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Synthesis of epoxy-mycolic acids

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Synthesis of Epoxy-Mycolic Acids

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by

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Contents

Abbreviations

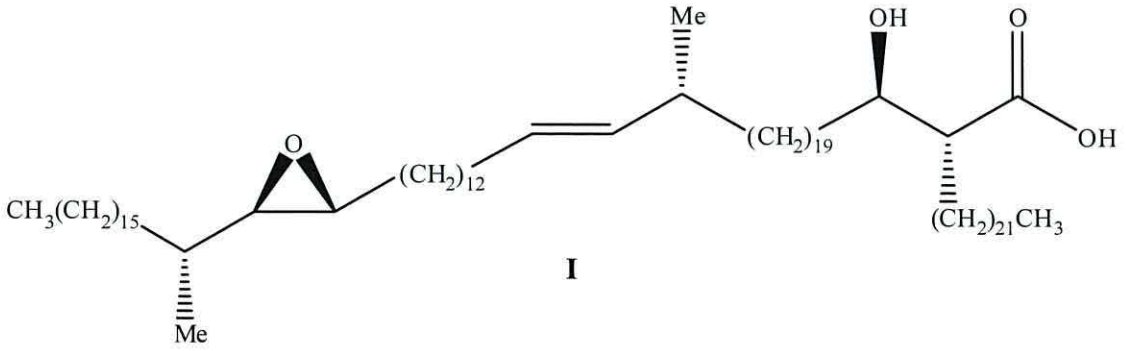
Ac	Acetyl
AIDS	Acquired Immune Deficiency Syndrome
aq.	Aqueous
BCG	Bacillus Calmette-Guérin
Bn	Benzyl
br.	Broad
°C	degrees Celsius
CID	Collision Induced Dissociation
d	doublet
DEAD	Diethyl azodicarboxylate
DIBAL-H	diisobutylaluminium hydride
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
d.p.	decimal places
Ether	diethyl ether
GC	Gas chromatography
h	hours
HIV	Human immunodeficiency virus
HMPA	Hexamethylphosphoric triamide
HPLC	High performance liquid chromatography
Hz	Hertz

IMS	industrial methylated spirits
IR	Infra-red
i-Pr	Isopropyl
J	coupling constant
LDA	lithium *N,N*-diisopropylamide
m	multiplet
MALDI	Matrix-assisted laser desorption/ionization
MALDI-TOF	Matrix Assisted Laser Desorption Ionization Time-Of-Flight
MCPBA	meta-chloroperoxybenzoic acid
MDR-TB	Multidrug-resistance Tuberculosis
Me	Methyl
MeLi	Methyllithium
MHz	Megahertz
min	minute
ml	milliters
mmol	millimols
m.p.	melting point
Ms	Mass spectrometry
M.Tb	*Mycobacterium tuberculosis*
NBS	*N*-bromosuccinimide
NMR	nuclear magnetic resonance
Oxone	$2KHSO_5.KHSO_4.K_2SO_4$ ($KHSO_5$: potassium peroxomonosulfate)
PCC	pyridinium chlorochromate

Petrol	petroleum spirit (boiling point 40 to 60 ^{o}C)
Ph	Phenyl
ppm	parts per million
PPTS	Pyridinium-*p*-toluenesulfonate
q	quartet
R	Rectus
r.t.	room temperature
s	singlet
S	Sinister
SAM	S-adenosyl-*L*-methionine
sat.	saturated
t	triplet
TBAF	tetra-n-butylammonium fluoride
TB	Tuberculosis
T-cells	T-Lymphocytes
TDM	trehalose dimycolate
THF	Tetrahydrofuran
THP	Tetrahydropyran
TLC	Thin layer chromatography
WHO	World Health Organisation
XDR-TB	Extensively drug-resistant tuberculosis

Abstract

Mycolic acids are major components of the cell wall of *Mycobacterium tuberculosis* and other mycobacteria. They are usually long chain high molecular mass branched β-hydroxy fatty acids, containing 70–90 carbons, α-alkyl (C_{22}–C_{24}) and containing different functionalities in the main chain. Their presence is thought to explain the characteristic resistance of these mycobacteria to most antibiotics and other chemotherapeutic agents. Synthetic mycolic acids have applications in the detection and treatment of tuberculosis and in the treatment of asthma. This project consists of three parts. The first part was to synthesise (*R,R*)-epoxy-*trans*-alkene-mycolic acid (**I**) and (*S,S*)-epoxy-*trans*-alkene-mycolic acid (**II**). The target for this part of the project was an epoxy-mycolate present in the cell wall of *Mycobacterium fortuitum*. Two synthetic stereoisomers (**I**) and (**II**) were compared by ^{1}H and ^{13}C NMR spectra with natural epoxy-*trans*-alkene mycolic acid and the stereochemistry of natural mycolic acid was also proved to be as in compound (**II**). The second part was to synthesise (*S,R*)-*cis*-cyclopropane (*R,R*)-epoxy-mycolic acid (**III**) and (*S,R*)-*cis*-cyclopropane (*S,S*)-epoxy-mycolic acid (**IV**) from *Mycobacterium smegmatis*. Two synthetic stereoisomers (**III**) and (**IV**) were compared by proton NMR spectroscopy with natural *cis*-cyclopropane epoxy mycolic acid isolated from *M. smegmatis*. The third part was to synthesise (*E/Z*)-(*R,R*)-epoxy-alkene mycolic acid methyl ester (**V**) and (*E/Z*)-(*S,S*)-epoxy-alkene mycolic acid methyl ester (**VI**) from *M. smegmatis*. An attempt was made to separate the (*E/Z*)-alkene-epoxy mycolic acid methyl esters by silver ion TLC but was not successful.



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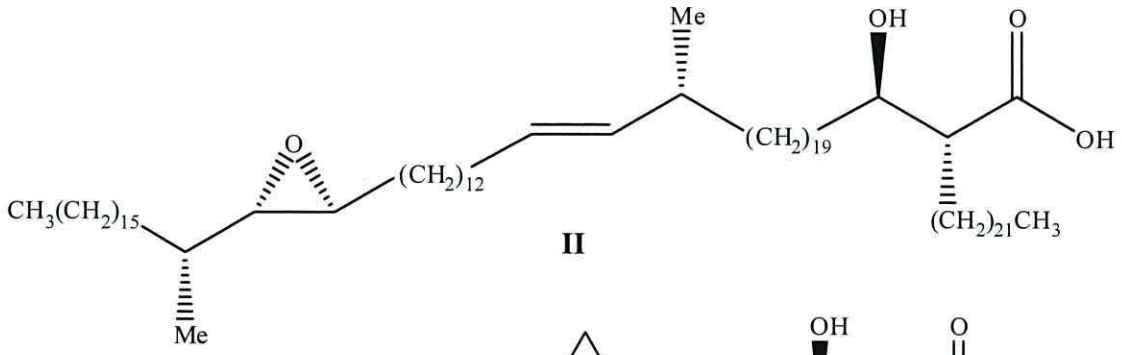

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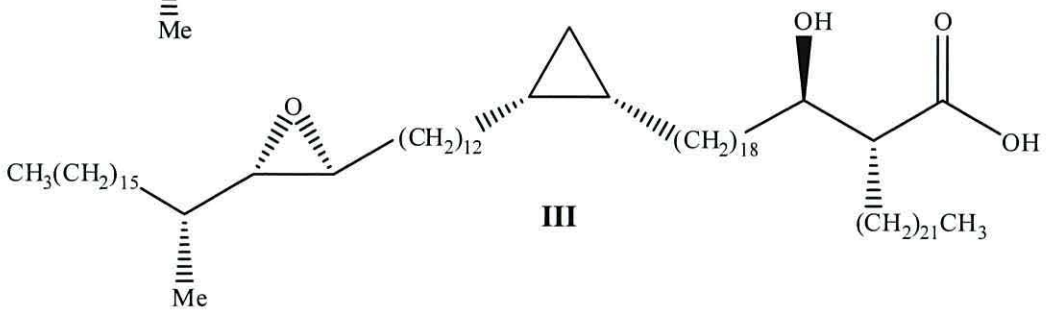

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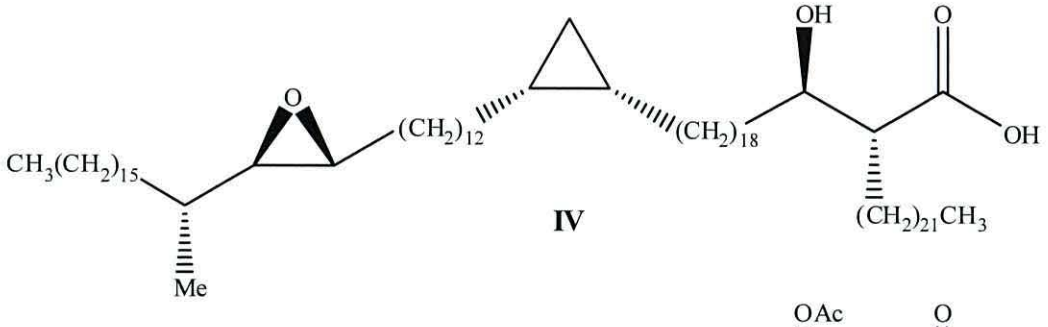

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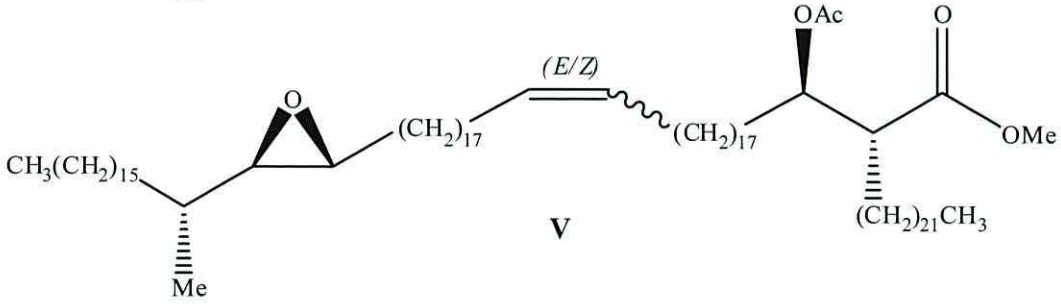

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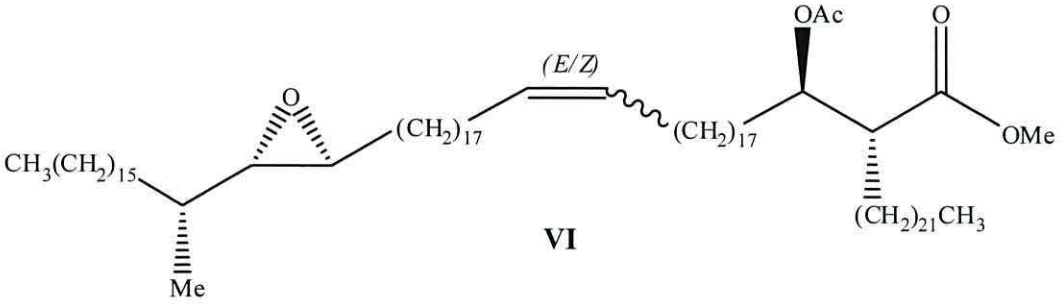


VI

1-Introduction

1.1-Tuberculosis (TB)

1.1.1-History of TB

Tuberculosis or TB is an infectious bacterial disease caused by *Mycobacterium tuberculosis*.[1] TB kills 3 million people each year and accounts for about 25 % of preventable deaths.[2,3,4] The first reference to TB in Egypt was documented more than 5000 years ago. It was found in Egyptian mummies and was clearly depicted in early Egyptian art.[5,6] Another reference of a disease called phthisis was found in ancient Greek literature by Hippocrates around 460 BCE.[7,8] Tuberculosis was well established in East Africa by the time Europeans reached the area in the 19th century.[9] There are also written texts describing tuberculosis in India as early as 3300 years ago and in China 2300 years ago.[10,11] It is thought that TB existed in the Americas with similar evidence to that found in Egypt, before the arrival of European explorers.[12,13] TB was first isolated in 1882 by a German physician named Robert Koch who received the Nobel Prize for this discovery.[14] However, tuberculosis became a recognised public problem during the Industrial Revolution, when cities were overcrowded and the public health care facilities were inadequate for the number of citizens. In the 18th and 19th century, up to 25 percent of deaths in Europe were caused by this disease.[15] The recorded mortality rate due to tuberculosis in England was 1,120 per 100,000 of the population. Early in the 19th century the mortality rate started to decline and the 19th century was called 'the century of tuberculosis'.[16]

Each year eight million people develop tuberculosis disease and almost three million die of tuberculosis. Over 95 % of these occur in developing countries. One billion people have died from tuberculosis in the 19th and 20th centuries, and 1.3 million cases and 450,000 deaths from tuberculosis occur annually in those under the age of 15 years, in developing countries worldwide. In 1990 there were 1.9 million deaths of patients over the age of five from tuberculosis compared with 1.1 million deaths from leprosy, malaria, tropical diseases, AIDS and diarrhoea combined.[17] In 1993, the world health Organization took the unprecedented step of declaring a tuberculosis global emergency. WHO estimated that, without immediate action, 1000 million people will be newly

infected, over 150 million will become ill and 36 million will die of TB between 2002 and 2020.[18]

The first genuine success in immunizing against tuberculosis was developed from attenuated bovine-strain tuberculosis by Albert Calmette and Camille Guérin in 1906. It was called "BCG" (*Bacille Calmette-Guérin*). The BCG vaccine was first used on humans in 1921 in France;[19] 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.[20]

The death toll began to fall as living standards improved at the start of the 20th century, and from the 1940s, effective medicines were developed. However, there are now more people in the world with TB than there were in 1950, and 3 million individuals will die this year from this disease mainly in less developed countries.[21] In developed countries, the incidence of TB decreased even more steadily after the introduction of streptomycin, in combination with other drugs, in the 1940s. The control of the spread of this disease seemed possible at least in "Western" countries.[22] One-third of the world's current population has been infected with *M. tuberculosis*, and infections occur at a rate of one per second.[23] The distribution of tuberculosis is not uniform across the globe with about 80 % of the population in many Asian and African countries testing positive, while only 5-10 % of the US population test positive.[23] The main rise in tuberculosis infection is through co-infection with Human Immunodeficiency Virus (HIV) and Acquired Immunodeficiency Syndrome (AIDS). Because HIV weakens the immune system, a person co-infected with HIV and *M. tuberculosis* is many times more likely to become sick with TB, than someone infected with *M. tuberculosis* but HIV-negative.[24,25] TB was the cause of death for 11 % of all adults with AIDS in 2000.[26] In addition, there has been a simultaneous increase in cases of drug-resistant tuberculosis, which is due to an ineffective administration of antibiotics and other chemotherapeutic agents.[27] Rates of multi-drug-resistant TB are high, especially in the former Soviet Union countries, and it is a significant threat for TB control efforts.[28] The World Health Organisation (WHO) estimates that up 50 million people worldwide may be infected with drug resistant strains of tuberculosis.[29] Another factor that helps the spread of TB is the movement of people, travellers, refugees or displaced people.

1.1.2-Antibiotics for Tuberculosis Treatment

Antibiotics are usually part of the therapy in people who have no symptoms and whose germs are in an inactive state. Antibiotics in this case are helpful in preventing the activation of infection. Streptomycin (**1**) was discovered in 1943 and introduced as the first antibiotic for the treatment of TB. It is derived from the actinobacterium *Streptomyces griseus*.[30] Isoniazid (**2**) is the first-line antituberculosis medication in prevention and treatment. It was introduced in 1952 and it was found to be effective against tuberculosis.[31] The rifamycins (**3**) are a family of antibiotics obtained by fermentation and chemical modification. It was first isolated in 1957 from a fermentation culture of *Streptomyces mediterranei*.[32] (**Fig. 1**).

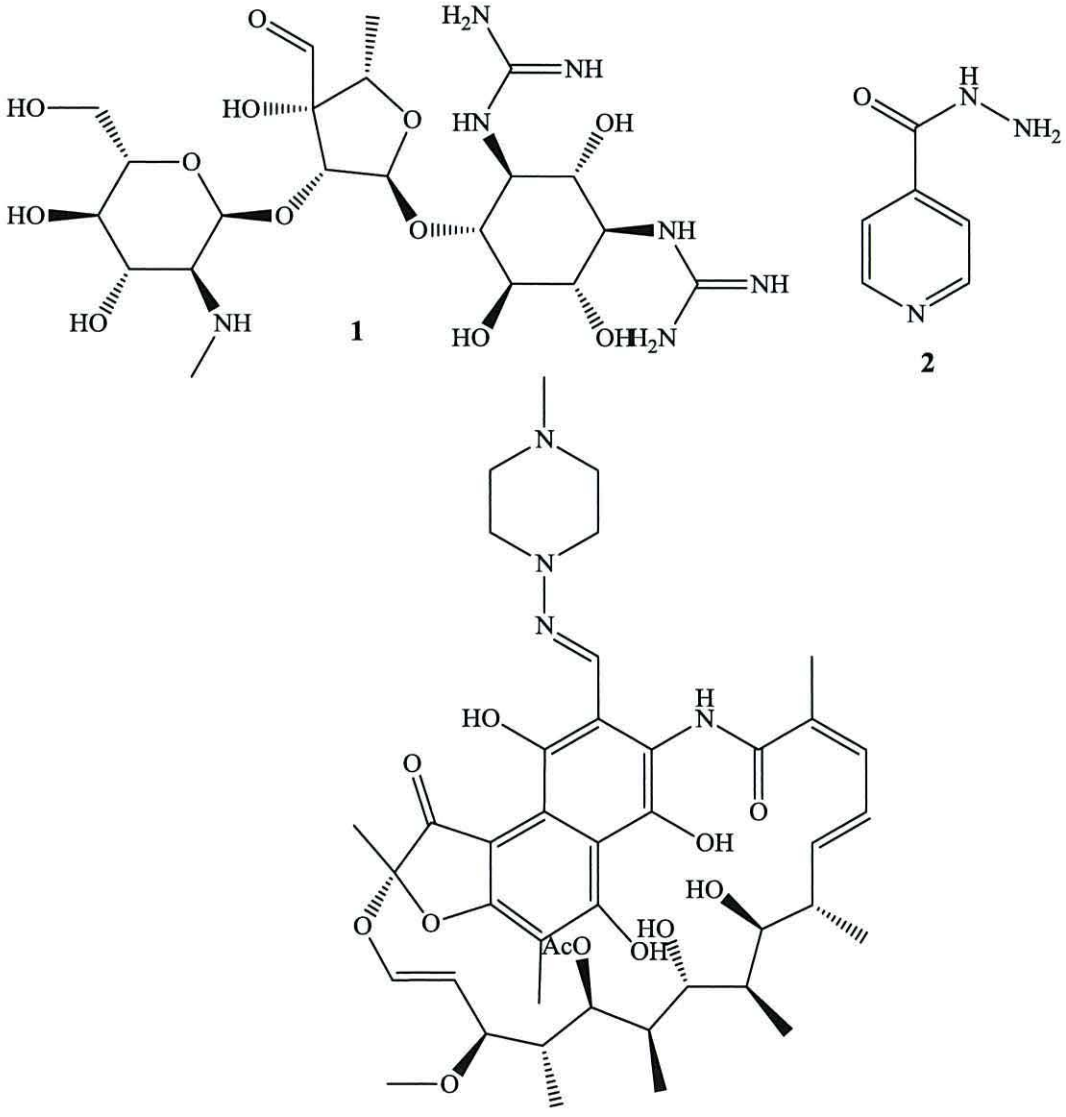


Fig. 1: Streptomycin (**1**), isoniazid (**2**) andrifamycins (**3**)

Another antibiotic, *para*-aminosalicylic-acid (**4**), is used to treat tuberculosis. It was discovered by the Swedish chemist Jörgen Lehmann in 1944.[33] Ethambutol (**5**) is also used to kill the bacteria that cause tuberculosis (TB). It was introduced in 1961 as a bacteriostatic first-line drug effective against actively growing mycobacteria.[34] (**Fig. 2**)

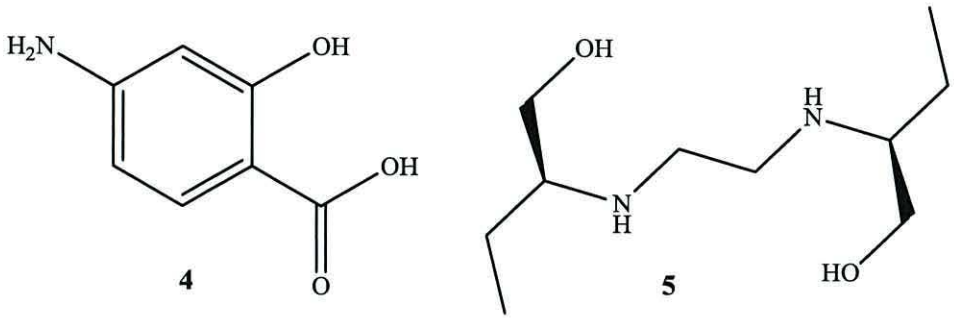


Fig. 2: *para*-Aminosalicylic acid (**4**) and ethambutol (**5**).

According to the WHO, the following drugs can be classified as second-line drugs: aminoglycosides (amikacin and kanamycin), fluoroquinolones ofloxacin, gatifloxacin and ciprofloxacin), polypeptides (viomycin, caperomycin and enviomycin), thionamides (ethionamide and prothionamide) and D-cycloserine.[35]

1.1.3-Tuberculosis Treatment

Mycobacteria are problematic as they are resistant to most commonly used antibiotics and chemotherapeutic agents. Treatment of TB consists of a combination of different antibiotics. The two classes of antibiotics are first-line drugs and second-line drugs. Usually, the treatment is given for six to nine months according to a therapy regime consisting of a two month course using the four first-line drugs isonazid, rifampcin, pyrazniamide and ethambutol or streptomycin, followed by a four month course of isoniazid and rifampcin.[36] The second-line drugs are for drug-resistant TB and for patients who cannot tolerate the first-line drugs; they are less effective, more toxic and require longer use than first-line drugs.[37] According to the World Health Organization, in 2007 the overall success rate for tuberculosis treatment was 70 %.[38]

1.1.4-Multidrug-resistance tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB)

Multidrug-resistant tuberculosis caused by *M. tuberculosis* shows high-level resistance to both isoniazid and rifampicin with or without resistance to other drugs.[39,40] The probability of resistance is much higher for less effective antitubercular drugs.[41] 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.[42]

Extensively drug-resistant tuberculosis (XDR-TB) is a form of TB caused by bacteria that are resistant to the most effective anti-TB drugs. XDR-TB is defined as TB that has developed resistance to the first line anti-TB drugs that define MDR-TB.[43] XDR-TB is associated with a much higher mortality rate than MDR-TB.[44]

1.1.5-Tuberculosis and HIV/AIDS

Tuberculosis is a major cause of death among people living with HIV/AIDS. The risk of developing TB is between 20 and 37 times greater in people living with HIV than among those who do not have HIV infection.[45] TB and HIV co-infection is recognized as a major setback to both TB and HIV infection control programmes. TB and HIV are not a good combination. It's a very aggressive infection and because of this can make the HIV virus multiply quicker, speeding up the deterioration of the body's vital immune system. The South East Asia region of the World Health Organization (WHO) accounts for nearly 40 % of all tuberculosis cases globally and 18 % of the world's HIV infected people also live in this region. In sub-Saharan Africa, HIV/AIDS and co-infection is becoming a growing problem. In 2006, about 85 % of all HIV-positive people with TB were found in Africa.[46]

1.2-Mycobacteria

Mycobacteria are a type of germ. There are many different kinds. The most well known one causes tuberculosis. Mycobacteria are found in many places in nature, including soil and water. Mycobacteria fall into two groups: the slow growers and the rapid growers. Over 70 *Mycobacterium* species have been defined, at least 30 of which cause disease in humans or animals.[47] In 1997, Hunter reported that eight species of mycobacteria have been associated with waterborne transmission of human disease. These species include *Mycobacterium avium complex, Mycobacterium fortuitum, Mycobacterium marinum, Mycobacterium ulcerans, Mycobacterium gordonae, Mycobacterium xenopi and Mycobacterium scrofulaceum.*[48] *M. tuberculosis* is related to other pathogenic mycobacteria, with some responsible for causing tuberculosis in other species of animal and some causing other non-tuberculosis diseases in humans.[49] *Mycobacterium leprae* is the cause of leprosy,[50] *Mycobacterium ulcerans* causes Buruli

ulcer,[51] *M. avium paratuberculosis* causes Johne's disease in animals[52] and possibly Crohn's disease in humans.[53] *M. marinum* is the causative agent of fish tuberculosis, infecting around 150 species of saltwater and freshwater fish all over the world.[54]
Finally, other mycobacteria such *as Mycobacterium kansasii* and *Mycobacterium chelonae* cause TB-like disease in immune deficient people, in particular in AIDS patients.[2]

1.2.1-*Mycobacterium tuberculosis*

M. tuberculosis is a pathogenic bacterial species in the genus *Mycobacterium* and the causative agent of most cases of tuberculosis.[55] *M. tuberculosis*is a small rod-shaped bacillus, approximately (1-4 x 0.3-0.6 μm) in size, which divides every 16 to 20 hours[2] (**See Fig. 3**), an extremely slow rate compared with other bacteria, such as *E. coli* that can divide roughly every 20 minutes.[56]

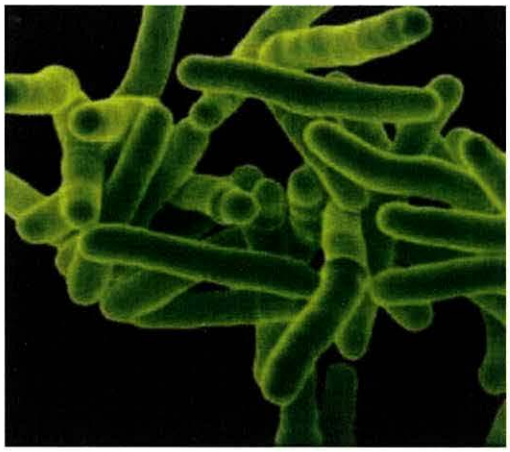


Fig. 3: Scanning electron micrograph of *M. tuberculosis*[57]

M. tuberculosis is a slow growing acid-fast bacterium, the causative agent of one of the most severe infections.[58] It is identified microscopically by its staining characteristics: it retains certain stains after being treated with acidic solution and is thus classified as an "acid-fast bacillus" or AFB.[59] *M. tuberculosis* is classified as a Gram-positive bacterium, as it contains only one phospholipid within its cell wall. However, due to the high lipid and mycolic acid content in its cell wall, *M. tuberculosis* stains very weakly in Gram-positive test.[59] The *M. tuberculosis* complex includes *M. tuberculosis, M. bovis, Mycobacterium microti and M. africanum,* which can all cause the disease tuberculosis.[60]

1.2.2-*Mycobacterium fortuitum*

M. fortuitum is a nontuberculous, rapidly growing *Mycobacterium*. It has been found in water and soil throughout the world. *M. fortuitum* was first isolated from an amphibian source in 1905 and it has been considered a pathogen for both animals and humans since its first isolation from a human abscess in 1938.[61,62] **(See Fig. 4)**

Fig. 4: Scanning electron micrograph of *M. fortuitum*[63]

Major types of disease caused by *M. fortuitum* include those of soft tissue, skin and lung.[64] The *M. fortuitum* group involving *M. fortuitum, Mycobacterium porcinium, Mycobacterium mageritense, Mycobacterium fortuitum third biovariant complex, Mycobacterium abscessus and M. chelonae* are species of rapidly growing mycobacteria (RGM).[65]

Treatment of *M. fortuitum* infections is often difficult as the organism is resistant to standard antitubercular agents and often to other antibiotics as well. In general, the organism is usually susceptible *invitro* to amikacin, cefoxitin, imipenem, sulfonamides and fluoroquinolones.[66,67]

1.2.3-*Mycobacterium smegmatis*

M. smegmatis is a species of rapidly growing *Mycobacterium*, and it has been considered to be a human pathogen. It is similar to *M. fortuitum and M. chelonae,* the other members of group 4 of the Runyon classification of non-tuberculosis mycobacteria.[68] *M. smegmatis* was first discovered and isolated in 1884 by Lustgarten, and lives in aggregate layers of cells attached to each other in a community called a biofilm. It is found in water, soil and plants.[69] *M. smegmatis* is 3.0 to 5.0 μm long with a bacillus shape, and is an acid-fast bacterial species in the phylum *Actinobacteria.*

However, some studies have shown that *M. tuberculosis* and *M. smegmatis* have some genomic similarities and, therefore, *M. smegmatis* has also been proposed as an appropriate model for studying some of the properties of these mycobacteria in general.[70,71] Even though *M. smegmatis* is pathogenic to animals and humans under some circumstances, this organism is generally considered to be a non-pathogenic species for the frog and the tissue culture model of infection. *M. smegmatis* is very useful for the research analysis of other species in the genus mycobacteria in cell culture laboratories. There are many species of *Mycobacterium* that are common, causing harmful diseases, such as *M. tuberculosis, M. bovis and M. leprae*. *M. smegmatis* is important because it is rapid growing and non-pathogenic compared to these species. Treatment of *M. smegmatis* group disease has generally included the same drugs as for treatment of the *M. fortuitum* group.[68]

1.2.4-The mycobacterial cell envelope

The mycobacteria have a cell envelope consisting of three structural features: the capsule, the wall and the plasma membrane as can be seen in **Figure 5**.

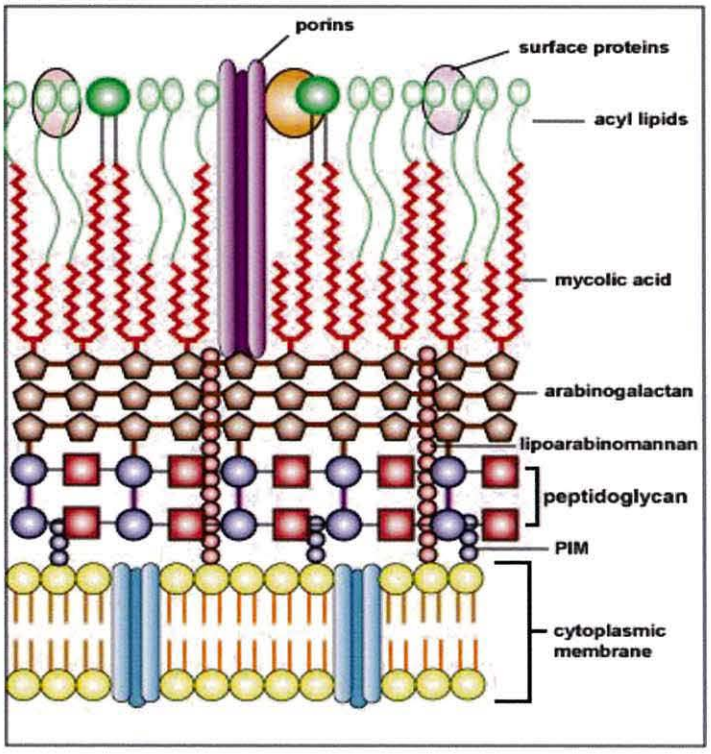


Fig. 5: The cell wall of mycobacteria[72]

The capsule-like layer consists of carbohydrates and proteins containing small amounts of lipids.[73] The plasma membrane is similar to that of other living organisms. It is not

symmetrical in cells carefully fixed from a viable state, in that the outer, electron-dense layer is thicker that the inner layer.[2] The cell wall is composed of three major parts: peptidoglycan, arabinogalatan and mycolic acid.[3]

The peptidoglycan comprises alternating N-acetylglucosamine (NAG) and N-acetylmuramic (NAM) saccharides.[74] The arabinogalactan is a complex hetropolysaccharide, composed of arabinan multi-branched chains.[75] Mycolic acids are high molecular weight long chain fatty acids alkylated in α-position and hydroxylated in β-position.[76]

Mycolic acids are the main constituent of the cell wall, and they occur along with large number of different lipids: for example several different lipids with multi-methyl branched fatty acids,[4,77] trehalose 6,6'-dimycolate and many others. 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.[78,79,80]

In fact, the general resistance of these cells to drugs is considered to be connected with the low permeability of the mycobacterial cell wall to hydrophilic compounds.[81]

Trehalose 6,6'-dimycolate (TDM), or "cord factor" is an interesting lipid component of the cell envelope, consisting of two mycolic acids esterified to trehalose at 6,6' positions.[82] (**Fig. 6**).

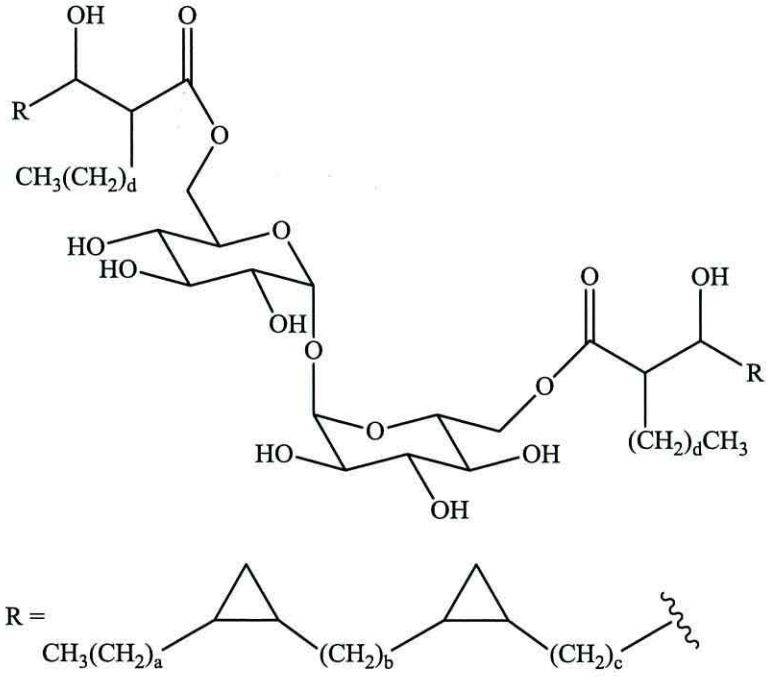


Fig. 6: An example of atrehalose 6,6'-dimycolate,"Cord factor"

Cord factor is considered a "free lipid" since it can be liberated from the cell wall by extraction in an appropriate solvent, while the other mycolic acids, linked by covalent bonds to the arabinogalactan complex, cannot be liberated so readily.[83] In fact, antibodies prepared against cord factor, showed greater reactivity for the types of mycolic acids contained in the glycolipids used as antigens, than other kinds of mycolic acids.[84,85,86]

The antibodies prepared with cord factors of *M. avium* and *M. tuberculosis* were able to distinguish between these two species by recognising their different mycolic acid subclasses.[87,88,89]

1.3-Asthma

Asthma is a chronic inflammatory disease of the airways. Airways become constricted with swelling and excessive mucous production, making it difficult to breathe. Symptoms of asthma are wheezing, shortness of breath, chest tightness, and coughing. The development of asthma has been linked to exposure to an environmental stimulant cold air, exercise, emotional stress, tobacco smoke. In children the most common triggers are viral illnesses such as those that cause the common cold.[90] The illness can be controlled by using medication and avoiding "attack triggers" such as cigarette smoke; allergens such as mold, pollen, animal dander, dust, food, feathers and cockroaches, respiratory infections and exposure to sudden temperature change or cold air.[91] During an asthma attack an environmental stimulant will react with the airway, leading to an immune response, restricting the airway and producing excess mucus. Hence an asthma attack is a result of the body's immune response, trying to prevent an external organism entering the body. This immune response is triggered because the patient has an inflamed airway and is hypersensitive to specific environmental triggers.[92] The cause of asthma is believed to be due to genetic and environmental factors.[91] Asthma cannot be cured, but it can be controlled through careful disease management and avoidance of asthma triggers. The general approach to asthma treatment is acute rescue treatment, controller treatment and preventing of long-term complications. Over the last century, it has been recognized that asthma may be precipitated by certain environmental exposures and that eliminating these exposures may be of value in asthma treatment.[93]

Mycolic acids are part of the cell wall of *M. tuberculosis*, and were shown to have potential as immunotherapeutic agents in a mouse model of asthma.[94] Infection with *M. tuberculosis*, the bacterium that causes TB, has been linked to a decreased risk of

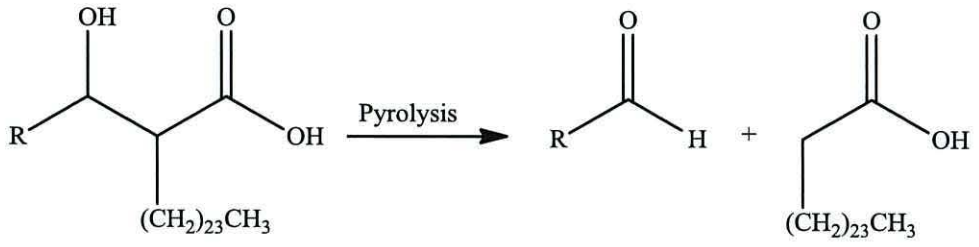
developing allergic asthma. Recently, Grooten and Baird have found that single synthetic analogues of molecules called mycolic acids present in the cell wall of the TB bacterium, have the ability to block immune responses that cause asthma symptoms.[95]

1.4-Mycolic acids

1.4.1-Overview

Mycolic acids were firstly defined as the major ether soluble components of the wax-like substance found in *M. tuberculosis* by Anderson *et al.*[96,97] during his classical systematic investigation of the chemistry of the lipids of mycobacteria; the overall formula $C_{88}H_{176}O_4$ proposed by Anderson is not far from reality.[98] Anderson *et al.* also elucidated structural details, reporting one carboxyl, one hydroxyl and one methoxyl group, and that pyrolysis under reduced pressure at 300 °C gives n-hexacosanoic acids.[97] Lesuk and Anderson prepared normycolic acid by reaction product also a small amount of a monohydroxy monocarboxylic acid with higher molecular weight than the original mycolic acid.[99] The acid had the approximate composition of $C_{104}H_{208}O_3$ and gave n-hexacosanoic acid on pyrolysis.

