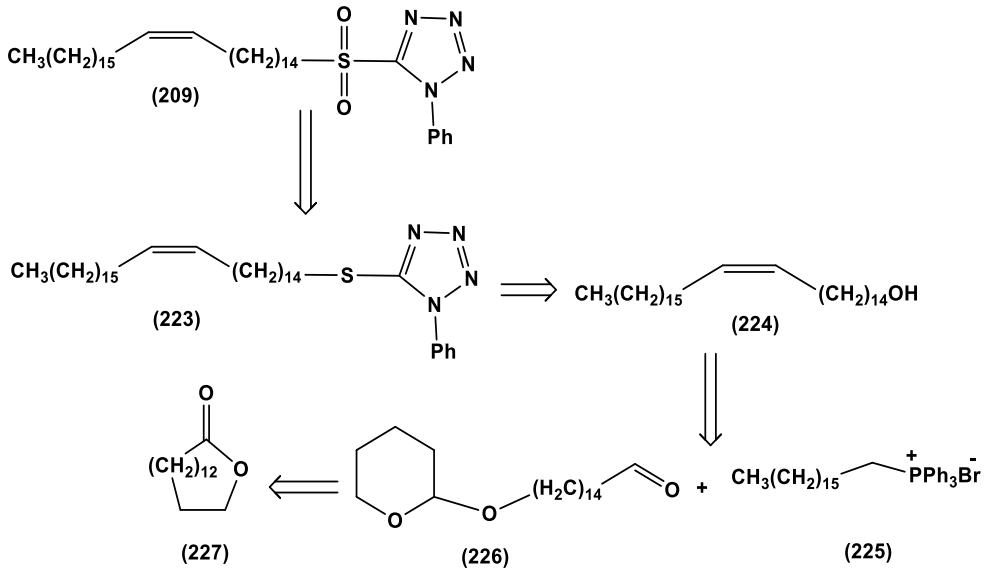
Scheme (38): Retrosynthesis of the fragment that will form the proximal position **(210 and 211)**.

The proximal position moiety could be synthesised from aldehyde **(218)** and sulfone **(217)**, which could themselves be obtained from and *D*-mannitol **(219)** and tetradecanedioic acid **(222)**, respectively **(Scheme 39)**.



Scheme (39): Retrosynthesis of the fragment **(214)**.

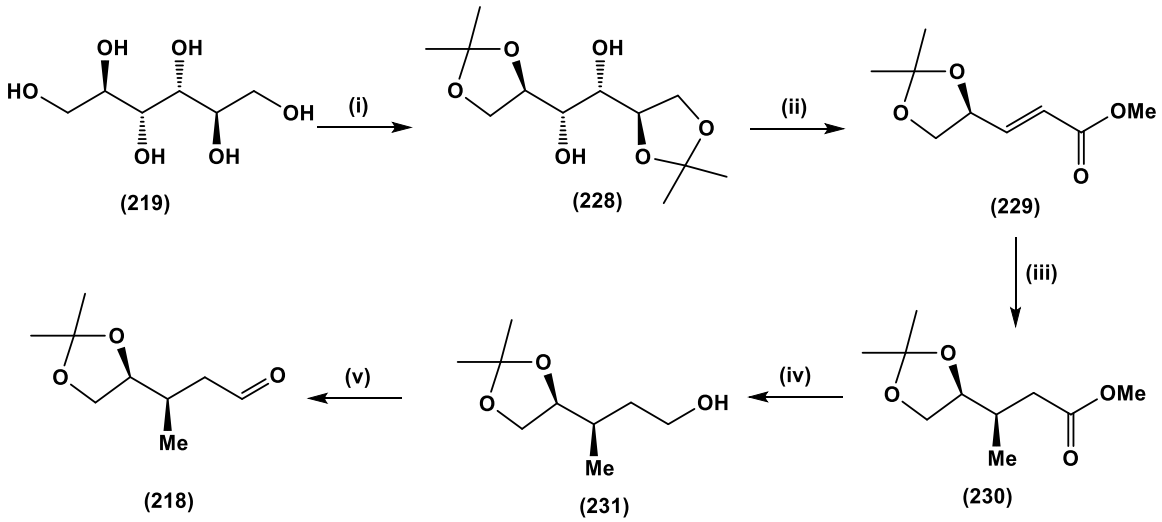
The *Z*- alkene unit at the distal position of the meromycolate chain **(209)** could be obtained from the commercially available cyclic lactone **(227) (Scheme 40)**.



Scheme (40): Retrosynthesis of the *Z*- alkene at the distal position.

2.3.1 Preparation of the aldehyde (218)

D-Mannitol (**219**) was protected using acetone in the presence of anhydrous zinc chloride to give the diol 1,2:5,6-di-*O*-isopropylidene-*D*-mannitol **(228)** in 86% yield.[265] Oxidative cleavage of diol **(228)** with sodium metaperiodate in aqueous sodium hydrogen carbonate gave the intermediate glyceraldehyde acetonide,[266] which was treated in *situ* with methyl diisopropyl-phosphinylacetate, and aqueous potassium carbonate to give the α,β-unsaturated ester (**229**), in 70% yield via a Horner-Wadsworth-Emmons reaction,[267,268] **(Scheme 41)**. The ^{1}H NMR spectrum of **(229)** showed the expected olefin signals as two double doublets at δ 6.9 (*J* 5.7 and 15.8 Hz, vicinal and *trans* coupling constant) and 6.11 (*J* 1.6 and 15.8 Hz, allylic and *trans* coupling constant), respectively. The methyl ester appeared as a singlet at δ 3.75. The ^{13}C NMR showed a signal at δ 110.2 for the acetal carbon.



Scheme (41): Reagents and conditions: (i) $ZnCl_2$, acetone 86% (ii) $NaIO_4$, $NaHCO_3$, H_2O, methyl diisopropyl-phosphinyl acetate, K_2CO_3 70%; (iii) MeLi, dry ether, 77%; (iv) $LiAlH_4$, THF, 87%; (v) PCC, CH_2Cl_2, 87%.

The diastereoselective conjugate addition of a methyl nucleophile to the α, β-unsaturated ester **(229)** was conducted as reported by Leonard and co-workers.[269] Thus, **(229)** was exposed to MeLi in diethyl ether at – 78 ^oC to give *syn*-adduct, **(230)** as a single diastereomer in 77% yield. As before, it is important to keep the temperature below – 78 ^oC. The added methyl group appeared in the ^1H NMR spectrum as a doublet at δ 0.98 (*J* 6.7 Hz). This, along with the disappearance of the alkene signals confirmed the formation of the product. The ^{13}C NMR spectrum showed signals at δ 173.0 for the carbonyl carbon and at δ 108.9 for the cyclic acetal carbon.[269] Again, there were no signals corresponding to the alkene group.

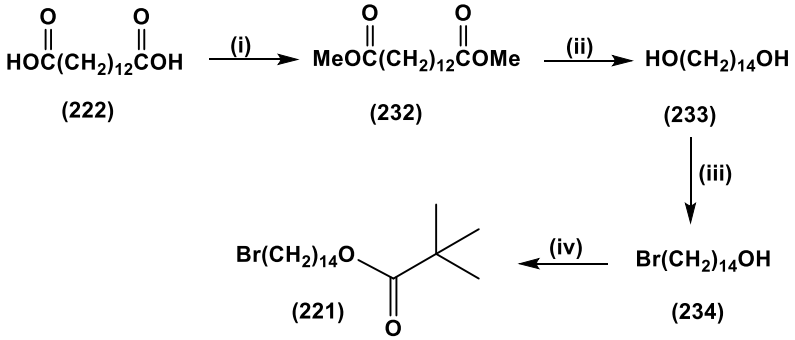
The ester **(230)** was reduced to the corresponding primary alcohol **(231)** with lithium aluminium hydride in dry THF, in good yield 87%. The IR spectrum showed a broad stretch at ν_{max} 3427 cm^{-1} for the O–H group.

Finally, the alcohol **(231)** was oxidised to the desired aldehyde **(218)** with pyridinium chlorochromate (PCC) in dichloromethane. The ^1H NMR spectrum showed a broad. triplet at δ 9.77 (*J* 1.8 Hz) for the aldehyde proton and in the ^{13}C NMR a signal appeared at δ 201.6 for the carbonyl carbon.

2.3.2 Preparation of the intermediate C-14 sulfone

To extend the carbon chain aldehyde **(218)** could be coupled with a sulfone of required length via a Julia reaction. For MA **(155)** a C-14 sulfone **(217)** therefore needed to be prepared. The Julia reaction was used to extend the chain rather than a Wittig coupling, because preparation of a long chain Wittig salt is difficult and also the yield is lower than that in the Julia reaction.

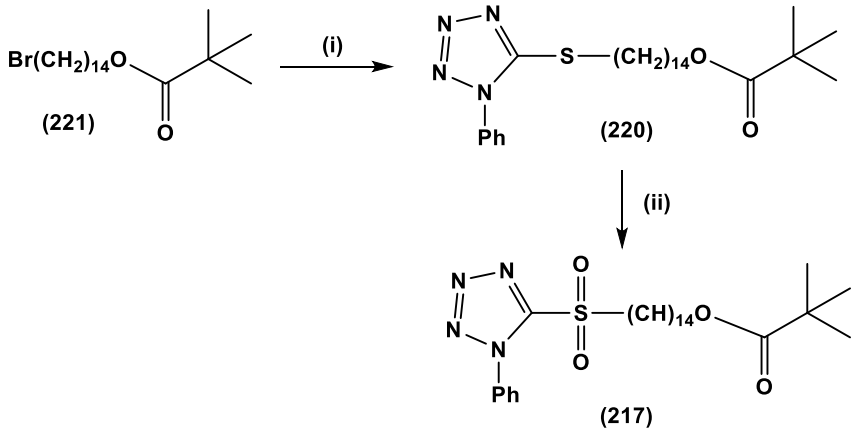
Tetradecanedioic acid **(222)** was converted into diester **(232)**, using conc. H_2SO_4 and methanol in a very good yield 98%. Reduction of the diester with $LiAlH_4$ in THF was then carried out to give the diol **(233)**. The ^1H NMR spectrum confirmed the formation of the product due to the disappearance of the signals corresponding to the ester groups and the appearance of a triplet at δ 3.65 (*J* 6.6 Hz) corresponding to the (2×C$\underline{H}_2$OH) integrating to four protons. The next step was monobromination of the diol **(233)** to 14-bromotetradecan-1-ol **(234)** with 48 % HBr in toluene under reflux. Again, the structure was proved by the ^1H NMR spectrum which showed a triplet at δ 3.64 (*J* 6.6 Hz) corresponding to the (-C$\underline{H}_2$OH) integrating to two protons and a triplet at δ 3.41 (*J* 6.9 Hz) corresponding to the (-C$\underline{H}_2$Br). The protection of the hydroxy group was then carried out using trimethyl acetyl chloride; this protection is very common and the deprotection is easy with a strong base such as KOH, or $LiAlH_4$. Understanding the ^1H and ^{13}C NMR spectra is also easy compared to it the THP protecting groups used. Consequently, compound **(221)** was obtained in 97% yield by protecting mono alcohol **(234)** with trimethyl acetyl chloride in the presence of triethylamine and catalytic 4-dimethylaminopyridine (DMAP) **(Scheme 42**).



Scheme (42): Preparation of compound **(221)**. Reagents and conditions: (i) H_2SO_4, MeOH, 98%; (ii) $LiAlH_4$, THF, 97%; (iii) HBr 48%, toluene, 85%; (iv) trimethyl acetyl chloride, Et_3N, DCM, 97%.

The ^{1}H NMR spectrum of the protected compound **(221)** showed a triplet at δ 4.04 (*J* 6.6 Hz) for the protons adjacent to the oxygen and a triplet at δ 3.40 (*J* 6.9 Hz) for the protons adjacent to the bromine substituent. The *tert*-butyl group protons appeared at δ 1.19 as a singlet. The ^{13}C NMR spectrum showed a signal at δ 178.6 for the carbonyl carbon, and a signal at δ 38.7 for the quaternary carbon of the protecting group, and a signal at δ 27.2 for the *tert*-butyl methyl carbons. The IR showed a peak at 1729 cm^{-1} for the carbonyl group C=O stretch.

The protected bromo compound **(221)** was reacted with 1-phenyl-1*H*-tetrazole-5-thiol in acetone to give the sulfide **(220) (Scheme 43)**. The proton NMR spectrum showed a multiplet at δ 7.71 – 7.41 for the phenyl group protons and a triplet at δ 3.38 (*J* 7.4 Hz) for the two protons ($C\underline{H}_2S$). Also, the carbon NMR spectrum showed five signals in the aromatic region: one at δ 154.5 for the tetrazole ring carbon, and another four at δ 133.7, 130.0, 129.7 and 123.8 for the phenyl group carbons.



Scheme (43): Preparation of sulfone C-14 **(217)**, reagents and conditions: (i) 1-phenyl-1*H*-tetrazole-5-thiol, K_2CO_3, acetone and THF reflux 86%; (ii) H_2O_2, $(NH_4)_6Mo_7O_{24}.4H_2O$, THF, IMS, 93%.

Finally, the sulfide **(220)** was oxidised to the desired sulfone **(217)**, as previously discussed and purified by column chromatography. The characteristic signal for the protons adjacent to the sulfonyl group appeared as a triplet at δ 3.77 – 3.69 (*J* 7.9 Hz) as shown in **Figure (43)**. The sulfone **(217)** was thus obtained in five steps in an overall yield of 55%.

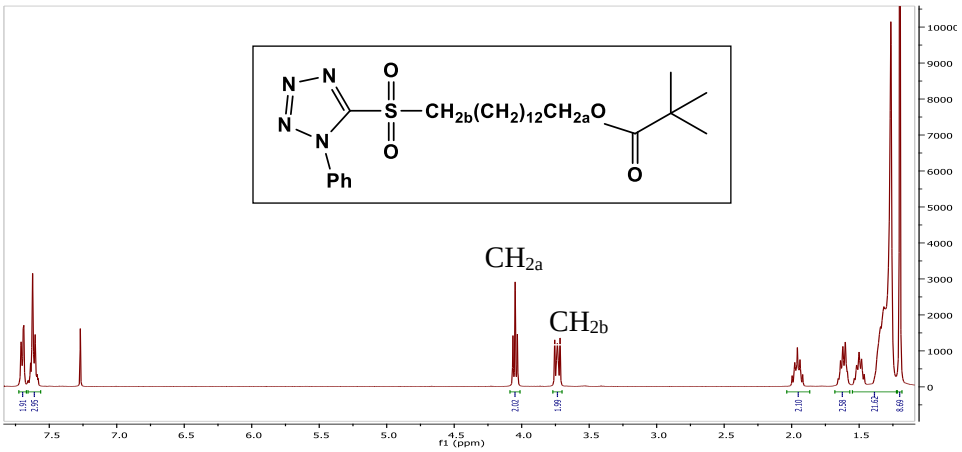
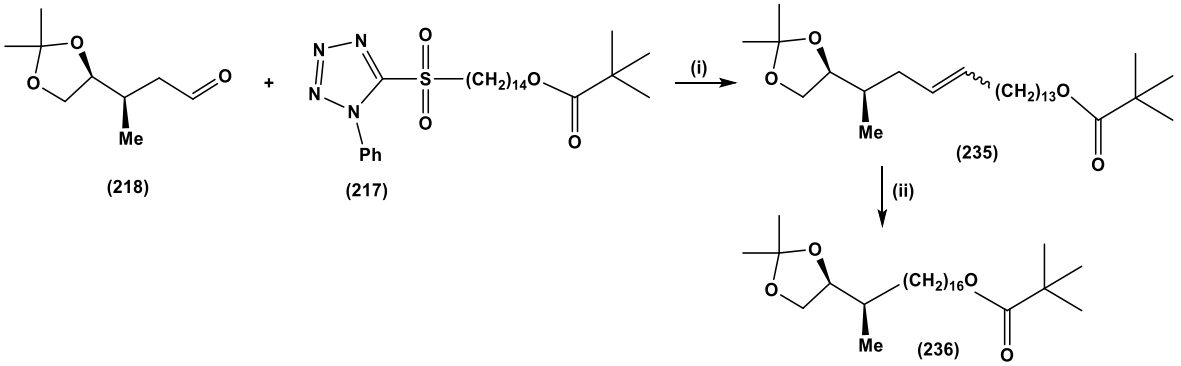


Figure (43): ^{1}H NMR spectrum of the intermediate sulfone **(217)**.

2.3.3 Coupling of aldehyde (218) with sulfone (217)

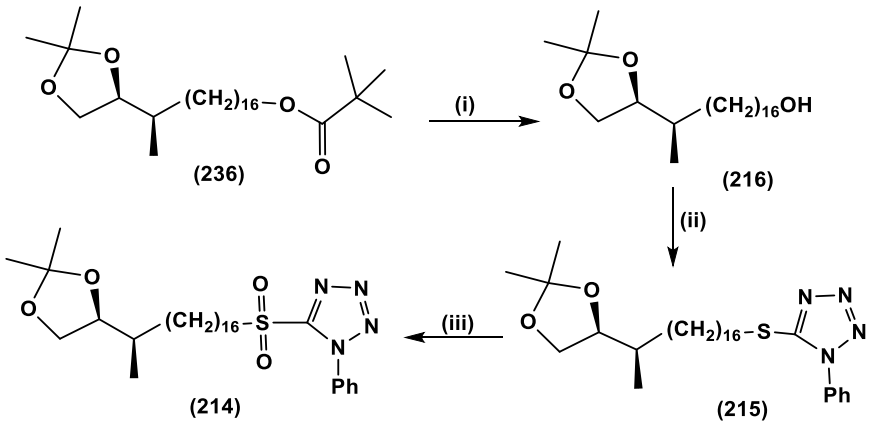
The aldehyde **(218)** was used as the source of the α-methyl *trans*-alkene unit, at the proximal position. In order to extend the length of the carbon chain, the aldehyde **(218)** was coupled to the sulfone **(217)** in a Julia reaction, using lithium bis(trimethylsilyl) amide at −10 °C. Subsequently, the reaction mixture was allowed to reach room temperature and stirred for 3 hours to complete the reaction. The reaction appeared to be very straightforward, giving the desired alkene in a 69% yield, as a mixture of *E* and *Z*-isomers. The purified product **(235)** gave a ^{1}H NMR spectrum which revealed peaks for the two alkene protons at δ 5.49 – 5.25 as a multiplet, and a multiplet at δ 1.31 – 1.22 for the long chain protons. The ^{13}C NMR spectrum showed two signals at δ 132.6 and 127.5 for the olefinic carbons. The cyclic acetal carbon appeared at δ 108.6 and two signals appeared at δ 79.9 and 67.8 for the carbons adjacent to the oxygen atoms. The alkene mixture was then hydrogenated with hydrogen gas in the presence of Pd (10%) on carbon in IMS and THF (1:1) to give the saturated compound **(236)** in a yield of 92% **(Scheme 44)**.



Scheme (44): Preparation of intermediate **(236)**, Reagents and conditions: (i) $LiN(SiMe_3)_2$, dry THF, 69%; (ii) H_2 gas, Pd (10%) on C, IMS and THF, 92%.

2.4 Preparation of the intermediate C-16 sulfone (214)

In order to couple the above compound **(236)** with the mycolic motif unit **(183)** it needed to be converted into sulfone **(214)**. Firstly, $LiAlH_4$ in THF was used to remove the protecting pivalate ester of **(236)** to give the corresponding alcohol **(216)** in an excellent yield, 96% **(Scheme 45)**. The ^1H NMR spectrum in $CDCl_3$ showed a triplet at δ 3.50 (*J* 6.7 Hz) for the two protons adjacent to the hydroxyl group. The IR spectrum showed a broad peak at 3400 cm^{-1} for the O–H stretch. The next stage was to perform a Mitsunobu reaction.[270] This reaction involves adding diethyl azodicarboxylate (DEAD) at 0 ºC under nitrogen gas dropwise to a solution of alcohol **(216)**, triphenylphosphine and 1-phenyl-1*H*-tetrazole-5-thiole in dry THF, and allowing the mixture to stir for 3 hours.

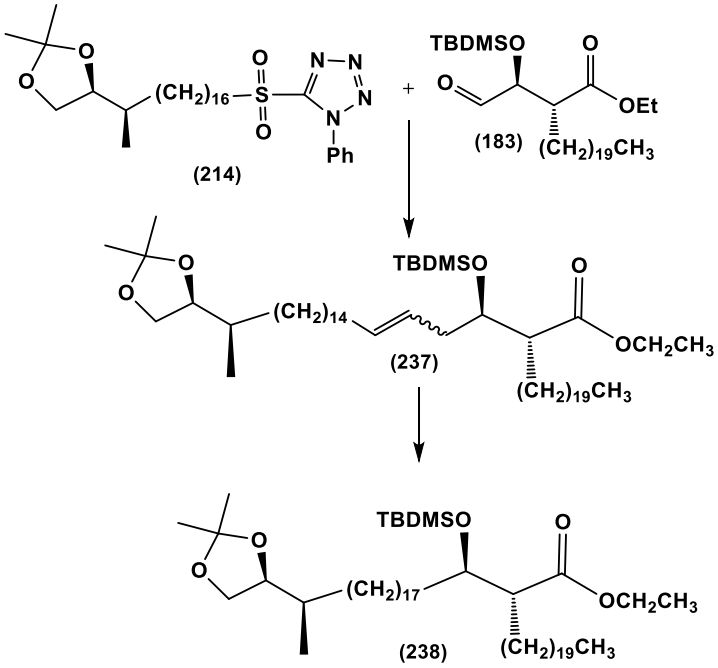


Scheme (45): Preparation of the intermediate C-16 sulfone **(214)**. Reagents and conditions: (i) $LiAlH_4$, THF 96%, (ii) diethyl azodicarboxylate, 1-phenyl-1*H*-tetrazole-5-thiol, PPh_3, dry THF, 83%, (iii) $(NH_4)_6Mo_7O_{24}.4H_2O$, H_2O_2, THF, IMS, 96%.

Finally, the sulfide **(215)** was oxidised to the corresponding sulfone **(214)** with hydrogen peroxide and ammonium molybdate (VI) tetrahydrate as shown in **Scheme (45)**. The protecting acetal group remained during the oxidation, which was confirmed by the NMR spectra. The ^1H NMR spectrum of the sulfone **(214)** showed a triplet at δ 3.74 (*J* 7.9 Hz) for the two protons next to the sulfonyl group, a doublet at δ 0.97 (*J* 6.7 Hz) for the chiral methyl protons and two singlets at δ 1.41 and δ 1.36 for the two methyls on the acetal protecting group. The acetal carbon signal appeared in the ^{13}C NMR spectrum at δ 108.4.

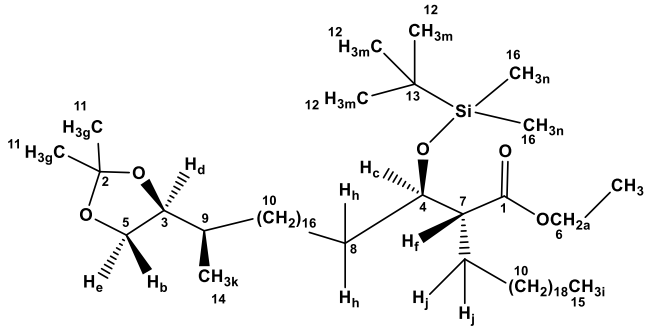
2.4.1 Extension of the mycolic motif chain by coupling with sulfone (214)

This step involved the coupling of the mycolic motif unit **(183)** with the long chain containing the methyl group which would finally be next to the *trans*-alkene unit. The mycolic motif unit and the meromycolate, the aldehyde **(183)** was coupled with sulfone **(214)** in a modified Julia reaction in the presence of lithium bis(trimethylsilyl) amide in dry THF to give the desired alkene **(237)** as a mixture of *E* and *Z*-isomers in 79% yield **(Scheme 46)**. The double bond was then hydrogenated using hydrogen gas in the presence of palladium on carbon (10%), to give the saturated compound **(238)** in 78% yield. The specific rotation was measured at $[\alpha]_D^{24}$ = + 6.37 (*c* 1.36 $CHCl_3$), which is close to the value for similar compounds that differ only in the chain lengths.[203] The structure of compound **(238)** was confirmed using NMR spectroscopy, this showed no signals in the olefinic region, which proved that the hydrogenation had been completed, see **Table (4)**.



Scheme (46): The coupling reaction between mycolic motif **(183)** and intermediate sulfone **(214)**, Reagents and conditions: (i) $LiN(SiMe_3)_2$, dry THF, 79%; (ii) H_2, Pd (10%) on C, IMS and THF, 78%.

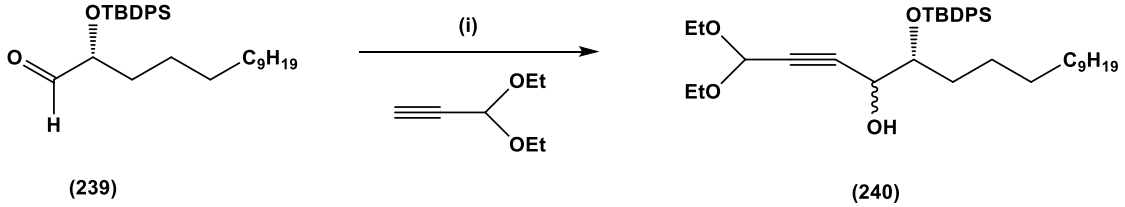
Table (4): ^{1}H NMR and ^{13}C NMR data analysis of compound **(238)**.



Proton	δ/ppm	Multiplicity	Integration	J (Hz)	Carbon	δ/ppm
H_a	4.12	m	2	-----	C_1	174.6
H_b	4.01	dd	1	6.2, 7.8	C_2	108.5
H_c	3.95 – 3.78	m	1	-----	C_3	80.4
H_d	3.87	br q	1	6.95	C_4	73.2
H_e	3.61	br t	1	7.7	C_5	67.8
H_f	2.50	ddd	1	3.8,7.0,10.8	C_6	59.9
H_h	1.59-1.52	m	2	----	C_7	51.6
H_j	1.49-1.42	m	2	-----	C_8	36.5
H_g	1.40, 1.36	s	2 × 3H	-----	C_9	33.7
-CH_2 chain	1.33 – 1.18	m	72	-----	C_{10}	32.7-22.7
H_k	0.97	d	3	6.7	C_{11}	25.8
H_i	0.89	t	3	4.9	C_{12}	25.5
H_m	0.87	s	9	-----	C_{13}	18.0
H_n	0.05,0.03	s	2 × 3H	-----	C_{14}	15.6
					C_{15}	14.1
				-----	C_{16}	-4.4, -5.0

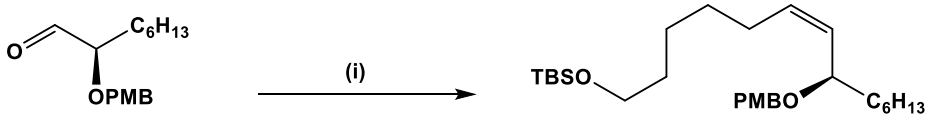
2.5 Synthesis of a model mycolic acid

Based on a number of literature methods concerning the synthesis and reaction of closely allied species,[271,272] we can be confident that no significant epimerisation occurs adjacent to the aldehyde during the above reaction **(Scheme 46)**. Two examples from the literature are shown below in **Schemes 47** and **48**.[271,272]



(239) (240)

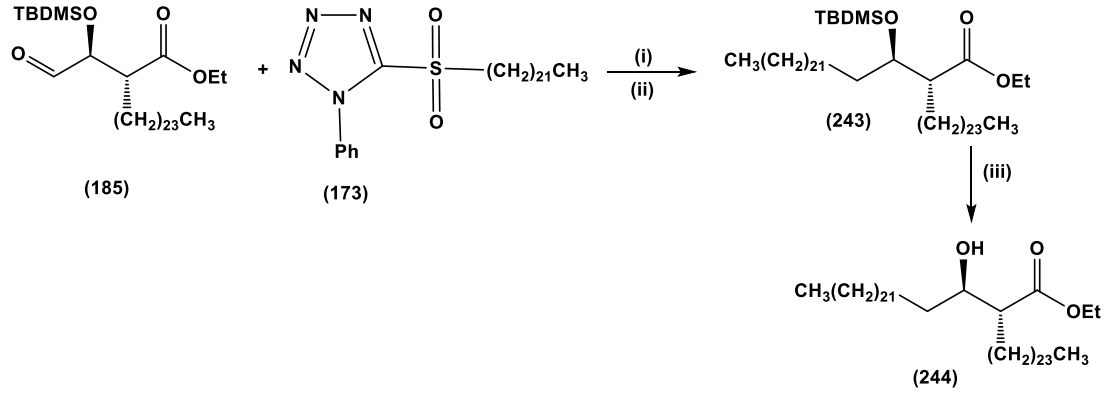
Scheme (47): Reagents and conditions, (i) n-BuLi, THF, -78 °C – 0 °C 89%.



(241) (242)

Scheme (48): Reagents and conditions, (i) n-BuLi, $TBSO(CH_2)_6P^+Ph_3Br^-$, THF, -78 °C to rt, 2hrs, 74%.

Furthermore, to confirm this, the synthesis of a model mycolic acid **(244)** was also carried out to confirm that no epimerisation occurred. Mycolic acid motif **(185)** was coupled with sulfone **(173)**, followed by the reduction of the double bond to give the protected model mycolic acid **(243)**. Deprotection of **(243)** using TBAF in dry THF gave hydroxy ester **(244)**, **Scheme (49)**.



(185) (173) (243) (244)

Scheme (49): Reagents and conditions: (i) $LiN(SiMe_3)_2$, dry THF, –15 °C, 78%; (ii) Pd on 10% C, THF and IMS 93%; (iii) TBAF, dry THF, 85%.

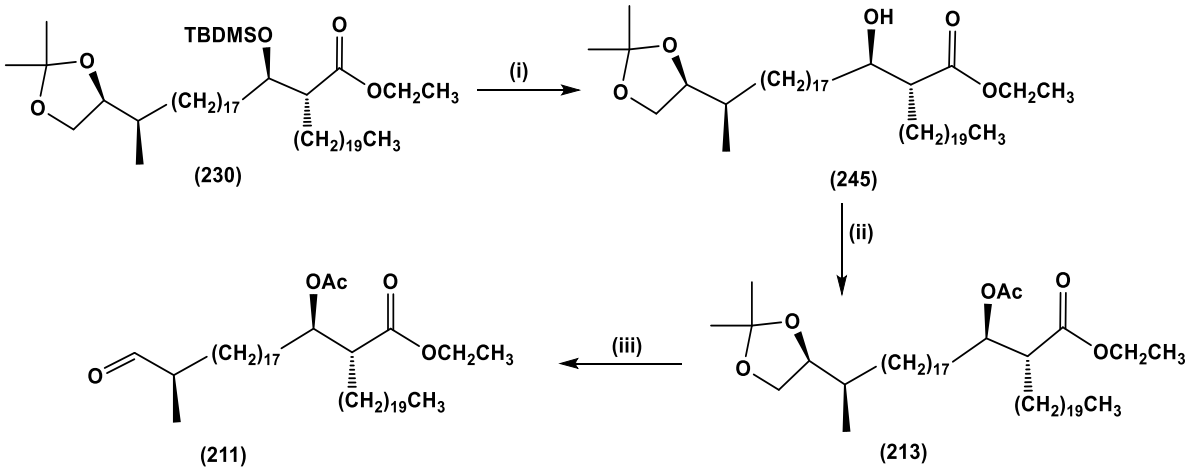
The molecular rotation ($[M]_D$) confirmed that during the reaction no epimerisation occurred; the molecular rotation is defined as one-hundredth of the product of the specific rotation and the relative molecular mass of an optically-active compound:

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

the specific rotation of the β-hydroxy ester (**244**) was $[\alpha]_D^{23}$ = + 5.3 (*c* 0.91 $CHCl_3$), therefore the molecular rotation of **(244)** was calculated to be + 41.0, which is very close to the calculated $[M]_D$ value for the corynomycolate reported in the literature (+ 40).[148] Had there been any epimerisation, the value for **(244)** would have been much lower.

2.6 Preparation of the intermediate aldehyde (211) for final coupling

In order to have compound **(211)** ready to be coupled to the meromycolate moiety to form the full MA, the acetonide protecting group needed to be removed and the secondary alcohol protecting group needed to be changed from the bulky TBDMS group to an acetate. The deprotection reagent *tetra-n*-butyl ammonium fluoride (TBAF) was used to remove the sterically hindered *tert*-butyldimethylsilyl. The product, secondary alcohol **(245)**, was obtained in 82% yield, with the IR showing a peak at 3520 cm^{-1} confirming the formation of the O–H bond. The NMR spectra did not show any signals for the *tert*-butyldimethylsilyl group and other signals were as expected. Protection of the alcohol as an acetoxy group using acetic anhydride and anhydrous pyridine was then carried out to give product **(213)** in 98% yield. The ^1H NMR spectrum showed that the proton adjacent to the acetoxy group had shifted downfield and appeared as a doublet of triplets at δ 5.10 (*J* 4.0, 7.5 Hz). The methyl protons on the acetyl group also appeared as a singlet at δ 2.03. Following this, oxidative cleavage of the cyclic acetal group **(213)** with periodic acid in dry ether led to the corresponding aldehyde **(211)** in 77% yield **(Scheme 50**).



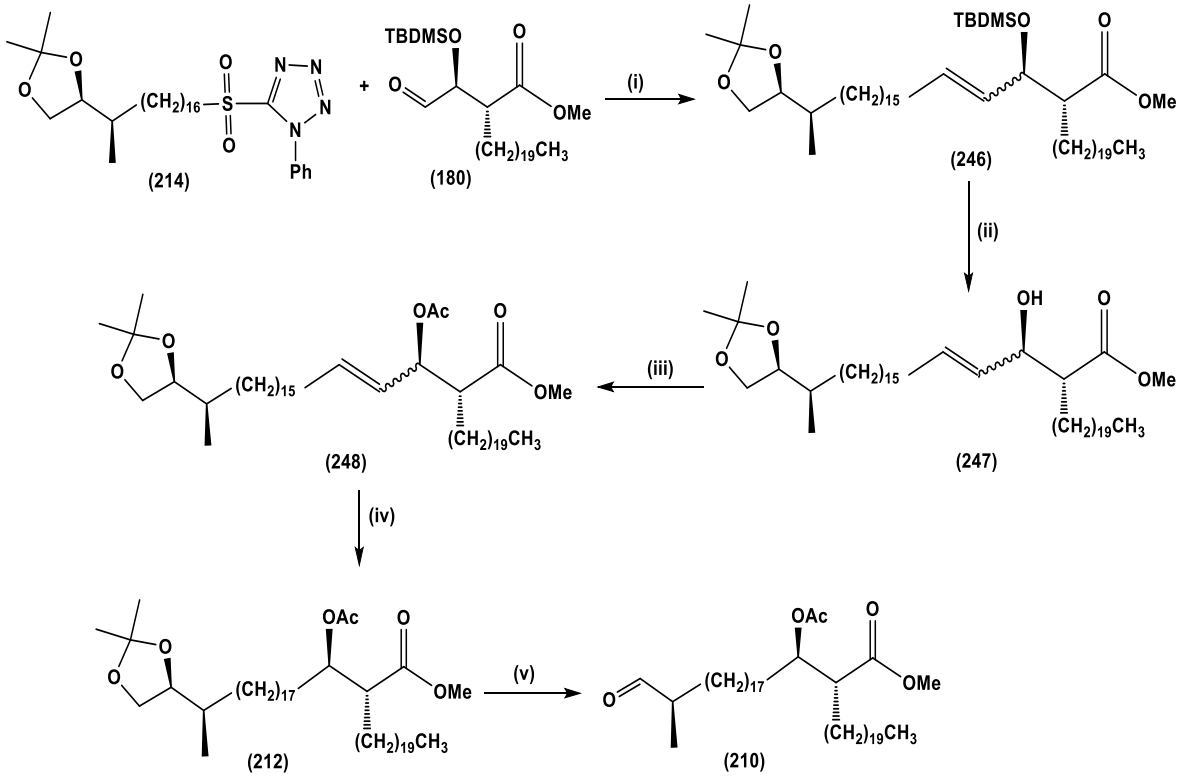
Scheme (50): The synthesis of α-methyl aldehyde **(211)**, reagent and conditions: (i) TBAF, pyridine, THF, 82%; (ii) acetic anhydride, pyridine, dry toluene,98%; (iii) periodic acid, dry ether, 77%.

The ^{1}H NMR spectrum showed a doublet at δ 9.61 (*J* 1.9 Hz) for the aldehyde proton, a multiplet at δ 2.38 – 2.29 for the proton next to the aldehyde and a doublet at δ 1.09 (*J* 7.0 Hz) for the methyl protons at the α-position to the aldehyde. The ^{13}C NMR spectra showed a signal at δ 205.3 for the carbonyl carbon of the aldehyde, a signal at δ 46.3 for the carbon next to the aldehydes. The disappearance of the signals of the protecting group in the proton and carbon NMR spectra confirmed the success of the reaction. The IR spectrum showed a peak at 1731cm^{-1} (C=O) and the specific rotation of **(211)** was $[\alpha]_{D}^{29}$ = + 6.0 (*c* 0.4, $CHCl_3$).

2.7 Preparation of methyl (2*R*,3*R*,21*R*)-3-acetoxy-2-eicosyl-21-methyl-22-oxodocosanoate (212)

The sulfone (**214**) and the mycolic motif unit aldehyde **(180)** were dissolved in THF, followed by addition of lithium *bis*(trimethylsilyl) amide at −15 °C. The reaction mixture was allowed to reach room temperature and stirred for 3 hours. The reaction afforded a mixture of the *E*/*Z* isomers in a ratio 2:1, in an 76% yield **Scheme (51**). As discussed previously (section **2.4.1**) we can be confident that no epimerisation has occurred during this coupling reaction. Again the ^{1}H NMR spectrum of **(246)** showed two multiplets at δ 5.47 – 5.40 and at δ 5.37 – 5.31 respectively for the two alkene protons. The ^{13}C NMR spectrum showed two signals at δ 132.7 and 127.5 for the olefinic carbons. This was followed by deprotection of the *tert*-butyldimethylsilyloxy group of compound **(246)** to

give the corresponding secondary alcohol **(247)** at the β-position. Protection of the alcohol as an acetoxy group by using acetic anhydride and anhydrous pyridine in dry toluene gave product **(248)** in 88% yield. Hydrogenation using palladium hydroxide on activated charcoal $Pd(OH)_2$, gave the saturated compound **(212)** in 96% yield. Following this, the oxidative cleavage of the cyclic acetal group with periodic acid in dry ether led to corresponding aldehyde **(210)** in 88% yield. It is important to note that during this synthesis the hydrogenation of the alkene **(246)** was initially attempted, a number of times, however it proved to be problematic, with the ^1H NMR spectrum indicating that a complex mixture had been formed, with loss of the β-hydroxy and methoxy groups. It was therefore necessary to deprotect the bulky TBDMS group and re-protect the alcohol with an acetate group before hydrogenation of alkene **(248)** was carried out successfully.



Scheme (51): The synthesis of compound **(210)**. Reagents and conditions: (i) $LiN(SiMe_3)_2$, dry THF 76%; (ii) TBAF, pyridine, THF, 88%; (iii) acetic anhydride, pyridine, dry tolene, 88%; (iv) H_2, Pd $(OH)_2$, IMS and THF 96%; (v) periodic acid, dry ether, 88%.

The ^{1}H NMR spectrum for compound **(210)** showed a doublet at δ 9.61 (*J* 1.7 Hz) for the aldehyde proton, a multiplet at δ 2.65 – 2.56 (1H, m) for the α-H next to the ester group, another multiplet at δ 2.39 – 2.26 for the proton next to the aldehyde group and a doublet at δ 1.09 (*J* 7.0 Hz) for the methyl protons at the *α*-position to the aldehyde **(Figure 44)**. The ^{13}C NMR spectrum showed a signal at δ 202.4 for the carbonyl carbon of the aldehyde and, a signal at δ 46.3 for the carbon next to the aldehyde **Table (5)**.

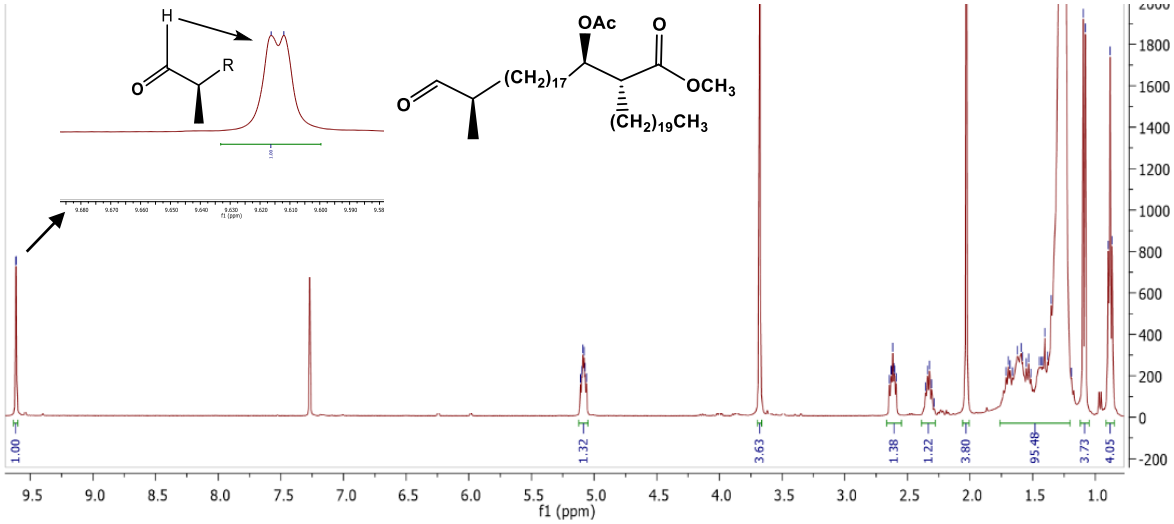
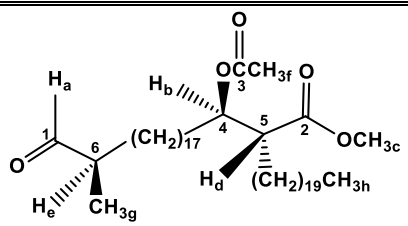


Figure (44): ^{1}H NMR spectrum of compound **(210)**.

Table (5): ^{1}H NMR and ^{13}C NMR data analysis of compound **(210)**.

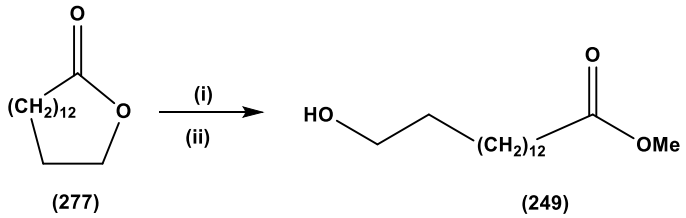


Proton	*δppm*	*Multiplicity*	*Integration*	*J (Hz)*	*Carbon*	*δppm*
H_a	9.61	d	1	1.7	C_1	205.4
H_b	5.08	dt	1	4.1,7.6	C_2	173.7
H_c	3.68	s	3	-----	C_3	170.4
H_d	2.65 – 2.56	m	1	-----	C_4	74.0
H_e	2.39 – 2.26	m	1	-----	C_5	51.5
H_f	2.03	s	3	-----	C_6	46.3
-CH_2 chain	1.73 – 1.19	m	92	----		
H_g	1.09	d	3	7.0	----	----
H_h	0.88	t	3	6.6	----	----

2.8 Preparation of *Z*-alkene sulfone (209) for final coupling

2.8.1 Ring opening of lactone for preparation of the 15-carbon chain

To complete the final coupling reaction for the synthesis of the *cis*-alkene-α-methyl-*trans*-alkene mycolic acid, the moremycolate moiety needed to be prepared. The synthesis started with the commercially available and inexpensive ω-pentadecalactone **(227)**. Ring opening of the *ω*-pentadecalactone **(227)** was achieved by adding it to freshly prepared sodium methoxide in methanol and stirring at 80 ^{o}C for 3 hours. The solution was acidified with aq. HCl (1N) and worked up. The proton NMR spectrum showed the product was a mixture of the desired methyl ester and the carboxylic acid. Therefore, the acid was esterified in methanol and a catalytic amount of H_2SO_4 by refluxing for 90 minutes to give 15-hydroxypentadecanoic acid methyl ester **(249)** in 86% yield **(Scheme 52**).[273]

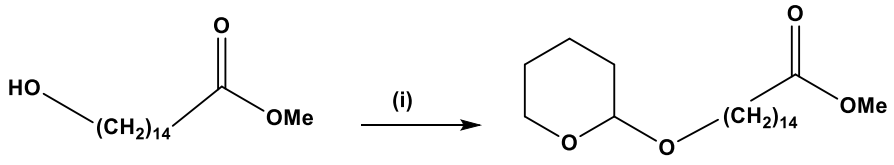


Scheme (52): Ring opening of the lactone **(277)**. Reagents and conditions: (i) NaOMe, MeOH, H_2SO_4, 80 °C, 2 hrs, (ii) MeOH, H_2SO_4, reflux 1 hr, 86%.

The ^{1}H NMR spectrum of **(249)** showed a singlet at δ 3.67 for the methyl ester protons, a triplet at δ 3.64 (*J* 6.5 Hz) for the two protons next to the hydroxy group, and a triplet at δ 2.31 (*J* 7.5 Hz) for the two protons next to the carbonyl group. The long chain protons appeared as a multiplet at δ 1.41 – 1.15. The ^{13}C NMR spectrum showed a carbonyl signal at δ 174.4, a signal at δ 63.0 for the carbon next to the hydroxy group, and a signal at δ 51.4 for the methyl ester carbon. The IR spectrum showed a broad peak at 3298 cm^{-1} for the O–H stretch and another broad peak at 1742 cm^{-1} for the C=O stretch.

2.8.2 Protection with a dihydropyranyl group

The hydroxyl group of the methyl 15-hydroxypentadecanoate **(249)** was protected with dihydropyran in the presence of pyridinium-*p*-toluene–sulfonate in dichloromethane to give methyl 15-((tetrahydro-2*H*-pyran-2-yl)oxy)pentadecanoate **(250)** in 94% yield **Scheme (53)**.[274]



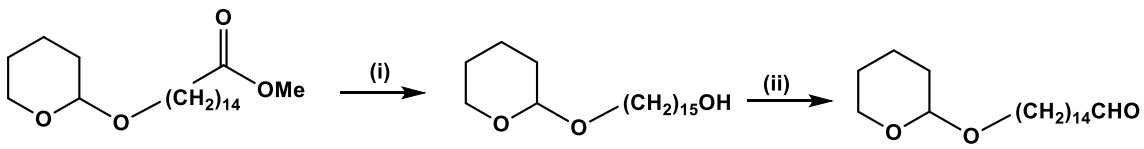
(249) **(250)**

Scheme (53): Protection the alcohol with a THP group. Reagents and conditions: (i) 3,4-dihydro-2*H*-pyran, PPTS, dry DCM, 94%.

The proton NMR showed the signals of the THP group with a broad multiplet at δ 4.58 ppm integrated to one proton corresponding to the acetal proton. There was a triplet at δ 2.30 ppm (*J* 7.5 Hz) integrating to two protons corresponding to the CH_2 adjacent to the carbonyl group, while the remaining signals of the OCH_3 group appeared at δ 3.67. The carbon NMR showed signals at δ 174.3 corresponding to the carbonyl group, and at δ 98.8 ppm for the carbon of the acetal group of the THP.

2.8.3 Reduction and oxidation

The protected methyl ester **(250)** was reduced with lithium aluminium hydride in THF and after quenching with a sat. aq. solution of sodium sulfate alcohol **(251)** was obtained, which was converted to the corresponding aldehyde **(226)** in 82% yield using PCC in dichloromethane **Scheme (54)**. PCC is a mild oxidizing agent and widely used for the oxidation of primary alcohols to aldehydes without further oxidation to carboxylic acids, and under neutral conditions.



(250) **(251)** **(226)**

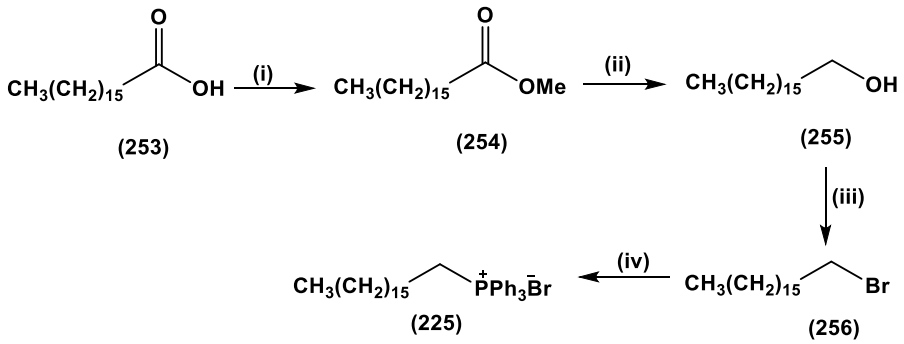
Scheme (54): Preparation of the chain extended α-methyl-*trans*-cyclopropane aldehyde. Reagents and conditions: (i) $LiAlH_4$, THF, 91%; (ii) PCC, DCM, 82%.

The proton NMR spectrum of compound **(251)** confirmed the disappearance of the sharp singlet belonging to the methoxy ester (OCH_3), and instead showed a broad triplet at δ 3.64 (*J* 5.3 Hz) for the two protons next to the alcohol. The carbon NMR spectrum also showed a signal at δ 63.0 for the carbon bonded to the hydroxyl group. The IR spectrum showed a broad peak at 3392 cm^{-1} due to the OH group. The ^{1}H NMR spectrum for aldehyde **(226)** showed a triplet at δ 9.75 (*J* 7.2 Hz), for the aldehyde and a doublet of triplets at δ 2.40 (*J* 1.7, 7.4 Hz), integrating to two protons for the adjacent to aldehyde proton. The most significant signal of the THP group was a multiplet at δ 4.56 integrating to one proton corresponding to the acetal THP. The ^{13}C NMR signal showed an aldehyde signal at δ 202.9, and the THP acetal carbon appeared at δ 98.8. The IR spectrum also confirmed the formation of the aldehyde due to the peak at 1727 cm^{-1} corresponding to the C=O group, while that for the O-H group had disappeared.

2.9 Synthesis of *Z*-alkene THP (252)

2.9.1 Preparation of phosphonium salt

In order to produce the desired *Z* alkene THP **(252)**, the Wittig salt **(225)** needed to be prepared. To obtain this esterification of heptadecanoic acid **(253)** using conc. H_2SO_4 and methanol was firstly carried out to give compound **(254)**, in 99% yield. This was then reduced to alcohol **(255)** using lithium aluminium hydride in THF. The alcohol was characterized by proton NMR, which showed a triplet at δ 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 group. The IR spectrum showed a peak at 3389 cm^{-1} belonging to O-H group in the compound. The alcohol **(255)** was converted into 1-bromoheptadecane **(256)** using *N*-bromosuccinimide with triphenylphosphine in dichloromethane. The heptadecyltriphenylphosphonium bromide **(225)** was prepared following the literature method.[275] It was obtained by refluxing the bromo-compound **(256)** and triphenylphosphine in toluene at 70°C for 4 days **(Scheme 55)**. The pure phosphonium salt **(225)** was obtained in 74% yield after purification by column chromatography, firstly eluting with petroleum ether and then dichloromethane/methanol (95:5), and then precipitation in dry ether.

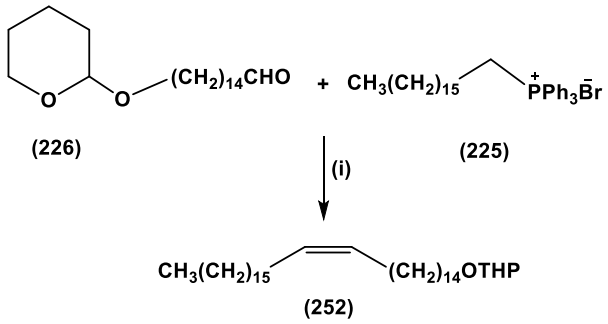


Scheme (55): Synthesis of alkyl phosphonium salt **(225)**, Reagents and conditions: (i) H_2SO_4, MeOH, 99%; (ii) $LiAlH_4$, THF, 95%; (iii) NBS, PPh_3, DCM, 92%; (iv) PPh_3, toluene, 74%.

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 **(225)** showed the aromatic protons as a broad multiplet between δ 7.91–7.66 integrating to fifteen protons, and the two protons next to the phosphorus appeared as a multiplet at δ 4.03 – 3.49. The carbon NMR also showed the aromatic carbons between δ 134.9–118.3.

2.9.2 Insertion of the first double bond via a Wittig reaction

The crucially important part of this synthesis was formation of the *Z*-alkene isomer. There is one well known literature method, coupling of an aldehyde with a phosphonium salt in the presence of sodium bis(trimethylsilyl)amide that gives the *Z*-stereoisomer with great selectivity. This was attempted using compounds **(226)** and **(225)** and gave the required *Z*-alkene **(252) (Scheme 56)**.



Scheme (56): Synthesis of *Z*-alkene **(252)**, reagents and conditions: (i) sodium bis(trimethylsilyl)amide, dry THF, 64%.

The proton NMR of **(252)** confirmed the success of the reaction, which showed the *cis* double bond protons as a broad triplet at δ 5.36 (*J* 4.7 Hz) integrating to two protons **Figure (45)**. The signals of the THP group showed a multiplet at δ 4.59 belonging to the acetal proton. The carbon NMR showed the alkene carbons at δ 129.9, and the THP acetal carbon at δ 98.8.

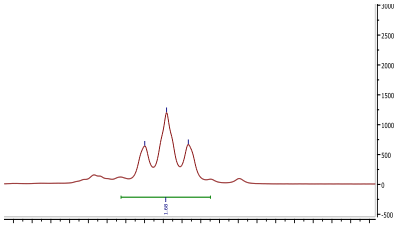
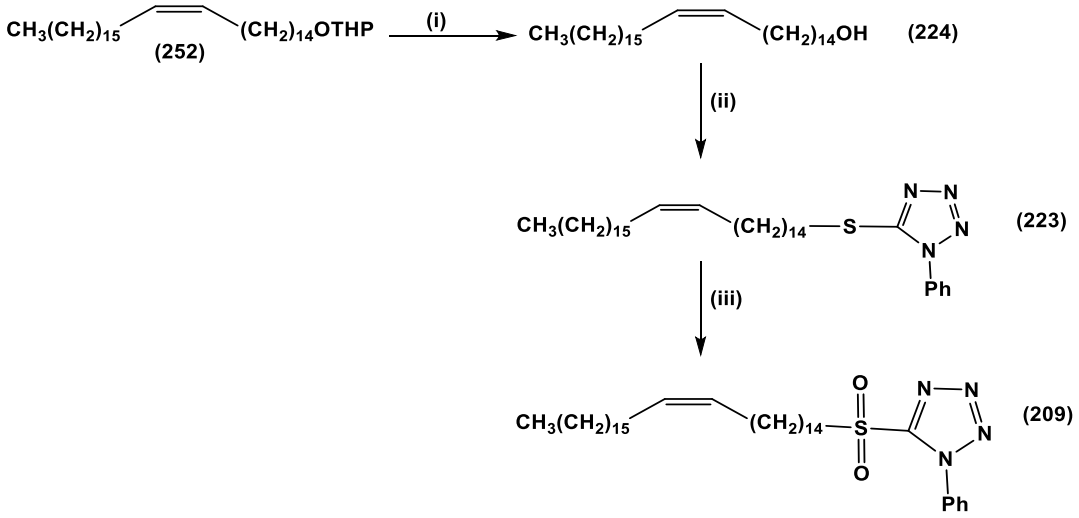


Figure (45): The proton NMR signal for the cis alkene protons of **(252)**.

2.9.3 Deprotection of *Z*-alkene THP (252)

The deprotection of the THP group in compound **(252)** was carried out using pyridinium-*p*-toluene sulfonate in a mixture of THF and MeOH stirring at 45 ^{o}C for six hours to give alcohol **(224)** in 71% yield, **(Scheme 57)**. The alcohol compound **(224)** was then treated with 1-phenyl-1*H*-tetrazole-5-thiol and triphenylphosphine in the presence of diethyl azodicarboxylate (DEAD) to give the *Z*- alkene sulfide **(223)** in a very good yield of 96%. Finally, the *Z*-alkene sulfone **(209)** was prepared via oxidation of sulfide **(223)** using hydrogen peroxide and ammonium molybdate in IMS and THF.



Scheme (57): Preparation of *Z*-alkene sulfone **(209)**. Reagents and conditions (i) PPTS, THF, MeOH, at 40 ^{o}C, 71%; (ii) 1-phenyl-1*H*-tetrazole-5-thiol, Ph_3P, DEAD, dry THF, 96%; (iii) hydrogen peroxide, $(NH_4)_6Mo_7O_{24}.4H_2O$, IMS and THF, 83%.

The ^{1}H spectrum of compound **(224)** showed a triplet at δ 3.65 (*J* 6.6 Hz) for the two protons belonging to the carbon next to the OH group and the carbon NMR showed a signal at δ 63.1 ppm for the same carbon, while the protons belonging to the THP group had disappeared from the spectrum. It has been found that there is a broad triplet at δ 3.40 (*J* 7.4 Hz) integrating to two protons belonging to the carbon next to the sulfur atom and the phenyl group protons appeared as a multiplet at δ 7.84-7.38 integrating to five protons for the sulfide compound **(223)**. The ^{1}H NMR spectrum of the *Z*-alkene sulfone **(209)** showed a broad triplet at δ 3.74 (*J* 7.9 Hz) integrating to the two protons next to the sulfonyl group. The sulfonyl carbon signal appeared in the carbon NMR spectrum at δ 56.0 **(Figure 46)**.