These were subsequently characterized by Asselineau and Lederer in the 1950s. They also carried out this pyrolysis confirmed the position of the hydroxyl group first reported by Anderson *et al.* to be in the β-position to the carboxylic acid, with a pyrolysis reaction of mycolic acid (**See Fig. 7**).[100]

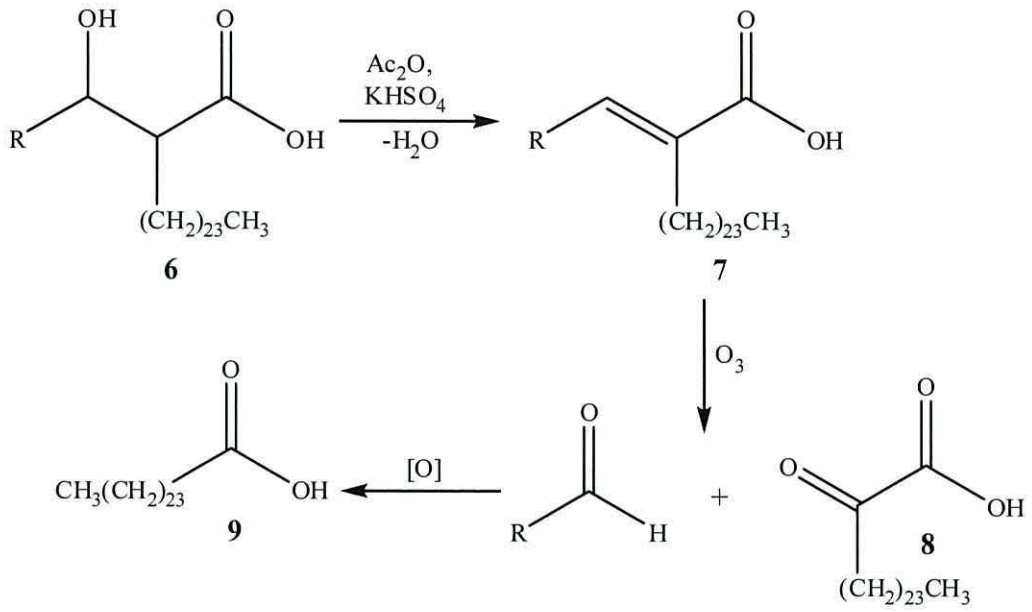


6

Fig. 7: Pyrolysis of mycolic acids

The real course of the reaction seems to be more complicated. However, when Asselineau and Lederer carried out this pyrolysis they reported no aldehyde, but a mixture of methoxy-free substance. They then set about confirming the structure (**6**) for mycolic acid through the following sequence of reactions, heating mycolic acid with acetic anhydride and 10 % potassium hydrogen sulfate to eliminate the β-hydroxyl group to give the α,β-unsaturated anhydro-mycolic acid (**7**). Ozonolysis of (**7**) converted

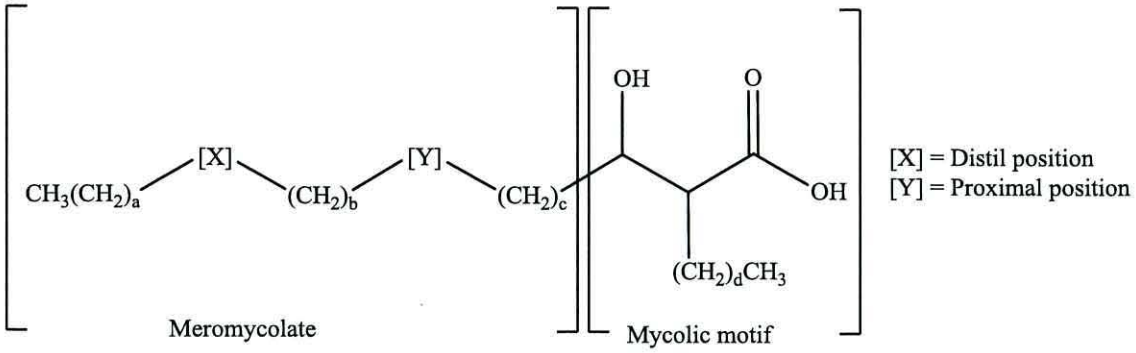
it into α-oxo-hexacosanoic acid (**8**) or, by further oxidation gives n-pentacosanoic acid (**9**)[100] (**Scheme 1).**



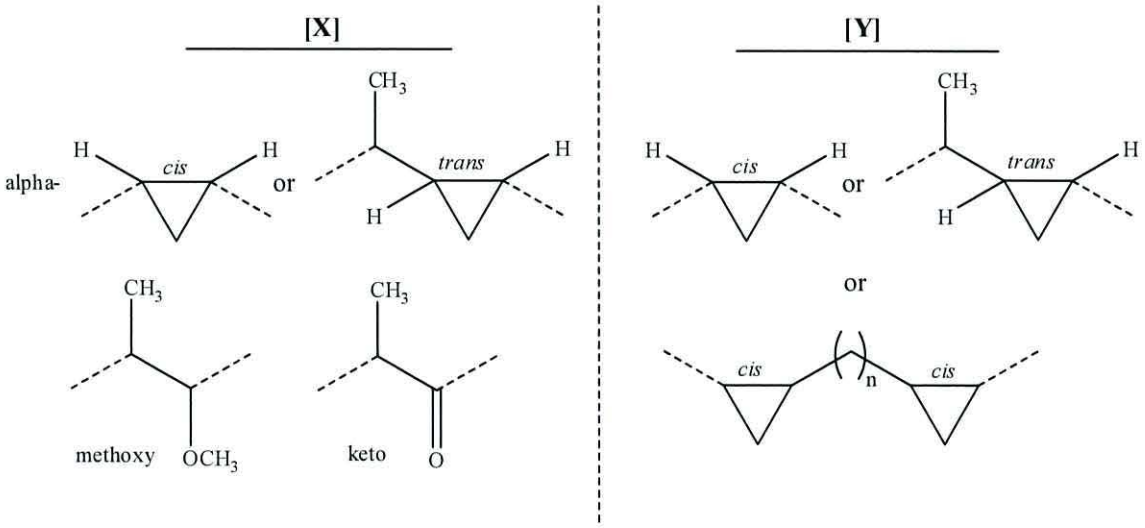
Scheme 1: Degradation of mycolic acid (**6**) to (**8**) or (**9**)

Mycolic acids are the major components of the cell wall of *M. tuberculosis* and other mycobacteria, some of which are pathogenic to animals and humans.[101,102,103] They are usually long chain high molecular mass contains 70–90 carbons, α-alkyl (C_{22}–C_{24}) branched β-hydroxy fatty acids, and contain different functionalities in the main chain (**Fig.8**).[2] These molecules represent major cell wall constituents, 40-60 % of the cell dry weight and are found covalently linked to the cell envelope arabinogalactan and to glycerol; both types of mycolic acid containing components are believed to play a crucial role in the structure and function of the mycobacterial cell wall.[104,3]

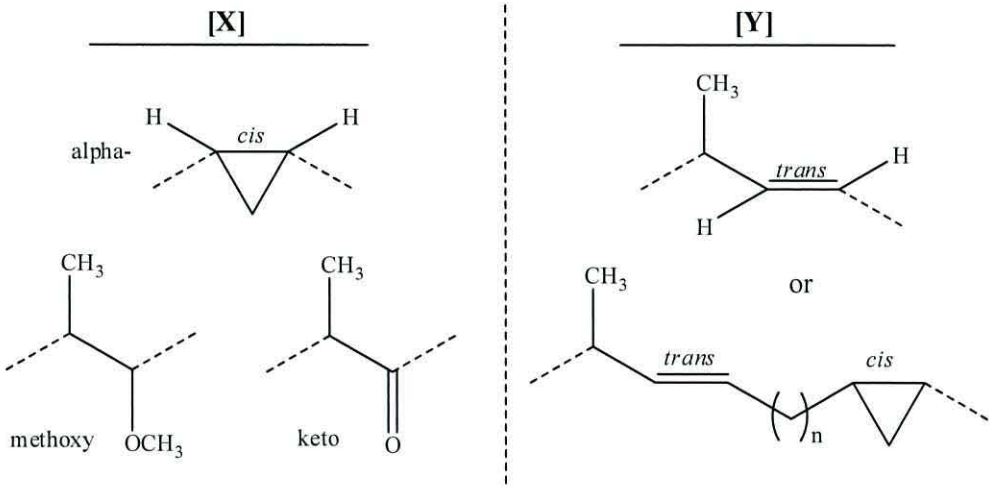
Mycolic acids can be divided into two parts: the mycolic motif and the meromycolate chain. The mycolic motif contains the α-alkyl β-hydroxy fatty acid functionality and the other functionality is found within the meromycolate (**See Fig. 8**).[105,106,107,108] The meromycolate chain from pathogenic mycobacteria normally has two intra-chain groups, the distal and proximal groups, that vary. As the functionality of these positions appears to play an important role in the mycobacterial cell envelope, the resultant mycolic acids have been catalogued by their functionality. These can be classified into three types: type-1 mycolates contain no olefin, type-2 includes all *trans* olefin mycolates and type-3 have only *cis* olefins as shown (**Fig. 8**).[102,103]



Type-1 mycolate



Type-2 mycolate



Type-3 mycolate

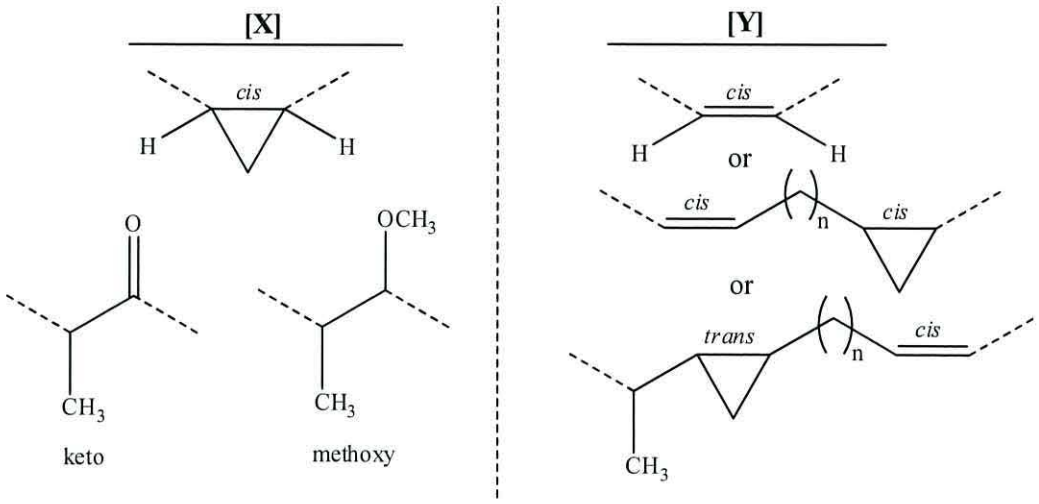


Fig. 8: Generalized structures of major mycobacterial mycolic acids and functional groups[102]

The structure of the α-alkyl-β-hydroxy fatty acid portion is common to each mycolic acid, except for minor variation in the length of the chain in the α-position with respect to the carboxylic end. The functional groups of the proximal or distal position contain *cis*- or *trans*- cyclopropane, *cis*- or *trans*- double bonds and epoxy rings in the variable regions termed [X] and [Y], as well as methyl, methoxyl and oxo groups at different sites.[101] They can be separated from each other by two-dimensional thin layer chromatography (TLC)[109,110,111] on silica gel, high-performance liquid chromatography (HPLC)[112,113,114] and gas chromatography (GC).[115] In association with mass spectrometry (MS), infrared spectroscopy (IR) and nuclear magnetic resonance (NMR) techniques, these have permitted the identification of several kinds of mycolic acids present in each *Mycobacterium*. Actually, HPLC patterns are characteristic for each *Mycobacterium* and they have been used as a rapid diagnostic tool for speciating mycobacteria.[114]

Mycolic acids are highly complex homologous mixtures but include long-chain (*R,R*)-β-hydroxy-acids ($RCH(OH)CH(R')CO_2H$, where *R* is a 'meromycolate' chain (mero-chain) consisting of 50-60 carbon and *R'* is a shorter aliphatic chain (α-chain) possessing 22-26 carbons. *M. tuberculosis* commonly contains *cis*-cyclopropanes, α-methyl-*trans*-cyclopropanes, α-methyl-β-keto- and α-methyl-β-methoxy-group in the *R*-chain and a simple long chain alkyl group in the *R'* position,[110,108,116] such as compounds (**10-14**) (**Fig. 9**).

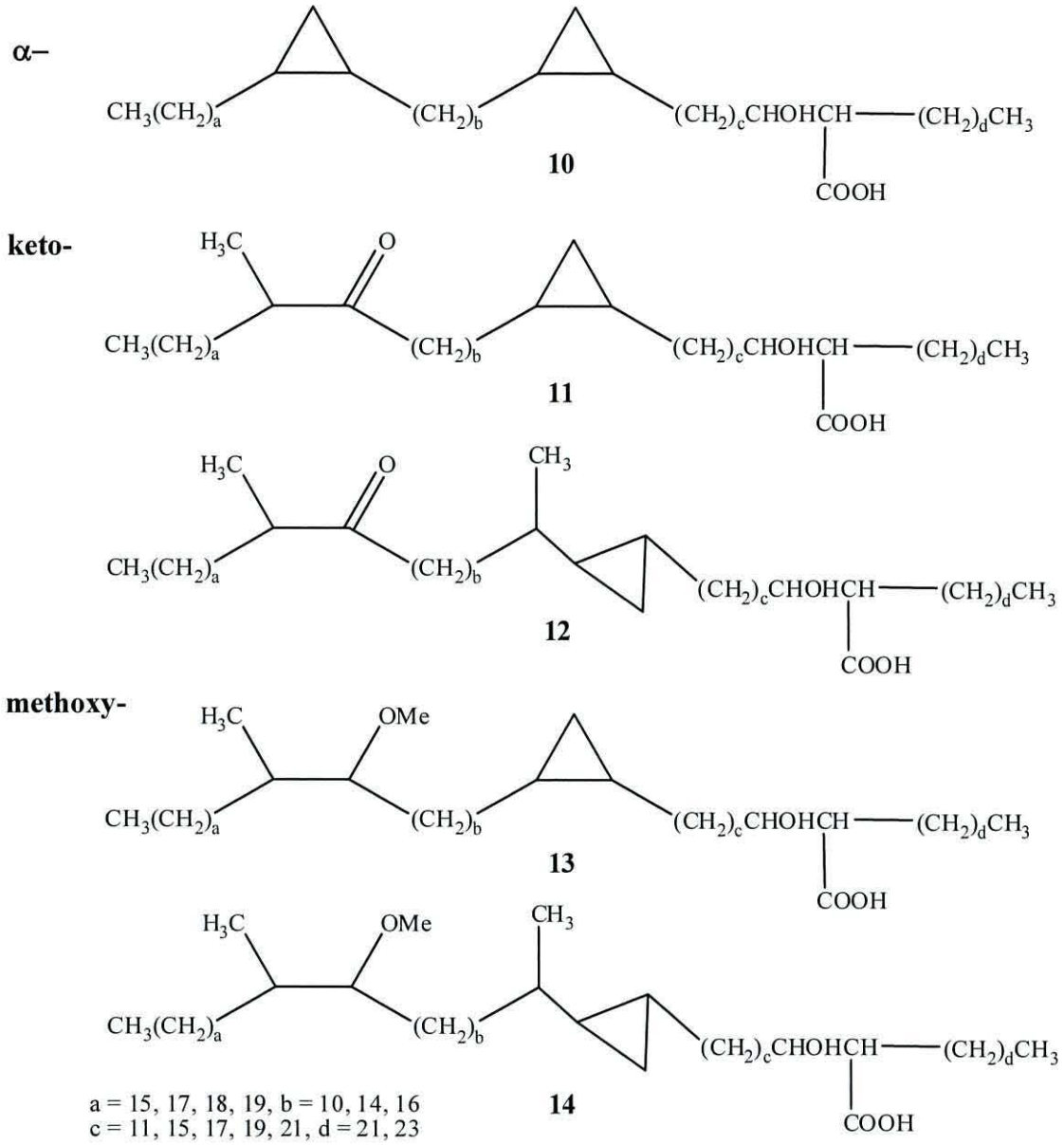


Fig. 9: Major types of mycolic acids from *M. tuberculosis* complex

In addition some mycobacteria contain completely different sets of mycolic acid (**See Fig. 10**). For example *M. smegmatis* contains α'(**15**) and α-mycolic acids (**16, 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*. *M. fortuitum* contains mycolic acids (**18-21**) with an epoxy ring.[117,118] Recently, more different oxygenated mycolic acids have been isolated in numerous mycobacteria; these include ω-carboxy-mycolic acid from *Mycobacterium phlei*,[119,120] ω-1-methoxy-mycolic acidfrom *Mycobacterium alvei*,[121] wax ester mycolic acid from *M. aurum*,[118] and hydroxy mycolic acid.[122] There are also a lot of mycolic acids with different

combinations of the distal group and proximal group in different mycobacteria (**Fig. 10**). Polar modifications are generally restricted to the distal position, whereas nonpolar modifications occur at both the distal and the proximal positions.

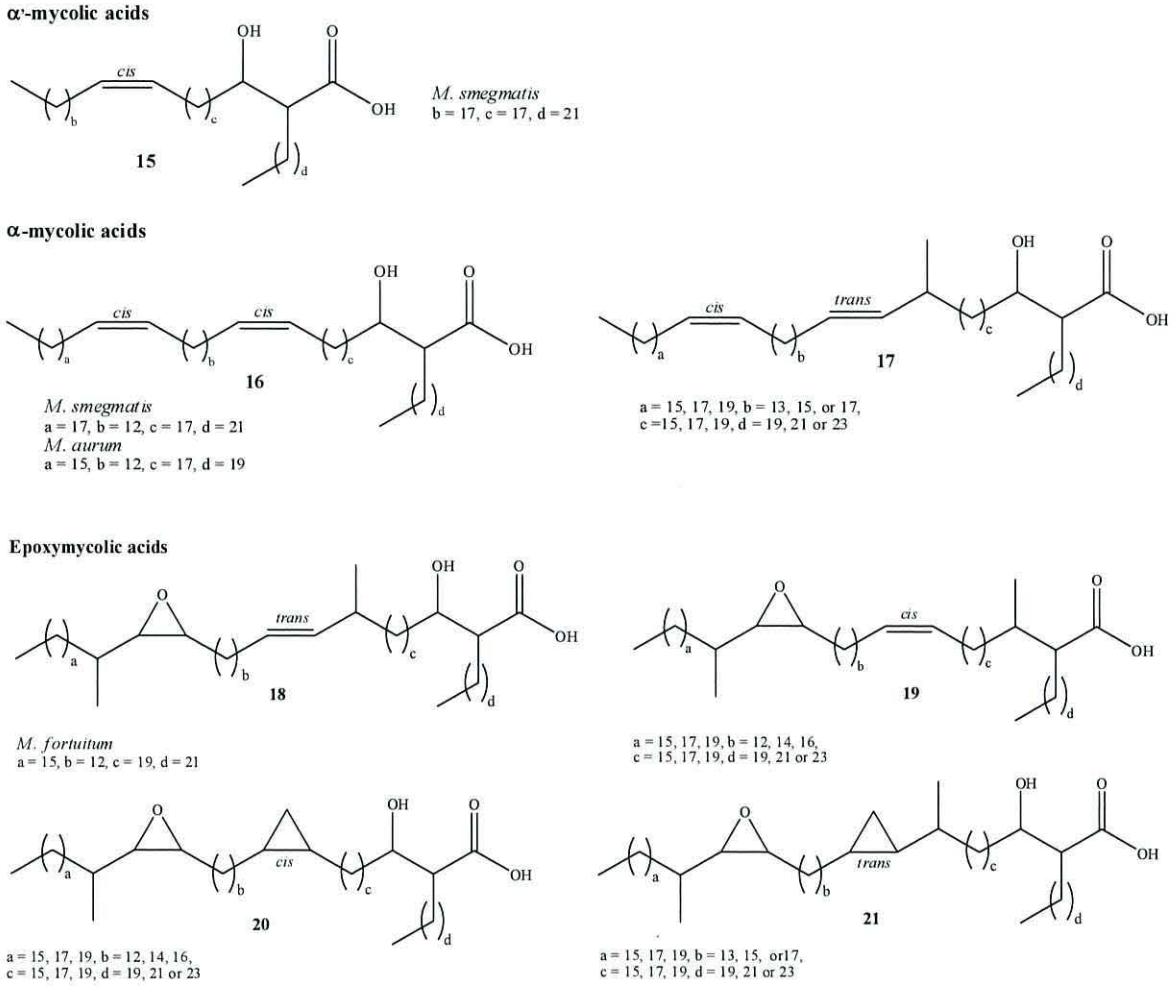


Fig. 10: Mycolic acids from other mycobacteria[118,120]

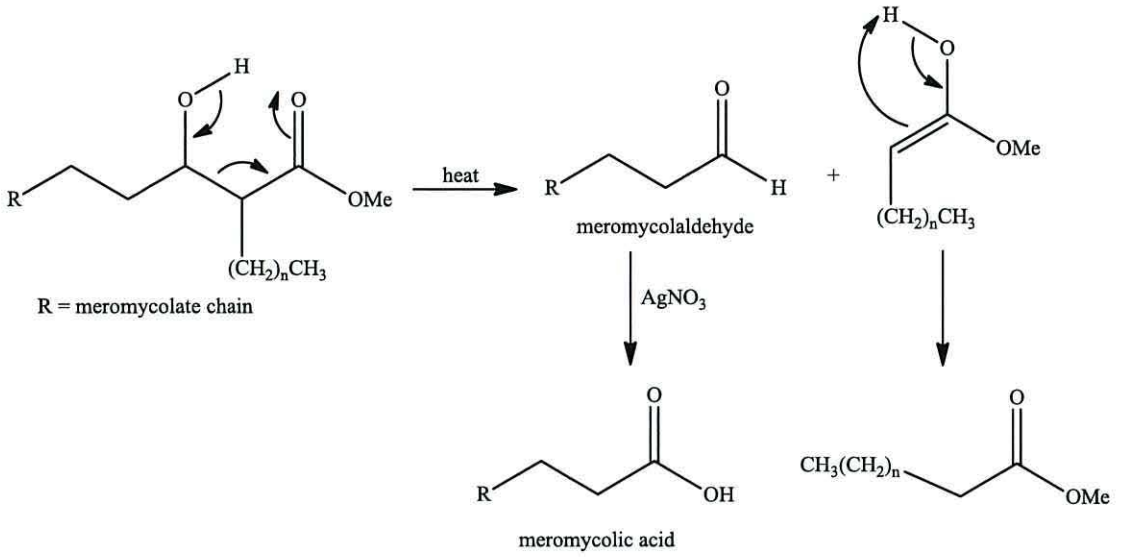
1.4.2-Chain length

In all mycobacteria, there are not only different types of mycolic acids, but also different homologues for each of them. In *M. tuberculosis* alone, a family of over 500 individual mycolic acids with closely related chemical structure has been recognised,[104] while *M. smegmatis* about 100 structural isomers are present in the mixture of α-mycolates.[123] These circumstances made the isolation of a single compound and the determination of its real structure extremely difficult. However, mycolic acids have been the focus of constant study since their discovery. This is not only because they are unique to this type of organism but also because of their importance for the survival and virulence of the mycobacteria.

Recently, MALDI-TOF mass spectrometry has provided a rapid and highly sensitive technique for analysis of mycolic acids and other lipids. Laval *et al.*[120] used this technique to analyse the length of the total carbon chain of the major types of mycolic acid of different mycobacteria; both pathogenic slow-growers, such as *M. tuberculosis* and non-pathogenic fast growers such as *M. smegmatis* produced a series of even carbon number (C_{74}–C_{82}) of α-mycolic acids. In addition, the main chain of oxygenated (methoxy and keto) mycolic acid from slow growers were four to six carbon atoms longer than the corresponding α-mycolic acids, whereas rapid growers elaborated oxygenated homologues possessing the same chain length as their α-mycolic acids.[124,2] The structure of mycolic acids lends itself to very efficient packing within the mycobacterial cell wall, resulting in a highly impermeable barrier. Excluding the β-hydroxy group, all other functionality leading to disruption of the highly ordered "linear" packing is found toward the distal end of the meromycolate chain.

Mycolic acids occur within all mycobacteria, in varying combinations of functionality type and chain length, and there are at least 54 reported species of mycobacteria. Therefore during diagnosis of mycobacterial disease, mycolic acid profiles can be useful in predicting if the disease is tubercular or non-tubercular. Methods have been developed using HPLC to identify characteristic mycolic acids specific to one mycobacteria.[114,125]

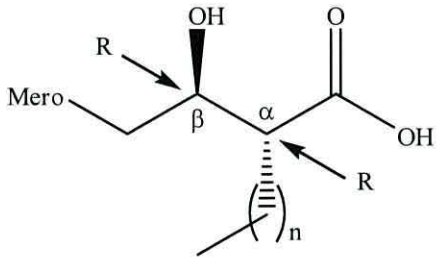
Watanabe *et al.* used MALDI spectrometry to study mycolic acids (both major and minor components) present in 19 strains of the *M. tuberculosis* complex. Combining this new methodology with CID mass spectroscopy they succeeded in locating, precisely, the functional groups in the meromycolate moiety of different type of mycolic acids.[103] In this study, the pyrolysis of mycolic acid methyl ester, at 300 °C to give meromycolaldehydes and carboxylic acid methyl esters, and subsequent oxidation of the meromycolaldehydes with silver nitrate to give the corresponding meromycolic acids (**Scheme 2).**



Scheme 2: Preparation of meromycolic acid

1.4.3-Stereochemistry of mycolic acids

The stereochemistry of the chiral centres contained in mycolic acid has still not been completely clarified. The two stereochemistries at the α- and β-position of the mycolic motif have been found to be both in the *R*-configuration for all mycolic acids examined, irrespective of the groups in the meromycolate chain.[126,127,128,129] The (*R,R*)-configuration was confirmed first for the corynomycolic acids (**22, Fig. 11**).[130,131] The formation of a hydrogen bond between the hydroxyl group and the carboxylic group has a stabilising effect for the aligned configuration between the two long chains.[132,133]



22

Fig. 11: The chiral centres in the β-hydroxy fatty acid moiety

The configuration at these two chiral centres is believed to play an important role in T cell recognition,[134] and the generation of an immune response by the host organism against pathogenic mycobacteria;[134] the same is also true for the antitumour properties of mycolic acid derivatives.[135]

Recent stereochemical studies suggested that, in the hydroxy (**23**), methoxy (**24**), and keto (**25**) groups in the mycolic acids, the methyl branch adjacent to the oxygenated functions is in the *S*-configuration (**See Fig. 12**). The formation of the wax ester (**26**) is also believed to be via an enzymatic oxidation of the *S*-keto-mycolic acid.[122,136]

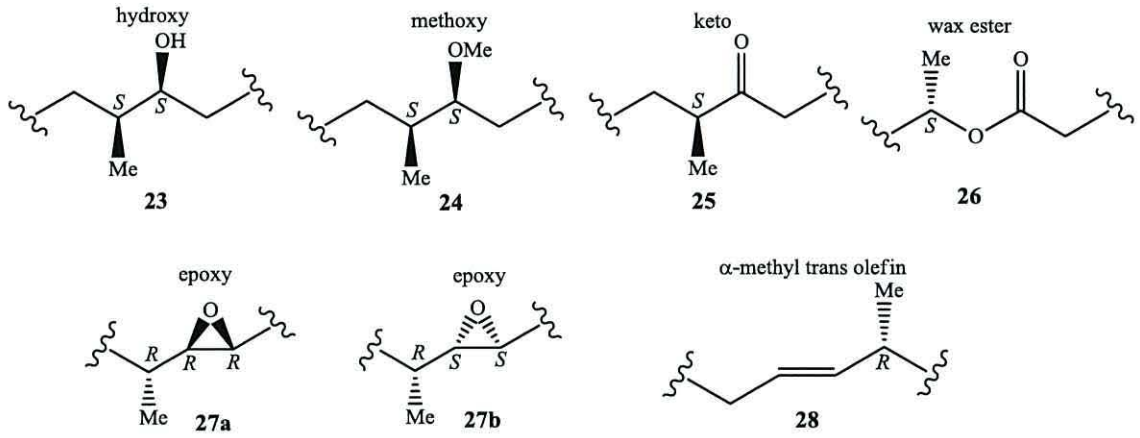


Fig. 12: The stereochemistry of some chiral centres of the mycolic acids

Other reports identify *R*-stereochemistry for the three stereocentres of the α-methyl-*trans*-epoxy unit in related mycolic acids (**27a**, **27b**).[136,129] Furthermore, the methyl branch next to the *trans*-alkene unit (**28**), present in mycolic acids is in the *R*-confugration.[136]

The determination of the chiralities of this functional group has been derived through comparison with a simpler, established compound, and subsequent modelling of the extent to which additional chiral centres would have changed the degree of optical rotation of the entire molecule.[129] The stereochemical results have also been obtained by fragmenting mycolic acids into smaller sub-units which could be compared with known compounds.[136] However, a report discussed a synthetic strategy targeting multiple diastereomers of the α-methyl-*trans*-cyclopropane unit. After analysis of the optical rotation, ^{1}H NMR and ^{13}C NMR, in comparison with work carried out by Anderson *et al*. and Al Dulayymi *et al.* deduced that the α-methyl-*trans*-cyclopropane unit is found in the *S,R,S*-configuration (**See Fig. 13**).[137, 138]

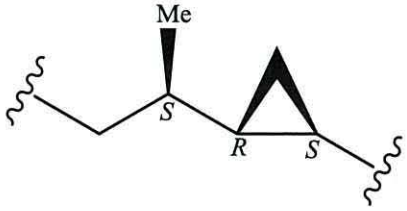


Fig. 13: α-Methyl-*trans*-cyclopropane mycolic acid configuration

1.4.4-Roles of different groups in the mycolic acids

The kinds of mycolic acids and their relative abundance depend upon growth conditions.[139,140,141] It has been discovered that the functionality present in the mycolic acid plays a role in the fluidity of the cell envelope and permeability and virulence of the pathogenic *Mycobacterium*.[142,143] Cyclopropanation occurs in slow-growing pathogenic mycobacteria like *M. tuberculosis*; it does not occur in environmental ones, such as *M. smegmatis*.[116] Cyclopropane fatty acids are less sensitive than unsaturated lipids to ozonolysis and other oxidative treatment.[144] In the biosynthetic growth of *trans*-cyclopropane containing mycolic acids a higher ratio of oxygenated mycolates are recorded, which provides evidence to suggest that *trans*-cyclopropanes and oxygenated functionalities are biosynthetically related.[116] Keto and methoxy mycolates are also critical for the virulence of mycobacteria.[145,146,147] Keto-mycolic acids have been shown to play key roles in the virulence and in regulating the fluidity of the cell envelope of *M. tuberculosis*.[145,146] It is also known that slow-growing pathogens such as *M. tuberculosis*, are able to manipulate the ratios between methoxy and keto-mycolates in order to adapt better to the environment. This enables the pathogen to control the permeability and fluidity of the cell envelope.[145] It is also known that keto-mycolic acids behave differently to α-mycolic acids when a high surface pressure is applied to the monolayer. Therefore, it is believed that keto-mycolic acids play an important role in permeability of the cell envelope.[148] In particular, it has been observed that keto-mycolates have an essential role in the growth of the organism within the natural host cell.[146] Conversely, it has been documented that loss of the methoxy-mycolic acids does not have an adverse affect on the pathogen permeability and hence its resistance to antibiotics.[149]

cis-Alkenes in the meromycolate chain have an effect on the packing, making the mycolates in the cell envelope less fluid. *trans*-Alkenes do not cause as much disruption in the chain, leading to tighter packing of the mycolic acids in the cell envelope.[104] This is confirmed by the fact that, in some mycobacterial species, the level of *trans*-alkenes rises if the *Mycobacterium* grows under increased temperature.[143] However, further understanding of the effects that functionality has on the fluidity and permeability of the cell envelope may give an insight into possible anti-*Mycobacterium* treatment.[146]

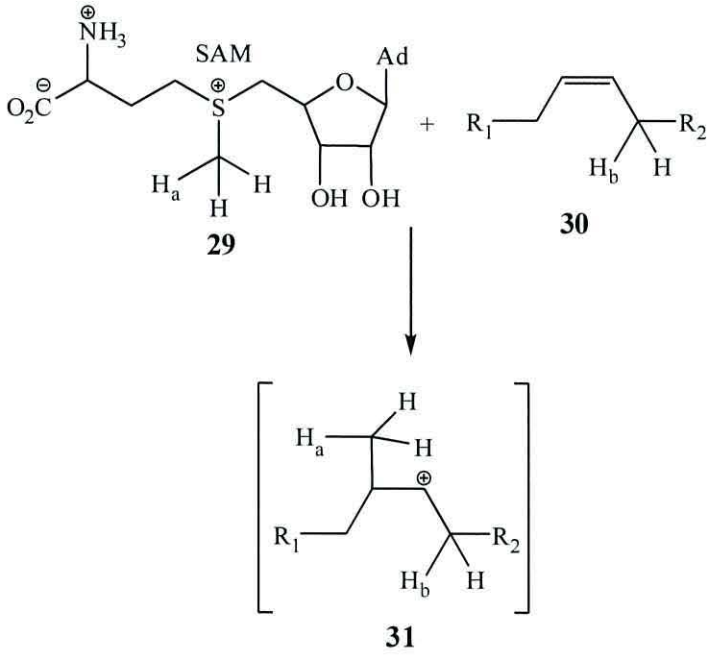
1.4.5-Biosynthesis of mycolic acids

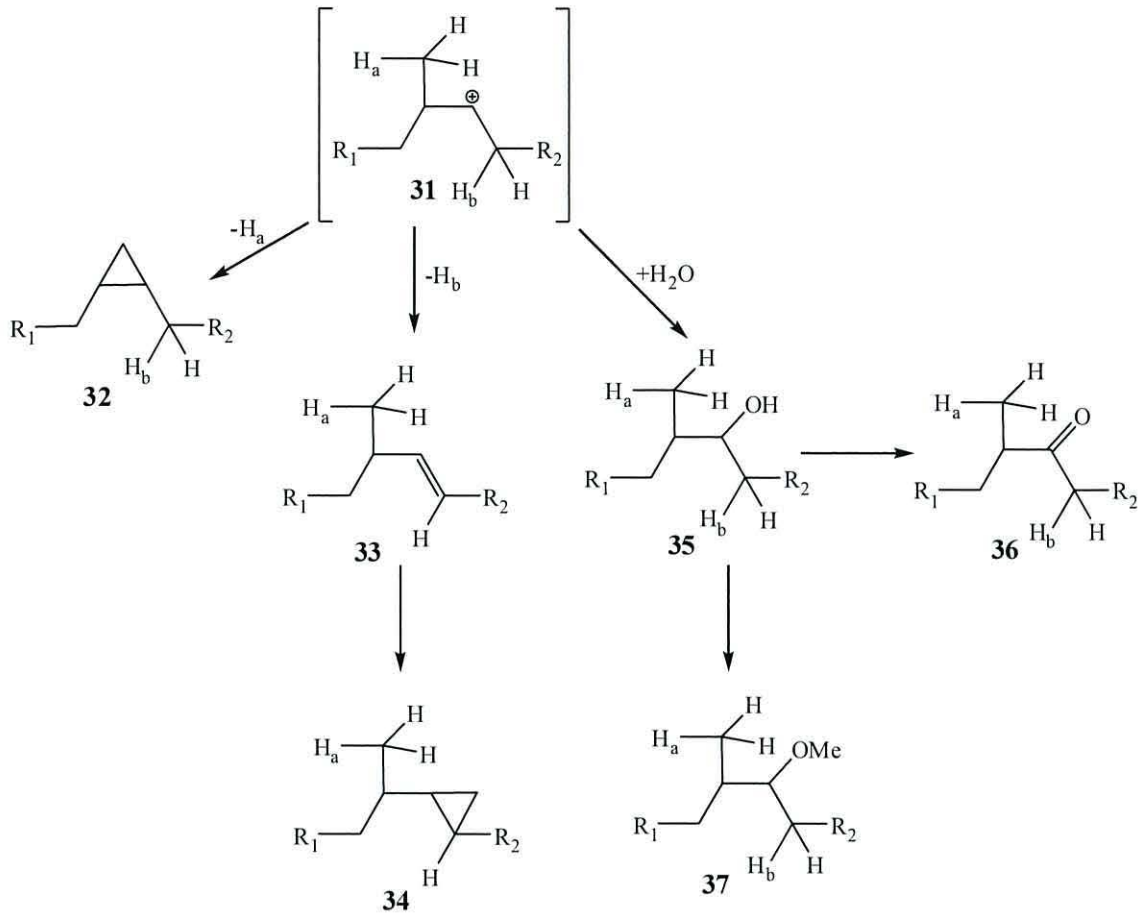
The biosynthesis of mycolic acids can be described in four steps: (a) synthesis of C_{24}-C_{26} straight chain saturated fatty acids to provide C_1 and C_2 atoms and an α-alkyl chain;

(b) synthesis of the backbone of meromycolic acids of C_{40}-C_{60}; (c) modification of meromycolate chain to introduce functional groups other than β-hydroxy; and (d) the final condensation step to produce mycolic acids. Many enzymes involved in catalyzing different steps in the biosynthesis of these molecules are targets to develop mechanism based antituberculosis drugs.[150,151]

Several hypotheses proposing different mechanisms for the processes of cyclopropanation, oxygenation and methylation have been put forward. Experiments in which mycobacteria are grown in the presence of labelled methionine indicate that the methyl group of methionine can become incorporated directly into mycolic acids. It has been shown that the bridging methylenes of the cyclopropane ring, the carbon of the methoxy functionality, and the methyl branches adjacent to *trans*-olefins, methoxy and keto moieties are all derived from methionine, presumably by S-adenosyl-*L*-methionine (SAM).[123,136,152,153]

Methylation of a *cis*-alkene (**30**), using SAM (**29**), gives the carbocation intermediate (**31**) involved in functionalization of the meromycolate chain and futher reaction to yield the various substituents found in mycobacterial mycolic acids (**Scheme 3)**.