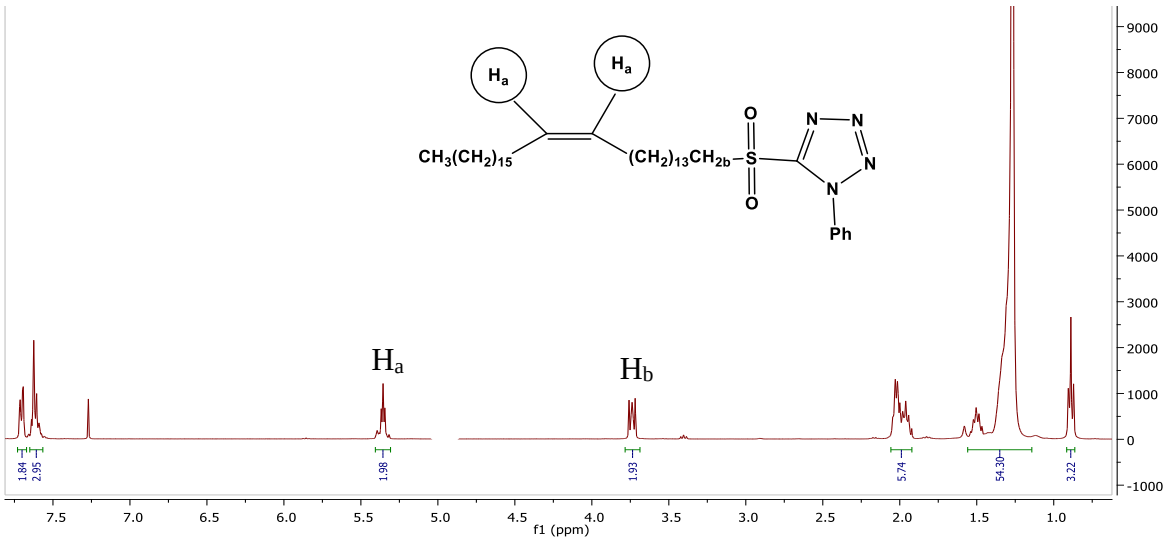
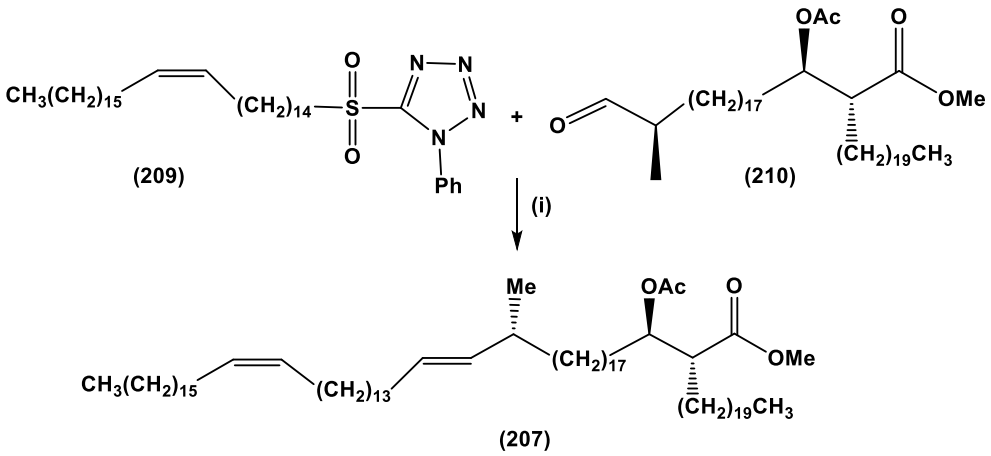


Figure (46): ^{1}H NMR spectrum of sulfone compound **(209)**.

2.10 Final coupling reaction between the meromycolate moiety and mycolic motif unit to form mycolic acid (207)

Recent studies suggested that the steroselectivity and yield of the Julia olefination is sensitive to base with the typical modified Julia-Kocienski olefination using lithium *bis*(trimethylsilyl)amide being likely to give the alkene product as a mixture of *cis*- and *trans*- stereoisomers. Therefore, as isolation of the *trans*-alkene would be highly problematic, lithium *bis*(trimethylsilyl)amide was not used as the base. Both Pospíšil *et al.*[276] and Kocienski *et al*. discussed the use of different bases, in particular potassium *bis*(trimethylsilyl)amide, to increase the stereoselectivity in favour of the *trans*-isomer. Koceinski *et al*. discovered that changing the base used increases stereoselectivity in the

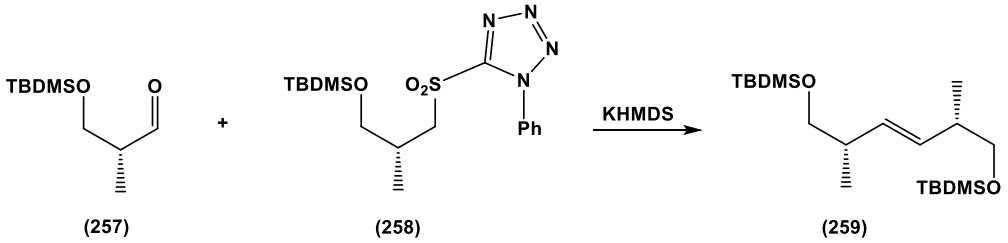
order Li<Na<K, and Pospíšil *et al*. gives evidence that increasing the size of the alkyl chain of the aldehyde leads to higher stereoselectivity. The coupling between the α-methyl substituted aldehyde **(210)** and the *Z*-alkene sulfone **(209)** to form the protected mycolic acid **(207)** was initially carried out in 1,2-dimethoxyethane with potassium *bis* (trimethylsilyl) amide (1 M in THF or 0.5 M in toluene). This reaction was attempted a number of times at various temperatures (between – 20 and 20 °C); however only a very small amount of product was formed (max 2% yield), with the ^{1}H NMR spectra indicating that a complex mixture was obtained in each case. The problem with this reaction was likely to be due to the poor solubility of the compounds in 1,2-dimethoxyethane. An alternative method, using dry THF as solvent in the presence of potassium *bis* (trimethylsilyl) amide (1 M in THF or 0.5 M in toluene) at −16 ºC was therefore used, which led to the formation of the required product **(207)** in 39% yield **(Scheme 58)**.



Scheme (58): Reagents and conditions: (i) potassium bis(trimethylsilyl)amide, dry THF, 39%.

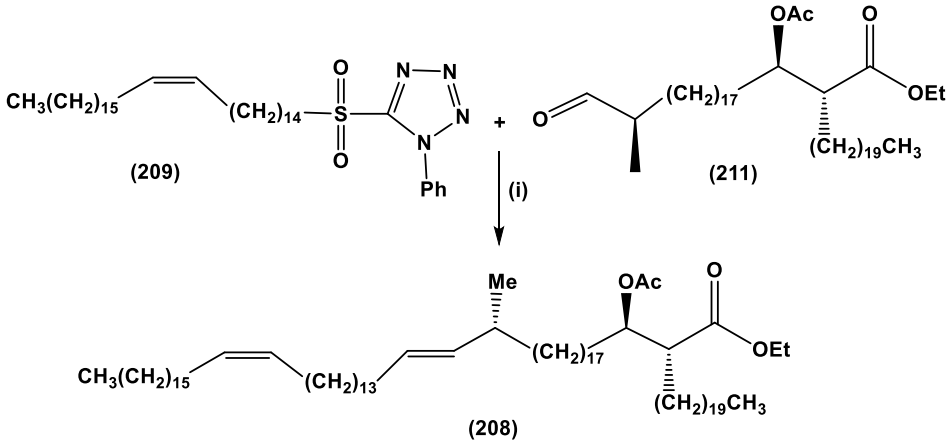
The stereoselective formation of the α-methyl *trans* alkene was confirmed by NMR spectroscopy. The specific rotation of compound **(207)** was $[\alpha]_{D}^{30}$ = + 11 (*c* 0.21 $CHCl_3$).

It is again essential that during the synthesis of **(207)** that no epimerisation occurs adjacent to the aldehyde. There are a number of examples of this kind of reaction where the chirality is retained,[277,278,279] for example **Scheme (59)**



Scheme (59): Literature example of the retention of chirality adjacent to the aldehyde.[280]

The coupling reaction was also carried out between the ethyl ester **(211)** and sulfone **(209)** to form protected mycolic acid **(208) (Scheme 60)**.

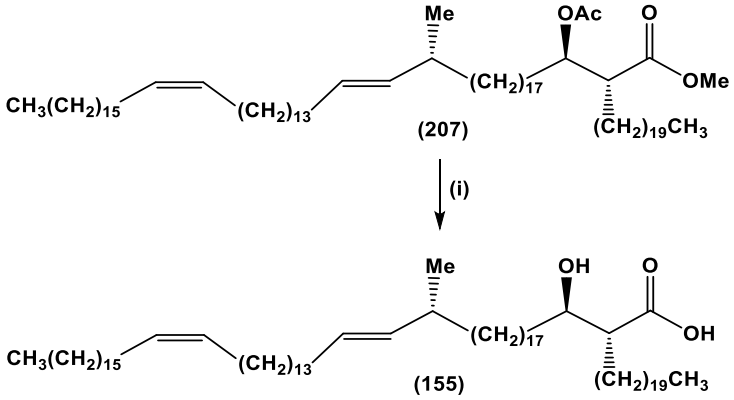


Scheme (60): Reagents and conditions: (i) potassium bis(trimethylsilyl)amide, THF, 47%.

The structure was confirmed by the ^{1}H NMR spectrum which showed a multiplet at δ 5.41 – 5.29 and a doublet of doublets at δ 5.24 (*J* 7.3, 15.4 Hz) corresponding to the alkene protons. The coupling constant of 15.4 Hz between the olefinic hydrogen atoms confirmed the formation of the *trans*-alkene. The ^{13}C NMR spectrum showed signals in the olefinic region at δ 136.4, 129.9 and 128.4 for the *cis* and *trans*-alkene carbons. The specific rotation of the product **(208)** was found to be $[\alpha]_{D}^{23}$ = + 11 (*c* 0.17 $CHCl_3$).

2.10.1 Deprotection and hydrolysis of (207) to produce free mycolic acid (155)

Finally, the resulting acetylated mycolic acid methyl ester **(207)** was hydrolysed using lithium hydroxide in a mixture of THF, methanol and water at 45 °C for 16 hours to give the free alkene mycolic acid **(155), Scheme (61)**.



Scheme (61): Reagents and conditions: (i) LiOH, MeOH, H_2O, THF, 45 °C, 16 h 71%.

After purification by re-crystallization from petrol/ethyl acetate (1:2) the MA **(155)** was obtained in a yield of 71%. The ^{1}H NMR spectrum confirmed the formation of the free MA due to the disappearance of the signal at δ 3.68 corresponding to the methyl ester and that at δ 5.09 (*J* 4.0,7.6 Hz) corresponding to the acetyl group at the β- position. The IR spectrum further confirmed the formation of free mycolic acid **(155)**, which showed a broad peak at 3585 cm^{-1} for the OH stretch. A detailed analysis of the spectroscopic data for **(155)** is given below.

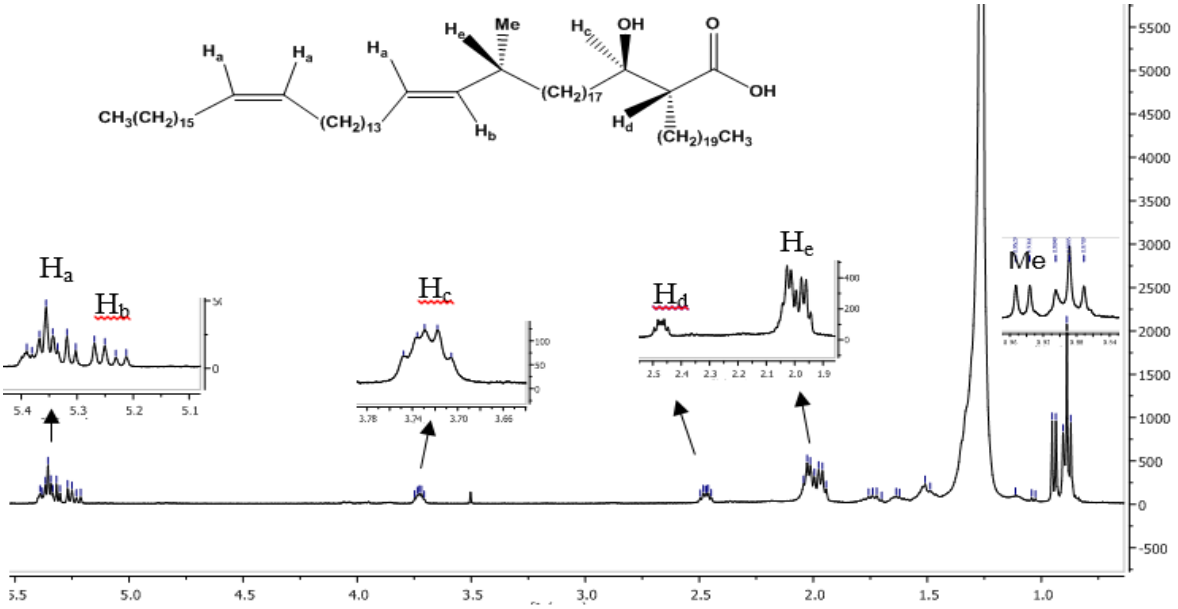


Figure (47): ^{1}H NMR spectrum of *cis*-alkene-α-methyl-trans-*alkene* mycolic acid **(155)**.

The ^{1}H NMR spectrum of the *cis*-alkene-α-methyl-*trans*-alkene mycolic acid **(155)** showed a multiplet at δ 5.41 – 5.29 (3H) corresponding to two protons of the *cis* double bond and one proton of the trans double bond, and a double doublet at δ 5.24 (*J* 7.5, 15.3 Hz) corresponding to the other proton of the *trans* double bound. The signals corresponding to H_c and H_d appeared as two double triplets at δ 3.73 and δ 2.47 respectively, while the proton adjacent to the α-methyl next to the trans double bond appeared as a multiplet at δ 2.08 – 1.93 with six protons corresponding to $C\underline{H_2}$ groups next to the double bonds. The Figure also shows a multiplet at δ 1.81 – 0.99 corresponding to the long-chain protons. Finally, the signals that occur as a doublet at δ 0.94 (*J* 6.7 Hz) and a triplet at δ 0.89 (*J* 6.5 Hz) correspond to the CH_3 next to the double bond and the two terminal CH_3 groups respectively. A summary of the proton and carbon NMR data is given in **Table (6)**. The 2D-NMR confirmed the structure of **(155)**, where the proton signals of the double bonds were correlated to their carbons throughout **(Figure 48)**.

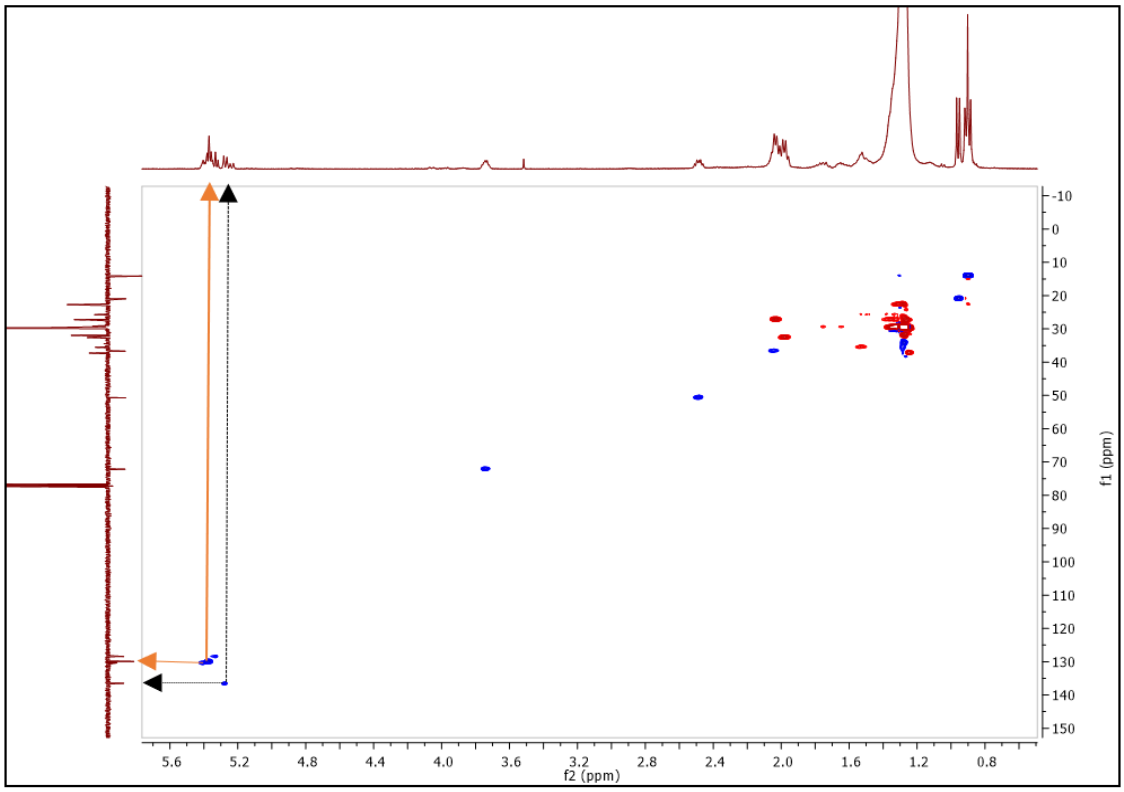
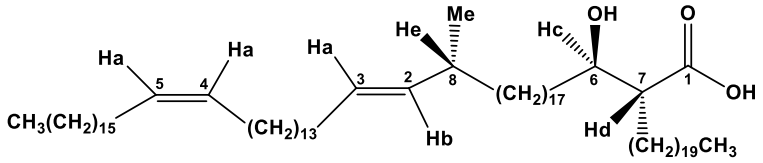


Figure (48): The 2D-NMR for free mycolic acid **(155)**.

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



Proton	*δ/ppm*	Multiplicity	Integration	*J*/ Hz	Carbon	*δ/ppm*
Ha	5.41 – 5.29	m	3	----	C1	178.5
Hb	5.24	dd	1	7.5,15.3	C2	136.5
Hc	3.73	dt	1	4.8, 9.4	C3	130.4
Hd	2.47	dt	1	5.2, 9.2	C4	129.9
He +6CH_2 adjacent to double bonds	2.08 – 1.93	m	7	----	C5	128.4
CH_3	0.94	d	3	6.7	C6	72.1
Terminal CH_3	0.89	t	6	6.8	C7	50.7

The IR data for the *cis*-alkene-α-methyl-*trans*-alkene mycolic acid **(155)** indicated the formation of the acid, **Table (7)**.

Table (7): IR analysis of *cis*- alkene-α- methyl-*trans*- alkene mycolic acid **(155)**.

Stretching	Wave number (cm^{-1})
O-H	3585
C-H	2915
C-H	2848
C=O	1686

The structure of **(155)** was also confirmed by mass spectrometry [TOF-MS-ASAP$^+$–Found $(M+H)^+$:1096.1323 $C_{75}H_{147}O_3$ requires: 1096.1350 **(Figure 49)**.

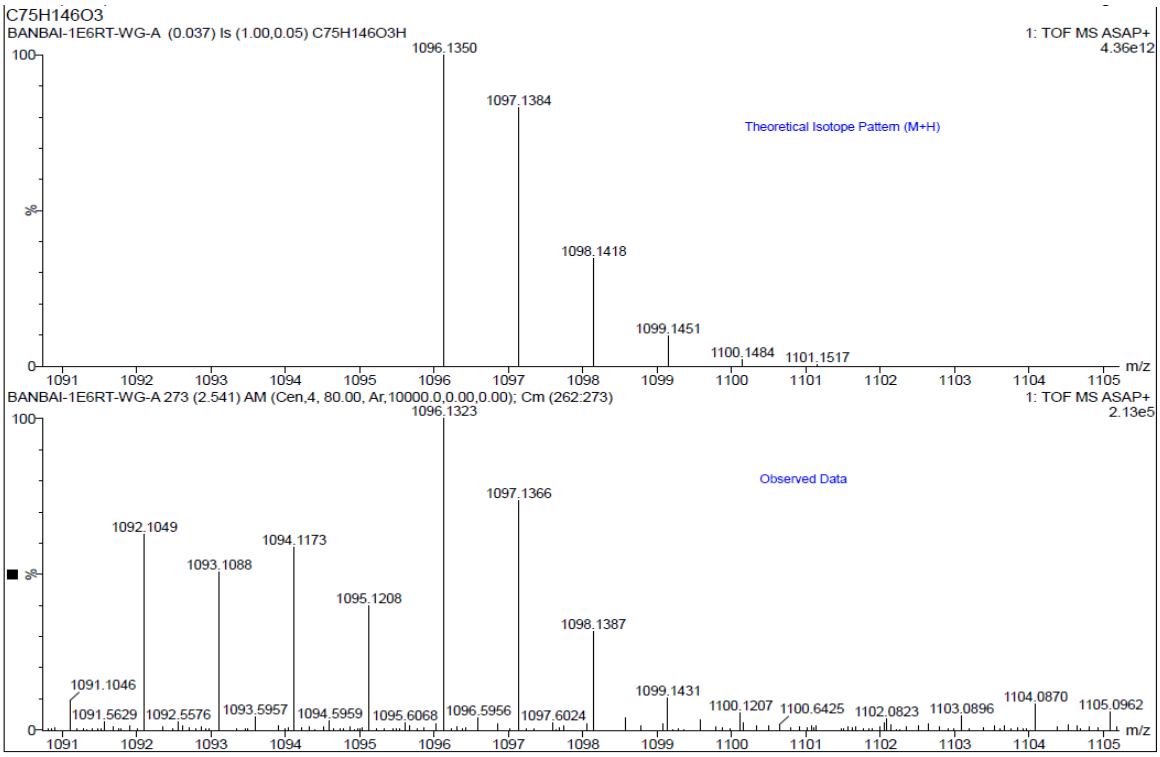
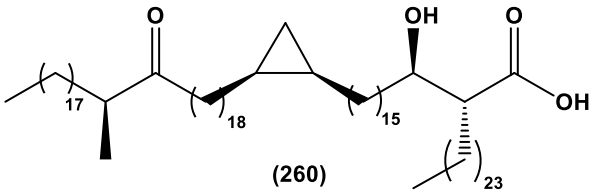


Figure (49): Mass spectrometry data for mycolic acid **(155)**.

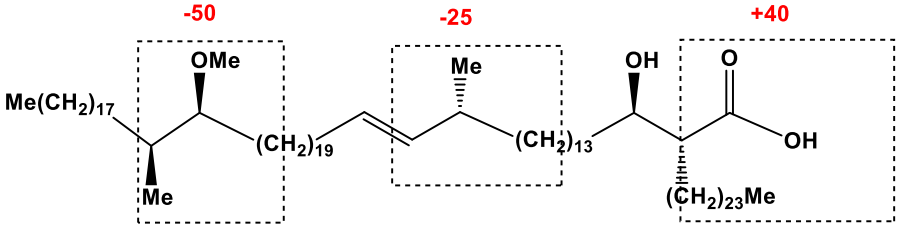
The molecular rotations (see page 85) of individual functional groups in natural mixtures of mycolic acids and in model compounds have been identified by Quémard *et. al.*[148] In general, the $[M]_D$ in such systems where a group of chiral centres are separated by long carbon chains are approximately additive; the individual values of the *R*,*R*-centres of the 2-hydroxy acid (ester) fragment in mycolates is estimated as + 40, the $RCH{=}CHCH_3$-fragment as – 25 (for 30% content). Therefore, the $[M]_D$ value of the free acid **(155)** can be estimated as +15 (-25+40) based on the sum of hydroxy acid and α-methyl *trans* alkene contributions. The specific rotation of acid **(155)** was determined to be +2.2 (*c* 0.92 $CHCl_3$), which corresponds to $[M]_D$ = +24 which is in agreement with the calculated value using the relationship above.

By comparison, the specific rotation of the keto mycolic acid **(260)** was determined to be +7.34 (*c* 0.79, $CHCl_3$),[269] which corresponds to $[M]_D$ = +92, which is in agreement with the sum of the contributions of an *S*-α-methylketone unit ($[M]_D$ = +44) and an *R*, *R*-hydroxy acid unit ($[M]_D$ = +40), assuming a very small contribution from the *cis*-cyclopropane component.



(260)

Another example for this occurs in the work of Hanan Ali [281] For the methoxy mycolic acid **(261)** the specific rotation of this acid was determined to be –1.6 (c = 0.99, $CHCl_3$), which corresponds to an $[M]_D$ = –24. The figure for the (*S*,*S*) α-methyl-methoxy fragment may be estimated from the present result as – 50, the $RCH{=}CHCH_3$- fragment as – 25 and *R*,*R*-centres of the 2-hydroxy acid (ester) fragment in mycolates is estimated as + 40. Therefore, the $[M]_D$ value can be estimated as –35 (–50–25+40) which is in agreement with the calculated value.



(261)

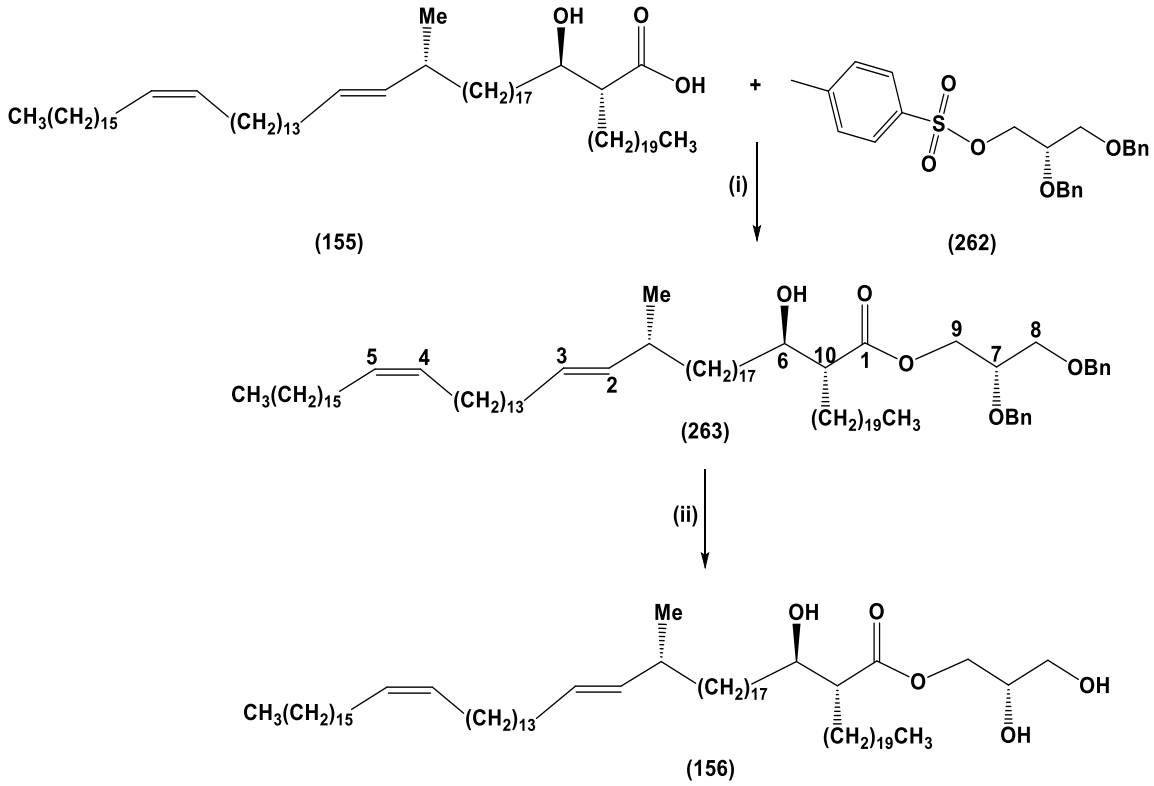
The amount of ethyl ester **(208)** obtained was not enough to carry out the same deprotection to the free acid **(155)** (see page 93).

2.11 Synthesis of sugar and glycerol esters of MA (155)

There are many lipids present within the cell wall of *M. tuberculosis*.[67] Mycobacterial cord factors are known as very interesting compounds due to their biological activity, which has been the subject of many investigations. Studies have shown that these components of mycobacteria have immune activity. The outer envelope of all mycobacteria consists of TDMs that have unique properties. 2% of the dry weight of *M. tuberculosis* is made of surface lipid making it the most abundant for extraction.[125,282] TDM is necessary for the survival of the mycobacteria inside macrophages, while GMM has immunological properties. GMMs have the capacity to produce T cell proliferative responses in a number of species including humans,[283] mice, guinea pigs, and cattle. GroMM has been identified *in vitro* as the most immunopotentiating compound among a number of different lipids isolated from the mycobacterial cell wall.[234] GroMM is a specific immune target in human individuals with latent, but not active, tuberculosis, but the *in vivo* response to GroMM and the relevance of it to latent infection remain poorly understood. GroMM is however known to be important in vaccines as an adjuvant. The methyl arabino-mycolates (MAM) is a part of the cell wall of *M. tuberculosis.* All these different kinds of sugar and glycerol mycolates could therefore play an important role in better understanding the properties of mycobacteria. In addition, investigating their biological activity could lead to improvements in the detection and diagnosis of mycobacterial diseases. The synthesis of a range of sugar esters of MA **(155)** will be discussed below.

2.11.1 Synthesis of GroMM

The first ester prepared was the GroMM **(156)**, by coupling free MA **(155)** with the protected glycerol **(262)**, followed by deprotection of the sugar moiety **(Scheme 62)**.



Scheme (62): Reagents and conditions: (i) cesium hydrogencarbonate, (*S*)-2,3-bis(benzyloxy)-propyl-4-methyl benzene sulfonate, DMF:THF (1:5), 70 ^{o}C, 65%; (ii) Na, NH_3, 1,4-dioxane, 46%.

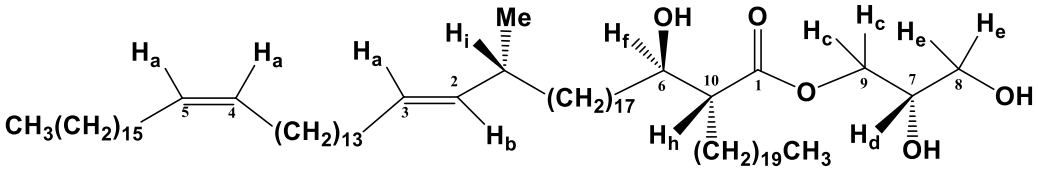
The coupling reaction between the MA **(155)** and protected glycerol **(262)**, supplied by Dr. J Al-Dulayymi, was carried out using cesium hydrogen carbonate to give protected GroMM **(263)** in 65% yield. The structure was confirmed by the ^{1}H NMR spectrum which showed a multiplet at δ 7.53 – 7.09 for the ten aromatic protons of the benzyl groups. Protons corresponding to the double bond were revealed as a multiplet at δ 5.42 – 5.29 (3H) and a double doublet at δ 5.24 (*J* 7.4, 15.3 Hz). Two doublets occurred at δ 4.68 (*J* 11.8 Hz) and δ 4.64 (*J* 11.8 Hz) for (PhC$\underline{H_2}$), and one broad singlet at δ 4.55 for the second (PhC$\underline{H_2}$). Two double doublets occurred at δ 4.43 (*J* 4.0, 11.7 Hz) and δ 4.22 (*J* 5.5, 11.7 Hz) for the protons next to the ester group, and a signal was present at δ 3.60 – 3.57 corresponding to the proton at the β-position. The signal corresponding to the proton at the α-position in the mycolate component appeared as a triplet of doublets at δ 2.43 (*J* 5.4, 9.3 Hz). Finally, a doublet at δ

0.95 (*J* 6.7 Hz) and a triplet at δ 0.89 (*J* 6.8 Hz) belonged to the methyl group and terminal (2 × CH_3) respectively. Formation of the protected compound **(263)**, was also proven by the ^{13}C NMR spectrum, which showed a signal for the carbonyl ester at δ 175.5 and the signals for the benzylic carbons at δ 73.5 and δ 72.3. The signals for C7, C9 and C8 in the glycerol unit appeared at δ 75.8 δ 69.6 and δ 63.5 respectively.

The next step was deprotection of the glycerol unit. The most common procedure used for the debenzylation is the use of palladium on carbon or palladium hydroxide as a catalyst under an atmosphere of hydrogen. This was however, not used for the deprotection of the benzyl groups in compound **(263)** due to the presence of the two double bonds in the mycolate compound of the GroMM, which would also be hydrogenated.

Selective removal of the benzyl groups in **(263)** was achieved using sodium in liquid ammonia, and gave deprotected GroMM **(156)** in 46% yield. The ^{1}H NMR spectrum confirmed that the deprotection of GroMM **(156)** had been achieved by showing that the signal pertaining to the ten aromatic protons at δ 7.53 – 7.09 had disappeared, while the two signals presenting as double doublets for the two protons next to the ester group had shifted to δ 4.26 (*J* 4.2, 11.5 Hz) and 4.19 (*J* 6.4, 11.5 Hz). Two signals seen as multiplets at δ 3.95 – 3.88 and δ 3.71 – 3.63 corresponded to the three protons next to the hydroxyl groups. Also, a broad double doublet at δ 3.56 *(J* 5.8,11.5 Hz) belonging to the proton at the β-position, a broad multiplet at δ 2.45 – 2.36 corresponded to the proton in the α- position of the mycolate, while the protons of the long chain appeared as a multiplet at δ 1.71 – 1.05. The ^{13}C NMR spectrum showed that the signals for the benzylic carbons at δ 7.53 – 7.09 had disappeared, but all signals expected for the carbons present in GroMM (**156**) were seen, **(Table 8**).

Table (8): The ^{1}H NMR and ^{13}C NMR spectrum analysis of GroMM **(156)**.

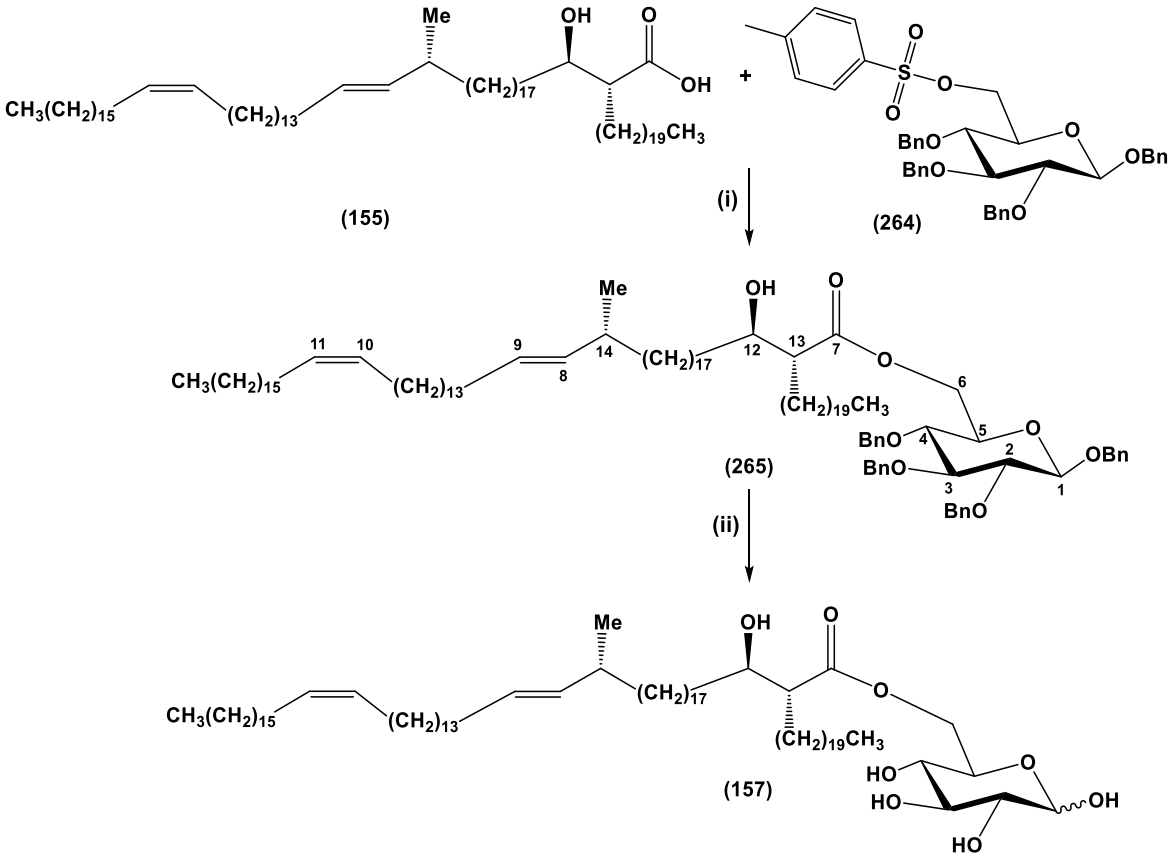


Proton	Integration	Multiplicity	*δ/ppm*	*J/* Hz	Carbon	*δ/ppm*
H_a	3	m	5.38 – 5.26	-----	C_1	175.5
H_b	1	dd	5.21	7.2, 15.4	C_2	136.4
H_c	1	dd	4.26	4.2, 11.5	C_3	130.3
H_c	1	dd	4.19	6.4, 11.5	C_4	129.8
H_d	1	m	3.95– 3.88	-----	C_5	128.4
H_e, H_e	2	m	3.71 – 3.63	-----	C_6	72.7
H_f	1	dd	3.56	5.8,11.5	C_7	69.8
H_h	1	m	2.45 – 2.36	-----	C_8	65.1
H_i and CH_2 next to the double bounds	7	m	2.03 – 1.90	-----	C_9	63.0
Alkyl chain	126	m	1.70 – 0.94	-----	C_{10}	52.4
CH_3 α-to the double bond	3	d	0.91	6.7	-----	------
Terminal CH_3	6	t	0.86	6.7	-----	-----

Nano-electrospray ionization (NSI) mass spectrometry was used to reduce fragmentation and enhance the abundance of the intact molecular ion of (**156**), giving $(M+H)^+$: 1170.1713 ($C_{78}H_{153}O_5$, requires:1170.1712].

2.11.2 Synthesis of GMM

The coupling between free mycolic acid **(155)** and protected glucose **(264)**, supplied by Dr. J. Al Dulayymi, to form the protected sugar ester, followed by deprotection of the sugar to give free GMM **(157)** was carried out as shown in **Scheme (63)**.



Scheme (63): Reagents and conditions: (i) cesium hydrogen carbonate, DMF:THF (1:5), 70 °C, 67%; (ii) Na, NH_3, 1,4-dioxane, 25%.

The protected GMM was obtained in a yield of 67% with the NMR spectra confirming the required product had been formed. The ^{1}H NMR spectrum showed a multiplet at δ 7.44 – 7.00 for the twenty aromatic protons and a multiplet at δ 5.42 – 5.30 and a double doublet at δ 5.25 (*J* 7.4, 15.4 Hz), corresponding to the protons of the two double bonds in the mycolate moiety. The signals for each of the CH_2 groups of the benzyl groups on the sugar appeared as doublets in the range δ 4.96– 4.61. A doublet occurred at δ 4.55 (*J* 11.2 Hz), corresponding to one proton attached to C-6. A broad doublet appeared at δ 4.53 (*J* 7.7 Hz), which corresponds to the β-anomeric proton attached to C-1 and a double doublet at δ 4.23

(*J* 4.5, 11.7 Hz) corresponds to the second proton attached to C-6. The spectrum also showed a broad triplet at δ 3.67 (*J* 7.6 Hz) associated with the proton attached to the C-2 and the C<u>H</u>-OH of the mycolate. A multiplet at δ 3.53–3.49 corresponds to protons attached to C-3, C-4 and C-5. The three protons of the α-methyl next to the *trans*-double bond appeared as a doublet at δ 0.95 (*J* 6.7 Hz).

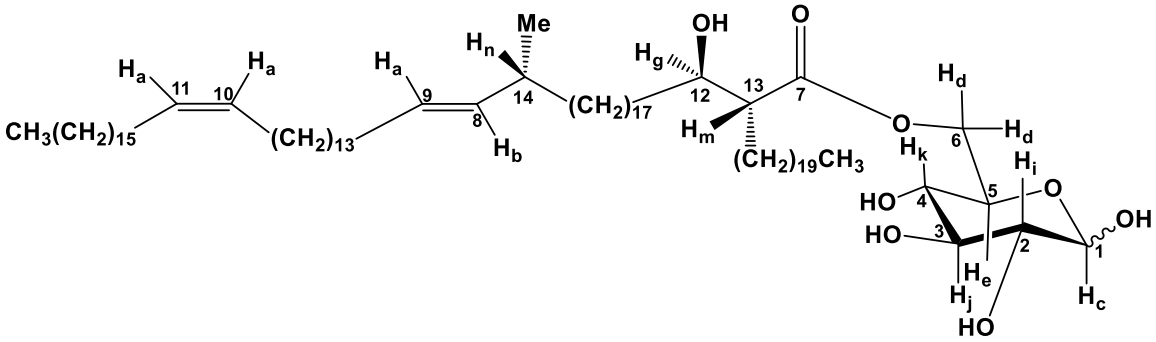
The ^{13}C NMR spectrum showed a signal for the carbonyl ester at δ 175.2 and the signals for the carbons of the two double bonds at δ 136.5, 130.4, 129.9 and 128.5. Also, the signals for the carbons of the benzyl groups appeared at δ 138.4 –127.7, while the carbons of the sugar appeared at δ 102.3, 84.5, 82.3, 77.8 and 72.9 from C1 to C5, and C6 at δ 62.9. Positions C10 and C11 (β-hydroxy position) appeared at δ 84.5 and δ 72.3 respectively.

Careful removal of the benzyl groups from the protected GMM **(265)** was achieved by using sodium in liquid ammonia to give deprotected GMM **(157)** as a mixture of α and β anomers in a ratio of (0.6:0.4) in 25% yield. The ^{1}H NMR spectrum confirmed that the deprotection of compound **(265)** had been successful; the signal pertaining to the twenty aromatic protons at δ 7.44–7.00 was absent from the spectrum of the free GMM (**157**), and the signals for the CH_2 of the benzylic protons were also not present.

The ^{1}H NMR spectrum showed a multplet at δ 5.37 – 5.23 (3H, m) and a double doublet at δ 5.19 (*J* 7.4, 15.4 Hz) corresponding to the protons of the two double bonds in the mycolate component. A doublet at δ 5.11 (*J* 3.3 Hz) corresponds to Hc-α, and another doublet at δ 4.46 (*J* 7.7 Hz) to Hc-β. The spectrum also showed a broad doublet at δ 4.41 (*J* 11.9 Hz) associated with Hd-α,β, a broad double doublet at δ 4.24 (*J* 5.9, 11.8 Hz) corresponding to Hd-α,β, a double double doublet at δ 3.95 (*J* 2.2, 5.8, 9.8 Hz) related to He-α, and another multiplet at δ 3.67 – 3.58 corresponding to Hk-α and CH-OH of the mycolate component. Another signal which appears as a double double doublet at δ 3.48–3.44 (*J* 2.2, 5.8, 9.8 Hz) is related to He-β; a multiplet at δ 3.43–3.26 corresponds to Hi,j-α and Hj,k-β. Another broad triplet occurs at δ 3.17 (*J* 8.4 Hz) corresponding to Hi-β. All these data confirmed the formation of the free GMM, and a detailed analysis of the ^{1}H NMR can be seen in **Table (9)** below. Formation of the deprotected GMM **(157)** was also demonstrated by the ^{13}C NMR spectrum, which showed that the signals for the carbon environments in the aromatic region were not present.

The analysis of the ^{13}C NMR spectrum showed all signals expected for the carbons present in free GMM **(157)**

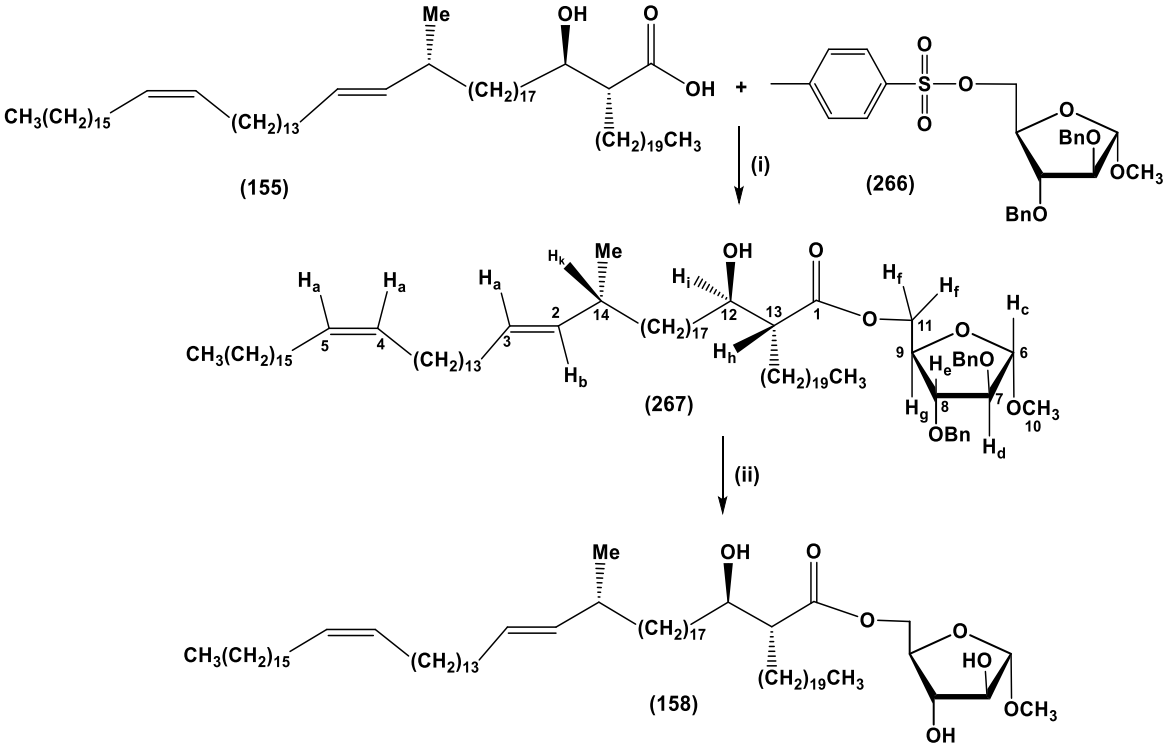
Table (9): ^{1}H and C^{13} NMR analysis of GMM **(157)**.



Proton	Integration	Multiplicity	*δ/ppm*	*J/* Hz	Carbon	*δ/ppm*
H_a	3	m	5.37 – 5.23	-----	C-1α	92.3
H_b	1	dd	5.19	7.4, 15.4	C-1β	96.6
Hc-α	1	d	5.11	3.3	C-2α	72.2
Hc-β	1	d	4.46	7.7	C-2β	74.6
H_d-α,β	1	br. d	4.41	11.9	C-3α	70.5
H_d-α,β	1	br.dd	4.24	5.9,11.8	C-3β	76.2
H_e-α	1	ddd	3.96-3.92	2.2, 5.8, 9.8	C-4α	73.7
H_e-β	1	ddd	3.48-3.44	2.2, 5.8, 9.8	C-4 β	70.3
Hk-α and Hg	2	m	3.43-3.26	-----	C-5α	69.2
Hi-β	1	br.t	3.17	8.4	C-5β	73.7
H_m	1	m	2.42 – 2.34	-----	C-6α/β	63.6
H_n and CH_2 next to the double bounds	7	m	2.07 – 1.84	-----	C-6α/β	63.5
CH_3 α-to the double bond	3	d	0.89	6.7	C 7	175.2
Terminal CH_3	6	t	0.83	6.7	C8	136.4
					C9	130.3
					C10	129.9
					C11	128.4
					C12	72.5
					C-13α/β	52.6
					C-13α/β	52.4

2.11.3 The synthesis of arabino-MA fragment from AG

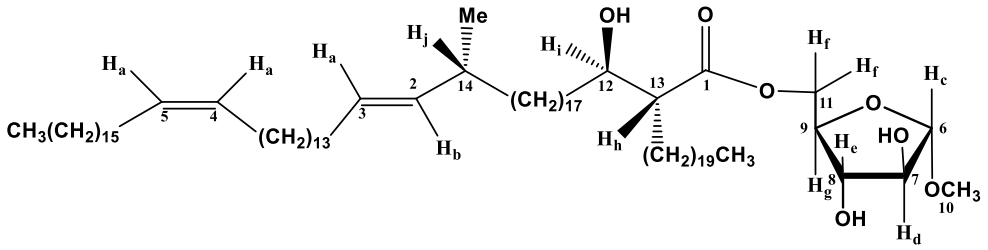
The esterification between the free mycolic acid **(155)** and protected arabinofuranoside **(266)** which was supplied by Dr. J. Al Dulayymi, was carried out using dry cesium hydrogen carbonate in a mixture of dry DMF:THF and gave the protected compound **(267)** in 58% yield, **(Scheme 64)**. The ^{1}H NMR spectrum of the protected compound **(267)** showed a multiplet at δ 7.45 – 7.20 for the ten aromatic protons, a multiplet at δ 5.41 – 5.29 and a double doublet at δ 5.25 (*J* 7.4, 15.4 Hz) corresponding to 3Ha and 1Hb respectively of the double bonds. A broad singlet at δ 4.92 corresponded to H_c, and the signals for CH_2 of the benzyl group appear as doublet in the range δ 4.58–4.48, together with a multiplet at δ 4.24 – 4.20 integrating to one to proton corresponding to H_g. Also, a doublet at δ 3.99 (*J* 2.3 Hz) corresponded to Hd and a double doublet at δ 3.84 (*J* 2.6, 6.5 Hz) corresponded to H_e. A multiplet appeared at δ 4.32-4.28 for H_f, while the singlet at δ 3.38 corresponded to the methoxy group. The ^{13}C NMR spectrum showed signals for the carbonyl ester at δ 175.0, while the two signals for the CH_2 of the benzyl group appeared at δ 72.4 and δ 72.1 together with a signal at δ 72.2 for C12, while C-6, C-7, C-8, C-9 and C-11 were seen at δ 107.2, 87.9, 83.7, 79.4 and 63.5 respectively. The O<u>C</u>H$_3$ group appeared at δ 54.9.



Scheme (64): Reagents and conditions: (i) cesium hydrogen carbonate, DMF:THF (1:5), 70 °C 58%; (ii) Na, NH_3, 1,4-dioxane 39%.

The removal of the benzyl groups in **(267)** was achieved using sodium in liquid ammonia to give the deprotected compound **(158)**, in 39% yield. The ^{1}H NMR spectrum of this showed that the signals corresponding to Hc had shifted upfield to δ 4.81 from δ 4.92 after losing the deshielding effect of the aromatic ring. The ^{1}H NMR spectrum confirmed that the deprotected compound **(158)** had been obtained successfully, **Table (10)**.

Table (10): NMR ^{1}H and ^{13}C analysis of fragment from AG **(158)**.

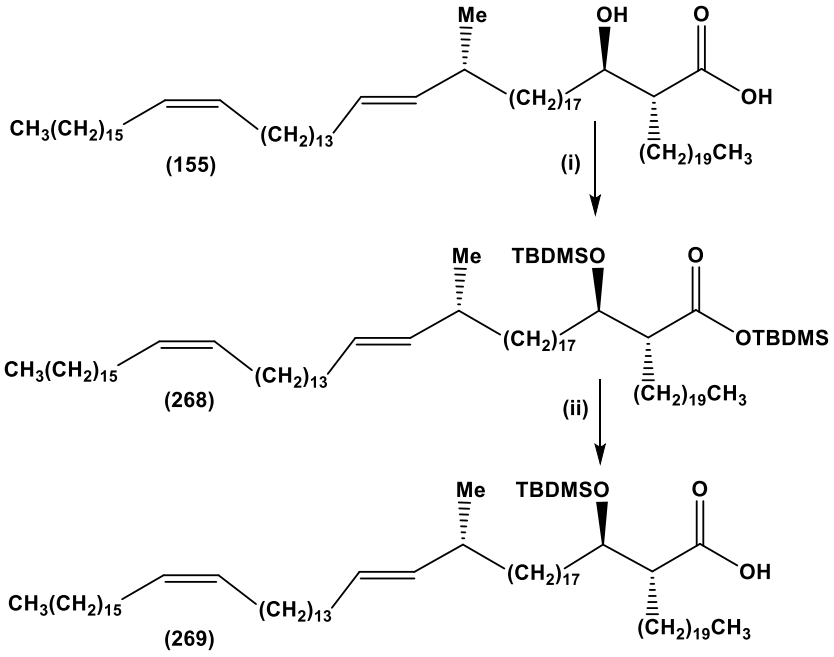


Proton	Integration	Multiplicity	*δ/ppm*	*J/* Hz	Carbon	*δ/ppm*
H_a	3	m	5.37 – 5.26	-----	C_1	175.0
H_b	1	dd	5.20	7.4, 15.4	C_2	136.4
H_c	1	br.s	4.81	-----	C_3	130.3
H_f	1	dd	4.32	4.7, 9.8	C_4	129.8
H_f	1	dd	4.28	4.9, 10.1	C_5	128.3
H_g	1	br.q	4.09	4.7	C_6	108.6
H_d	1	dd	4.0	1.9, 3.7	C_7	82.0
H_e	1	dd	3.87	2.7, 4.7	C_8	81.2
H_i	1	m	3.69 – 3.55	-----	C_9	77.9
OCH_3	3	s	3.36	-----	C_{10}	55.0
H_h	1	m	2.45 – 2.36	-----	C_{11}	63.4
CH_3 α-to the double bond	3	d	0.90	6.7	C_{12}	72.5
Terminal CH_3	6	t	0.85	6.8	C_{13}	52.5

2.12 Synthesis of trehalose esters (TDM and TMM) of the *cis*-alkene-α-methyl-*trans*-alkene mycolic acid

The synthetic *cis*-alkene-α-methyl-*trans*-alkene mycolic acid **(155)** was esterified with trehalose to produce trehalose dimycolate (TDM) and trehalose monomycolate (TMM). In order to prepare a TDM from the unsaturated mycolic acid **(155)**, it was necessary to protect the β-hydroxyl group with a TBDMS protecting group. The mycolic acid **(155)** was stirred with imidazole, *tert*-butyldimethylsilyl chloride and DMAP in a mixture of DMF/toluene at 70 °C for 18 hours.

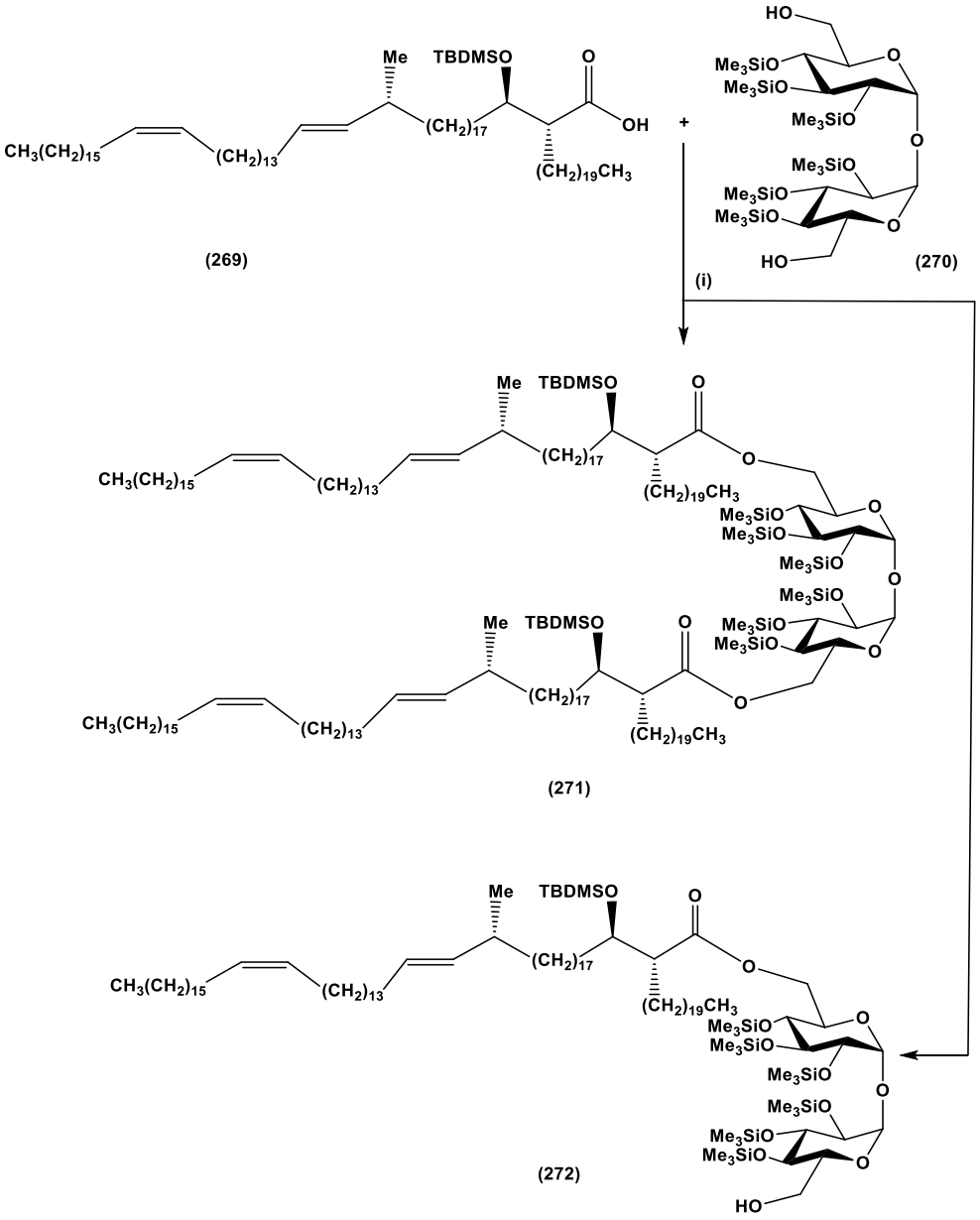
The reaction usually leads to the protection of both the β-hydroxy group and the carboxylic acid to give **(268)**. The next step was therefore the selective deprotection of the carboxylic acid group, by stirring it with K_2CO_3 in a mixture of THF/H_2O/MeOH for 3 hours at 45 °C, to give the target compound **(269)**, **(Scheme 65)**.



Scheme (65): Reagents and conditions: (i) imidazole, DMF, toluene, *tert*-butyldimethylsilyl chloride, 4-dimethylaminopyridine 70 °C, 18 hrs (ii) THF/H_2O/MeOH, K_2CO_3, 80%.

The proton NMR spectrum of compound **(269)** showed the silyl protecting group protons as a singlet at δ 0.91 integrating to 9H and two singlets at δ 0.12 and δ 0.10, each integrating to 3H, for the six protons of the two methyl groups. The ^{13}C NMR spectrum also confirmed the protection, which showed that the signals that related to the methyl carbons on the *tert*-butyl

group appeared at δ 25.7, the signal for the quaternary carbon at δ 17.9 and the two signals for the methyl groups bonded to silicon at δ – 4.3 and δ – 5.0. The esterification reaction between the protected mycolic acid **(269)** and protected trehalose **(270)**, which was supplied by Dr. J. Al Dulayymi, was achieved successfully after 6 days at room temperature under nitrogen gas using coupling reagents 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) and DMAP in CH_2Cl_2. The protected cord factors (TDM and TMM), were then separated by column chromatography **Scheme (66)**.



Scheme (66): Reagents and conditions (i) DMAP/EDCI/CH_2Cl_2, TDM 17%, TMM 70%.