Scheme 3: The insertion of the non-oxygenated functional groups in mycolic acids

Addition of a methyl group from SAM generates the carbenium ion shown which can then be deprotonated to form a *cis*-cyclopropane (**32**). The removal of a proton from the methyene group in the α-position yields the α-methyl-*trans*-olefin unit (**33**). The *trans*-olefin could be the substrate for a second SAM-dependent methylation to form the α-methyl-*trans*-cyclopropane unite (**34**) seen in the oxygenated mycolates of *M. tuberculosis*. If the carbocation intermediate (**31**) undergoes a hydration reaction the hydroxy-mycolate (**35**) is formed, which is a precursor for the biosynthesis of the corresponding methoxy (**37**) and keto (**36**) mycolates.[104,147] (**Scheme 3**).

With the discovery of the cyclopropane synthases in *M. tuberculosis*, it has been demonstrated that the proteins involved in the formation of functional groups require S-adenosyl-*L*-methionine as cofactor.[154] This is of particular importance as it confirms the hypothesized biosynthetic relationship between the different functionalities. The biosynthesis of mycolic acids is of great importance as this provides us with a better understanding of the stereochemistry of mycolates. Conversely, through a more accurate analysis of the stereochemistry of the chiral centres present in the meromycolate chain,

important information about the biosynthesis of these compounds might also be acquired.

1.4.6-Mycolic acids folding

Grant *et al.*[155] suggested a possible reason for the stronger recognition by T cell receptor of oxygenated mycolic acids. They suggested that keto and methoxy-mycolates fold in a way that allows the three polar functions of the lipid chain to be in proximity and to form epitope, acting as a site for recognition within the immune system (**Fig. 14**).

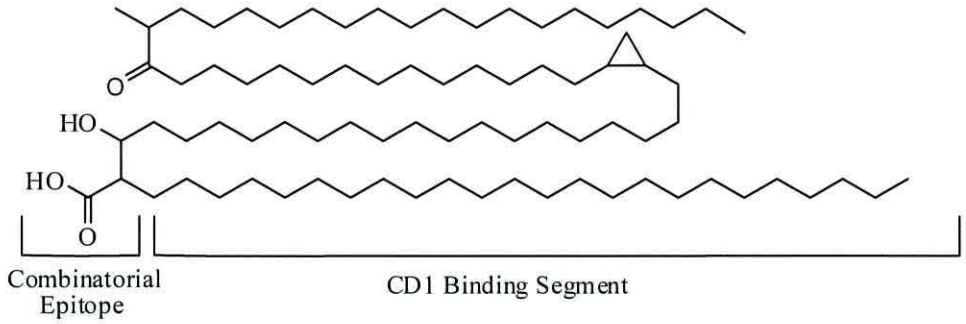


Fig. 14: Modified from Grant *et al.*[155]

Villeneuve *et al.*[156,157] both discussed the changes which occur in the configuration of the alkyl chains when put under varying temperatures and pressure. This suggests that at low temperature and pressure the folded conformation observed above (**Fig. 14**) is retained, however as temperature and pressure is increased this folded conformation is lost.

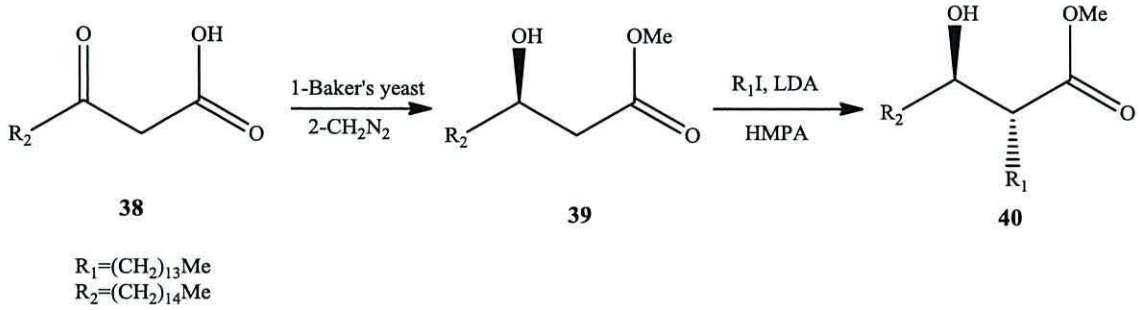
Finally, synthetic mycolic acids may be utilised for the preparation of a simple model of the multi-layer structure present in the *M. tuberculosis* cell wall. This method has already been used to determine the relationships between monolayer properties and the chemical structures of different natural types of mycolic acids.[158,159] The importance of mycolic acid folding has been revealed by cryo-electron microscopy,[160,161] which showed the presence of a distinct mycobacterial outer membrane. To correlate with the dimensions of this outer membrane, folding of mycolic acids is necessary.[161]

1.4.7-Previous syntheses of corynomycolate analogues

Lederer *et al.* described the first synthesis for this kind of compound in 1952.[162] Thesame group, followed by others, using similar methods based on the Claisen condensation, prepared other corynomycolate analogues, but always as a mixture of diastereoisomers.[163,164]

Subsequently, Kitano *et al.*[165,166] synthesized an enantiomerically pure corynomycolate. This method was a key feature of the preparation optically active compounds with the correct stereochemistry in the α-position.

Utaka *et al.*[167] described an approach, in which initially there was the introduction of the hydroxyl group at the β-position by a stereoselective reduction of the β-keto ester (**38**) with Baker's yeast (**Scheme 4**).[168] This approach used a Fräter reaction,[169] where the hydroxyl group in the molecule (**39**) forced the insertion of the alkyl chain with the correct configuration. The α-alkyl-β-hydroxy carboxylate (**40**) was directly obtained with the correct chain length and with the two chiral centres in the correct configuration.



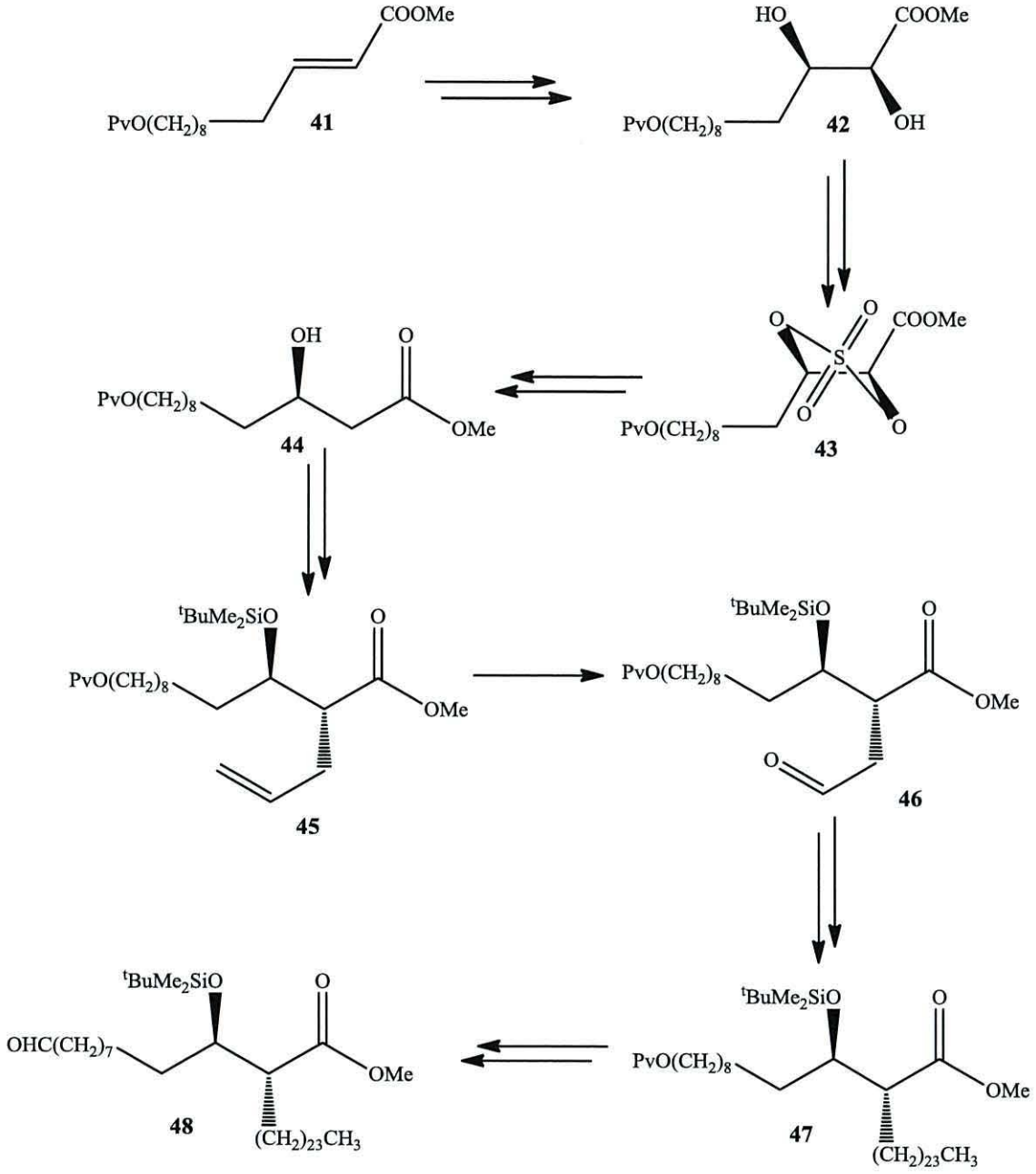
38 **39** **40**

$R_1=(CH_2)_{13}Me$
$R_2=(CH_2)_{14}Me$

Scheme 4: The Utaka *et al.* method

Recently, a slightly different method for the preparation of corynomycolate analogues has been reported.[170] In this method for the synthesis of the α-alkyl β-hydroxy unit (**48**), a short chain allyl iodide is used as an alkylation agent and then chain extended using a Julia-Kocienski olefination. This reaction starts from very simple materials to prepare the *E*-α,β-unsaturated ester (**41**).[170] After four steps this is transformed into the diol (**42**) using a Sharpless dihydroxylation.[171] The diol is converted into the sulfate (**43**) and then regioselectively reduced and hydrolysed to give the β-hydroxy ester (**44**).

Subsequently, a Fräter alkylation[169] with allyl iodide introduced an allyl chain at the α-position and the hydroxyl group was protected to give alkene (**45**). The interesting aspect to this new method of synthesis is that the generation of the aldehyde (**46**) allows the introduction of any chain length desired at the α-position (**Scheme 5**).



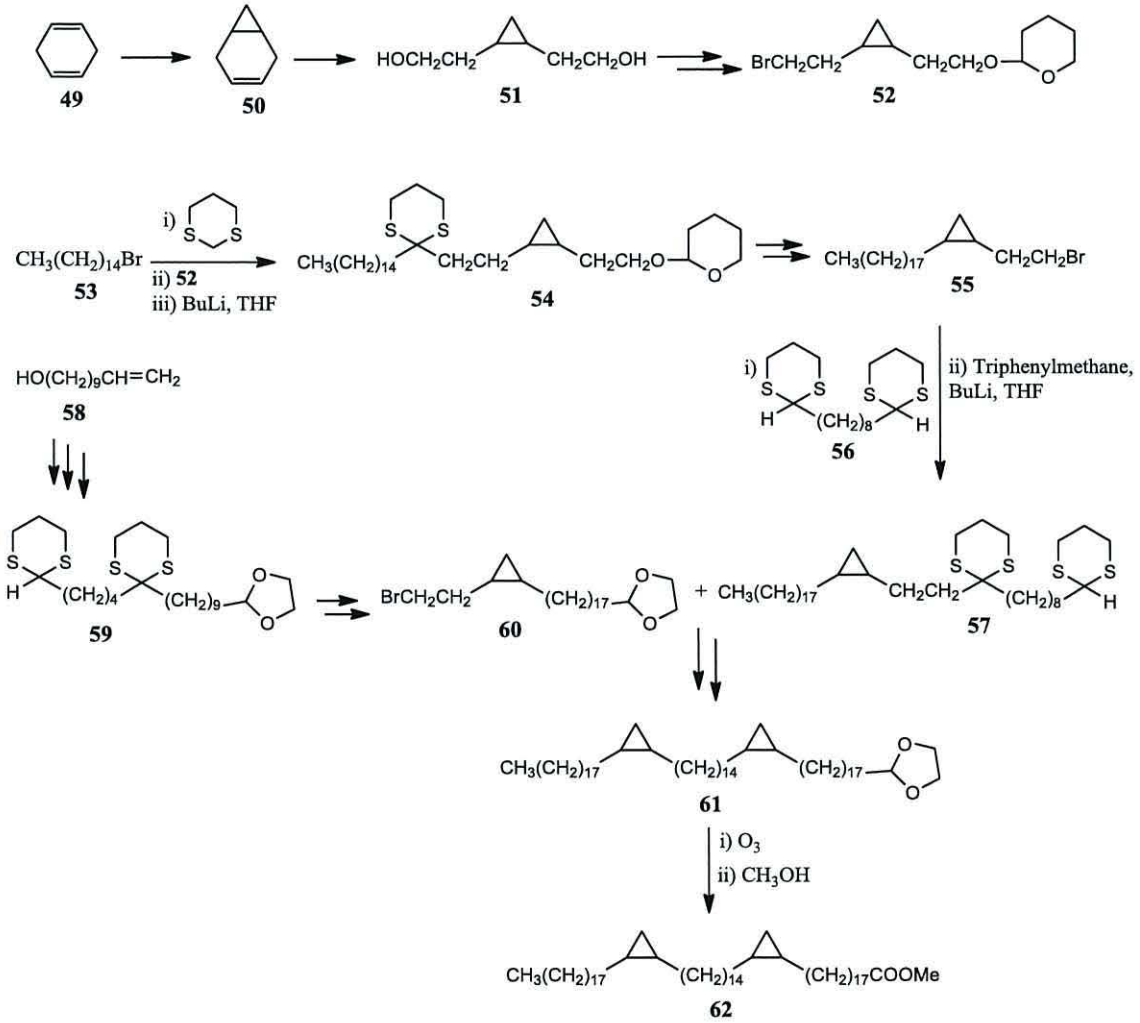
Scheme 5: An improved method to prepare the mycolic acid moeity

Also another method for the introduction of this hydroxyl group into the β-position with the required chirality has been recently reported by Al Dulayymi *et al.*[172] (See later, **Scheme 9**).

1.4.8-Previous synthesis of meromycolic acids

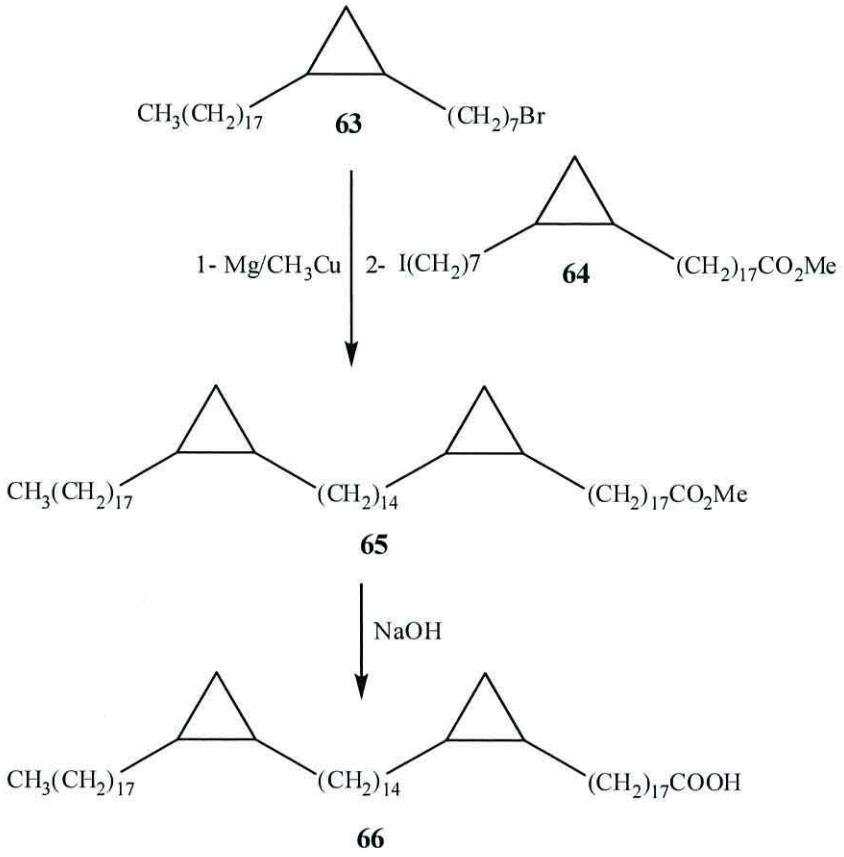
Meromycolic acid without stereochemical definition was first synthesised by Gensler *et al.* in 1977.[173] Norcarene (**50**), was prepared from 1,4-cyclohexadiene (**49**), followed by reduction of the ozonide from norcarene (**50**) to diol (**51**).[174] The diol (**51**) was protected with a tetrahydropyranyl group and bromination then gave the cyclopropane containing

bromide (**52**). The cyclopropane (**55**) was formed by alkylation of pentadecyl bromide (**53**) with (**52**), desulfurization with Raney nickel,[175] hydrolysis and bromination. Further chain extension using the bisdithiane (**56**) gave (**57**), called "methyl end", which was one of the two major parts making up the meromycolic acid product.[173] The synthesis that was realized started with the ozonolysis of 10-undecenol (**58**), to 10-hydroxydecanal, after many stepsconverted into the protected compound (**59**) which in an alkylation reaction again with (**52**) led to the second intermediate (**60**) called "the carboxyl end" of the meromycolic acid. Coupling of the lithio derivative of bisdithiane intermediate (**57**) with alkyl bromide (**60**), followed by desulfurization gave the expected product (**61**). Ozonolysis of (**61**) gave methyl meromycolate (**62**) containing two *cis*-cyclopropane rings[173] (**Scheme 6**).



Scheme 6: The first synthesised diastereomeric mixture of meromycolic acids

Subsequently, Gensler *et at.*[176] provided the second approach to the meromycolic acid (**66**), which combined different fragments. The method is relatively short and it could be easily scaled up (**Scheme 7**).

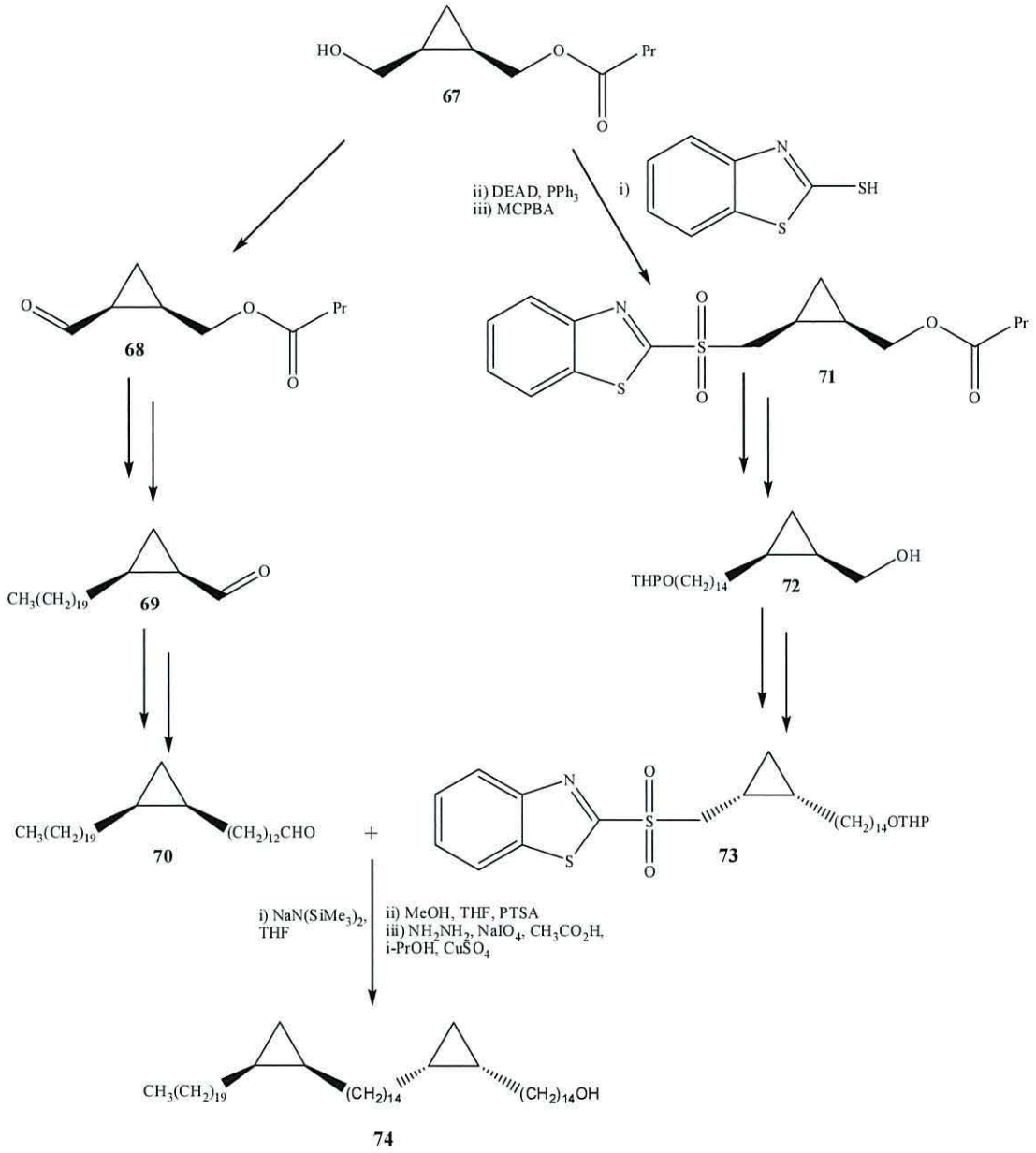


Scheme 7: The second Gensler *et al.* approach to meromycolic acids

The Grignard reagent prepared from the alkyl bromide (**63**) on reaction with the alkyl iodide (**64**) yielded the desired product (**66**). This method is an improvement over the first approach, also by Gensler, but still presents some problems because the final coupling gives a very poor yield and gives several other compounds and does not provide any control over the absolute stereochemistry.

Recently, another approach for the synthesis of a single enantiomer of an analogue of meromycolic acid was reported by Al Dulayymi *et al.*[177] They set about preparing single enantiomers of cyclopropane intermediate, then successfully coupled the intermediates together with no loss of stereochemistry. The aldehyde (**68**) was derived from the anhydride of cyclopropane-*cis*-1,2-dicarboxylic acid.[178] A Wittig reaction of this with nonadecyltriphenylphosphonium bromide and *n*-butyl lithium, and reduction by lithium aluminium hydride gave alcohol as a mixture of *Z*- and *E*-isomers. The alkene was saturated by di-imide and oxidation of the alcohol led to aldehyde (**69**). A second Wittig reaction was coupled between (**69**) and phosphonium salt, followed

saturation and oxidation led to the aldehyde (**70**). A Julia reaction of sulphone (**71**) with 13-tetra-hydropyranyloxy-tridecanal were prepared protecting alcohol (**72**), further converted into the sulphone (**73**). The important feature of this method is the coupling reaction, which is used to link the different units in many stages, securing the final desired stereochemistry. The enantiomer (**74**) was prepared from the Julia reaction between aldehyde (**70**) and sulfone (**73**) as a mixture of *E* and *Z*-alkenes. The subsequent deprotection and saturation of the alkene with di-imide to give the enantiomerically pure alcohol (**74**) (**Scheme 8**).



Scheme 8: Al Dulayymi *et al.* approach

Also in recent studies it has been reported that various meromycolic acids which contain an α-methyl-*trans*-cyclopropane unit, have been synthesised.[137,179] The meromycolates (**76**, **77**, **78**, **Fig.15**) are derived from ω-carboxy-mycolic acid. These methods offer important advantages: a better overall yield and the control of the absolute stereochemistry.

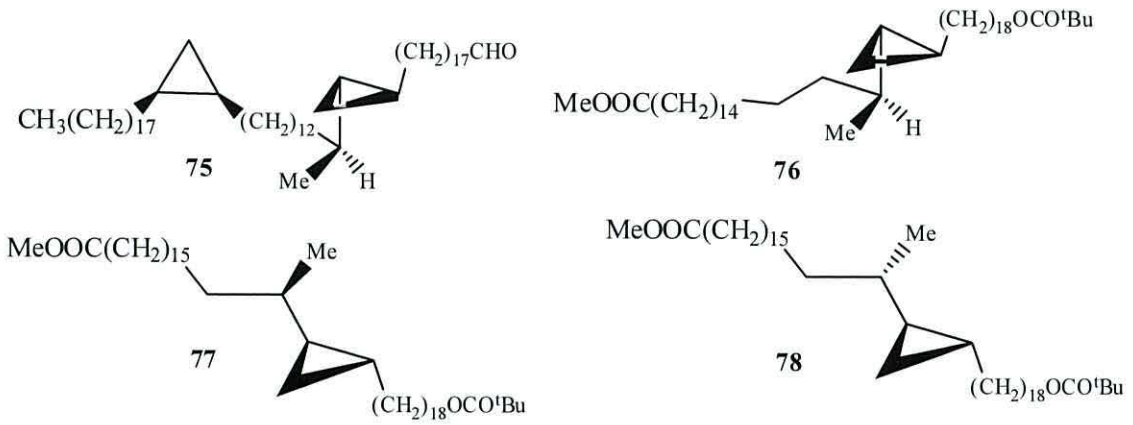
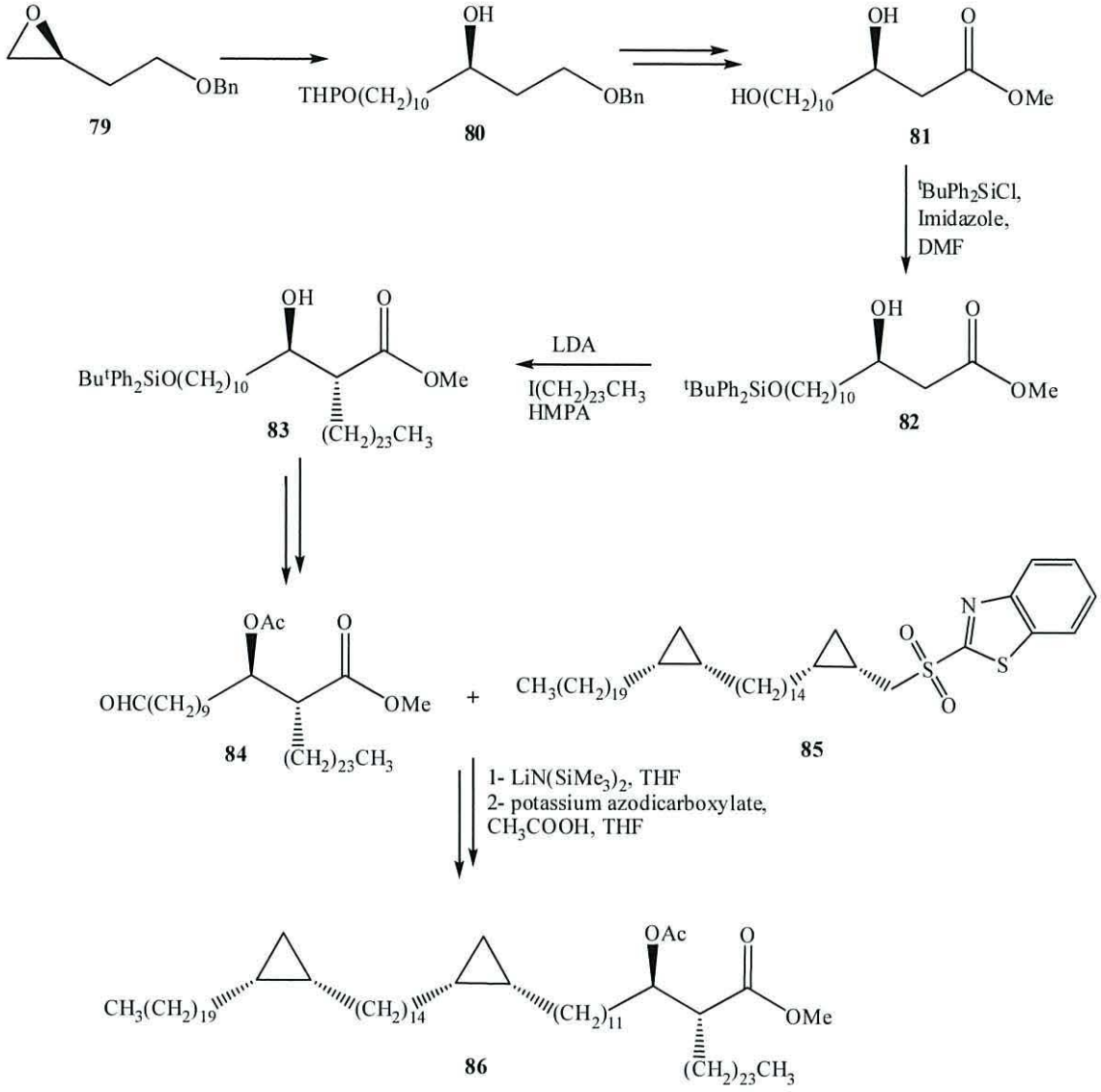


Fig. 15: Meromycolic acids containing α-methyl-*trans*-cyclopropane

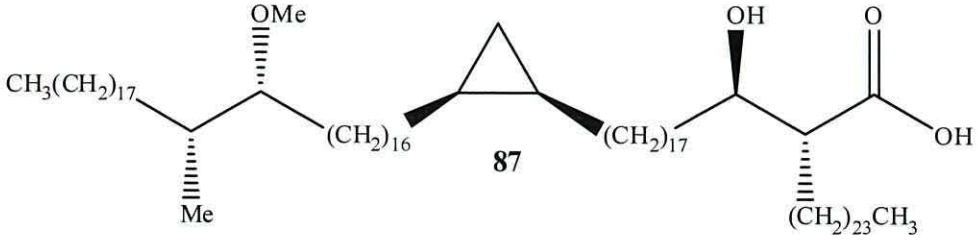
1.4.9-The synthesis of whole mycolic acids

Al Dulayymi *et al.*[172] described the synthesis of a single enantiomer of a major α-mycolic acid of *M. tuberculosis*. Ring opening of the epoxide (**79**)[180] with a Grignard reagent prepared from 9-bromononan-1-ol tetrahydropyranyl ether led to a single enantiomer of the monoprotected diol (**80**). This was transformed, in a few steps, to the diol (**81**) (**Scheme 9**).[181] The next step was the protection of the primary alcohol and the alkylation of the α-carbon to give the hydroxy ester (**82**). Protection of the secondary alcohol in (**83**) as the acetate, deprotection of the primary alcohol and oxidation led to the aldehyde (**84**), which was coupled to the dicyclopropane sulfone (**85**) in a modified Julia reaction to give the protected α-mycolic acid (**86**).



Scheme 9: A synthesis of an α-mycolic acid by Al Dulayymi *et al.*[172]

Most recently, the synthesis of a series of three stereoisomers of a complete methoxy mycolic acid (**87**, **88**, **89**, **Fig.16**) was reported.[182] This type of molecule is also isolated from *M. tuberculosis*,[103,107] and the different effects biological of these acids and of their stereoisomers may be determined.