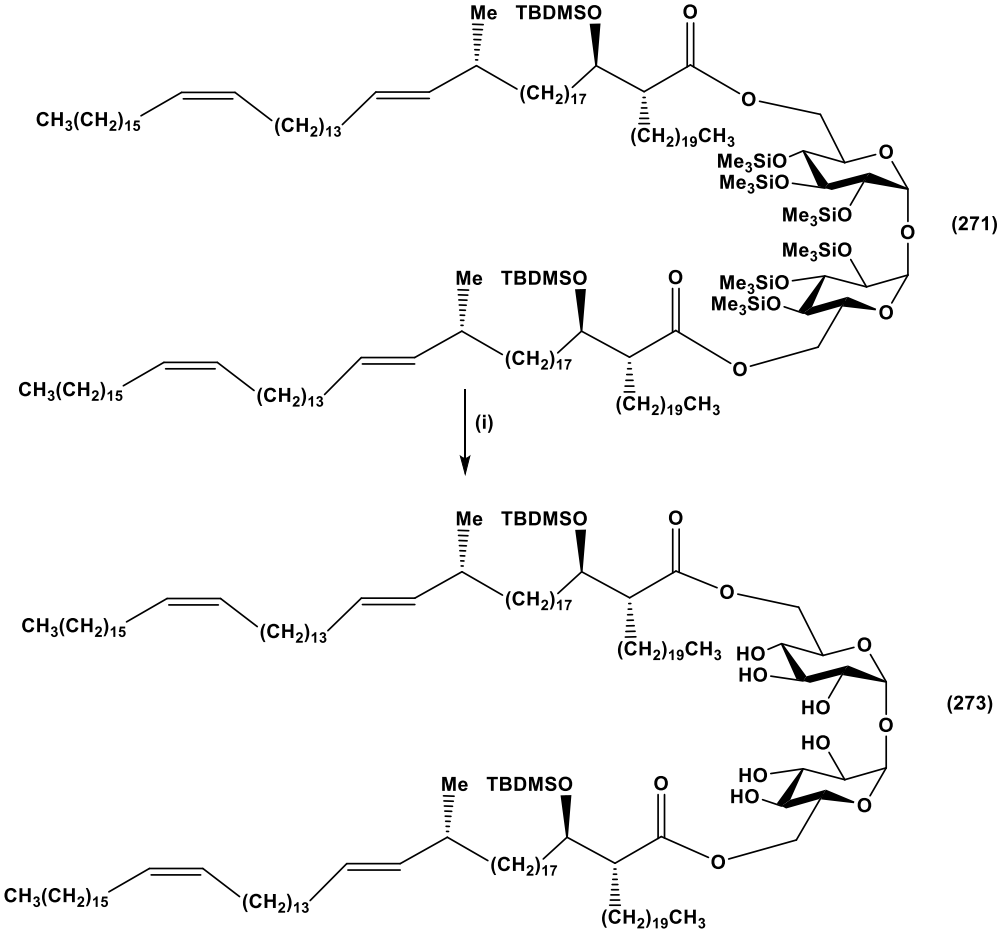
The first product to be obtained was the TDM **(271)** in 17% yield with the structure being confirmed by ^{1}H and ^{13}C NMR spectroscopy and MALDI mass spectrometry. The ^{1}H NMR spectrum of TDM **(271)** displayed signals for the *tert*-butyl protons of the silyl protecting group as a singlet at δ 0.88 (18H), with the multiplet at δ 5.41 – 5.30 and the double doublet at δ 5.24 (*J* 7.4, 15.4 Hz) corresponding to the eight alkene protons. A doublet at δ 4.85 (*J* 2.9 Hz) corresponded to the two acetal protons of the trehalose. The remaining trehalose protons, including the β-hydroxyl protons resonated at δ 4.37, 4.01, 3.93, 3.90, 3.53 and 3.38. The α- proton of the mycolic acid appeared as a multiplet at δ 2.61 – 2.51, and a multiplet appeared at δ 2.10 – 1.92, integrating to fourteen protons, which corresponded to those next to the double bonds and the proton adjacent to the α-methyl next to the *trans* double bond. The methyl group appeared at δ 0.94 (*J* 6.7 Hz) as a doublet integrating to six protons, while the terminal methyl groups of the alkyl chains appeared as a triplet at δ 0.89 *(J* 6.6 Hz). The protons of the trimethylsilyl protecting groups on the trehalose gave three singlets at δ 0.16, 0.15, and 0.14 each integrating to eighteen protons. The methyl groups of the TBDMS protecting group showed a singlet at δ 0.06 integrating to twelve protons.

The ^{13}C NMR spectrum showed a signal at δ 173.8 for the carbonyl carbon. The double bond carbon signals appeared at δ 136.5, 130.4, 129.9, and 128.4. Also, the remainder of the sugar carbons gave signals between δ 73.4 – 70.7. The methyl carbon signals of the protecting silyl groups of the sugar appeared at δ 1.09, 0.94 and 0.16. Additionally, the carbons of the two methyl groups bonded to silicon in the silyl groups of the mycolate component appeared at δ – 4.5 and δ – 4.7.

The second fraction was the TMM **(272)**, obtained in a yield of 70%. The hemiacetal protons appeared as two doublets at δ 4.92 (*J* 3.0 Hz) and δ 4.85 (*J* 2.9 Hz) with an integration of one proton for each, here the signals were more complicated due to the loss of symmetry. The remaining sugar protons appeared between δ 4.36 – 3.4. It was found that the methyl signals for the protecting groups on the sugar were divided into six groups, one for each (Me) situated at C2-C3-C4, between δ 0.17 – 0.13, in each case they integrated to 9H and to 54 hydrogens in total. The signals for the protecting groups were similar to those found for the TDM **(271)**, but with half the number of the protons relating to mycolic acid sub class. The ^{13}C NMR spectrum displayed signals at δ 174.1 for the ester group and at δ 94.5 and 94.4 for the C1 in the sugar cores. Signals at δ – 4.5 and – 4.7 were displayed for the two methyl groups bound to the silicon in the TBDMS group.

2.12.1 Trehalose deprotection of TDM (271) and TMM (272)

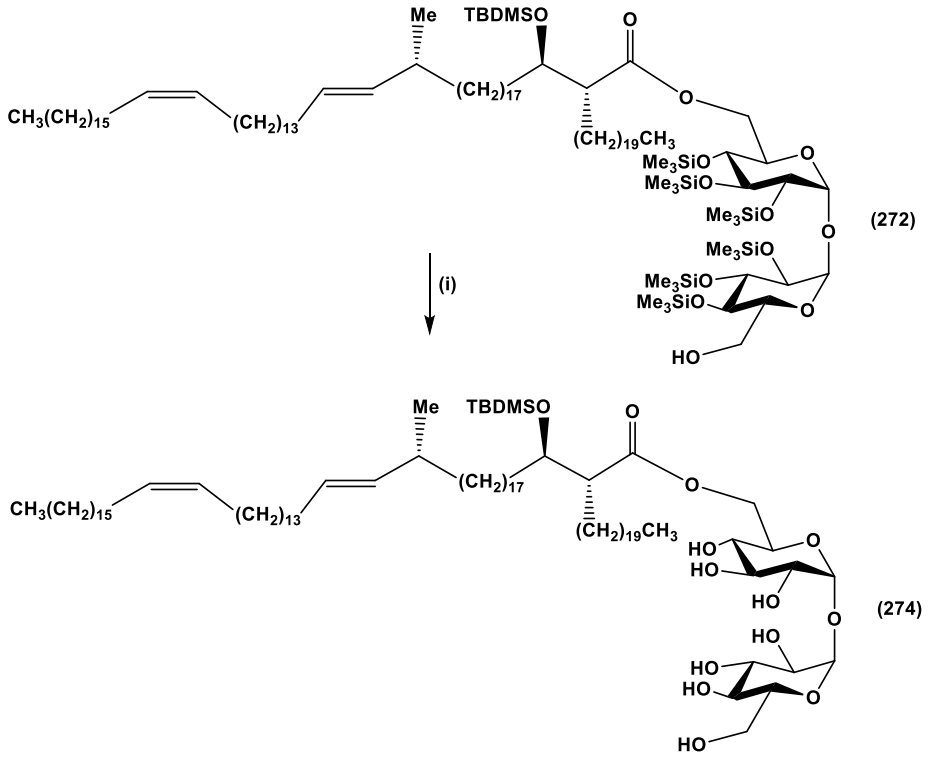
The trimethylsilyl protecting groups on the trehalose moiety of both TDM **(271)** and TMM **(272)** were then removed. This deprotection was carried out using tetra-*n*-butyl ammonium fluoride (TBAF) to give the silyl-protected TDM **(273) (Scheme 67)** and silyl-protected TMM **(274)**, **(Scheme 68)**.



Scheme (67): Reagents and conditions: (i) tetrabutylammonium fluoride, THF, TDM, 15%.

The ^{1}H NMR spectrum of silyl-protected TDM **(273)** showed a multiplet at δ 5.36 – 5.21 and a double doublet at δ 5.17 (*J* 7.7, 14.0 Hz) for the protons of the double bonds while the doublet for the hemiacetal protons was shifted to δ 4.98 (*J* 3.2 Hz). The remaining signals for the sugar protons and the β-hydroxyl protons resonated at δ 4.35, 4.20, 3.91, 3.85, 3.73, 3.46 and 3.34, and were also shifted. The proton at the α-position appeared as a multiplet at δ 2.42 – 2.30, while the doublet at δ 0.88 (*J* 6.7 Hz) corresponded to the α-methyl *trans* group. The terminal methyl groups gave a triplet at δ 0.84 (*J* 6.3 Hz) and the *tert*-butyl

groups showed as a singlet at δ 0.81 with an integration of eighteen protons. The signals corresponding to the trimethylsilyl protecting groups on the sugar were not present in the spectrum, which confirmed that the reaction had been successful.

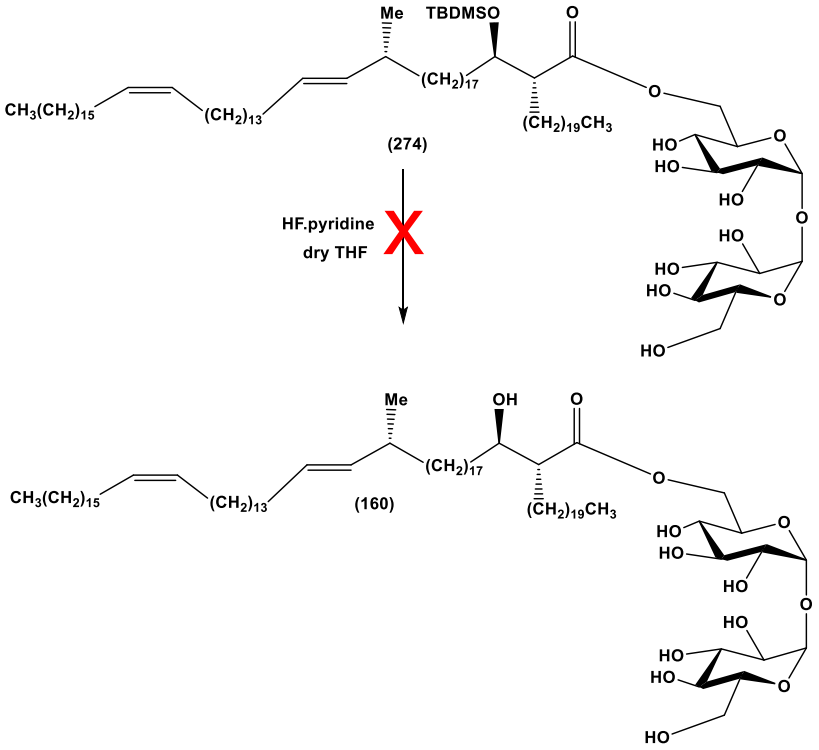


Scheme (98): Reagents and conditions: (i) tetrabutylammonium fluoride, THF, TMM 30%.

The ^{1}H NMR spectrum of the silyl-protected TMM **(274)** showed a multiplet at δ 5.38 – 5.26 and a double doublet at δ 5.20 (*J* 7.4, 15.3 Hz) for the protons of the double bonds, while the hemiacetal protons appeared as a broad triplet at δ 5.06 (*J* 3.5 Hz) and a double doublet at δ 4.28 (*J* 3.7, 10.6 Hz) each with an integration of one proton. The remaining sugar protons resonated between δ 4.23 – 3.46. The signals belonging to the trimethylsilyl protecting groups of the sugar at δ 0.17, 0.16 and 0.15 were not present in the ^{1}H NMR spectrum, and the signals at δ 1.1, δ 1.0 and δ 0.9, corresponding to the carbons of the trimethylsilyl groups, were also absent from the ^{13}C NMR spectrum, which indicated that the removal of the silyl groups from the sugar had been successful.

2.12.2 Deprotection of the hydroxy acid part of TDM (273) and TMM (274)

The final step in the synthesis of TDM **(159)** and TMM **(160)** was the removal of the TBDMS groups from β-position of compounds **(273)** and **(274)**. Due to the small amount of material for TDM **(273)** this reaction was not attempted. The deprotection of TMM **(274)** was attempted in dry THF using HF.Pyridine in pyridine, however the compound was lost during the purification, **Scheme (69**).



Scheme (69): Desilylation of the hydroxy acid of TMM **(274)**.

Neither the free TDM **(159)** nor TMM **(160)** were prepared here, however the deprotection of the TBDMS group, using HF.Pyridine, has been carried out successfully for similar TDM and TMM compounds. It is therefore likely had more material been available this deprotection would yield the required products, **(159)** and **(160)**.

Chapter 3

3. Conclusion and further work

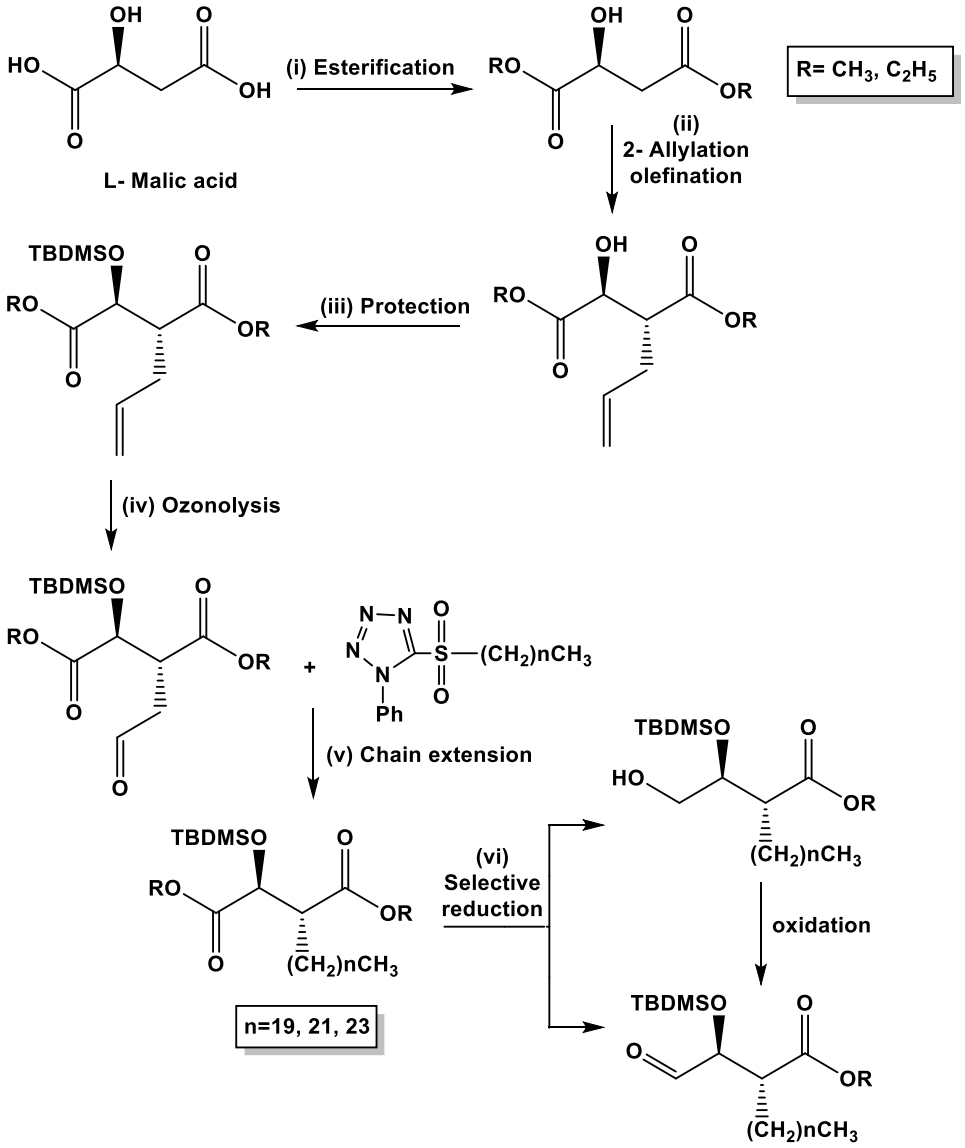
3.1 Conclusion

The three main amis of this project wrere: to develop an improved method for the synthesis of the α-alkyl β-hydroxy (mycolic motif) unit; to use this for the synthesis of a single enantiomer of a *cis*-alkene-α-methyl-*trans*-alkene mycolic acid, *i.e.* found in *M. brumae*; and to prepare various sugar and glycerol esters of this specific M.A.

Thus, initial work focussed on the development of an improved method for the synthesis of the mycolic motif unit. The preparation of this intermediate unit has so far proved to be the most difficult part of the mycolic acid synthesis. Many methods have been reported for the preparation of α-alkyl-β-hydroxy esters, Al Dulayymi *et al.*[251] used (*S*)-(2-benzeneoxyethyl) oxirane **(161)** as a chiral intermediate prepared from commercially available *D*-aspartic acid based on a method developed by Frick *et al*.[252] In a different approach, Toschi *et al.,*[202] used a short chain allylation at the α- position in the initial step followed by further elongation of this by a modified Julia-Kocienski reaction to achieve the intended chain length.

More recently Koza *et al.,*[203] used *L*-aspartic acid as the starting material for the synthesis of the (*R*)-(2-benzeneoxyethyl)oxirane intermediate **(92)** in four steps, followed by a short chain Fräter allylation and a further Julia-Kocienski chain extension at the α-position. Khan *et al.*[206] on the other hand prepared the β-hydroxy ester using *L*-malic acid as starting material. This was esterified to give diethyl (*S*)-malate, which was then alkylated with different allylic halides by Fräter allylation followed by selective reduction with BH_3·DMS to give diol.

In this thesis an alternative method that involved fewer steps was targeted, using the cheaper, commercially available *L*-malic acid as starting material, **Scheme (70**).

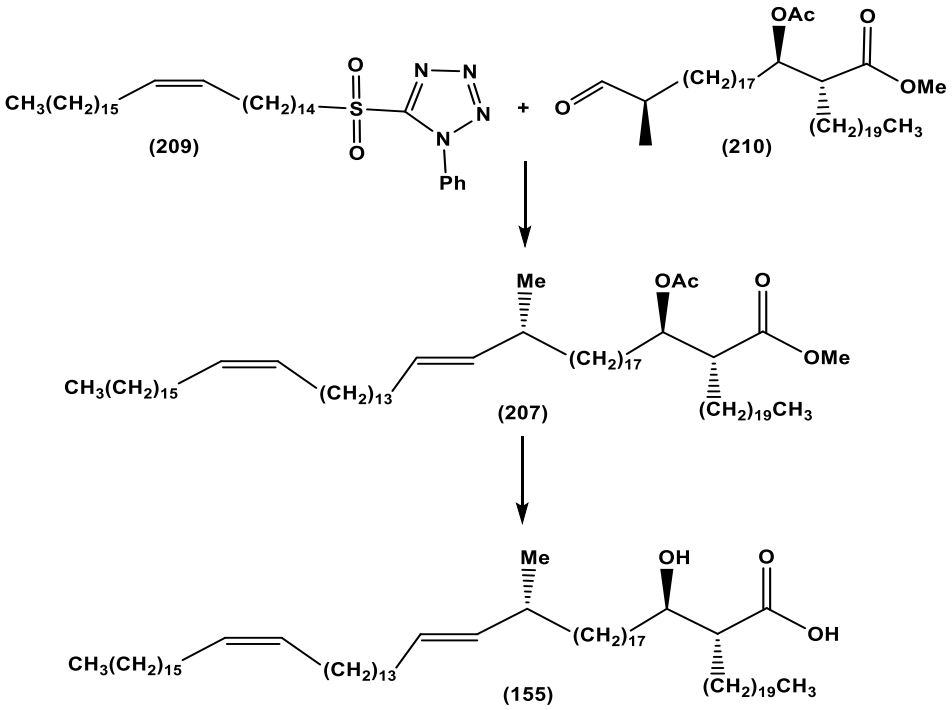


Scheme (70): A new method for the preparation of the mycolic motif unit. Reagents and conditions: (i) 1- $SOCl_2$/ ROH; (ii) LDA/ AllBr/THF; (iii) TBDMSCl / imidazole/ DMF; (iv) O_3/O_2 ; (v) (1)$LiN(SiMe_3)_2$ (2)/ H_2/Pd/C; (vi) DIBAL/$MgBr_2.Et_2O$.

Following this approach the required mycolic motif unit was successfully obtained in 6 steps in an overall yield of 27%. This is a significant improvement compared to previous methods starting from *L*-aspartic acid (14 steps, 10% yield) and *L*-malic acid (10% yield over 10 steps), as the number of reaction steps has been reduced and the overall yield of the product improved.

Moreover, there was no need to use HMPA in the preparation of the mycolic motif unit in this synthesis (the yield of the allylation step was found to be the same with or without using HMPA). Also, as the mycolic motif unit is common to all mycolic acids, having an improved route to this will be beneficial in the synthesis of all mycolic acids.

The next part of this work involved the use of the mycolic motif unit discussed above to prepare the *cis*- alkene-α-methyl- *trans*-alkene MA **(155)**, which has been isolated from *M. brumae.* The synthesis of this MA **(155)** was achieved over a multi step sequence from the commercially available starting materials *D*-mannitol **(219)**, tetradecanedioic acid **(222)** and lactone **(227)**. These were used as precursors for various aldehyde and sulfone compounds, which were then coupled together to give the target MA. The final coupling step, between the proximal position **(209)** and meromycolate moiety **(210)** is shown in **Scheme (71)**.



Scheme (71): The coupling to form a mycolic acid.

The studies carried out to optimise the yield of the α-methyl-*trans*-alkene proved that the yield of the modified Julia olefination and the stereoselectivity in the final coupling reaction were sensitive to the base used to deprotonate the sulfone and the solvent polarity. Using 1-phenyl-1*H*-tetrazole-5-yl sulfones with potassium *bis*(trimethylsilyl)amide as base and 1,2-dimethoxyethane or tetrahydrofuran as solvent gave high *E*-stereoselectivity. It was confirmed that if the sulfone or

aldehyde was α-substituted (*i.e.* **210**) the reaction was completely sterioselective for the formation of the *E*-isomer. This can be seen in **Scheme (71)** where the coupling reaction between the aldehyde **(210)** and the sulfone **(209)** using potassium bis(trimethylsilyl)amide in THF gave the desired alkene **(207)** as only the *E*-diastereomer in a yield of 35%. This coupling formed the whole structure of the *cis*-alkene-α-methyl-*trans*-alkene mycolic acid and, following hydrolysis, the free mycolic acid **(155)** was obtained in the correct stereochemistry.

Having successfully prepared MA **(155)**, the final part of this project involved its use in the preparation of a range of sugar and glycerol esters. Esterification of the free mycolic acid **(155)** with protected glycerol **(262)**, glucose tosylate **(264)** and arabinose tosylate **(266)** gave the protected GroMM **(263)**, GMM **(265)** and ArM **(267)**. The harder step was the debenzylation of the sugar moieties in the presence of the two double bonds, in the MA moiety. This was achieved using sodium in liquid ammonia to give free GroMM **(156)**, GMM **(157)** and MAM **(158)** in yields of 46, 25 and 39% respectively **Figure (50)**.

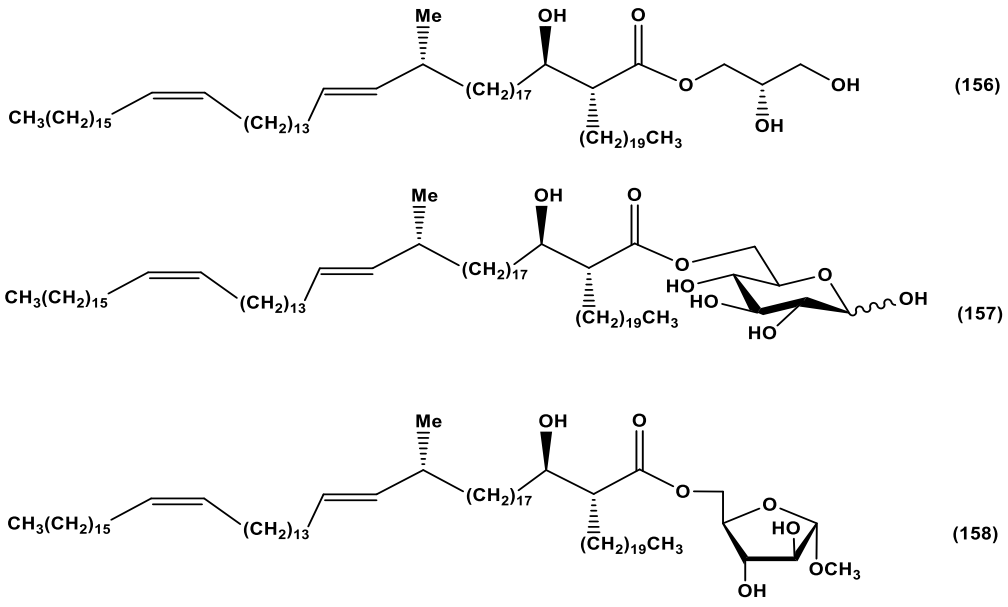
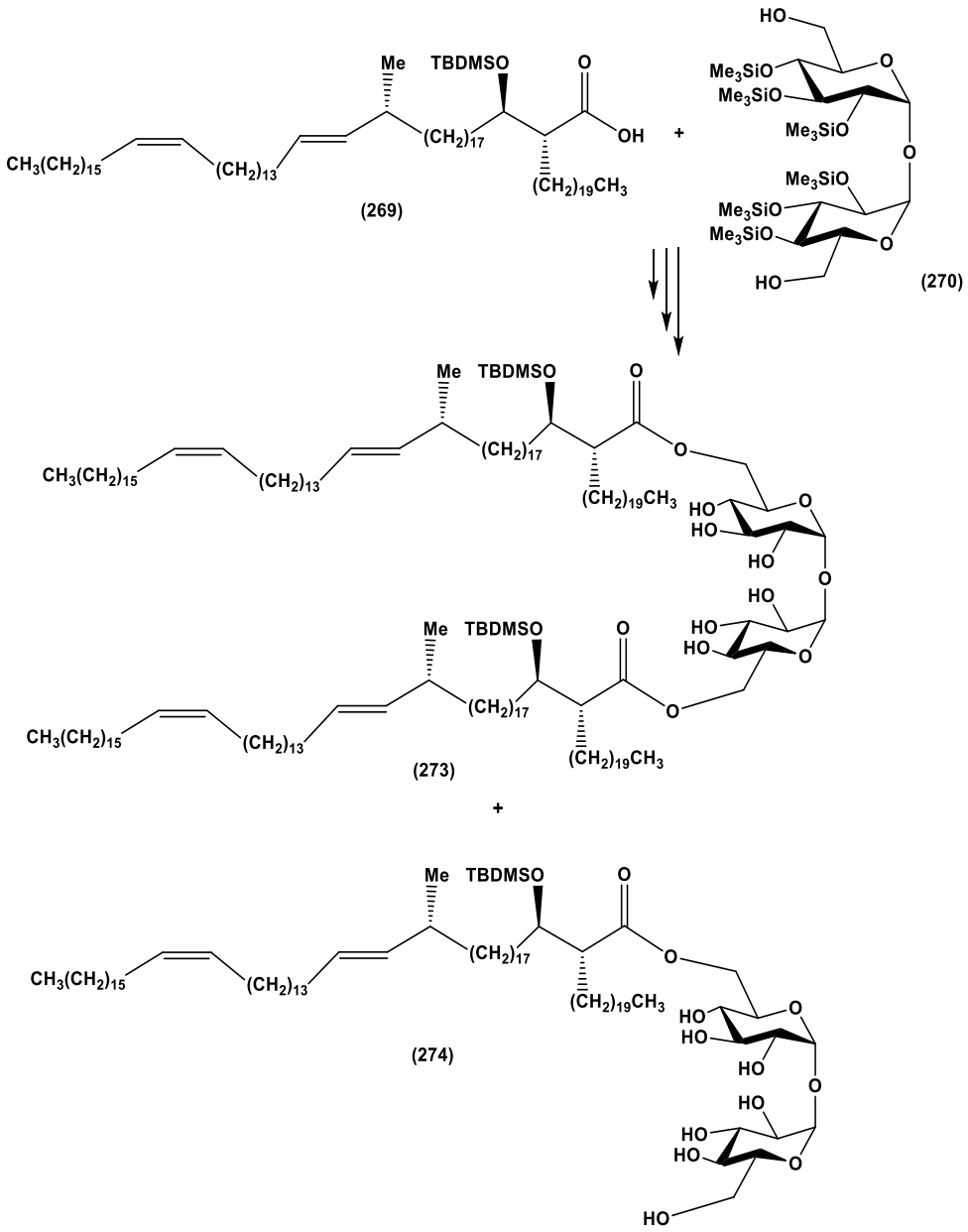


Figure (50): Sugar and glycerol esters of mycolic acid **(155)**.

The synthesis of the TDM and TMM cord factors of MA **(155)** was also attempted. The secondary alcohol of MA **(155)** was firstly protected as a TDMS group and this was then coupled to the protected trehalose **(270)**, which after purification by column chromatography gave TDM **(271)** and TMM **(272)**. Deprotection of the sugar moiety of both these compounds gave TDM **(273)** and TMM **(274)** in 15% and 30% yields respectively **Scheme (72)**.



Scheme (72): Preparation of protected TMM and TDM.

Due to the small amount of material obtained for TDM **(273)**, removal of the TBDMS group was not attempted. This reaction was attempted for TMM **(274)** using HF. Pyridine in pyridine, however, unfortunately the material was lost during purification.

In summary a new shorter method for the synthesis of the mycolic motif unit has been successfully developed. The synthesis of the free MA **(155)** and its sugar and glycerol esters **(156)**, **(157)** and **(158)** has also been achieved. The biololgical activity of these new antigens can now be investigated and compared to that of other MA cord factor antigens.

3.2 Further work

1. Preparation of a number of different ester mycolates of MA **(155)** through coupling with different sugars, *e.g* arabino glycerol, di-arabino glycerol, methoxy tri-arabinose and tri-arabino glycerol, to investigate their biological activity.

2. Synthesis of an α-mycolic acid of *Mycobacterium brumae* containing a *trans*-alkene and an α-methyl-*cis*-alkene (reverse stereochemistry of the synthesised mycolic acid (**155**)), to compare the biological activity for both compounds.

3. Determine and compare the biological activity of the synthesised compounds. Indeed, the choice of new target molecules in each of the sections of this thesis will be determined by the results of detailed on-going biological assays. These compounds could then be utilised to study the immunological properties of synthetic analogs, which would then allow for the determination of those structural features, which are responsible for their biological activity.

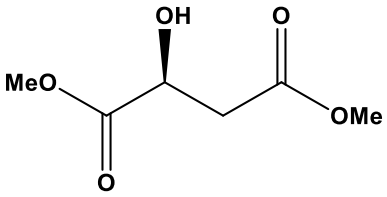
Chapter 4

4. Experimental

4.1 General considerations

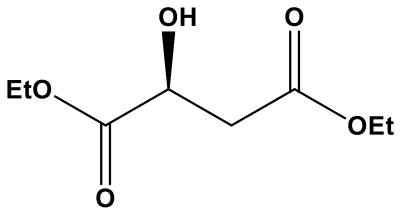
All chemicals and reagents were purchased from chemical companies (Sigma-Aldrich, Alfa Aesar, and Acros). THF was distilled over sodium and benzophenone under nitrogen, while dichloromethane, diisopropylamine and HMPA were distilled over calcium hydride. Petrol refers to the fraction b.p. 40–60 °C. Organic solutions were dried over anhydrous magnesium sulfate and solvents were removed at 14 mmHg; residual traces of solvent were finally removed at 0.1 mmHg. All reactions were performed using glassware dried in an oven at (120 ºC). Reactions carried out under inert conditions were carried out under a slow stream of nitrogen and for low temperature reactions liquid nitrogen/IMS was used to cool the solutions, the temperature being controlled using a digital thermometer. Solid compounds were purified by re-crystallisation; column chromatography and distillation were used for purification and separation of oily compounds. Column chromatography was conducted under medium pressure using silica gel (BDH, particle size 33–70 mm); TLC was carried out on pre-coated Kieselgel 60 F254 (Art. 5554; Merck) plates; separated components were detected using UV light, I_2 or phosphomolybdic acid solution in IMS followed by charring. Melting points were measured using a Gallenkamp melting point apparatus. Optical rotations were measured as solutions of known concentration in chloroform using a Polar 2001 automatic polarimeter. Ozonolysis was carried out by using ANSEROS GENERATOR COM, ozone generator series com-AD, ozongenerator COM-AD-01. All NMR samples were recorded on Bruker Avance (400 MHz) spectrometer and were prepared as solutions in deuterated $CDCl_3$ or CD_3OD unless otherwise indicated. Chemical shifts are quoted in δ relative to the trace resonance of proton chloroform (δ_H 7.27 ppm, δ_C 77.0 ppm), and the resonances of methanol (δ_H 4.87 and 3.31 ppm, δ_C 49.00 ppm). IR (infrared) spectra were performed on a Perkin-Elmer 1600 FTIR, and using KBr disc for the solid compounds. NSI and MALDI mass spectrometry data was obtained from the EPSRC UK National Mass Spectrometry Facility at Swansea University and Dr Paul Gates (Bristol University). A laboratory book was filled in including chemical safety information following COSHH regulations.

Experiment 1: Dimethyl (*S*)-2-hydroxysuccinate (164)



Thionyl chloride $SOCl_2$ (59.5 mL, 0.820 mol) was slowly added to a stirred solution of *L*-malic acid **104** (50.0 g, 0.372 mol) in methanol (300 mL) at 0 ºC under N_2 and the mixture was stirred overnight at room temperature. The reaction mixture was neutralized with 5% aqueous $NaHCO_3$ and the excess methanol was evaporated. The aqueous layer was re-extracted with CH_2Cl_2 (2 × 200 mL) and then the combined organic layers were dried over $MgSO_4$, filtered and concentrated. The crude product was purified by flash distillation at 90 – 120 ºC to give a colourless oil, dimethyl (*S*)-2-hydroxysuccinate **164** (59 g, 98%) $[\alpha]_D^{23}$ = +5.4 (c 0.56, $CHCl_3$) (*lit.*[152] $[\alpha]_D^{22}$ = + 8.6 (c 1.2, EtOH)). This showed δ_H (400 MHz, $CDCl_3$): 4.50 (1H, dd, *J* 5.0, 10.3 Hz), 3.80 (3H, s), 3.70 (3H, s), 3.30 (1H, d, *J* 5.0 Hz), 2.86 (1H, dd, *J* 4.4,16.4 Hz), 2.78 (1H, dd, *J* 6.2, 16.4 Hz); δ_C (101 MHz, $CDCl_3$): 173.7, 171.0, 67.2, 52.8, 52.0, 38.4; ν_{max}: 3489, 3023 ,2956, 2852, 1741, 1439, 1368, 1273, 1219, 1173, 1108, 1045 cm^{-1}. All data matched those in the literature.[152]

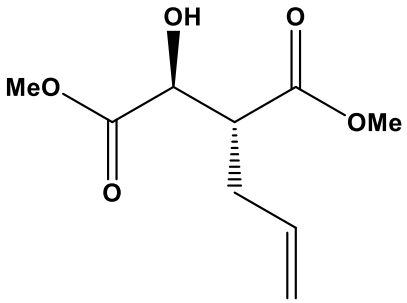
Experiment 2: Diethyl (*S*)-2-hydroxysuccinate (105)



The above experiment was repeated using thionyl chloride $SOCl_2$ (59.5 mL, 0.820 mol) *L*-malic acid **104** (50.0 g, 0.372 mol) in ethanol (300 mL) at 0 ºC. The reaction mixture was worked up and purified as above to give a colourless oil diethyl (*S*)-2-hydroxysuccinate **105** (70 g, 99%) $[\alpha]_D^{22}$ = + 8.2 (c 0.71, $CHCl_3$). (*lit.*[256] $[\alpha]_D^{22}$ = + 9.1 (*c* 2.2, EtOH)). This showed δ_H (400 MHz, $CDCl_3$): 4.49 (1H, dd, *J* 5.6, 10.3 Hz), 4.33 – 4.23 (2H, m), 4.18 (2H, q, *J* 7.1 Hz), 3.22 (1H, d, *J* 5.4 Hz), 2.86 (1H, dd, *J* 4.5, 16.3 Hz), 2.79 (1H, dd, *J* 6.0, 16.3 Hz), 1.29 (6H, t, *J* 7.1 Hz); δ_C (101 MHz, $CDCl_3$): 173.6, 170.5, 67.5, 61.9, 60.6, 38.7, 13.2; ν_{max}: 3485, 3020, 2958,

2849, 1743, 1436, 1370, 1270, 1220, 1176, 1109, 1049 cm^{-1}. All data matched those in the the literature.[256]

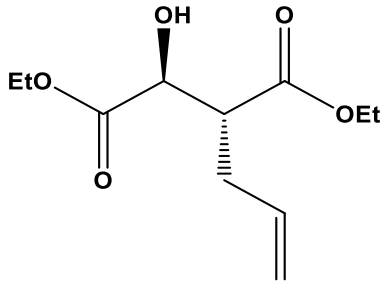
Experiment 3: Dimethyl (*2R,3S*)-2-allyl-3-hydroxysuccinate (165)



Methyl lithium (407 mL, 0.610 mol, 1.5 M) was added dropwise to a stirred solution of dry diisopropylamine (57.0 mL, 41.1 g, 0.407 mol) in dry THF (150 mL) at −78 ºC which was then allowed to reach room temperature and stirred for 30 min. The solution was cooled down again to −61 ºC and dimethyl (*S*)-2-hydroxysuccinate **164** (30 g, 0.18 mol) in dry THF (40 mL) was slowly added. The temperature was allowed to rise gradually up to −45 ºC and stirring was continued for 1 h whilst maintaining the temperature at −45 ºC, then at −20 ºC for 40 min, and between −20 ºC and −10 ºC for another 20 min. The mixture was re-cooled to −62 ºC and allyl bromide (23.9 mL, 33.5 g, 0.277 mol) in dry THF (20 mL) was slowly added. Again, the temperature gradually rose to −45 ºC and the reaction was stirred for 1 h at this temperature. TLC analysis showed a trace of starting material was left so another portion of allyl bromide (22.3 mL, 31.3 g, 0.250 mol) was added and stirring was continued at −20 ºC for 1 h and then at 0 ºC for 2 h. The reaction was quenched with a sat. aqueous solution of NH_4Cl (50 mL) and extracted with ethyl acetate (3 × 150 mL). The combined organic layers were dried over $MgSO_4$, filtered and the solvent was evaporated. The crude product was purified by column chromatography, eluting with petrol/ethyl acetate (1:1) to give a thick yellow oil, dimethyl (*2R,3S*)-2-allyl-3-hydroxysuccinate **165** (22 g, 58%), $[\alpha]_D^{23}$ = + 11 (c 1.5, $CHCl_3$), {MALDI-Found $[M+H]^+$: 203.0914; $C_9H_{15}O_5$ requires: 203.0914}. This showed δ_H (400 MHz, $CDCl_3$): 5.88 – 5.73 (1H, m), 5.17 (1H, dd, *J* 1.4, 17.1 Hz), 5.12 (1H, d, *J* 10.2 Hz), 4.31 (1H, dd, *J* 3.2, 7.1 Hz), 3.80 (3H, s), 3.69 (3H, s), 3.17 (1H, br.d, *J* 7.1 Hz), 2.99 (1H, ddd, *J* 3.2, 6.6, 8.7 Hz), 2.62 (1H, dt, *J* 6.3, 8.1 Hz),

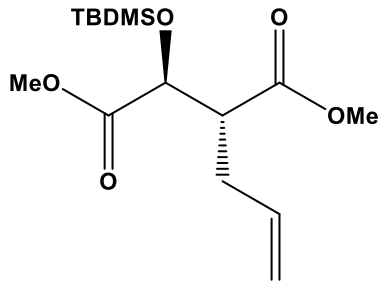
2.50 – 2.40 (1H, m); δ_C (101 MHz, $CDCl_3$): 173.8, 172.4, 134.6, 117.8, 70.0, 52.5, 51.8, 48.0, 31.9; ν_{max}: 3468, 3020, 2954, 1741, 1642, 1438, 1218, 921 cm^{-1}.

Experiment 4: Diethyl (*2R,3S*)-2-allyl-3-hydroxysuccinate (166)



The above experiment was repeated using diethyl (*S*)-2-hydroxysuccinate **105** (50.0 g, 0.262 mol), diisopropyl amine (81.0 mL, 58.5 g, 0.578 mol), butyl lithium (542 mL, 0.860 mol, 1.6 M) and allyl bromide (34.0 mL, 47.7 g, 0.394 mol). The reaction mixture was worked up and purified as above to give diethyl (2*R*,3*S*)-2-allyl-3-hydroxysuccinate **166** as a thick yellow oil (32 g, 53%) $[\alpha]_D^{23}$ = + 13.4 (c 1.79, $CHCl_3$) (*lit.*[206] $[\alpha]_D^{20}$ = + 11.3 (c 2.23, $CHCl_3$)). This showed δ_H (400 MHz, $CDCl_3$): 5.88 – 5.75 (1H, m), 5.16 (1H, dd, *J* 1.4, 17.1 Hz), 5.10 (1H, br.d, *J* 10.1 Hz), 4.29 (1H, dd, *J* 5.2, 9.5 Hz), 4.27 – 4.20 (2H, m), 4.13 (2H, q, *J* 7.1 Hz), 3.19 (1H, d, *J* 7.0 Hz), 2.95 (1H, ddd, *J* 3.1, 6.7, 8.5 Hz), 2.62 (1H, dt, *J* 6.4, 8.2 Hz), 2.48 – 2.38 (1H, m), 1.30 (3H, t, *J* 7.1 Hz), 1.23 (3H, t, *J* 7.1 Hz); δ_C (101 MHz, $CDCl_3$): 173.5, 171.9, 134.9, 118.0, 70.2, 61.8, 60.8, 48.1, 32.1, 14.1, 14.0; ν_{max}: 3494, 3030, 2951, 2863, 1735, 1643, 1438,1132,1099 cm^{-1}. All data matched those in the literature.[206]

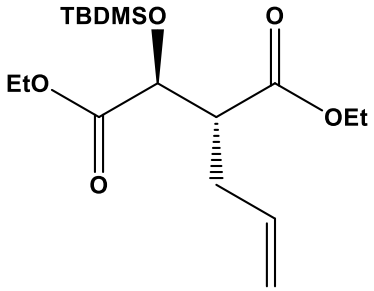
Experiment 5: Dimethyl (*2R,3S*)-2-allyl-3-((*tert*-butyldimethylsilyl) oxy)succinate (167)



tert-Butyldimethylchlorosilane (24.5 g, 0.162 mol) was added to a stirred solution of dimethyl (*2R,3S*)-2-allyl-3-hydroxysuccinate **165** (25.3 g, 0.125 mol) in dry DMF (150 mL) and imidazole (17 g, 0.25 mol) at 0 ºC. The cooling bath was removed, and the reaction

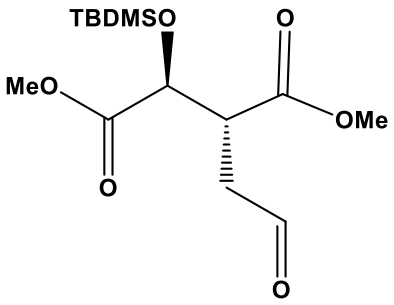
mixture was stirred at 45 ºC for 20 h, when TLC showed no starting material. DMF was removed by flash distillation and water (100 mL) was added and the product was extracted with dichloromethane (3 × 200 mL). The combined organic layers were dried over $MgSO_4$, filtered and the solvent was evaporated. The crude product was purified by column chromatography, eluting with petrol/ethyl acetate (10:1) to give a thick colorless oil, dimethyl (*2R,3S*)-2-allyl-3-((*tert*-butyldimethylsilyl)oxy)succinate **167** (34 g, 86%) $[\alpha]_D^{23} = -50$ (c 1.5, $CHCl_3$), {MALDI-Found $[M+H]^+$: 317.1775; $C_{15}H_{29}O_5Si$ requires: 317.1779}. This showed δ_H (400 MHz, $CDCl_3$): 5.75 (1H, ddt, *J* 6.9, 10.2, 17.1 Hz), 5.13 (1H, dd, *J* 1.5, 17.1 Hz), 5.05 (1H, br.d *J* 10.2 Hz), 4.37 (1H, d, *J* 6.0 Hz), 3.75 (3H, s), 3.67 (3H, s), 2.97 (1H, dt, *J* 6.1, 8.6 Hz), 2.50 – 2.41 (1H, m), 2.29 – 2.19 (1H, m), 0.88 (9H, s), 0.07 (3H, s), 0.05 (3H, s); δ_C (101 MHz, $CDCl_3$): 172.3, 172.2, 134.8, 117.2, 72.9, 51.9, 51.6, 50.0, 31.8, 25.5, –5.1, –5.5; ν_{max}: 3155, 2954, 2859, 1738, 1640, 1437, 1389, 1255, 1195 cm^{-1}.

Experiment 6: Diethyl (*2R,3S*)-2-allyl-3-((*tert*-butyldimethylsilyl) oxy)succinate (168)



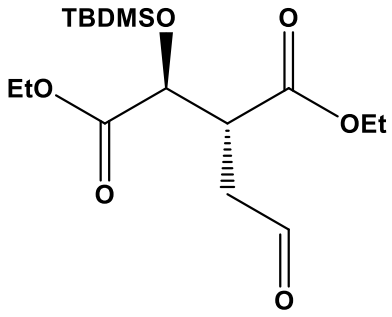
The above experiment was repeated using imidazole (20.6 g, 0.304 mol), *tert*-butyldimethylchlorosilane (29.7 g, 0.197 mol) and diethyl (*2R,3S*)-2-allyl-3-hydroxysuccinate **166** (35.0 g, 0.152 mol). The mixture was worked up and purified as above to give diethyl (*2R,3S*)-2-allyl-3-((*tert*-butyldimethylsilyl)oxy)succinate **168** (45 g, 86%) as a thick colorless oil $[\alpha]_D^{22} = -44$ (c 0.81, $CHCl_3$), {Found $[M+Na]^+$: 367.2; $C_{17}H_{32}NaO_5Si$ requires: 367.2}. This showed δ_H (400 MHz, $CDCl_3$): 5.77 (1H, ddt, *J* 6.9, 10.2, 17.1 Hz), 5.11 (1H, dd, *J* 1.5, 17.1 Hz), 5.05 (1H, d, *J* 10.2 Hz), 4.34 (1H, d, *J* 5.5 Hz), 4.28 – 4.04 (4H, m), 2.96 (1H, dt, *J* 6.0, 8.5 Hz), 2.52 – 2.42 (1H, m), 2.29 – 2.18 (1H, m), 1.30 (3H, t, *J* 7.1 Hz), 1.24 (3H, t, *J* 7.1 Hz), 0.89 (9H, s), 0.07 (3H, s), 0.04 (3H, s); δ_C (101 MHz, $CDCl_3$): 172.0, 171.7, 135.0, 117.1, 72.9, 61.0, 60.5, 49.9, 31.9, 25.6, 14.1, –4.9, –5.4; ν_{max}: 3155, 2958, 2859, 1730, 1643, 1447, 1389, 1255, 1192 cm^{-1}.

Experiment 7: Dimethyl (*2S,3R*)-2-((*tert*-butyldimethylsilyl)oxy)-3-(2-oxoethyl)succinate (169)



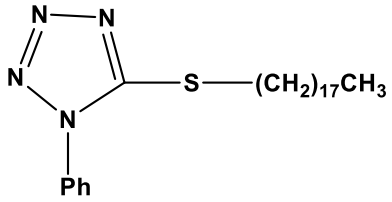
O_3 and O_2 were bubbled through a stirred solution of dimethyl (*2R,3S*)-2-allyl-3-((*tert*-butyldimethylsilyl)oxy)succinate **167** (30 g, 0.094 mol) in dichloromethane (500 mL) at –78 ºC for 30 min, until a blue color appeared. After 30 min, the mixture was quenched with triphenyl phosphine (37.3 g, 0.142 mol) then the reaction mixture allowed to reach room temperature, when TLC showed no starting material. The solvent was evaporated, and the crude product was purified by column chromatography eluting with petrol/ethyl acetate (5:1) to give a thick colorless oil, dimethyl (*2S,3R*)-2-((*tert*-butyldimethylsilyl)oxy)-3-(2-oxoethyl)succinate **169** (28 g, 93%) $[\alpha]_D^{23} = -38$ (c 0.72, $CHCl_3$), {Found $[M+H]^+$:319.1572 ; $C_{14}H_{27}O_6Si$ requires: 319.1571}.This showed δ_H (400 MHz, $CDCl_3$): 9.81 (1H, br. s), 4.53 (1H, d, *J* 4.3 Hz), 3.74 (3H, s), 3.70 (3H, s), 3.50 (1H, dt, *J* 4.4, 8.9 Hz), 3.02 (1H, dd, *J* 9.0, 15.4 Hz), 2.69 (1H, dd, *J* 4.6, 15.4 Hz), 0.90 (9H, s), 0.10 (3H, s), 0.07 (3H, s); δ_C (101 MHz, $CDCl_3$): 199.4, 171.5, 170.9, 72.0, 52.0, 51.9, 43.8, 40.9, 25.4, 25.3, –5.1, –5.63; ν_{max}: 3020, 2954, 2845, 1741, 1474, 1437, 1318, 1215, 1182, 1095, 1045, 1006 cm^{-1}.

Experiment 8: Diethyl (*2S,3R*)-2-((*tert*-butyldimethylsilyl)oxy)-3-(2-oxoethyl)succinate (170)



The above (experiment 7) was repeated using diethyl (*2R,3S*)-2-allyl-3-((*tert*-butyldimethylsilyl)oxy)succinate **168** (47.6 g, 0.138 mol) and triphenyl phosphine (54.2 g, 0.207 mol). The mixture was worked up and purified as above to give diethyl (*2S,3R*)-2-((*tert*-butyldimethylsilyl)oxy)-3-(2-oxoethyl)succinate **170** (40 g, 84%) as a thick colorless oil $[\alpha]_D^{23} = -41$ (c 2.2, $CHCl_3$), { Found $[M+Na]^+$: 369.2; $C_{16}H_{30}O_6SiNa$ requires: 369.2}. This showed δ_H (400 MHz, $CDCl_3$): 9.81 (1H, br. s), 4.48 (1H, d, *J* 4.0 Hz), 4.25 – 4.09 (4H, m), 3.49 (1H, dt, *J* 4.3, 8.8 Hz), 3.03 (1H, dd, *J* 9.1, 15.3 Hz), 2.65 (1H, dd, *J* 4.6, 15.2 Hz), 1.28 (3H, t, *J* 7.1 Hz), 1.24 (3H, t, *J* 7.1 Hz), 0.90 (9H, s), 0.10 (3H, s), 0.06 (3H, s); δ_C (101 MHz, $CDCl_3$): 199.8, 171.2, 170.5, 72.2, 61.2, 61.1, 44.0, 41.1, 25.6, 14.0, 14.01, –4.9, –5.5; ν_{max}: 3088, 2980, 2845, 2732, 1738, 1459, 1381, 1318, 1249, 1182, 1095, 1045, 1006 cm^{-1}.

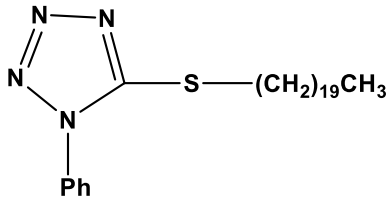
Experiment 9: 5-(Octadecylthio)-1-phenyl-1*H*-tetrazole (190)



1-Phenyl-1*H*-tetrazole-5-thiol (29.4 g, 0.165 mol) was added to a stirred solution of 1-bromooctadecane **188** (50.0 g, 0.150 mol) and anhydrous potassium carbonate (83 g, 0.60 mol) in acetone (400 mL) and THF (100 mL) at room temperature. The reaction mixture refluxed for 3 h, then worked up and purified as before to give a white solid 5-(octadecylthio)-1-phenyl-1*H*-tetrazole **190** (57 g, 89%), m.p. 68-69 ºC, {Found $[M+H]^+$:431.3199; $C_{25}H_{43}N_4S$ requires: 431.3203}. This showed δ_H (400 MHz, $CDCl_3$): 7.68 – 7.47 (5H, m), 3.40 (2H, t, *J* 7.4 Hz), 1.82 (2H, m), 1.49 – 1.18 (30H, m), 0.89 (3H, t, *J* 6.8 Hz); δ_C (101 MHz, $CDCl_3$): 154.5 , 133.8,

130.0, 129.7, 123.8, 33.4, 31.9, 29.63, 29.61, 29.57, 29.5, 29.4, 29.3, 29.0, 28.99, 28.6, 22.7, 14.1; ν_{max}: 2914, 2847, 1595, 1499, 1471, 1341, 1273, 1129 cm^{-1}.

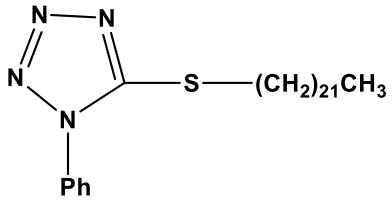
Experiment 10: 5-(Eicosylthio)-1-phenyl-1*H*-tetrazole (195)



(1): Triphenyl phosphine (52 g, 0.20 mol) was added to a stirred solution of eicosan-1-ol **191**(50.0 g, 0.167 mol) in CH_2Cl_2 (400 mL). The mixture was cooled to 0 ºC and *N*-bromosuccinamide (38.6 g, 0.217 mol) was added in portions and the resulting mixture was stirred at room temperature for 3 h. Saturated aqueous sodium bisulphate (150 mL) was then added and the product was extracted with CH_2Cl_2 (2 × 200 mL). The combined organic layers were dried over $MgSO_4$, filtered and the solvent was evaporated to give a residue, which was refluxed in petrol (150 mL) for 30 min. The triphenyl phosphonium oxide was filtered on a pad of celite and washed with petrol (100 mL). The solvent was evaporated and the crude product was purified by recrystallization with petrol to give a white solid, 1-bromoeicosane **193** (54 g, 90%) which showed δ_H (400 MHz, $CDCl_3$): 3.43 (2H, t, *J* 6.9 Hz), 1.92 – 1.80 (2H, m), 1.48 – 1.16 (34H, m), 0.89 (3H, t, *J* 6.7 Hz); δ_C (126 MHz, $CDCl_3$): 34.1, 33.0, 32.1, 29.9, 29.8, 29.7, 29.6, 29.5, 28.9, 28.3, 22.8, 14.2; ν_{max}: 2928, 2854, 1464, 1256, 1057cm^{-1}.

(**2):** To a stirred solution of 1-bromoicosane **193** (50.0 g, 0.138 mol) and anhydrous potassium carbonate (76.3 g, 0.550 mol) in acetone (400 mL) and THF (100 mL). 1-Phenyl-1*H*-tetrazole-5-thiol (25.8 g, 0.140 mol) was added, the reaction mixture was refluxed for 3 h. After this time TLC showed no staring material was left and solvent was evaporated. The crude product was purified by recrystallization from MeOH/acetone (1:1) to give a white solid 5-(eicosylthio)-1-phenyl-1*H*-tetrazole **195** (55 g, 88%),[205] m.p. 63-65 ºC. This showed δ_H (400 MHz, $CDCl_3$): 7.65 – 7.48 (5H, m), 3.40 (2H, t, *J* 7.4 Hz), 1.89 – 1.78 (2H, m), 1.58 – 1.08 (34H, m), 0.89 (3H, t, *J* 6.8); δ_C (101 MHz; $CDCl_3$): 154.5, 133.8, 130.0, 127.7, 123.9, 33.4, 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 29.1, 29.0, 28.6, 22.7, 14.1; ν_{max}: 2916, 2850, 1597, 1502, 1473, 1420, 1390, 1247, 1091cm^{-1}. All data matched those in the literature.[205]

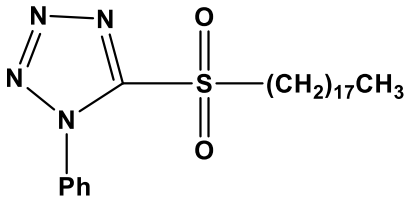
Experiment 11: 5-(docosylthio)-1-phenyl-1*H*-tetrazole (196)



(1): Triphenyl phosphine (48.2 g, 0.183 mol) was added to a stirred solution of docosanol-1-ol **192** (50.0 g, 0.153 mol) in CH_2Cl_2 (400 mL). The mixture was cooled to 0 ºC and *N*-bromosuccinamide (35.4 g, 0.199 mol) was added in portions and the resulting mixture was stirred at room temperature for 3 h. The reaction mixture was worked up and purified as before to give 1-bromodocosane **194** as a white solid (55 g, 93%) which showed δ_H (400 MHz, $CDCl_3$): 3.42 (2H, t, *J* 6.9 Hz), 1.93 – 1.80 (2H, m), 1.49 – 1.12 (38H, m), 0.89 (3H, t, *J* 6.7 Hz); δ_C (101 MHz, $CDCl_3$): 34.0, 32.9, 31.9, 29.7, 29.6, 29.5, 29.4, 28.8, 28.2, 22.7, 14.1; ν_{max}: 2920, 2850, 1463, 1255cm^{-1}.