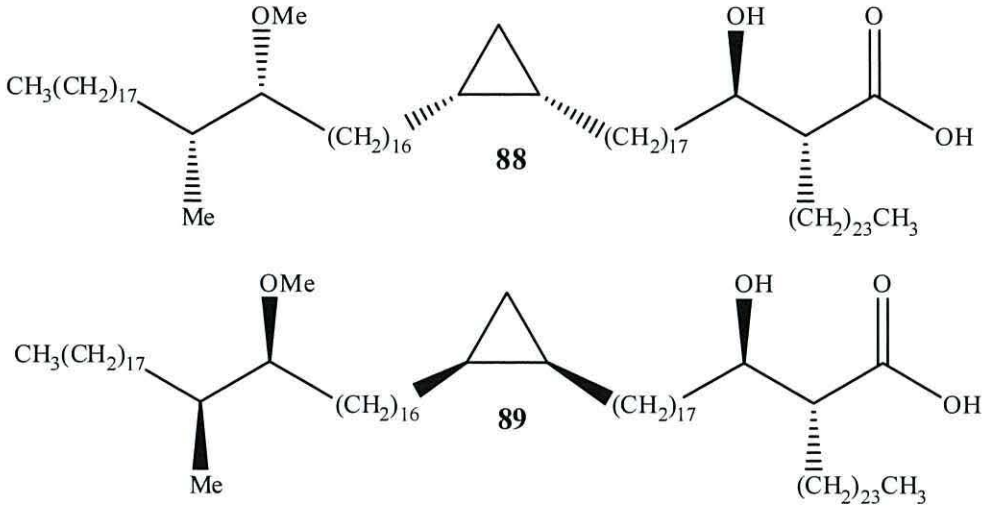


Fig. 16: Synthetic methoxy-mycolic acids by Al Dulayymi *et al.*[182]

Another approach for the synthesis of ketomycolates containing both α-methyl-*trans* and *cis*-cyclopropane fragments (**90** and **91**), can be adjusted to produce a variety of absolute stereochemistries and chain lengths (**Fig.17**).[183]

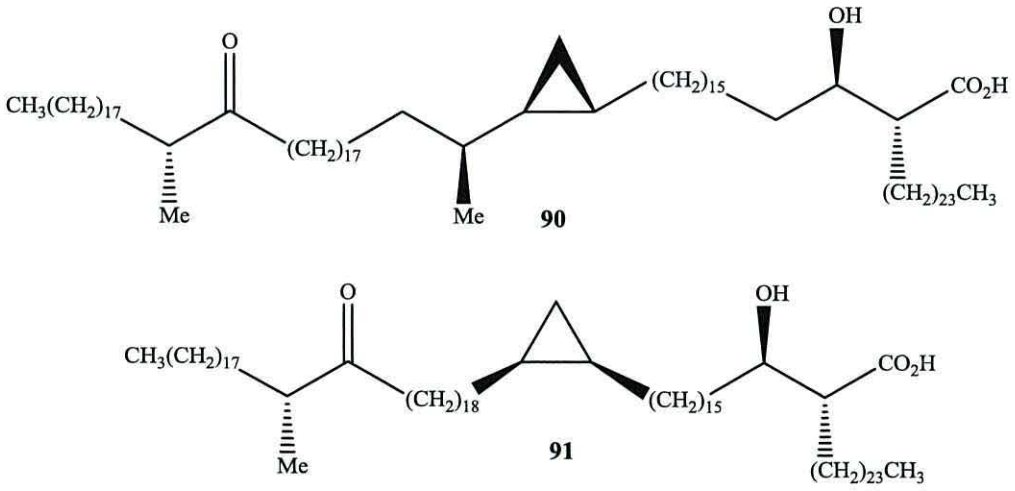
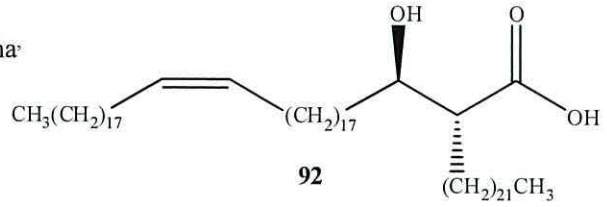


Fig.17: Synthesis keto-mycolic acids by Koza *et al.*[183]

Baird *et al.* have made a considerable contribution to the area of complete synthesis of mycolic acids, where they have published several routes to obtain enantiomerically pure mycolic acids. Same examples of which are shown below. (**Fig. 18**).[182184,185,186,187]

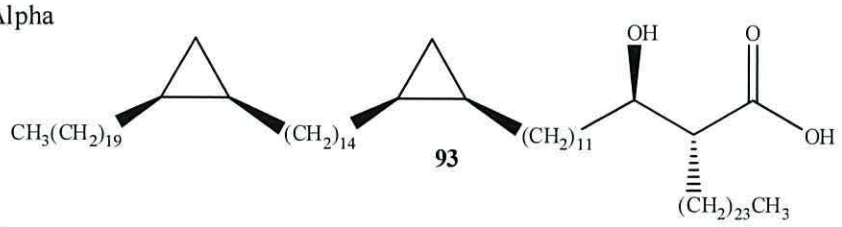
Alpha



92

Muzael, M. *et al.*[184]

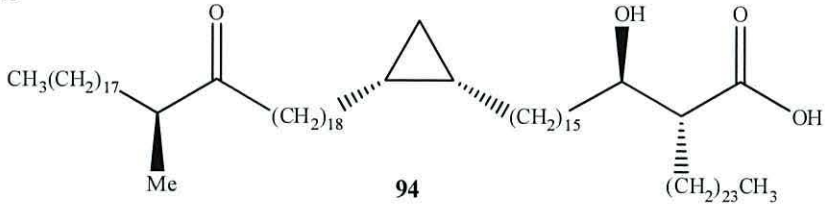
Alpha



93

Al Dulayymi *et al.*[186]

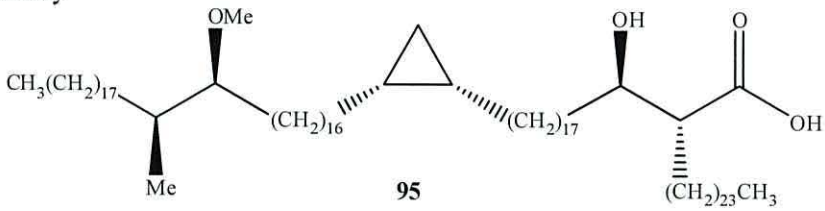
Keto



94

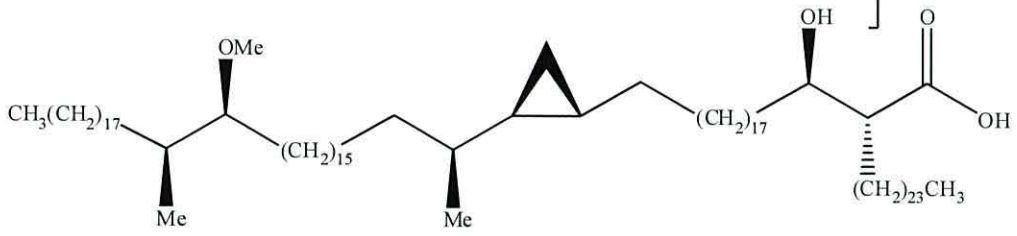
Koza, G. *et al.*[185]

Methoxy



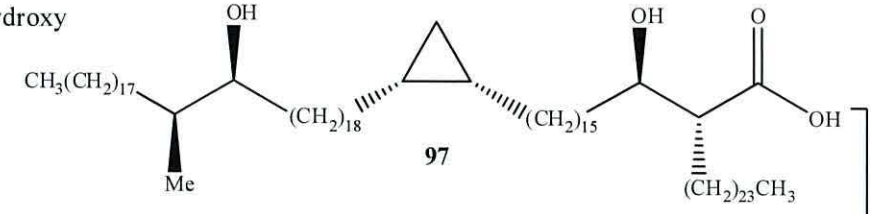
95

Rowels, R.[187]



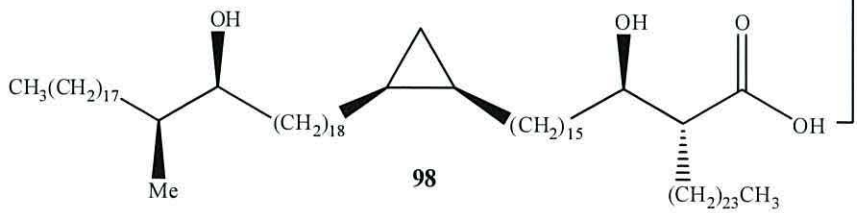
96

Hydroxy



97

Koza, G. *et al.*[185]



98

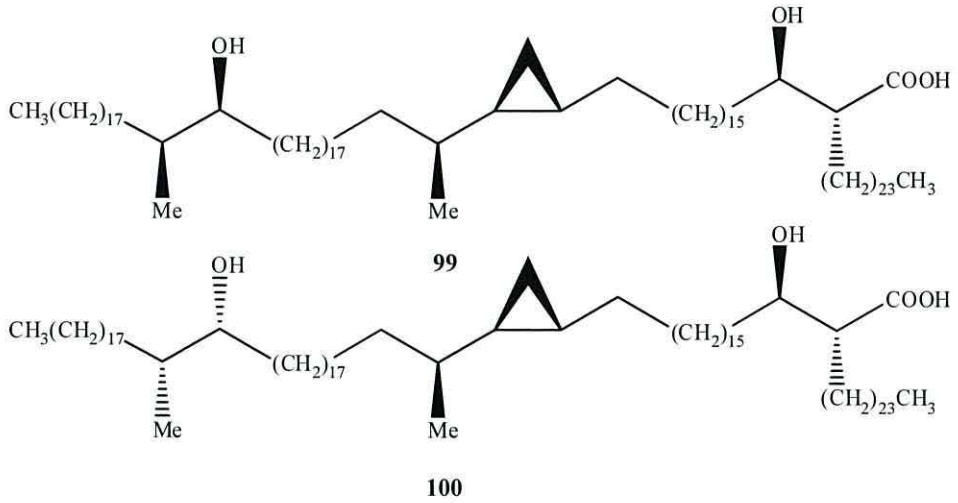
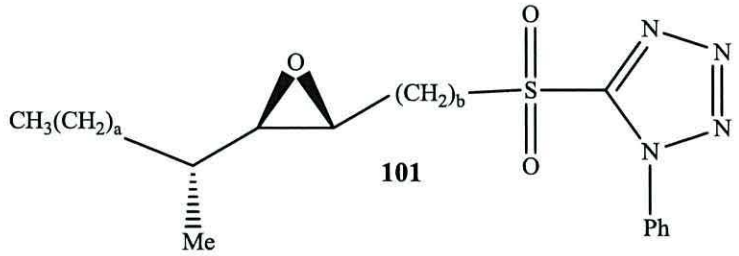


Fig. 18: Synthesis of mycolic acids

2. Results and Discussion

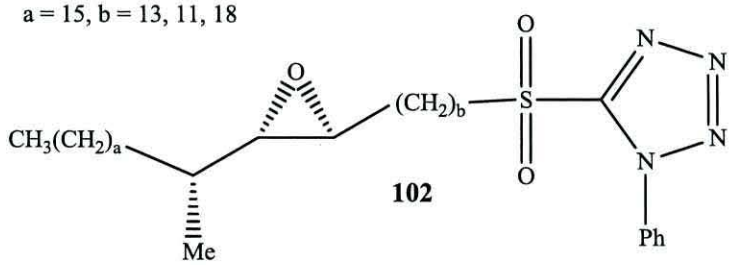
2.1- Aim of the project

This project consists of four parts. The aim of first part was to synthesise (*R,R*)-epoxy sulfone (**101**) and (*S,S*)-epoxy sulfone (**102**) (**Section 2.2**). The second part was to synthesise (*R,R*)-epoxy-*trans*-alkene-mycolic acid (103) and (*S,S*)-epoxy-*trans*-alkene-mycolic acid (**104**) (**Section 2.3**). The target for this part of the project is an epoxy-mycolate present in the cell wall of *Mycobacterium fortuitum* (**Fig. 19**). The third part was to synthesise (*S,R*)-*cis*-cyclopropane (*R,R*)-epoxy-mycolic acid (**105**) (**Section 2.6**) and (*S,R*)-*cis*-cyclopropane (*S,S*)-epoxy-mycolic acid (**106**) (**Section 2.7**) from *Mycobacterium smegmatis*. The fourth part was to synthesise (*E/Z*) (*R,R*)-epoxy-alkene mycolic acid (**107**) and (*E/Z*) (*S,S*)-epoxy-alkene mycolic acid (**108**) from *M. smegmatis* (**Fig. 19**) (**Section 2.8**). The synthesis of these molecules will be important for the identification of their exact structures and the stereochemistry of natural mycolic acids as well as in studying their biochemical properties.



101

a = 15, b = 13, 11, 18



102

a = 15, b = 13, 11, 18

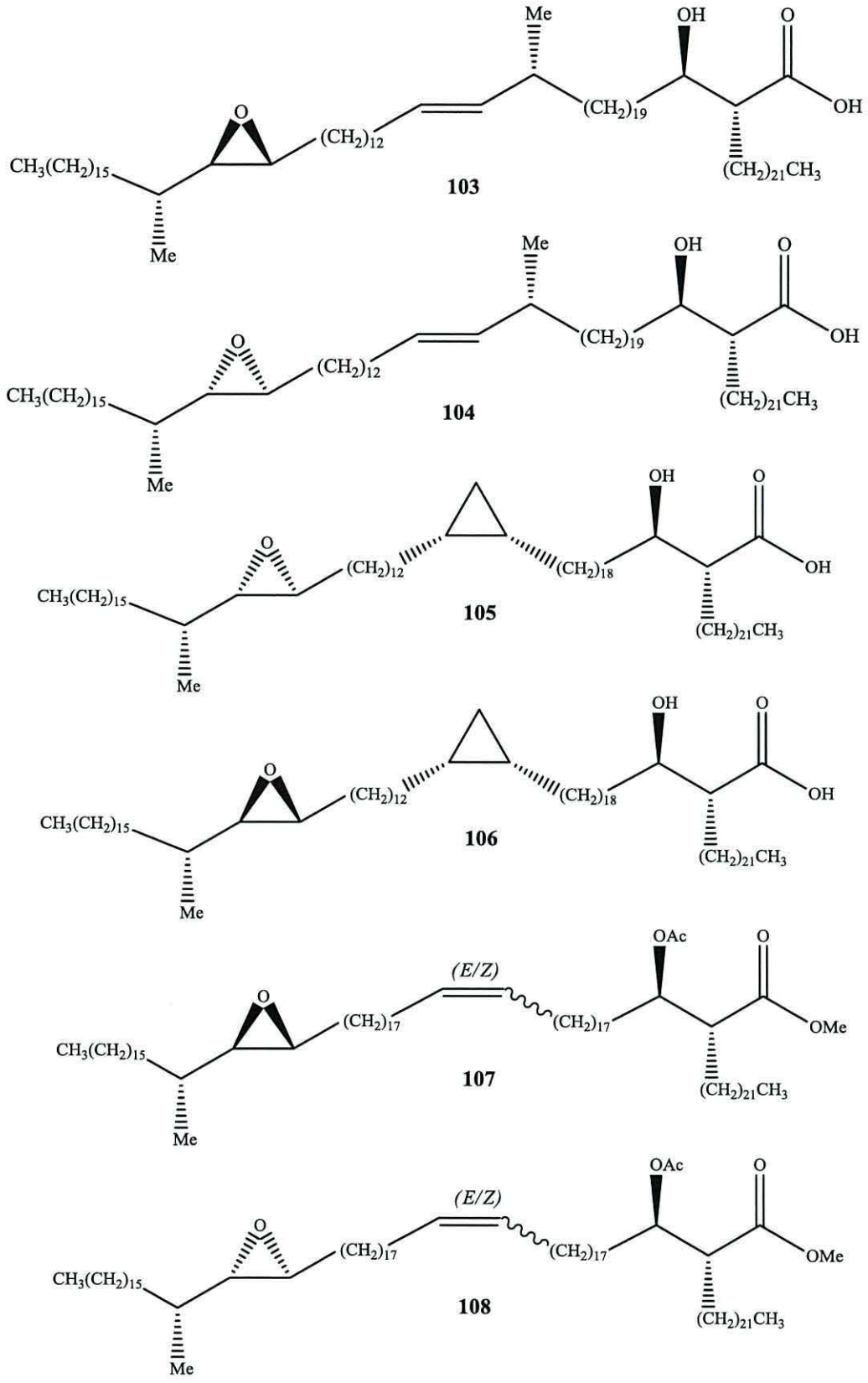


Fig. 19: The target mycolic acids

2.2-The synthesis of epoxy sulfones (157 / 158)

In order to synthesise complete epoxy mycolic acids, it was first necessary to make the epoxy sulfones (**157**) and (**158**) (**See Figure 20**).

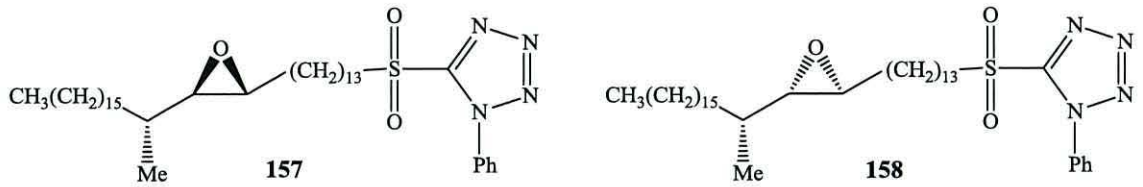
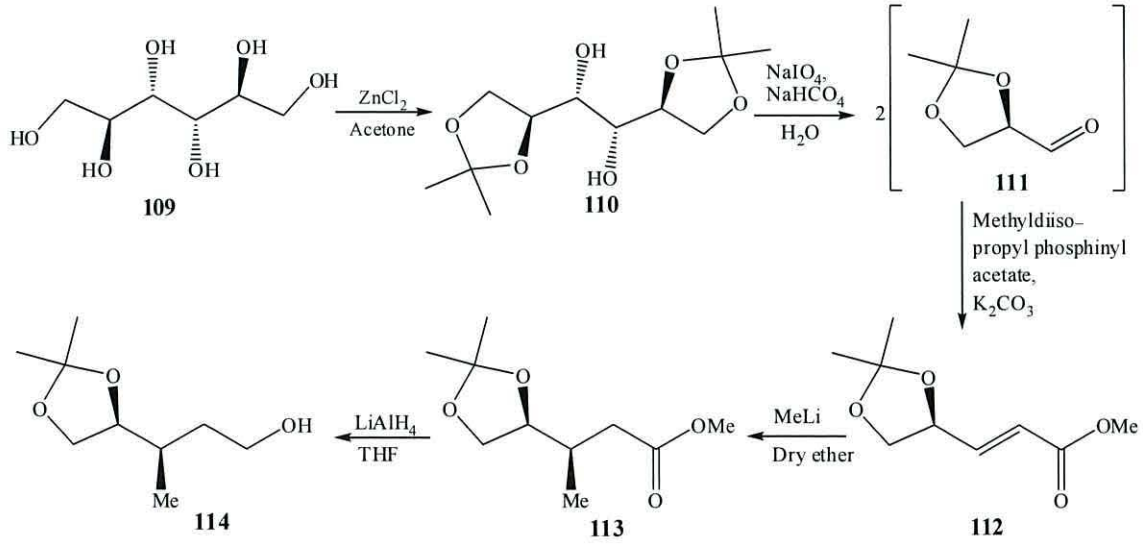
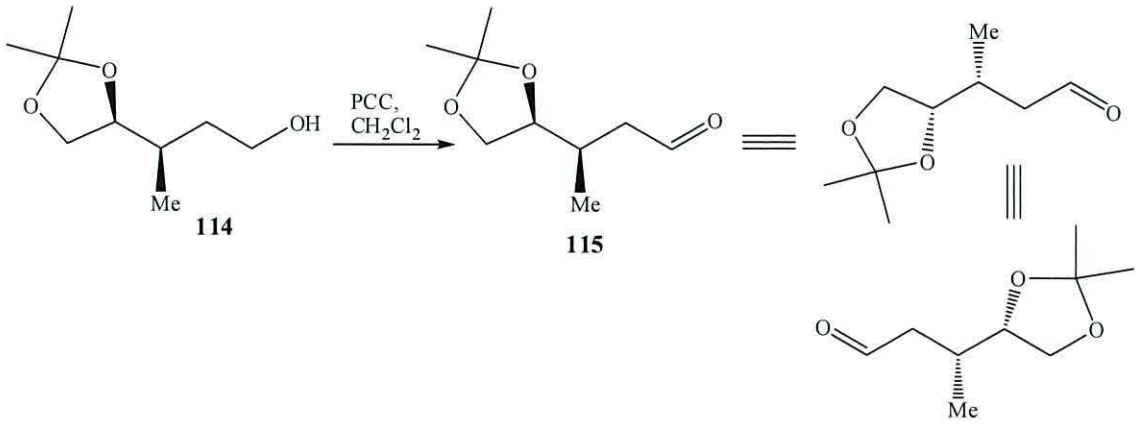


Fig. 20: The epoxy sulfones (**157**) and (**158**)

2.2.1- Preparation of α-methyl-β-hydroxy unit

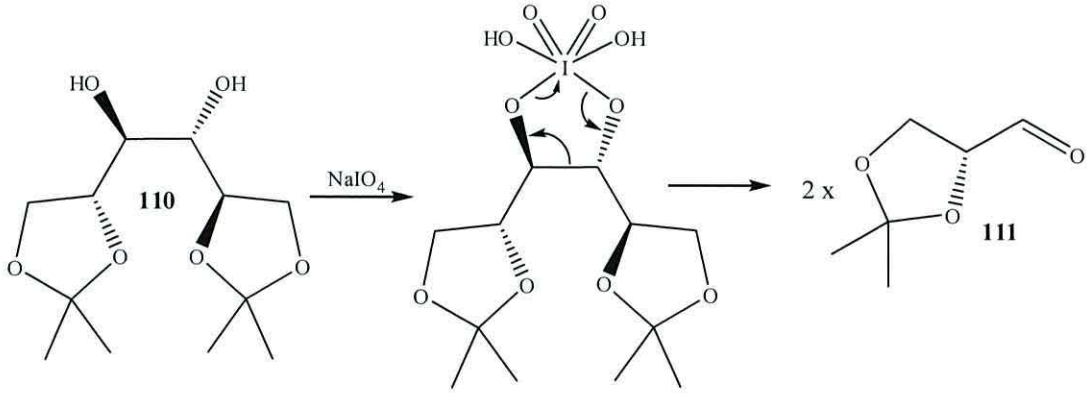
D-Mannitol (**109**), a naturally occurring cheap polyhydroxy compound, was used as a starting material for the *R*-form of the molcule. *D*-mannitol (**109**) was protected as 1,2:5,6-di-*O*-isopropylidene-*D*-mannitol (**110**) in 55 % yield by using acetone in the presence of anhydrous zinc chloride and employing the literature method.[188,189] The 1,2-diol (**110**) was cleaved with sodium metaperiodate in aqueous sodium hydrogen carbonate to give the intermediate glyceraldehyde acetonide (**111**)[190] and due to the instability of this aldehyde it was reacted immediately with methyldiisopropyl phosphinylacetate and aqueous potassium carbonate to give the α,β-unsaturated ester (**112**)[191,192] in 72 % yield via a Horner-Emmons reaction.[190,193,194] The major product was *E*-alkene and a very small amount *Z*-alkene was separated by chromatography **(Scheme 10)**.





Scheme 10: Preparation of the aldehyde (**115**) from (**109**)

The oxidative cleavage is believed to go by the following mechanism (**Scheme 11**).

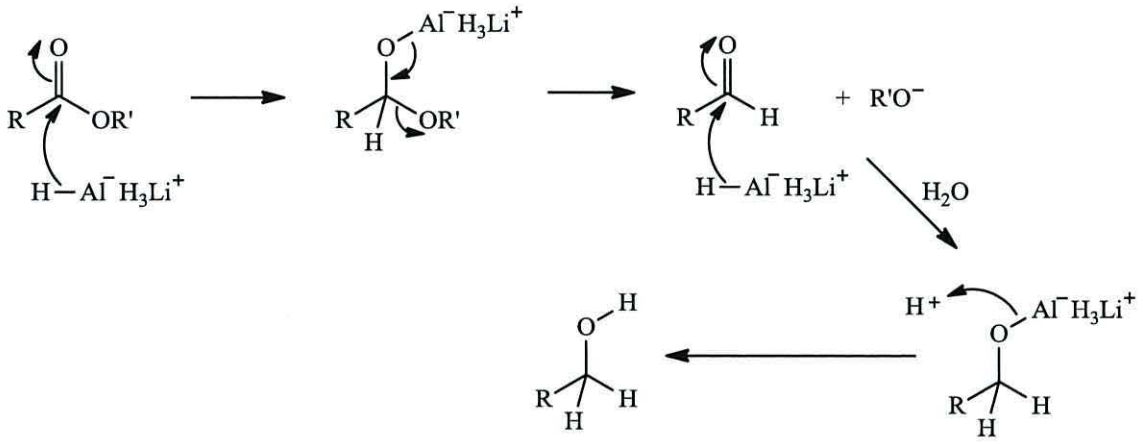


Scheme 11: Mechanism of oxidative cleavage of (**110**)

In the ^{1}H NMR spectrum of (**112**), the olefinic signals appeared as two doublets of doublets at δ 6.77 (*J* 5.68, 15.7 Hz, vicinal and *trans* coupling constant) and δ 5.99 (*J* 1.26, 15 Hz, allylic and *trans* coupling constant), respectively. The methyl group of the ester showed a singlet at δ 3.62. The ^{13}C NMR showed a signal at δ 109.96 for the acetal carbon and one at δ 166.2 for the carbon of the carbonyl group.

The diastereoselectivity of conjugate additions to α,β-unsaturated ester derived from glyceraldehydes is known.[195] The α,β-unsaturated ester (**112**) was treated with methyl lithium in diethyl ether at - 78 °C to give *syn*-product, (*R*)-3-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-butric acid methyl ester (**113**) in 68 % overall yield. The ^{1}H NMR spectrum showed a doublet at δ 0.9 (*J* 6.65 Hz) for the methyl group. The carbon NMR spectrum showed signals at δ 172.8 for the carbonyl carbon and at δ 108.6 for the cyclic acetal carbon. The IR spectrum showed a broad signal at ν_{max} 1739 cm^{-1} for the C=O stretch.

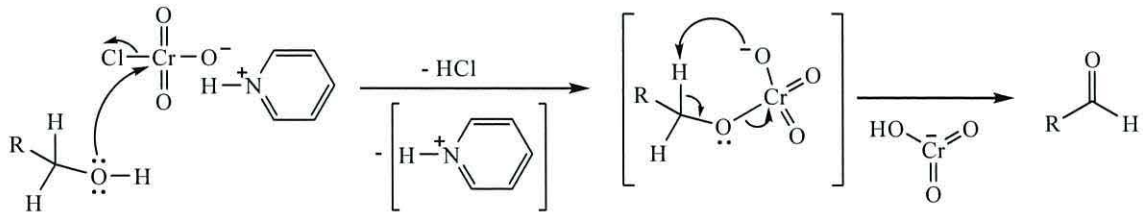
The ester (**113**) was reduced with lithium aluminium hydride in THF, and quenched with saturated aqueous sodium sulfate decahydrate to give alcohol (**114**) in a yield of 97 %. The ^{1}H NMR spectrum of the hydroxyl group ($C\underline{H}_2OH$) of alcohol (**114**) appeared as a multiplet including a triplet at δ 3.56–3.5 and the IR spectrum showed a broad band at ν_{max} 3418 cm^{-1} for the OH stretch. In the $LiAlH_4$ reduction, the ester was first reduced to give aldehyde and using another equivalent of $LiAlH_4$ to give another metal alkoxide complex, and when quenched was protonated on the alkoxide oxygen, producing the alcohol **(Scheme 12)**.



Scheme 12: The mechanism of $LiAlH_4$ reduction of esters

Finally, oxidation of the alcohol (**114**) with pyridinium chlorochromate (PCC) in dichloromethane gave aldehyde (**115**). The ^{1}H NMR spectrum showed a triplet at δ 9.67 (*J* 1.9 Hz) for the aldehyde proton and the ^{13}C NMR a signal appeared at δ 201.4 for the carbonyl carbon.

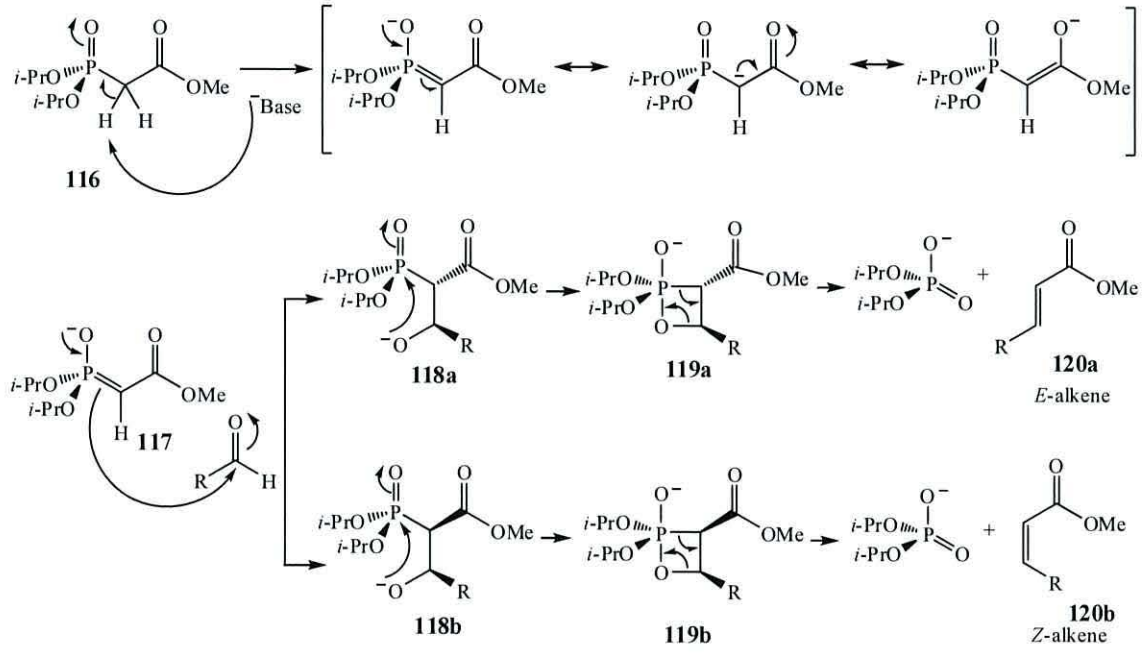
PCC was used to oxidize the primary alcohol to the aldehyde; the mechanism of oxidation by PCC is believed to be as follows **(Scheme 13)**.



Scheme 13: The mechanism of oxidation by PCC

2.2.2-The Horner-Wadsworth-Emmons reaction

Aldehydes or ketones react with stabilized phosphorus ylides to give *trans*-alkenes in excellent selectivity. The Horner-Wadsworth-Emmons reaction begins with the deprotonation of the phosphonate to give the phosphonate carbanion (**117**), whose resultant resonance effect gives it its stability. Nucleophilic addition of the carbanion onto the aldehyde produces the intermediates (**118a**) and (**118b**),[196] and then these can interconvert to the intermediate (**119a**) and (**119b**). Finally, the fragmentation of (**119a**) and (**119b**) gives two isomers, *E*-alkene (**120a**) and the *Z*-alkene (**120b**).[197] (**Scheme 14**). However, the reaction favours for formation of the *trans*-alkenes.



Scheme 14: Mechanism of the Horner-Wadsworth-Emmons Reaction

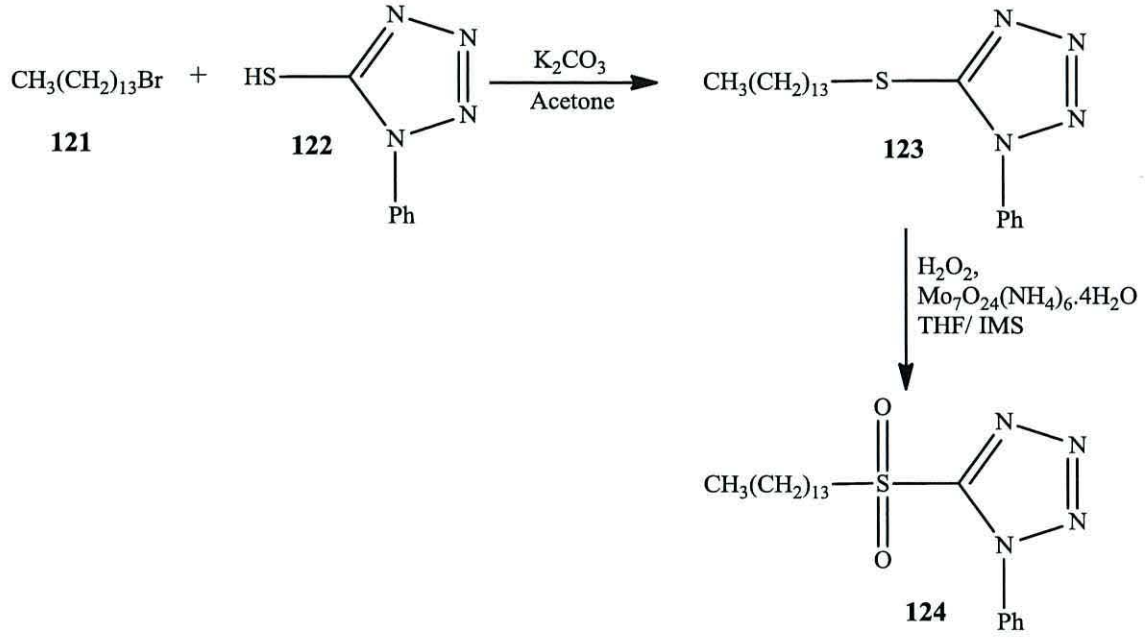
2.2.3-Preparation of 5-(tetradecane-1-sulfonyl)-1-phenyl-1*H*-tetrazole (124)

Firstly, the 5-tetradecylsulfanyl-1-phenyl-1*H*-tetrazole (**123**) was prepared by reaction of 1-phenyl-1*H*-tetrazole-5-thiol (**122**) with 1-bromotetradecane (**121**), using potassium carbonate and acetone. The crude product was purified by re-crystallisation from acetone and diluted with methanol to give a white solid in a good yield (**Scheme 15**).

The ^{1}H NMR spectrum of (**123**) showed a multiplet at δ 7.6–7.51 for the five aromatic protons, a triplet at δ 3.39 (*J* 7.25 Hz) for (-CH_2S-) and a triplet at δ 0.88 (*J* 6.6 Hz) for the methyl group. The ^{13}C NMR showed a signal at δ 154.5 for the carbon in the tetrazole ring, four the signals for the aromatic carbons at δ 133.8, 130.0, 129.7 and 123.8 and signals at δ 33.4 for the ($\underline{C}H_2$S) and at δ 14.1 for the methyl ($\underline{C}H_3$).

The subsequent oxidation of the sulfide with hydrogen peroxide in the presence of ammonium molybdate (VI) tetrahydrate in THF and IMS gave the sulfone (**124**) (**Scheme 15**).[182]

The ^{1}H NMR spectrum of the sulfone (**124**) included a multiplet at δ 7.72–7.69 (two protons aromatic), another multiplet at δ 7.65–7.58 (three protons aromatic), and a triplet at δ 3.73 (*J* 7.85 Hz) for the two protons (-C<u>H</u>$_2$SO$_2$-), which confirmed the success of the reaction. The ^{13}C NMR showed a signal at δ 56.0 for the (-<u>C</u>H$_2$SO$_2$-).



Scheme 15: Preparation of the sulfone (**124**)

The sulfone showed a distorted triplet at δ 3.73 for two protons (H_A and $H_{A'}$) adjacent to sulfonyl group. The signal observed is a characteristic 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. This can be shown by a Newman Projection, where H_A will show *cis*- splitting to H_B and *trans*- to $H_{B'}$ (**See Fig. 21**).

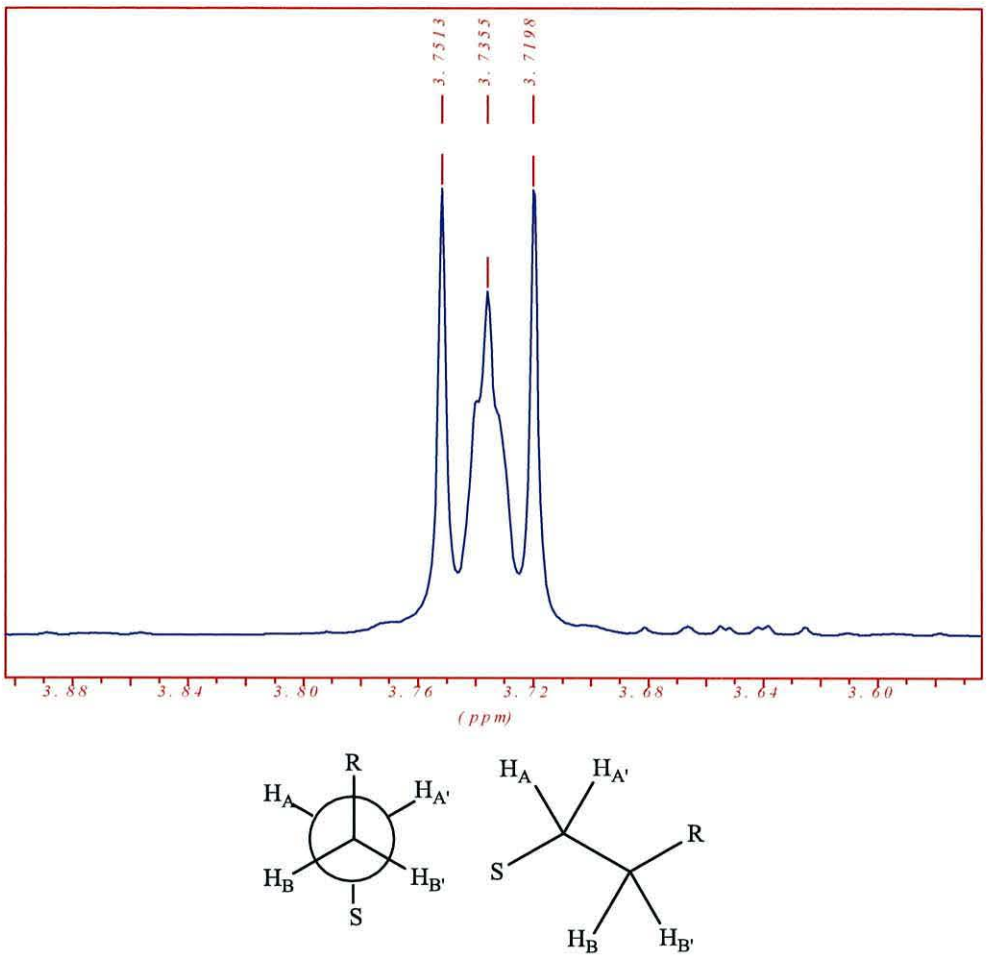
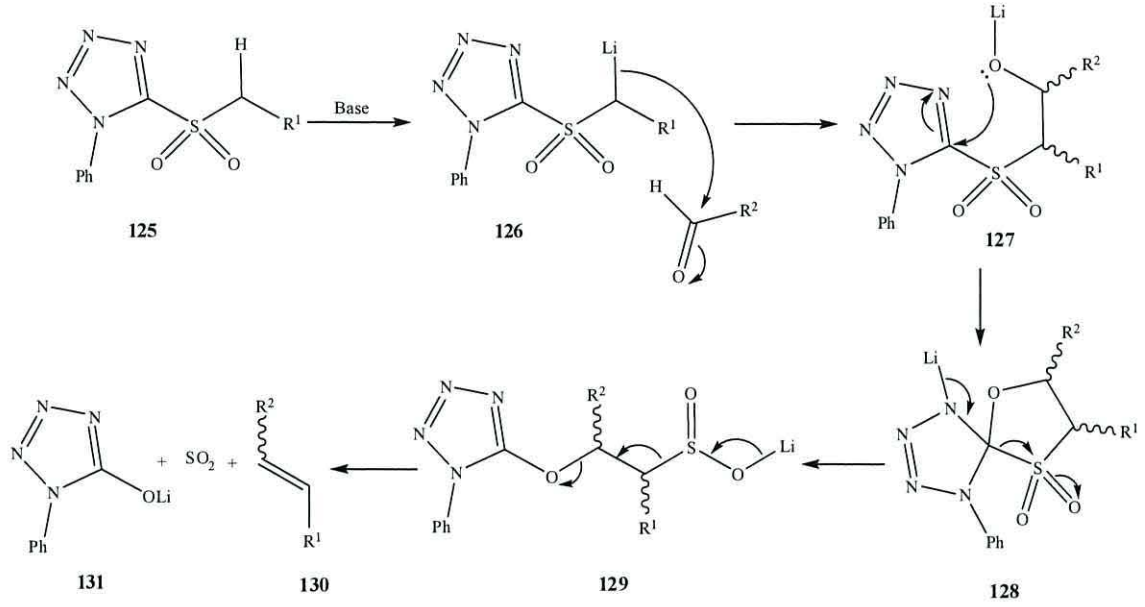


Fig. 21: The characteristic signal of the protons (H_A and $H_{A'}$) adjacent to a sulfonyl group

2.2.4-Mechanism of the Julia-Kocienski reaction

Marc Julia and Jean-Marc Paris first published the formation of an alkene using a phenylsulfone and aldehyde in a reaction now called the Julia olefination.[198] The method was later significantly developed by Kocienski *et al.* and the modification of this method has led to it becoming an important reaction in organic synthesis.[199]

The Julia reaction occurs between a hetroarylsulfone and a carbonyl compound.[200] In the classical Julia, with simple phenylsulfones, four different steps are required to obtain the double bond.[198] The sulfone (**125**) with an aldehyde (**115**) in the presence base makes the intermediate β-alkoxysulfone (**127**). However, this β-alkoxysulfone is inherently unstable and it therefore readily undergoes a Smiles rearrangement.[201] The rearrangement occurs via a possible spirocyclic intermediate (**128**) and results in the transfer of the hetrocycle from sulfur to oxygen to yield sulfinate salt (**129**). Spontaneous elimination of sulfur dioxide and lithium 1-phenyl-1*H*-tetrazole (131) from (**129**), then leads to thedesired (*E/Z*)-alkene (**130**, **Scheme 16**).[200]

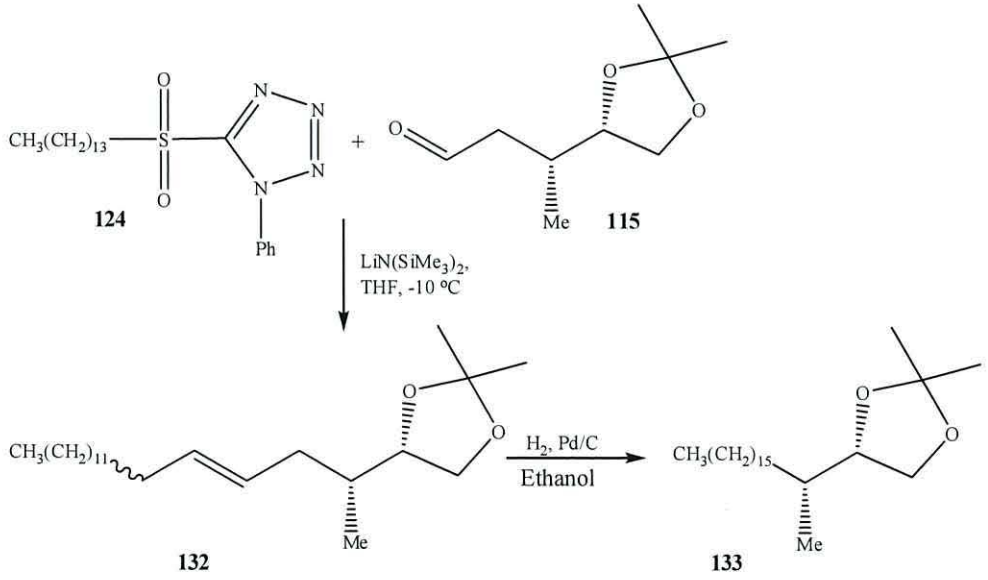


Scheme 16: Mechanism of Julia-Kocienski olefination

2.2.5-The Julia reaction between (124) and (115)

The aldehyde (**115**) was dissolved in dry THF and the sulfone (**124**) was added. The coupling was starting by addition of the base lithium bis(trimethylsilyl)amide at - 10 °C. Further, it was allowed to reach room temperature and stirred for 3 hours and worked up to complete the reaction. The product was formed as a mixture of *E*- and *Z*-alkene (**132**) stereoisomers in a ratio of 2:1 (**Scheme 17**).

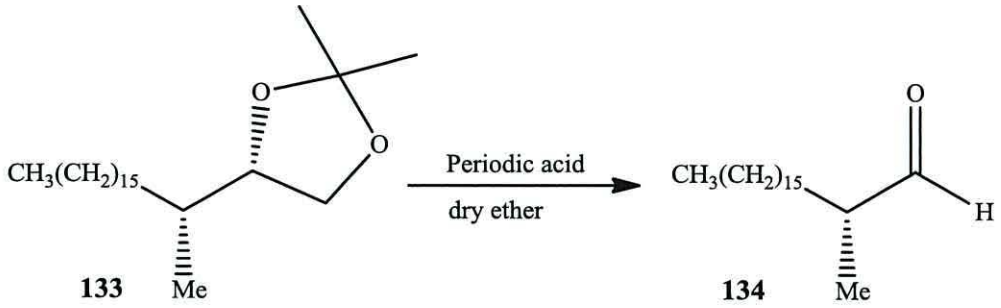
For hydrogenation, the (*E/Z*)-alkene was dissolved in ethanol and stirred with palladium on carbon (10 %) as a catalyst and hydrogen, to give a saturated compound (**133**). The proton and carbon NMR spectrum showed no signal in the olefin region. This confirmed the hydrogenation was complete.



Scheme 17: The Julia reaction between aldehyde (**115**) and sulfone (**124**) and formation of (**133**)

2.2.6-Preparation of the intermediate aldehyde

The compound (**133**) was oxidised with periodic acid in dry ether and cleavage of the cyclic acetal group led to the aldehyde (**134**) in 77 % yield (**Scheme 18**).



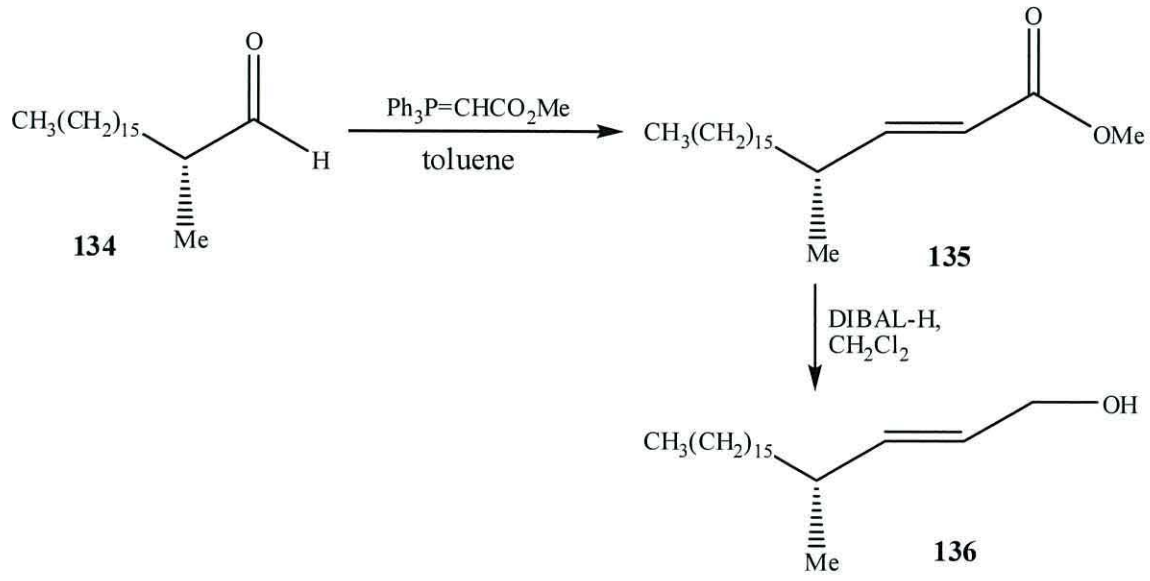
Scheme 18: Preparation of aldehyde (**134**) from (**133**)

The ^{1}H NMR spectrum showed a doublet at δ 9.6 (*J* 1.9 Hz) for the aldehyde proton. The ^{13}C NMR spectra showed a signal at δ 205.3 for the carbonyl carbon of the aldehyde.

2.2.7-Preparation of allylic alcohol (136)

The Wittig reaction between the (*R*)-2-methyl-octadecanal (**134**) and methyl (triphenylphosphoranylidene) acetate in toluene at room temperature gave α,β-unsaturated ester (**135**) mainly as the *E*-isomer[202,203] and the small amount of the *Z*-isomer was separated by column chromatography. The α,β-unsaturated ester (**135**) was then reduced to the corresponding allylic alcohol (**136**) with diisobutylaluminium hydride (DIBAL-H) in dry dichloromethane at - 60 °C because the reaction was extremely exothermic. The reaction was stirred overnight at room temperature and then quenched by adding saturated aqueous ammonium chloride at - 30 °C. Subsequently, hydrochloric acid (5 %) was added until it became a clear solution. The crude product was purified by column chromatography to give the allylic alcohol (**136**) in 95 % yield (**Scheme 19**). The ^{1}H NMR spectrum of compound (**135**) showed the expected olefinic signals as two doublets of doublets δ 6.86 (*J* 7.9, 15.45 Hz, vicinal and *trans* coupling constant) and δ 5.77 (*J* 0.95, 15.75 Hz, allylic and *trans* coupling constant) respectively. The high coupling constant proved the formation of the *E*-isomer (generally the coupling constant for (*E*)-alkenes is between 10 Hz and 18 Hz while for (*Z*)-alkenes it is between 6 Hz and 12 Hz).[204] The methyl on the ester appeared as a singlet at δ 3.72. The ^{13}C NMR spectrum included signals at δ167.3 for the carbon of the carbonyl group, 155.0 for (CH=CHCO$_2$Me) and 119.1 for (CH=CHCO$_2$Me). The ^{1}H NMR spectrum of compound (**136**) showed a multiplet at δ 5.62–5.57 for the olefinic protons (-CH=CH-)

and two protons (-C<u>H</u>$_2$OH) appeared as a doublet at δ 4.09 (*J* 5.05 Hz). The ^{13}C NMR spectrum confirmed the result because the carbon of the carbonyl group had disappeared and it showed two signals for olefinic carbons at δ 139.4, δ 127.0 and a signal at δ 63.9 for (<u>C</u>H$_2$OH). The specific rotation was $[\alpha]_D^{26} = -15.0$ (*c* 1.4, $CHCl_3$).



Scheme 19: Preparation of allylic alcohol (**136**)

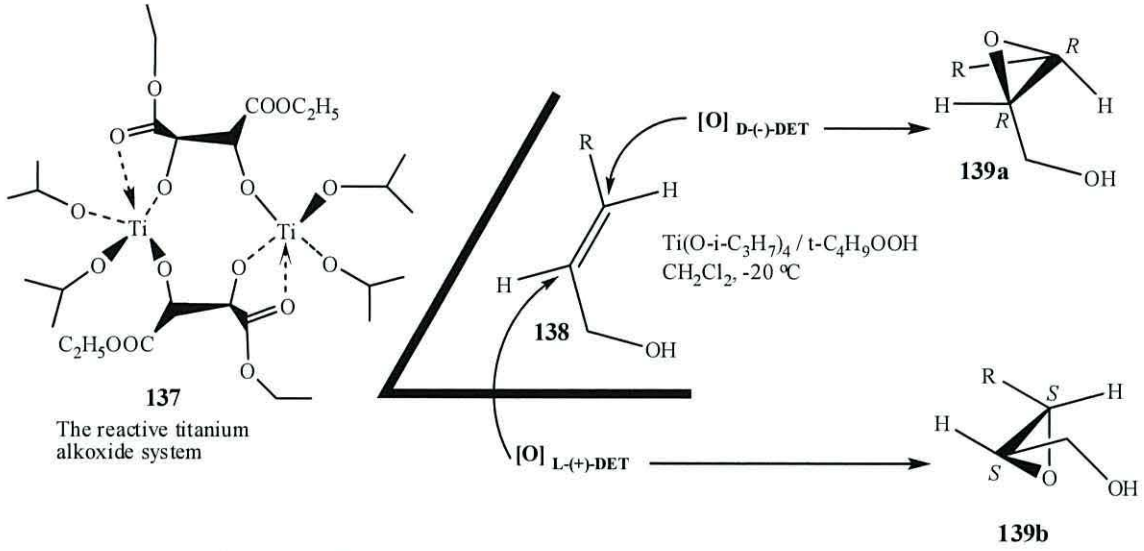
2.2.8-The Sharpless epoxidation

2.2.8a-Overview

This reaction was reported for the first time by Katsuki and Sharpless in the 1980s,[205] and it has been employed for the preparation of many natural compounds. Allylic alcohols are very reactive toward epoxidation by *tert*-butyl hydroperoxide in the presence of titanium tetraisopropoxide and (*R*,*R*)-diethyl tartrate or (*S*,*S*)-diethyl tartrate to give epoxides in high yield (50–90 %) and high with optical induction.[206,207] The mechanism of the epoxidation involves reaction of titanium tetraisopropoxide with tartaric esters to give a titanium (epoxo)(alkoxide) complex, which loses the oxygen atom as an epoxide and leaves a titanium alkoxide system. The catalytically active species is thought to be a dimer in solution (**137**, **Scheme 20**).[207]

This structure has not been completely established but the compound can coordinate the allylic alcohol and oxidant, at the titanium atom of the *tert*-butyl hydroperoxide and activate, to a certain extent, a coordinated alkylperoxide towards nucleophilic attack by the double bond of the allylic alcohol (**138**). The catalyst is sensitive to water and anhydrous reagents and conditions must be used.[208] The stereoselectivity is governed by the chiralty of the tartaric ester. If allylic alcohol (**138**) is drawn as in **Scheme 20**, the

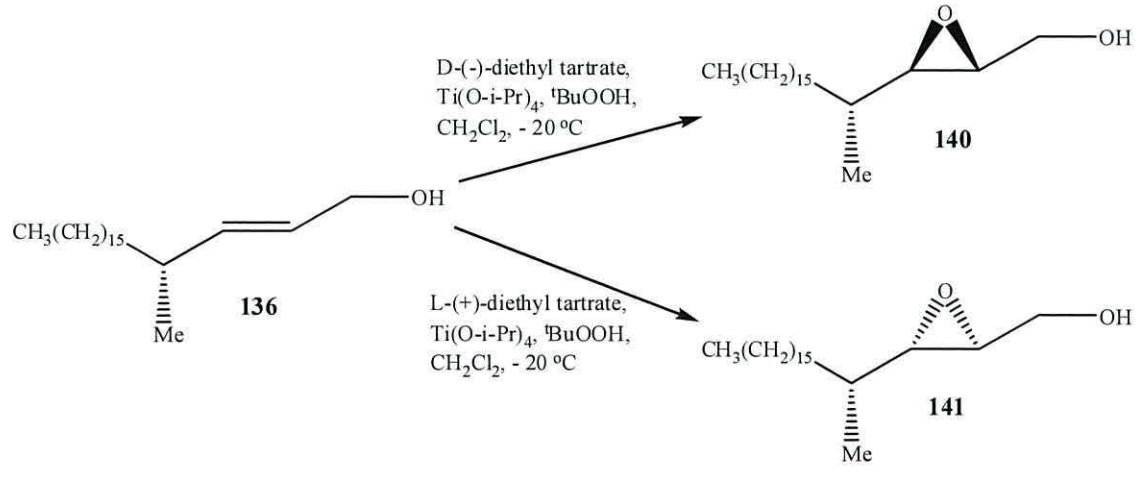
oxygen is delivered from bottom using L-(+)-diethyl tartrate, while if D-(-)-diethyl tartrate is used, the epoxide oxygen is added from the top. The reaction can thus give two different stereoisomers (**139a**) and (**139b**).[209,210]



Scheme 20: The general mechanism of the Sharpless epoxidation

2.2.8b-Sharpless epoxidation of (E)-(R)-4-methyl-eicos-2-en-1-ol

The allylic alcohol (**136**) was subjected to asymmetric Sharpless epoxidations with both D-(-)-DET and L-(+)-DET in order to understand the properties of the catalyst.[207,211] For this reaction a mixture was prepared of one of the diethyl tartrates and titanium tetraiopropoxide in dry dichloromethane in the presence of molecular sieves under nitrogen at - 20 °C and *tert*-butylhydroperoxide was added. The reaction with the alkene was allowed to continue for 4.5 hours, when TLC showed no starting material, after which it was left overnight at - 20 °C, and then quenched with water and a 30 % solution of sodium hydroxide, in aqueous saturated sodium chloride. The two reactions gave epoxy alcohols (**140**) and (**141**) in 67 and 75 % yield respectively.[203] (**Scheme 21**).



Scheme 21: The Sharpless epoxidation of the allylic alcohol (**136**) to epoxides (**140**, **141**)

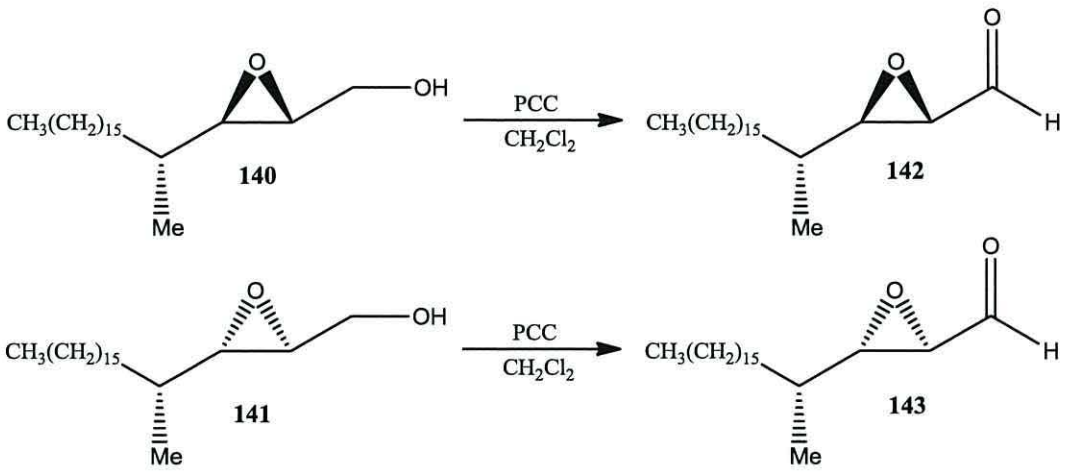
The ^{1}H NMR spectrum of (**140**) included a doublet of doublets of doublets at δ 3.9 (*J* 2.5, 5.56, 12.6 Hz) and δ 3.6 (*J* 4.45, 6.95, 12 Hz) for one of the protons next to the hydroxyl group ($C\underline{H}_2OH$), a multiplet at δ 2.94–2.92 and a double doublet at δ 2.76 (*J* 2.2, 7.25 Hz) for the two protons in the epoxide ring. The ^{1}H NMR spectrum of (141) showed a doublet of doublets of doublets at δ 3.88 (*J* 2.2, 5.65, 12.6 Hz), a multiplet at δ 3.58–3.53 for ($C\underline{H}_2OH$), a broad pentet at δ 2.94 (*J* 2.5 Hz), and a double doublet at δ 2.68 (*J* 2.55, 7.25 Hz) for the two protons in the epoxide ring.

The ^{13}C NMR spectrum of (**140**) showed signals at δ 61.9 for ($\underline{C}H_2OH$), δ 60.6 and δ 57.0 for the two carbons of the epoxide ring, while in the IR spectrum a peak appeared at 3431 cm^{-1} for the OH stretch. In the same way compound (**141**) showed a peak at δ 61.8 for ($\underline{C}H_2OH$), and two signals at δ 60.6 and δ 58.5 for the epoxide ring. Finally, the specific rotation of (**140**) was $[\alpha]_D^{28} = +\ 18.13$. In the literature, the use of the same catalyst was reported for the synthesis of ((2*R*,3*R*)-3-dodecyl-oxiranyl)-methanol with $[\alpha]_D^{19} = +\ 21$. The specific rotation of (**141**) was measured as $[\alpha]_D^{22} = -\ 21.16$. In the literature the $[\alpha]_D^{28}$ was - 25.5.[212]

2.2.8c-Oxidation of the alcohols

The primary alcohols (**140**, **141**) were oxidised to give aldehydes (**142**, **143**) using PCC in dichloromethane. The reaction was continued for 2 hours without heating and the products were purified by column chromatography to give white solids in 75 and 82 % yield, respectively.[213] (**Scheme 22**).

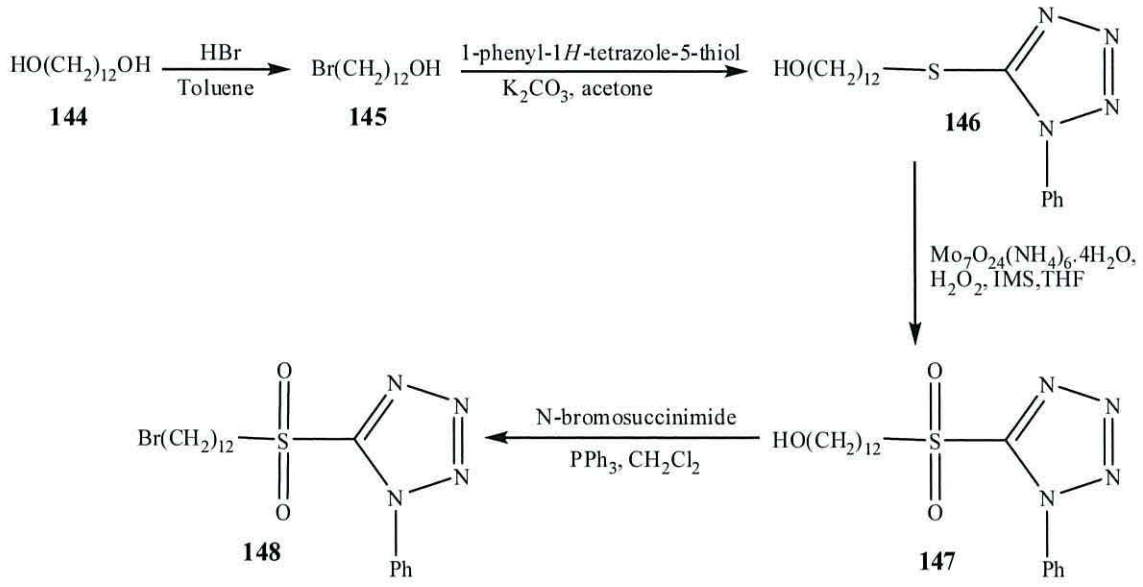
The ^{1}H NMR spectrum of (**142**) or (**143**) showed a doublet at δ 9.02 (*J* 6.3 Hz) for the proton of the aldehyde, and the ^{13}C NMR spectra showed a signal at δ 198.6 for the carbon of the aldehyde. The (2*R*,3*S*)-3-((*R*)-1-methyl-heptadecyl)-oxirane-2-carbaldehyde (**142**) gave $[\alpha]_D^{28} = -\ 63.5$, while the (2*S*,3*R*)-3-((*R*)-1-methyl-heptadecyl)-oxirane-2-carbaldehyde (**143**) had an $[\alpha]_D^{20} = +\ 53.5$.



Scheme 22: Oxidation of the alcohols (**140**, **141**) to aldehydes (**142**, **143**)

2.2.8d-Preparation of C_{12} sulfone

Twelve carbons were added to the side chains of aldehydes (**142** / **143**). The bromo-sulfone (**148**) was prepared from 1,12-dodecandiol (**144**). The diol (**144**) was reacted with aqueous hydrobromic acid (48 %, w.w.) by refluxing in toluene to give 12-bromododecan-1-ol (**145**),[214] followed by reaction with 1-phenyl-1*H*-tetrazole-5-thiol to give the sulfide (**146**). The sulfide was oxidised with hydrogen peroxide gave the sulfone (**147**). Finally, the sulfone (**147**) was converted into bromo-compound (**148**) with N-bromosuccinimide and triphenylphosphine in dichloromethane. (**Scheme 23**).



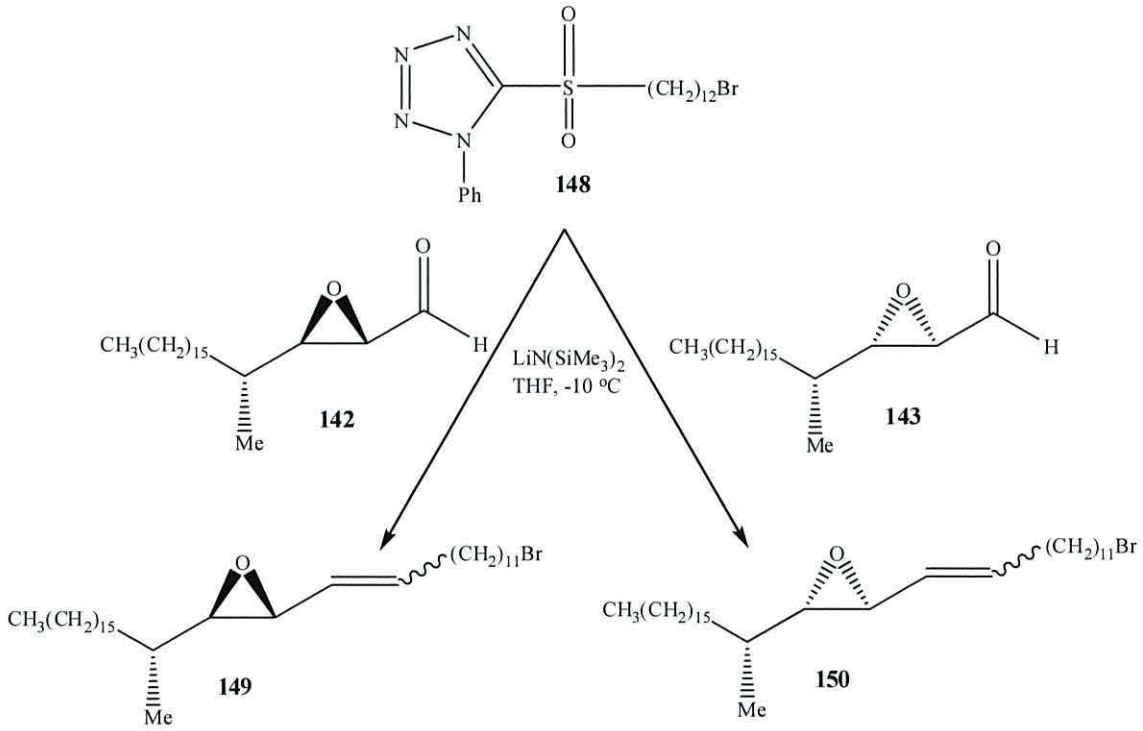
Scheme 23: Preparation of C_{12} sulfone (**148**)

The ^{1}H NMR spectrum of compound (**148**) showed a multiplet at δ 7.7–7.68 (two protons aromatic) and another multiplet at δ 7.64–7.57 (three protons aromatic), a

tripletat δ 3.72 (*J* 7.9 Hz) for the two protons ($C\underline{H}_2SO_2$) and a triplet at δ 3.4 (*J* 6.95 Hz) for the ($C\underline{H}_2Br$).

2.2.8e-The coupling reaction between aldehydes (142, 143) and sulfone (148)

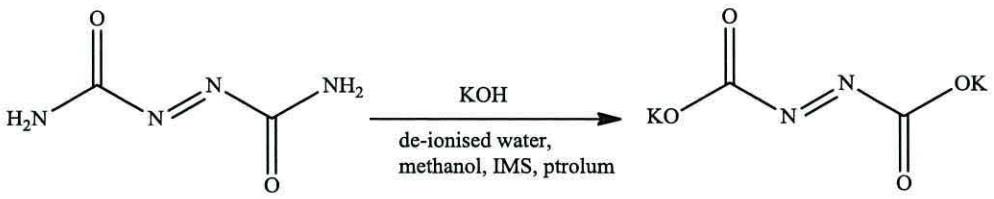
This reaction involved the coupling of the aldehydes (**142**, **143**) and the sulfone (**148**) in the presence of lithium bis(trimethylsilyl)amide in THF and gave the alkenes (**149**, **150**), each as a mixture of *E* and *Z*-isomers in ratio 1.6:1, in 79 and 71 % yield, respectively (**Scheme 24).**



Scheme 24: The coupling reaction

2.2.8f-Hydrogenation of the alkenes (149, 150) by dipotassium azodicarboxylate

Hydrogenation of the alkenes formed above, containing an epoxide ring requires milder condition than catalytic palladium on carbon and hydrogen. Dipotassium azodicarboxylate was prepared from potassium hydroxide and azodicarbonamide at 0 °C in de-ionised water to give a yellow solid product and stored in a freezer (**Scheme 25**).[215]



Scheme 25: The formation of dipotassium azodicarboxylate

Di-imide (HN=NH) was generated by decarboxylation of potassium azodicarboxylate with glacial acetic acid and methanol.[216,217]

Hydrogenation of an alkene using di-imide goes via the following mechanism (**Fig. 22**).

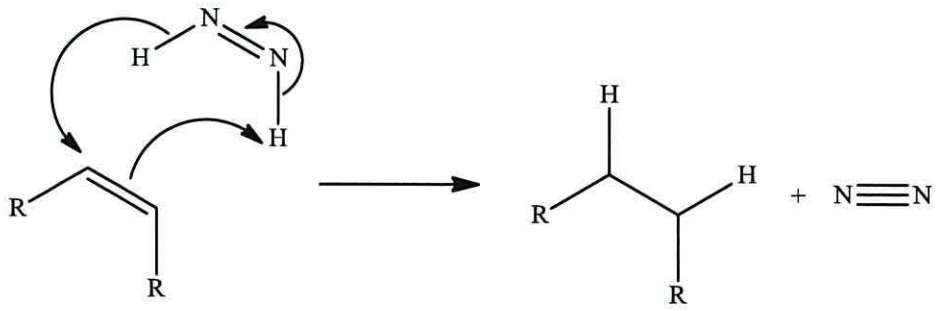
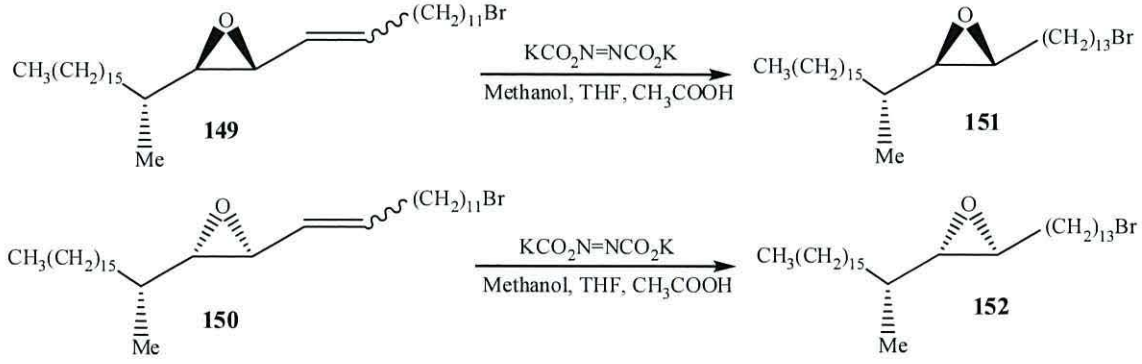


Fig 22: Hydrogenation of an alkene with di-imide mechanism

For hydrogenation, the alkenes (**149**, **150**) were dissolved in THF and methanol and an excess of dipotassium azodicarboxylate was added at 0 °C under nitrogen. A solution of glacial acetic acid in THF was added dropwise over 12 hours. The mixture was stirred for another 24 hours. After work up, the ^{1}H NMR spectrum showed there was still a small amount of unsaturated compound present, so the procedure was repeated for another 24 hours to give saturated products (**151**, **152**) each as a white solid in 93 and 83 % yield, respectively (**Scheme 26**).



Scheme 26: Hydrogenation of (**149**) and (**150**) with dipotassium azodicarboxylate

The ^{1}H NMR spectrum of (**151**) showed no signal in the double bond region and two protons appeared at δ 3.41 as a triplet (*J* 6.95 Hz) for (C<u>H</u>$_2$Br), and a doublet of triplets at δ 2.67 (*J* 2.25, 5.7 Hz) for the proton (-CHOC<u>H</u>-) of the epoxide ring and a doublet of doublets at 2.46 (*J* 2.2, 7.25 Hz) for the other proton of the epoxide ring. Compound (**152**) showed signals at δ 3.41 as a triplet (*J* 6.95 Hz) for (C<u>H</u>$_2$Br), a double of triplet at δ 2.71 (*J* 2.2, 5.35 Hz) for the proton of the epoxide ring and a doublet of doublets at δ

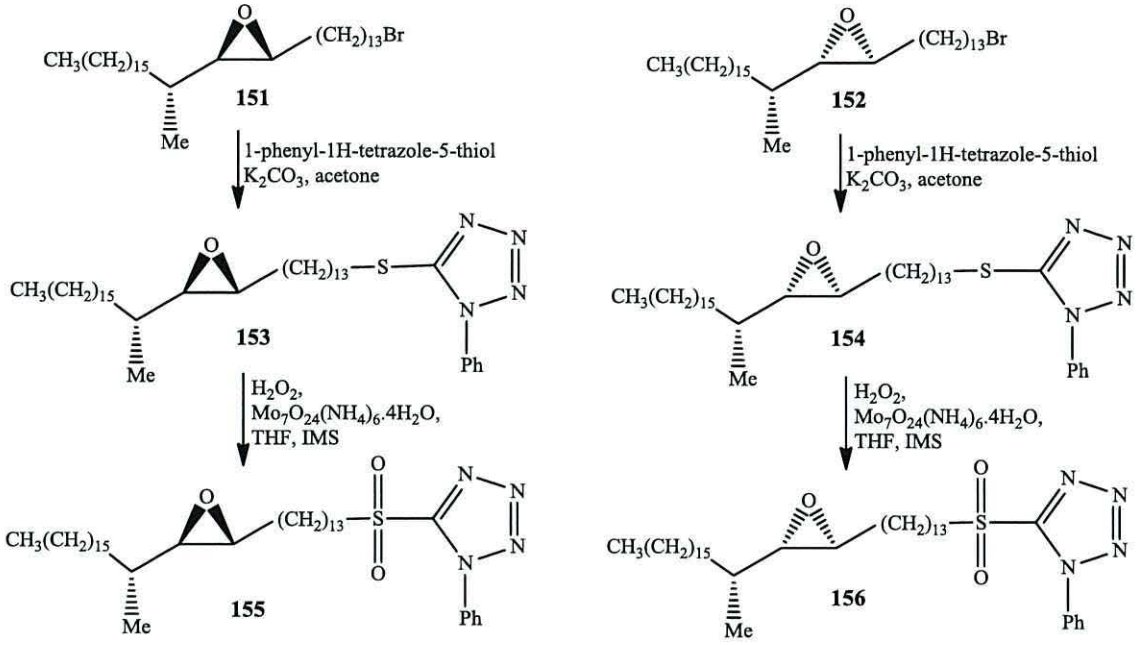
2.41 (*J* 1.9, 6.95 Hz) for the other proton of the epoxide ring. The ^{13}C NMR spectra of (**151**) or (**152**) showed signals at δ 63.8, δ 57.5 and δ 63.8, δ 58.8, respectively for the carbons of the epoxide ring. Also compounds (**151**) and (**152**) showed opposite specific rotations of $[\alpha]_D^{26} = +10.4$ and $[\alpha]_D^{24} = -13.13$, respectively.