(2): To a stirred solution of 1-bromodocosane **194** (50.0 g, 0.128 mol), anhydrous potassium carbonate (70.7 g, 0.514 mol) and1-phenyl-1*H*-tetrazole-5-thiol (25.2 g, 0.141 mol) in acetone (400 ml) and THF (100 mL). The reaction mixture was refluxed for 3 h then worked up and purified as before to give a white solid 5-(docosylthio)-1-phenyl-1*H*-tetrazole **196** (58 g, 93%).[202] This showed δ_H (400 MHz, $CDCl_3$): 7.66 – 7.46 (5H, m), 3.40 (2H, t, *J* 7.4 Hz), 1.90 – 1.76 (2H, m), 1.49 – 1.18 (38H, m), 0.89 (3H, t, *J* 6.7 Hz); δ_C (101 MHz; $CDCl_3$): 154.5, 133.8, 130.0, 129.7, 123.9, 33.9, 31.9, 29.7, 29.65, 29.60, 29.53, 29.43, 29.35, 29.1, 29.0, 28.6, 22.8, 14.10; ν_{max}: 3017, 2925, 2853, 1598, 1500 cm^{-1}. All data matched those in the literature.[202]

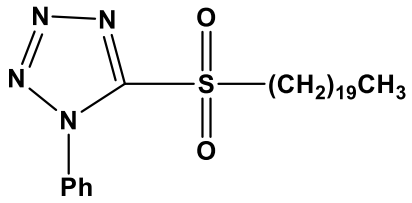
Experiment 12: 5-(Octadecylsulfonyl)-1-phenyl-1*H*-tetrazole (171)



A solution of ammonium molybdate (VI) tetrahydrate (64.5 g, 0.052 mol) was dissolved in cold hydrogen peroxide (35%, 120 mL) and was gradually added to a stirred solution of 5-

(octadecylthio)-1-phenyl-1*H*-tetrazole **190** (50 g, 0.11 mol) in IMS (400 mL) and THF (200 mL) at 5 ºC. The reaction mixture was allowed to reach room temperature and stirred for 2 h, and then another portion of ammonium molybdate (VI) tetrahydrate (24.3 g, 0.010 mol) in cold hydrogen peroxide was added (35 %, 70 mL). The reaction mixture was stirred for 18 h. The mixture was poured into water (2.5 litres) and extracted with dichloromethane (3 × 400 mL). The compound organic layers were washed with more water (2 × 400 mL) and dried. The solvent was evaporated and the crude product was purified by column chromatography, eluting with with petrol/ethyl acetate (5:1), to give a semi solid, 5-(octadecylsulfonyl)-1-phenyl-1*H*-tetrazole **171** (52 g, 97%), m.p. 77-78 ºC {Found $[M+H]^+$:463.3095; $C_{25}H_{43}O_2N_4S$ requires: 463.3101}, which showed δ_H (400 MHz, $CDCl_3$): 7.77 – 7.67 (2H, m), 7.66 – 7.57 (3H, m), 3.79 – 3.69 (2H, distorted t, *J* 7.9 Hz), 1.96 (2H, m), 1.56 – 1.19 (30H, m), 0.89 (3H, t, *J* 6.8 Hz); δ_C (101 MHz, $CDCl_3$): 153.3, 133.0, 131.4, 129.9, 129.7, 125.0, 56.0, 52.9, 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 28.9, 28.1, 22.7, 21.9, 14.1; ν_{max}: 2960, 2847, 1593, 1498, 1470, 1341, 1152 cm^{-1}.

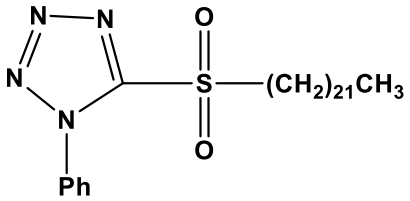
Experiment 13: 5-(Eicosylsulfonyl)-1-phenyl-1*H*-tetrazole (172)



m-Chloroperoxybenzoic acid (62.7 g, 0.270 mol) in dichloromethane (50 mL) was added to a stirred solution of 5-(eicosylthio)-1-phenyl-1*H*-tetrazole **195** (50 g, 0.10 mol) in dichloromethane (300 mL) and $NaHCO_3$ (36.6 g, 0.430 mol) at 0 ºC. The reaction mixture was stirred at room temperature for 18 h then quenched with NaOH solution (200 mL 10%) and stirred for 2 h. The organic was separated and the aqueous layer was re-extracted with dichloromethane (2 × 200 mL). The combined organic layers were dried over $MgSO_4$, filtered and evaporated to give a white solid, which was recrystallized from MeOH/Acetone (1:1) to give a white solid of 5-(eicosylsulfonyl)-1-phenyl-1*H*-tetrazole **172** (51 g, 96%).[205] This showed δ_H (400 MHz, $CDCl_3$): 7.73 – 7.69 (2H, m), 7.66 – 7.57 (3H, m), 3.74 (2H, distorted t, *J* 7.9 Hz), 2.01 – 1.91 (2H, m,), 1.53 – 1.19 (34H, m), 0.89 (3H, t, *J* 6.8 Hz); δ_C (101 MHz; $CDCl_3$): 153.5, 133.1, 131.4, 129.7, 125.1, 56.0, 31.9, 29.7, 29.65, 29.6, 29.5,

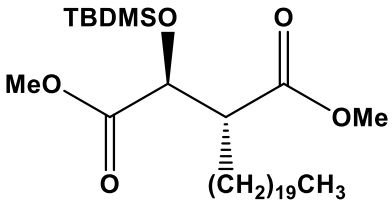
29.4, 29.3, 29.2, 28.9, 28.1, 22.7, 21.9, 14.1; ν_{max}: 2920, 2849, 1595, 1499, 1470, 1338, 1153 cm^{-1}. All data were identical to the literature.[205]

Experiment 14: 5-(docosylsulfonyl)-1-phenyl-1*H*-tetrazole (173)



The above experiment was repeated using 5-(docosylthio)-1-phenyl-1*H*-tetrazole **196** (50.0 g, 0.102 mol), *m*-chloroperoxybenzoic acid (80.7 g, 0.257 mol) and $NaHCO_3$ (34.5 g, 0.411 mol). The reaction mixture was worked up and purified as above to give a white solid, 5-(docosylsulfonyl)-1-phenyl-1*H*-tetrazole **173** (51 g, 96%).[202] This showed δ_H (400 MHz, $CDCl_3$) 7.73 – 7.68 (2H, m), 7.65 – 7.59 (3H, m), 3.73 (2H, distorted t, *J* 8.1 Hz), 2.02 – 1.90 (2H, m), 1.53 – 1.20 (38H, m), 0.89 (3H, t, *J* 6.7 Hz); δc (101 MHz; $CDCl_3$): 153.5, 133.1, 131.4, 129.7, 125.1, 56.0, 31.9, 29.7, 29.65, 29.62, 29.55, 29.44, 29.35, 29.2, 28.9, 28.14, 22.7, 21.9, 14.1; ν_{max}: 3018, 2926, 2854, 1498, 1342, 1152 cm^{-1}. All data data matched those in the literature.[202]

Experiment 15: Dimethyl (2*S*,3*R*)-2-((*tert*-butyldimethylsilyl)oxy)-3-icosylsuccinate (174)

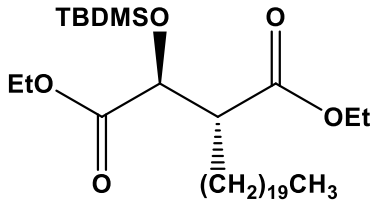


Lithium bis-(trimethylsilyl) amide (90.2 mL, 0.090 mol) was added to a stirred solution of dimethyl (2*S*,3*R*)-2-((*tert*-butyldimethylsilyl)oxy)-3-(2-oxoethyl)succinate **169** (15.3 g, 0.048 mol) and 5-(octadecylsulfonyl)-1-phenyl-1*H*-tetrazole **171** (27.8 g, 0.060 mol) in dry THF (250 mL) at –20 °C under nitrogen atmosphere. The mixture was allowed to reach room temperature and stirred for 3 h. TLC showed that the reaction was complete. It was quenched with sat. aqueous NH_4Cl (250 mL). The product was extracted with petrol/ethyl acetate (10:1, 3 × 150 mL). The combined organic layers were dried over $MgSO_4$, 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 yellow oil, dimethyl (2*S*,3*R*)-2-((*tert*-butyldimethylsilyl)oxy)-3-((*E/Z*)-docos-2-en-1-yl)succinate **206** (20 g,76%) as a mixture of *E/Z* isomers which showed δ_H (400 MHz, $CDCl_3$): 5.53 – 5.39 (1H, m), 5.36 – 5.24 (1H, m), 4.37 (1H, dd, *J* 6.4, 15.6 Hz), 3.74 (3H, s), 3.66 (3 H, s), 2.90 (1H, m), 2.39 (1H, m), 2.17 (1H, m), 1.99 (1H, m), 1.37 – 1.23 (31 H, m), 0.87 (12H, s including t, corresponding to CH_3), 0.06 (3H, s), 0.04 (3H, s); δ_C (125 MHz, $CDCl_3$): 172.5, 172.4, 133.8, 132.8, 129.9, 128.6, 73.0, 51.9, 50.7, 41.4, 32.5 – 11.4, -5.0, -5.4.

Palladium on carbon (10%, 2.0 g) was added to a stirred solution of the above alkene in THF/IMS (500 mL, 1:1) and the suspension was stirred overnight under hydrogen atmosphere. The suspension was then quenched with a few drops of water and filtered over a bed of celite and washed with ethyl acetate (150 mL). The filtrate was concentrated and purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a thick oil, dimethyl (2*S*,3*R*)-2-((*tert*-butyldimethylsilyl)oxy)-3-eicosylsuccinate **174** (19.8 g, 99%) $[\alpha]_D^{22} = -27$ (c 0.69, $CHCl_3$) {Found $[M+Na]^+$: 579.4400; $C_{32}H_{64}O_5SiNa$ requires: 579.4415}. This showed δ_H (400 MHz, $CDCl_3$): 4.34 (1H, d, *J* 7.2 Hz), 3.73 (3H, s), 3.67 (3H, s), 2.83 (1H, ddd, *J* 4.6, 7.2, 10.2 Hz), 1.78 – 1.17 (38H, m), 0.88 (3H, t, *J* 6.4 Hz), 0.87 (9H, s) 0.05 (3H, s), 0.04 (3H, s); δ_C (101 MHz, $CDCl_3$): 173.2, 172.4, 73.8, 51.8, 51.5, 50.6, 31.9, 29.7, 29.6, 29.5, 29.3, 27.6, 27.3, 25.5, 14.0, –5.2, –5.6; ν_{max}: 2935, 2856, 1760, 1740, 1473, 1439, 1352 cm^{-1}.

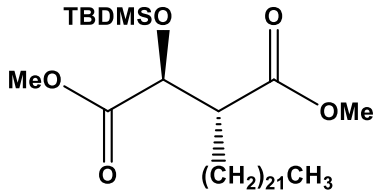
Experiment 16: Diethyl (2*S*,3*R*)-2-((*tert*-butyldimethylsilyl)oxy)-3-eicosylsuccinate (177)



Lithium bis-(trimethylsilyl) amide (95.2 mL, 0.092 mol) was added to a stirred solution of diethyl (*2S,3R*)-2-((*tert*-butyldimethylsilyl)oxy)-3-(2-oxoethyl)succinate **170** (20.0 g, 0.057 mol) and 5-(octadecylsulfonyl)-1-phenyl-1*H*-tetrazole **171** (29.3 g, 0.063 mol)) in dry THF (250 mL) at –10 ºC under nitrogen atmosphere. The mixture was worked up, purified and hydrogenation as above to give a thick oil of diethyl (2*S*,3*R*)-2-((*tert*-butyldimethylsilyl)oxy)-3-icosylsuccinate **177** (26, 77%)

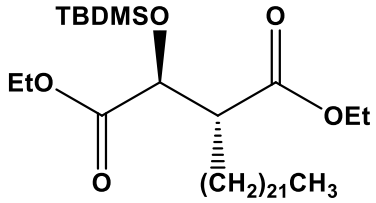
$[\alpha]_D^{22}$ = – 24 (c 0.91, $CHCl_3$), {Found $[M+Na]^+$: 607.5; $C_{34}H_{68}O_5SiNa$ requires: 607.5}. This showed δ_H (400 MHz, $CDCl_3$): 4.32 (1H, d, *J* 6.8 Hz), 4.27 – 4.05 (4H, m), 2.81 (1H, ddd, *J* 4.7, 6.7,11.4 Hz), 1.78 – 1.08 (44H, m, including 6H for 2 × CH_3), 0.89 (3H, t, *J* 7.3 Hz), 0.87(9H, s), 0.06 (3H, s), 0.04 (3H, s); δ_C (101 MHz, $CDCl_3$): 172.7, 172.0, 73.8, 60.9, 60.4, 50.5, 31.9, 29.68, 29.7, 29.6, 29.5, 29.4, 29.3, 27.7, 27.3, 25.5, 14.2, 14.1, 14.0, –5.0, –5.5; ν_{max}: 2950, 2925, 2855, 1719, 1468, 1459, 1377, 1180 cm^{-1}.

Experiment 17: Dimethyl (*2S,3R)-2-((tert*-butyldimethylsilyl)oxy)-3-docosylsuccinate (175)



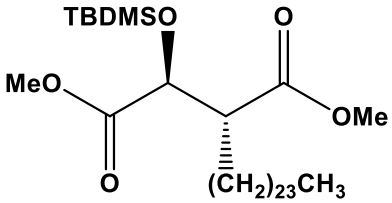
Lithium bis-(trimethylsilyl) amide (82.9 mL, 0.082 mol) was added a stirred solution of dimethyl *(2S,3R)*-2-((*tert*-butyldimethylsilyl)oxy)-3-(2-oxoethyl)succinate **169** (16.0 g, 0.050 mol) and 5-(icosylsulfonyl)-1-phenyl-1*H*-tetrazole **172** (27.1 g, 0.055 mol) in dry THF (250 mL) at –10 ºC under nitrogen atmosphere. The mixture was allowed to reach room temperature and stirred for 3 h. then worked up, purified and hydrogenated as before to give a thick yellow oil, dimethyl (*2S,3R*)-2-((*tert*-butyldimethylsilyl)oxy)-3-docosylsuccinate **175** (22 g, 76%) $[\alpha]_D^{22}$ = – 22 (c 0.91, $CHCl_3$) {Found $[M+H]^+$: 585.4905; $C_{34}H_{69}O_5Si$ requires: 585.4909}. This showed δ_H (400 MHz, $CDCl_3$): δ_H (400 MHz, $CDCl_3$): 4.34 (1H, d, *J* 7.3 Hz), 3.74 (3H, s), 3.68 (3H, s), 2.83 (1H, ddd, *J* 4.8, 7.4, 10.4 Hz), 1.71 – 1.05 (42H, m), 0.89 (3H, t, *J* 6.6 Hz), 0.87 (9H, s), 0.05 (3H, s), 0.04 (3H, s); δ_C (101 MHz; $CDCl_3$): 172.9, 172.2, 74.0, 61.0, 60.6, 50.6, 32.1, 29.9, 29.8, 29.7, 29.6, 3.7, 29.5, 29.4, 27.8, 27.4, 25.7, 22.8, 14.4, 14.3, 14.2, –4.9, –5.4; ν_{max}: 2931, 2850, 1767, 1746, 1472, 1434, 1350 cm^{-1}.

Experiment 18: Diethyl (*2S,3R*)-*2*-((*tert*-butyldimethylsilyl)oxy)-3-docosylsuccinate (178)



Lithium bis-(trimethylsilyl) amide (71.4 mL, 0.070 mol) was added to a stirred solution of diethyl (*2S,3R*)-2-((*tert*-butyldimethylsilyl)oxy)-3-(2-oxoethyl)succinate **170** (15 g, 0.04 mol) and sulfone **172** (23.3 g, 0.040 mol) in dry THF (250 mL) at −10 ºC under nitrogen atmosphere. The mixture was allowed to reach room temperature and stirred for 3 h. The reaction was worked up, purified and hydrogenated as before to give a thick yellow oil, diethyl (*2S,3R)-2-((tert*-butyldimethylsilyl)oxy)-3-docosylsuccinate **178** (20 g, 76%) $[\alpha]_D^{22}$ = – 29 (c 0.99, $CHCl_3$) {Found $[M\text{-}^tBu]^+$: 555.4434; $C_{32}H_{63}O_5Si$ requires: 555.4443}. Which showed δ_H (400 MHz, $CDCl_3$): 4.33 (1H, d, *J* 6.8 Hz), 4.26 – 4.10 (4H, m), 2.83 (1H, dt, *J* 6.8, 10.1 Hz), 1.79 – 1.18 (48H, m, including 6H for 2 × CH_3), 0.91 (3H, t, *J* 5.2 Hz), 0.89 (9H, s), 0.08 (3H, s), 0.06 (3H, s); δ_C (101 MHz, $CDCl_3$): 172.9, 172.2, 74.0, 61.0, 60.6, 50.6, 32.1, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 27.8, 27.4, 25.7, 22.8, 14.4, 14.3, 14.2, –4.9, –5.4; ν_{max}: 2931, 2850, 1767, 1746, 1472, 1434, 1350 cm^{-1}.

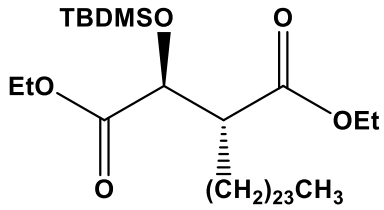
Experiment 19: Dimethyl (2*S*,3*R*)-2-((*tert*-butyldimethylsilyl)oxy)-3-tetracosylsuccinate (176)



Lithium bis-(trimethylsilyl) amide (17.2 mL, 0.017 mol) was added to a stirred solution of dimethyl (*2S,3R*)-2-((*tert*-butyldimethylsilyl)oxy)-3-(2-oxoethyl)succinate **169** (3.34 g, 0.014 mol) and 5-(docosylsulfonyl)-1-phenyl-1*H*-tetrazole **173** (5.98 g, 0.011 mol) in dry THF (50 mL) at −10 ºC under nitrogen atmosphere and stirred for 3 h. The mixture was worked up, purified and hydrogenated as before to give a thick colorless oil, dimethyl (*2S,3R*)-2-((*tert*-butyldimethylsilyl)oxy)-3-tetracosylsuccinate **176** (4.0 g, 77%), $[\alpha]_D^{23}$ = – 22 (c 1.3, $CHCl_3$) {Found $[M+Na]^+$: 635.5013; $C_{36}H_{72}O_5SiNa$ requires: 635.5047}. This showed δ_H (400 MHz, $CDCl_3$): 4.35 (1H, d, *J* 7.2 Hz), 3.74 (3H, s), 3.68 (3H, s), 2.84 (1H,

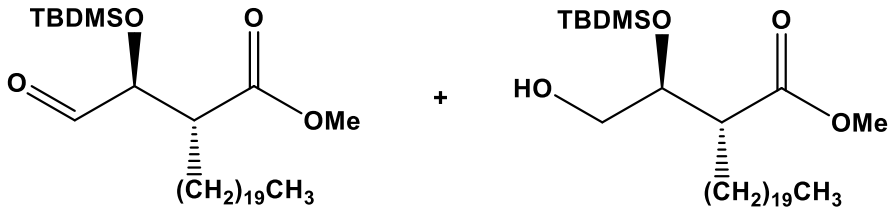
ddd, *J* 4.6, 7.2, 10.0 Hz), 1.40 – 1.21 (46H, m), 0.90 (3H, t, *J* 7.2 Hz), 0.87 (9H, s), 0.06 (3H, s), 0.04 (3H, s); δ_C (101 MHz, $CDCl_3$): 173.2, 172.4, 73.8, 51.9, 51.6, 50.6, 31.9, 29.7, 29.6, 29.5, 29.4, 27.7, 27.3, 25.5, 22.7, 18.0, 14.1, –5.2, –5.6; ν_{max}: 2927, 2855, 1735, 1467, 1378, 1252, 1166, 908, 736 cm^{-1}.

Experiment 20: Diethyl(2*S*,3*R*)-2-((*tert*-butyldimethylsilyl)oxy)-3-tetracosylsuccinate (179)



Lithium bis-(trimethylsilyl) amide (32.5 mL, 0.032 mol) was added to a stirred solution of diethyl (*2S,3R*)-2-((*tert*-butyldimethylsilyl)oxy)-3-(2-oxoethyl)succinate **170** (6.83 g, 0.019 mol) and sulfone **173** (11.2 g, 0.021 mol) worked up and purified as before to give after the hydrogenation diethyl (*2S,3R*)-2-((*tert*-butyldimethylsilyl)oxy)-3-tetracosylsuccinate **179** (9.0 g, 75%), $[\alpha]_D^{23} = -22$ (c 1.61, $CHCl_3$), {Found $[M-^tBu]^+$: 583.4758; $C_{34}H_{68}O_5Si$ requires: 584.4836}. This showed δ_H (400 MHz, $CDCl_3$): 4.32 (1H, d, *J* 6.8 Hz), 4.25 – 4.08 (4H, m), 2.82 (1H, dt, *J* 4.7, 6.8 Hz), 1.78 – 1.13 (52H, m, including 6H for 2 × CH_3), 0.89 (3H, t, *J* 6.2 Hz), 0.88 (9H, s), 0.06 (3H, s), 0.04 (3H, s); δ_C (101 MHz, $CDCl_3$): 172.9, 172.2, 74.0, 61.0, 60.6, 50.6, 32.1, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 27.8, 27.4, 25.7, 22.8, 14.3, 14.2, 14.1, –4.9, –5.4; ν_{max}: 2925, 2855, 1719, 1468, 1377, 1180 cm^{-1}.

Experiment 21: Methyl (*R*)-2-((*S*)-1-((*tert*-butyldimethylsilyl)oxy)-2-oxoethyl)docosanoate (180) and methyl (*R*)-2-((*S*)-1-((*tert*-butyldimethylsilyl)oxy)-2-hydroxyethyl)docosanoate (180a)

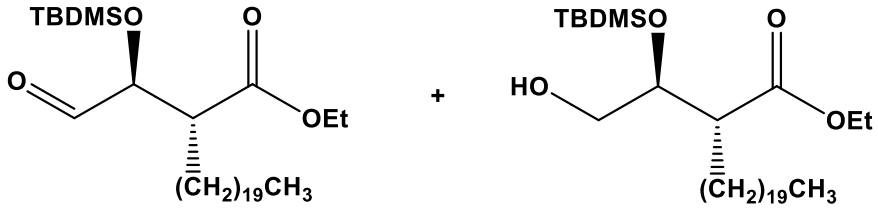


A solution of dimethyl (2*S*,3*R*)-2-((*tert*-butyldimethylsilyl)oxy)-3-icosylsuccinate **174** (5.0 g, 0.0090 mol) and magnesium bromide dietherate (3.9 g, 0.01 mol) in dichloromethane (150 mL) was stirred vigorously at room temperature for 1 h and then cooled to –38 ºC.

Diisobutylaluminum hydride (15.6 mL, 0.010 mol 1 M solution in toluene) was added dropwise at −38 ºC ensuring that vigorous stirring was maintained throughout the addition and that the internal temperature remained constant (colour change noted: orange to grey/orange). The reaction mixture was then stirred for a period of 1 h, with the internal temperature being maintained in the range −38 to −34 ºC. The mixture was then quenched at −34 ºC by the addition of methanol (10 mL) (colour change noted: initial orange colour restored) and after having warmed to room temperature the reaction mixture was quenched with a freshly prepared saturated aqueous solution of sodium sulfate (10 mL). The resulting mixture was stirred at room temperature for a period of 1 – 1.5 h until the coagulation of the aluminium salts was apparent. Magnesium sulfate (10 g) was then added and the mixture was stirred for a further of 10 min prior to being filtered, under vacuum, through a small plug of celite. The filter-cake was washed thoroughly with dichloromethane (75 mL) and the filtrate was concentrated to afford a pale yellow oil. The product was purified by column chromatography, eluting with petrol/ethyl acetate (20:1) to give the first fraction a semi-solid methyl (*R*)-2-((*S*)-1-((*tert*-butyldimethylsilyl)oxy)-2-oxoethyl)docosanoate **180** (3.8 g, 78%) $[\alpha]_D^{20} = -18$ (c 0.56, $CHCl_3$), {Found $[M+Na]^+$: 549.4; $C_{31}H_{62}O_4SiNa$ requires: 549.4}. This showed δ_H (400 MHz, $CDCl_3$): 9.63 (1H, d, *J* 1.9 Hz), 4.07 (1H, dd, *J* 1.9, 5.6 Hz), 3.71 (3H, s), 2.80 (1H, dt, *J* 5.7, 8.8 Hz), 1.87 – 1.17 (38H, m), 0.92 (3H, t, *J* 9.3 Hz), 0.91 (9H, s), 0.09 (3H, s), 0.08 (3H, s); δ_C (101 MHz, $CDCl_3$): 202.1, 173.1, 78.0, 60.7, 49.1, 32.1, 29.9, 29.8, 29.7, 29.6, 29.4, 29.5, 27.3, 27.2, 25.7, 22.8, 14.4, 14.3, –4.4, –5.0; ν_{max}: 2925, 2854, 1739, 1464 cm^{-1}.

The second fraction was methyl (*R*)-2-((*S*)-1-((*tert*-butyldimethylsilyl)oxy)-2-hydroxyethyl)-docosanoate **180a** (0.5 g, 10%) $[\alpha]_D^{20} = -9.6$ (c 0.56, $CHCl_3$), {Found $[M+Na]^+$: 551.4; $C_{31}H_{64}O_4SiNa$ requires: 551.4}. This showed δ_H (400 MHz, $CDCl_3$): 3.91 (1H, dt, *J* 3.6, 7.4 Hz), 3.66 (3H, s), 3.60 (2H, br. d, *J* 3.6 Hz), 2.76 – 2.68 (1H, m), 1.66 – 1.08 (39H, m), 0.87 (12H, s including t, *J* 6.6 Hz for terminal CH_3), 0.08 (3H, s), 0.05 (3H, s); δ_C (101 MHz, $CDCl_3$): 173.9, 73.8, 63.6, 51.4, 49.1, 31.9, 29.6, 28.3, 27.5, 25.7, 22.7, 14.1, –4.5, –5.1; ν_{max}: 3365, 2929, 2858, 1740, 1465, 1254, 1052 cm^{-1}.

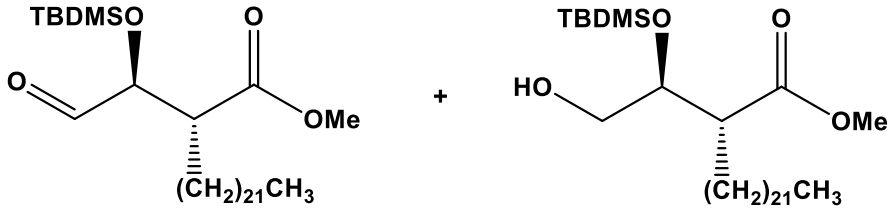
Experiment 22: Ethyl (*R*)-2-((*S*)-1-((*tert*-butyldimethylsilyl)oxy)-2-oxoethyl)docosanoate (183) and ethyl (*R*)-2-((*S*)-1-((*tert*-butyldimethylsilyl)oxy)-2-hydroxyethyl)docosanoate (183a)



The above experiment was repeated using diethyl (2*S*,3*R*)-2-((*tert*-butyldimethylsilyl)oxy)-3-icosylsuccinate **177** (5.0 g, 0.008 mol), magnesium bromide dietherate (3.7 g, 0.013 mol) in dichloromethane (120 mL) and diisobutylaluminum hydride (14.8 mL, 0.014 mol 1 M solution in toluene). The mixture was worked up and purified as above to give ethyl (*R*)-2-((*S*)-1-((*tert*-butyldimethylsilyl)oxy)-2-oxoethyl)docosanoate **183** (3.5 g, 74%) $[\alpha]_D^{19} = -15.7$ (c 2.65, $CHCl_3$), {Found $[M+Na]^+$: 563.4; $C_{32}H_{64}O_4SiNa$ requires: 563.4}. This showed δ_H (400 MHz, $CDCl_3$): 9.61 (1H, d, *J* 1.7 Hz), 4.21 – 4.05 (2H, m), 4.03 (1H, dd, *J* 1.7, 5.2 Hz), 2.75 (1H, dt, *J* 5.7, 8.6 Hz), 1.84 – 1.15 (41H, m, including 3H for CH_3), 0.89 (9H, s), 0.87 (3H, t, *J* 7.0), 0.05 (3H, s), 0.05 (3H, s); δ_C (101 MHz, $CDCl_3$): 202.8, 171.9, 77.9, 60.7, 49.0, 31.9, 29.7, 27.4, 27.3, 25.6, 22.7, 14.2, 14.0, –4.6, –5.2; ν_{max}: 2925, 2858, 1742, 1467 cm^{-1}.

The second fraction was **183a** (0.45 g, 9 %) $[\alpha]_D^{22} = -8$ (*c* 0.8, $CHCl_3$), {Found $[M+Na]^+$:565.5; $C_{32}H_{66}O_4SiNa$ requires: 565.5}. This showed δ_H (400 MHz, $CDCl_3$): 4.19 – 4.06 (2H, m), 3.93 (1H, dt, *J* 3.7, 7.5 Hz), 3.62 (2H, d, *J* 3.6 Hz), 2.74 – 2.65 (1H, m), 1.53 – 1.13 (42H, m, including 3H, for CH_3), 0.88 (12H, s, including t, *J* 7.1 Hz for terminal CH_3), 0.09 (3H, s), 0.06 (3H, s); δ_C (101 MHz, $CDCl_3$): 177.8, 74.3, 69.7, 58.4, 45.9, 31.9, 29.7, 29.6, 29.3, 27.6, 25.5, 23.5, 18.4, 14.0, –4.5, –5.2 ; ν_{max}: 3463, 2926, 2854, 1730, 1463, 1252, 1059 cm^{-1}.

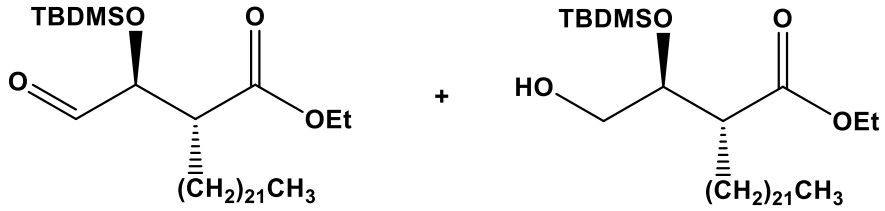
Experiment 23: Methyl (*R*)-2-((*S*)-1-((*tert*-butyldimethylsilyl)oxy)-2-oxoethyl)tetracosanoate (181) and methyl (*R*)-2-((*S*)-1-((*tert*-butyldimethylsilyl)oxy)-2-hydroxyethyl)tetracosanoate (181a)



A solution of dimethyl (2*S*,3*R*)-2-((*tert*-butyldimethylsilyl)oxy)-3-docosylsuccinate **175** (4.0 g, 0.06 mol) and magnesium bromide dietherate (2.6 g, 0.01 mol) in dry dichloromethane (120 mL) was stirred vigorously at room temperature for 1 h and then cooled to −38 ºC. Diisobutylaluminum hydride (11.6 mL, 0.01 mol 1 M solution in toluene) was added dropwise at −38 ºC ensuring that vigorous stirring was maintained throughout the period of addition and that the internal temperature remained constant (colour change noted: orange to grey/orange). The mixture was then stirred for 1 h, with the internal temperature being maintained in the range −38 to −34ºC. The reaction was worked up and purified as before to give the first fraction, a semi-solid methyl (*R*)-2-((*S*)-1-((*tert*-butyldimethylsilyl)oxy)-2-oxoethyl)hexacosanoate **181** (2.8 g, 75%) $[\alpha]_D^{19} = -18.7$ (c 1.93, $CHCl_3$), {Found $[M+Na]^+$: 577.4612; $C_{33}H_{66}O_4SiNa$ requires: 577.4623}. This showed δ_H (400 MHz, $CDCl_3$): 9.61 (1H, d, *J* 1.9 Hz), 4.05 (1H, dd, *J* 1.9, 5.6 Hz), 3.69 (3H, s), 2.78 (1H, dt, *J* 5.7, 8.9 Hz), 1.86 – 1.18 (42H, m), 0.90 (9H, s), 0.88 (3H, t, *J* 6.9 Hz), 0.07 (3H, s), 0.06 (3H, s); δ_C (101 MHz, $CDCl_3$): 202.7, 172.1, 78.3, 60.4, 49.2, 31.9, 29.7, 29.6, 29.5, 29.4, 27.4, 25.6, 22.7, 14.1, –4.5, –5.2; ν_{max}: 2924, 2854, 1739, 1464 cm^{-1}

The second fraction was methyl *(R)-2-((S)-1-((tert*-butyldimethylsilyl)oxy)-2-hydroxyethyl) hexacosanoate **181a** (0.45 g, 12%) $[\alpha]_D^{20} = -6.6$ (c1.9, $CHCl_3$), {Found $[M+Na]^+$: 579.5; $C_{33}H_{68}O_4SiNa$ requires: 579.5}. This showed δ_H (400 MHz, $CDCl_3$): 3.95 – 3.89 (1H, m), 3.68 (3H, s), 3.62 (2H, d, *J* 3.4 Hz), 2.77 – 2.68(1H, m), 1.80 – 1.10 (43H, m), 0.88 (12H, s, including t, *J* 6.4 Hz for terminal CH_3), 0.09 (3H, s), 0.06 (3H, s); δ_C (101 MHz $CDCl_3$): 174.9, 74.1, 64.2, 51.2, 47.9, 31.9, 29.7, 29.6, 29.4, 25.6, 23.5, 22.7, 14.1, –4.5, –5.2; ν_{max}: 3453, 2924, 2854, 1732, 1464, 1255, 1056 cm^{-1}.

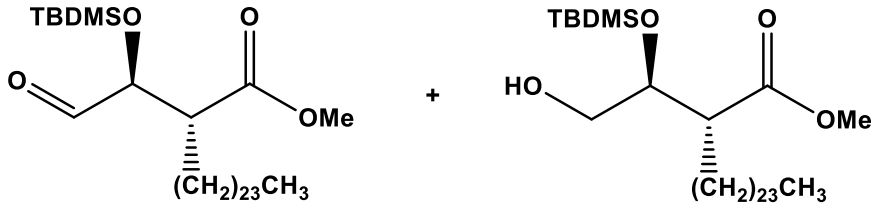
Experiment 24: Ethyl (*R*)-2-((*S*)-1-((*tert*-butyldimethylsilyl)oxy)-2-oxoethyl)tetracosanoate (184) and ethyl (*R*)-2-((*S*)-1-((*tert*-butyldimethylsilyl)oxy)-2-hydroxyethyl)tetracosanoate (184a)



The above experiment was repeated using diethyl (*2S,3R)-2-((tert*-butyldimethylsilyl)oxy)-3-docosylsuccinate **178** (3.0 g, 4.9 mmol), magnesium bromide dietherate (1.80 g, 7.34 mmol) in dichloromethane (90 mL) and diisobutylaluminum hydride (8.3 mL, 8.3 mmol 1 M solution in toluene). The reaction mixture was worked up and purified as before to give ethyl (*R*)-2-((*S*)-1-((*tert*-butyldimethylsilyl)oxy)-2-oxoethyl)tetracosanoate **184** (2.0 g, 72%) $[\alpha]_D^{19} = -14.2$ (c 1.86, $CHCl_3$) {Found $[M+Na]^+$: 591.5; $C_{34}H_{68}O_4SiNa$ requires: 591.5}. This showed δ_H (400 MHz, $CDCl_3$): 9.63 (1H, d, *J* 1.8 Hz), 4.26 – 4.06 (2H, m), 4.05 (1H, dd, *J* 1.7, 5.2 Hz), 2.77 (1H, dt, *J* 5.7, 8.5 Hz), 1.86 – 1.10 (45H, m, including t, *J* 6.7 Hz for CH_3), 0.91 (9H, s), 0.88 (3H, t, *J* 7.0 Hz), 0.08 (3H, s), 0.07 (3H, s); δ_C (101 MHz, $CDCl_3$): 202.9, 172.3, 78.2, 60.8, 49.5, 32.1, 29.9, 29.8, 29.7, 29.6, 29.5, 27.5, 27.4, 25.7, 22.8, 14.4, 14.3, –4.4, –5.0; ν_{max}: 2924, 2855, 1740, 1460 cm^{-1}.

The second fraction was **184a** (0.70 g, 15%) $[\alpha]_D^{24} = -5.5$ (c 1.46, $CHCl_3$), {Found $[M+Na]^+$: 593.5; $C_{34}H_{70}O_4SiNa$ requires: 593.5}. This showed δ_H (400 MHz, $CDCl_3$): 4.20 – 4.08 (2H, m), 3.92 (1H, dt, *J* 3.6,7.4 Hz), 3.62 (2H, d, *J* 3.6 Hz), 2.74 – 2.65 (1H, m), 1.53 – 1.13 (46H, m, including triplet with coupling constant 6.5 Hz, for CH_3), 0.89 (12H, s, including t, *J* 7.2 Hz for terminal CH_3), 0.09 (3H, s), 0.06 (3H, s); δ_C (101 MHz, $CDCl_3$): 175.9, 73.2, 65.7, 52.4, 45.9, 31.9, 29.7, 29.6, 29.3, 27.6, 25.5, 23.5, 18.4, 14.0, –4.5, –5.2 ; ν_{max}: 3454, 2924, 2854, 1731, 1464, 1254, 1056 cm^{-1}.

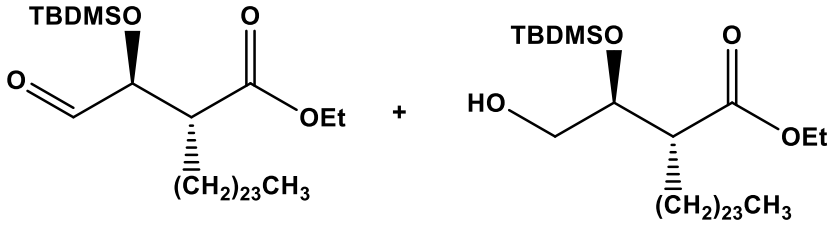
Experiment 25: Methyl (*R*)-2-((*S*)-1-((*tert*-butyldimethylsilyl)oxy)-2-oxoethyl)hexacosanoate(182) and methyl (*R*)-2-((*S*)-1-((*tert*-butyldimethylsilyl)oxy)-2-hydroxyethyl)hexacosanoate and (182a)



A solution of dimethyl (*2S,3R*)-2-((*tert*-butyldimethylsilyl)oxy)-3-tetracosylsuccinate **176** (5.0 g, 8.2 mmol) and magnesium bromide dietherate (3.1 g, 12 mmol) in dichloromethane (150 mL) was stirred vigorously at room temperature for 1 h and then cooled to –38 ºC. Diisobutylaluminum hydride (13.8 mL, 13.9 mmol 1 M solution in toluene) was added dropwise at –38 ºC ensuring that vigorous stirring was maintained throughout the period of addition and that the internal temperature remained constant (colour change noted: orange to grey/orange). The reaction mixture was then stirred for 1 h, with the internal temperature being maintained in the range –38 to –34 ºC. The reaction was worked up and purified as before to give the first fraction as a semi-solid methyl (*R*)-*2*-((*S)*-1-((*tert*-butyldimethylsilyl)oxy)-2-oxoethyl)hexacosanoate **182** (3.0 g, 71%) $[\alpha]_D^{24}$ = – 15.9 (c 2.51, $CHCl_3$), {Found $[M+H]^+$: 583.5112; $C_{35}H_{71}O_4Si$ requires: 583.5116}. This showed δ_H (400 MHz, $CDCl_3$): 9.61 (1H, d, *J* 1.9 Hz), 4.05 (1H, dd, *J* 1.9, 5.6 Hz), 3.69 (3H, s), 2.79 (1H, dt, *J* 5.7,8.9 Hz), 1.61 – 1.19 (46H, m), 0.90 (9H, s), 0.88 (3H, t, *J* 5.2 Hz), 0.07 (3H, s), 0.07 (3H, s); δ_C (101 MHz, $CDCl_3$): 201.3, 174.1, 68.8, 52.6, 49.0, 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 27.4, 27.3, 25.6, 22.7, 14.1, –4.5, –5.3; ν_{max}: 2924, 2855, 1740, 1460 cm^{-1}.

The second fraction was methyl *(R)-2-((S)-1-((tert*-butyldimethylsilyl)oxy)-2-hydroxyethyl) hexacosanoate **182a** (0.70 g, 15%) $[\alpha]_D^{22}$ = – 9.9 (c 0.99, $CHCl_3$), {Found $[M+Na]^+$: 607.5081; $C_{35}H_{72}O_4SiNa$ requires: 607.5092}, which showed δ_H (400 MHz, $CDCl_3$): 3.95 – 3.90 (1H, m), 3.69 (3H, s), 3.62 (2H, br. d, *J* 3.3 Hz), 2.77 – 2.70 (1H, m), 1.89 (1H, dd, *J* 5.3, 7.6 Hz), 1.26 (46H, m), 0.89 (12H, s, including t, *J* 6.7 Hz for terminal CH_3), 0.10 (3H, s), 0.06 (3H, s); δ_C (101 MHz, $CDCl_3$): 174.9, 73.9, 63.7, 51.4, 49.0, 31.9, 29.7, 28.3, 27.6, 25.7, 22.7, 14.1, –4.5, –5.1; ν_{max}: 3363, 2925, 2854, 1738, 1465, 1254, 1056 cm^{-1}.

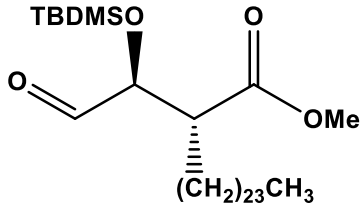
Experiment 26: Ethyl (*R*)-2-((*S*)-1-((*tert*-butyldimethylsilyl)oxy)-2-oxoethyl)hexacosanoate (185) and ethyl (*R*)-2-((*S*)-1-((*tert*-butyldimethylsilyl)oxy)-2-hydroxyethyl)hexacosanoate (185a)



The above experiment was repeated using diethyl (*2S,3R*)-2-((*tert*-butyldimethylsilyl)oxy)-3-tetracosylsuccinate **179** (0.5 g, 0.7 mmol) magnesium bromide dietherate (0.30 g, 1.1 mmol) in dichloromethane (5 mL) and diisobutylaluminum hydride (1.3 mL, 0.001 mol 1 M solution in toluene). The reaction mixture was worked up and purified as before to give ethyl (*R*)-2-((*S*)-1-((*tert*-butyldimethylsilyl)oxy)-2-oxoethyl)hexacosanoate **185** (0.30 g, 61%) $[\alpha]_D^{19}$ = – 15.7 (c 2.65, $CHCl_3$), {Found $[M+Na]^+$: 619.5; $C_{36}H_{72}O_4SiNa$ requires: 619.5}. This showed δ_H (400 MHz, $CDCl_3$): 9.63 (1H, d, *J* 1.7 Hz), 4.23 – 4.07 (2H, m), 4.05 (1H, dd, *J* 1.7, 5.2 Hz), 2.77 (1H, dt, *J* 5.7, 8.6 Hz), 1.53 – 1.13 (49H, m including 3H for CH_3), 0.90 (9H, s), 0.89 (3H, t, *J* 7.5 Hz), 0.07 (3H, s), 0.06 (3H, s); δ_C (101 MHz, $CDCl_3$): 203.0, 172.1, 78.0, 60.8, 49.2, 32.1, 29.9, 29.8, 29.7, 29.6, 29.5, 27.5, 27.4, 25.7, 22.8, 14.4, 14.3, –4.4, –5.0; ν_{max}: 2924, 2855, 1740, 1460 cm^{-1}.

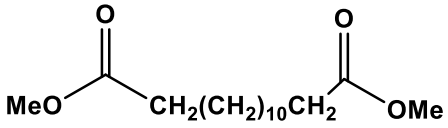
The second fraction was **185a** (0.11 g, 24%) $[\alpha]_D^{19}$ = – 10.6 (c 1.55, $CHCl_3$), {Found $[M+Na]^+$: 621.5; $C_{36}H_{74}O_4SiNa$ requires: 621.5}. This showed δ_H (400 MHz, $CDCl_3$): 4.15 – 4.10 (2H, m), 3.93 (1H, dt, *J* 3.5, 7.3 Hz), 3.63 (2H, d, *J* 3.7 Hz), 2.70 (1H, ddd, *J* 4.2, 7.6,11.4 Hz), 1.34 – 1.22 (50H, m including triplet *J* 6.5 Hz for CH_3), 0.89 (12H, s including t, *J* 7.4 Hz for terminal CH_3), 0.09 (3H, s), 0.06 (3H, s); δ_C (101 MHz, $CDCl_3$): 177.9, 74.3, 69.8, 63.8, 60.3, 58.5, 45.9, 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 28.2, 27.6, 27.5, 25.7, 25.6, 23.5, 22.7, 14.1, –4.5, –5.2; ν_{max}: 3454, 2924, 2854, 1732, 1464, 1255, 1053 cm^{-1}.

Experiment 27: Methyl (*R*)-2-((*S*)-1-((*tert*-butyldimethylsilyl)oxy)-2-oxoethyl)hexacosa-noate(185)



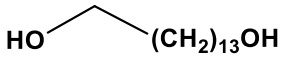
Methyl (*R*)-2-((*S*)-1-((*tert*-butyldimethylsilyl)oxy)-2-hydroxyethyl)hexacosanoate **185a** (0.5 g, 0.83 mmol) in dichloromethane (4 mL) was added to a stirred suspension of pyridinium chlorochromate (0.3 g, 1.66 mmol) in dichloromethane (20 mL) in portions at room temperature. The mixture was stirred for 2 h. TLC showed no starting material was left. The mixture was diluted with petrol/ether (5:1, 200 mL) and filtered through a bed of celite on silica. The filtrate was evaporated and concentrated to obtain the crude product. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) gave a semi-solid **185** methyl (*R*)-2-((*S*)-1-((*tert*-butyldimethylsilyl)oxy)-2-oxoethyl)hexacosanoate (0.4 g, 82%), which showed ^{1}H, ^{13}C and IR spectra matched those in experiment **25**.

Experiment 28: Dimethyl tetradecanedioate (**232)**



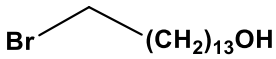
Conc. H_2SO_4 (5 mL) was added to a stirred solution of tetradecanoic acid **222** (25.0 g, 0.096 mol) in methanol (150 mL) and the reaction mixture was refluxed for 3 h when TLC showed there was no staring material was left. The excess methanol was evaporated and then sat. aq. $NaHCO_3$ (100 mL) was added to neutralize the acid. The product was extracted with CH_2Cl_2 (3 × 200 mL) then the combined organic layers were dried over $MgSO_4$ and concentrated. The crude product was purified by column chromatography, eluting with petrol/ethyl acetate (5:1) to give a white solid of dimethyl tetradecanedioate **232** (27 g, 98%) {Found $[M+Na]^+$: 309.2; $C_{16}H_{30}O_4Na$ requires: 309.2}.This showed δ_H (400 MHz, $CDCl_3$): 3.55 (6H, s), 2.19 (4H, t, *J* 7.5 Hz), 1.57 – 1.09 (20H, m); δ_C (101 MHz, $CDCl_3$): 173.9, 51.0, 33.8, 29.3, 29.2, 29.0, 28.9, 24.7; ν_{max}: 2929, 2856, 1731, 1438 cm^{-1}.

Experiment 29: Tetradecane-1,14-diol (233)



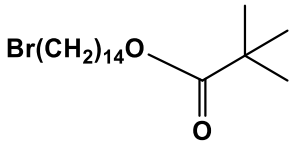
A solution of the dimethyl ester **232** (27.0 g, 0.094 mol) in dry THF (40 mL) was added to a stirred suspension solution of $LiAlH_4$ (8.8 g, 0.23 mol) in dry THF (250 mL) at –10 °C and the mixture was refluxed for 3 h. When TLC showed no starting material was left, the reaction mixture was quenched with sat. aq. sodium sulfate (20 mL) at –10 °C until a white precipitate was formed and then $MgSO_4$ (20 g) was added. The mixture was filtered through a pad of celite, and washed with THF and the solvent evaporated. The product was purified by column chromatography, eluting with petrol/ethyl acetate (5:1) to give a white solid, tetradecane-1,14-diol **233** (21 g, 97%).[284] This showed δ_H (400 MHz, $CDCl_3$): 3.65 (4H, t, *J* 6.6 Hz), 1.42 – 1.21 (26H, m); δ_C (101 MHz, $CDCl_3$): 62.6, 32.5, 29.5, 29.3, 25.6; ν_{max}: 3410, 2927, 2852, 1424 cm^{-1}.

Experiment 30: 14-Bromotetradecan-1-ol (234)



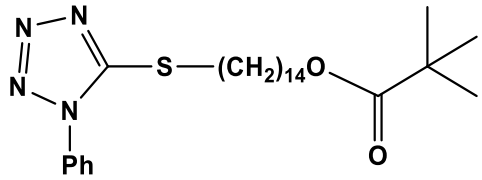
Tetradecane-1,14-diol **233** (19 g, 0.082 mol) was dissolved in toluene (150 mL) and aqueous HBr (30 mL, 48%) was added then the mixture was refluxed for 18 h. The reaction mixture was cooled to room temperature and the organic layer was separated. The aqueous layer was extracted with toluene (2 × 100 mL) and the combined organic layers were evaporated. The brown oil residue was dissolved in CH_2Cl_2 (300 mL) and washed with sat. aq. sodium bicarbonate (200 mL). The aqueous layer was re-extracted with CH_2Cl_2 (2 × 100 mL). The combined organic layers were dried, evaporated. The crude product was purified by column chromatography, eluting petrol/ethyl acetate (10:1, then 5:1) to give 14-bromotetradecan-1-ol **234** (21 g, 85%). {Found $[M+Na]^+$: 315.1309; $C_{14}H_{29}BrNaO$ requires: 315.1299}. This showed δ_H (400 MHz, $CDCl_3$): 3.64 (2H, t, *J* 6.6 Hz), 3.41 (2H, t, *J* 6.9 Hz), 1.91 – 1.80 (2H, m), 1.65 – 1.20 (23H, m); δ_C (101 MHz, $CDCl_3$): 63.0, 34.0, 32.8, 29.6, 29.5, 29.4, 28.8, 28.2, 25.8; ν_{max}: 3583, 3020, 2929, 1523, 1027 cm^{-1}.

Experiment 31: 14-Bromotetradecyl pivalate (221)



A solution of trimethylacetyl chloride (11.1 mL ,10.8 g, 0.090 mol, 1.2 mol eq.) in CH_2Cl_2 (25 mL) was added to a stirred solution of 14-bromotetradecan-1-ol **234** (17.6 g, 0.060 mol), triethylammine (12.1 g, 0.120 mol, 2 mol eq.) and 4-dimethylaminopyridine (1g) in CH_2Cl_2 (150 mL), over a period of 15 min. at 5 ºC. The mixture was allowed to reach room temperature and stirred for 18 h, then quenched with dilute HCl (150 mL). The organic layer was separated, and the aqueous layer was re-extracted with CH_2Cl_2 (2 × 100 mL). The combined organic layers were washed with brine (2 × 200 mL). The organic phase was dried, and the solvent was evaporated. The product was purified by column chromatography, eluting with petrol/ethyl acetate (5:1) to give a colourless oil 14-bromotetradecyl pivalate **221** (22 g, 97%).[202] This showed δ_H (400 MHz, $CDCl_3$): 4.04 (2H, t, *J* 6.6 Hz), 3.40 (2H, t, *J* 6.9 Hz), 1.91 – 1.79 (2H, m), 1.67 – 1.56 (2H, m), 1.47 – 1.23 (20H, m), 1.19 (9H, s); δ_C (101 MHz, $CDCl_3$): 178.6, 64.4, 45.1, 38.7, 34.0, 32.9, 29.6, 29.5, 29.4, 29.2, 28.7, 28.6, 28.2, 27.2, 25.9; ν_{max}: 2927, 2854, 1729, 1479, 1462, 1285, 1158 cm^{-1}. All data data matched those in the literature.[202]

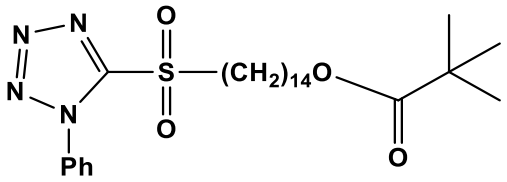
Experiment 32: ((1-Phenyl-1*H*-tetrazol-5-yl)thio)tetradecyl pivalate (220)



14-Bromotetradecyl pivalate **221** (14.1 g, 0.037 mol) was added to a stirred solution of anhydrous potassium carbonate (20.7 g, 0.150 mol) and 1-phenyl-1*H*-tetrazole-5-thiol (7.37 g, 0.041 mol) in acetone (100 mL) and THF (50 mL) at room temperature then the mixture was refluxed for 3 h. The reaction mixture was worked up and purified as before to give a white solid of ((1-phenyl-1*H*-tetrazol-5-yl)thio)tetradecyl pivalate **220** (15 g, 86%).{Found $[M+Na]^+$: 497.3; $C_{26}H_{42}O_2N_4SNa$ requires: 497.3}. This showed δ_H (400 MHz, $CDCl_3$): 7.71 – 7.41 (5H, m), 4.03 (2H, t, *J* 6.6 Hz), 3.38 (2H, t, *J* 7.4 Hz), 1.88 – 1.71 (2H, m), 1.66 – 1.52 (2H, m), 1.48 – 1.21 (20H, m), 1.18 (9H, s); δ_C (101 MHz, $CDCl_3$): 178.6, 154.5,

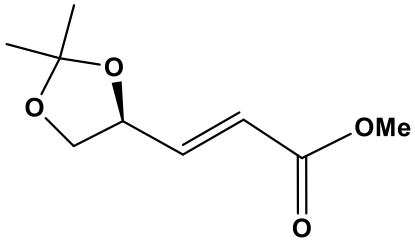
133.7, 130.0, 129.7, 123.8, 64.4, 38.7, 33.3, 29.5, 29.4, 29.2, 29.1, 29.0, 28.6, 28.5, 27.2, 25.8; ν_{max}: 2929, 2856, 1717, 1501, 1480, 1462, 1388, 1215, 1159 cm^{-1}.

Experiment 33: 14-((1-Phenyl-1*H*-tetrazol-5-yl)sulfonyl)tetradecyl pivalate (217)



Ammonium molybdate (VI) tetrahydrate (16.1 g, 0.013 mol) was dissolved in cold hydrogen peroxide (35%, 30 mL) and was gradually added to a stirred solution of ((1-phenyl-1*H*-tetrazol-5-yl)thio)tetradecyl pivalate **220** (13.7 g, 0.0280 mol) in IMS (200 mL) and THF (100 mL) at 0 ºC. The mixture was allowed to reach room temperature and stirred for 2 h, and then another portion of ammonium molybdate (VI) tetrahydrate (6.1 g, 0.0040 mol) in cold aqueus hydrogen peroxide (35 %, 15 mL) was added and stirred for 18 h. The mixture was worked up and purified as before to give 14-((1-phenyl-1*H*-tetrazol-5-yl)sulfonyl)- tetradecyl pivalate **217** (13 g, 93%), {Found $[M+Na]^+$: 529.2806; $C_{26}H_{42}O_4N_4SNa$ requires: 529.2819}. This showed δ_H (400 MHz, $CDCl_3$): 7.70 (2H, m), 7.61 (3H, m), 4.05 (2H, t, *J* 6.6 Hz), 3.74 (2H, distorted t, *J* 7.9 Hz), 1.96 (2H, m), 1.68 – 1.57 (2H, m), 1.55 – 1.22 (20H, m), 1.20 (9H, s); δ_C (101 MHz, $CDCl_3$): 178.6, 153.5, 133.0, 131.4, 129.7, 125.0, 64.4, 56.0, 38.7, 29.5, 29.4, 29.2, 29.1, 28.9, 28.6, 28.1, 27.2, 26.0, 21.9 ; ν_{max}: 2929, 2856, 1722, 1455, 1338, 1151 cm^{-1}.