2.2.8h-Preparation of the intermediate epoxy sulfones (155, 156)

The (2*R*,3*R*)-2-(13-bromo-tridecyl)-3-((*R*)-1-methyl-heptadecyl)-oxirane (**151**) and (2*S*,3*S*)-2-(13-bromo-tridecyl)-3-((*R*)-1-methyl-heptadecyl)-oxirane (**152**) were converted into the sulfides (**153**), (**154**) with 1-phenyl-1*H*-tetrazole-5-thiol and potassium carbonate in acetone. The crude products were purified by column chromatography to give a white solid in each case (yield 75, 82 %, respectively) (**Scheme 27**).

The ^{1}H NMR spectrum of (**153**) or (**154**) showed a multiplet at δ 7.6–7.51, δ 7.60–7.52 for the five aromatic protons, a triplet at δ 3.39, δ 3.40 for (-C$\underline{H_2}$S-), a doublet of triplets at δ 2.66 (*J* 2.2, 5.65 Hz), a doublet of triplets at δ 2.71 (*J* 2.2, 5.65 Hz) for the proton of the epoxide ring and a doublet of doublets at δ 2.45 (*J* 2.25, 7.25 Hz), δ 2.41 (*J* 2.2, 7.25 Hz) for the second proton of the epoxide ring, respectively. The ^{13}C NMR spectrum of (**153**) or (**154**) included signals at δ 63.8, 57.4 and δ 63.8, δ 58.8, respectively for the carbons of the epoxide ring. Compounds (**153**) and (**154**) again showed opposite specific rotations, $[\alpha]_D^{20} = +6.85$, $[\alpha]_D^{24} = -12.47$, respectively.

The sulfides (**153**, **154**) were oxidised to the sulfones (**155**, **156**) and purified by column chromatography to give pure products as white solids (69, 67 %, respectively) (**Scheme 25**).



Scheme 27: Preparation of the intermediate epoxy sulfones (**155**) and (**156**)

The ^{1}H NMR spectrum of (**155**) showed a distorted triplet at δ 3.72 (*J* 7.9 Hz) for the (-C$\underline{H_2}$SO$_2$-). The two signals in **Figure 23a** show a doublet of triplets at δ 2.66 (*J* 1.9, 5.65 Hz) for H_a, and a doublet of doublets at δ 2.45 (*J* 2.25, 7.25 Hz) for H_b of the epoxide ring. Proton H_c is shown in **Figure 23b** to give a multiplet at δ 1.98-1.91 and other signals appeared as a broad multiplet at δ 1.58-1.25, including a broad singlet at δ 1.25 for the long chain, a doublet at δ 0.91 (*J* 6.6 Hz) for CH_{3d} next to the epoxide ring and a triplet at δ 0.87 (*J* 6.6 Hz) for the CH_{3e}. The ^{13}C NMR spectrum showed signals at δ 63.74 and 57.41 for the two carbons of the epoxide ring (**Table 1**). The ^{1}H NMR spectrum of (156) included a triplet at δ 3.73 for the (-C$\underline{H_2}$SO$_2$-), a doublet of triplets at δ 2.72 (*J* 2.55, 5.7 Hz) for one proton of the epoxide ring and a doublet of doublets at δ 2.41 (*J* 2.2, 6.95 Hz) for the other proton of the epoxide ring (**Fig.24**). The ^{13}C NMR spectrum showed signals at δ 63.8 and 58.8 for two carbons of the epoxide ring. Compounds (**155**) and (**156**) again showed opposite specific rotations $[\alpha]_D^{20} = +6.25$ and $[\alpha]_D^{23} = -8.45$, respectively.

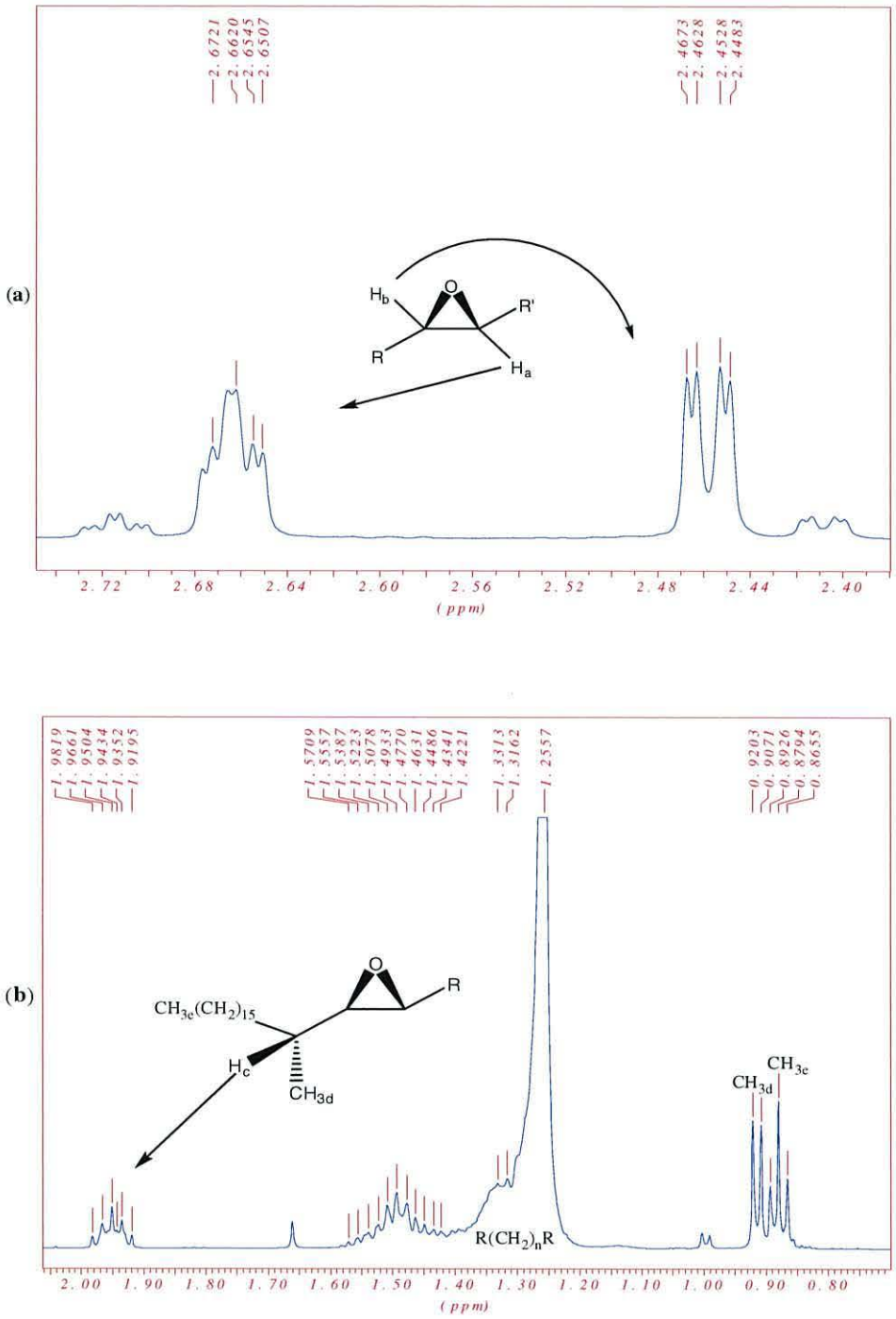
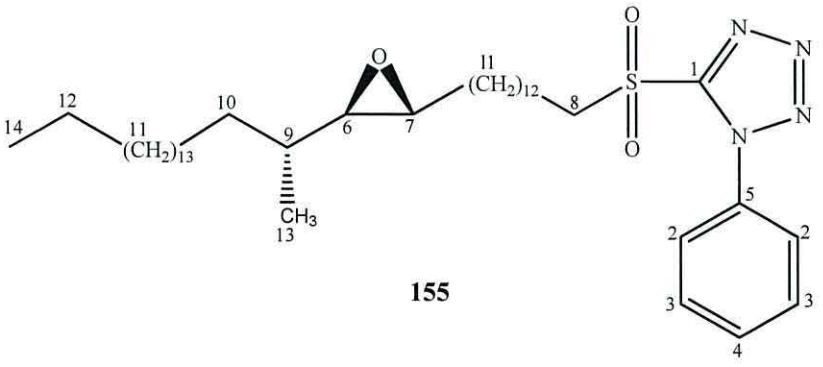


Fig. 23a,b: ^{1}H NMR spectrum of the intermediate epoxy sulfone (**155**)



155

Carbon	δ	Carbon	δ
C_1	153.5	C_8	56.0
C_2	133.0	C_9	35.8
C_3	131.4	C_{10}	34.6
C_4	129.6	C_{11}	32.2-22.6
C_5	125.0	C_{12}	21.9
C_6	63.74	C_{13}	15.1
C_7	57.41	C_{14}	14.1

Table 1: ^{13}C NMR data analysis for compound (**155**)

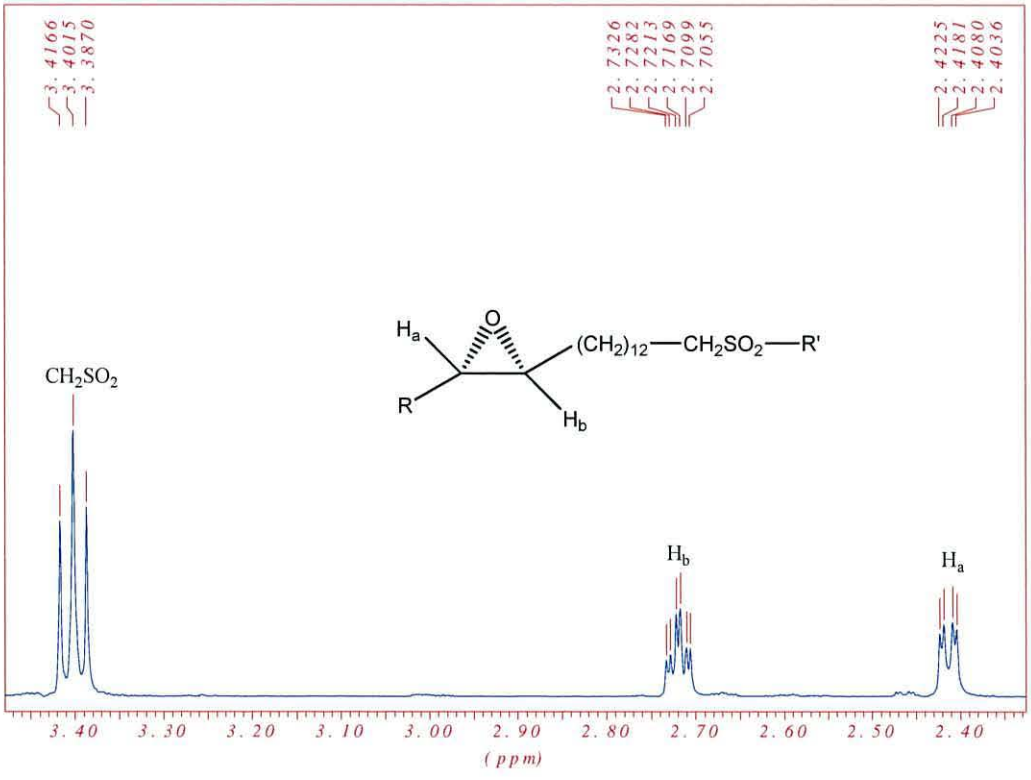
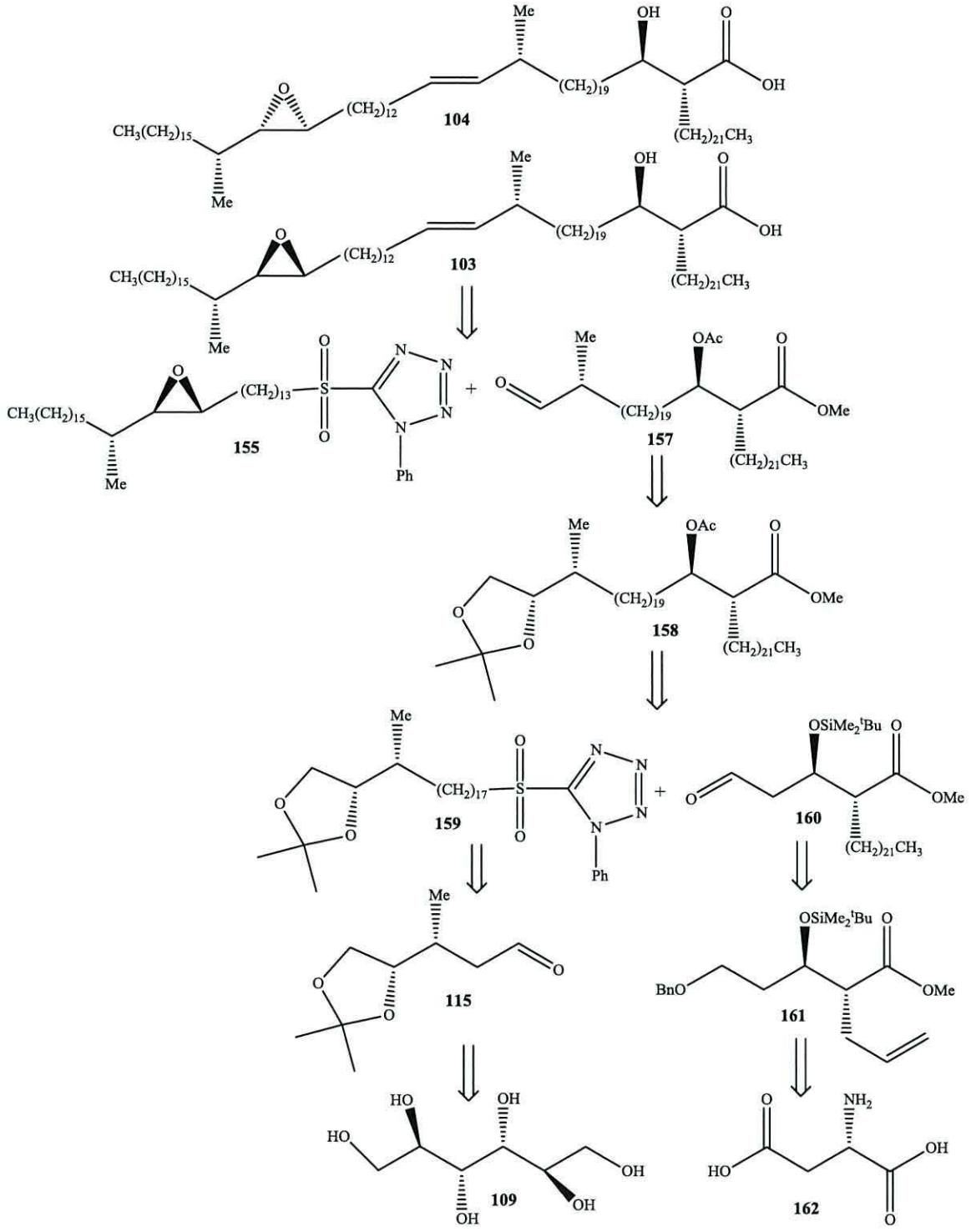


Fig 24: ^{1}H NMR spectrum of the sulfone (**156**)

2.3-The synthesis of epoxy-*trans*-alkene-mycolic acids

The synthesis of two stereoisomeric epoxymycolic acids (**103**) and (**104**) containing an (*R*)-α-methyl-*trans*-alkene at the proximal position. The reactions are summarized in **Scheme 28**.

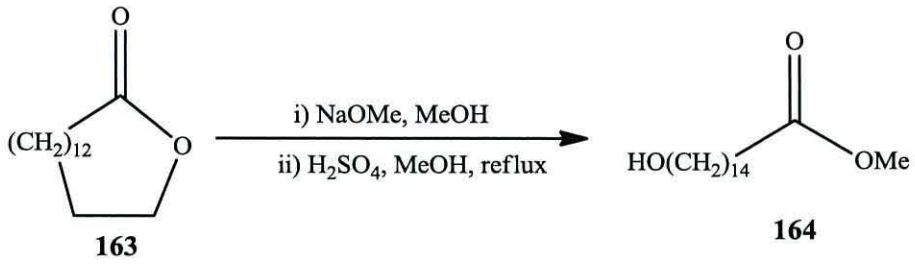


Scheme 28: Retrosynthesis of epoxy-*trans*-alkene mycolic acids (**103**, **104**)

2.3.1-Preparation of (*R*)-α-methyl sulfone (159)

2.3.1a-Ring opening of lactone for preparation of the 15 carbon chain

The 15-hydroxypentadecanoic acid methyl ester (**164**) was prepared from ω-pentadecalactone (**163**) and added to sodium methoxide solution which was prepared by addition of a small piece of sodium to dry methanol. The lactone's ring was opened by reaction with the sodium methoxide solution and stirred at 80 °C for 2 hours. The solution was acidified with aqueous (1N) HCl. The proton NMR spectrum showed the product was a mixture of the methyl ester and the carboxylic acid. Therefore, the acid was esterified in methanol and a catalytic amount of H_2SO_4 and refluxed for 90 minutes to give 15-hydroxypentadecanoic acid methyl ester (**164**) in 85 % yield (**Scheme 29**).[218]



163 **164**

Scheme 29: Ring opening of the lactone (**163**)

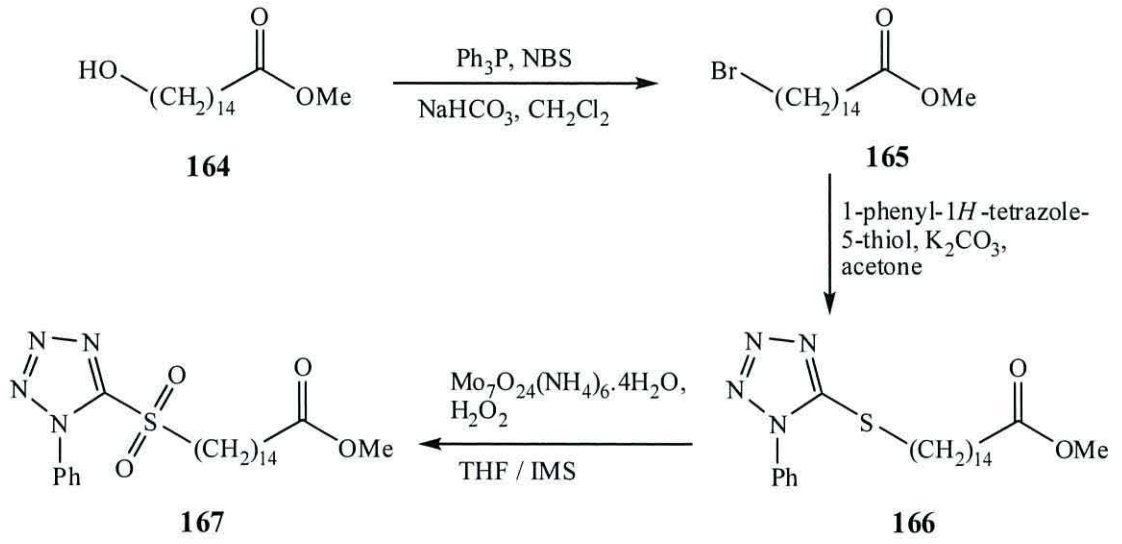
The ^{1}H NMR spectrum of (**164**) showed a singlet at δ 3.67 OC<u>H</u>$_3$ and a triplet at δ 3.64 (*J* 6.6 Hz) (C<u>H</u>$_2$OH). The ^{13}C NMR spectrum showed a signal at δ 174.3 for the carbonyl carbon at δ 63.1 for the carbon next to the hydroxyl group (<u>C</u>H$_2$OH) and a signal at δ 51.4 (O<u>C</u>H$_3$). Similar NMR spectra were also reported in the literature.[218] The IR spectrum showed a broad band at 3298 cm^{-1} for the OH stretch and the broad peak at 1742 cm^{-1} for the C=O stretch.

2.3.1b-Preparation of sulfone (167)

The 15-hydroxypentadecanoic acid methyl ester (**164**) was converted into the corresponding 15-bromopentadecanoic acid methyl ester (**165**) by using N-bromosuccinimide and triphenylphosphine in dichloromethane (**Scheme 28**).[218] The ^{1}H NMR spectrum included a triplet at δ 3.41 (*J* 6.6 Hz) for the protons next to bromine (C<u>H</u>$_2$Br).

The bromo-ester (**165**) was converted into the sulfide (**166**) with 1-phenyl-1*H*-tetrazole-5-thiol and potassium carbonate in acetone with 90 % yield. The ^{1}H NMR spectrum showed a multiplet at δ 7.75–7.60 for five aromatic protons and a triplet at δ 3.39 (*J* 7.3 Hz) for two protons next to the sulfanyl group. Lastly, the sulfide (**166**) was oxidised to

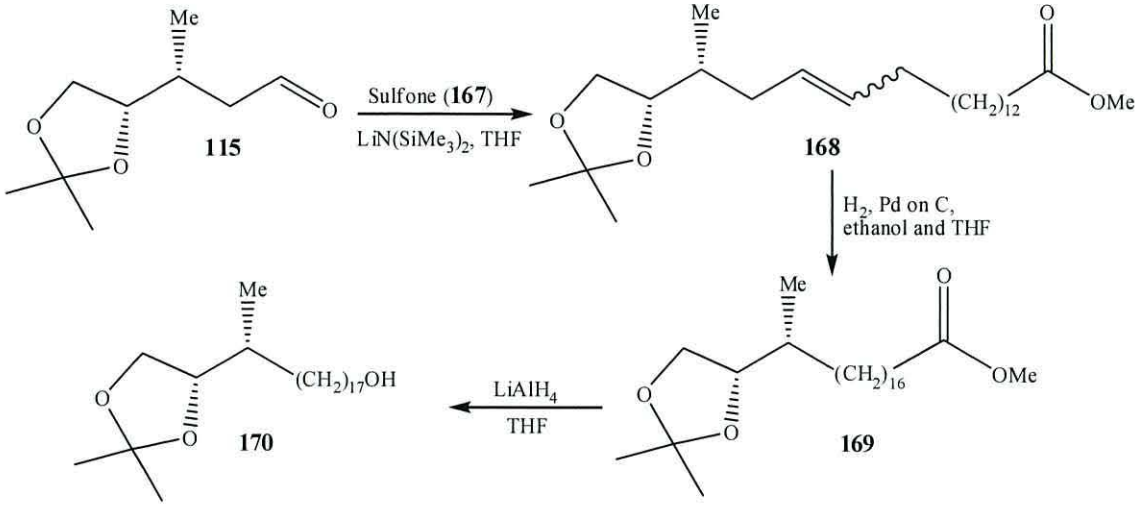
the corresponding sulfone (**167**) with hydrogen peroxide and ammonium molybdate tetrahydrate in IMS and THF in 91 % yield (**Scheme 30**). The ^{1}H NMR spectrum included two signals as a multiplet at δ 7.71–7.68 for two aromatic protons and δ 7.63–7.58 for three aromatic protons and a distorted triplet at δ 3.73 ($C\underline{H}_2SO_2$).



Scheme 30: Preparation of the sulfone (**167**)

2.3.2-The chain extension

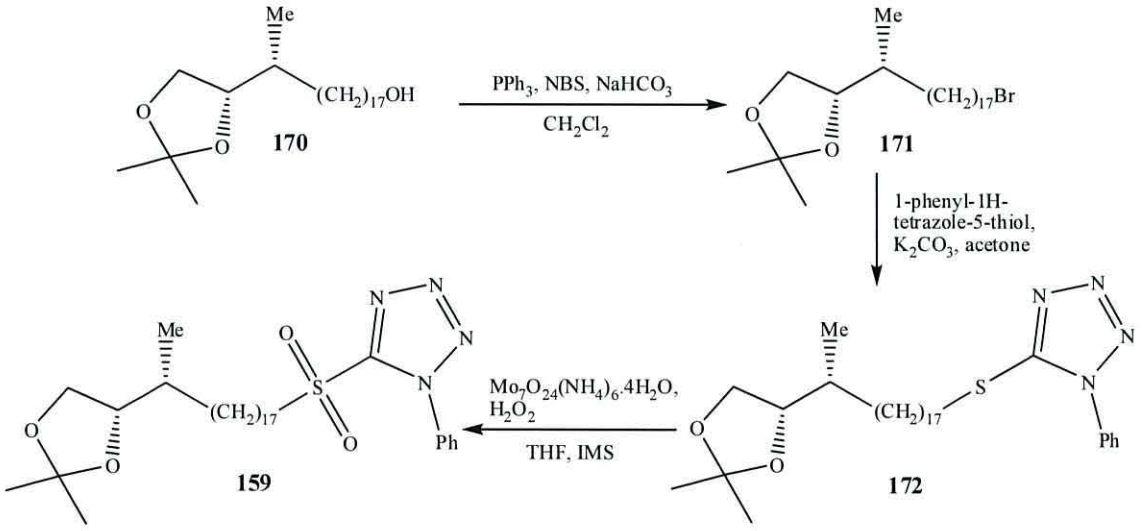
The aldehyde (**115**) was prepared as discussed in **Section 2.2.1**. A Julia reaction of the aldehyde (**115**) with the sulfone (**167**) gave the alkene (**168**) as two isomers, *E* and *Z*, in ratio 2:1. Hydrogenation with palladium on carbon in ethanol and THF and hydrogen gave a white solid (**169**) in 93 % yield. The methyl ester (**169**) was reduced to the corresponding alcohol (**170**) with $LiAlH_4$ in THF in 91 % yield (**Scheme 31**). The ^{1}H NMR spectrum of (**170**) showed a triplet at δ 3.63 (*J* 6.6 Hz) for the two protons adjacent to the hydroxyl group. The IR included a broad peak at 3448 cm^{-1} for the OH stretch.



Scheme 31: Chain extension of the aldehyde (**115**) to (**170**)

2.3.3-Preparation of the intermediate sulfone (159)

The bromo-compound (**171**) was prepared from alcohol (**170**) with NBS and PPh_3 in dichloromethane. Sodium bicarbonate was added to the mixture for neutralisation of any acid formed because the acetal protecting group is very acid sensitive. The bromo-compound (**171**) was converted into the sulfide (**172**) with 1-phenyl-1*H*-tetrazole-5-thiol. Lastly, the sulfide (**172**) was oxidised with hydrogen peroxide to give the sulfone (**159**) in 92 % yield. (**Scheme 32**).



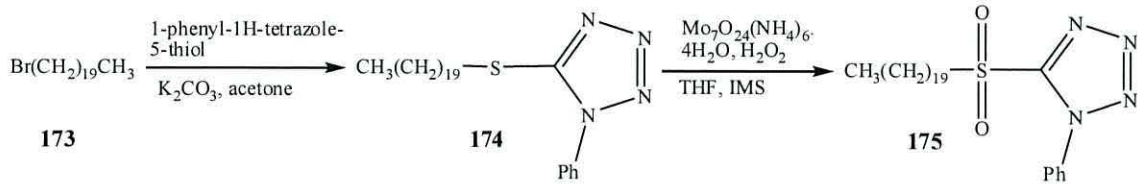
Scheme 32: Preparation of the sulfone (**159**) from (**170**)

The ^1H NMR spectrum of the sulfone (**159**) included a triplet at δ 3.73 (*J* 7.85 Hz) ($C\underline{H}_2SO_2$), two singlets at δ 1.40 and δ 1.35 for two methyl on the acetal protecting group and a doublet at δ 0.96 (*J* 6.65 Hz) for the chiral methyl protons. The ^{13}C NMR spectrum of (**159**) showed a signal at δ 108.5 for the carbon of the acetal group.

2.3.4-Preparation of the intermediate corynomycolate moiety (157)

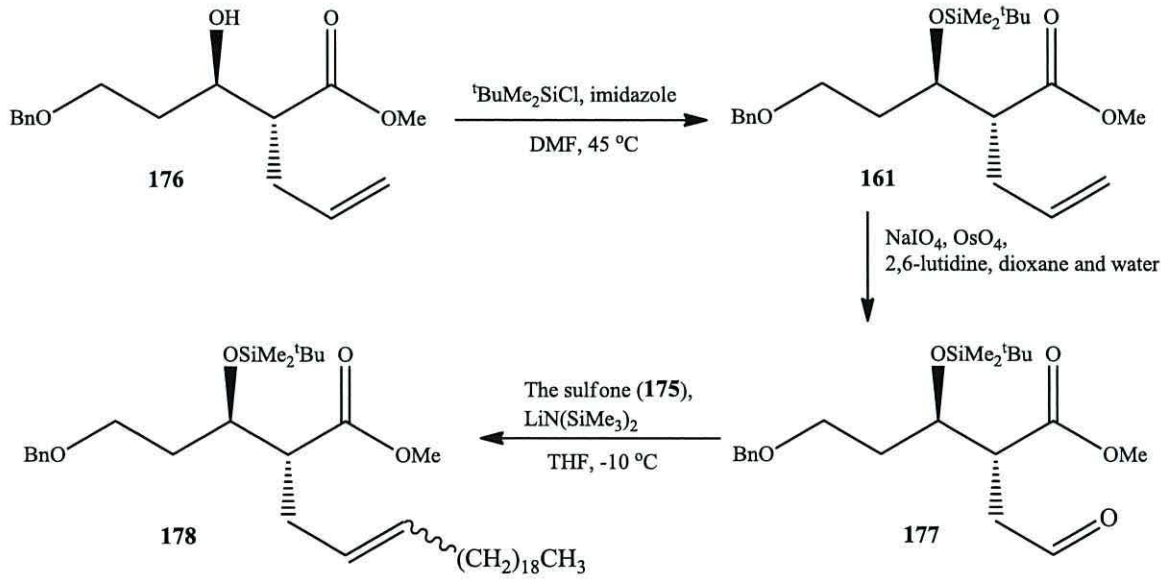
2.3.4.1a-The chain extension

The sulfone (**175**) was prepared with 1-bromoeicosane (**173**) using the method explained in **Section 2.3.1b** (**Scheme 33**).



Scheme 33: Preparation of the sulfone (**175**)

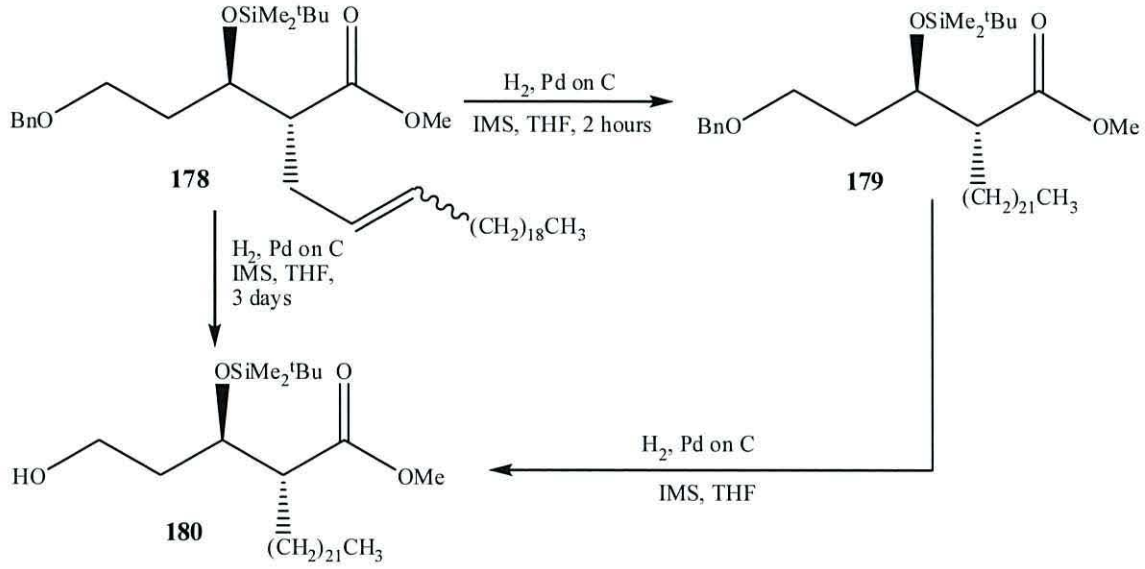
For protection, secondary alcohol (**176**)[181,172] was treated with imidazole and *tert*-butyldimethylchlorosilane in DMF stirring at 45 °C for 18 hours to give compound (**161**) in 76 % yield. The protecting group protons showed in the proton NMR as a singlet at δ 0.87 for the *tert*-butyl group and a singlet at δ 0.05 for the two methyls. The oxidation of alkene (**161**) with 2,6-lutidine, OsO_4 and $NaIO_4$ in 1,4-dioxane-water (3:1) led to the aldehyde (**177**) in 95 % yield (**Scheme 34**). The reaction was carried out in the presence of 2,6-lutidine, improving the cleavage of the olefin.[219] The ^1H NMR of the aldehyde (**177**) showed a singlet at δ 9.80 for the proton of the aldehyde and the ^{13}C NMR spectrum showed a signal at δ 200.4 for the carbonyl carbon. The Julia reaction between the aldehyde (**177**) and the sulfone (**175**) in the presence of lithium bis(trimethylsilyl)amide in THF gave the *E*- and *Z*-alkenes (**178**) in 74 % yield as a mixture in ratio 2:1 (**Scheme 34**).



Scheme 34: The chain extension of (**176**) to (**178**)

2.3.4.1b-Hydrogenation and debenzylation

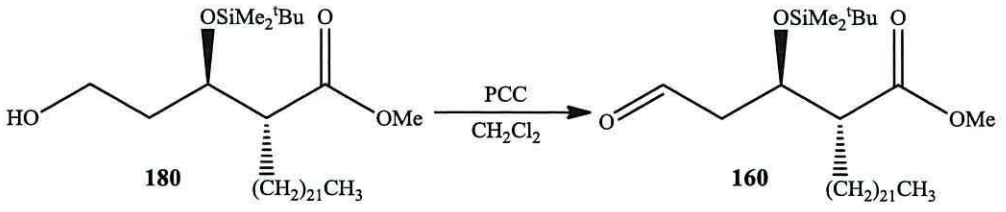
The alkene (**178**) was hydrogenated with Pd (10 %) on carbon as a catalyst in IMS and THF in presence hydrogen gas for 2 hours to give the saturated compound (**179**). Following this, hydrogenolysis of compound (**179**) converted it into the saturated and debenzylated compound (**180**) in 84 % yield (**Scheme 35**). The ^{1}H NMR spectrum of alcohol (**180**) showed a multiplet at δ 4.29–4.25 for the proton adjacent to the *tert*-butyldimethylsilanyloxy group and a doublet of doublets of doublets at δ 2.30 (*J* 3.15, 5.0, 8.15 Hz) for the proton the α-position. The ^{13}C NMR spectrum showed signals at δ 65.9 for the carbon next to the silyl group and the carbon next to the hydroxyl group appeared at δ 64.7. The specific rotation of the alcohol (**180**) was $[\alpha]_D^{22} = -8.3$ (*c* 0.4, C_6H_6).



Scheme 35: Hydrogenation and debenzylation of (**178**) to (**180**)

2.3.4.1c-Oxidation of the alcohol

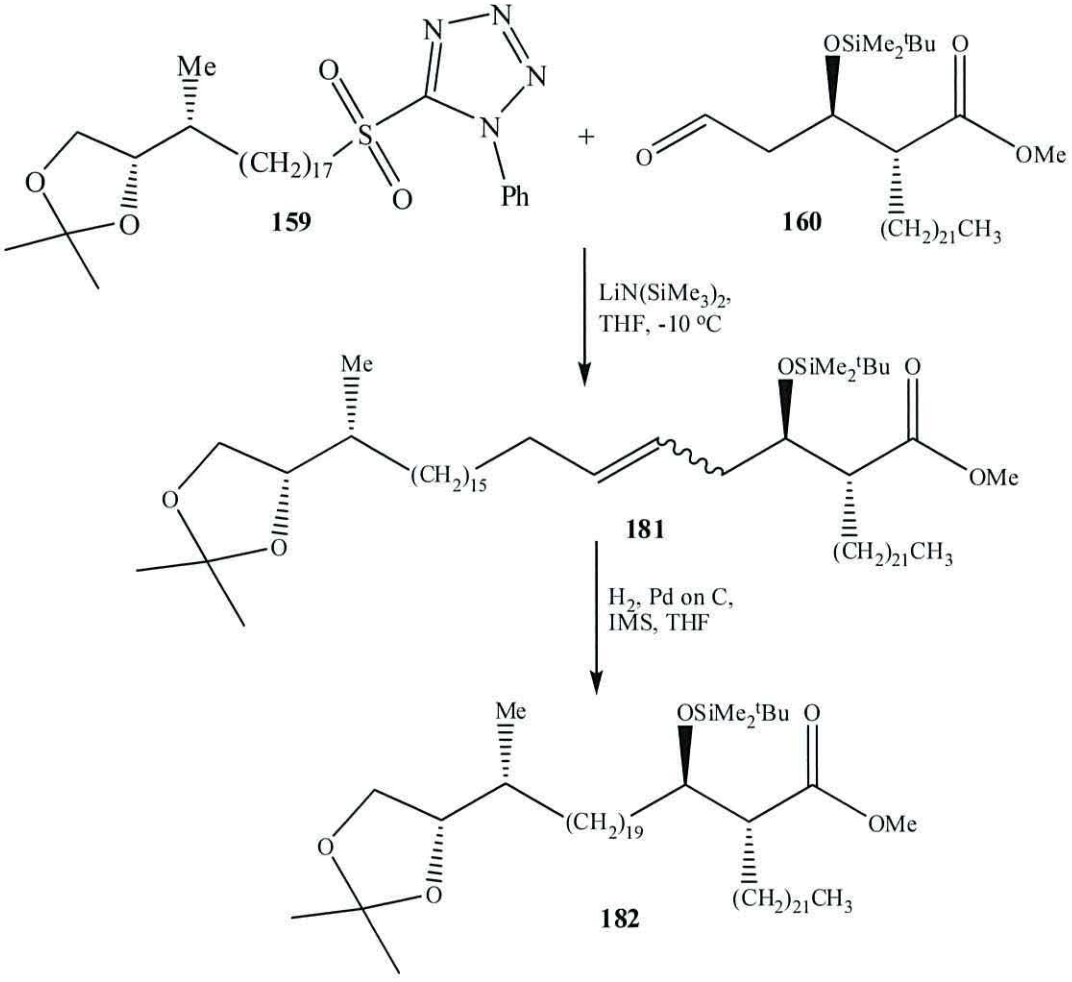
The oxidation of the alcohol (**180**) using PCC in dichloromethane led to the corresponding aldehyde (**160**) in 93 % yield (**Scheme 36**). The ^{1}H NMR spectrum of this showed a triplet at δ 9.8 (*J* 1.6 Hz) for the proton of the aldehyde and the optical rotation was measured as $[\alpha]_D^{26} = -5.0$ (*c* 1.23, $CHCl_3$).



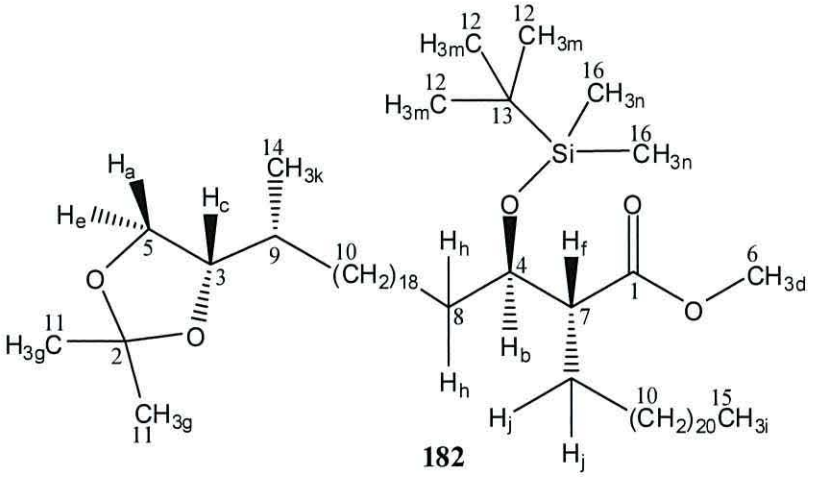
Scheme 36: Oxidation of the alcohol (**180**) to aldehyde (**160**)

2.3.4.2-The Julia reaction

This reaction involved the coupling between the corynomycolate moiety with the long chain compound containing the methyl group. In the coupling reaction, the aldehyde (**160**) was reacted with sulfone (**159**) in the presence of lithium bis(trimethylsilyl)amide in THF to obtain the *E*- and *Z*-alkenes (**181**) as a mixture of isomers in ratio 2:1 in 89 % yield. Hydrogenation of the alkenes (**181**) used Pd on carbon as a catalyst in IMS and THF in the presence of hydrogen gas to give the corresponding saturated product (**182**) in 98 % yield (**Scheme 37**). The specific rotation was measured at $[\alpha]_D^{20} = +4.41$ (*c* 1.62, $CHCl_3$). The NMR spectra were as expected (**Table 2**).



Scheme 37: The coupling reaction of (**159**) with (**160**) to produce (**182**)



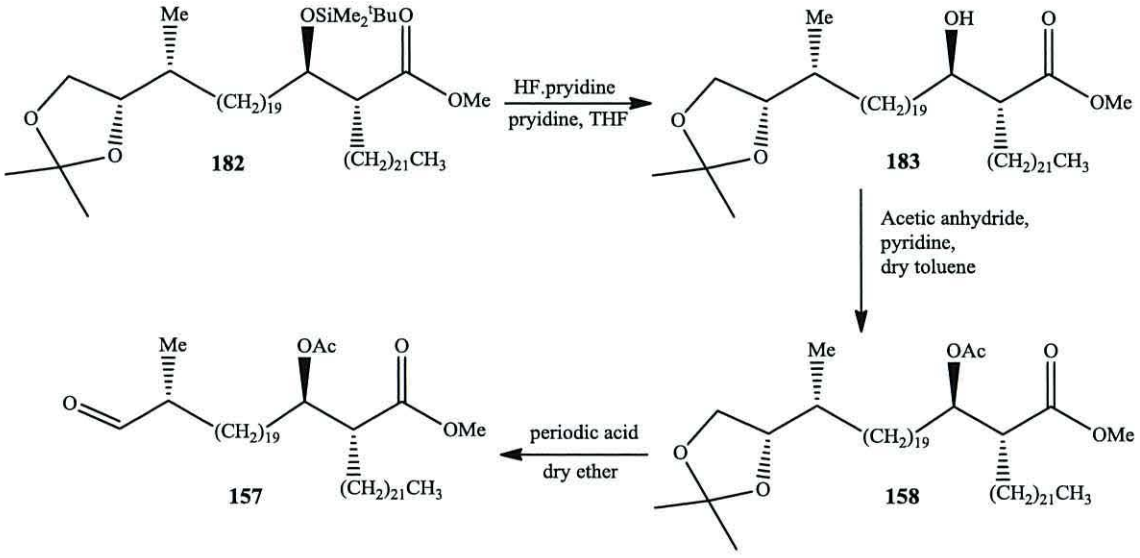
182

Proton	δ	Multiplicity	Integration	J (Hz)	Carbon	δ
H_a	4.0	dd	1	6.3, 7.55	C_1	175.1
H_b	3.92-3.9	m	1	-	C_2	108.5
H_c	3.87	br q	1	6.95	C_3	80.4
H_d	3.65	S	3	-	C_4	73.2
H_e	3.6	br t	1	7.9	C_5	67.8
H_f	2.52	ddd	1	3.75, 7.25, 11.0	C_6	51.91
H_h	1.59-1.53	m	2	-	C_7	51.56
H_j	1.49-1.42	m	2	-	C_8	36.5
H_g	1.40, 1.35	s	2 × 3H	-	C_9	33.7
$R(C\underline{H}_2)_vR'$	1.29-1.22	br m	77	-	C_{10}	32.7-22.7
H_k	0.96	d	3	6.65	C_{11}	25.75
H_i	0.88	t	3	7.25	C_{12}	25.5
H_m	0.86	s	9	-	C_{13}	18.0
H_n	0.047,0.023	s	2 × 3H	-	C_{14}	15.6
-	-	-	-	-	C_{15}	14.1
-	-	-	-	-	C_{16}	-4.37, -4.9

Table 2: ^{1}H NMR and ^{13}C NMR data analysis of compound (**182**)

2.3.4.3-Preparation of the intermediate aldehyde (157)

The aldehyde (**157**) was prepared from compound (**182**). This reaction involved changing the silyl protecting group at this step to an acetyl group. This was because the mycolic acid contained epoxide ring in structure, and the epoxide ring may be sensitive to the acid. The compound (**182**) was dissolved with THF in a polyethylene vial and pyridine with HF.pyridine was added. The mixture was stirred at 40 °C for 17 hours. This reaction removed the *tert*-butyldimethylsilyl group to give secondary alcohol (**183**) in 80 % yield. The IR spectrum of (**183**) included a broad peak at 3369 cm^{-1} for the OH stretch. Protection of the alcohol as an acetoxy group by using acetic anhydride and anhydrous pyridine in dry toluene gave product (**158**) in 98 % yield. Following this, the oxidative cleavage of the cyclic acetal group (**158**) with periodic acid in dry ether led to corresponding aldehyde (**157**) in 64 % yield (**Scheme 38**).



Scheme 38: The synthesis of aldehyde (**157**)

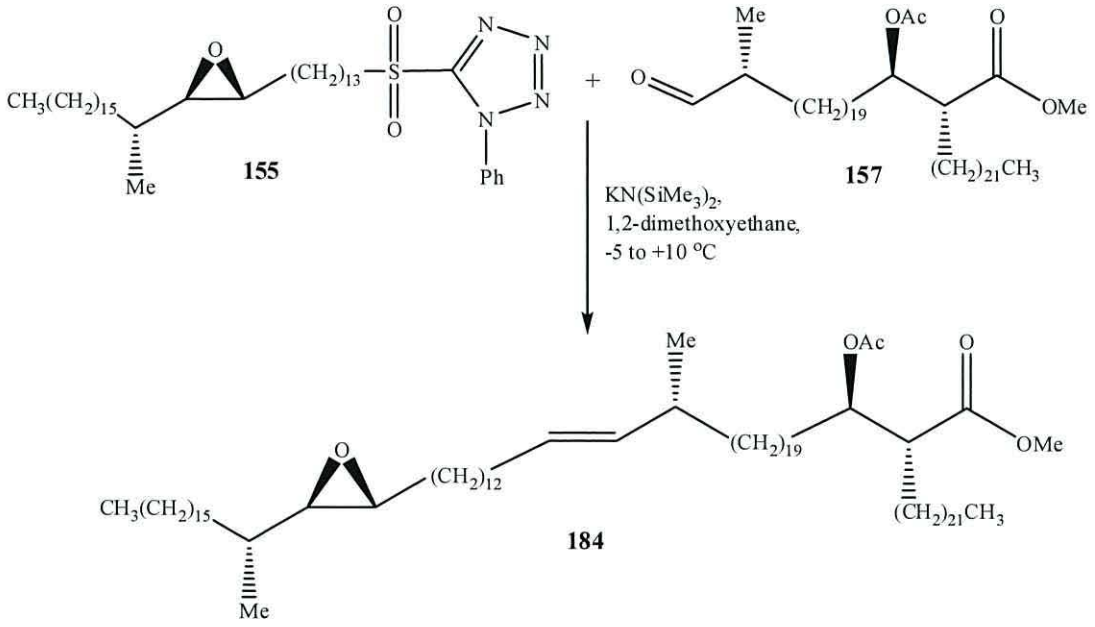
The ^{1}H NMR spectrum of (**158**) showed a doublet of quartets at δ 5.08 (*J* 4.1, 8.2 Hz) for the proton as a next acetyl group and a singlet at δ 2.03 for the methyl acetyl group. The ^{13}C NMR spectrum of (**158**) showed signals at δ 173.6 and δ 170.3 for two carbonyl carbons. The specific rotation was measured as $[\alpha]_D^{25} = +15.40$ (*c* 1.1, $CHCl_3$) and the mass spectrum of (**158**) showed the correct molecular ion [$(M + Na)^+$: 871.5758, $C_{54}H_{104}NaO_6$ requires: 871.7725]. The ^{1}H NMR, ^{13}C NMR, IR and optical rotation were used to confirm the structure of the corynomycolaldehyde (**157**). Compound (**157**) showed a doublet at δ 9.61 (*J* 2.2 Hz) for the aldehyde proton, a multiplet at δ 2.37–2.29

for the proton next to the aldehyde proton and a doublet at δ 1.09 (*J* 6.95 Hz) for the methyl group next to the aldehyde proton. The ^{13}C NMR of (**157**) showed signals at δ 205.4 for the carbonyl carbon of the aldehyde, at δ 46.3 for the carbon next to the aldehyde carbon and a signal at δ 13.3 for carbon of the methyl at the α-position to the aldehyde. The IR spectrum showed a peak at 1745 cm^{-1} and the specific rotation of (**157**) was $[\alpha]_D^{25} = +4.90$ (*c* 1.02, $CHCl_3$).

2.3.5-Final coupling reaction to form (*R,R*)-epoxy-*trans*-alkene

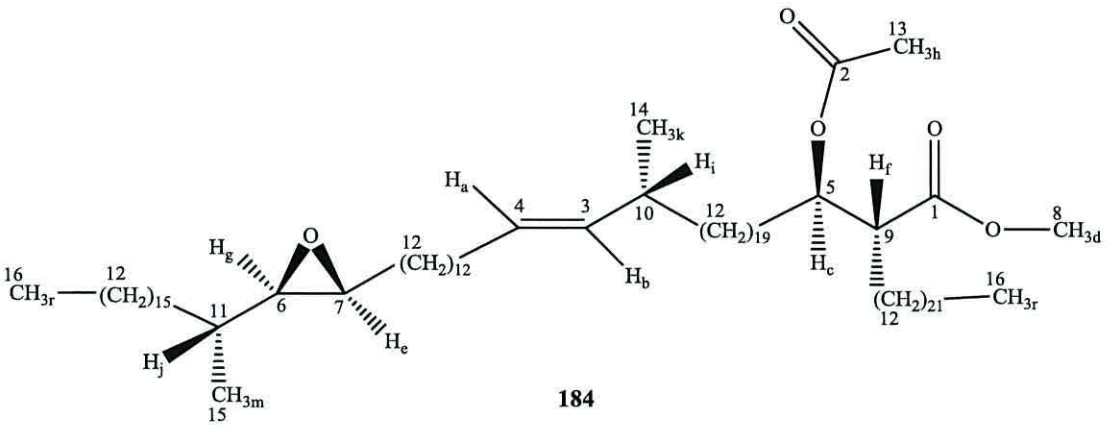
Recent studies suggested that the steroselectivity and yield of the Julia olefination is sensitive to base such as potassium bis(trimethylsilyl)amide and solvent polarity.[172,220] This reaction led only to *E*-isomer especially if the aldehyde or sulfone were α-substituted.[221,222]

The aldehyde (**157**) was coupled with the (*R,R*)-epoxy of the sulfone (**155**) in 1,2-dimethoxyethane using potassium bis(trimethylsilyl)amide at - 5 °C. The mixture was allowed to reach + 10 °C. The crude product was purified by column chromatography to give alkene (**184**) as just the *E*-isomer in 32 % yield. This reaction generated the whole structure of the (*E*)-(2*R*,3*R*,23*R*)-3-acetoxy-23-methyl-37-[(2*R*,3*R*)-3-((*R*)-1-methyl-heptadecyl)-oxiranyl]-2-docosyl-heptatriacont-24-enoic acid methyl ester (**184**) (**Scheme 39**). The ^1H NMR spectrum showed a doublet of triplets at δ 5.33 (*J* 6.6, 15.45 Hz) and a doublet of doublets at δ 5.24 (*J* 7.55, 15.45 Hz) for the alkene protons. The coupling constant of 15.45 Hz between the olefinic protons confirmed the formation of the *trans*-alkene. The two protons on the epoxide ring appeared as a doublet of triplets at δ 2.66 (*J* 2.2, 5.65 Hz) and a doublet of doublets at δ 2.46 (*J* 2.2, 7.25 Hz). The ^{13}C NMR spectrum showed two signals at δ 136.5 and δ 128.4 for the *trans*-alkene carbons. The specific rotation was measured as $[\alpha]_D^{18} = +7.15$ (*c* 1.02, $CHCl_3$) and mass spectrometry gave a molecular ion with the expected mass.



Scheme 39: The coupling reaction to form (*R,R*)-epoxide of *trans*-alkene (**184**)

More detail of the ^{1}H NMR and ^{13}C NMR data analysis for compound (**184**) can be seen in **Table 3**.



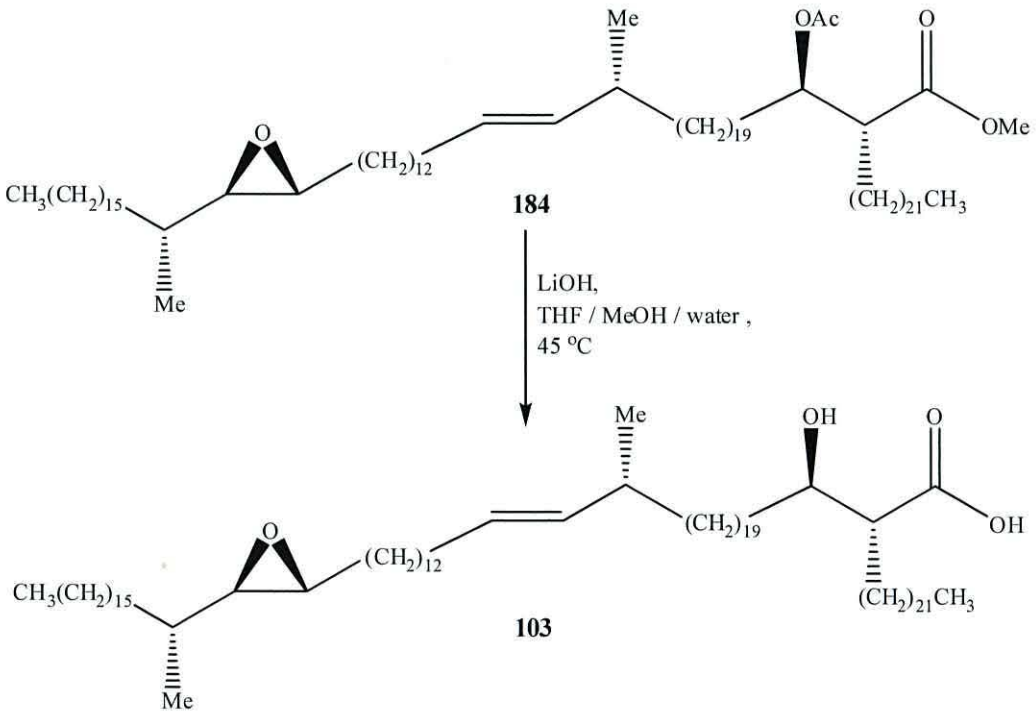
Proton	δ	Multiplicity	Integration	J (Hz)	Carbon	δ
H_a	5.33	dt	1	6.6,15.45	C_1	173.6
H_b	5.24	dd	1	7.55, 15.45	C_2	170.3
H_c	5.09	br dq	1	3.8, 7.9	C_3	136.5
H_d	3.68	s	3	-	C_4	128.4
H_e	2.66	dt	1	2.2, 5.65	C_5	74.1
H_f	2.62	ddd	1	4.4, 6.95, 11.05	C_6	63.8
H_g	2.46	dd	1	2.2, 7.25	C_7	57.5
H_h	2.03	s	3	-	C_8	51.5
H_i, H_j	1.99-1.94	m	2	-	C_9	49.6
$R(C\underline{H}_2)_nR$	1.69-1.13	br m	134	-	C_{10}	37.24
H_k	0.94	d	3	6.95	C_{11}	36.7
H_m	0.92	d	3	6.6	C_{12}	35.83-22.7
H_r	0.88	t	2 × CH_3	6.95	C_{13}	21.0
-	-	-	-	-	C_{14}	20.9
-	-	-	-	-	C_{15}	16.0
-	-	-	-	-	C_{16}	14.1

Table 3: ^1H and ^{13}C NMR data of (**184**)

2.3.6-The hydrolysis of the mycolic acid methyl ester

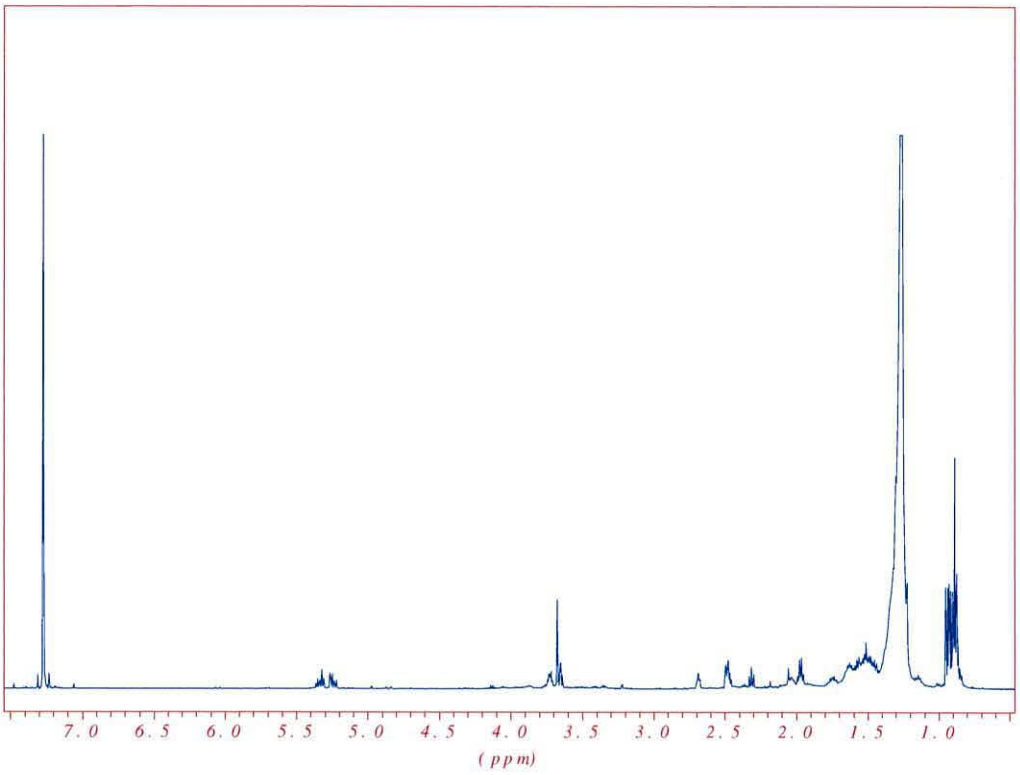
The hydrolysis of the (*R,R*)-epoxy-*trans*-alkene-myclic acid methyl ester (**184**) to give acid (**103**) was achieved by using an excess of lithium hydroxide monohydrate in THF, methanol and water. The mixture was stirred at 45 °C for 18 hours to give epoxy mycolic acid (**103**) (**Scheme 40**). The proton NMR spectrum (**Fig. 25a**) of the synthetic (*R,R*)-epoxy-*trans*-alkene-mycolic acid (**103**). The proton H_a is shown in **Figure 25b** to give a doublet of triplets at δ 5.33 (*J* 6.6, 15.1 Hz) and a doublet of doublets at δ 5.24 (*J*

7.55, 15.15 Hz) is seen for the H_b. The coupling constant of 15.1 Hz between the olefinic protons confirmed the formation of the *trans*-alkene. The protons on the epoxide ring appeared as two multiplets at δ 2.69-2.67 for H_c and δ 2.49–2.45 for H_e. The α-proton of free acid (**103**) appeared as a multiplet at δ 2.49–2.45 for H_d (**Fig. 25c**). The three signals in **Figure 25d** show doublet at δ 0.94 for the CH_{3f}, a doublet at δ 0.92 for CH_{3g} and a triplet at δ 0.88 for the CH_{3h}. The IR spectrum showed a broad peak at 3392 cm^{-1} for the OH stretch. The specific rotation of the protected (*R,R*)-epoxy-alkene-mycolic acid methyl ester (**184**) was measured $[\alpha]_D^{18} = +7.15$ (*c* 1.02 $CHCl_3$), and for the corresponding free mycolic acid (**103**) was $[\alpha]_D^{20} = +6.02$ (*c* 0.52, $CHCl_3$). The mass spectrum of the (*R,R*)-epoxy-*trans*-alkene-mycolic acid (**103**, **Fig. 27**) showed a molecular ion $[M + Na]^+$: 1204.1843, while the major compound of the natural mycolic acid was reported to show a molecular ion at $[M + Na]^+$: 1204.19 (**Fig. 26**).[223]

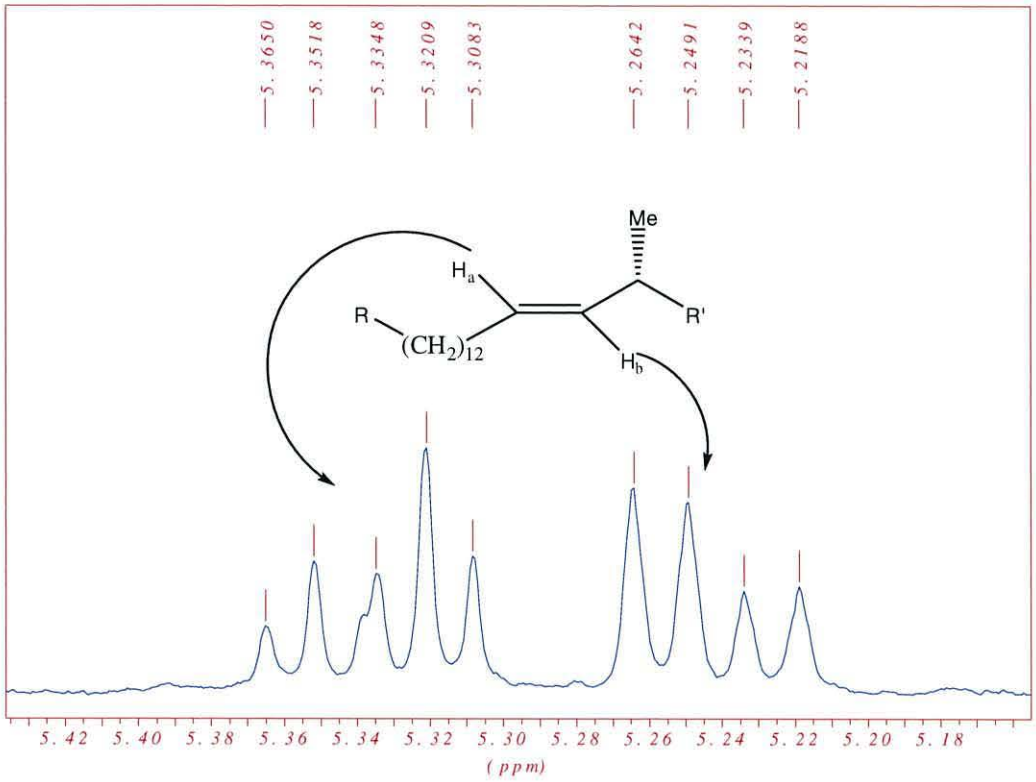


Scheme 40: Hydrolysis of the methyl mycolate (**184**) to free acid (**103**) with LiOH

(**a**)


7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
(ppm)

(**b**)


5.3650
5.3518
5.3348
5.3209
5.3083
5.2642
5.2491
5.2339
5.2188
Me
Ha
R
(CH2)12
R'
Hb
5.42 5.40 5.38 5.36 5.34 5.32 5.30 5.28 5.26 5.24 5.22 5.20 5.18
(ppm)

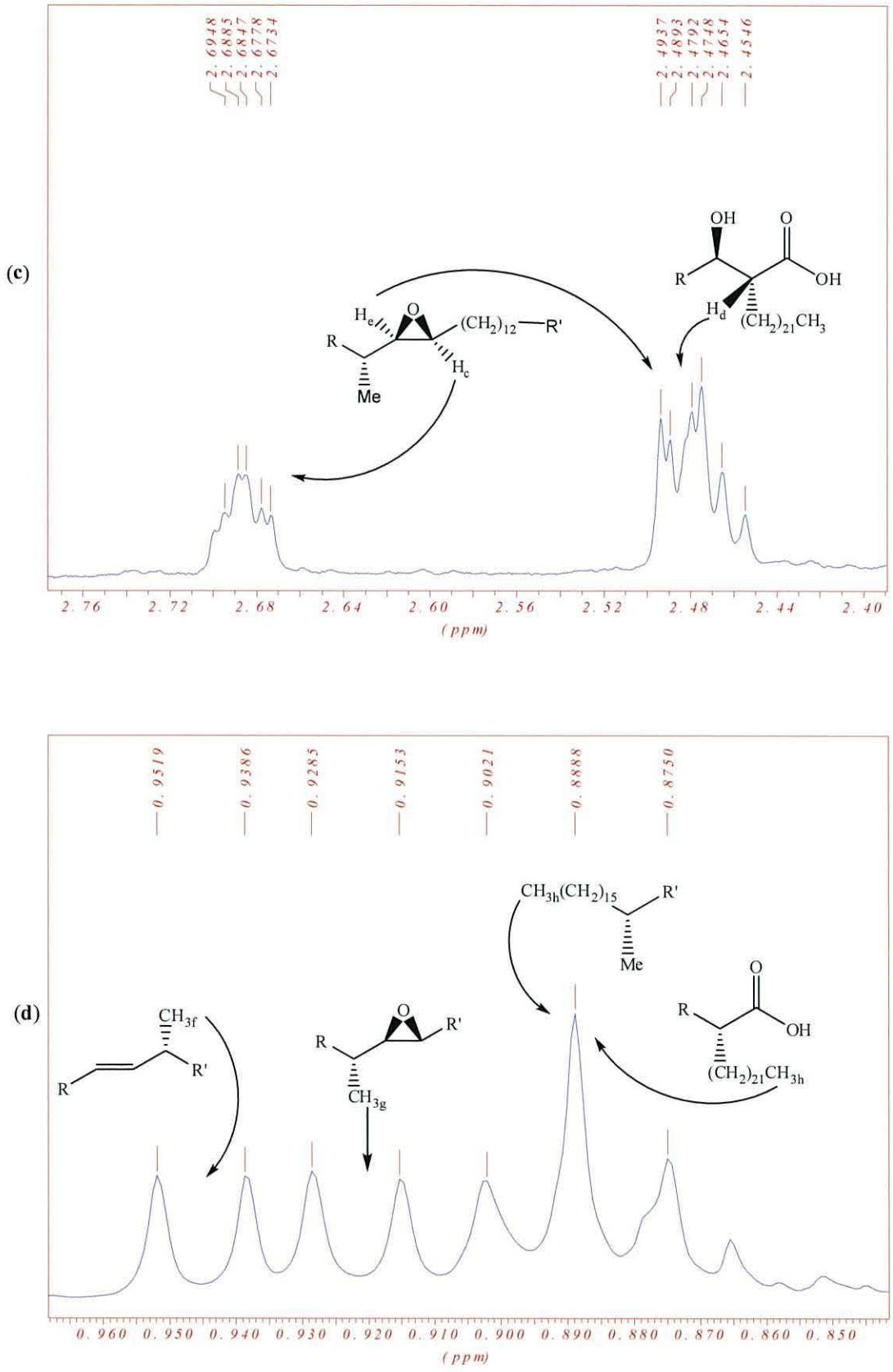


Fig. 25a-d: The ^{1}H NMR spectrum of the synthetic (*R,R*)-*epoxy-trans*-alkene-mycolic acid (**103**)

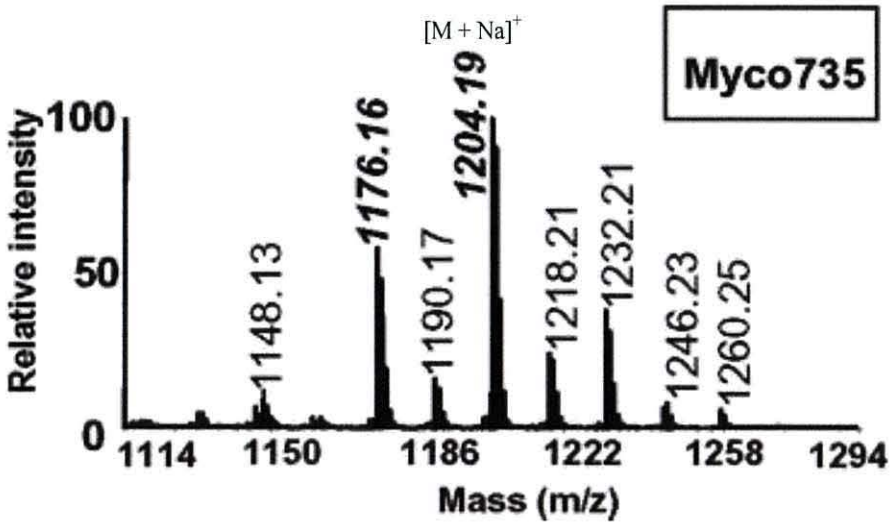


Fig. 26: MALDI-TOF mass spectra of natural epoxy-alkene-mycolic acid[223]

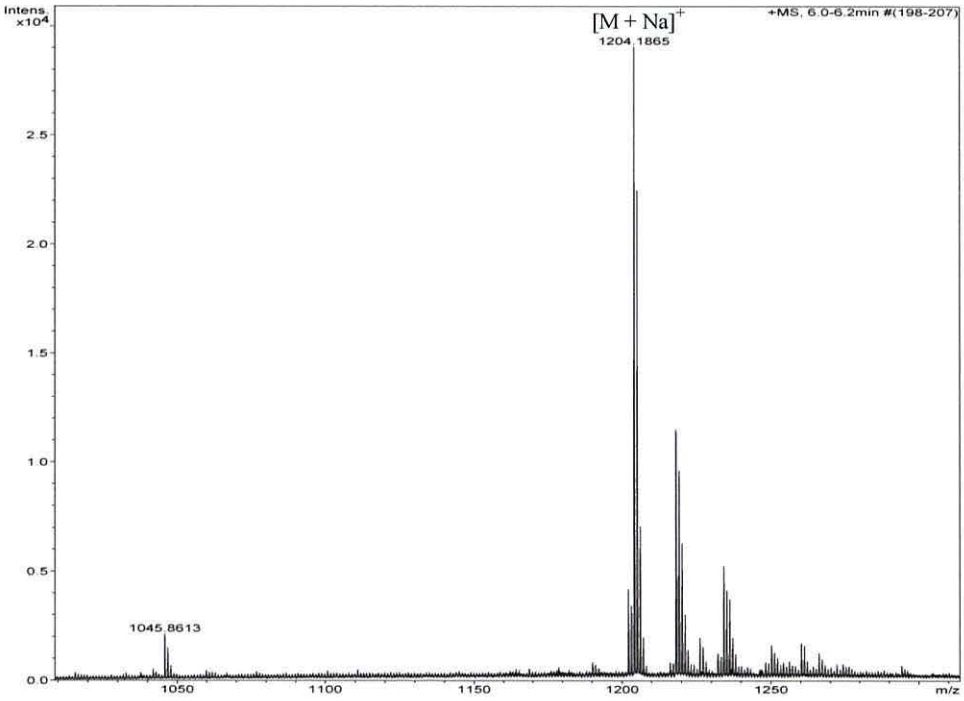
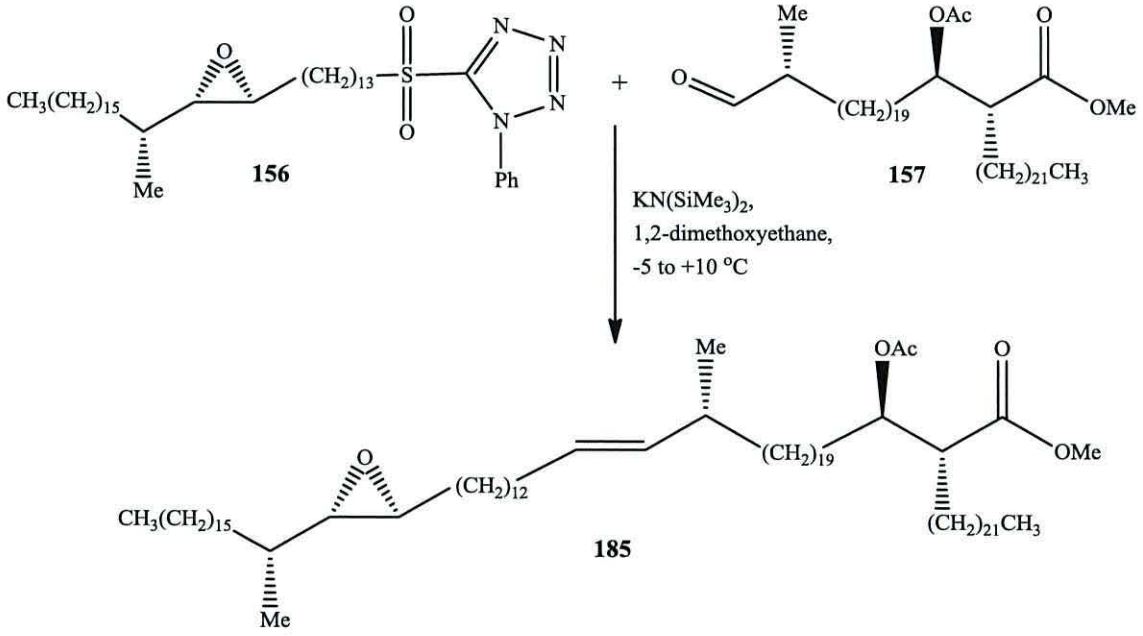


Figure 27: Mass spectrum of the (*R,R*)-epoxy-*trans*-alkene-mycolic acid (**103**)

2.3.7-Final coupling reaction to form (*S,S*)-epoxy-*trans*-alkene

The Julia reaction was carried out between aldehyde (**157**) and the (*S,S*)-epoxy of the sulfone (**156**) in 1,2-dimethoxyethane and potassium bis(trimethylsilyl) amide was added at - 5 °C. The crude product was purified by column chromatography to give alkene (**185**) as just the *E*-isomer. This reaction gave the complete structure of the (*E*)-(2*R*,3*R*,23*R*)-3-acetoxy-23-methyl-37-[(2*S*,3*S*)-3-((*R*)-1-methyl-heptadecyl)-oxiranyl]-

2-docosyl-heptatriacont-24-enoic acid methyl ester (**185**) (**Scheme 41**). The ^{1}H NMR spectrum showed a doublet of quartets at δ 5.33 (*J* 6.65, 15.45 Hz) and a doublet of doublets at δ 5.24 (*J* 7.6, 15.45 Hz) for the alkene protons. The coupling constant of 15.45 Hz between the olefinic protons confirmed the formation of the *trans*-alkene. The two protons of the epoxide ring appeared as a doublet of triplets at δ 2.71 (*J* 2.2, 5.35 Hz) and a doublet of doublets at δ 2.40 (*J* 5.05, 7.25 Hz). The α-proton appeared as a doublet of doublets of doublets at δ 2.62 (*J* 4.1, 6.6, 10.7 Hz). The ^{13}C NMR spectrum showed two signals at δ 136.5 and δ 128.4 for the *trans*-alkene carbons. The specific rotation was $[\alpha]_D^{25} = -2$ (*c* 1.0, $CHCl_3$) and the mass spectrum of (**185**) gave the correct molecular ion.