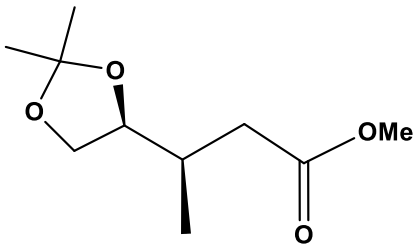
Experiment 34: Methyl (*S*,*E*)-3-(2,2-dimethyl-1,3-dioxolan-4-yl)acrylate (229)



A solution of $NaIO_4$ (19.6 g, 0.091 mol) in water (100 mL) was added drop wise to a stirred solution of 1,2:5,6-*O*-isopropylidene-*D*-mannitol **219** (20 g, 0.076 mol) in 5% $NaHCO_3$ (200 mL) at 0 ºC and stirring was continued for 1 h at room temperature. The mixture was cooled to 0 ºC and (diisopropoxyphosphoryl)-acetic acid methyl ester (40.0 g, 0.168 mol) was added with stirring followed by a 6 M solution of aq. K_2CO_3 (260 mL) at 0

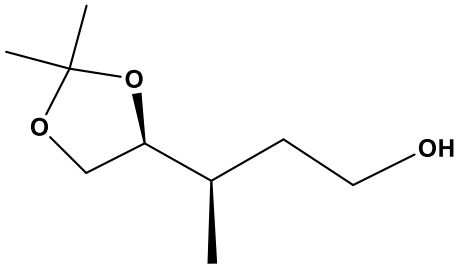
– 4 ºC. The mixture was allowed to reach room temperature and stirring was continued for 20 h. then extracted with CH_2Cl_2 (3 × 300 mL), the combined organic extracts were dried and the solvent was evaporated. The product was purified by column chromatography eluting with petrol/ethyl acetate (5:1) to give a colourless oil, (*E*)-3-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-acrylic acid methyl ester **229** (19 g, 70%) $[\alpha]_D^{24}$ = + 45.2 (c 1.25, $CHCl_3$). (*lit.* $[\alpha]_D^{24}$ = + 40.4 (c 1.09, $CHCl_3$)). The NMR and IR data match those in the literature.[269]

Experiment 35: Methyl (*R*)-3-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)butanoate (230)



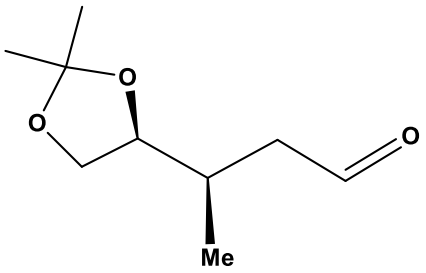
Methyl lithium (77 mL, 0.107 mol, 1.4M) was added drop wise to a stirred solution of (*E*)-3-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-acrylic acid methyl ester **229** (10.0 g, 0.053 mol) in dry ether (300 mL) at −78 ºC under nitrogen atmosphere. The mixture was maintained at −78 ºC for 2.5 h, and then allowed to gradually warm up to −60 ºC when water (10 mL) was added. After 5 min, sat. aq. NH_4Cl (60 mL) was added, whereupon the temperature rose to −40 ºC. The cooling bath was removed and the temperature of the mixture brought to 0 ºC by addition of water (100 mL). The organic layer was separated and the aqueous layer was extracted with ether (100 mL). The combined organic phases were washed with brine (2 × 100 mL), dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol /ether (2:1) to give a colourless oil, (*R*)-3-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-butyric acid methyl ester **230** (8.0 g, 77%) $[\alpha]_D^{23}$ = + 8.6 (*c* 1.1, $CHCl_3$) (*litt*. $[\alpha]_D^{22}$ = + 8.34 (*c* 1.12, $CHCl_3$)). All data data matched those in the literature.[269]

Experiment 36: (*R*)-3-((*S*)-2,2-Dimethyl-[1,3]dioxolan-4-yl)-butan-1-ol (231)



A solution of methyl (*R*)-3-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)butanoate **230** (10.0 g, 0.049 mol) in dry THF (40 mL) was added to a stirred suspension solution of $LiAlH_4$ (2.8 g, 0.074 mol) in dry THF (150 mL) at −10 ºC then the mixture was allowed to reach room temperature and refluxed for 1 h. The reaction mixture was worked up and purified as before to give a colourless oil, (*R*)-3-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-butan-1-ol **231** (7.5 g, 87%) $[\alpha]_D^{25}$ = + 19.2 (*c* 1.12, $CHCl_3$). (*lit*. $[\alpha]_D^{24}$ = + 18.1 (*c* 1.35, $CHCl_3$)). The NMR and IR data matched those in the literature.[285]

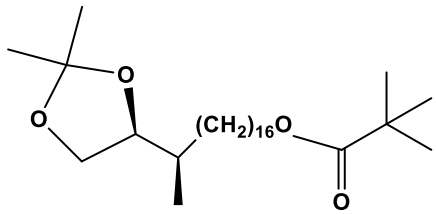
Experiment 37: (*R*)-3-((*S*)-2,2-Dimethyl-[1,3]dioxolan-4-yl)-butyraldehyde (218)



A solution of the primary alcohol **231** (7.0 g, 0.04 mol) in CH_2Cl_2 (30 mL) was added to a stirred solution of PCC (17.3 g, 0.080 mol) in CH_2Cl_2 (250 mL). Addition was done portion and portion, and during the addition a black colour appeared. The reaction was stirred for 3 h. The reaction diluted first with petrol/ethyl acetate (10:1, 250 mL). The solution was filtered through a bed of silica and the solvent was evaporated. The product was purified by column chromatography eluting with petrol/ethyl acetate (5:1) to give a colourless oil, (*R*)-3-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-butyraldehyde **218** (6 g, 87%) $[\alpha]_D^{23}$ = + 8.24 (c1.06, $CHCl_3$).[285] This showed δ_H (400 MHz, $CDCl_3$): 9.77 (1H, br. t, *J* 1.8 Hz), 4.11 – 4.05 (1H, m), 3.99 (1H, dd, *J* 6.7, 8.2 Hz), 3.64 (1H, dd, *J* 7.3, 8.2 Hz), 2.56 (1H, ddd, *J* 1.3, 5.1, 16.4 Hz), 2.44 – 2.33 (1H, m), 2.28 (1H, ddd, *J* 2.1, 8.0, 16.4 Hz), 1.41 (3H, s), 1.35 (3H, s), 1.0 (3H, d, *J* 6.8 Hz); δ_C (101 MHz, $CDCl_3$): 201.6, 78.6, 66.3, 46.6, 30.4, 26.2, 25.1, 22.6,

15.5; ν_{max}: 2985, 1725, 1371, 1215, 1066, 859 cm^{-1}. All data data matched those in the literature.[285]

Experiment 38: (*R*)-17-((*S*)-2,2-Dimethyl-1,3-dioxolan-4-yl)octadecyl pivalate (236)

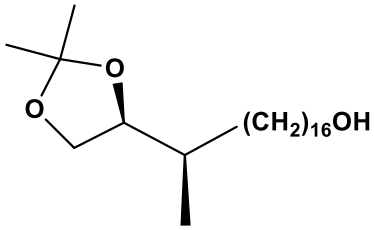


Lithium bis (trimethylsilyl) amide (49.8 mL, 0.049 mol, 1.0 M) was added to a stirred solution of 14-((1-phenyl-1*H*-tetrazol-5-yl)sulfonyl)tetradecyl pivalate **217** (16.9 g, 0.033 mol) and (*R*)-3-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-butyraldehyde **218** (5.2 g, 0.03 mmol) in dry THF (100 mL) at −10 ºC under nitrogen atmosphere. The solution was allowed to reach room temperature and stirred for 3 h, then quenched with sat. aq. NH_4Cl (100 mL). The product was extracted with ethyl acetate (3 × 150 mL). The combined organic layers were dried, and the solvent was evaporated. The product was purified by column chromatography, eluting with petrol/ethyl acetate (20:1) to give a colourless oil (*R*)-17-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)octadec-14-en-1-yl pivalate **235** (10 g, 69%) as a mixture of two isomers. This showed δ_H (400 MHz, $CDCl_3$): 5.49 – 5.25 (2H, m), 4.04 (2H, t, *J* 6.6 Hz), 4.01 (1H, m), 3.89 (1H, br.q, *J* 6.8 Hz), 3.61 (1H, br.t, *J* 7.6Hz), 2.02 (2H, m), 1.94 – 1.74 (1H, m), 1.69 – 1.55 (2H, m), 1.39 (3H, s), 1.34 (3H, s), 1.31 – 1.22 (22H, m), 1.19 (9H, s), 0.96 (3H, d, *J* 6.7 Hz); δ_C (101 MHz, $CDCl_3$): 179.6, 132.6, 127.5, 108.6, 79.9, 67.8, 64.5, 41.3, 36.9, 36.2, 32.6, 30.9, 30.6, 29.7, 29.6, 29.5, 29.2, 29.1, 28.6, 27.2, 26.6, 25.9, 25.6, 22.6, 20.4, 19.4, 15.7; ν_{max}: 2922, 2853, 1463, 1065, 860 cm^{-1}.

Palladium 10% on carbon (1.5 g) was added to a stirred solution of the alkenes **235** (10.0 g, 0.020 mol) in IMS (150 mL) and THF (150 mL) under hydrogen atmosphere. Hydrogenation was carried out for 16 h, the solution was filtered over a bed of celite and the solvent was evaporated. The product was purified by column chromatography, eluting with petrol/ethyl acetate (20:1) to give (*R*)-17-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)octadecyl pivalate **236** (9.0 g, 92%) $[\alpha]_D^{26}$ = + 12.4 (*c* 0.180 $CHCl_3$), {Found $[M+Na]^+$: 477.3905; $C_{28}H_{54}O_4Na$ requires: 477.3914}, This showed δ_H (400 MHz, $CDCl_3$): 4.04 (2H, t, *J* 6.6 Hz), 3.99 (1H, dd, *J* 6.3, 7.8 Hz), 3.86 (1H, br. q, *J* 6.9 Hz), 3.59 (1H, br.t, *J* 7.7 Hz), 1.61 (2H, m), 1.40 (3H, s), 1.35 (3H, s), 1.32 – 1.23 (34H, m), 1.19 (9H, s), 1.1

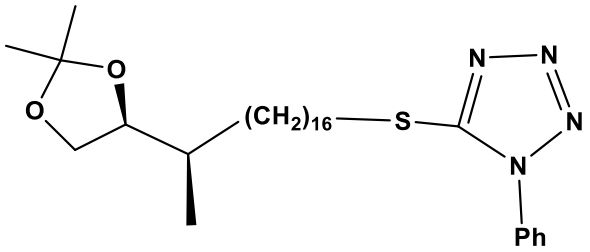
– 1.07 (1H, m), 0.96 (3H, d, *J* 6.7 Hz) ; δ_C (101 MHz, $CDCl_3$): 178.6, 108.5, 80.4, 67.8, 64.4, 38.7, 36.5, 32.7, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.2, 28.6, 27.2, 26.6, 25.9, 25.5, 15.6; ν_{max}: 2926, 2854, 1731, 1463, 1397, 1284, 1157 cm^{-1}.

Experiment 39: (*R*)-17-((*S*)-2,2-Dimethyl-1,3-dioxolan-4-yl) octadecan-1-ol (216)



A solution of dimethyl-1,3-dioxolan-4-yl)octadecyl pivalate **236** (9.2 g, 0.01 mol) in dry THF (40 mL) was added to a stirred suspension solution of $LiAlH_4$ (1.0 g, 0.02 mol) in dry THF (150 mL) at −10 ºC and the mixture was refluxed for 3 h. The mixture was worked up and purified as before to give a white solid, (*R*)-17-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl) octadecan-1-ol **216** (7 g, 96%) $[\alpha]_D^{25}$ = + 19 (*c* 0.18 $CHCl_3$), {Found $[M+Na]^+$: 393.3334; $C_{23}H_{46}O_3Na$ requires: 393.3339}. This showed δ_H (400 MHz, $CDCl_3$): 4.00 (1H, dd, *J* 6.2, 7.8 Hz), 3.87 (1H, br. q, *J* 6.9 Hz), 3.65 (1H, br. t, *J* 7.7 Hz), 3.50 (2H, t, *J* 6.7 Hz), 1.60 (2H, m), 1.41 (3H, s), 1.36 (3H, s), 1.34 – 1.22 (30H, m), 1.08 (1H,m), 0.97 (3H, d, *J* 6.7 Hz); δ_C (101 MHz, $CDCl_3$): 108.5, 80.4, 67.8, 63.0, 36.5, 32.8, 32.7, 29.9, 29.7, 29.6, 29.4, 27.0, 26.6, 25.7, 25.5, 15.6; ν_{max} 3400, 2916, 2850, 1469, 1370, 1265, 1207, 1158, 1056 cm^{-1}.

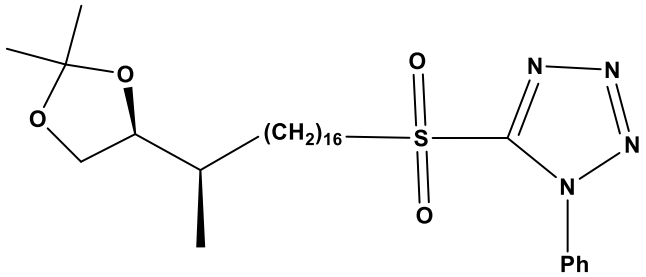
Experiment 40: 5-(((*R*)-17-((*S*)-2,2-Dimethyl-1,3-dioxolan-4-yl)octadecyl)thio)-1-phenyl-1*H*-tetrazole(215)



Diethyl azodicarboxylate (DEAD) (4.4 g, 0.020 mol) in dry THF (10 mL) was added to a stirred solution of (*R*)-17-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)octadecan-1-ol **216** (7.3 g, 0.01 mol). Triphenylphosphine (6.7 g, 0.02 mol) and 1-phenyl-1*H*-tetrazole-5-thiol (4.5 g, 0.02 mol) in dry THF (70 mL) at 0 ºC under nitrogen atmosphere. The mixture was allowed

to reach room temperature and then stirred for 3 h. The solvent was evaporated and the residue was stirred with petrol/ethyl acetate (10:1, 150 mL) for 30 min and then filtered through a pad of celite. The filtrate was evaporated, and the crude product was purified by column chromatography, eluting with petrol/ethyl acetate (5:1) to give compound **215** (8.7 g, 83%) $[\alpha]_D^{26}$ = + 13 (*c* 0.17 $CHCl_3$),{ Found $[M+Na]^+$: 553.3534; $C_{30}H_{50}O_2SN_4Na$ requires: 553.3547}. This showed δ_H (400 MHz, $CDCl_3$): 7.63 – 7.49 (5H, m), 3.99 (1H, dd, *J* 6.2, 7.8 Hz), 3.86 (1H, br. q, *J* 6.9 Hz), 3.59 (1H, br. t, *J* 7.7 Hz), 3.39 (2H, t, *J* 7.4 Hz), 1.80 (2H, m), 1.56 (1H, m), 1.43 (1H,m), 1.40 (3H, s), 1.35 (3H, s), 1.33 – 1.19 (26H, m), 1.08 (1H, m), 0.96 (3H, d, *J* 6.7 Hz); δ_C (101 MHz, $CDCl_3$): 154.5, 133.7, 130.0, 129.7, 123.8, 108.4, 80.4, 67.8, 36.5, 33.3, 32.7, 29.8, 29.6, 29.5, 29.4, 29.0, 28.6, 26.9, 26.6, 25.5, 15.6; ν_{max}: 2975, 2854, 1644, 1501, 1464, 1380, 1245, 1161, 1065 cm^{-1}.

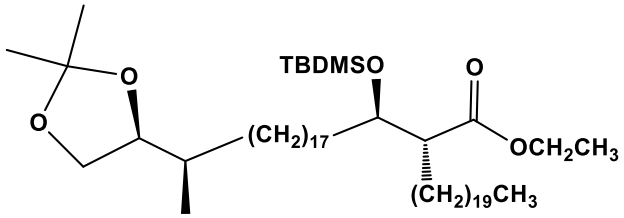
Experiment 41: 5-(((*R*)-17-((*S*)-2,2-Dimethyl-1,3-dioxolan-4-yl)octadecyl)sulfonyl)-1-phenyl-1*H*-tetrazole (214)



Ammonium molybdate VI tetrahydrate (9.1 g, 0.07 mol) was dissolved in cold aqueous hydrogen peroxide in (35 %, w/w, 30 mL) and gradually added to a stirred solution of 5-(((*R*)-17-((*S*)-2,2-Dimethyl-1,3-dioxolan-4-yl)octadecyl)thio)-1-phenyl-1*H*-tetrazole **215** (8.7 g, 0.01 mol) in IMS (150 mL) and THF (75 mL). The mixture was stirred for 2 h, and then another portion of ammonium molybdate VI tetrahydrate (3.4 g, 0.002 mol) in cold aqueous hydrogen peroxide in (35 %, w/w, 12 mL) was added and stirred at room temperature for 18 h. The reaction mixture was worked up and purified as before to give 5-(((*R*)-17-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)octadecyl)sulfonyl)-1-phenyl-1*H*-tetrazole **214** (8.9 g, 96%) $[\alpha]_D^{25}$ = + 18 (*c* 0.19 $CHCl_3$), {Found $[M+H]^+$: 563.3683; $C_{30}H_{51}N_4O_4S$ requires: 563.3626}. This showed δ_H (400 MHz, $CDCl_3$): 7.73 – 7.68 (2H, m), 7.65 – 7.57 (3H, m), 4.00 (1H, dd, *J* 6.2, 7.8 Hz), 3.87 (1H, br. q, *J* 6.9 Hz), 3.74 (2H, t, *J* 7.9 Hz), 3.60 (1H, br. t, *J* 7.7 Hz), 1.96 (2H, m), 1.53 – 1.45 (2H, m), 1.41 (3H, s), 1.36 (3H, s), 1.33 – 1.23 (26H, m), 1.11-1.08 (1H, m),

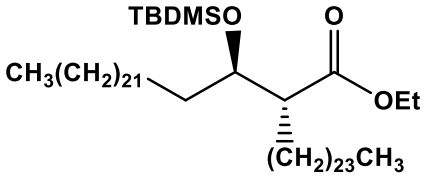
0.97 (3H, d, *J* 6.7 Hz); δ_C (101 MHz, $CDCl_3$): 153.5, 131.4, 129.7, 125.0, 108.4, 80.4, 67.8, 56.0, 36.5, 32.7, 29.8, 29.6, 29.5, 29.4, 29.2, 28.9, 28.1, 27.0, 26.6, 25.5, 21.9, 15.6; ν_{max}: 2928, 2855, 1597, 1498, 1464, 1343, 1259, 1150, 1071 cm^{-1}.

Experiment 42: Ethyl (2*R*,3*R*,21*R*)-3-((*tert*-butyldimethylsilyl)oxy)-21-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-eicosyldocosanoate (238)



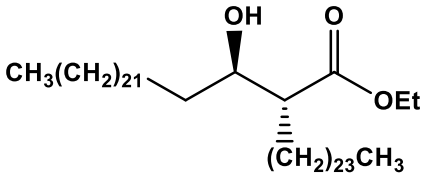
Lithium bis(trimethylsilyl) amide (8.3 mL, 0.008 mol, 1.0 M) was added drop wise to a stirred solution of ethyl (*R*)-2-((*S*)-1-((*tert*-butyldimethylsilyl)oxy)-2-oxoethyl)docosanoate **183** (2.5 g, 0.004 mol) and 5-(((*R*)-17-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)octadecyl)sulfonyl)-1-phenyl-1*H*-tetrazole **214** (3.1 g, 0.005 mol) in dry THF (50 mL) under nitrogen at –15 ºC. The mixture was allowed to reach room temperature, and stirred for 2 h. The mixture was worked up and purified as before to give ethyl *E/Z*-(2*R*,21*R*)-3-((*tert*-butyldimethylsilyl)oxy)-21-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-eicosyldocos-4-enoate **237** (3.2 g, 79%) as a mixture in ratio (2.5:1). Palladium 10% on carbon (0.5 g) was added to a stirred solution of the alkenes **237** (3.2 g, 0.003 mol) in IMS (100 mL) and THF (100 mL) under hydrogen atmosphere. Hydrogenation was carried out 6 h. The mixture was worked up and purified as before to give ethyl (2*R*,3*R*,21*R*)-3-((*tert*-butyldimethylsilyl)oxy)-21-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-eicosyldocosanoate **238** (2.5 g, 78%) $[\alpha]_D^{24}$ = + 6.4 (*c* 1.4 $CHCl_3$), {Found $[M+Na]^+$: 901.7999; $C_{55}H_{110}O_5NaSi$ requires: 901.8015}. This showed δ_H (400 MHz, $CDCl_3$): 4.12 (2H, m), 4.01 (1H, dd, *J* 6.2, 7.8 Hz), 3.95 – 3.78 (1H, m), 3.87 (1H, br. q, *J* 7.0 Hz), 3.61 (1H, br. t, *J* 7.7 Hz), 2.50 (1H, ddd, *J* 3.8, 7.0, 10.8 Hz), 1.59 – 1.52 (2H, m), 1.49 – 1.42 (2H m), 1.41 (3H, s), 1.36 (3H, s), 1.33 – 1.18 (72H, m), 0.97 (3H, d, *J* 6.7 Hz), 0.89 (3H, t, *J* 4.9 Hz), 0.87 (9H, s), 0.05 (3H, s), 0.03 (3H, s); $\delta_{C:}$ (101 MHz, $CDCl_3$): 174.6, 108.5, 80.4, 73.2, 67.8, 65.1, 60.4, 60.0, 59.9, 51.6, 36.5, 33.7, 32.7, 31.9, 30.9, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 27.8, 27.4, 27.1, 27.0, 26.6, 25.8, 25.5, 23.8, 22.7, 18.0, 15.6, 14.3, 14.1, –4.4, –5.0; ν_{max}: 2928, 2855, 1725, 1466, 1381, 1095 cm^{-1}.

Experiment 43: Ethyl (2*R*,3*R*)-3-((*tert*-butyldimethylsilyl)oxy)-2-tetracosylhexacosanoate (243)



Lithium bis(trimethylsilyl) amide (2.8 mL, 0.003 mol, 1.06 M) was added dropwise to a stirred solution of the aldehyde **185** (1.0 g, 1.67 mmol) and 5-(docosylsulfonyl)-1-phenyl-1*H*-tetrazole **173** (1.0 g, 2.0 mmol) in dry THF (15 mL) under nitrogen at –15 ºC. The mixture was allowed to reach room temperature, and stirred for 2 h. The reaction mixture was worked up and purified as before to give methyl- *E/Z*- (2*R*,3*R*)-3-((*tert*-butyl dimethylsilyl)oxy)-2-tetracosylheptacos-6-enoate **275** as a mixture of two isomers (1.1 g, 74%). Palladium 10% on carbon (0.2 g) was added to a stirred solution of the above alkenes **275** (1.0 g, 0.0011 mole) in IMS (20 mL) and THF (10 mL) under hydrogen atmosphere for 16 h. The product was purified by column chromatography, eluting with petrol/ethyl acetate (20:1) to give a white solid, **243** (0.8 g, 80%) {Found $[M+H]^+$: 891.8918; $C_{58}H_{119}O_3Si$ requires: 891.8923}. This showed δ_H (400 MHz, $CDCl_3$): 4.20 (2H, q, *J* 7.1 Hz), 3.90 (1H, dt, *J*, 5.8,7.6 Hz), 2.61 (1H, m), 2.18 (3H, s), 1.84 – 1.15 (92H, m), 0.89 (6H, t, *J* 6.6 Hz); δ_C (101 MHz, $CDCl_3$): 178.6, 73.4, 60.4, 51.6, 50.2, 31.9, 29.7, 29.6, 29.5, 29.4, 28.8, 22.7, 14.1; ν_{max}: 2915, 2848, 1740, 1467, 1371, 1234, 1169, 1096, cm^{-1}.

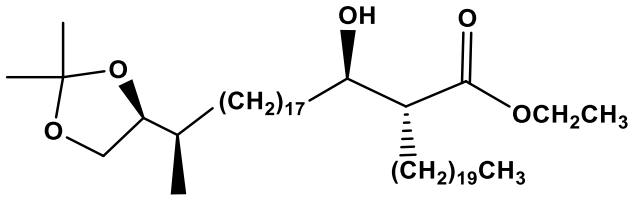
Experiment 44: Ethyl (2*R*,3*R*)-3-hydroxy-2-tetracosylhexacosanoate (244)



Tetrabutylammonium fluoride (1.12 mL, in 1.0 M THF, 1.121 mmol) was added dropwise to a stirred solution of **243** (0.5 g, 0.56 mmol) in anhydrous THF (5 mL) at 0 ºC under nitrogen atmosphere. The mixture was allowed to reach room temperature and stirred for 18 h when TLC showed no starting material was left. The solvent was evaporated to give the residue which was purified by column chromatography on silica gel eluting with petrol/ethyl acetate (5:1) to give a white semi-solid, **244** (0.2 g, 47%) $[\alpha]_D^{23}$ = + 5.27 (*c* 0.91 $CHCl_3$), {Found $[M+Na]^+$: 799.7870; $C_{52}H_{104}O_3Na$ requires: 799.7878}. This showed δ_H

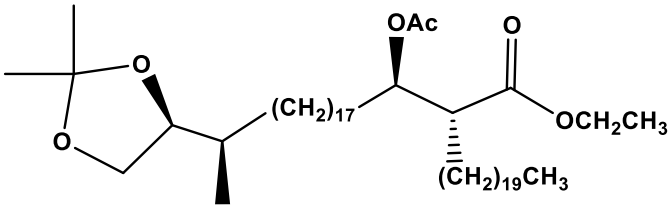
(400 MHz, $CDCl_3$): 4.19 (2H, q, *J* 7.1 Hz), 3.65 (1H, dt, *J*, 5.8,7.6), 2.45 – 2.38 (1H, m), 2.18 (3H, s), 1.61 – 1.20 (91H, m), 0.86 (6H, t, *J* 6.7 Hz); δ_C (101 MHz, $CDCl_3$): 175.9, 72.3, 60.4, 50.8, 41.4, 31.8, 29.7, 29.6, 29.5, 29.4, 29.3, 27.3, 25.7, 22.7, 22.5, 20.4, 19.4, 14.3, 14.1; ν_{max}: 3389, 2928, 2855, 1725, 1466 cm^{-1}.

Experiment 45: Ethyl (2*R*,3*R*,21*R*)-21-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)-3-hydroxy-2-eicosyldocosanoate (245)



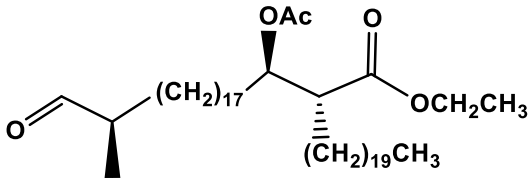
Tetrabutylammonium fluoride (10.4 mL, in 1.0 M THF, 0.010 mol) was added dropwise to a stirred solution of ethyl (2*R*,3*R*,21*R*)-3-((*tert*-butyldimethylsilyl)oxy)-21-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-icosyldocosanoate **238** (2.3 g, 0.002 mol) in anhydrous THF (15 mL) at 0 ºC under nitrogen atmosphere. The mixture was allowed to reach room temperature and stirred for 16 h when TLC showed no starting material. The solvent was evaporated to give the residue which was purified by column chromatography on silica gel eluting with hexane/ethyl acetate (5:1) to give ethyl (2*R*,3*R*,21*R*)-21-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)-3-hydroxy-2-eicosyldo-cosanoate **245** (1.64 g, 82%) $[\alpha]_D^{24}$ = + 17.5 (*c* 1.83 $CHCl_3$), {Found $[M+Na]^+$: 787.7137; $C_{49}H_{96}O_5Na$ requires: 787.7150}. This showed δ_H (400 MHz, $CDCl_3$): 4.19 (2H, q, *J* 7.1 Hz), 4.01 (1H, dd, *J* 6.5, 7.4 Hz), 3.87 (1H, br. q, *J* 6.9 Hz), 3.65 (1H, m), 3.61 (1H, br. t, *J* 7.7 Hz), 2.45 – 2.38 (1H, m), 1.79 – 1.65 (2H, m), 1.65 – 1.52 (2H, m), 1.41 (3H, s), 1.36 (3H, s), 1.34 – 1.17 (73H, m), 1.15 – 1.02 (1H, m), 0.97 (3H, d, *J* 6.6 Hz), 0.89 (3H, t, *J* 6.6 Hz) ; δ_C (101 MHz, $CDCl_3$): 175.8, 108.5, 80.4, 72.3, 68.0, 67.8, 60.4, 50.8, 36.5, 35.7, 32.7, 31.9, 29.9, 29.7, 29.6, 29.5, 29.4, 29.3, 27.0, 26.9, 26.6, 25.7, 25.6, 25.5, 22.7, 15.6, 14.3, 14.0; ν_{max}: 3520, 2924, 2854, 1710, 1461, 1377, 1264, 1189, 1164 cm^{-1}.

Experiment 46: Ethyl (2*R*,3*R*,21*R*)-3-acetoxy-21-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-eicosyldocosanoate (213)



A mixture of acetic anhydride (10 mL) and anhydrous pyridine (10 mL) was added to stirred solution of the alcohol **245** (1.64 g, 0.002 mol) at room temperature and the mixture was stirred for 18 h, then diluted with toluene (10 mL) and the solvent evaporated. The product was purified by column chromatography, eluting with petrol/ethyl acetate (5:1) to give a white solid, ethyl (2*R*,3*R*,21*R*)-3-acetoxy-21-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-eicosyldocosanoate **213** (1.7 g, 98%) $[\alpha]_D^{24}$ = + 16.5 (*c* 1.75 $CHCl_3$), {MALDI-Found $[M+Na]^+$: 829.7246; $C_{51}H_{98}O_6Na$ requires: 829.7256}. This showed δ_H (400 MHz, $CDCl_3$): 5.10 (1H, td, *J* 4.0, 7.5 Hz), 4.15 (2H, q, *J* 7.1 Hz), 4.01 (1H, dd, *J* 6.3, 7.7 Hz), 3.88 (1H, br. q, *J* 7.0 Hz), 3.61 (1H, br. t, *J* 7.7 Hz), 2.66 – 2.53 (1H, m), 2.03 (3H, s), 1.59 – 1.52 (4H, m), 1.41 (3H, s), 1.36 (3H, s), 1.34 – 1.16 (71H, m), 1.10 – 1.08 (1H, m), 0.97 (3H, d, *J* 6.7 Hz), 0.89 (3H, t, *J* 6.8 Hz); δ_C (101 MHz, $CDCl_3$): 173.1, 170.3, 108.5, 80.4, 74.1, 67.8, 60.3, 49.6, 36.5, 32.7, 31.9, 31.7, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 28.1, 27.4, 27.0, 26.6, 25.5, 24.9, 22.7, 22.6, 21.0, 15.6, 14.3, 14.0; ν_{max}: 2927, 2855, 1732, 1467, 1379, 1248, 1163, 1097 cm^{-1}.

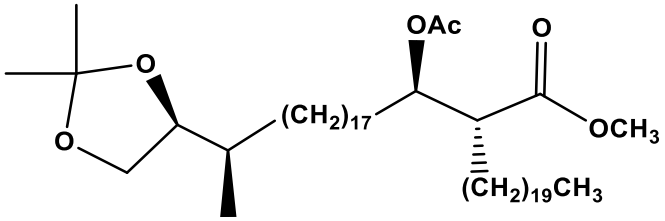
Experiment 47: Ethyl (2*R*,3*R*,21*R*)-3-acetoxy-2-eicosyl-21-methyl-22-oxodocosanoate (211)



Periodic acid (1.0 g, 4.6 mmol) was added to a stirred solution of ethyl (2*R*,3*R*,21*R*)-3-acetoxy-21-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-eicosyldocosanoate **213** (1.5 g, 1.85 mmol) in dry ether (75 ml) at room temperature under nitrogen. The mixture was stirred for 16 h, when TLC showed no starting material. The precipitate was filtered through a bed of celite and washed with ether. The solvent was evaporated and the crude product was purified by column chromatography, eluting with petrol/ethyl acetate (10:1) to give a white semi-solid, ethyl (2*R*,3*R*,21*S*)-3-acetoxy-2-

eicosyl-21-methyl-22-oxodocosanoate **211** (1.1 g, 77%) $[\alpha]_D^{24}$ = + 6 (*c* 0.4 $CHCl_3$) {Found $[M+Na]^+$: 757.6659; $C_{47}H_{90}O_5Na$ requires: 757.6680}. This showed δ_H (400 MHz, $CDCl_3$): 9.61 (1H, d, *J* 1.9 Hz), 5.09 (1H, td, *J* 4.0,7.7 Hz), 4.14 (2H, q, *J* 7.1 Hz), 2.60 (1H, ddd, *J* 5.5, 9.1, 10.8 Hz), 2.38 – 2.29 (1H, m), 2.02 (3H, s), 1.46 – 1.17 (95H, m), 1.09 (3H, d, *J* 7.0 Hz), 0.88 (3H, t, *J* 6.7 Hz); δ_C (101 MHz, $CDCl_3$): 205.3, 173.1, 170.3, 74.2, 60.3, 49.6, 46.3, 31.9, 31.7, 30.5, 29.7, 29.6, 29.5, 29.4, 29.3, 28.2, 27.4, 26.9, 24.9, 22.7, 21.0, 14.3, 14.0, 13.3; ν_{max}: 2916, 2855, 1731, 1465, 1375, 1247, 1183, 1025 cm^{-1}.

Experiment 48: Methyl (2*R*,3*R*,21*R*)-3-acetoxy-21-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-eicosyldocosanoate (212)

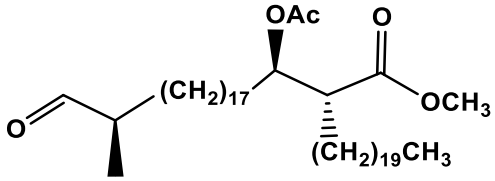


Lithium bis(trimethylsilyl) amide (9.3 mL, 0.009 mol, 1.0 M) was added drop wise to a stirred solution of methyl (*R*)-2-((*S*)-1-((*tert*-butyldimethylsilyl)oxy)-2-oxoethyl)docosanoate **180** (3.0 g, 0.005 mol) and 5-(((*R*)-17-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)octadecyl)sulfonyl)-1-phenyl-1*H*-tetrazole **214** (3.5 g, 0.006 mol) in dry THF (50 mL) under nitrogen at –15 ºC. The mixture was allowed to reach room temperature, and stirred for 2 h. The mixture was worked up and purified as before to give (3.8 g, 76%), as a mixture of methyl *E/Z*- (2*R*,21*R*)-3-((*tert*-butyldimethylsilyl)oxy)-21-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-eicosyldocos-4-enoate **246**. This showed 5.47 – 5.40 (1H, m), 5.37 – 5.31(1H, m), 4.00 (1H, dd, *J* 6.3, 7.9 Hz), 3.98–3.89 (1H, m), 3.89 (1H, br.q, *J* 6.9 Hz), 3.66 (3H, s), 3.61 (1H, br.t, *J* 7.9 Hz), 2.56–2.50 (1H, m), 2.35–2.16 (2H, m), 2.01–1.98 (2H, m), 1.59–1.51 (3H, m), 1.41 (3H, s), 1.36 (3H, s), 1.39–1.26 (48H, m, v.br.), 1.12–1.07 (1H, m), 0.97 (3H, d, *J* 6.6 Hz), 0.89 (3H, t, *J* 6.9 Hz), 0.87 (9H, s), 0.06 (3H, s), 0.02 (3H, s); δ_C: 175.1, 132.7, 127.5, 108.5, 80.4, 73.2, 67.8, 51.4, 51.16, 36.5, 32.8, 32.7, 31.9, 29.9, 29.7, 29.66, 29.64, 29.62, 29.60, 29.57, 29.53, 29.49, 29.43, 29.36, 29.25, 27.7, 27.6, 27.0, 26.6, 25.7, 25.5, 22.7, 18.0, 15.6, 14.1, - 4.3, - 5.0; ν_{max}: 2925, 2854, 1740, 1465, 1368, 1253, 1165, 1071cm^{-1}.

Tetrabutylammonium fluoride (17.6 mL, in 1.0 M THF, 0.010 mol) was added dropwise to a stirred solution of the above alkenes **246** in anhydrous THF (15 mL) at 0 ºC under nitrogen atmosphere.

The mixture was allowed to reach room temperature and stirred for 16 h. The reaction mixture was worked up and purified as before to give a white semi-solid, methyl *E/Z*-(2*R*,21*R*)-21-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)-3-hydroxy-2-eicosyldocos-4-enoate **247** (2.8 g, 88%). A mixture of acetic anhydride (12 mL) and anhydrous pyridine (12 mL) was added to stirred solution of the above alcohol (2.8 g, 3.0 mmol) at room temperature and stirred for 18 h. The mixture was worked up and purified as before to give a white semi-solid methyl *E/Z*-(2*R*,21*R*)-3-acetoxy-21-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-eicosyl docos-4- enoate **248** (2.6 g, 88%). Palladium hydroxide on activated charcoal (10% $Pd(OH)_2$-C 0.5g) was added to a stirred solution of the above alkenes (2.6 g, 3.340 mmol) in ethyl acetate (50 mL) under hydrogen atmosphere for 2 h. The mixture was worked up and purified as before to give a white solid, methyl (2*R*,3*R*,21*R*)-3-acetoxy-21-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-icosyldocosanoate **212** (2.5 g, 96%) $[\alpha]_D^{23}$ = + 17.5 (*c* 0.92 $CHCl_3$), {MALDI-Found $[M+Na]^+$: 815.7093; $C_{50}H_{96}O_6Na$ requires: 815.7099}. This showed δ_H (400 MHz, $CDCl_3$): 5.09 (1H, td, *J* 4.1, 7.6 Hz), 4.01 (1H, dd, *J* 6.4, 7.7 Hz), 3.87 (1H, br. q, *J* 6.9 Hz), 3.68 (3H, s), 3.61 (1H, br. t, *J* 7.7 Hz), 2.67 – 2.57 (1H, m), 2.35 (1H, m), 2.04 (3H, s), 1.41 (3H, s), 1.36 (3H, s), 1.35 – 1.20 (72H, m), 0.97 (3H, d, *J* 6.7 Hz), 0.89 (3H, t, *J* 6.6 Hz); δ_C (101 MHz, $CDCl_3$): 173.7, 170.4, 108.5, 80.4, 74.1, 67.8, 51.6, 49.6, 36.5, 32.7, 31.9, 31.7, 29.9, 29.6, 29.5, 29.4, 29.3, 28.1, 27.5, 27.1, 26.6, 25.5, 24.9, 22.7, 21.0, 15.6, 14.6, 14.1; ν_{max}: 2916, 2855, 1731, 1465, 1375, 1247, 1183, 1025 cm^{-1}.

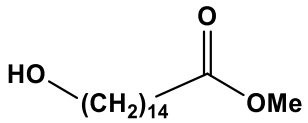
Experiment 49: Methyl (2*R*,3*R*,21*R*)-3-acetoxy-2-icosyl-21-methyl-22-oxodocosanoate (210)



Periodic acid (1.7 g, 0.0070 mol) was added to a stirred solution of methyl (2*R*,3*R*,21*R*)-3-acetoxy-21-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-eicosyldocosanoate **212** (2.5 g, 0.0030 mol) in dry ether (125 mL) at room temperature under nitrogen. The mixture was stirred for 16 h. The mixture was worked up and purified as before to give a white semi-solid, methyl (2*R*,3*R*,21*R*)-3-acetoxy-2-eicosyl-21-methyl-22-oxodocosanoate **210** (2.0 g, 88%) $[\alpha]_D^{23}$ = + 6.7 (c 0.53, $CHCl_3$) {Found $[M+Na]^+$: 743.6506; $C_{46}H_{88}O_5Na$ requires: 743.6524}. This showed δ_H (400 MHz, $CDCl_3$): 9.61 (1H, d, *J* 1.7 Hz), 5.08 (1H, dt, *J* 4.1,7.6 Hz), 3.68 (3H, s), 2.65 – 2.56 (1H, m), 2.39 – 2.26 (1H, m), 2.03 (3H, s), 1.73 – 1.19 (92H, m), 1.09 (3H,

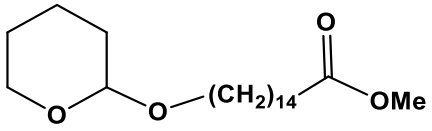
d, *J* 7.0 Hz), 0.88 (3H, t, *J* 6.6 Hz); δ_C (101 MHz, $CDCl_3$): 205.4, 173.7, 170.4, 74.0, 51.5, 49.6, 46.3, 31.9, 31.7, 30.5, 29.7, 29.6, 29.5, 29.4, 29.3, 28.1, 27.5, 26.9, 25.0, 22.7, 21.0, 14.0, 13.3; ν_{max}: 2916, 2849, 1731, 1439, 1373, 1235, 1197, 1021 cm^{-1}.

Experiment 50: Methyl 15-hydroxypentadecanoate (249)



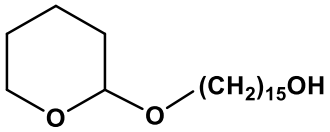
Sodium (11.8 g, 0.513 mol) was added to a stirred solution of methanol (400 mL) at 0 ºC. The mixture was allowed to reach room temperature until all of the sodium was consumed. *ω*- Pentadecalactone **227** (50 g, 0.20 mol) was added, and the mixture was heated to 80 ºC and stirred for 2 h. The reaction was cooled to room temperature and quenched with aq. HCl (250 mL, 1N) and water (200 mL). 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 (2 mL) was added. The mixture was refluxed for 1 h until no acid was left, then cooled to room temperature 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 with ethyl acetate. The solvent was evaporated and the product was purified by recrystallization using petroleum ether to give methyl 15-hydroxypentadecanoate **249** (49 g, 86%),[273] m.p. 45 – 47 ºC (*lit.* 47 – 48 °C). This showed δ_H (400 MHz, $CDCl_3$): 3.67 (3H, s), 3.64 (2H, t, *J* 6.5 Hz), 2.31 (2H, t, *J* 7.5 Hz), 1.70 – 1.49 (4H, m), 1.41 – 1.15 (21H, m); δ_C (101 MHz, CDCl3): 174.4, 63.0, 51.4, 34.1, 32.8, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 25.7, 24.9; ν_{max}: 3298, 2919, 2850, 1742, 1464, 1178 cm^{-1}. All data were identical to the literature.[273]

Experiment 51: Methyl 15-((tetrahydro-2*H*-pyran-2-yl)oxy)pentadecanoate (250)



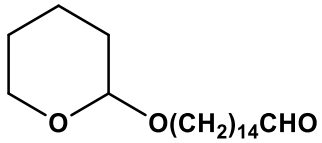
3,4-Dihydro-2*H*-pyran (18.5 g, 0.224 mol) and pyridinium-*p*-toluene-sulfonate (8.3 g, 0.033 mol) were added to a stirred solution of 15-hydroxypentadecanoate **249** (30 g, 0.11 mol) in dry CH_2Cl_2 (250 mL) under nitrogen at room temperature. The mixture was stirred for 3 h when TLC showed no starting material was left, then quenched by adding sat. aq. $NaHCO_3$ (150 mL), and extracted with CH_2Cl_2. The solvent was evaporated and the product was purified by column chromatography, eluting with petrol/ethyl acetate (5:2) to give methyl 15-((tetrahydro-2*H*-pyran-2-yl)oxy)pentadecanoate **250** (37g, 94%).[202] This showed δ_H (400 MHz, $CDCl_3$): 4.58 (1H, m), 3.87 (1H, m), 3.73 (1H, dt, *J* 6.9, 9.3 Hz), 3.67 (3H, s), 3.50 (1H, m), 3.38 (1H, dt, *J* 6.7, 9.4 Hz), 2.30 (2H, t, *J* 7.5 Hz), 1.83 (1H, m), 1.72 (1H, m), 1.59 (4H, m), 1.56 – 1.19 (24H, m); δ_C (101 MHz, $CDCl_3$): 174.3, 98.8, 67.7, 62.3, 51.4, 34.1, 30.8, 29.7, 29.6, 29.5, 29.4, 29.2, 29.1, 26.2, 25.5, 24.9, 19.7; ν_{max}: 2929, 2855, 1731, 1438 cm^{-1}. All data matched those in the literature.[202]

Experiment 52: 15-((Tetrahydro-2*H*-pyran-2-yl)oxy)pentadecan-1-ol (251)



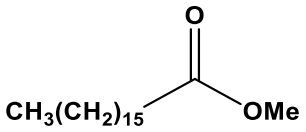
A solution of the methyl 15-((tetrahydro-2*H*-pyran-2-yl)oxy)pentadecanoate **250** (30.0g, 0.084 mol) in THF (50 mL) was added to a stirred suspension solution of $LiAlH_4$ (4.7g, 0.12 mol) in THF (250 mL) at −10 °C and the mixture was refluxed for 3 h. The mixture was worked up and purified as before to give 15-((tetrahydro-2*H*-pyran-2-yl)oxy)pentadecan-1-ol **251** (26 g, 91%) {Found $[M+NH_4]^+$: 346.3318; $C_{20}H_{44}O_3N$ requires: 346.3316}. This showed δ_H (400 MHz, $CDCl_3$): 4.58 (1H, m), 3.87 (1H, dt, *J* 5.8, 7.5 Hz), 3.73 (1H, dt, *J* 3.4,7.4 Hz), 3.64 (2H, br.t, *J* 5.3 Hz), 3.50 (1H, m), 3.39 (1H, dt, *J* 4.1,7.2 Hz), 1.84 (1H, m), 1.72 (1H, m), 1.58 (4H, m), 1.40 – 1.20 (27H, m); δ_C (101 MHz, $CDCl_3$): 98.8, 67.7, 63.0, 62.3, 32.8, 30.8, 29.7, 29.6, 29.5, 29.4, 26.2, 25.7, 25.5, 19.7; ν_{max}: 3392, 2923, 2852, 1468 cm^{-1}.

Experiment 53: 15-((Tetrahydro-2*H*-pyran-2-yl)oxy)pentadecanal (226)



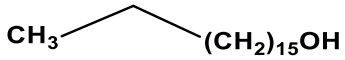
A solution of the primary alcohol **251** (4.0 g, 0.01 mol) in CH_2Cl_2 (20 mL) was added to a stirred solution of PCC (5.2 g, 0.02 mol) in CH_2Cl_2 (100 mL), a black colour appearing during the addition. The reaction was stirred for 3 h then worked up and purified as before to give 15-((tetrahydro-2*H*-pyran-2-yl)oxy)pentadecanal **226** (3.2 g, 82%) {Found $[M+Na]^+$: 349.3; $C_{20}H_{38}O_3Na$ requires: 342.3}. This showed δ_H (400 MHz, $CDCl_3$): 9.75 (1H, t, *J* 7.2 Hz), 4.56 (1H, m), 3.86 (1H, m), 3.71 (1H, dt, *J* 6.9, 9.5 Hz), 3.53 – 3.43 (1H, m), 3.36 (1H, dt, *J* 6.7, 9.5 Hz), 2.40 (2 H, dt, *J* 1.7, 7.4 Hz), 1.82 (1H, m), 1.69 (1H, m), 1.53 (4H, m), 1.38 – 1.16 (24H, m); δ_C (101 MHz, $CDCl_3$): 202.9, 98.8, 67.6, 62.3, 43.9, 30.7, 30.6, 29.7, 29.6, 29.51, 29.5, 29.4, 29.36, 29.3, 29.1, 26.2, 25.7, 25.5, 22.0, 19.6; ν_{max}: 2926, 2854, 1727, 146 cm^{-1}.

Experiment 54: Methyl heptadecanoate (254)



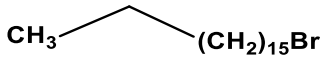
Conc. H_2SO_4 (3 mL) was added to a stirred solution of heptadecanoic acid (commercially available) **253** (25.0 g, 0.092 mol) in methanol (150 mL) and the mixture was refluxed for 3 h. The reaction was monitored by TLC until no starting material was left. The excess of methanol was evaporated and then sat. aq. $NaHCO_3$ (100 mL) was added to neutralize the acid. The product was separated, and aqueous layer was extracted with CH_2Cl_2 (3 × 200 mL) then the combined organic layers were dried over $MgSO_4$ and concentrated. The crude product was purified by recrystallization from petrol to give a white solid, methyl heptadecanoate **254** (26 g, 99%). This showed δ_H (400 MHz, $CDCl_3$): 3.67 (3H, s), 2.31 (2H, t, *J* 7.6 Hz), 1.68 – 1.17 (28H, m), 0.88 (3H, t, *J* 4.9 Hz); δ_C (101 MHz, $CDCl_3$): 177.4, 51.4, 34.1, 31.9, 29.7, 29.6, 29.4, 29.1, 25.0, 22.7, 14.1. ν_{max}: 2921, 2850,1744 cm^{-1}.

Experiment 55: Heptadecan-1-ol (255)



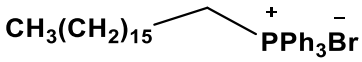
A solution of the methyl heptadecanoate **254** (36.0 g, 0.126 mol) in THF (30 mL) was added to a stirred suspension solution of $LiAlH_4$ (7.1 g, 0.18 mol) in THF (200 mL) at −10 ºC and the mixture was refluxed for 3 h. The mixture was worked up and purified as before to give a colourless oil of heptadecan-1-ol **255** (31 g, 95%).[201] This showed δ_H (400 MHz, $CDCl_3$): 3.65 (2H, t, *J* 6.6 Hz), 1.66 – 1.13 (31H, m), 0.89 (3H, t, *J* 6.8 Hz); δ_C: (101 MHz, $CDCl_3$) 68.0, 63.0, 32.8, 31.9, 29.7, 29.6, 29.4, 29.3, 25.7, 25.6, 22.7, 14.0. ν_{max}: 3389, 2925, 2856 cm^{-1}. All data matched those in the literature.[201]

Experiment 56: 1-Bromoheptadecane (256)



Triphenyl phosphine (38.0 g, 0.144 mol) was added to a stirred solution of heptadecan-1-ol **255** (30.9 g, 0.120 mol) in CH_2Cl_2 (300 mL). The mixture was cooled to 0 ºC and *N*-bromosuccinamide (27.9 g, 0.157 mol) was added in portions and the resulting mixture was stirred at room temperature for 3 h. The mixture was worked up and purified as before to give a white solid, 1-bromoheptadecane **256** (36 g, 92%).[201] This showed δ_H (400 MHz, $CDCl_3$): 3.42 (2H, t, *J* 6.9 Hz), 1.92 – 1.81 (2H, m), 1.48 – 1.19 (26H, m), 0.89 (3H, t, *J* 6.8 Hz); δ_C (101 MHz, $CDCl_3$): 34.0, 32.8, 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 28.8, 28.1, 22.7, 14.1; ν_{max}: 2916, 2848, 1433, 1110 cm^{-1}. All data matched those in the literature.[201]

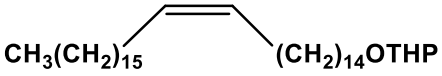
Experiment 57: Heptadecyltriphenylphosphonium bromide (225)



1-Bromoheptadecane **256** (20.0 g, 0.062 mol) was added to a stirred solution of triphenyl-phosphine (24.6 g, 0.0940 mol) in toluene (150 mL). The mixture was refluxed for 120 h. The solvent was evaporated and petroleum ether (100 mL) was added and again evaporated.

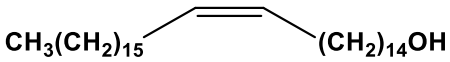
The residue was treated with diethyl ether (150 mL) and stirred for 1 h; by this time a slurry of fine crystals had formed. These were filtered, washed well with ether and dried the product was further purified by column chromatography, firstly eluting with petroleum ether and then CH_2Cl_2/MeOH (95:5) to give a white solid, heptadecyltriphenylphosphonium bromide **225** (27 g, 74%),[201] which showed δ_H (400 MHz, $CDCl_3$): 7.91 – 7.66 (15H, m), 4.03 – 3.49 (2H, m), 1.79 – 1.05 (30H, m), 0.87 (3H, t, *J* 6.7 Hz); δ_C (101 MHz, $CDCl_3$): 134.9, 133.8, 133.7, 130.5, 130.4, 118.3, 31.9, 30.5, 30.3, 29.7, 29.6, 29.5, 29.3, 29.2, 23.0, 22.7, 22.5, 14.0; ν_{max}: 2916, 2848, 1433, 1110 cm^{-1}. All data matched those in the literature.[201]

Experiment 58: (Z)-2-(Dotriacont-15-en-1-yloxy)tetrahydro-2*H*-pyran (252)



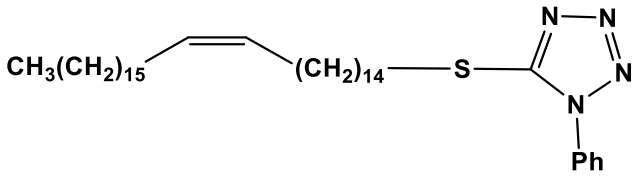
Sodium bis(trimethylsilyl)amide, (25.0 mL, 0.025 mol, 1.0 M in THF) was added to a stirred solution of heptadecyltriphenylphosphonium bromide **225** (9.70g, 0.016 mol) in dry THF (50 mL) at room temperature under nitrogen atmosphere. The mixture was stirred for 30 min and then aldehyde **226** (2.7 g, 0.008 mol) in dry THF (10 mL) was added at room temperature. The mixture was stirred for 3 h, then 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 and evaporated to give the crude product, which was purified by column chromatography, eluting with petrol/ethyl acetate (20:1) to give (*Z*)-2-(dotriacont-15-en-1-yloxy)tetrahydro-2*H*-pyran **252** (2.9 g, 64%), {Found $[M+NH_4]^+$: 566.5869; $C_{37}H_{76}O_2N$ requires: 566.5871}. This showed δ_H (400 MHz, $CDCl_3$): 5.36 (2H, br.t, 4.7 Hz), 4.59 (1H, m), 3.88 (1H, m), 3.74 (1H, dt, *J* 6.9, 9.5 Hz), 3.51 (1H, m), 3.39 (1H, dt, *J* 6.7, 9.6 Hz), 2.02 (4H, m), 1.84 (1H, m), 1.73 (1H, ddt, *J* 3.2, 5.9, 11.9 Hz), 1.65 – 1.50 (4H, m), 1.46 – 1.19 (52H, m, *J* 70.1), 0.89 (3H, t, *J* 6.8); δ_C (101 MHz, $CDCl_3$): 133.8, 133.6, 129.9, 128.7, 128.5, 128.4, 98.8, 67.7, 62.3, 31.9, 30.8, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 27.2, 26.2, 25.5, 22.7, 19.7, 14.1; ν_{max}: 2925, 2853, 1464, 1352 cm^{-1}.

Experiment 59: (*Z*)-Dotriacont-15-en-1-ol (224)



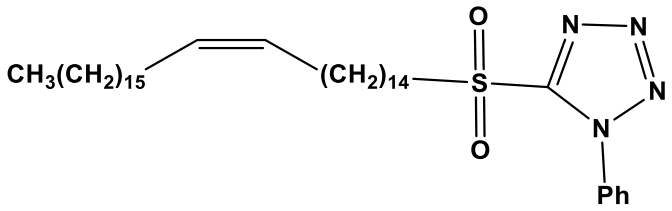
Pyridinum-*p*-toluenesulfonate (5.6 g, 0.02 mol) was added to a stirred solution of (Z)-2-(dotriacont-15-en-1-yloxy)tetrahydro-2*H*-pyran **252** (2.5 g, 4.0 mmol) in THF (40 mL), methanol (16 mL) and stirred at 40 ºC for 6 h. Sat. aqueous $NaHCO_3$ (30 mL) and water (20 mL) were added and the product was extracted with 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 (10:1) to give (*Z*)-dotriacont-15-en-1-ol **224** (1.5 g, 71%), {Found $[M+NH_4]^+$: 482.5292; $C_{32}H_{68}ON$ requires: 482.5295}. This showed δ_H (400 MHz, $CDCl_3$): 5.36 (2H, br.t, 4.7 Hz), 3.65 (2H, t, *J* 6.6 Hz), 2.02 (4H, m), 1.62 – 1.20 (53H, m), 0.88 (3H, t, *J* 6.9 Hz); δ_C (101 MHz, $CDCl_3$): 129.9, 63.1, 32.8, 32.6, 31.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 27.2, 25.7, 22.7, 14.1; ν_{max}: 3394, 2928, 2855, 1520, 1423 cm^{-1}.

Experiment 60: (*Z*)-5-(Dotriacont-15-en-1-ylthio)-1-phenyl-1*H*-tetrazole (223)



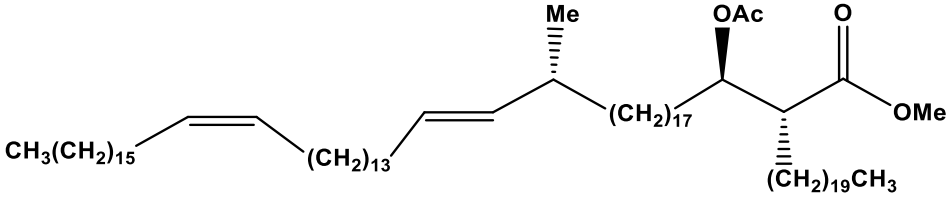
Diethyl azodicarboxy (0.64 g, 0.003 mol), (*Z*)-dotriacont-15-en-1-ol **224** (1.32 g, 0.002 mol). Triphenylphosphine (0.96 g, 0.003 mol) and 1-phenyl-1*H*-tetrazole-5-thiol (0.65 g, 0.003 mol) in dry THF (20 mL) at 0 ºC under nitrogen atmosphere. The mixture was allowed to reach room temperature and then stirred for 3 h. The reaction was worked up and purified as before to give a thick oil, (*Z*)-5-(dotriacont-15-en-1-ylthio)-1-phenyl-1*H*-tetrazole **223** (1.7 g, 96%), {Found $[M+H]^+$: 625.5230; $C_{39}H_{69}N_4S$ requires: 625.5237}. This showed δ_H (400 MHz, $CDCl_3$): 7.84 – 7.38 (5H, m), 5.36 (2H, br.t, *J* 4.7 Hz), 3.40 (2H, t, *J* 7.4 Hz), 2.02 (4H, m), 1.82 (2H, m), 1.51 – 1.17 (50H, m), 0.89 (3H, t, *J* 6.8 Hz); δ_C (101 MHz, $CDCl_3$): 154.5, 130.0, 129.9, 129.8, 129.7, 123.8, 33.4, 31.2, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.0, 27.2, 22.7, 14.10; ν_{max}: 3020, 2928, 1523, 1476, 1423, 1215, 1019 cm^{-1}.

Experiment 61: (*Z*)-5-(Dotriacont-15-en-1-ylsulfonyl)-1-phenyl-1*H*-tetrazole (209)



Ammonium molybdate (VI) tetrahydrate (3.1 g, 0.0020 mol) was dissolved in cold hydrogen peroxide (35%, 20 mL) and the solution was gradually added to a stirred solution of (*Z*)-5-(dotriacont-15-en-1-ylthio)-1-phenyl-1*H*-tetrazole **223** (3.5, 0.0050 mol) in IMS (100 mL) and THF (50 mL) at 0 °C. The mixture was allowed to reach room temperature and stirred for 2 h; then another portion of ammonium molybdate (VI) tetrahydrate (1.1 g, 0.00090 mol) in cold hydrogen peroxide was added (35 %, 7 mL). The mixture was stirred for 18 h. After that it was worked up and purified as before to give a white solid, **(***Z***)**-5-(dotriacont-15-en-1-ylsulfonyl)-1-phenyl-1*H*-tetrazole **209** (3.0 g, 83%), {Found $[M+H]^+$: 657.5140; $C_{39}H_{69}N_4O_2S$ requires: 657.5136}. Which showed δ_H (400 MHz, $CDCl_3$): 7.70 (2H, m), 7.61 (3H, m), 5.36 (2H,br.t, 4.7 Hz), 3.74 (2H, distorted t, *J* 7.9 Hz), 2.08 – 1.90 (4H, m), 1.56 – 1.11 (52H, m), 0.89 (3H, t, *J* 6.8 Hz); δ_C (101 MHz, $CDCl_3$): 153.5, 133.0, 131.4, 130.3, 129.9, 129.8, 129.7, 125.0, 123.8, 56.0, 33.4, 32.6, 31.9, 29.8, 29.7, 29.63, 29.6, 29.5, 29.4, 29.3, 29.2, 29.0, 28.9, 28.1, 27.2, 22.7, 21.9, 14.1; ν_{max}: 3020, 2919, 2850, 1596, 1469, 1344, 1215, 1019 cm^{-1}.

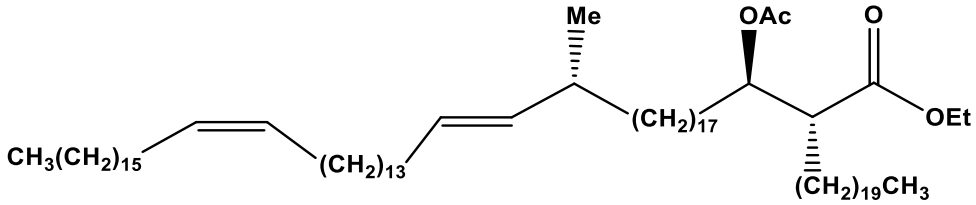
Experiment 62: Methyl (2*R*,3*R*,21*R*,22*E*,37*Z*)-3-acetoxy-2-eicosyl-21-methyltetrapentaconta-22,37-dienoate (207)



The aldehyde **210** (0.66 g, 0.91 mmol) was added to a stirred solution of the sulfone **209** (0.69 g, 1.05 mmol) in dry THF (50 mL) under nitrogen at room temperature. The mixture was cooled to –16 ºC and potassium bis (trimethylsilyl) amide (1.5 mL, 1.57 mmol, 1 M in THF) was added. The mixture was allowed to reach room temperature and stirred for 3 h. Sat. aq. NH_4Cl (15 mL) followed by petrol/ethyl acetate (1:1, 50 mL) and the organic layer

was separated. The aq. layer was re-extracted with ethyl acetate (2 × 50 mL), the combined organic layers were dried and evaporated. The product was purified by column chromatography, eluting with petrol/ethyl acetate (20:1) to give a white solid, compound **207** (0.41 mg, 39%) $[\alpha]_D^{30}$ = + 11.4 (*c* 0.21 $CHCl_3$) {MALDI-Found $[M+Na]^+$:1174.1419; $C_{78}H_{150}O_4Na$ requires: 1174.1410}. This showed δ_H (400 MHz, $CDCl_3$): 5.41 – 5.28 (3H, m), 5.24 (1H, dd, *J* 7.4, 15.3 Hz), 5.09 (1H, td, *J* 4.0,7.6 Hz), 3.68 (3H, s), 2.62 (1H, ddd, *J* 4.3, 6.8,10.8 Hz), 2.04 (3H, s), 2.02 – 1.93 (7H, m), 1.66 – 1.17 (122H, m), 0.94 (3H, d, *J* 6.7 Hz), 0.89 (6H, t, *J* 6.8 Hz); δ_C (101 MHz, $CDCl_3$): 173.6, 170.3, 136.5, 130.4, 129.9, 128.4, 74.1, 51.5, 49.6, 37.2, 36.7, 32.6, 32.5, 31.9, 31.7, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 28.1, 27.5, 27.4, 27.2, 25.0, 22.7, 21.0, 14.1; ν_{max}: 2915, 2848, 1737, 1468, 1375, 1237, 1199, 1021 cm^{-1}.

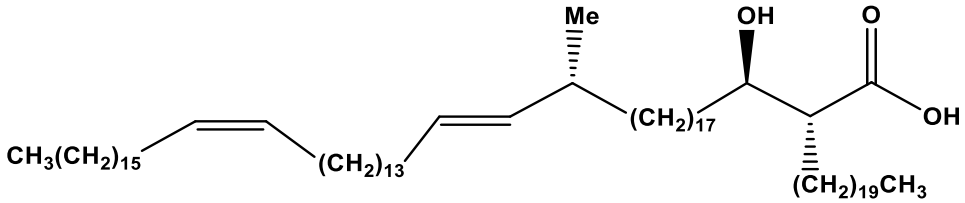
Experiment 63: Ethyl (2*R*,3*R*,21*R*,22*E*,37*Z*)-3-acetoxy-2-eicosyl-21-methyltetrapentaconta-22,37-dienoate (208)



The aldehyde **211** (50.0 mg, 0.068 mmol) in dry THF (1 mL) was added to a stirred solution of the sulfone **209** (49.1 mg, 0.074 mmol) in dry THF (4 mL) under nitrogen at room temperature. The mixture was cooled to 10 ºC and potassium bis (trimethylsilyl) amide (0.22 mL, 0.111 mmol, 0.5 M in toluene) was added and the mixture was allowed to reach room temperature and stirred for 2 h. Sat. aq. NH_4Cl (8 mL) and a mixture of petrol/ethyl acetate (1:1, 50 mL) were added and the organic layer was separated. The aqueous layer was re-extracted with petrol/ethyl acetate (1:1, 2 × 20 mL), the combined organic layers were dried, and the solvent was evaporated. The product was purified by column chromatography, eluting with petrol/ethyl acetate (20:1) to give a white semi-solid, the title compound **208** (20 mg, 47%) $[\alpha]_D^{23}$ = +10.6 (*c* 0.170 $CHCl_3$) {MALDI-Found $[M+Na]^+$:1188.1580; $C_{79}H_{152}O_4Na$ requires: 1188.1583}. This showed δ_H (400 MHz, $CDCl_3$): 5.41 – 5.29 (3H, m), 5.24 (1H, dd, *J* 7.3, 15.4 Hz), 5.10 (1H, td, *J* 4.1,7.7 Hz), 4.15 (2H, q, *J* 7.1 Hz), 2.64 – 2.55 (1H, m), 2.10 – 1.92 (10H, m), 1.69 – 1.12 (122H, m), 0.95 (3H, d, *J* 6.7 Hz), 0.88 (9H, t, *J* 6.8 Hz); δ_C (101 MHz, $CDCl_3$): 173.1, 170.3, 136.4, 129.9, 128.4, 74.1, 58.3, 49.6,

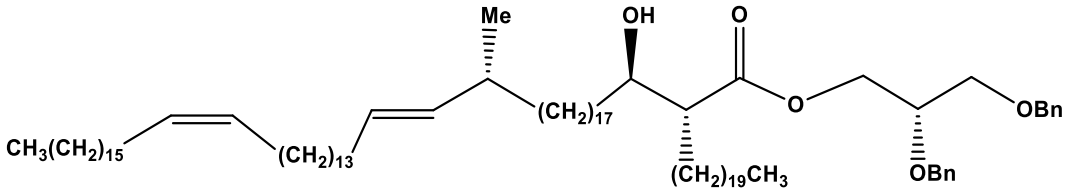
37.2, 36.7, 32.6, 31.9, 31.7, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 27.3, 27.1, 24.9, 22.8, 21.0, 20.9, 15.2, 14.3, 14.0; ν_{max}: 2924, 2853, 1744, 1464, 1370, 1235, 1177, 1023 cm^{-1}.

Experiment 64: (2*R*,3*R*,21*R*,22*E*,37*Z*)-3-Hydroxy-2-eicosyl-21-methyltetrapentaconta-22,37-dienoic acid (155)



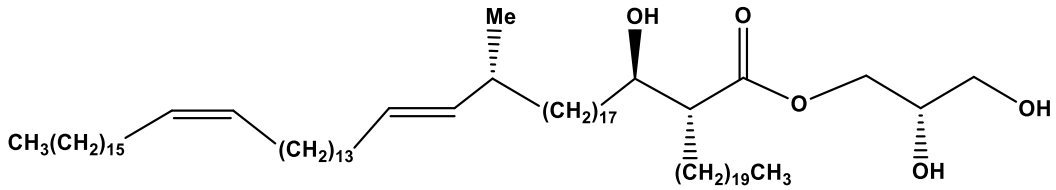
Lithium hydroxide monohydrate (0.21 g, 5.2 mmol) was added to a stirred solution of methyl (2*R*,3*R*,21*R*,22*E*,37*Z*)-3-acetoxy-2-icosyl-21-methyltetrapentaconta-22,37-dienoate **207** (0.4 g, 0.34 mmol) in THF (15 mL), water (2 mL) and MeOH (1.5 mL). The mixture was heated at 45 ºC for 16 h. When TLC showed no starting material, the mixture was diluted with petrol/ethyl acetate (5:1, 20 mL), and then acidified with sat.aq.solution of $KHSO_4$. The product was extracted with warm petrol/ethyl acetate (5:1, 3 × 50 mL), and the combined organic layers were dried over $MgSO_4$, evaporated to give a crude product, which was purified by re-crystallization from petrol/ethyl acetate (1:2, 50 mL) to give a white solid of (2*R*,3*R*,21*R*,22*E*,37*Z*)-3-hydroxy-2-eicosyl-21-methyltetrapentaconta-22,37-dienoic acid **155** (0.27 g, 71%), m.p. 235-237 ºC, $[\alpha]_D^{20}$ = + 2.2 (*c* 0.92 $CHCl_3$) {MALDI-Found $[M+Na]^+$:1096.1323; $C_{75}H_{147}O_3$ requires: 1096.1350}. This showed δ_H (400 MHz, $CDCl_3$): 5.41 – 5.29 (3H, m), 5.24 (1H, dd, *J* 7.5, 15.3 Hz), 3.73 (1H, dt, *J* 4.8, 9.4 Hz), 2.47 (1H, dt, *J* 5.2, 9.2 Hz), 2.08 – 1.93 (7H, m), 1.81 – 0.99 (124H, m), 0.94 (3H, d, *J* 6.7 Hz), 0.89 (6H, t, *J* 6.8 Hz); δ_C (101 MHz; $CDCl_3$): 178.5, 136.5, 130.4, 129.9, 128.4, 72.1, 50.7, 37.2, 36.7, 35.5, 32.6, 31.9, 29.8, 29.7, 29.6, 29.58, 29.5, 29.50, 29.4, 29.4, 29.3, 29.1, 27.4, 27.3, 27.2, 25.7, 22.7, 21.0, 14.1; ν_{max}: 3585 (broad), 2915, 2848, 1686, 1468, 1376, 720 cm^{-1}.

Experiment 65: (*S*)-2,3-Bis(benzyloxy)propyl(2*R*,3*R*,21*R*,22*E*,37*Z*)-3-hydroxy-2-icosyl-21-methyltetrapentaconta-22,37-dienoate (263)



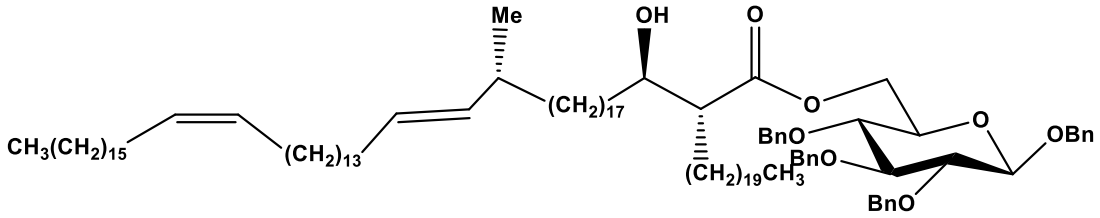
Dry cesium hydrogen carbonate (29.0 mg, 0. 150 mmol) was added to a stirred solution of (*S*)-2,3-bis(benzyloxy)propyl 4-methylbenzenesulfonate **262** (0.014 mg, 0.033 mmol; supplied by Dr. J. Al Dulayymi) and (2*R*,3*R*,21*R*,22*E*,37*Z*)-3-hydroxy-2-eicosyl-21-methyltetrapentaconta-22,37-dienoic acid **155** (33.0 mg, 0.030 mmol) in dry THF and DMF (5:1, 1.5 mL) at room temperature. The mixture was brought to 70 °C and stirred at this temperature for two days. TLC showed no starting material. Ethyl acetate (20 mL) and water (10 mL) were added. The organic layer was separated and the aqueous layer was re-extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with water (10 mL) and brine (10 mL), dried over $MgSO_4$, filtered and the solvent was evaporated. The product was purified by column chromatography, eluting with petrol/ethyl acetate (5:1) to give a semi-solid (*S*)-2,3-bis(benzyloxy)propyl-(2*R*,3*R*,21*R*,22*E*,37*Z*)-3-hydroxy-2-eicosyl-21-methyltetrapentaconta-22,37-dienoate **263** (26 mg, 65%), $[\alpha]_D^{20}$ = + 8.5 (c 0.33, $CHCl_3$), {MALDI-Found $[M+Na]^+$: 1372.1; $C_{92}H_{164}O_5Na$ requires: 1372.2}. This showed δ_H (400 MHz, $CDCl_3$): 7.53 – 7.09 (10H, m), 5.42 – 5.29 (3H, m), 5.24 (1H, dd, *J* 7.4, 15.3 Hz), 4.68 (1H, d, *J* 11.8 Hz), 4.64 (1H, d, *J* 11.8 Hz), 4.55 (2H, br. s), 4.43 (1H, dd, *J* 4.0, 11.7 Hz), 4.22 (1H, dd, *J* 5.5, 11.7 Hz), 3.87 (1H br. pent, *J* 5.4 Hz), 3.60 – 3.57 (3H, m), 2.43 (1H, dt, *J* 5.4, 9.3 Hz), 2.09 – 1.91 (7H, m), 1.86 – 1.00 (123H, m), 0.95 (3H, d, *J* 6.7 Hz), 0.89 (6H, t, *J* 6.8 Hz); δ_C (101 MHz, $CDCl_3$): 175.5, 138.0, 137.9, 136.5, 130.4, 129.9, 128.4, 128.3, 127.7, 127.6, 75.8, 73.5, 72.3, 72.2, 69.6, 63.5, 51.4, 37.2, 36.7, 35.5, 34.4, 32.58, 31.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.1, 27.5, 27.4, 27.2, 26.7, 25.8, 22.7, 20.9, 14.1; ν_{max}: 3519 (broad), 2922, 2853, 1736, 1463, 1369, 1115, 968, 906 cm^{-1}.

Experiment 66: (*S*)-2,3-Dihydroxypropyl-(2*R*,3*R*,21*R*,22*E*,37*Z*)-3-hydroxy-2-icosyl-21-methyltetrapentaconta-22,37-dienoate (156)



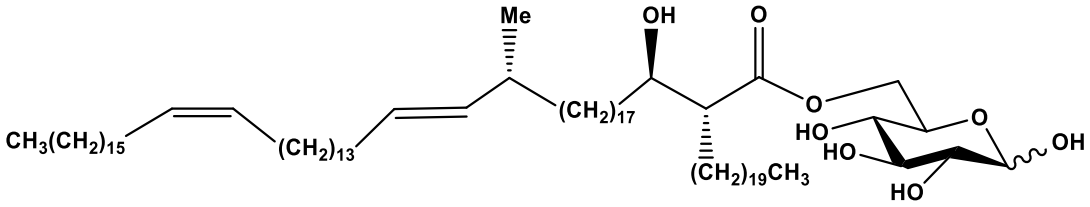
Liquid ammonia (100 mL) was decanted into a (250 mL) two necked flask under a liquid nitrogen/methylated spirit condenser protected by a soda lime guard tube. Sodium (~ 50 mg) was added until blue colour of solution persisted. The compound **263** (26.0 mg, 0.019 mmol) in 1,4-dioxane (2 mL) was added; then the reaction mixture was stirred for 4-5 min until the blue colour disappeared. After this the reaction was quenched with NH_4Cl (5 mL) and ether (30 mL). The ammonia was allowed to evaporate, and the organic layer was separated and the aqueous layer was re-extracted with ether (2 × 30 mL). The combined organic layers were dried and evaporated. The residue was purified by column chromatography on silica gel eluting with $CHCl_3$/MeOH (20:1) to give (*S*)-2,3-dihydroxypropyl(2*R*,3*R*,21*R*,22*E*,37*Z*)-3-hydroxy-2-eicosyl-21-methyltetrapentaconta-22,37-dienoate **156** (10.4 mg, 46%) $[\alpha]_D^{24}$ = + 18.3 (*c* 0.109 $CHCl_3$) {MALDI-Found $[M+H]^+$: 1170.1713; $C_{78}H_{153}O_5$ requires: 1170.1712}. This showed δ_H (400 MHz, $CDCl_3$+ few drops of CD_3OD): 5.38 – 5.26 (3H, m), 5.21 (1H, dd, *J* 7.2, 15.4 Hz), 4.26 (1H, dd, *J* 4.2, 11.5 Hz), 4.19 (1H, dd, *J* 6.4, 11.5 Hz), 3.95–3.88 (1H, m), 3.71–3.63 (2H, m), 3.56 (1H, br.dd, *J* 5.8,11.5 Hz), 2.45 – 2.36 (1H, m), 2.03 – 1.90 (7H, m), 1.70 – 0.94 (126H, m), 0.91 (3H, d, *J* 6.7 Hz), 0.86 (6H, t, *J* 6.7 Hz); δ_C (101 MHz, $CDCl_3$): 175.5, 136.4, 130.3, 129.8, 128.4, 72.7, 69.8, 65.1, 63.0, 52.4, 37.2, 36.6, 35.0, 32.5, 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 29.0, 27.4, 27.3, 27.1, 25.3, 22.6, 20.9, 14.0; ν_{max}: 3365 (broad), 2919, 2850, 1719, 1654, 1466,1054 cm^{-1}. The starting material (5 mg) was recovered.

Experiment 67: Benzyl-2,3,4-tri-*O*-benzyl-6-*O*-(2*R*,3*R*,21*R*,22*E*,37*Z*) -3-hydroxy-2-eicosyl-21-methyltetrapentaconta-22,37-dienoate-β-D-glucopyranoside (265)



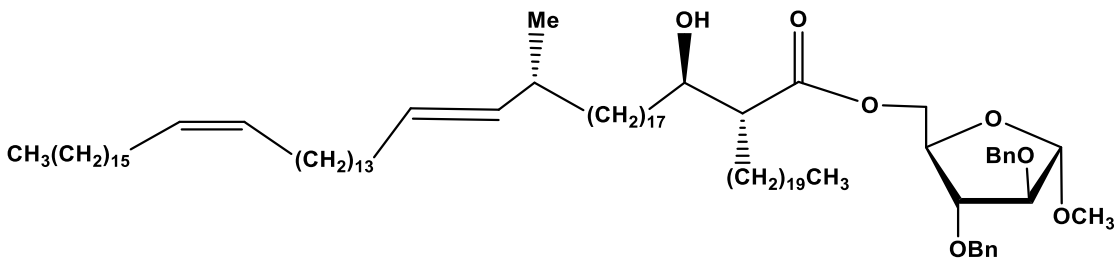
(2*R*,3*R*,21*R*,22*E*,37*Z*)-3-Hydroxy-2-eicosyl-21-methyltetrapentaconta-22,37-dienoic acid **155** (35 mg, 0.031 mmol) was dissolved in a mixture of THF and DMF (5:1, 1.5 mL) at room temperature, and then gently heated until all had dissolved. Dry cesium hydrogen carbonate (30.9 mg, 0.159 mmol) and benzyl-2,3,4-tri-*O*-benzyl-6-*O*-tosyl-β-D-glucopyranoside **264** (24.4 mg, 0.035 mmol; supplied by Dr. J. Al Dulayymi) were added. The mixture was brought to 70 °C and stirred at this temperature for 18 h. After TLC showed no starting material was left, the reaction was quenched with water (5 mL). The product was extracted with dichloromethane (3 × 25 mL). 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 (5:1) to give a semi-solid, the title compound **265** (34.5 mg, 67%) $[\alpha]_D^{20}$ = + 12.7 (*c* 0.730, $CHCl_3$), {MALDI-Found $[M+Na]^+$: 1640.1; $C_{109}H_{180}O_8Na$ requires: 1640.4}. This showed δ_H (400 MHz, $CDCl_3$): 7.44 – 7.00 (20H, m), 5.42 – 5.30 (3H, m), 5.25 (1H, dd, *J* 7.4, 15.4 Hz), 4.96 (1H, d, *J* 11.0 Hz), 4.95 (1H, d, *J* 11.0 Hz), 4.93 (1H, d, *J* 12.5 Hz), 4.90 (1H, d, *J* 11.9 Hz), 4.79 (1H, d, *J* 10.9 Hz), 4.72 (1H, d, *J* 10.9 Hz), 4.71 (1H, d, *J* 11.0 Hz), 4.64 (1H, d, *J* 12.5 Hz), 4.61 (1H, d, *J* 11.9 Hz), 4.55 (1H, d, *J* 11.0 Hz), 4.53 (1H,br. d, *J* 7.7 Hz), 4.23 (1H, dd, *J* 4.5, 11.7 Hz), 3.67 (2H, br.t, *J* 7.6 Hz), 3.53 – 3.49 (3H, m), 2.48 (1H, dt, *J* 5.4, 9.1 Hz), 2.09 – 1.92 (7H, m), 1.86 – 1.01 (123H, m), 0.95 (3H, d, *J* 6.7 Hz), 0.89 (6H, t, *J* 6.8); δ_C (101 MHz, $CDCl_3$): 175.2, 138.4, 138.3, 137.7, 137.2, 136.5, 130.4, 129.9, 128.5, 128.4, 128.3, 128.1, 128.0, 127.9, 127.8, 127.7, 102.3, 84.5, 82.3, 77.8, 75.7, 75.1, 74.9, 72.9, 72.3, 71.1, 62.9, 51.3, 37.2, 36.7, 35.6, 32.6, 31.9, 29.8, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 27.5, 27.4, 27.2, 25.9, 22.7, 20.9, 14.1; ν_{max}: 3495 (broad), 2916, 2848, 1735, 1465, 1369, 1111,967, 903 cm^{-1}.

Experiment 68: 6-*O*-(2*R*,3*R*,21*R*,22*E*,37*Z*)-3-hydroxy-2-eicosyl-21-methyltetrapentaconta-22,37-dienoate-α/β-D-glucopyranoside (157)



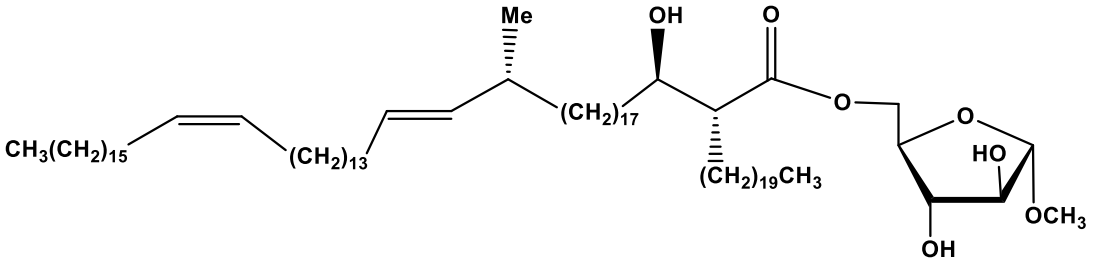
Liquid ammonia (100 mL) was condensed into a two neck flask (250 mL) under a liquid nitrogen/methylated spirit condenser protected by a soda lime guard tube. Sodium (~ 50 mg) was added until the blue colour of the solution persisted. Compound **265** (33 mg, 0.020 mmol) in 1,4-dioxane (2 mL) was added and the reaction mixture was stirred for 4-5 min until the blue colour disappeared. The reaction was quenched with NH_4Cl (5 mL) and ether (30 mL) was added. The ammonia was allowed to evaporate, and the organic layer was separated and the aqueous layer re-extracted with ether (2 × 30 mL). The combined organic layers were dried and evaporated. The residue was purified by column chromatography on silica gel, eluting with $CHCl_3$/MeOH (10:1) to give the title compound **157** (6.5 mg, 25%) $[\alpha]_D^{24}$ = + 4.9 (*c* 0.65 $CHCl_3$), {Found $[M+NH_4]^+$: 1275.2135; $C_{81}H_{160}O_8N$ requires: 1275.2138}. This showed δ_H (α, β isomers) in 6:4 ratio (400 MHz, $CDCl_3$+ few drops of CD_3OD): 5.37 – 5.23 (3H, m), 5.19 (1H, dd, *J* 7.4, 15.4 Hz), 5.11 (0.6H, d, *J* 3.3 Hz, H-1α), 4.46 (0.4H, d, *J* 7.7 Hz, H-1β), 4.41 (1H, br. d, *J* 11.9 Hz, H-6α,β), 4.24 (1H, br. dd, *J* 5.9, 11.8 Hz, H-6 α,β), 3.96 – 3.92 (0.6H, ddd, *J* 2.2, 5.8, 9.8 Hz, H-5α), 3.67 – 3.58 (1.6H, m, H-4α + CH-OH mycolic acid), 3.48 – 3.44 (0.4H, ddd, *J* 2.2, 5.8, 9.8 Hz, H-5β), 3.43 – 3.26 (2H, m H-2,3α, H-3,4β), 3.17 (0.4H, br. t, *J* 8.4 Hz H-2β), 2.42 – 2.34 (1H, m), 2.07 – 1.84 (7H, m), 1.70 – 0.97 (121H, m), 0.89 (3H, d, *J* 6.7 Hz), 0.83 (6H, t, *J* 6.7 Hz); δ_C for α, β isomers (400 MHz, $CDCl_3$ + few drops of CD_3OD): 175.2, 136.4, 130.3, 129.9, 128.4, 96.6 (C-1β), 92.3 (C-1α), 85.5, 76.2, 74.6, 73.7, 72.5, 72.2, 70.5, 70.3, 69.2, 65.7, 65.6, 63.6, 63.5, 52.6, 52.4, 37.2, 36.6, 35.0, 32.5, 31.9, 29.6, 29.5, 29.4, 29.3, 29.2, 29.0, 27.3, 27.1, 25.4, 22.6, 20.9, 14.0; ν_{max}: 3389 (broad), 2919, 2850, 1735, 1466, 1376, 1030cm^{-1}. The starting material (4 mg) was recovered.

Experiment 69: Methyl 2,3-di-*O*-benzyl-5-*O*-[(2*R*,3*R*,21*R*,22*E*,37*Z*)-3-hydroxy-2-eicosyl-21-methyltetrapentaconta-22,37-dienoate]-α-D-arabinofuranosiode (267)



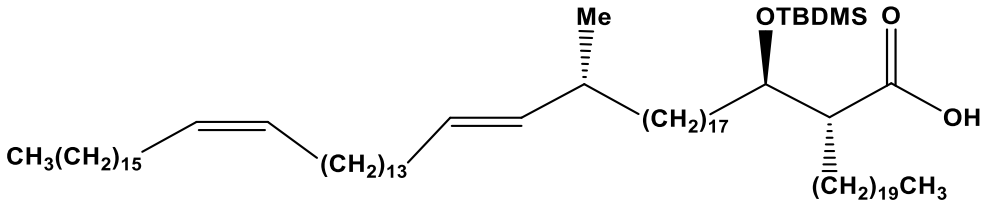
Dry cesium hydrogen carbonate (43 mg, 0.22 mmol) was added to a stirred solution of arabinofuranosiode **266** [286] (23.8 mg, 0.047 mmol; supplied by Dr. J. Al Dulayymi) and (2*R*,3*R*,21*R*,22*E*,37*Z*)-3-hydroxy-2-eicosyl-21-methyltetrapentaconta-22,37-dienoic acid **155** (35 mg, 0.031 mmol) in dry THF and DMF (5:1, 1.5 mL) at room temperature. The mixture was brought to 70 °C and stirred at this temperature for 4 days. After TLC showed no starting material, the reaction was quenched with water (5 mL). The product was extracted with dichloromethane (3 × 25 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was evaporated. The product was purified by column chromatography, eluting with petrol/ethyl acetate (5:1) to give a semi-solid methyl 2,3-di-*O*-benzyl-5-*O*-[(2*R*,3*R*,21*R*, 22*E*,37*Z*)-3-hydroxy-2-eicosyl-2-methyltetrapentaconta-22,37-dienoate]-α-D-arabinofuranoside **267** (26 mg, 58%) $[\alpha]_D^{21}$ = + 13.9 (c 0.72, $CHCl_3$), {MALDI-Found $[M+Na]^+$: 1444.2697; $C_{95}H_{168}O_7Na$ requires: 1444.2682}. This showed δ_H (400 MHz, $CDCl_3$) 7.45 – 7.20 (10H, m), 5.41 – 5.29 (3H, m), 5.25 (1H, dd, J 7.4, 15.4 Hz), 4.92 (1H, br. s), 4.58 (1H, d, J 12.0 Hz), 4.56 (1H, d, J 11.7 Hz), 4.51 (1H, d, J 11.9 Hz), 4.48 (1 H, d, J 11.9 Hz), 4.32 – 4.28 (2H, m), 4.24 – 4.20 (1H, m), 3.99 (1H, d, J 2.3 Hz), 3.84 (1H, dd, J 2.6, 6.5 Hz), 3.66 – 3.60 (1H, m), 3.38 (3H, s), 2.44 (1H, dt, J 5.5, 9.2 Hz), 2.07 – 1.94 (7H, m), 1.73 – 1.01 (123H, m), 0.95 (3H, d, J 6.7 Hz), 0.89 (6H, t, J 6.7 Hz); δ_C (101 MHz, $CDCl_3$): 175.0, 137.5, 137.3, 136.5, 130.4, 129.9, 129.7, 128.5, 128.4, 128.0, 127.9, 107.2, 87.9, 83.7, 79.4, 72.4, 72.2, 72.1, 63.5, 54.9, 51.5, 37.2, 36.7, 35.5, 32.6, 31.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 27.4, 27.3, 27.2, 25.8, 22.7, 20.9, 14.1; ν_{max}: 3520 (broad), 2921, 2852, 1734, 1465, 1369, 1110, 965, 908 cm^{-1}.

Experiment 70: Methyl-5-*O*-[(2*R*,3*R*,21*R*,22*E*,37*Z*)-3-hydroxy-2-eicosyl-21-methyltetra pentaconta-22,37-dienoate]-α-D-arabinofuranosiode (158)



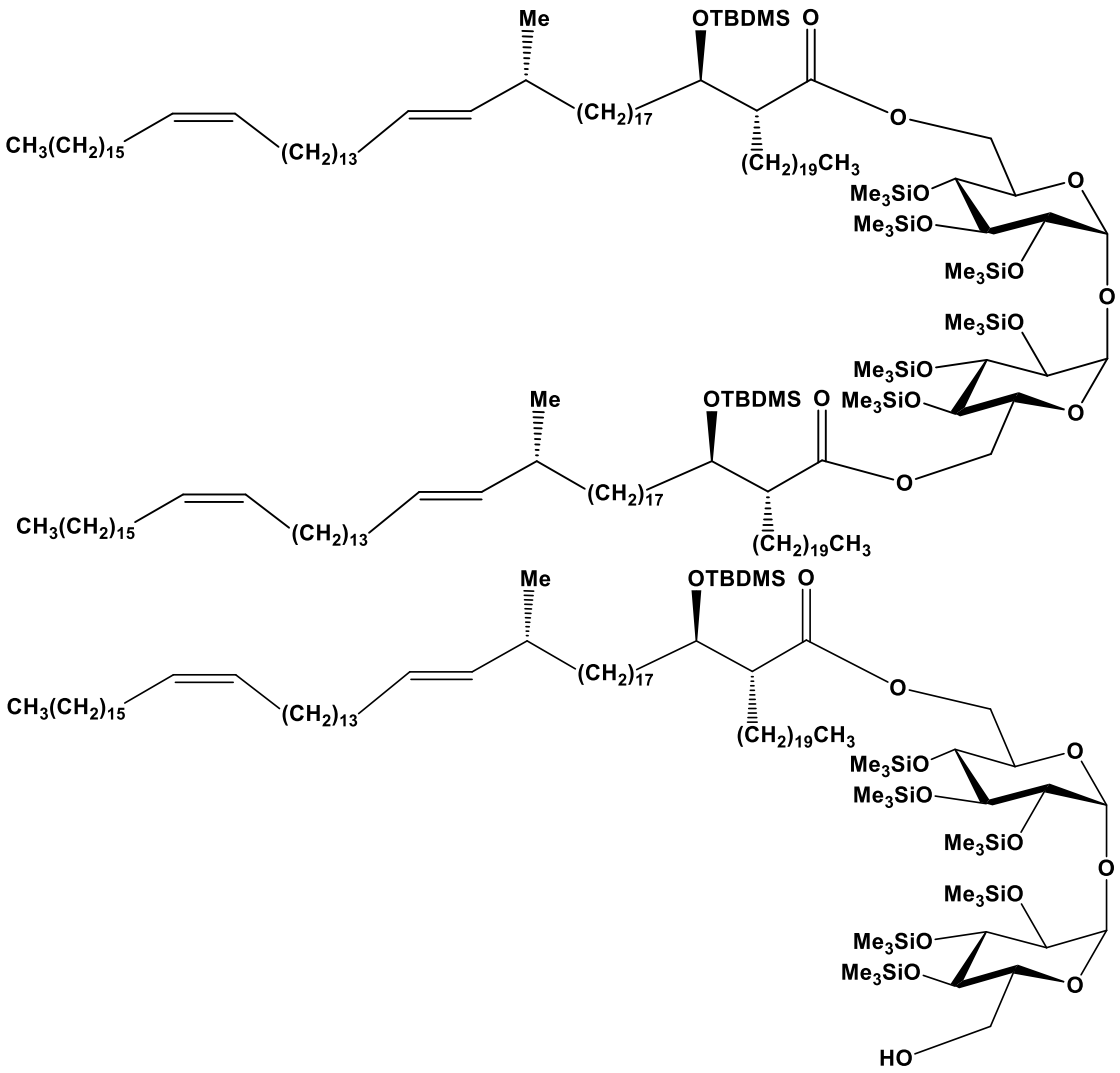
Liquid ammonia (100 mL) was condensed into a two neck flask (250 mL) under a liquid nitrogen/methylated spirit condenser protected by a soda lime guard tube. Sodium (~50 mg) was added until the blue colour of the solution persisted. Compound **267** (25 mg, 0.017mmol) in 1,4-dioxane (2 mL) was added and the reaction mixture was stirred for 4-5 min until the blue colour disappeared. The reaction was quenched with NH_4Cl (5 mL) and ether (30 mL). The ammonia was allowed to evaporate and the organic layer was separated and the aqueous layer re-extracted with ether (2 × 30 mL). The combined organic layers were dried and evaporated. The residue was purified by column chromatography on silica gel, eluting with $CHCl_3$/MeOH (20:1) to give the title compound **158** (8.6 mg, 39%) $[\alpha]_D^{21}$ = + 14.85 (*c* 0.7, $CHCl_3$). {MALDI-Found $[M+Na]^+$: 1264.1761; $C_{81}H_{156}O_7Na$ requires: 1264.1749}. This showed δ_H (400 MHz, $CDCl_3$): 5.37 – 5.26 (3H, m), 5.20 (1H, dd, *J* 7.4, 15.4 Hz), 4.81 (1H, br. s), 4.32 (1H, dd, *J* 4.7, 9.8 Hz), 4.28 (1H, dd, *J* 4.9, 10.1 Hz), 4.09 (1H, br.q *J* 4.7 Hz), 4.0 (1H, dd, *J* ,1.9, 3.7 Hz), 3.87(1H, dd, *J* 2.7, 4.7 Hz), 3.69 – 3.55 (1H, m), 3.36 (3H, s), 2.45 – 2.36 (1H, m), 1.96 (7H, m), 1.59 – 1.01 (125H, m), 0.90 (3H, d, *J* 6.7 Hz), 0.85 (6H, t, *J* 6.8 Hz); δ_C (101 MHz, $CDCl_3$): 175.0, 136.4, 130.3, 129.8, 128.3, 108.6, 84.1, 82.0, 81.2, 77.9, 72.5, 63.4, 57.9, 55.0, 52.5, 37.1, 36.6, 35.4, 34.9, 32.6, 32.48, 31.9, 31.3, 30.2, 29.9, 29.8, 29.6, 29.3, 28.5, 27.3, 27.2, 27.1, 27.0, 26.8, 26.6, 25.6, 25.3, 24.4, 22.6, 20.8, 14.6, 14.0; ν_{max}: 3402, 2923, 2850, 1726, 1465, 1217, 1096 cm^{-1}. Starting material (6 mg) was also recovered.

Experiment 71: (2*R*,3*R*,21R,22E,37Z)-3-((*tert*-Butyldimethylsilyl)oxy)-2-icosyl-21-methyltetrapentaconta-22,37-dienoic acid (269)



Imidazole (0.19 g, 0.0029 mol) was added to a stirred solution of mycolic acid **155** (0.23 g, 2.0 mmol) in dry DMF (2 mL) and dry toluene (1.5 mL) at room temperature followed by the addition of *tert*-butyldimethylsilylchloride (0.44 g, 0.002 mol) and 4-DMAP (50 mg). The reaction mixture was stirred at 70 °C for 24 h. The solvent was removed under high vacuum and the residue was diluted with petrol/ethyl acetate (10:2, 50 mL) and water (20 mL). The organic layer was separated and the aqueous layer was re-extracted with petrol/ethyl acetate (10:2, 3 × 20 mL). The combined organic layers were washed with water, dried and evaporated to give a colourless oil residue. The residue was dissolved in THF (6 mL), water (0.7 mL), and methanol (0.7 mL), and to this was added potassium carbonate (150 mg). The mixture was stirred at 45 °C for 3 h, diluted with petrol/ethyl acetate (5:2, 30 mL) and water (2 mL) then acidified with potassium hydrogen sulfate to pH 2. The organic layer was separated and the aqueous layer was re-extracted with petrol/ethyl acetate (2 × 20 mL). The combined organic layers were washed with water, dried and evaporated to give a residue, which was purified by column chromatography, eluting with petrol/ethyl acetate (20:1) to give compound **269** (0.2 g, 80%) $[\alpha]_D^{23}$ = + 3.3 (c 0.84, $CHCl_3$), {MALDI-Found $[M+Na]^+$: 1232.2020; $C_{81}H_{160}O_3SiNa$ requires: 1232.2029}. This showed δ_H (400 MHz, $CDCl_3$): 5.42 – 5.30 (3H, m), 5.25 (1H, dd, *J* 7.4, 15.4 Hz), 3.88 (1H, dt, *J* 5.2, 10.3 Hz), 2.54 (1H, dt, *J* 4.7, 9.7 Hz), 2.10 – 1.92 (7H, m), 1.71 – 1.10 (123H, m), 0.95 (3H, d, *J* 6.7 Hz), 0.91 (9H, s), 0.89 (6H, t, *J* 7.0 Hz), 0.12 (3H, s), 0.10 (3H, s); δ_C (101 MHz, $CDCl_3$): 177.9, 136.5, 130.3, 129.9, 128.4, 73.5, 50.8, 37.3, 36.7, 34.9, 32.6, 32.0, 31.6, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 27.5, 27.4, 27.2, 25.7, 24.5, 22.7, 22.6, 21.0, 17.9 ,14.1, –4.3, –5.0; ν_{max}: 2919, 2850, 1708, 1464, 1361, 1252, 1075 cm^{-1}.

Experiment 72: 6,6'-*bis*-*O*-(2*R*,3*R*,21*R*,22*E*,37*Z*)-3-((*tert*-Butyldimethylsilyl)oxy)-2-eicosyl-21-methyltetrapentaconta-22,37-dienoic-2,3,4,2',3',4'-hexakis-*O*-(trimethylsilyl)-α,α'-trehalose (271) and 6-*O*-(2*R*,3*R*,21*R*,22*E*,37*Z*)-3-((*tert*-Butyldimethylsilyl)oxy)-2-eicosyl-21-methyltetrapentaconta-22,37-dienoic-2,3,4,2',3',4'-hexakis-*O*-(trimethylsilyl)-α,α'-trehalose (272)

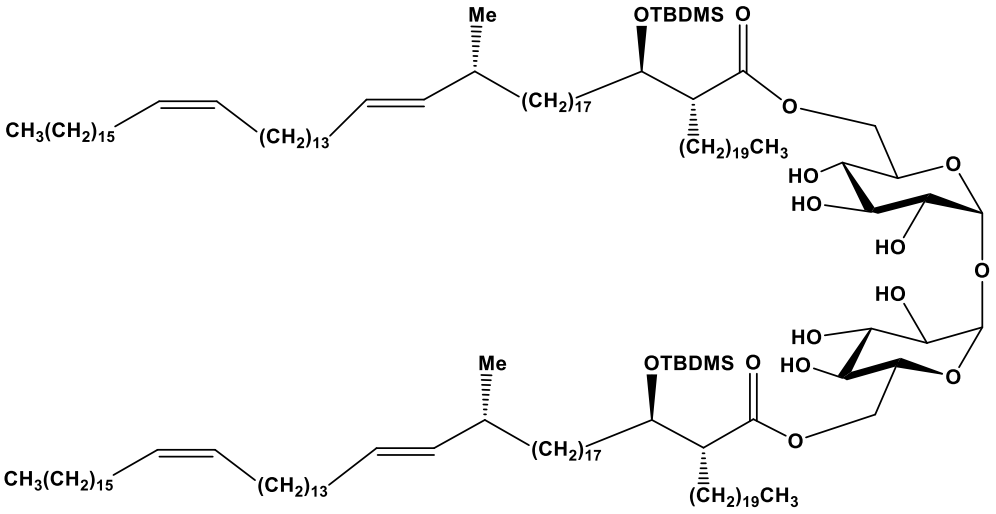


1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (0.14g, 0.76 mmol) and DMAP (0.09 g, 0.76 mmol) were added to a stirred solution of **269** (0.19 g, 0.16 mmol) and protected α,α'-trehalose **270**[287] (74.0 mg, 0.09 mmol) and powdered 4 Å molecular sieves in dry dichloromethane (4 mL) at room temperature under an atmosphere nitrogen atmosphere. The mixture was stirred for 6 days at room temperature, then diluted with dichloromethane (5 mL) and silica gel (1 g) was added. The mixture was evaporated under

reduced pressure to give a residue, which was purified by column chromatography, eluting with petrol/ethyl acetate (20:1) to give the first fraction, **TDM 271**, as a colourless thick oil (50.0 mg, 17%) $[\alpha]_D^{23}$ = + 9.2 (c 0.24, $CHCl_3$), {MALDI-Found $[M+Na]^+$:3179.9; $C_{192}H_{386}O_{15}Si_8Na$ requires: 3179.7}. This showed δ_H (400 MHz, $CDCl_3$): 5.41 – 5.30 (6H, m), 5.24 (2H, dd, *J* 7.4, 15.4 Hz), 4.85 (2H, br. d, *J* 2.9 Hz), 4.37 (2H, br. d, *J* 10.3 Hz), 4.01 (2H, br. dt, *J* 3.1, 10.7 Hz), 3.93 (2H, br. pent, *J* 5.2 Hz), 3.90 (2H, br. t, *J* 8.9 Hz), 3.53 (2H, br. t, *J* 9.1 Hz), 3.38 (2H, dd, *J* 2.8, 9.3 Hz), 2.61 – 2.51 (2H, m), 2.10 – 1.92 (14H, m), 1.66 – 1.02 (244H, m), 0.94 (6H, d, *J* 6.7 Hz), 0.89 *(*12H, t, *J* 6.6 Hz), 0.88 (18H, s), 0.16 (18H, s), 0.15 (18H, s), 0.14 (18H, s), 0.06 (12H, s); δ_C (101 MHz, $CDCl_3$): 173.8, 136.5, 130.4, 129.9, 128.4, 94.8, 85.4, 73.4, 72.8, 71.8, 70.7, 66.2, 62.4, 57.7, 51.9, 37.3, 36.7, 33.4, 32.6, 31.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 28.1, 27.4, 27.2, 25.8, 22.7, 20.9, 14.1, 1.09, 0.94, 0.16, –4.5, –4.7; ν_{max}: 2927, 2880, 1745,1466, 1379, 1273,1163, 1100 cm^{-1}.

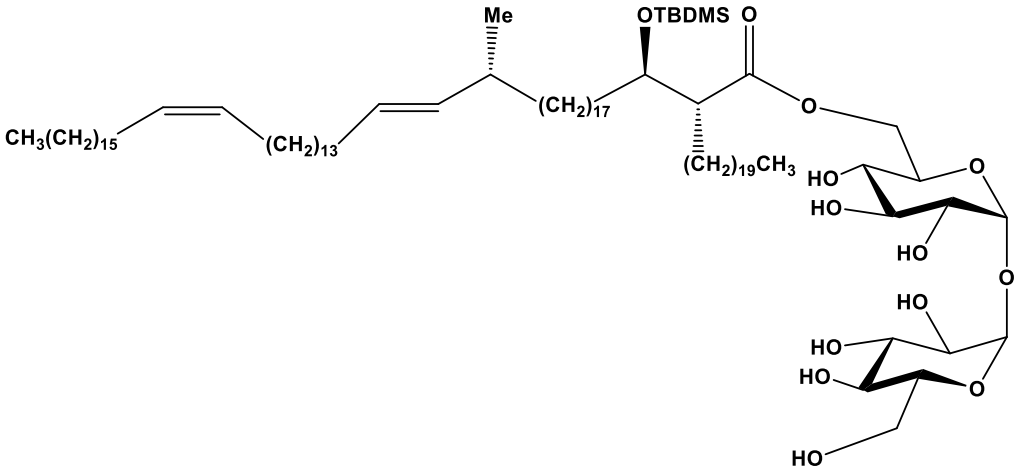
The second fraction, **TMM 272**, was a thick oil (0.13 g, 70%) $[\alpha]_D^{23}$ = + 30.2 (c 0.39, $CHCl_3$), {MALDI-Found $[M+Na]^+$: 1988.5422; $C_{111}H_{228}O_{13}Si_7Na$ requires: 1988.5457}. This showed δ_H (400 MHz, $CDCl_3$): 5.47 – 5.29 (3H, m), 5.24 (1H, dd, *J* 7.4, 15.4 Hz), 4.92 (1H, d, *J* 3.0 Hz), 4.85 (1H, d, *J* 2.9 Hz), 4.36 (1H, dd, *J* 1.9, 11.7 Hz), 4.08 (1H, dd, *J* 4.0, 11.7 Hz), 4.0 (1H, td, *J* 2.2, 9.5 Hz), 3.97 – 3.91 (2H, m), 3.88 (1H, dd, *J* 5.1, 8.9 Hz), 3.84 (1H, td, *J* 3.1, 9.3 Hz), 3.75 – 3.63 (2H, m), 3.5 (1H, dd, *J* 3.7, 9.0 Hz), 3.49 (1H, dd, *J* 3.7, 9.0 Hz), 3.47 (1H, dd, *J* 3.7, 9.0 Hz), 3.43 (1H, dd, *J* 3.0, 9.3 Hz), 3.4 (1H, dd, *J* 3.0, 9.3 Hz), 2.64 – 2.49 (1H, m), 2.10 – 1.87 (7H, m), 1.78 – 0.98 (123H, m), 0.95 (3H, d, *J* 6.7 Hz), 0.88 (6H, t, *J* 6.6 Hz), 0.87 (9H, s), 0.17 (9H, s), 0.16 (9H, s), 0.155 (9H, s), 0.15 (18H, s), 0.13 (9H, s), 0.06 (3H, s), 0.055 (3H, s); δ_C (101 MHz, $CDCl_3$): 174.1, 136.5, 130.4, 129.9, 128.4, 94.5, 94.4, 73.4, 73.3, 72.9, 72.8, 72.7, 72.0, 71.4, 70.7, 62.5, 61.7, 51.8, 37.3, 36.7, 33.4, 32.6, 32.6, 31.9, 30.0, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 28.1, 27.5, 27.5, 27.4, 27.2, 26.4, 25.9, 25.8, 24.8, 22.7, 21.0, 18.0, 14.1, 1.1, 1.0, 0.9, 0.85, 0.17, 0.04, –4.5, –4.7; ν_{max}: 3607, 2921, 2852, 1741, 1461, 1250,1109, 1077, 836 cm^{-1}.

Experiment 73: 6,6'-*bis*-*O*-(2*R*,3*R*,21*R*,22*E*,37*Z*)-3-((*tert*-Butyldimethylsilyl)oxy)-2-icosyl-21-methyltetrapentaconta-22,37-dienoic-α,α'-trehalose (273)



Tetrabutylammonium fluoride (0.12 mL, 0.12 mmol, 1.0 M) was added to a stirred solution of **TDM 271** (47.9 mg, 0.015 mmol) in dry THF (2 mL) at 5 °C under a nitrogen atmosphere. The mixture was allowed to reach room temperature and stirred for 1 h, then evaporated to give a residue, which was purified by column chromatography, eluting with $CHCl_3$/MeOH (10:1) to give the title compound **273** as a colourless thick oil (6 mg, 15%) $[\alpha]_D^{23}$ = + 10.4 (*c* 0.5, $CHCl_3$). {Found $[M+Na]^+$: 2747.5; $C_{174}H_{338}O_{15}Si_2Na$ requires: 2747.5}. This showed δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 5.36 – 5.21 (6H, m), 5.17 (2H, dd, *J* 7.7, 14.0 Hz), 4.98 (2H, d, *J* 3.2 Hz), 4.35 (2H, br.dd, *J* 3.1, 10.7 Hz), 4.20 (2H, br. d, *J* 10.5 Hz), 3.91 (2H, br. d, *J* 9.4 Hz), 3.85 (2H, br. q, *J* 5.0 Hz), 3.73 (2H, br. t, *J* 9.3 Hz), 3.46 (2H, dd, *J* 3.8, 9.3 Hz), 3.34 (2H,br. m), 2.42 – 2.30 (2H, m), 2.03 – 1.86 (14H, m), 1.76 – 1.09 (250H, m), 0.88 (6H, d, *J* 6.7 Hz), 0.84 (12H, t, *J* 6.3 Hz), 0.81 (18H, s), -0.06 (6H, s), -0.07 (6H, s); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 175.4, 136.3, 130.2, 129.8, 128.3, 94.4, 85.5, 72.5, 71.3, 70.9, 69.9, 66.0, 63.9, 52.4, 37.1, 36.5, 34.7, 34.5, 34.4, 32.4, 31.8, 29.5, 29.4, 29.3, 29.2, 29.0, 28.9, 28.8, 27.2, 27.1, 27.0, 26.0, 25.7, 25.2, 25.0, 22.5, 20.7, 19.9, 13.9, 13.3, –0.3, –6.0.; ν_{max}: 3401, 2925, 2856, 1734, 1459, 1053 cm^{-1}.

Experiment 74: 6-*O*-(2*R*,3*R*,21*R*,22*E*,37*Z*)-3-((*tert*-Butyldimethylsilyl)oxy)-2-eicosyl-21-methyltetrapentaconta-22,37-dienoic-α,α'-trehalose (274)



Tetrabutylammonium fluoride (0.5mL, 0.52 mmol, 1.0 M) was added to a stirred solution of **TMM 272** (0.13 g, 0.06 mmol) in dry THF (3 mL) at 5 °C under a nitrogen atmosphere. The mixture was allowed to reach r.t. and stirred for 3 h, then evaporated to give a residue, which was purified by column chromatography, eluting with $CHCl_3$/MeOH (5:1) to give the title compound **274** (30.0 mg, 30%) $[\alpha]_D^{23}$ = + 18.2 (c 0.45, $CHCl_3$), {MALDI-Found $[M+Na]^+$:1556.3071; $C_{93}H_{180}O_{13}SiNa$ requires: 1556.3085}. This showed δ_H (400 MHz, $CDCl_3$ + few drop of CD_3OD): 5.38 – 5.26 (3H, m), 5.20 (1H, dd, *J* 15.3, 7.4 Hz), 5.06 (2H, br. t, *J* 3.5 Hz), 4.28 (1H, dd, *J* 3.7, 10.6 Hz), 4.21 (1H, dd, *J* 4.5, 11.8 Hz), 3.9 (1H, br. d, *J* 8.3 Hz), 3.87-3.84 (1H, m), 3.81-3.71 (4H, m), 3.65 (1H, dd, *J* 5.2, 11.6 Hz), 3.47 (1H, dd, *J* 3.9, 5.9 Hz), 3.46 (1H, dd, *J* 5.9, 9.7 Hz), 2.44 – 2.35 (1H, m), 2.03 – 1.89 (7H, m), 1.83 – 0.94 (131H, m), 0.90 (3H, d, *J* 7.8 Hz), 0.83 (6H, t, *J* 7.0 Hz), 0.81 (9H, s), –0.003 (3H, s), –0.024 (3H, s); δ_C (101 MHz, $CDCl_3$+ few drop of CD_3OD): 175.5, 136.4, 130.3, 129.8, 128.3, 94.4, 93.4, 73.2, 72.4, 71.4, 71.2, 70.9, 70.0, 66.1, 65.5, 64.3, 63.2, 62.2, 52.3, 50.1, 37.2, 36.6, 35.5, 35.3, 34.9, 34.6, 34.4, 32.5, 31.8, 29.6, 29.5, 29.4, 29.3, 29.2, 29.0, 28.9, 27.3, 27.2, 27.0, 26.8, 26.6, 26.0, 25.8, 25.5, 25.4, 25.0, 22.6, 20.8, 17.7, 14.0, –5.9; ν_{max}: 3319, 2973, 2844, 1733, 1467, 1087 cm^{-1}.

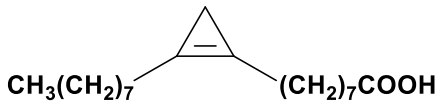
Chapter 5

5. Cyclopropene Fatty Acids

5.1 Introduction

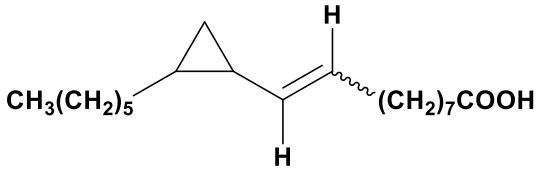
5.2 Cyclopropene Fatty Acids

In 1952 Nunn and his group separated the first naturally occurring cyclopropene fatty acid from the seed oil of the *Sterculia foetida* tree.[288] The structure was suggested to be 8-(2-octyl-cycloprop-1-enyl)-octanoic acid, namely sterculic acid **(276)**.



(276)

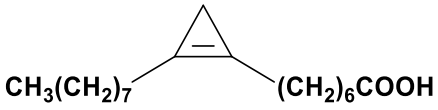
Verma *et al.* questioned this structure **(276)** due to inaccurate analysis of the infra-red data and rather proposed the structure to be 10-(2-hexylcycloprop-1-enyl) dec-9-enoic acid **(277)**.[289]



(277)

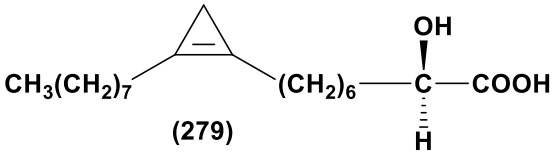
The value of this structure was however reduced when it was found that the absorption signal in the IR spectrum, which characterises the existence of a di-substituted double bond, was found to be due to a polymeric impurity.[290] Several other studies also rejected Verma's structure in favour of Nunn's original structure, which was confirmed by X-ray diffraction.[291] This was done by hydrogenation the natural sterculic acid **(276)** to give the unnatural *cis*-9,10-methylene octadecanoic acid and analysing it by X-ray crystallography. ^{1}H NMR spectroscopy was also used to prove the structure by analysis of the compound. Gensler *et al.,* also synthesised and confirmed the structure by preparing the methyl ester of sterculic acid **(276)** successfully in 1969.[292] In 1956 the second cyclopropene fatty acid was isolated from the seed oil of different plants in the *Malvaceae* family,[293] which was called

malvalic acid **(278)**, and the study of its IR spectrum proposed that it was a homologue of sterculic acid.[294] Analysis of the crystal structure of dihydromalvalic acid showed that malvalic acid **(278)** was certainly a homologue of sterculic acid **(276)** and had the structure shown below. Conformation of this was obtained in 1970 by the synthesis of its methyl ester.[295]

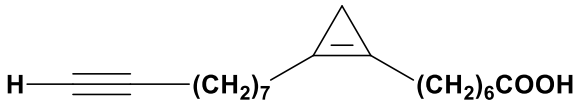


(278)

Other naturally occurring cyclopropene fatty acids that have been isolated, include *D*-2-hydroxysterculic acid **(279)**,[296] which is believed to be an intermediate in the malvalic acid **(278)** biosynthesis, and sterculynic acid **(280)**[297] which was isolated from *Sterculia alata*.



(279)



(280)

5.3 Cyclopropene fatty acids occurrence and conformation

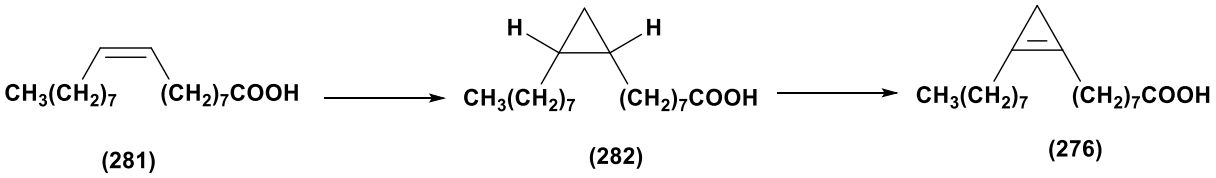
The first isolation of cyclopropene fatty acids was from a specific number of plant families including *Malvaceae* and *Sterculiaceae.* However, nowadays they have been isolated from different types of plants beside these **(**Table **11)**. *Sterculia foetida* contains the largest amount of cyclopropene fatty acids in comparison with other families of plant and, it was isolated from this plant first. The amount of cyclopropene fatty acid content in the plant also varies with season,[298] location within the plant and plant maturity.

Table (11): Percentage of malvalic acid **(278)** and sterculic acid **(276)** in several plant species.

			% Fatty acid in seed oil	
Order	**Family**	**Species**	**Malvalic Acid (278)**	**Sterculic Acid (276)**
Malvaceae	*Sterculiaceae*	*Sterculia foetida*[299]	6.7	54.5
		Sterculia oblonga[300]	-	19.0
	Malvaceae	*Gossypium hirsutum*[300]	1.1	0.4
		Triumfetta pilosa[301]	2.4	6.6
	Sarcolaenaceae	*Schizolaena rosea*[302]	0.8	0.4
Urticales	*Moraceae*	*Ficus benghalensis*[303]	3.7	1.6
Fabales	*Leguminosae*	*Cassia corymbose*[304]	3.2	2.8
		Telia babul[305]	2.5	2.1
Not known	*Boraginaceae*	*Trichodesma zeylanicum*[306]	8.2	7.8

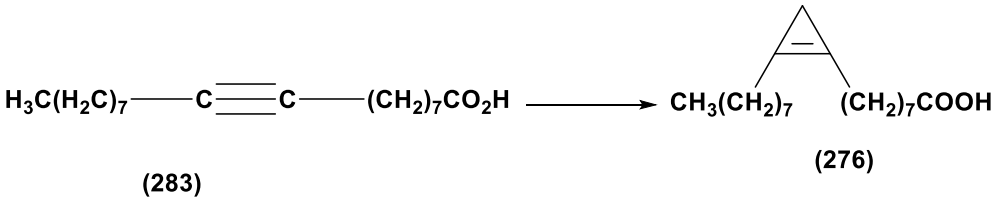
5.4 Biosynthesis of cyclopropene fatty acids

There are not many studies concerning the biosynthesis of cyclopropene fatty acids. Wilson[307] demonstrated the co-occurrence of sterculic acid **(276)** with its corresponding cyclopropane acid **(282)** suggesting that the cyclopropane is first formed by methylene addition to oleic acid **(281)**, followed by dehydrogenation of dihydorsterculic acid **(282)** to sterculic acid, **Scheme (73)**.



Scheme (73): Biosynthesis of sterculic acid.

In 1965 Smith *et al.*[308] discovered traces of stearolic acid **(283)** in sources of sterculic acid and suggested that the cyclopropene fatty acid was obtained directly by addition of a methylene unit to the internal acetylene, **Scheme (74)**.

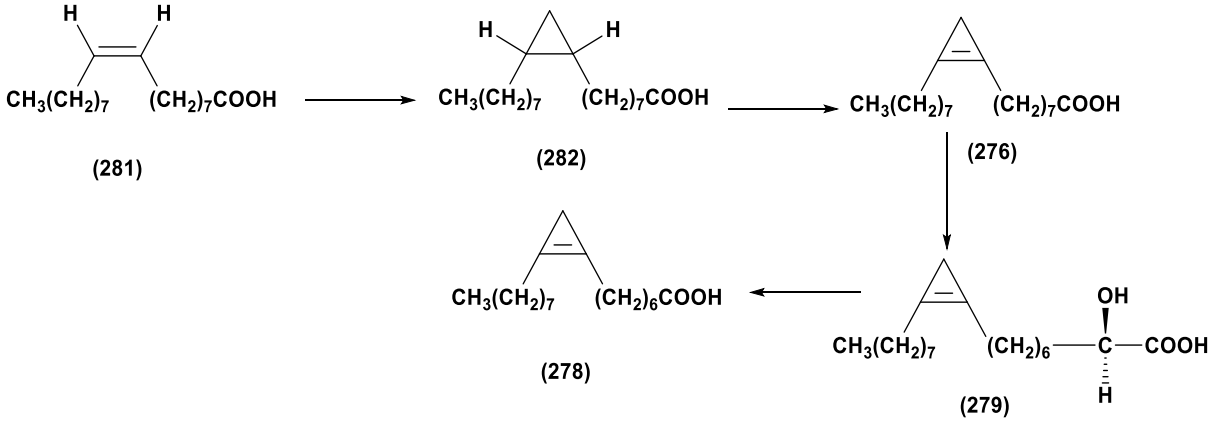


(283) (276)

Scheme (74): Biosynthesis of sterculic acid.

Yano[309] and his group, however, showed that incubation of the methylene source with acetylenic fatty acids gave no cyclopropene fatty acid, but reaction with oleic acid yielded the cyclopropane. This therefore proved that oleic acid **(281)** was the substrate in preference to stearolic acid **(283)**.

Smith *et al.*[310] proposed that the carbon chains, but not the ring methylene group of sterculic and malvalic acids were derived from acetate units. This suggested that malvalic acid is biosynthesised from sterculic acid by a one carbon degradation. The subsequent isolation of *D*-2-hydroxysterculic acid **(279)**[311] suggested this was the intermediate in the oxidation degradation. The conversion of sterculic acid to malvalic acid was shown to be due to an α-oxidative system found in tissues capable of biosynthesising cyclopropene fatty acids. Time studies indicated that the α-oxidation occurs after ring formation, but the exact mode of action is unknown.



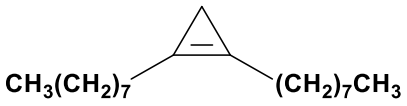
Scheme (75): Biosynthesis of sterculic acid **(276)** and malvalic acid **(278)**.

5.5 The biological effects of cyclopropene fatty acids

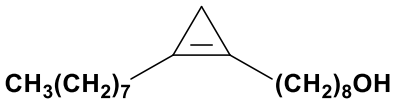
Cyclopropene fatty acids have significant biological properties that have been known for a long time. Feeding pigs on a diet containing cotton seed oils led to them producing a solid fat, while the use of milk from cows fed on a similar diet produced butter with a high m.pt. The cotton plant *Gossypium hirsutus* contains cyclopropene fatty acids which explains the existence of the cyclopropene fatty acids in the cotton seed oil as a part of the animal's diets. Furthermore, hens fed on a diet containing cotton seed oil produced eggs with pink coloured and pasty yolks.[312] The pasty yolk is believed to be due to an increase in the proportion of saturated to unsaturated fatty acids in the yolk and the pink white disorder is believed to be due to an increase in the permeability of the vitellin membrane surrounding the yolk.[313] These effects were later discovered to be due to the cyclopropene fatty acids in the cotton seed oil,[314] which led to the realisation that cyclopropene fatty acids could potentially be very important as desaturase inhibitors.

5.6 Cyclopropene fatty acids as desaturase inhibitors

When rats were given dietary sterculic acid it caused a dramatic difference in the ratios of stearic acid to oleic acid, which proved that cyclopropene fatty acids prevented this conversion.[315] Other studies on hen livers revealed that both malvalic acid **(278)** and sterculic acid **(276)** prevented the Δ^9 desaturation of stearic acid.[316] In this case, the amount of inhibition was found to depend on the concentration, and a greater inhibition came from the methyl esters of the cyclopropene compounds. It has been found that sterculyl-CoA is the actual inhibitor of Δ^9 desaturase instead of the free acid.[315] This proposes that the reason for the inability of sterculine **(284)** and sterculyl alcohol **(285)** to inhibit Δ^9 desaturase to the same degree is, because they are not able to form acyl-CoA.



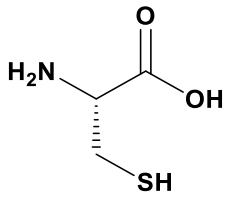
(284)



(285)

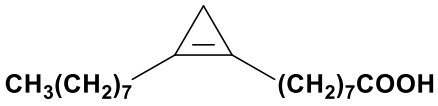
The specific mechanism of cyclopropene fatty acids to inhibit Δ^9 desaturase is still unclear. It has been found that for the compound to be able to inhibit Δ^9 desaturase, the cyclopropene ring should be seen at the C9 and / or C10 position. It is proposed that the

inhibition effect of sterculic acid **(276)** is related to the cyclopropene ring as 1,2-dihydroxy sterculene presented very little or no effect as an inhibitor.[317] The rapid reaction of methyl sterculate with thiols, suggests that the inhibiting ability of Δ^9 desaturase by sterculic acid occurs through reaction with a thiol group within the enzyme. This suggestion is supported by the fact that the enzyme is inhibited by compounds such as *N*-ethylmaleimide which is a thiol specific reagent, therefore showing that the thiol groups are important binding sites within the active site of Δ^9 desaturase.[315] Testing of diverse amino acid mimics showed that cysteine **(286)**, was the only one that reacted with cyclopropene containing compounds, thus suggests that the inhibition is irreversible and that cyclopropene fatty acids can only react with cysteine **(286)** unit at the active site of the enzyme.[318]

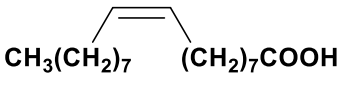


(286)

Because of the similarity of sterculic acid **(276)** and oleic acid **(281)** in basic shape, it is believed that sterculic acid has good inhibition properties towards Δ^9 desaturase.



(276)



(281)

5.7 Uses of cyclopropene fatty acids

As sterculic acid **(276)** has been shown to be a good inhibitor of Δ^9 desaturase many industrial uses for the cyclopropene containing fatty acid have been identified.

Cyclopropene fatty acids have been used in the food industry, such as cocoa butter which is not only significant but also very expensive. Using the fact of inhibition of Δ^9 desaturase by the sterculic acid a substitute oil could be produced. Using cheaper carbohydrate feedstocks for fermenting yeast gives huge quantities of fats counting 5% stearic acid and around 50% oleic acid **(281)**. A fat of the same composition to cocoa butter should be produced by adding sterculic acid to the yeast culture due to the high levels of stearic acid and low levels of oleic acid in cocoa butter, and this was found to be the case. Another major motivation for using sterculic acid is in the medical field due to its significant ability to regulate body weight. Research conducted on rats by disturbing Δ^9 desaturase has shown that increasing expenditure of energy, and reducing adiposity in the body are resistant to diet induced obesity.[319] Therefore, this mutation in the Δ^9 desaturase gene has led to global changes in the gene expression and altered the metabolic activity which accounts for the loss of body fat. The disruption in Δ^9 desaturase is therefore a promising therapeutic target in the treatment of the metabolic syndrome.[320] As sterculic acid **(276)** is an inhibitor of Δ^9 desaturase, cells were treated with sterculic acid and this was found to reduce their adiposity.[321] As obesity has now reached epidemic proportions with high levels of obesity related diseases this research is very significant.

Human tumour cells are known to contain higher levels of oleic acid **(281)** due to the higher levels and activity of Δ^9 desaturase. This leads to a more permeable membrane which leads to an increase in the metabolic rate and cell division which causes the development of cancers. However, until recently the relationship between Δ^9 desaturase and cancer remained unknown. It has now been shown that in lung cancer cells when the Δ^9 desaturase expression was reduced, the biosynthesis of fatty acids was also significantly impaired which shows a causal relationship between Δ^9 desaturase and abnormal lipid metabolism in cancer cells.[322] Numerous different tumour cells exhibit higher levels and higher activity of Δ^9 desaturase such as in human larynx tumour cells[323] and in simian transformed human lung fibroblast.[324] Research has also shown that males with prostatic malignancy also had higher levels of Δ^9 desaturase and a threefold increase in some monounsaturated fatty acids. Δ^9 Desaturase inhibitors could therefore be promising anti-cancer agents.[325] Interferon[326] is a commercially available drug used to treat certain types of cancers and studies have shown that it increases the amount of saturated fatty acids *in vitro* by inhibiting the action of Δ^9

desaturase. This inhibition decreases membrane fluidity, making the membrane denser.[327] This increase in the membrane rigidity may explain the capacity to inhibit virus release and possibly cell division. Interferon's inhibitory properties may therefore be responsible for its anti-cancer properties. Conjugated linoleic acid has also been found to possess some inhibitory effects on some forms of mammary carcinogens by decreasing the levels and activity of Δ^9 desaturase.[328] As sterculic acid **(276)** is a more potent inhibitor of Δ^9 desaturase it is believed that it would have a greater effect on the fluidity of the membrane and would therefore be a better anti-cancer agent. Sterculic acid has already displayed some anti-tumour effects *in vitro* and in a tumour model in rats.[329]

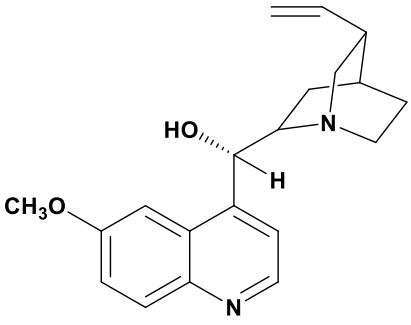
Sterculic acid may also be effective in the treatment of tuberculosis. The thiourea drug isoxyl is used for the treatment of tuberculosis, and has been found to be active against multi drug resistant tuberculosis.[330] Isoxyl decreases the levels of oleic acid and therefore tuberculostearic acid (TBSA) and has some effect on the synthesis of mycolic acids. This suggests that isoxyl works by inhibiting Δ^9 desaturase. By comparing the effect of isoxyl with that of sterculic acid on *M. bovis* BCG it was shown that both have the same effect on the levels of oleic acid. However, sterculic acid had no effect on the synthesis of mycolic acids which suggests that isoxyl has different effects on different fatty acids. However, the primary effect of isoxyl is to inhibit oleic acid biosynthesis, which is a vital part of the mycobacterial membrane. This suggests that alternative Δ^9 desaturase inhibitors could also be a potential drug target for the treatment of tuberculosis.

5.8 Malaria

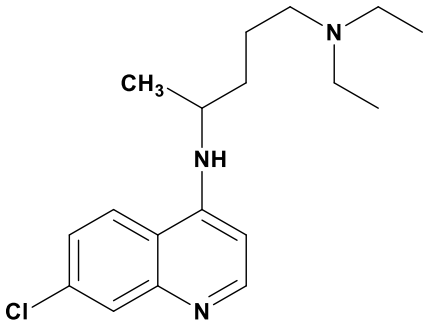
The parasite *Plasmodium falciparum* causes the most severe form of human malaria; which results in approximately a million deaths annually from about 250 million cases. Children living in Africa represent the majority of the deaths[331] caused by the *P. falciparum* parasite. Every 45 seconds malaria causes the death of child in Africa, and this accounts for 20% of all childhood deaths. Transmission of malaria is through the bites of infected Anopheles mosquitoes. Transmission occurs in the intensive places and it likes to bite people instead of animals. In places where high levels of malaria transmit, the immunity of people is a significant factor for malaria development. The immunity of individuals is testified development from time to time of exposure and this explain why the most deaths in Africa happen in young kids who have not yet development this immunity agent's malaria.

5.8.1 Treatment

Quinine **(287)** was the first efficient medicine for the treatment of *P. falciparum* malaria and it was first isolated from the bark of the Cinchona tree in 1820.[332] It was later placed by chloroquine **(288)** and another quinine derivatives.[333] The first discovery of chloroquine **(288)** was in 1934 but it was not used for treatment against malaria until 1947.



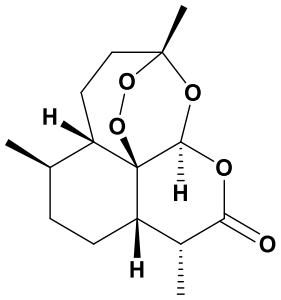
(287)



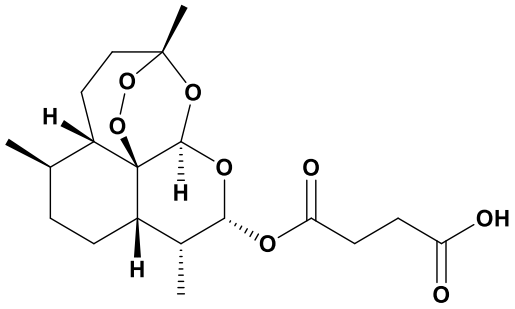
(288)

The subsequent resistance of *P. falciparum*[333,334] to chloroquine has however led to other drugs being investigated. Currently an artemisinin **(289)** based combination therapy is recommended for the treatment of *P. falciparum* malaria. In 1972, artemisinin **(289)** was discovered in the leaves of the common herb plant *Artemisia annua.* Since then derivatives of artemisinin have been synthesised, such as artesunate **(290)**, to improve the efficiency as an anti-malarial therapy. Artemisinin and its derivatives have a short half-life therefore they

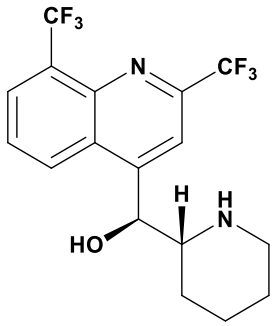
are recommended to be taken in combination with another partner therapy such as mefloquine **(291)**, which has a longer half-life.[335]



(289)



(290)



(291)

5.9 Fatty acids in *Plasmodium falciparum*

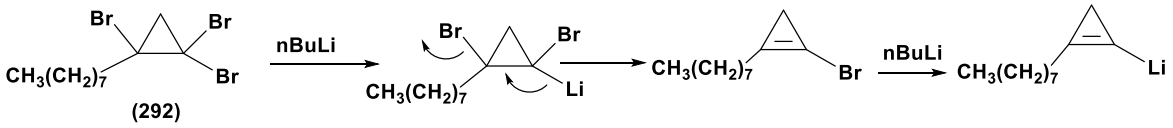
A red blood cells infected has seen a large rise in the fat content during *P. falciparum* infection[336] It has been mentioned that *P. falciparum* has a restricted capacity to protract fatty acids itself during infection. Fatty acids could also get from human serum and experiments have shown that the parasite growing is not continuous in a culture without them. The oleic acid **(281)** especially is vital for parasite growth.[337] It is believed that these fatty acids may be incorporated by passive diffusion through the membranes of *P. falciparum*'s infected red blood cells.[338] Research has shown the parasite contains members of the type II fatty acid biosynthetic pathway which contradicts earlier reports and suggests that *P. falciparum* can indeed synthesise fatty acids *de novo*.[339] It has been reported that this pathway is only active in the liver stages and that it is not vital for the blood stage parasite. It can therefore be concluded that blood stage *P. falciparum* depends on the hosts fatty acids, but not much research has been done to see if these fatty acids can be modified by the parasite. The Δ^9desaturase was subsequently discovered to be present in the parasite, which suggests that the fatty acids can be modified. Further investigations were made into the importance of Δ^9 desaturase to the *P. falciparum* parasite.[340] This study showed that *P. falciparum* can desaturate scavenged stearic acid and that the activity of Δ^9 desaturase is essential for parasite growth in the blood stages.

5.10 Results and discussion

The initial aim of the project was the development of a flexible route to sterculic acid and analogous CPFAs. Studies of the inhibitory effects of these analogues on Δ^9 desaturase could then be carried out. As it has been discovered that unsaturated fatty acids are essential for *P. falciparum* growth, this strengthens the impetus to investigate Δ^9 desaturase inhibitors as potential anti-malarial drugs. As sterculic acid **(276)** is a potent inhibitor of Δ^9 desaturase, analogues of sterculic acid **(276)** will be synthesised in order to try and maximise the inhibition against *P. falciparum* Δ^9 desaturase. This could then guide us towards a better inhibitor and may also convey more information about the active site of the enzyme and could be an important target in anti malarial treatment.

5.11 Synthesis of sterculic acid analogues

For the synthesis of all the analogues discussed below, 1,1,2-tribromo-2-octyl cyclopropane **(292)**, which was supplied by Dr. J. Al-Dulayymi, was used as the basic bulding block; this forms the alkyl chain as well as allowing for the formation of the cyclopropene ring from the cyclopropane via a coupling reaction with an electrophile.



Scheme (76): Formation of a lithiated cyclopropene.

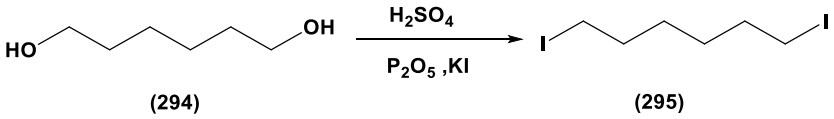
The 1,1,2-tribromo-2-octyl cyclopropane **(292)** is firstly treated with two equivalents of *n*-butyl lithium to give lithiated cyclopropane, followed by reaction with an electrophile to give desired compound.

5.11.1 Synthesis of 7-(2-octyl-cycloprop-1-enyl)heptanoic acid methyl ester (293)

7-(2-Octyl-cycloprop-1-enyl)heptanoic acid methyl ester **(293)**[341] or to give its common name, methyl malvalate, was the first analogue synthesised. This is also one of the few naturally occurring cyclopropene fatty acids and contains one less carbon atom in its acid side

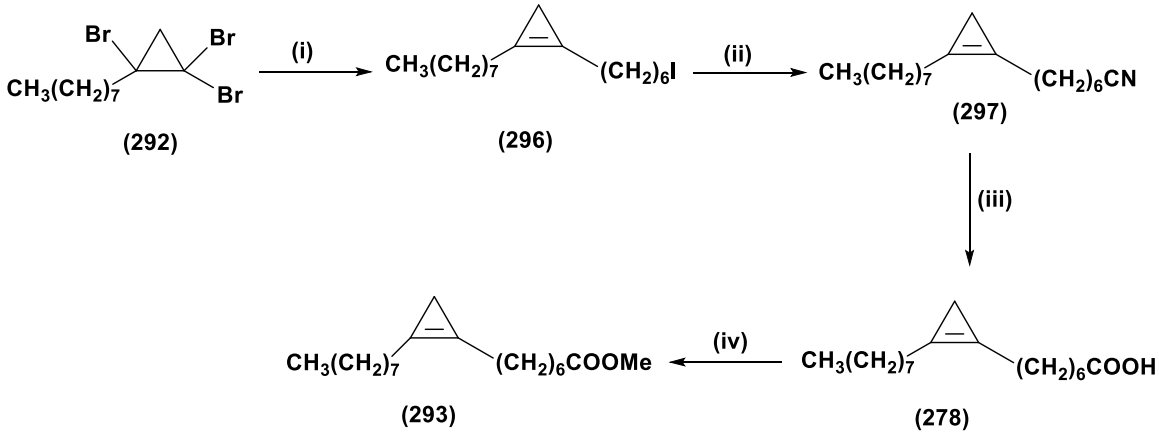
chain compared to sterculic acid **(276)** but it still has the second cyclopropene carbon at the C-9 position.

1,6-Diiodo-hexane **(295)** was synthesised from the commercially available 1,6-hexanediol in one step using phosphoric acid, phosphorus pentoxide and potassium iodide. The reaction mixture was stirred at 120 °C for 6 hours to give, after work up, **(295)** in 74% yield, **Scheme (77)**.



Scheme (77): Synthesis of 1,6 di-iodo-hexane **(295)**.

The ^{1}H NMR spectrum of compound **(295)** confirmed that the reaction had been successful due to the triplet at δ 3.20 (*J* 7.0 Hz) integrating to four protons corresponding to the (2×CH_2I). This diiodide **(295)** was then coupled to the lithiocyclopropene derived by reaction of 1,1,2-tribromo-2-octyl cyclopropane **(292)** with methyllithium, in the first step of the synthesis of **(293)**, **Scheme (78)**.



Scheme (78): Synthesis of 7-(2-octyl-cycloprop-1-enyl)-heptanoic acid methyl ester. (i) n-BuLi, HMPA, $I(CH_2)_6I$ **(295)**, 23%; (ii) NaCN, DMSO, 83%; (iii) NaOH, EtOH/H_2O, TBAH, DCM, 53%; (iv) ether, diazomethane 80%.

The coupling was carried out in the presence of HMPA to form 1-(6-iodo-hexyl)-2-octyl-cyclopropene **(296)** in 23% yield. The presence of the cyclopropene ring in this product could be easily confirmed by the ^{1}H NMR spectrum which showed a singlet at δ 0.78 integrating to

two cyclopropene protons. Thene was also a triplet at δ 3.20 (*J* 7.0 Hz) for the methylene group adjacent to the iodine. The double bond of the cyclopropene ring could also be easily identified in the ^{13}C NMR spectrum with two peaks present at δ 109.0 and δ 109.6.

Iodide **(296)** was then heated to 60 °C with sodium cyanide in DMSO.[342] This converted the iodide into the nitrile to form 7-(2-octyl-cycloprop-1-enyl)-heptanenitrile **(297)**. The formation of the nitrile could again be easily confirmed due to the characteristic peaks in the IR spectrum (2245 cm^{-1}) and ^{13}C NMR spectrum at (δ 119.8), respectively. All remaining data were as expected for cyanide **(297)** with the only other significant peak in the ^{1}H NMR spectrum occurring as a triplet at δ 2.35 (*J* 7.1 Hz) for the methylene group adjacent to the cyanide.

The next stage was to convert the nitrile into the corresponding acid **(278)** by treating **(297)** with sodium hydroxide and TBAH in DCM to give the required product in 53% yield. This conversion was confirmed by the ^{1}H NMR and ^{13}C NMR spectra which showed signals at δ 2.37 (t, *J* 7.5 Hz) corresponding to the (<u>CH$_2$</u>COOH) and δ 179.8 corresponding to the carbonyl group, respectively. Also, the IR spectrum showed a peak for the OH at 3027 cm^{-1}.

The final step was the conversion of the free malvalic acid **(278)** to the corresponding methyl ester through the reaction of the acid with diazomethane in presence of ether. The esterification was confirmed by the ^{1}H NMR and ^{13}C NMR spectra which showed a singlet at δ 3.68 corresponding to the methyl ester and a signal at δ 174.3 corresponding to the carbonyl group respectively **(Figure 51)**.

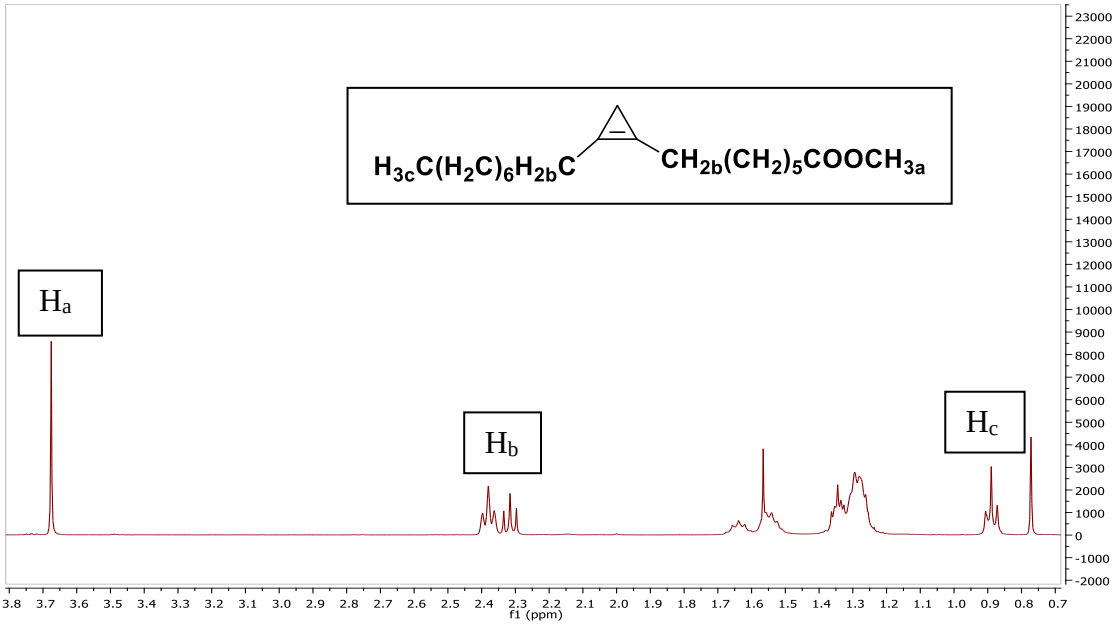
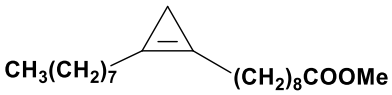


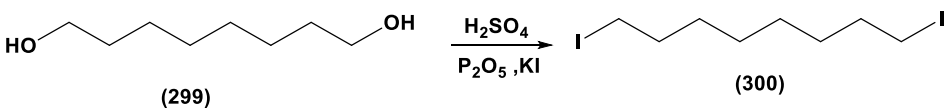
Figure (51): ^{1}H NMR spectrum of the compound **(278)**.

5.11.2 The synthesis of 9-(2-octyl-cycloprop-1-enyl)-nonanoic acid methyl ester (298)



(298)

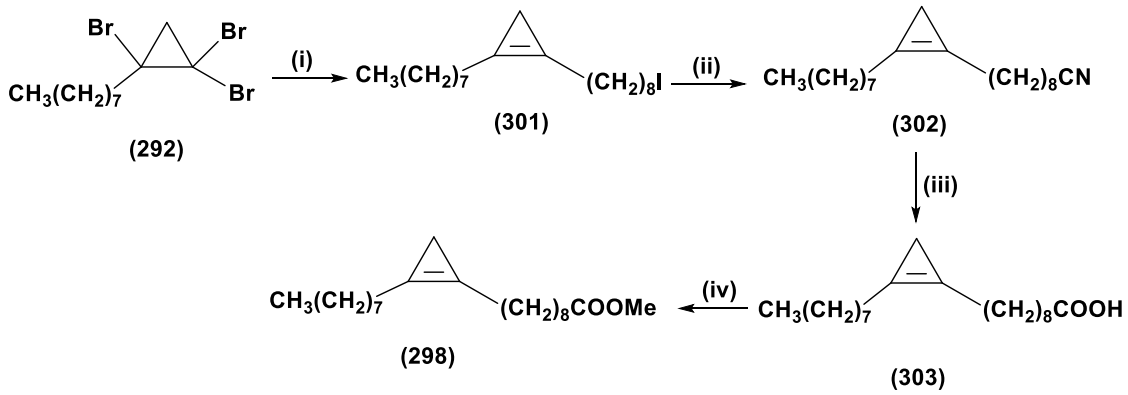
9-(2-Octyl-cycloprop-1-enyl)-nonanoic acid methyl ester **(298)** was the next analogue prepared to see if increasing the number of carbon atoms would cause a difference in the inhibitory capacity of the analogue. Compound **(298)** also contains the cyclopropene at the C_{10} position and therefore should exhibit inhibitory qualities. Before this could be prepared, 1,8-diiodo octane was prepared from the corresponding diol (1,8-octane diol) **(299)**, following the same method as was used for the synthesis of 1,6-diiodo-hexane **(295) (**see **Scheme 78)**. Diiodo octane **(300)** was obtained in an overall yield of 78% **(Scheme 79)**.



(299) **(300)**

Scheme (79): Synthesis of 1,8-diiodo- octane.

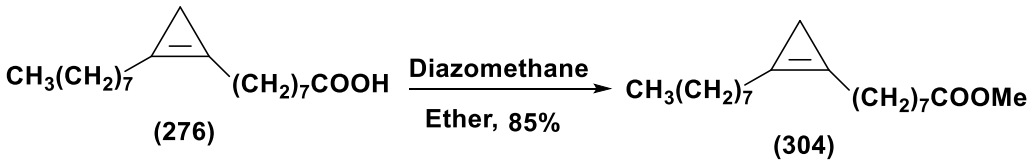
The synthesis of **(298)** then proceeded as shown in **Scheme (80)**, giving the required product in an overall yield of 6%, compared to 8% for the previous analogue **(292)**.



Scheme (80): Reagents and conditions: (i) n-BuLi, HMPA, $I(CH_2)_6I$, 21%; (ii) NaCN, DMSO, 78%; (iii) NaOH, $EtOH/H_2O$, TBAH, DCM, 44%; (iv) ether, diazomethane 83%.

5.11.3 Esterification of free sterculic acid

Having prepared methyl esters **(293)** and **(298)** natural sterculic acid **(276)** was also converted to its methyl ester. Natural sterculic acid, provided by Dr. J. Al-Dulayymi, was esterified using an excess solution of diazomethane in ether to give **(304)** in 85% yield **Scheme (81)**[341,343]. The ^{1}H and ^{13}C NMR spectra were as expected, and confirmed the formation of the methyl ester.

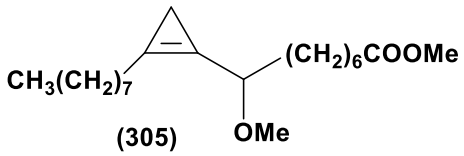


Scheme (81): Esterification of free sterculic acid.

5.12 The synthesis of 8-methoxy-8-(2-octyl-cycloprop-1-enyl)-octanoic acid methyl ester (344)

5.12.1 Introduction of new functional groups

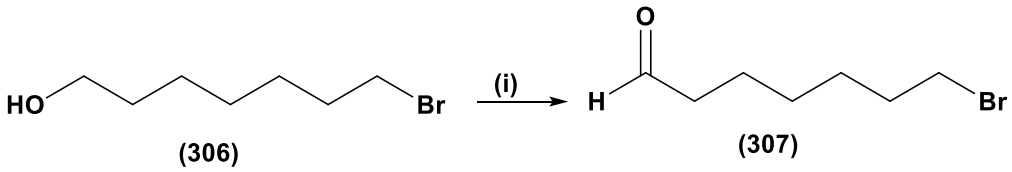
The next analogue to be prepared **(305)** incorporated a functional group into the carboxylic acid side chain of the sterculic acid **(276)**. Again, this was prepared to investigate their effects on the level of inhibition.



(305)

The OMe group would be included at the C-8 position, which is adjacent to the cyclopropene ring. It is known that the cyclopropene reacts with the enzymes active site, therefore, the C-8 position should also be close to or part of the active site. Adding functional groups at this position might therefore have a profound effect in one way or another on the compounds ability to inhibit Δ^9 desaturase. The chain length in this case would be identical to that of sterculic acid as changing more than one aspect of the molecule would make it difficult to know what effect each individual aspect of the molecule would have on the inhibitory qualities of the molecule.

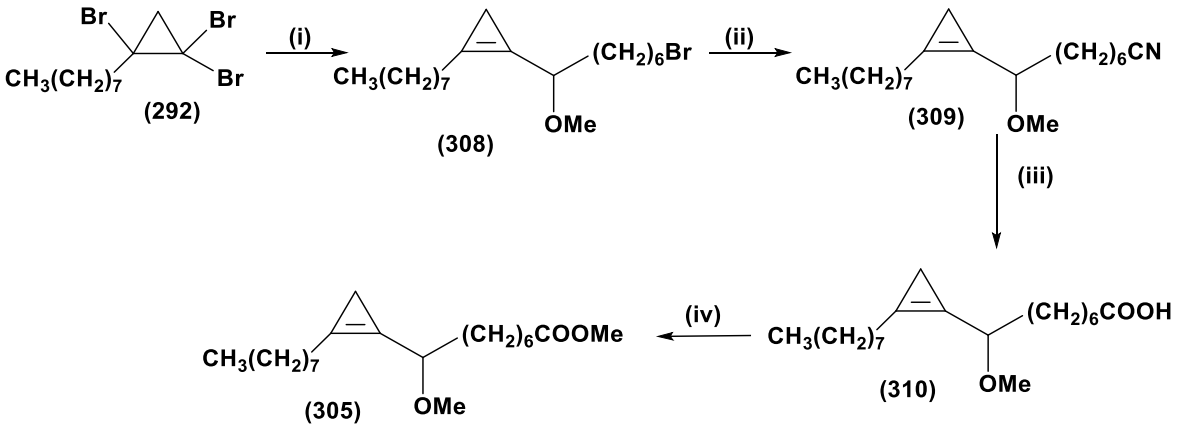
To prepare 8-methoxy-8-(2-octyl-cycloprop-1-enyl)-octanoic acid methyl ester **(305)**, 7-bromo heptanal **(307)** needed to be prepared **(Scheme 82)**.



(306) (307)

Scheme (82): Synthesis of 7-bromo heptanal (i) PCC, CH_2Cl_2, 77%.

This was obtained by oxidising **(306)** in 77% yield and the structure was confirmed by NMR. The ^{1}H NMR spectrum showed a triplet at δ 9.78 (*J* 1.7 Hz) corresponding to the aldehyde proton and another triplet at δ 3.42 (*J* 6.8 Hz) belonging to the CH_2 adjacent to the bromine atom. Also, the ^{13}C showed a signal at δ 202.5 for aldehyde carbon. This aldehyde was then used immediately as the electrophile in the coupling reaction with the lithiocyclopropene derived from 1,1,2-tribromo-2-octylcyclopropane **(292)**, **(Scheme 83)**.



Scheme (83): Synthesis of 8-methoxy-8-(2-octyl-cycloprop-1-enyl)-octanoic acid methyl ester (i) n-BuLi; HMPA, $Br(CH_2)_6COH$; MeI, 46%; (ii) NaCN, DMSO, 43%; (iii) NaOH, EtOH, H_2O; TBAH, CH_2Cl_2, 93%; (iv) ether, diazomethane 80%.

The formation of compound **(308)** was confirmed by the H^1 NMR spectrum, which indicated the presence of a methoxy group due to a three proton singlet at δ 3.31. The protons on the carbon atom next to the bromide were seen as a triplet at δ 3.18 (*J* 7.0 Hz) integrating to two protons as expected. Two peaks were observed in the ^{13}C NMR spectrum at δ 108.6 and δ 114.1 representing the two carbon atoms of the double bond of the cyclopropene ring. However, the characteristic singlet at around δ 0.78 in the ^1H NMR spectrum was not apparent. In this case a doublet was seen at δ 0.94 (*J* 8.5 Hz). The inclusion of a functional group at the C_8 position therefore caused the two cyclopropene protons to become non-equivalent.

1-(7-Bromo-1-methoxy-heptyl)-2-octyl-cyclopropene **(308)** was then heated at 60 °C with sodium cyanide in DMSO as before. This formed nitrile **(309)** which again showed the distinctive peaks at 2246 cm^{-1} in the IR spectrum and at δ 119.8 ppm in the ^{13}C NMR spectrum, whilst all other peaks were as for the starting material with the two cyclopropene protons again observed as a doublet and the methoxy group as a singlet integrating to three protons.

8-Methoxy-8-(2-octyl-cycloprop-1-enyl)-octanenitrile **(309)** was therefore reacted with aqueous sodium hydroxide and heated under reflux to give the carboxylate. TBAH and dichloromethane were then added, and the mixture was stirred. The disappearance of the peaks representing the nitrile was evident in both the IR and ^{13}C NMR spectra. The ^1H

NMR spectrum showed a triplet at δ 2.45 (*J* 7.2 Hz) corresponding to the $\underline{CH_2}COOH$ while the ^{13}C NMR spectrum showed a signal at δ 174.2 corresponding to the carbonyl group in the acid **(310)**. The next step was esterification of the acid using diazomethane in ether to give 8-methoxy-8-(2-octyl-cycloprop-1-enyl)-octanoic acid methyl ester **(305)**. The ^1H NMR data showed two singlets at δ 3.66 and δ 3.30 representing the two methoxy groups. The presence of the cyclopropene could also be confirmed with a characteristic peak in the IR spectrum at 1863 cm^{-1}, two signals in the ^{13}C NMR spectrum at δ 114.0 ppm and δ 108.6 ppm and doublet at δ 0.93 (*J* 8.5 Hz) ppm in the ^1H NMR spectrum confirming that the ring did not decompose during this reaction.

5.13 Effects of the synthesised cyclopropene fatty acids on *Toxoplasma gondii*

Toxoplasma gondii is a zoonotic pathogen that is the causative agent of toxoplasmosis, a disease which affects both humans and animals. Stearoyl-CoA desaturase (SCD), which is a key enzyme for the synthesis of unsaturated fatty acids, is predicted to exist in *T. gondii* and the inhibition of this enzyme by sterculic acid has been shown. Analogues of sterculic acid *i.e.* **(293)**, **(298)**, **(304)** and **(305)**, were therefore prepared during this project so that their anti-parasitic effects against *T. gondii* could also be investigated.

Three assays were carried out using these compounds, by Qun Liu and co-workers at China Agricultural University: a tachyzoite release assay; a tachyzoite proliferation assay; and an MTS assay. The results of these assays have been published[344] and will be discussed below.

(i) Tachyzoite release assay

This assay investigated the effect of the test compounds on tachyzoite release from host cells. The effect on the intracellular *T. gondii* was tested by adding a 1mM concentration of each compound or 0.1% DMSO, as a control, to the cell cultures. Cell rupture and tachyzoite release was then observed by microscope at 0, 12, 24 and 36 hours. Due to a 1mM concentration of sterculic acid leading to cell death within 12 hours, the concentration was reduced to 0.1mM; that of the analogues was kept at 1mM. In the culture treated with DMSO tachyzoite release started after 24 hours and after 36 hours only a small number of intact cells remained. In contrast, most of the cells treated with sterculic acid and the related methyl esters were unchanged after 36 hours, with only a small amount of tachyzoite

release being observed. Of all the test compounds **(305)** led to the least amount of tachyzoite release.

(ii) Tachyzoite proliferation assay

For this assay tachyzoites were fluorescently labelled and were observed under a microscope. Freshly released parasites were used to infect cell monolayers and were allowed to replicate for 18 hours in the culture medium with sterculic acid and the related methyl esters; DMSO was again used as a control. After this time the number of parasitophorous vacuoles containing 1 to 8 tachyzoites were counted. The results showed that all the methyl ester compounds inhibited the tachyzoite proliferation, with **(305)** again being the most effective, with only a small percentage (3%) of the parasitophorous vacuoles containing 8 tachyzoites, compared to DMSO, where there were 40 %.

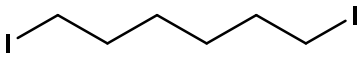
(iii) MTS assay

Cells infected with *T. gondii* tachyzoites were grown in culture medium with different concentrations of sterculic acid and the methyl ester analogues. After 24 hours anti-*T. gondii* and host cell cytotoxicity were measured. Cells grew in the presence of all the methyl ester analogues, with **(305)** promoting cell growth. As was previously observed, sterculic acid was cytotoxic to the cells. Increasing the concentration of the test compounds increased the anti-*T. gondii* activity of the cells. The EC_{50} values (*i.e.* concentration of test compound that inhibits the *T. gondii* induced cytopathic effect by 50%) for the methyl ester analogues ranged from 248μM **(305)** to 428 μM **(298)**, again suggesting that **(305)** was the most effective compound of those tested.

The above assays have shown that sterculic acid and all the methyl ester analogues, **(293), (298), (304)** and **(305)**, inhibited *T. gondii* growth *in vitro*. In all the assays, **(305)**, with a methoxy substituent at the C-8 position, was most effective. It is believed that the compounds inhibited growth by targeting the synthesis of unsaturated fatty acid. Future work could be to investigate the *in vivo* effects of the analogues used in this study. In addition, other analogues with different substituents at the C-8 position could be prepared to see if they exhibit similar effects to **(305)**.

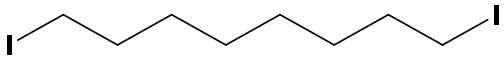
5.14 Experimental

Preparation of 1,6–diiodohexane (295)[345]



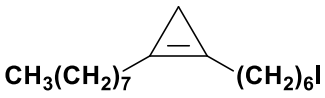
Hexane 1,6-diol **294** (12 g, 0.10 mol) was added to a stired solution of phosphoric acid (27 mL, 45g, 0.40 mol), potassium iodide (66 g, 0.40 mol) and phosphorous pentoxide (13 g, 91 mol) and the reaction mixture was stirred at 120 ^{o}C for 6 h. The cooled product mixture was poured into water (250 mL) and extracted with petrol (3 × 100 mL). The combined organic layers were then washed with water (100 mL), saturated sodium bicarbonate (100 mL), saturated sodium thiosulphate (50 mL) and brine (100 mL), dried over $MgSO_4$ and evaporated. The crude product was purified by column chromatography, eluting with petrol to afford the product **295** (28 g, 74%). This showed δ_H (400 MHz, $CDCl_3$): 3.20 (4H, t, *J* 7.0 Hz), 1.91 – 1.78 (4H, m), 1.48 – 1.37 (4H, m); δ_C (101 MHz, $CDCl_3$): 6.8, 29.4, 33.2; ν_{max}: 2926, 2851, 1736, 14560, 1423, 1175, 720 cm^{-1}.

Preparation of 1, 8–diiodooctane (300)



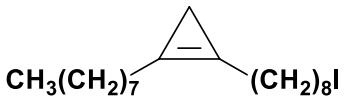
1,8- Octane diol **299** (14 g, 0.10 mol) was added to a stired solution of phosphoric acid (27 mL, 45 g, 0.40 mol), phosphorous pentoxide (13 g, 91 mol) and potassium iodide (66 g, 0.40 mol) at room temperature. The mixture was worked up and purified as before to give 1, 8–diiodooctane **300** (26 g, 78%). This showed δ_H (400 MHz, $CDCl_3$): 3.19 (4H, t, *J* 7.0 Hz), 1.87 – 1.77 (4H, m), 1.44 – 1.20 (8H, m); δ_C (101 MHz, $CDCl_3$): 33.5, 30.4, 28.4, 7.6; ν_{max}: 2928, 2853 ,1734,1459,1424,1176, 720 cm^{-1}. All data matched those in the literature.[345]

1-(6-Iodo-hexyl)-2-octyl-cycloprop-1-ene (296)



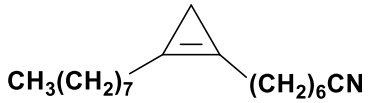
To a stirred solution of 1,1,2-tribromo-2-octyl-cyclopropane **293** (3.0 g, 0.0076 mol) in dry ether (50 mL), *n*-butyl lithium (1.6 M, 12 mL, 0.019 mol) was added dropwise under N_2 (g) at −78 °C. The mixture was allowed to reach room temperature, stirred for 30 min and then cooled to 0 °C. HMPA (2.64 g, 2.72 mL, 0.0152 mol) was then added dropwise followed by 1,6-diiodo-hexane **295** (2.6 g, 7.7 mmol). The mixture was stirred overnight at room temperature and then cooled to 0 °C. Water (50 mL) was added and the product was extracted with ether (2 × 100 mL). The combined organic layers were washed with water (2 × 20 mL), dried and evaporated. Column chromatography eluting with petrol gave 1-(6-iodo-hexyl)-2-octyl-cyclopropene **296** (0.64 g, 23%) as a colourless oil. This showed δ_H (400 MHz, $CDCl_3$): 3.20 (2H, t, *J* 7.0 Hz), 2.43 – 2.36 (4H, m), 1.90 – 1.81 (2H, m), 1.62 – 1.50 (2H, m), 1.52 – 1.23 (16H, m), 0.89 (3H, t, *J* 6.9 Hz), 0.78 (2H, s); δ_C (101 MHz, $CDCl_3$): 109.6,109.0, 45.1, 33.9, 33.5, 32.8, 31.9, 29.4, 29.3, 28.5, 28.0, 27.4, 27.2, 26.0, 22.7, 14.1, 7.4; ν_{max}: 2928, 2857, 1872, 1462 cm^{-1}. All data matched those in the literature.[346]

1-(8-Iodo-octyl)-2-octyl-cyclopropene (301)



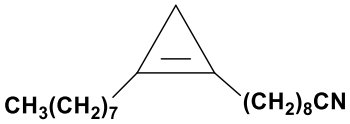
The above experiment was repeated using *n*-butyl lithium (1.6 M, 12 mL, 0.019 mol) and 1,1,2-tribromo-2-octyl cyclopropane (3.0 g, 0.0076 mol) in dry ether (50 mL). HMPA (2.64 mL, 2.72 g, 0.0152 mol) was added dropwise followed by 1,8-diiodo-octane **300** (2.9 g, 0.0078 mol) and the reaction mixture was worked up and purified as before, to give 1-(8-iodo-octyl)-2-octyl-cyclopropene **301** (0.64 g, 21%) as a colourless oil. This showed δ_H (400 MHz, $CDCl_3$): 3.42 (2H, t, *J* 6.9 Hz), 2.38 (4H, t, *J* 7.1 Hz), 1.85 (2H, p, *J* 7.1 Hz), 1.59 – 1.49 (4H, m), 1.48 – 1.36 (2H, m), 1.37 – 1.23 (16H, m), 0.89 (3H, t, *J* 6.8 Hz), 0.78 (2H, s); δ_C (101 MHz, $CDCl_3$): 109.4, 109.2, 45.1, 32.7, 31.9, 29.4, 29.3, 29.3, 29.2, 28.9, 27.4, 27.3, 26.9, 26.1, 26.0, 22.7, 14.1, 7.4; ν_{max}: 2927, 2856, 1872, 1464 cm^{-1}. All data matched those in the literature.[346]

7-(2-Octyl-cycloprop-1-enyl)-heptanenitrile (297)



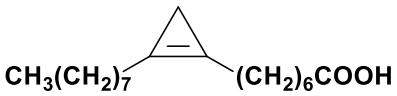
Sodium cyanide (0.25g, 0.0051 mol) was added to a stirred solution of 1-(6-iodo-hexyl)-2-octyl-cyclopropene **296** (0.64 g, 0.0017 mol) in DMSO (40 mL). The mixture was heated to 60 °C for 4 h or until TLC showed no starting material was left. Water (15 mL) was added to the cooled solution and the mixture was extracted with ether (2 × 100 mL). The combined organic layers were washed with saturated aqueous NH_4Cl (30 mL), dried, filtered and evaporated. Column chromatography, eluting with petrol/ethyl acetate (5:2) gave 7-(2-octyl-cycloprop-1-enyl)-heptanenitrile **297** (0.4 g, 83%) as a yellow oil, which showed δ_H (400 MHz, $CDCl_3$): 2.40 (4H, t, *J* 7 Hz), 2.35 (2H, t, *J* 7.1 Hz), 1.68 (2H, p, *J* 7.2 Hz), 1.62 – 1.54 (6H, m), 1.53 – 1.44 (2H, m), 1.40 – 1.19 (10H, m), 0.89 (3H, t, *J* 7.0 Hz), 0.78 (2H, s); δ_C (101 MHz, $CDCl_3$): 119.8, 109.8, 108.9, 31.9, 29.4, 29.4, 29.3, 28.5, 27.4, 27.0, 26.0, 25.8, 25.3, 22.3, 17.1, 14.1, 7.4; ν_{max}: 2925, 2857, 2245, 1872, 1462 cm^{-1}. All data matched those in the literature.[346]

9-(2-Octyl-cycloprop-1-enyl)-nonanenitrile (302)



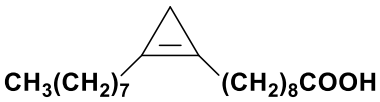
The above experiment was repeated using sodium cyanide (0.21 g, 0.0025 mol) and 1-(8-chloro-octyl)-2-octyl-cyclopropene **301** (0.32 g, 0.00081 mol) in DMSO (40 mL). The reaction mixture was worked up and purified as before to give 9-(2-octyl-cycloprop-1-enyl)-nonanenitrile **302** (0.18 g, 78%) as a yellow oil. This showed δ_H (400 MHz, $CDCl_3$): 2.39 (4H, br.t, *J* 7.1 Hz), 2.34 (2H, t, *J* 7.1Hz), 1.67 (2H, p *J* 7.4 Hz), 1.59 – 1.50 (4H, m), 1.49 – 1.40 (2H, m), 1.37 – 1.23 (16H, m), 0.89 (3H, t, *J* 6.9 Hz), 0.78 (2H, s); δ_C (101 MHz, $CDCl_3$): 119.8, 109.5, 109.2, 31.9, 29.4, 29.3, 29.2, 29.1, 28.8, 28.7, 27.4, 27.3, 26.1, 26.0, 25.4, 22.7, 17.1, 14.1, 7.4; ν_{max}: 2928, 2854, 2247, 1462 cm^{-1}. All data matched those in the literature.[346]

8-(2-Octylcycloprop-1-enyl) heptanoic acid (278)



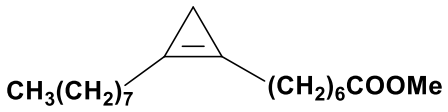
Sodium hydroxide (0.25 g, 0.0064 mol) was dissolved in a mixture of ethanol (3.5 mL) and water (0.5 mL). 7-(2-Octylcycloprop-1-enyl)heptanenitrile **297** (0.31g, 0.0011mol) was then added and the mixture was heated under reflux under N_2 for 8 h. The ethanol was evaporated and to the residue 0.1 M TBAH solution, (20 mL) was added. Dichloromethane (20 mL) was then added and the mixture was stirred for 12 h. The solution was acidified to ~pH 3 with aqueous potassium hydrogen sulfate. The mixture was separated, and the organic solution was washed with water (3 × 30 mL), dried, filtered and evaporated. Column chromatography eluting with petrol/ethyl acetate (5:2) gave 8-(2-octylcycloprop-1-enyl)heptanoic acid **278** (0.3 g, 53%) as a colourless oil. This showed δ_H (400 MHz, $CDCl_3$): 2.39 (4H, t, *J* 7.1 Hz), 2.37 (2H, t *J* 7.5 Hz), 1.65 (2H, m), 1.58 – 1.50 (4H, m), 1.38 – 1.24 (15H, m), 0.87 (3H, t, *J* 3.4 Hz), 0.77 (2H, s); δ_C (101 MHz, $CDCl_3$): 179.8, 109.5, 34.0, 31.9, 29.4, 29.3, 29.2, 29.1, 29.0, 27.4, 27.3, 26.0, 25.9, 24.6, 22.7, 14.1, 7.4; ν_{max}: br. 3027, 2926, 2850, 1711, 1464 cm^{-1}. All data matched those in the literature.[346]

9-(2-Octylcycloprop-1-enyl)nonanoic acid(303)



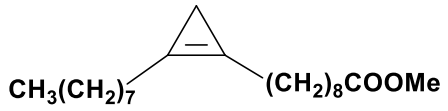
The above experiment was repeated using sodium hydroxide (0.19 g, 0.0048 mol) and 9-(2-octyl-cycloprop-1-enyl)-nonanenitrile **302** (0.24g, 0.00082 mol) in a mixture of ethanol (3.5 mL) and water (0.5 mL). The reaction mixture was worked up and purified as before to give 9-(2-octylcycloprop-1-enyl)decanoic acid **303** (0.11 g, 44%) as a colourless oil. This showed δ_H (400 MHz, $CDCl_3$): 2.39 (4H, t, *J* 7.2 Hz), 2.34 (2H, t, *J* 7.7 Hz), 1.68 – 1.60 (2H, m), 1.59 – 1.49 (4H, m), 1.37 – 1.23 (19H, m), 0.89 (3H, t, *J* 7.0 Hz), 0.77 (2H, s); δ_C (101 MHz, $CDCl_3$): 179.8, 109.5, 109.2, 34.0, 31.9, 29.4, 29.3, 29.2, 29.1, 29.0, 27.4, 27.3, 26.0, 25.9, 24.6, 22.7, 14.1, 7.4; ν_{max}: br. 3030, 2927, 2857, 1711, 1464 cm^{-1}. All data matched those in the literature.[346]

7-(2-Octyl-cycloprop-1-enyl)-heptanoic acid methyl ester (293)[341]



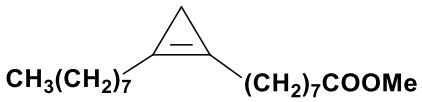
To a stirred solution of 8-(2-octylcycloprop-1-enyl) heptanoic acid **278** (0.3 g, 0.0010 mol) in ether (5 mL) an excess of diazomethane solution in ether (5 mL) was added dropwise, and reaction mixture was then stirred for 10–15 min. When TLC showed no starting material was left, the solution was evaporated to give a pure product, 7-(2-octyl-cycloprop-1-enyl)-heptanoic acid methyl ester **292** (0.25 g, 80%). This showed δ_H (400 MHz, $CDCl_3$): 3.68 (3H, s), 2.38 (4H, t, *J* 6.8 Hz), 2.32 (2H, t, *J* 7.6 Hz), 1.69 – 1.57 (2H, m), 1.59 – 1.49 (4H, m), 1.39 – 1.22 (14H, m), 0.89 (3H, t, *J* 6.9 Hz), 0.77 (2H, s); δ_C(101 MHz, $CDCl_3$): 174.3, 109.4, 109.2, 45.1, 32.7, 31.9, 29.4, 29.3, 29.3, 29.2, 28.9, 27.4, 27.3, 26.9, 26.1, 26.0, 22.7, 14.1, 7.4; ν_{max}: 2927, 2856, 1872, 1742, 1464 cm^{-1}. All data matched those in the literature.[346]

9-(2-Octyl-cycloprop-1-enyl)-nonanoic acid methyl ester (298)



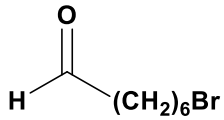
To a stirred solution of 8-(2-octylcycloprop-1-enyl)nonanoic acid **303** (0.11 g, 0.00035 mol) (5 mL) ether an excess of diazomethane solution in ether (3 mL) was added dropwise, and reaction mixture was then stirred for 10–15 min. When TLC showed no starting material was left, the solution was evaporated to give a pure product, 7-(2-octyl-cycloprop-1-enyl)-nonanoic acid methyl ester **298** (0.1 g, 83%) This showed δ_H (400 MHz, $CDCl_3$): 3.68 (3H, s), 2.38 (4H, t, *J* 7.1Hz), 2.32 (2H, t, *J* 7.6 Hz), 1.66 – 1.50 (6H, m), 1.39 – 1.22 (18H, m), 0.89 (3H, t, *J* 6.8 Hz), 0.78 (2H, s); δ_C(101 MHz, $CDCl_3$): 174.3, 109.4, 109.3, 51.4, 34.1, 31.9, 29.4, 29.3, 29.2, 29.1, 27.4, 27.3, 26.1, 26.0, 25.0, 22.7, 14.1, 7.4; ν_{max}: 2927, 2855, 1869, 1744, 1464 cm^{-1}. All data matched those in the literature.[346]

8-(2-Octylcycloprop-1-enyl)octanoic acid methyl aster (304)



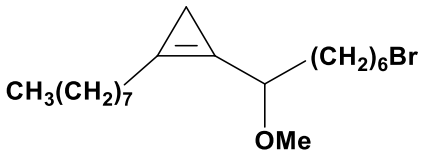
To a stirred solution of -8-(2-octyl-cycloprop-1-enyl)-octanoicacid **276** (0.4 g, 0.0013mol) in (5 mL) ether, an excess of diazomethane solution in ether (6 mL) was added dropwise, and the reaction mixture then stirred for 10–15 min. When TLC showed no starting material was remaining, the solution was evaporated to give an essentially pure product, 7-(2-octyl-cycloprop-1-enyl)-nonanoic acid methyl ester **304** (0.4 g, 85%). This showed δ_H(400 MHz, $CDCl_3$): 3.67 (3H, s), 2.38 (2H, t, *J* 7.1 Hz), 2.31 (2H, t, *J* 7.5 Hz), 1.69 – 1.49 (6H, m), 1.39 – 1.18 (18H, m), 0.89 (3H, t, *J* 6.6 Hz), 0.77 (2H, s); δ_C (101 MHz, $CDCl_3$): 174.3, 109.4, 109.3, 109.3, 51.4, 34.1, 31.9, 29.4, 29.3, 29.2, 29.1, 27.4, 27.3, 26.0, 26.0, 25.0, 22.7, 14.1, 7.4; ν_{max}: 2929, 2858, 1865, 1745, 1464cm^{-1}. All data matched those in the literature.[346]

7-Bromo-heptanal (307)



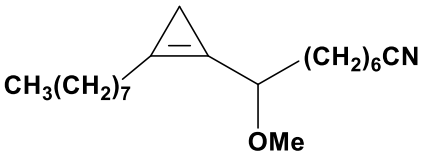
A solution of 7-bromo-heptan-1-ol **306** (3.25 g, 0.0167 mol) in dichloromethane (20 mL) was added dropwise to a stirred suspension of PCC (7.18 g, 0.0334 mol) in dichloromethane (100 mL). During the addition a black colour appeared and the reaction mixture was then stirred for 2 h after which time TLC showed that no starting material was left. Petrol/ethyl acetate (5:1) (70 mL) was added to the reaction mixture, forming a thick black precipitate, which was removed by filtration through a bed of silica gel/celite, and the solvent was removed by rotary evaporation. Column chromatography, eluting with petrol/ethyl acetate (5:1) gave 7-bromo-heptanal **307** (2.5g, 77%) as a colourless oil. This showed δ_H (400 MHz, $CDCl_3$): 9.78 (1H, t, *J* 1.7 Hz), 3.42 (2H, t, *J* 6.8 Hz), 2.46 (2H, td, *J* 1.7, 7.3 Hz), 1.88 (2H, p, *J* 7.2 Hz), 1.66 (2H, p, *J*, 7.3), 1.52 – 1.43 (2H, m), 1.41 – 1.31 (2H, m); δ_C (101 MHz, $CDCl_3$): 202.5, 43.7, 33.7, 32.5, 28.3, 27.9, 21.8; ν_{max}: 2935, 2858, 1725, 1461 cm^{-1}. All data matched those in the literature.[346]

1-(7-Bromo-1-methoxyheptyl)-2-octyl-cyclopropene (308)



n-Butyl lithium (1.6 M, 12 mL, 0.019 mol) was added dropwise to a stirred solution of 1,1,2-tribromo-2-octyl cyclopropane **293** (3.0 g, 0.0076 mol) in dry ether (50 mL) under N_2 at –78 °C. The mixture was allowed to reach room temperature, stirred for 30 min and then cooled to 0 °C, after which HMPA (2.72 g, 2.64 mL, 0.0150 mol) was added dropwise followed by 7-bromo-heptanal **307** (1.6 g, 0.0083 mol). The mixture was then stirred for 3 h and iodomethane (4.75 g, 10.8 mL, 0.0761 mol) was added. The mixture was stirred overnight at room temperature, cooled to 0 °C and water (70 mL) was added. The resulting solution was extracted with ether (2 × 100 mL) and the combined organic layers washed with water (2 × 20 mL), dried, filtered and evaporated. Column chromatography, eluting with petrol/ethyl acetate (5:1) gave (±)-1-(7-bromo-1-methoxy-heptyl)-2-octyl-cyclopropene **308** (1.3 g, 46%) as a colourless oil. This showed δ_H(400 MHz, $CDCl_3$): 4.19 (1H, t, *J* 6.3 Hz), 3.31 (3H, s), 3.18 (2H, t, *J* 7.0 Hz), 2.45 (2H, dt, *J* 0.6, 7.3 Hz), 1.81 (2H, m), 1.68 (2H, m), 1.57 (2H, p, *J* 7.3 Hz), 1.30 (16H, m), 0.94 (2H, d, *J* 8.5 Hz), 0.88 (3H, t, *J* 6.9 Hz); δ_C (101 MHz, $CDCl_3$): 114.1, 108.6, 76.3, 33.4, 33.3, 31.9, 30.4, 29.4, 29.3, 29.2, 28.5, 27.2, 26.6, 26.1, 25.1, 22.7, 14.1, 7.5, 7.0; ν_{max}: 2925, 2854, 1463cm^{-1}. All data matched those in the literature.[346]

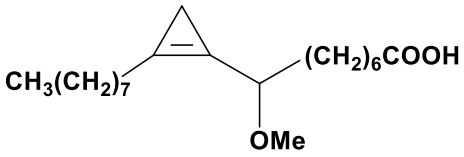
8-Methoxy-8-(2-octyl-cycloprop-1-enyl)-octanenitrile (309)



Sodium cyanide (0.40 g, 0.00810 mol) was added to a stirred solution of (±)-1-(7-bromo-1-methoxy-heptyl)-2-octyl-cyclopropene **308** (1.2 g, 0.0034 mol) in DMSO (40 mL). The mixture was heated to 60 °C for 3 h or until the TLC showed no starting material was left. Water (15 mL) was added to the cooled solution and the product was extracted with ether (2

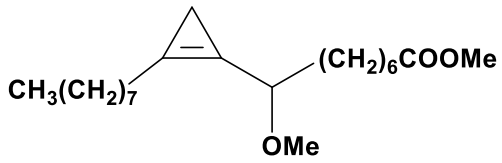
× 100 mL). The combined organic layers were washed with a saturated aqueous solution of NH_4Cl (30 mL), dried, filtered and evaporated. Column chromatography, eluting with petrol/ethyl acetate (5:2) gave 8-methoxy-8-(2-octyl-cycloprop-1-enyl)-octanenitrile **309** (0.46 g, 43%) as a yellow oil This showed δ_H(400 MHz, $CDCl_3$): 4.20 (1H, t, *J* 6.6 Hz), 3.32 (3H, s), 2.45 (2H, t, *J* 7.3 Hz), 2.33 (2H, t, *J* 7.1 Hz), 1.68 (4H, m), 1.58 (2H, m), 1.46 (2H, m), 1.30 (14H, m), 0.95 (2H, d, *J* 8.5 Hz), 0.89 (3H, t, *J* 6.8 Hz); δ_C (101 MHz, $CDCl_3$): 119.8, 114.2, 108.6, 76.3, 56.6, 39.4,33.3, 31.8, 29.4, 29.3, 28.7, 28.6, 27.2, 26.1, 25.3, 25.0, 22.7, 17.1, 14.1, 7.5; ν_{max}: 2925, 2854, 2246, 1865, 1465 cm^{-1}. All data matched those in the literature.[346]

8-Methoxy-8-(2-octyl-cycloprop-1-enyl)-octanoicacid (310)



Sodium hydroxide (0.35 g, 0.0087 mol) was dissolved in a mixture of ethanol (3.5 mL) and water (0.5 mL). 8-Methoxy-8-(2-octyl-cycloprop-1-enyl)-octanenitrile **309** (0.46 g, 0.00150 mol) was then added and the mixture heated under reflux under N_2 for 8 h. The ethanol was evaporated and to the residue (0.1 M TBAH solution 40 mL) was added. Dichloromethane (20mL) was then added and the mixture was stirred for 12 h. The solution was acidified to ~pH 3 with aqueous potassium hydrogen sulfate. The mixture was separated, and the organic solution was washed with water (3 × 30 mL) and the solution was dried, filtered and evaporated. Column chromatography, eluting with petrol/ethyl acetate (5:2) gave 8-methoxy-8-(2-octyl-cycloprop-1-enyl)-octanoic acid **310** (0.43 g, 93%). This showed δ_H (400 MHz, $CDCl_3$) 4.20 (2H, t, *J* 6.4 Hz), 3.32 (3H, s), 2.45 (2H, t, *J* 7.2 Hz), 2.36 (2H, t, *J* 7.5 Hz), 1.74 – 1.43 (6H, m), 1.42 – 1.18 (16H, m), 0.94 (2H, d, *J* 8.5 Hz), 0.89 (3H, t, *J* 6.9 Hz); δ_C (101 MHz, $CDCl_3$): 174.2, 108.6, 114.0, 55.6, 33.5, 33.3, 31.9, 29.4, 30.0, 29.2, 29.0, 27.2, 26.0, 25.8, 25.1, 24.6, 20.0, 14.0, 7.5; ν_{max}: 2927, 2855, 1863, 1743, 1464 cm^{-1}. All data matched those in the literature.[346]

8-Methoxy-8-(2-octyl-cycloprop-1-enyl)-octanoic acid methyl ester (305)



To a stirred solution of 8-methoxy-8-(2-octyl-cycloprop-1-enyl)-octanoicacid **310** (0.43 g, 0.00131 mol) and ether (5 mL) an excess of diazomethane solution in ether (5 mL) was added dropwise. The mixture was then stirred for 10-15 min, then TLC give a pure product, 7-(2-octyl-cycloprop-1-enyl)-nonanoic acid methyl ester **305** (0.4 g, 80%). This showed δ_H (400 MHz, $CDCl_3$):4.19 (1H, t, *J* 6.5 Hz), 3.66 (3H, s), 3.30 (3H, s), 2.44 (2H, t, *J* 7.25 Hz), 2.29 (2H, t, *J* 7.6 Hz), 1.61 (6H, m), 1.31 (16H, m), 0.93 (2H, d, *J* 8.5 Hz), 0.88 (3H, t, *J* 6.9 Hz); δ_C (101 MHz, $CDCl_3$): 174.2, 114.0, 108.6, 76.4, 56.5, 51.4, 34.0, 33.3, 31.9, 29.4, 29.3, 29.2, 29.1, 27.2, 26.1, 25.2, 24.9, 22.6, 14.1, 7.5; ν_{max}: 2927, 2855, 1863, 1743, 1464 cm^{-1}. All data matched those in the literature.[346]

References

1 V. Kumar, A. K. Abbas, N. Fausto and R. N. Mitchell, *Robbins Basic Pathology*, Saunders Elsevier, California, San Francisco, 8th edn., 2007.

2 B. M. Rothschild, L. D. Martin, G. Lev, H. Bercovier, G. K. Bar-Gal, C. Greenblatt, H. Donoghue, M. Spigelman and D. Brittain, *Clin. Infect. Dis.*, 2001, **33**, 305–311.

3 P. Kandola and L. S. Meena, *adv. Mater.Rev. 2014*, 2014, **1**, 13–19.

4 G. M. Harisinghani, T. C. McLoud, J. A. O. Shepard, J. P. Ko, M. M. Shroff and P. R. Mueller, *Radiographics*, 2000, **20**, 449–470.

5 J. M. Grange, *Mycobacteria and Human Disease*, Edward Arnold Ltd, London, 1988.

6 G. N. Meachen, *A Short History of Tuberculosis*, John Bale, Sons & Danielsson Ltd, London, 1936.

7 I. Hershkovitz, H. D. Donoghue, D. E. Minnikin, G. S. Besra, O. Y. C. Lee, A. M. Gernaey, E. Galili, V. Eshed, C. L. Greenblatt, E. Lemma, G. K. Bar-Gal and M. Spigelman, *PLoS One*, 2008, **3**, 1–6.

8 G. M Cristina, S. Brisse, R. Brosch, M. Fabre, B. Omaïs, M. Marmiesse, P. Supply and V. Vincent, *PLoS Pathog.*, 2005, **1**, 0055–0061.

9 A. J. E. Cave and A. Demonstrator, *British J. Tuberc.*, 1939, **33**, 142–152.

10 D. Brothwell and A. T. Sandison, *Diseases in Antiquity. A Survey of the Diseases, Injuries and Surgery of Early Populations*, Springfield, Ill: Charles C. Thomas, 1967.

11 L. Brown, *The Story of Clinical Pulmonary Tuberculosis*, The Radiological Society of North America, 1941.

12 T. M. Daniel, *Int. J. Tuberc. Lung Dis.*, 2000, **4**, 395–400.

13 C. A. Roberts and J. E. Buikstra, *The Bioarchaeology of Tuberculosis: A Global View on a Reemerging Disease*, University Press of Florida, 2003.

14 T. M. Daniel, *Respir. Med.*, 2006, **100**, 1862–1870.

15 K. Sakamoto, *Vet. Pathol.*, 2012, **49**, 423–439.

16 E. Grigg, *Am Rev Tuberc*, 1958, **78**, 583–603.

17 R. Koch, *Artic. Rev.*, 1982, **4**, 1270–1274.

18 I. E. Alcamo, *Fundamentals of Microbiology*, Benjamin/Cummings Pub. Co., University of Michigan, 4th edn., 1994.

19 P. R. Donald and P. D. van Helden, *N. Engl. J. Med.*, 2009, **360**, 2393–2395.

20 N. Nair and S. Sahu, *World Health*, 2010, **88**, 9–10.

21 D. S. Burke, *Vaccine*, 1993, **11**, 795–804.

22 M. D. A. Calmette, *Br. J. Tuberc.*, 1928, **22**, 161–165.

23 C. Bonah, *The 'Experimental Stable' of the BCG Vaccine: Safety, efficacy, proof, and standards, 1921-1933*, 2005, vol. 36.

24 G. W. Comstock, *Clin. Infect. Dis.*, 1994, **19**, 528–540.

25 M. Kwiatkowska and N. Ayas, *Thorax*, 2010, **65**, 1035–1036.

26 G. W. Comstock, R. S. Howard, L. H. Moulton, E. R. Rhoades, L. H. Harrison and A. C. A. Ue, *J. Am. Med. Assoc.*, 2004, **291**, 2086.

27 C. F. von Reyn and A. I. Zumla, *Brit. Med. J.*, 2008, **337**, 257–264.

28 C. F. von Reyn and J. M. Vuola, *Clin. Infect. Dis.*, 2002, **35**, 465–474.

29 G. P. Youmans, W. R. Barclay, R. S. Berghoff, R. G. Bloch, H. W. Bosworth, H. N. Bundesen, W. C. Davison, H. F. Dowling, R. H. Ebert, H. C. Hinshaw, E. E. Irons, A. C. Ivy, M. R. Lichtenstein, E. T. McEnery, K. A. Meyer, K. F. Meyer, R. S. Mitchell, J. E. Perkins, K. H. Pfuetze, H. M. Riggins, S. R. Rosenthal, H. J. Shaughnessy, S. J. Shipman, D. T. Smith, D. T. Smith and W. H. Theobald, *JAMA*, 1957, **164**, 951–954.

30 A. A. Husain, R. S. Kashyap, D. R. Kalorey, S. R. Warke, H. J. Purohit, G. M. Taori and H. F. Daginawala, *Indian J. Exp. Biol.*, 2011, **49**, 7–10.

31 C. Grüber, M. Kulig, R. Bergmann, I. Guggenmoos-Holzmann, *Pediatrics*, 2001, **107**, 3–9.

32 R. J. Sylvester, A. P. M. van der Meijden. and D. L. Lamm, *J. Urol.*, 2002, **168**, 1964–1970.

33 I. Olivier and D. T. Loots, *J. Microbiol. Methods*, 2012, **88**, 419–426.

34 A. Kochi, *Tuberculosis as a global emergency*, 1994, **vol**. 71.

35 WHO, *Global Tuberculosis Control*, 2009.

36 WHO, *Global Tuberculosis Control*, 2010.

37 WHO, *Global Tuberculosis Report*, 2016.

38 WHO, *Global Tuberculosis Report*, 2017.

39 A. Zumla, P. Mwaba, J. Huggett, N. Kapata, D. Chanda and J. Grange, *Lancet Infect. Dis.*, 2009, **9**, 197–202.

40 K. R. Uma Devi, B. Ramalingam and A. Raja, *Diagn. Microbiol. Infect. Dis.*, 2003, **46**, 205–209.

41 WHO, *Global Tuberculosis Report*, 2013.

42 WHO, *Global Tuberculosis Control*, 2006.

43 L. Aaron, D. Saadoun, I. Calatroni, O. Launay, N. Memain, V. Vincent, G. Marchal, B. Dupont, O. Bouchaud, D. Valeyre and O. Lortholary, *Eur. Soc. Clin. Microbiol. Infect. Dis.*, 2004, **10**, 388–98.

44 R. Colebunders and M. L. Lambert, *Brit. Med. J.*, 2002, **324**, 802–803.

45 R. E. Chaisson and N. A. Martinson, *N. Engl. J. Med.*, 2008, **358**, 1089–1092.

46 W. J. Burman and B. E. Jones, *Am. J. Respir. Crit. Care Med.*, 2001, **164**, 7–12.

47 R. P. Tripathi, N. Tewari, N. Dwivedi and V. K. Tiwari, *Med. Res. Rev.*, 2005, **25**, 93–131.

48 J. M. Grange, *Mycobacteria and Human Disease*, Hodder Arnold, London-United Kingdom, 2nd edn., 1996.

49 Y. L. Janin, *Bioorg. Med. Chem.*, 2007, **15**, 2479–2513.

50 G. G. Gallo, E. Martinelli, V. Pagani and P. Sensi, *Tetrahedron*, 1974, **30**, 3093–3097.

51 D. A. Mitchison, *Int. J. Tuberc. Lung Dis.*, 2000, **4**, 796–806.

52 H. Rée, *Treatment of tuberculosis: Guidelines for national programmes*, 2003, vol. 93.

53 M. D. Iseman, *Eur. Respir. J.*, 2002, **20**, 87s–94s.

54 F. Ryan, *Tuberculosis: the greatest story never told: the human story of the search for the cure for tuberculosis and the new global threat*, Swift Publishers, Bromsgrove, Worcs., UK, 1992.

55 B. P. Vareldzis, J. Grosset, I. de Kantor, J. Crofton, A. Laszlo and M. Felten, *Tuber. Lung Dis.*, 1994, **75**, 1–7.

56 M. A. Espinal, *Tuberculosis*, 2003, **83**, 44–51.

57 T. Shimao, *Tubercle*, 1987, **68**, 5–15.

58 C. Dye, M. A. Espinal, C. J. Watt, C. Mbiaga and B. G. Williams, *J. Infect. Dis.*, 2002, **185**, 1197–1202.

59 WHO, *WHO Global Task Force outlines measures to combat XDR-TB worldwide*, Geneva, 2006.

60 *Centers for Disease Control. 'Emergence of Mycobacterium tuberculosis with extensive resistance to second-line drug–Worldwide, 2000–2004'. MMWR Weekly*, 2006, **55**, 301–5.

61 V. M. Katoch, *Indian J. Med. Res.*, 2004, **120**, 290–304.

62 H. C. King, T. Khera-Butler, P. James, B. B. Oakley, G. Erenso, A. Aseffa, R. Knight, E. M. Wellington and O. Courtenay, *PLoS One*, 2017, **12**, 1–15.

63 R. Brosch, S. V. Gordon, M. Marmiesse, P. Brodin, C. Buchrieser, K. Eiglmeier, T. Garnier, C. Gutierrez, G. Hewinson, K. Kremer, L. M. Parsons, A. S. Pym, S. Samper, D. van Soolingen and S. T. Cole, *Proc. Natl. Acad. Sci.*, 2002, **99**, 3684–3689.

64 S. T. Cole, R. Brosch, J. Parkhill, T. Garnier, C. Churcher, D. Harris, S. V. Gordon, K. Eiglmeier, S. Gas, C. E. Barry, F. Tekaia, K. Badcock, D. Basham, D. Brown, T. Chillingworth, R. Connor, R. Davies, K. Devlin, T. Feltwell, S. Gentles, N. Hamlin, S. Holroyd, T. Hornsby, K. Jagels, A. Krogh, J. McLean, S. Moule, L. Murphy, K. Oliver, J. Osborne, M. A. Quail, M. A. Rajandream, J. Rogers, S. Rutter, K. Seeger, J. Skelton, R. Squares, S. Squares, J. E. Sulston, K. Taylor, S. Whitehead and B. G. Barrell, *Nature*, 1998, **393**, 537–544.

65 Epicentre, Rigged model of *Mycobacterium tuberculosis*, https://www.turbosquid.com/3d-models/3d-mycobacterium-tuberculosis-model/683105, (accessed 16 July 2017).

66 B. A. Forbes, D. F. Sahm and A. S. Weissfeld, *Baily & Scott's Diagnostic Microbiology*, Houston, United States., 12th edn., 2007.

67 P. J. Brennan and H. Nikaido, *Annu. Rev. Biochem.*, 1995, **64**, 29–63.

68 J. H. Patterson, R. F. Waller, D. Jeevarajah, H. Billman-Jacobe and M. J. McConville, *Biochem. J.*, 2003, **372**, 77–86.

69 J. M. Grange, M. D. Yates and I. N. de Kantor, *Guidelines for speciation within the Mycobacterium tuberculosis complex*, 1996, vol. 4.

70 L. M. O'Reilly and C. J. Daborn, *Tuber. Lung Dis.*, 1995, **76 suppl 1**, 1–46.

71 V. B. D. Skerman, V. McGowan and P. H. A. Sneath, *Int. J. Syst. Bacteriol.*, 1980, **30**, 225–420.

72 M. Castets, N. Rist and H. Boisvert, *Med. Afr. Noire*, 1969, **16**, 321–322.

73 F. Portaels, J. Aguiar, M. Debacker, a Guédénon and W. M. Meyers, *Infect Immun*, 2004, **72**, 62–65.

74 M. S. Setia, C. Steinmaus, C. S. Ho and G. W. Rutherford, *Lancet Infect. Dis.*, 2006, **6**, 162–170.

75 D. van Soolingen, A. G. M. van der Zanden, P. E. W. de Haas, G. T. Noordhoek, A. Kiers, N. A. Foudraine, F. Portaels, A. H. J. Kolk, K. Kremer and J. D. A. van Embden, *J. Clin. Microbiol.*, 1998, **36**, 1840–1845.

76 J. D. Aronson, *J. Infect. Dis.*, 1926, **39**, 314–320.

77 C. Le Dantec, J. Duguet, A. Montiel, N. Dumoutier, S. Dubrou and V. Vincent, *Appl. Environ. Microbiol.*, 2002, **68**, 1025–1032.

78 D. E. Griffith, T. Aksamit, B. A. Brown-Elliott, A. Catanzaro, C. Daley, F. Gordin, S. M. Holland, R. Horsburgh, G. Huitt, M. F. Iademarco, M. Iseman, K. Olivier, S. Ruoss, C. F. Von Reyn, R. J. Wallace and K. Winthrop, *Am. J. Respir. Crit. Care Med.*, 2007, **175**, 367–416.

79 A. I. Moore JE, Kruijshaar ME, Ormerod LP, Drobniewski F, *BMC Public Health*, 2010, **10**, 612.

80 M. Al Houqani, F. Jamieson, P. Chedore, M. Mehta, K. May and T. K. Marras, *Can. Respir. J.*, 2011, **18**, 19–24.

81 W.-J. Koh, O. J. Kwon and K. S. Lee, *J. Korean Med. Sci.*, 2005, **20**, 913–925.

82 J. Maugein, M. Dailloux, B. Carbonnelle, V. Vincent, J. Grosset, M. L. Abalain, R. Bauriaud, P. Bemer, A. Bourgoin, L. Brasme, E. Cambau, A. Carricajo, C. Carriere, B. Cattier, S. Coignard, M. Chomarat, G. Couetdic, M. F. David, L. Deforges, F. Doucet-Populaire, J. M. Duez, M. C. Gutierrez, J. L. Herrmann, V. Jarlier, F. Jaureguy, V. Lalande, L. Landraud, L. Lebrun, N. Lemaitre, C. Lorain, B. Malbruny, C. Martin, C. Offredo, M. Pestet-Caron, G. Richalet, J. Robert, J. P. Romaszko, E. Ronco, A. Rossier, D. Sicard, C. Trufot-Pernot, A. Vachée and V. Vincent, *Eur. Respir. J.*, 2005, **26**, 1092–1096.

83 M. Luquin, V. Ausina, V. Vincent-Levy-Frebault, M. A. Laneelle, F. Belda, M. Garcia-Barcelo, G. Prats and M. Daffe, *Int. J. Syst. Bacteriol.*, 1993, **43**, 405–413.

84 M. S. Glickman, J. S. Cox and W. R. Jacobs, *Mol. Cell*, 2000, **5**, 717–727.

85 C. Brambilla, A. Sánchez-Chardi, M. Pérez-Trujillo, E. Julián and M. Luquin, *Microbiol.*, 2012, **158**, 1615–1621.

86 Z. S. Yuksel, E. Buber, T. Kocagoz, A. Alp, Z. Saribas and N. L. Acan, *J. Mol. Microbiol. Biotechnol.*, 2011, **20**, 24–28.

87 E. Noguera-Ortega, S. Secanella-Fandos, H. Eraña, J. Gasión, R. M. Rabanal, M. Luquin, E. Torrents and E. Julián, *Eur. Urol. Focus*, 2016, **2**, 67–76.

88 E. Noguera-Ortega, N. Blanco-Cabra, R. M. Rabanal, A. Sánchez-Chardi, M. Roldán, S. Guallar-Garrido, E. Torrents, M. Luquin and E. Julián, *Sci. Rep.*, 2016, **6**, 1–12.

89 C. Linares, A. Bernabéu, M. Luquin and P. L. Valero-Guillén, *Microbiology*, 2012, **158**, 2878–2885.

90 R. E. Gordon and M. M. Smith, *J. Bacteriol.*, 1953, **66**, 41–48.

91 R. J. J. Wallace, D. R. Nash, M. Tsukamura, Z. M. Blacklock and V. A. Silcox, *J. Infect. Dis.*, 1988, **158**, 52–59.

92 B. A. Brown-Elliott and J. Richard J. Wallace, *Clin. Microbiol. Rev.*, 2002, **15**, 716–746.

93 S. H. Abidi, K. Ahmed, S. K. Sherwani, N. Bibi and S. U.Kazmi, *Int. J. Curr. Microbiol. Appl. Sci.*, 2014, **3**, 801–812.

94 D. E. Dykhuizen and G. Baranton, *Trends Microbiol.*, 2001, **9**, 472–473.

95 J. S. Tyagi and D. Sharma, *Trends Microbiol.*, 2002, **10**, 68–69.

96 https://www.google.co.uk/search *M. smegmatis*, (accessed 19 October 2017).

97 C. Foti, V. Sforza, C. Rizzo, G. De Pascale, D. Bonamonte, A. Conserva, A. Tarantino, C. Stella, S. Cantore, R. Grassi, A. Ballini, D. De Vito and G. Angelini, *Cases J.*, 2009, **2**, 6828.

98 M. L. Bagarazzi and David B. Weiner, *Clin. Infect. Dis.*, 1996, **22**, 1124–1125.

99 F. G. Weinberger, M., Berg, S. L., Feuerstein, I. M., Pizzo, P. A. and Witebsky, *Clin. Infect. Dis.*, 1992, **14**, 1229–39.

100 L. N. Jarikre, *Genitourin. Med.*, 1992, **68**, 45–46.

101 C. P. Busillo, K. D. Lessnau, V. Sanjana, S. Soumakis, M. Davidson, M. P. Mullen and W. Talavera, *Chest*, 1992, **102**, 797–801.

102 E. Bonnet, P. Massip, R. Bauriaud, L. Alric and J.-C. Auvergnat, *Clin. Infect. Dis.*, 1996, **23**, 644–645.

103 T. W. Barber, D. E. Craven and H. W. Farber, *Chest*, 1991, **100**, 716–720.

104 C. G. Ingram CW, Tanner DC, Durack DT, Kernodle GW Jr, *Clin Infect Dis*, 1993, **16**, 463.

105 D. E. Zoutman and L. Sigler, *J. Clin. Microbiol.*, 1991, **29**, 1855–1859.

106 http://www.snipview.com/q/Mycobacterium%20gordonae,(accessed 12 October 2017).

107 C. O. Thoen, A. G. Karlson and Elmer M. Himes, *Rev. Infect. Dis.*, 1981, **3**, 960–972.

108 https://commons.wikimedia.org/wiki/File:*Mycobacterium_avium-intracellulare*,(accessed 3 November 2017).

109 E. Tortoli, *Clin. Microbiol. Rev.*, 2003, **16**, 319–354.

110 C. Y. Turenne, R. Wallace and M. A. Behr, *Clin. Microbiol. Rev.*, 2007, **20**, 205–229.

111 L. G. Wayne and H. A. Sramek, *Clin. Microbiol. Rev.*, 1992, **5**, 1–25.

112 M. F. Thorel, M. Krichevsky and V. Vincent Levy-Frebault, *Int. J. Syst. Bacteriol.*, 1990, **40**, 254–260.

113 R. W. Brooks, B. C. Parker, H. Gruft and J. O. Falkinham, *Am. Rev. Respir. Dis.*, 1984, **130**, 630–633.

114 J. O. Falkinham, C. D. Norton and W. Mark, *Appl. Environ. Microbiol.*, 2001, **67**, 1225–1231.

115 R. J. Arsenault, Y. Li, K. Bell, K. Doig, A. Potter, P. J. Griebel, A. Kusalik and S. Napper, *Infect. Immun.*, 2012, **80**, 3039–3048.

116 S. Masala, D. Cossu, A. Pacifico, P. Molicotti and L. A. Sechi, *Gut Pathog.*, 2012, **4**, 4–7.

117 Y. Johnson-Ifearulundu, J. Kaneene and J. LIoyd, *J. Am. Vet. Med. Assoc.*, 1999, **214**, 822–825.

118 C. R. Horsburgh and R. M. Selik, *Am. Rev. Respir. Dis.*, 1989, **139**, 4–7.

119 D. E. Griffith, *Clin. Chest Med.*, 2002, **23**, 613–621.

120 J. E. Steadham, *J. Clin. Microbiol.*, 1980, **11**, 496–498.

121 H. W. Engel, L. G. Berwald and A. H. Havelaar, *Tubercle*, 1980, **61**, 21–26.

122 W. R. Waters, M. V. Palmer, T. C. Thacker, J. B. Payeur, N. B. Harris, F. C. Minion, R. Greenwald, J. Esfandiari, P. Andersen, J. McNair, J. M. Pollock and K. P. Lyashchenko, *Clin. Vaccine Immunol.*, 2006, **13**, 611–619.

123 T. Parish and N. G. Stoker, *Mol. Biotechnol.*, 1999, **13**, 191–200.

124 N. L. Rose, G. C. Completo, S. J. Lin, M. McNeil, M. M. Palcic and T. L. Lowary, *J. Am. Chem. Soc.*, 2006, **128**, 6721–6729.

125 C. Ratledge and J. L. Stanford, *The biology of the mycobacteria*, Academic Press, 1982.

126 A. Ortalo-Magne, M. A. Dupont, A. Lemassu, A. B. Andersen, P. Gounon and M. Daffe, *Microbiology*, 1995, **141**, 1609–1620.

127 E. Lederer, *Pure Appl. Chem.*, 1971, **25**, 135–165.

128 D. E. Minnikin, *Lipids:Complex lipids, their chemistry, biosynthesis and roles. In: The Biology of the Mycobacteria*, Academic Press, London-United Kingdom, 1982.

129 D. Chatterjee and K. H. Khoo, *Glycobiology*, 1998, **8**, 113–120.

130 P. E. Kolattukudy, N. D. Fernandes, K. Azad, M. Fitzmaurice and T. D. Sirakova, *Mol. Microbiol.*, 1997, **24**, 263–270.

131 H. Noll, H. Bloch, J. Asselineau and E. Lederer, *Biochim. Biophys. Acta*, 1956, **20**, 299–309.

132 D. E. Minnikin, L. Kremer, L. G. Dover and G. S. Besra, *Chem. Biol.*, 2002, **9**, 545–553.

133 R. Almog and C. A. Mannella, *Biophys. J.*, 1996, **71**, 3311–3319.

134 K. Takayama and E. L. Armstrong, *Biochemistry*, 1976, **15**, 441–447.

135 J. T. Belisle, V. D. Vissa, T. Sievert, K. Takayama, P. J. Brennan and G. S. Besra, *Science (80-.).*, 1997, **276**, 1420–1422.

136 B. G. Schroeder and C. E. Barry, *Bioorg. Chem.*, 2001, **29**, 164–177.

137 F. H. Stadolah, A. Lesuk and R. J. Anderson, *J. Biol. Chem.*, 1938, **126**, 505–513.

138 R. J. Anderson, *Chem. Rev.*, 1941, **29**, 225–243.

139 R. J. Anderson, *J. Biol. Chem.*, 1927, **74**, 525–535.

140 J. Asselineau, *Nature*, 1950, **166 (4227)**, 782–783.

141 D. E. Minnikin and N. Polgar, *Chem. Commun.*, 1967, 312–314.

142 D. E. Minnikin and N. Polgar, *Tetrahedron Lett.*, 1966, **7**, 2643–2647.

143 D. E. Minnikin and N. Polgar, *Chem. Commun.*, 1967, **18**, 916–918.

144 D. E. Minnikin and N. Polgar, *Chem. Commun.*, 1967, 1172–1174.

145 L. A. Davidson, P. Draper and D. E. Minnikin, *J. Gen. Microbiol.*, 1982, **128**, 823–828.

146 D. E. Minnikin and N. Polgar, *Chem. Commun.*, 1966, **18**, 648–649.

147 C. E. Barry, R. E. Lee, K. Mdluli, A. E. Sampson, B. G. Schroeder, R. A. Slayden and Y. Yuan, *Prog. Lipid Res.*, 1998, **37**, 143–179.

148 M. Quémard, A., Lanéelle, M., Marrakchi, H., Promé, D., Dubnau, E. and Daffé, *Eur. J. Biochem.*, 1997, **250**, 758–763.

149 A. Q. E. Dubnau, M. Lanéelle, S. Soares, A. Benichou, T. Vaz, D. Promé, J. Promé, M. Daffé, *Mol. Microbiol.*, 1997, **23**, 313–322.

150 R. Toubiana, J. Berlan, H. Sato and M. Strain, *J. Bacteriol.*, 1979, **139**, 205–11.

151 M. Watanabe, Y. Aoyagi, M. Ridell and D. E. Minnikin, *Microbiology*, 2001, **147**, 1825–1837.

152 M. Watanabe, Y. Aoyagi, H. Mitome, T. Fujita, H. Naoki, M. Ridell and D. E. Minnikin, *Microbiology*, 2002, **148**, 1881–1902.

153 P. J. Brennan and H. Nikaido, *Annu. Rev. Biochem.*, 1995, **64**, 29–63.

154 P. R. Wheeler, G. S. Besra, D. E. Minnikin and C. Ratledge, *Biochim. Biophys. Acta.*, 1993, **1167**, 182–188.

155 D. E. Minnikin, S. M. Minnikin and M. Goodfellow, *Biochim. Biophys. Acta/Lipids Lipid Metab.*, 1982, **712**, 616–620.

156 M. Luquin, J. Roussel, F. Lopez-Calahorra, G. Lanéelle, V. Ausina and M. Lanéelle, *Eur. J. Biochem.*, 1990, **192**, 753–759.

157 M. A. Lanéelle and G. Lanéelle, *Eur. J. Biochem*, 1970, **12**, 296–300.

158 B. Heyn and S. T. Cole, *Res. Microbiol.*, 1992, **143**, 721–730.

159 S. Toriyama, S. Imaizumi, I. Tomiyasu, M. Masui and I. Yano, *Biochim. Biophys. Acta*, 1982, **712**, 427–429.

160 G. Fráter, *Tetrahedron Lett.*, 1981, **22**, 425–428.

161 S. J. Danielson and G. R. Gray, *J. Biol. Chem.*, 1982, **257**, 12196–12203.

162 Y. Yuan, D. C. Crane, J. M. Musser, S. Sreevatsan and C. E. Barry, *J. Biol. Chem.*, 1997, **272**, 10041–10049.

163 K. Kaneda, S. Naito, S. Imaizumi, I. Yano, S. Mizuno, I. Tomiyasu, B. Tsuneko, E. Kusunose and M. Kusonose, *J. Clin. Microbiol.*, 1986, **24**, 1060–1070.

164 K. Kaneda, S. Imaizumi and I. Yano, *Microbiol. Immunol.*, 1995, **39**, 563–570.

165 G. Toschi, PhD Thesis, Bangor University, 2005.

166 F. Laval, M. A. Lanéelle, C. Déon, B. Monsarrat and M. Daffé, *Anal. Chem.*, 2001, **73**, 4537–4544.

167 C. Asselineau, J. Asselineau, G. Lanéelle and M. A. Lanéelle, *Prog. Lipid Res.*, 2002, **41**, 501–523.

168 E. Woermann and A. Muan, *J. Inorg. Nucl. Chem.*, 1970, **32**, 1455–1459.

169 C. Asselineau and J. Asselineau, *Bull. Soc. Chim. Fr.*, 1966, 1992–1999.

170 J. F. Tocanne and C. Asselineau, *Bull. Soc. Chim. Fr.*, 1968, 4519–4525.

171 M. Villeneuve, M. Kawai, M. Watanabe, Y. H. Y. Aoyagi, K. Takeya, H. Gouda, S. Hirono, D. E. Minnikin and H. Nakahara, *Biochim. Biophys. Acta*, 2007, **1768**, 1717–1726.

172 M. Villeneuve, M. Kawai, H. Kanashima, M. Watanabe, D. E. Minnikin and H. Nakahara, *Biochim. Biophys. Acta - Biomembr.*, 2005, **1715**, 71–80.

173 H. Durand, M. Gillois, J. F. Tocanne and G. Lanéelle, *Eur. J. Biochem.*, 1979, **94**, 109–118.

174 H. Durand, M. Welby, G. Lanéelle and J. F. Tocanne, *Eur. J. Biochem.*, 1979, **93**, 103–112.

175 D. B. Moody, T. Ulrichs, W. Mühlecker, D. C. Young, S. S. Gurcha, E. Grant, J. P. Rosat, M. B. Brenner, C. E. Costello, G. S. Besra and S. A. Porcelli, *Nature*, 2000, **404**, 884–888.

176 R. Watanabe, Y. C. Yoo, K. Hata, M. Mitobe, Y. Koike, M. Nishizawa, D. M. Garcia, Y. Nobuchi, H. Imagawa, H. Yamada and I. Azuma, *Vaccine*, 1999, **17**, 1484–1492.

177 M. Daffé, M. A. Lanéelle and C. Lacave, *Res. Microbiol.*, 1991, **142**, 397–403.

178 J. Asselineau and G. Lanéelle, *Front. Biosci.*, 1998, **3**, e164–e174.

179 A. H. Etemadi and J. Gasche, *Bull. Soc. Chim. Biol.*, 1965, **47**, 2095–2104.

180 C. Lacave, C., Laneelle, M. A., Daffé, M., Montrozier, H., Rols, M. P. and Asselineau, *Eur. J. Biochem.*, 1987, **163**, 369–378.

181 D. Z. Al Kremawi, J. R. Al Dulayymi and M. S. Baird, *Tetrahedron Lett.*, 2010, **51**, 1698–1701.

182 A. Quemard, C. Lacave and G. Laneelle, *Antimicrob. Agents Chemother.*, 1991, **35**, 1035–1039.

183 K. M. George, Y. Yuan, D. R. Sherman and C. E. Barry, *J. Biol. Chem.*, 1995, **270**, 27292–27298.

184 K. Takayama, C. Wang and G. S. Besra, *Society*, 2005, **18**, 81–101.

185 C. Vilchèze, H. R. Morbidoni, T. R. Weisbrod, H. Iwamoto, M. Kuo, J. C. Sacchettini and W. R. Jacobs, *J. Bacteriol.*, 2000, **182**, 4059–4067.

186 K. Mdluli, J. Swanson, E. Fischer, R. E. Lee and C. E. Barry, *Mol. Microbiol.*, 1998, **27**, 1223–1233.

187 S. J. Senior, P. A. Illarionov, S. S. Gurcha, I. B. Campbell, M. L. Schaeffer, D. E. Minnikin and G. S. Besra, *Bioorganic Med. Chem. Lett.*, 2003, **13**, 3685–3688.

188 Y. Yuan and C. E. Barry, *Proc. Natl. Acad. Sci. U. S. A.*, 1996, **93**, 21233–21282.

189 C. Don Lawson, M. Maza-Iglesias, M. Sirhan, J. Al Dulayymi and M. Baird, *SynOpen*, 2017, **1**, 0103–0116.

190 M. S. Glickman, S. M. Cahill and W. R. Jacobs, *J. Biol. Chem.*, 2001, **276**, 2228–2233.

191 D. E. Minnikin, O. Y. C. Lee, H. H. T. Wu, V. Nataraj, H. D. Donoghue, M. Ridell, M. Watanabe, L. Alderwick, A. Bhatt and G. S. Besra, in *Pathophysiological Implications of Cell Envelope Structure in Mycobacterium tuberculosis and Related Taxa. In Tuberculosis - Expanding Knowledge*, Ribon, W., Croatia, 2015, pp. 145–175.

192 G. S. Besra and L. Kremer, *Expert Opin. Investig. Drugs*, 2002, **11**, 153–157.

193 E. Lederer, V. Portelance and K. Serck-Hanssen, *Bull. Soc. Chim. Fr.*, 1952, 413–417.

194 J. Pudles and E. Lederer, *Biochim. Biophys. Acta.*, 1953, **11**, 602–603.

195 W. J. Gensler, I. Alam, R. S. Prasad, A. I. Radhakrishna and A. P. Chaudhuri, *Tetrahedron*, 1979, **35**, 2595–2600.

196 Y. Kitano, Y. Kobayashi and F. Sato, *J. Chem. Soc. Chem. Commun.*, 1985, 498–499.

197 Y. Kobayashi, Y. Kitano and F. Sato, *Tetrahedron*, 1986, **42**, 2937–2943.

198 M. Utaka, H. Higashi and A. Takeda, *J. Chem. Soc. Chem. Commun.*, 1987, **4**, 1368–1369.

199 M. Utaka, H. Watabu, H. Higashi, T. Sakai, S. Tsuboi and S. Torii, *J. Org. Chem.*, 1990, **55**, 3917–3921.

200 G. Fráter, U. Müller and W. Günther, *Tetrahedron*, 1984, **40**, 1269–1277.

201 J. R. Al Dulayymi, M. S. Baird and E. Roberts, *Tetrahedron*, 2005, **61**, 11939–11951.

202 G. Toschi and M. S. Baird, *Tetrahedron*, 2006, **62**, 3221–3227.

203 G. Koza, PhD Thesis, Bangor University, 2007.

204 K. Mori, Y. Shikichi, S. Shankar and J. Y. Yew, *Tetrahedron*, 2010, **66**, 7161–7168.

205 G. Koza, C. Theunissen, J. R. Al Dulayymi and M. S. Baird, *Tetrahedron*, 2009, **65**, 10214–10229.

206 A. A. Khan, S. H. Chee, B. L. Stocker and M. S. M. Timmer, *European J. Org. Chem.*, 2012, 995–1002.

207 S. Saito, T. Ishikawa, A. Kuroda, K. Koga and T. Moriwake, *Tetrahedron*, 1992, **48**, 4067–4086.

208 W. J. Gensler, J. P. Marshall, J. J. Langone and J. C. Chen, *J. Org. Chem.*, 1977, **42**, 118–125.

209 S. Winstein and J. Sonnenberg, *J. Am. Chem. Soc.*, 1961, **83**, 3235–3244.

210 G. R. Pettit and E. E. Van Tamelen, *Org. Synth.*, 1962, **12**, 356.

211 J. R. Al Dulayymi, M. S. Baird and E. Roberts, *Tetrahedron Lett.*, 2000, **41**, 7107–7110.

212 J. R. Al Dulayymi, M. S. Baird, E. Roberts, M. Deysel and J. Verschoor, *Tetrahedron*, 2007, **63**, 2571–2592.

213 G. Koza and M. S. Baird, *Tetrahedron Lett.*, 2007, **48**, 2165–2169.

214 M. Muzael, G. Koza, J. J. Al Dulayymi and M. S. Baird, *Chem. Phys. Lipids*, 2010, **163**, 678–684.

215 R. Ryll, Y. Kumazawa and I. Yano, *Microbiol. Immunol.*, 2001, **45**, 801–811.

216 C. S. Andersen, E. M. Agger, I. Rosenkrands, J. M. Gomes, V. Bhowruth, K. J. C. Gibson, R. V. Petersen, D. E. Minnikin, G. S. Besra and P. Andersen, *J. Immunol.*, 2009, **182**, 424–432.

217 R. Toubiana, J. Berlan, H. Sato and M. Strain, *J. Bacteriol.*, 1979, **139**, 205–211.

218 H. Bloch, *J. Exp. Med.*, 1952, **97**, 1–16.

219 M. B. Goren, *Bacteriol. Rev.*, 1972, **36**, 33–64.

220 A. D. Elbein, Y. T. Pan, I. Pastuszak and D. Carroll, *Glycobiology*, 2003, **13**, 17–27.

221 J. Müller, T. Boller and A. Wiemken, *Plant Sci.*, 1995, **112**, 1–9.

222 P. J. Woodruff, B. L. Carlson, B. Siridechadilok, M. R. Pratt, R. H. Senaratne, J. D. Mougous, L. W. Riley, S. J. Williams and C. R. Bertozzi, *J. Biol. Chem.*, 2004, **279**, 28835–28843.

223 E. Julián, M. Roldán, A. Sánchez-Chardi, O. Astola, G. Agustí and M. Luquin, *J. Bacteriol.*, 2010, **192**, 1751–1760.

224 G. Middlebrook, R. J. Dubos and C. Pierce, *J. Exp. Med.*, 1947, **86**, 175–184.

225 H. Bloch, *J. Exp. Med.*, 1950, **91**, 197–218.

226 M. Senn, T. Ioneda, J. Pudles and E. Lederer, *Eur. J. Biochem.*, 1967, **1**, 353–356.

227 T. Ioneda, M. Lenz and J. Pudles, *Biochem. Biophys. Res. Commun.*, 1963, **13**, 110–114.

228 E. Vilkas, A. Adam and M. Senn, *Chem. Phys. Lipids*, 1968, **2**, 11–16.

229 J. C. Promé, C. Lacave, A. Ahlbo-Coffy and A. Savagnac, *Eur. J. Biochem.*, 1976, **63**, 543–552.

230 K. Takayama and E. L. Armstrong, *J. Bacteriol.*, 1977, **130**, 569–571.

231 Y. Fujita, T. Naka, T. Doi and I. Yano, *Microbiology*, 2005, **151**, 1443–1452.

232 J. R. Al Dulayymi, M. S. Baird, M. Maza-Iglesias, S. Vander Beken and J. Grooten, *Tetrahedron Lett.*, 2009, **50**, 3702–3705.

233 E. Layre, A. Collmann, M. Bastian, S. Mariotti, J. Czaplicki, J. Prandi, L. Mori, S. Stenger, G. De Libero, G. Puzo and M. Gilleron, *Chem. Biol.*, 2009, **16**, 82–92.

234 C. A. S. Andersen, I. Rosenkrands, A. W. Olsen, P. Nordly, D. Christensen, R. Lang, C. Kirschning, J. M. Gomes, V. Bhowruth, D. E. Minnikin, G. S. Besra, F. Follmann, P. Andersen and E. M. Agger, *J. Immunol.*, 2009, **183**, 2294–2302.

235 Y. Hattori, I. Matsunaga, T. Komori, T. Urakawa, T. Nakamura, N. Fujiwara, K. Hiromatsu, H. Harashima and M. Sugita, *Biochem. Biophys. Res. Commun.*, 2011, **409**, 304–307.

236 H. Yu, J. Zhou, H. Takahashi, W. Yao, Y. Suzuki, X. Yuan, S. H. Yoshimura, Y. Zhang, Y. Liu, N. Emmett, V. Bond, D. Wang, X. Ding, K. Takeyasu and X. Yao, *J. Biol. Chem.*, 2014, **289**, 33333–33342.

237 Y. Yang, K. Kulka, R. C. Montelaro, T. A. Reinhart1, J. Sissons, A. Aderem and A. K. Ojha, *Cell Host Microbe*, 2014, **15**, 153–163.

238 P. J. Brennan, D. P. Lehane and D. W. Thomas, *Eur. J. Biochem.*, 1970, **13**, 117–123.

239 A. P. Lawton and M. Kronenberg, *Immunol. Cell Biol.*, 2004, **82**, 295–306.

240 Rajni, N. Rao and L. S. Meena, *Biotechnol. Res. Int.*, 2011, **2011**, 1–7.

241 I. Matsunaga, T. Naka, R. S. Talekar, M. J. McConnell, K. Katoh, H. Nakao, A. Otsuka, S. M. Behar, I. Yano, D. B. Moody and M. Sugita, *J. Biol. Chem.*, 2008, **283**, 28835–28841.

242 D. M. Zajonc and M. Kronenberg, *Curr. Opin. Struct. Biol.*, 2007, **17**, 521–529.

243 J. Prandi, *Carbohydr. Res.*, 2012, **347**, 151–154.

244 T. K. A. Nguyen, A. P. Koets, W. J. Santema, W. van Eden, V. P. M. G. Rutten and I. Van Rhijn, *Vaccine*, 2009, **27**, 4818–4825.

245 E. Girardi, J. Wang, T. Mac, C. Versluis, V. Bhowruth, A. J. R. Heck, I. Van Rhijn and D. M. Zajonc, *J. Immunol*, 2010, **185**, 376–386.

246 Y. Rombouts, B. Brust, A. K. Ojha, E. Maes, B. Coddeville, E. Elass-Rochard, L. Kremer and Y. Guerardel, *J. Biol. Chem.*, 2012, **287**, 11060–11069.

247 M. McNeil, S. J. Wallner, S. W. Hunter and P. J. Brennan, *Carbohydr. Res.*, 1987, **166**, 299–308.

248 M. Miyauchi, Y. Uenishi, I. Yano, M. Murata, M. Sunagawa, K. Shibuya, E. Koga-Yamakawa, N. Kusunose and Y. Kashiwazaki, *Drug Discov. Ther.*, 2011, **5**, 130–135.

249 S. Berg, D. Kaur, M. Jackson and P. J. Brennan, *Glycobiology*, 2007, **17**, 35R–56R.

250 M. Maza Iglesias, PhD Thesis, Bangor University, 2010.

251 R. Al Dulayymi, M. S. Baird and E. Roberts, *Chem. Commun.*, 2003, 228–229.

252 J. A. Frick, J. B. Klassen, A. Bathe, J. M. Abramson and H. Rapoport, *Synthesis*, 1992, **7**, 621–623.

253 Ch. D. Don-Lawson, PhD Thesis, Bangor University, 2012.

254 M. M. Sahb, PhD thesis, Bangor University, Bangor, UK, 2014.

255 S. Zhang, J. Yang, X. Liu, J. Chang and A. Cao, *Biomacromolecules*, 2003, **4**, 437–445.

256 K. Mori, *Tetrahedron*, 1975, **31**, 3011–3012.

257 P. J. Kocienski, B. Lythgoe and S. Ruston, *J. Chem. Soc. Perkin Trans. 1*, 1979, **1**, 1290–1293.

258 P. J. Kocienski, B. Lythgoe and I. Waterhouse, *J. Chem. Soc., Perkin Trans. 1*, 1980, 1045–1050.

259 P. J. Kocienski, B. Lythgoe and S. Ruston, *J. Chem. Soc.*, 1978, **8**, 829–834.

260 J. B. Baudin, G. Hareau, S. A. Julia and O. Ruel, *Tetrahedron Lett.*, 1991, **32**, 1175–1178.

261 P. R. Blakemore, *J. Chem. Soc., Perkin Trans. 1*, 2002, 2563–2585.

262 P. R. Blackemore, W. J. Coke, P. J. Kocienski and A. Morley, *Synlett.*, 1998, 26–28.

263 G. E. Keck, M. B. Andrus and D. R. Romer, *J. Org. Chem.*, 1991, **56**, 417–420.

264 M. A. Brimble, O. C. Finch, A. M. Heapy, J. D. Fraser, D. P. Furkert and P. D. O'Connor, *Tetrahedron*, 2011, **67**, 995–1001.

265 J. Kuszmann, É. Tomori and I. Meerwald, *Carbohydr. Res.*, 1984, **128**, 87–99.

266 J. Leonard, S. Mohialdin and A. Swain, *Synth. Commun.*, 1989, **19**, 3529–3534.

267 S. Takano, A. Kurotaki, M. Takahashi and K. Ogasawara, *Synthesis*, 1986, **5**, 403–406.

268 E. J. Kang, E. J. Cho, M. K. Ji, Y. E. Lee, D. M. Shin, S. Y. Choi, Y. K. Chung, J. Kim, H. Kim, S. Lee, M. S. Lah and E. Lee, *J. Org. Chem.*, 2005, **70**, 6321–6329.

269 J. Leonard, S. Mohialdin, D. Reed, G. Ryan and P. A. Swain, *Tetrahedron*, 1995, **51**, 12843–12858.

270 F. Buckel, *Synthesis*, 1999, **6**, 953–958.

271 M. González, Z. Gándara, B. Covelo, G. Gómez and Y. Fall, *Tetrahedron Lett.*, 2011, **52**, 5983–5986.

272 R. D. Kaduskar, A. A. Dhavan, S. Dallavalle, L. Scaglioni and L. Musso, *Tetrahedron*, 2016, **72**, 2034–2041.

273 E. D. Hostetler, S. Fallis, T. J. Mcarthy, M. J. Welch and J. A. Katzenellenbogen, *J. Org. Chem.*, 1998, **63**, 1348–1351.

274 L. Crombie and R. Denman, *Tetrahedron Lett.*, 1984, **25**, 4267–4270.

275 M. Salman, P. J. Brennan and J. T. Lonsdale, *Biochim. Biophys. Acta*, 1999, **1437**, 325–332.

276 Y. Katz, F. X. Lebas, H. V. Medley and R. Robson, *Clin. Ther.*, 1998, **20**, 424–437.

277 D. Takano, T. Nagamitsu, H. Ui, K. Shiomi, Y. Yamaguchi, R. Masuma, I. Kuwajima and S. Omura, *Org. Lett.*, 2001, **3**, 2289–2291.

278 M. Lautens, J. T. Colucci, S. Hiebert, N. D. Smith and G. Bouchain, *Org. Lett.*, 2002, **4**, 1879–1882.

279 A. Pollex, L. Abraham, J. Müller and M. Hiersemann, *Tetrahedron Lett.*, 2004, **45**, 6915–6918.

280 T. Brandl and R. W. Hoffmann, *European J. Org. Chem.*, 2004, 4373–4378.

281 H. M. Ali, PhD Thesis, Bangor University, 2015.

282 H. Noll and H. Bloch, *J. Biol. Chem.*, 1955, **214**, 251–265.

283 Y. Kinjo, E. Tupin, D. Wu, M. Fujio, R. Garcia-Navarro, M. R. E. I. Benhnia, D. M. Zajonc, G. Ben-Menachem, G. D. Ainge, G. F. Painter, A. Khurana, K. Hoebe, S. M. Behar, B. Beutler, I. A. Wilson, M. Tsuji, T. J. Sellati, C. H. Wong and M. Kronenberg, *Nat. Immunol.*, 2006, **7**, 978–986.

284 M. Muzael, Ph.D Thesis, Bangor University, 2012.

285 R. Munakata, T. Ueki, H. Katakai, K. I. Takao and K. I. Tadano, *Org. Lett.*, 2001, **3**, 3029–3032.

286 M. O. Mohammed, M. S. Baird and J. R. Al Dulayymi, *Tetrahedron Lett.*, 2015, **56**, 3268–3272.

287 J. R. Al Dulayymi, M. S. Baird, M. Maza-Iglesias, R. T. Hameed, K. S. Baols, M. Muzael and A. D. Saleh, *Tetrahedron*, 2014, **70**, 9836–9852.

288 J. R. Nunn, *J. Chem. Soc.*, 1952, **66**, 313–318.

289 G. Dijkstra and H. J. Duin, *Nature*, 1955, **176**, 71–72.

290 H. W. Kircher, *J. Org. Chem.*, 1964, **29**, 1979–1982.

291 F. L. Carter and V. L. Frampton, *Chem. Rev.*, 1964, **64**, 497–525.

292 W. J. Gensler, M. B. Floyd, R. Yanase and K. Pober, *J. Am. Chem. Soc.*, 1969, **91**, 2397–2398.

293 F. S. Shenstone and J. R. Vickery, *Nature*, 1956, **177**, 94.

294 J. J. Macfarlane, F. S. Shenstone and J. R. Vickery, *Nature*, 1957, **179**, 830–831.

295 W. J. Gensler, M. B. Floyd, R. Yanase and K. Pober, *J. Am. Chem. Soc.*, 1970, **92**, 2472–2475.

296 L. J. Morris, R. V. Harris, W. Kelly and A. T. James, *Biochem. Biophys. Res. Commun.*, 1967, **28**, 904–908.

297 A. W. Jevans and C. Y. Hopkins, *Tetrahedron Lett.*, 1968, **9**, 2167–2170.

298 H. E. Nordby and G. Yelenosky, *Phytochemistry*, 1987, **26**, 3151–3157.

299 T. L. Wilson, C. R. Smith Jr and K. L. Mikolajczak, *J. Am. Oil Chem. Soc.*, 1961, **38**, 696–699.

300 I. O. Roberts, PhD Thesis, Bangor University, 2006.

301 K. M. Hosamani and H. S. Ramesh, *Ind. Crops Prod.*, 2003, **17**, 53–56.

302 A. R. P. Gaydou, and E. M., Ramanoelina, *Phytochemistry*, 1983, **22**, 1725–1728.

303 K. M. Hosamani and R. S. Pattanashettar, *Ind. Crops Prod.*, 2003, **18**, 139–143.

304 K. M. Hosamani and R. M. Sattigeri, *Ind. Crops Prod.*, 2003, **17**, 57–60.

305 K. M. Hosamani, A. S. Patil and R. S. Pattanashettar, *Ind. Crops Prod.*, 2002, **15**, 131–137.

306 K. M. Hosamani, *Phytochemistry*, 1994, **37**, 1621–1624.

307 T. L. Wilson, C. R. Smith and K. L. Mikolajczar, *J. Am. Oil Chem. Soc.*, 1961, **38**, 696.

308 G. N. Smith and J. D. Bu'Lock, *Chem. Ind.*, 1965, 1840.

309 I. Yano, L. J. Morris, B. W. Nichols and A. T. James, *Lipids*, 1972, **7**, 35.

310 G. N. Smith and J. D. Bu'Lock, *Biochem. Biophys. Res. Commun.*, 1964, **17**, 433.

311 R. Reiser, C. K. Parekh and W. W. Menke, *Biological Problems of Lipids*, Elsevier, New York, 1963, vol. 1.

312 P. W. Lorenz, *Poult. Sci.*, 1939, **18**, 295–300.

313 P. J. Schaible and S. L. Bandemer, *Poult. Sci.*, 1946, **25**, 456–459.

314 J. C. Masson, M. G. Vavich, B. W. Heywang and A. R. Kemmerer, *Science (80-.).*, 1957, **126**, 751.

315 P. K. Raju and J. R. Reiser, *Biol. Chem.*, 1967, **242**, 379–384.

316 A. R. Johnson, J. A. Pearson, F. S. Shenston and A. C. Fogerty, *Nature*, 1967, **214**, 1244–1245.

317 A. T. James, P. Harris and J. Bezard, *Eur. J. Biochem.*, 1968, **3**, 318–325.

318 J. Quintana, M. Barrot, G. Fabrias and F. Camps, *Tetrahedron*, 1998, **54**, 10187–10198.

319 A. Dobrzyn and J. M. Ntambi, *Trends Cardiovasc. Med.*, 2004, **14**, 77–81.

320 J. M. Ntambi and M. Miyazaki, *Prog. Lipid Res.*, 2004, **43**, 91–104.

321 F. E. Gomez, D. E. Bauman, J. M. Ntambi and B. G. Fox, *Biochem. Biophys. Res. Commun.*, 2003, **300**, 316–326.

322 M. Issandou, A. Bouillot, J. Brusq, M. Forest, D. Grillot, R. Guillard, S. Martin, C. Michiels, T. Sulpice and A. Daugan, *Eur. J. Pharmacol.*, 2009, **618**, 28–36.

323 R. A. Igal, *Carcinogenesis*, 2010, **31**, 1509–1515.

324 L. Albino, M. P. Polo, M. G. De Bravo and M. J. T. De Alaniz, *Prostaglandins Leukot. Essent. Fat. Acids*, 2001, **65**, 295–300.

325 N. Scaglia, J. M. Caviglia and R. A. Igal, *Biochim. Biophys. Acta*, 2005, **1687**, 141–51.

326 G. Mamalakis, A. Kafatos, N. Kalogeropoulos, N. Andrikopoulos, G. Daskalopulos and A. Kranidis, *Prostaglandins Leukot. Essent. Fat. Acids*, 2002, **66**, 467–477.

327 K. Apostolov and W. Barker, *Infect Immun*, 1982, **38**, 843–847.

328 H. Dahl and M. Degre, *J. Pathol. Microbiol. Immunol.*, 1976, **84B**, 285–292.

329 Y. Choi, Y. Park, J. M. Storkson, M. W. Pariza and J. M. Ntambia, *Acad. Press*, 2002, **294**, 785–790.

330 B. F. Fermor, J. R. W. Masters, C. B. Wood, J. Miller, K. Apostolov and N. A. Habib, *Eur. J. Cancer*, 1992, **28**, 1143–1147.

331 B. Phetsuksiri, M. Jackson, H. Scherman, M. McNeil, G. S. Besra, A. R. Baulard, R. A. Slayden, A. E. DeBarber, C. E. Barry, M. S. Baird, D. C. Crick and P. J. Brennan, *J. Biol. Chem.*, 2003, **278**, 53123–53130.

332 World Health Organization, *World health statisitics*, 2011.

333 D. J. Sullivan and S. Krishna, *Malaria : Drugs, Disease and Post-genomic Biology*, Berlin : Springer-Verlag, 2005.

334 I. W. Sherman, *Malaria :Parasite Biology, Pathogenesis, and Protection*, ASM Press, Washington, DC, 1998.

335 R. A. Bayoumi, H. A. Babiker, S. M. Ibrahim, H. W. Ghalib, B. O. Saeed, S. Khider, M. Elwasila and E. A. Karim, *Acta Trop.*, 1989, **46**, 157–165.

336 R. N. Price, F. Nosten, C. Luxemburger, M. VanVugt, L. Phaipun and T. C. N. J. White, *Trans. R. Soc. Trop. Med. Hyg.*, 1997, **91**, 574–577.

337 K. Na-Bangchang, R. Ruengweerayut, P. Mahamad, K. Ruengweerayut and W. Chaijaroenkul, *Malar. J.*, 2010, **9**, 1–10.

338 S. Déchamps, S. Shastri, K. Wengelnik and H. J. Vial, *Int. J. Parasitol.*, 2010, **40**, 1347–1365.

339 T. Mitamura, K. Hanada, E. P. Ko-Mitamura, M. Nishijima and T. Horii, *Parasitol. Int.*, 2000, **49**, 219–229.

340 J. Mazumdar and B. Striepen, *Eukaryot. Cell*, 2007, **6**, 1727–1735.

341 M. S. Baird, C. M. Dale, W. Lytollis and M. J. Simpson, *Tetrahedron Lett.*, 1992, **33**, 1521–1522.

342 M. Thoroughly and J. Baumann, *J. Lipid Res.*, 1968, **9**, 287.

343 M. S. Baird and B. Grehan, *J. Chem. Soc. Perkin Trans. 1*, 1993, 1547–1548.

344 P. Hao, I. Q. M. Alaraj, J. R. Al Dulayymi, M. S. Baird, J. Liu and Q. Liu, *Korean J Parasitol*, 2016, **54**, 139–145.

345 N. Munehiro and T. Hase, *Bull. Chem. Soc. Jpn.*, 1991, **64**, 3084–3088.

346 E. H. Huws, PhD Thesis, Bangor University, 2011.