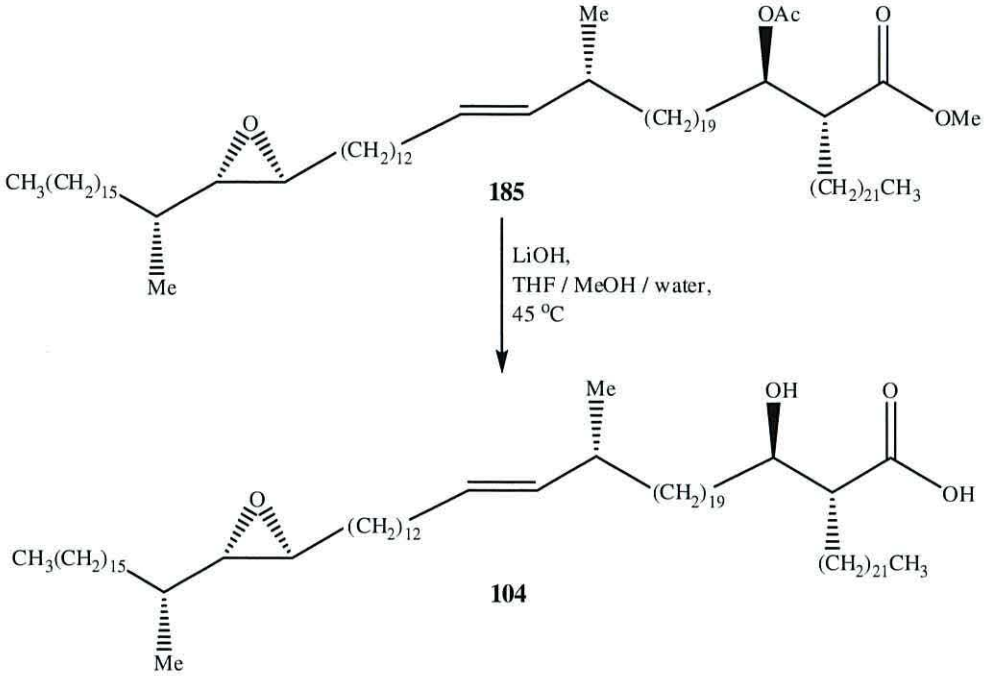


Scheme 41: A coupling reaction of (**156**) and (**157**) to form (*S,S*)-epoxy of *trans*-alkene (**185**)

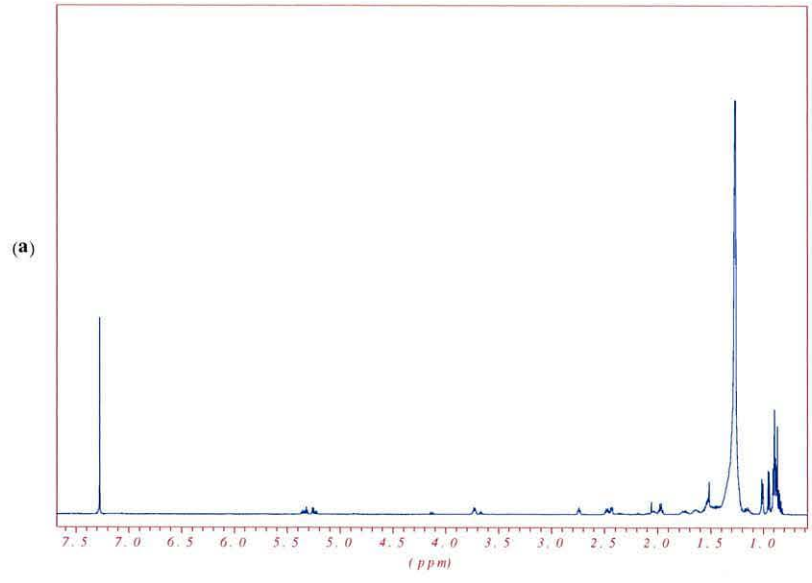
2.3.8-The hydrolysis of the mycolic acid methyl ester

The methyl ester and acetate groups of (**185**) were deprotected by using an excess of lithium hydroxide monohydrate in THF, methanol and water. The mixture was stirred at 45 °C for 18 hours to give (*S,S*)-epoxy-*trans*-alkene-mycolic acid (**104**) (**Scheme 42**). The proton NMR spectrum (**Fig. 28a**) of (**104**). Proton H_a is shown in **Figure 28b** to give a doublet of triplets at δ 5.33 (*J* 6.3, 15.15 Hz) and a doublet of doublets at δ 5.24 (*J* 7.55, 15.1 Hz) is seen for H_b. The coupling constant of 15.1 Hz between the olefinic protons confirmed the formation of the *trans*-alkene. The two protons on the epoxide ring appeared as a doublet of triplets at δ 2.73 (*J* 2.2, 5.35 Hz) for H_c and a doublet of

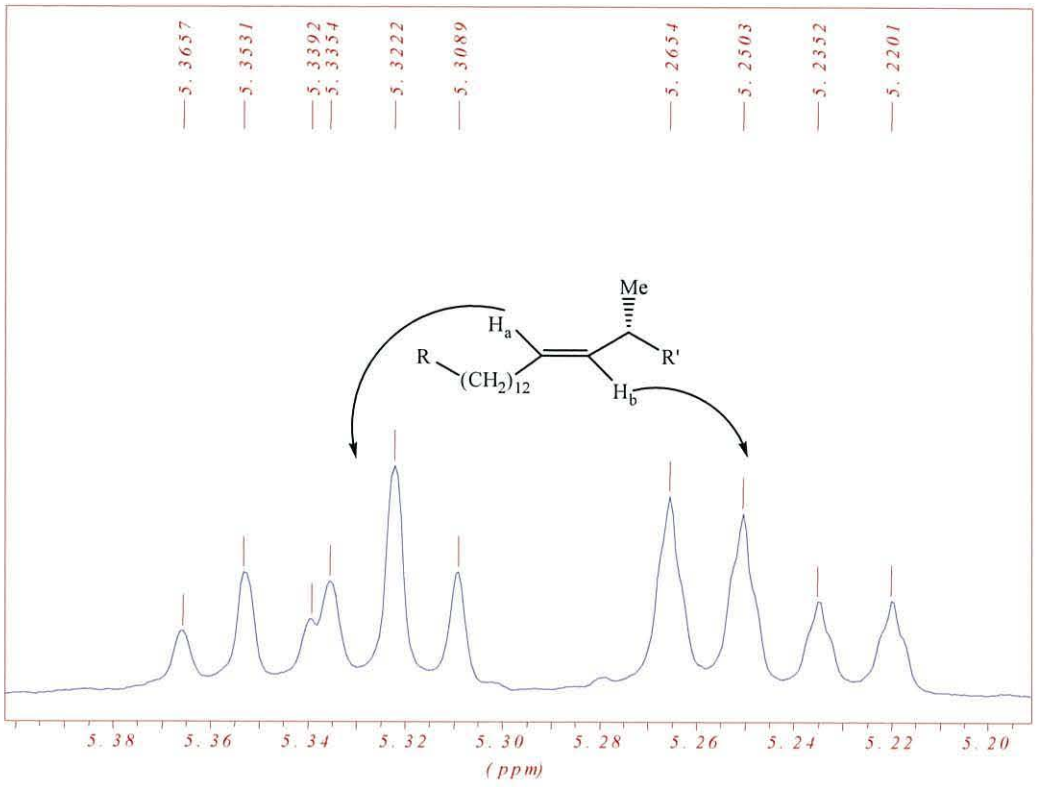
doublets at δ 2.43 (*J* 2.2, 7.25 Hz) for H_e. The α-proton of the free acid (**104**) appeared as a multiplet at δ 2.49–2.45 for H_d (**Fig. 28c**). The three signals in **Figure 28d** show a doublet at δ 1.0 for CH_{3f}, a doublet at δ 0.94 for CH_{3g} and a triplet at δ 0.89 for CH_{3h}. The IR spectrum showed a broad peak at 3368 cm^{-1} for the OH stretch. The specific rotation was measured $[\alpha]_D^{20} = -5.49$ (*c* 0.74, $CHCl_3$). The mass spectrum of (**104**) (**Fig. 29**) showed a molecular ion $[M + Na]^+$: 1204.1912, while again the major compound of the natural mycolic acid showed a molecular ion $[M + Na]^+$: 1204.19 as a major peak (**Fig. 26**).



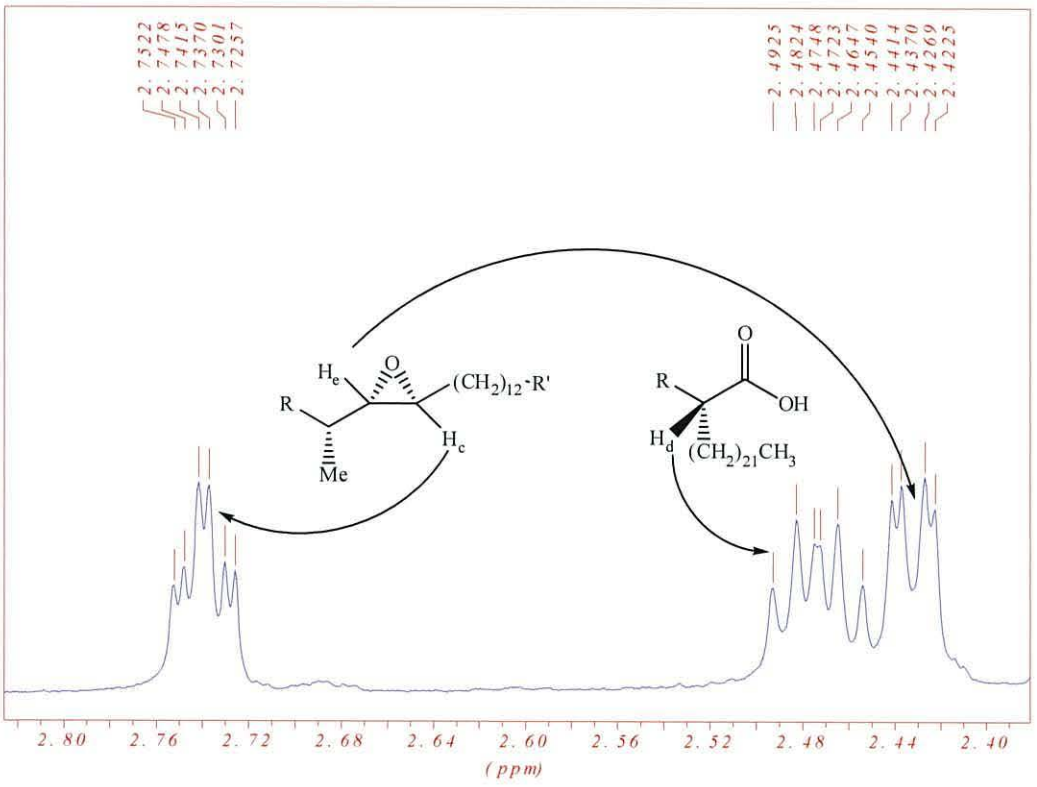
Scheme 42: Hydrolysis of the mycolic acid to free acid with LiOH



(**b**)


5.3657
5.3531
5.3392
5.3354
5.3222
5.3089
5.2654
5.2503
5.2352
5.2201
Me
H_a
R
$(CH_2)_{12}$
R'
H_b
5.38
5.36
5.34
5.32
5.30
5.28
5.26
5.24
5.22
5.20
(ppm)

(**c**)


2.7522
2.7478
2.7415
2.7370
2.7301
2.7257
2.4925
2.4824
2.4748
2.4723
2.4647
2.4540
2.4414
2.4370
2.4269
2.4225
H_e
O
$(CH_2)_{12}$-R'
R
Me
H_c
O
OH
R
H_d
$(CH_2)_{21}CH_3$
2.80
2.76
2.72
2.68
2.64
2.60
2.56
2.52
2.48
2.44
2.40
(ppm)

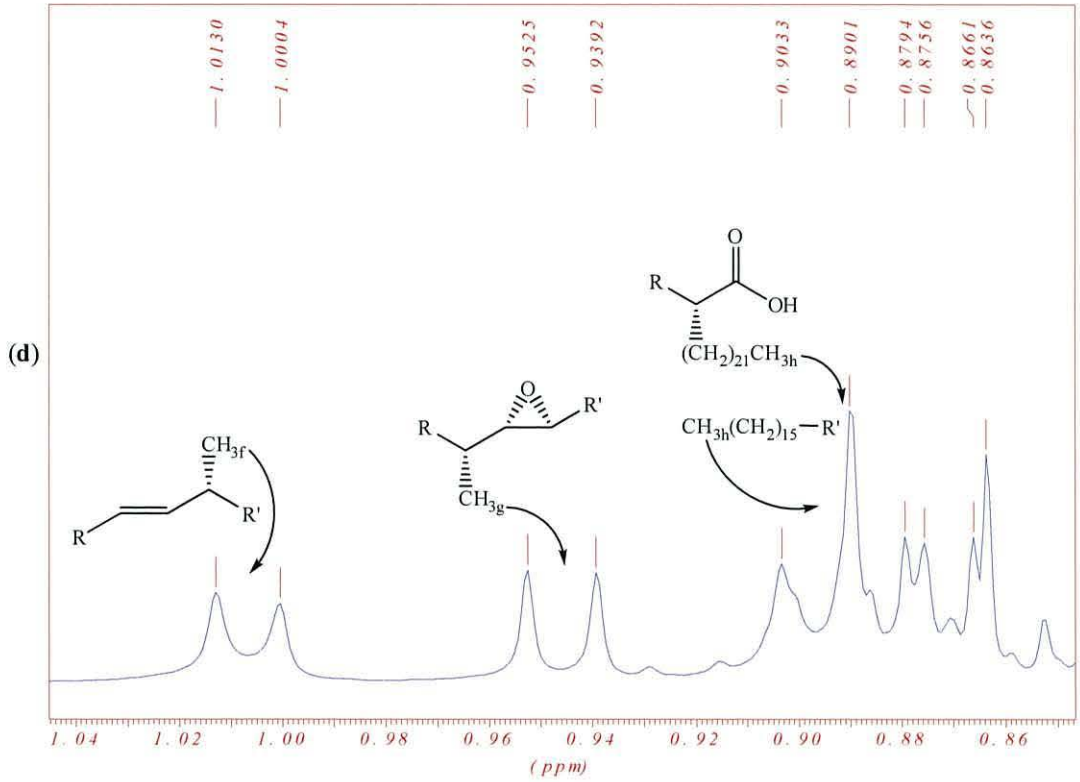


Fig. 28a-d: The ^{1}H NMR spectrum of the synthetic (*S,S*)-epoxy-*trans*-alkene-mycolic acid (**104**)

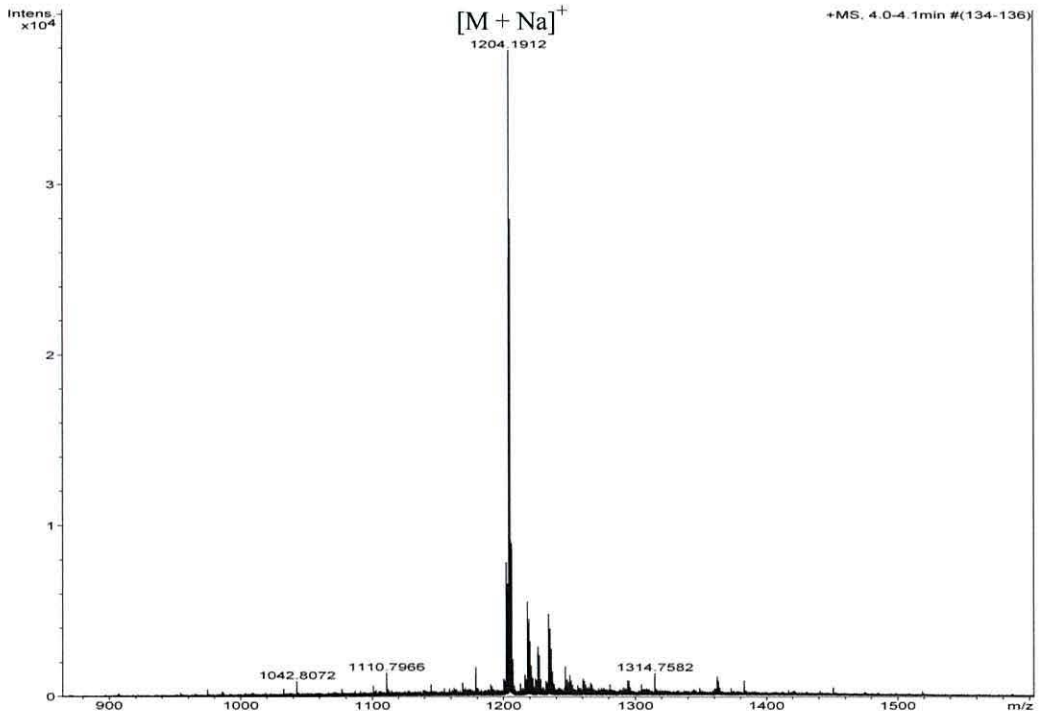
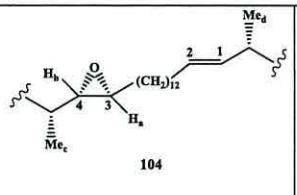
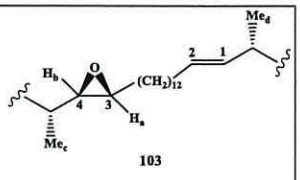


Fig. 29: Mass spectrum of the (*S,S*)-epoxy-*trans*-alkene-mycolic acid (**104**)

NMR	Natural[117] epoxy mycolic acid	Natural[147] epoxy mycolic acid	 104	 103
^{13}C NMR				
3	δ 63.69	-	δ 63.99	δ 63.91
4	δ 58.63	-	δ 59.0	δ 57.60
1	δ 136.40	-	δ 136.46	δ 136.45
2	δ 128.31	-	δ 128.41	δ 128.41
^{1}H NMR				
H_a	δ 2.695	δ 2.72*	δ 2.73**	δ 2.69-2.67**
H_b	δ 2.392	δ 2.43	δ 2.43	δ 2.49-2.45
CH_{3d}	δ 0.979	δ1.02	δ 1.0	δ 0.94
CH_{3c}	δ 0.919	δ 0.92	δ 0.94	δ 0.92
$[\alpha]_D$	-		- 5.49	+ 6.02

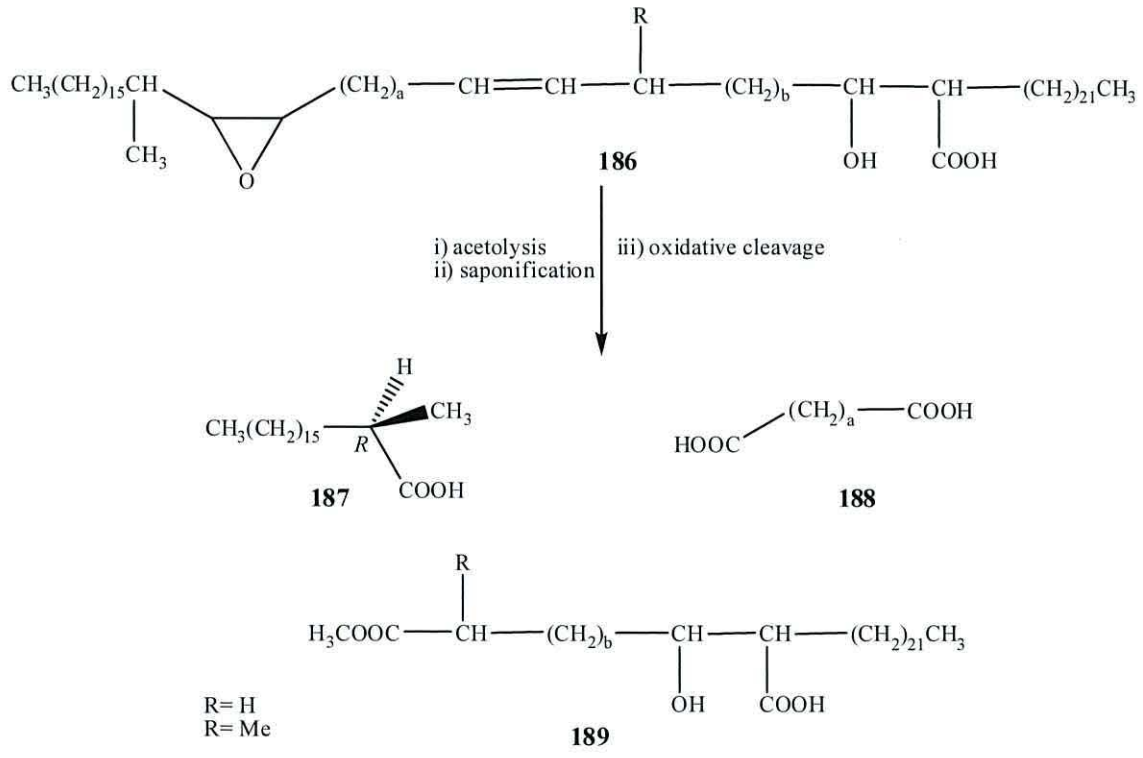
Spectra (500MHz) were collected in deuterochloroform, and *shift values are reported in ppm relative to internal $CHCl_3$ (δ 7.26 ppm)[147] and **shift values are reported in ppm relative to internal $CHCl_3$ (δ 7.27 ppm).

Table 4: Selected ^{13}C and ^{1}H NMR shifts and $[\alpha]_D$ for natural sample and epoxy mycolic acid compared to (**103**) and (**104**).

The proton and carbon NMR spectra of the synthetic epoxymycolic acids (**103**) and (**104**) were almost identical to each other and to those of a sample extracted from *M. smegmatis*.[223] (**Table 4**). The mass spectra of (**103**) and (**104**) gave a molecular ion pattern (**Fig. 29**) which corresponded to the major isomer of the natural sample (**Fig. 26**) of *M. smegmatis*.[223]

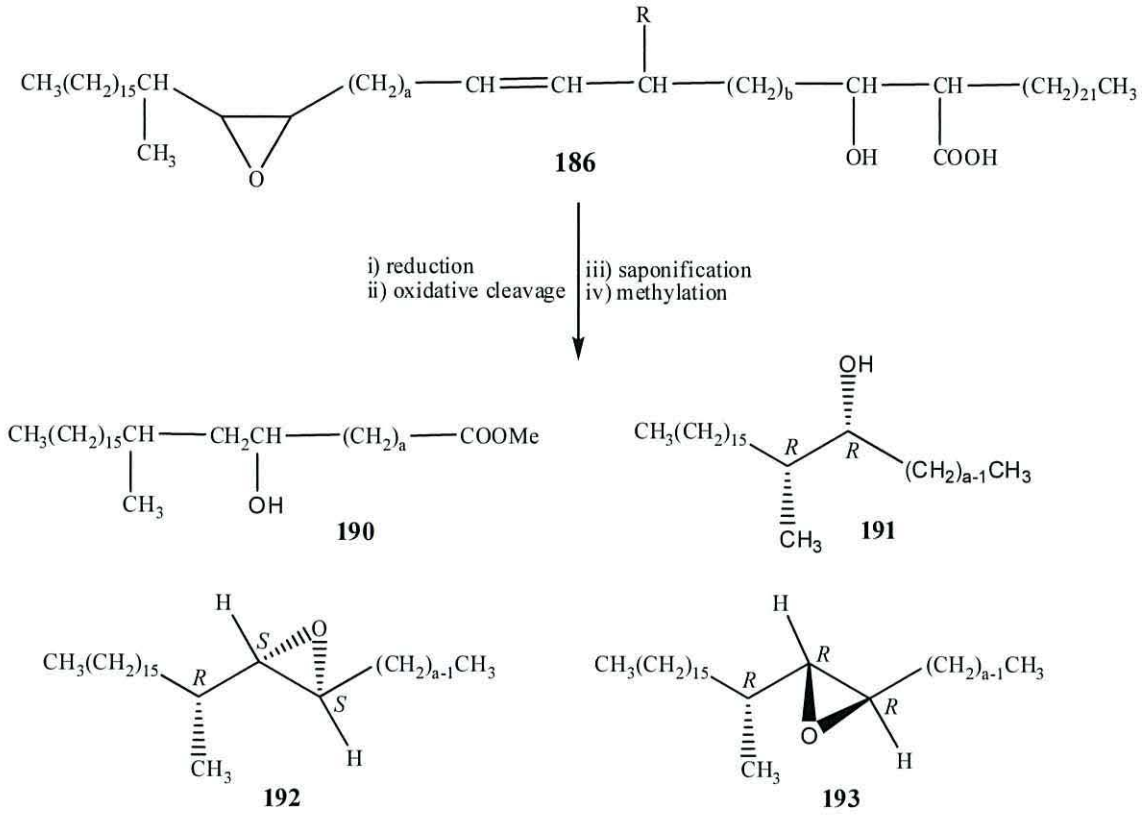
Compound (**104**) showed signals for the two epoxide hydrogens resonated at δ 2.73 (1H, dt, *J* 2.2, 5.35 Hz) and 2.43 (1H, dd, *J* 2.2, 7.25 Hz), together with two doublets at

δ 1.00 and 0.94 for the two methyl groups. The carbons of the epoxide appeared at δ 63.99 and 59.00 (**Table 4**). Epoxymycolates present in *M. smegmatis* showed a double doublets at δ 2.43 and a broad triplet at 2.72 for the hydrogens of the epoxide ring, moreover, Minnikin and co-workers reported that the chemical shift of the hydrogens of the epoxide ring resonated at δ 2.39 and δ 2.695, and that the signals of the methyl adjacent to the epoxide appearing at δ 0.98 and that adjacent to the alkene at δ 0.92. The close agreement between the ^{1}H and ^{13}C spectra obtained for (**104**) and that reported by Minnikin[117] and Yuan[147] for a natural epoxide mycolic acid suggests that the stereochemistry of the epoxide ring is that of (**104**). Moreover, the stereochemistry of epoxy mycolates containing a proximal alkene has been probed by two methods.[136] Firstly opening of the epoxide (**186**) by acetolysis followed by saponification and oxidative cleavage of both the 1,2-diol and the alkene leads to three products including *R*-acid (**187**). (**Scheme 43**).



Scheme 43: Opening of the epoxide ring by acetolysis, saponification and oxidative cleavage

Moreover, reductive ring-opening of the epoxide (**186**) followed by oxidative cleavage of the proximal alkene, saponification and methylation led to the two acids (**190**) and (**191**) (**Scheme 44**). The latter was shown to have *R*,*R*-stereochemistry. On this basis the authors assigned all the stereocentres in the epoxy-mycolic acid as *R*.[136]

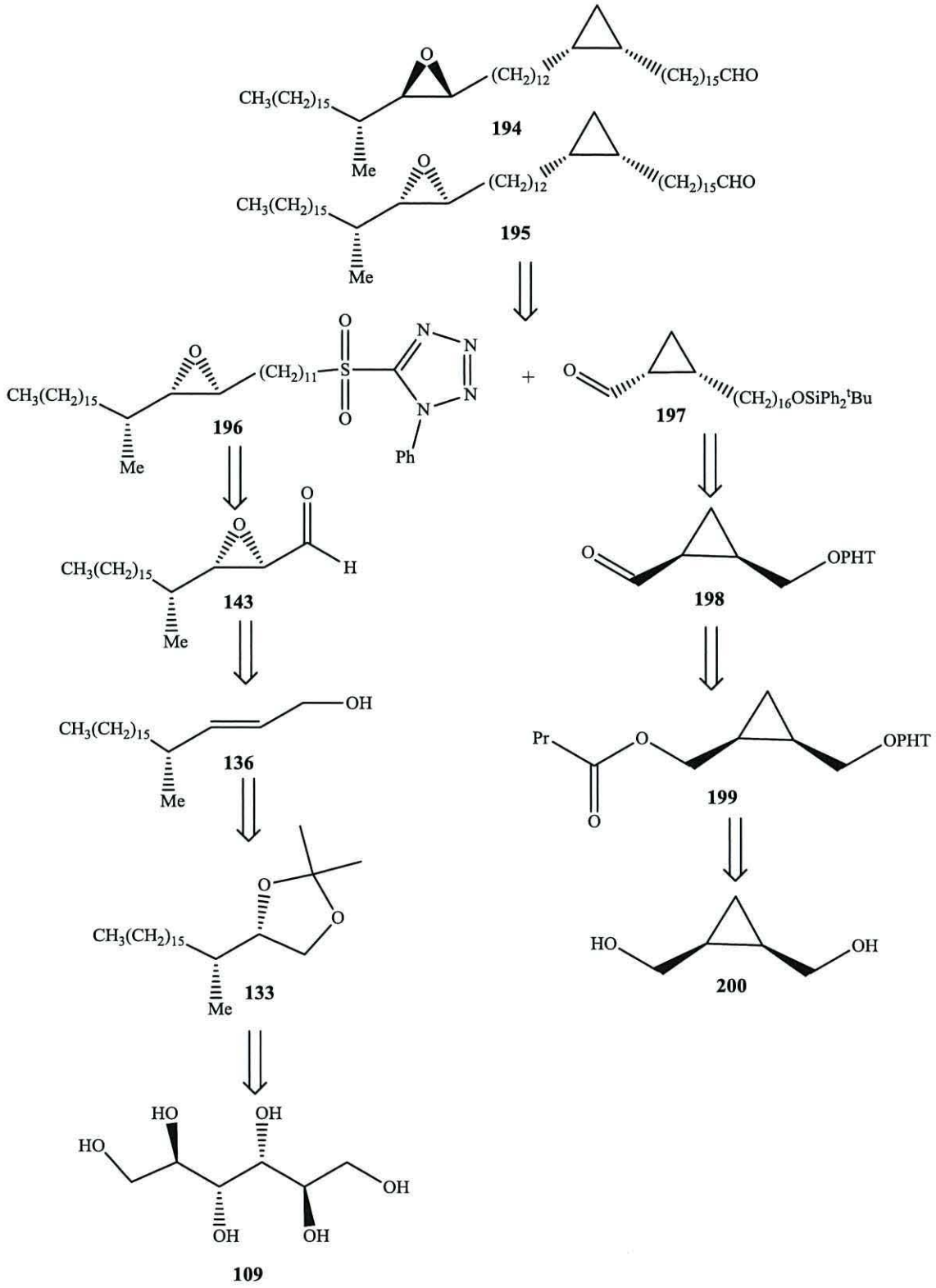


Scheme 44: Reduction, oxidatative cleavage, saponification and methylation of compound (**186**)

However, it seems clear in fact that the result actually suggests the epoxy fragment is *R*,*S*,*S* as in (**192**) rather than *R*,*R*,*R* as in (**193**), the priorities in the epoxide are different from those in the ring-opened alcohol.

2.4-The synthesis of *cis*-cyclopropane epoxy-meromycolaldehyde

The method used to prepare the epoxy-cyclopropane meromycolaldehydes (**194**) and (**195**) could be analysed as shown in **Scheme 45**.

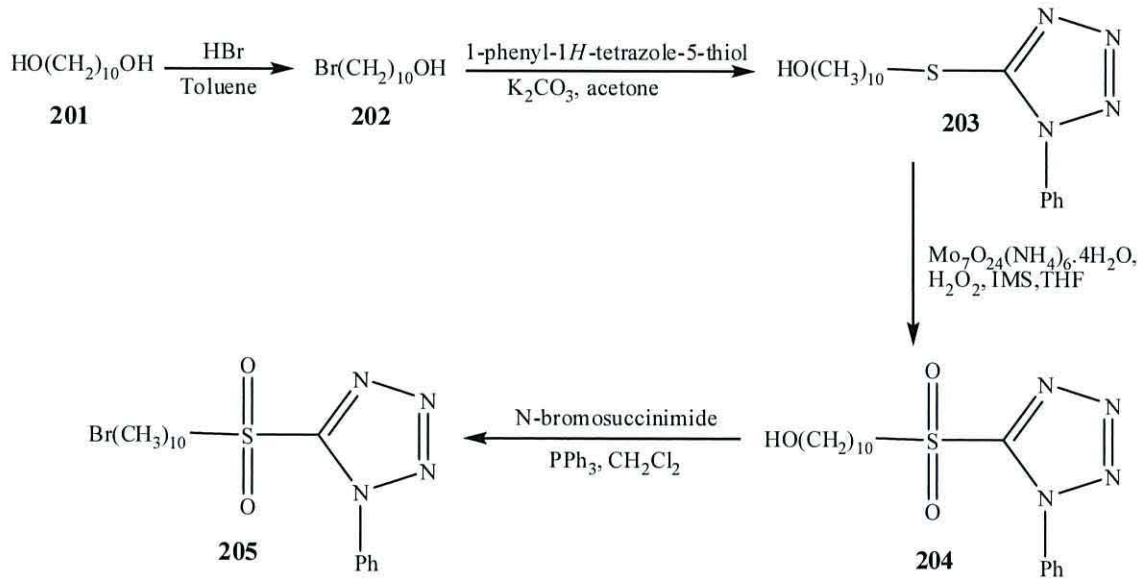


Scheme 45: The proposed preparation of (*R,R*) and (*S,S*)- epoxy-cyclopropane meromycolaldehydes (**194** and **195**)

2.4.1-The synthesis of (*R,R*) and (*S,S*)-epoxy sulfones (211, 195)

2.4.1a-Preparation of C_{10} sulfone (205)

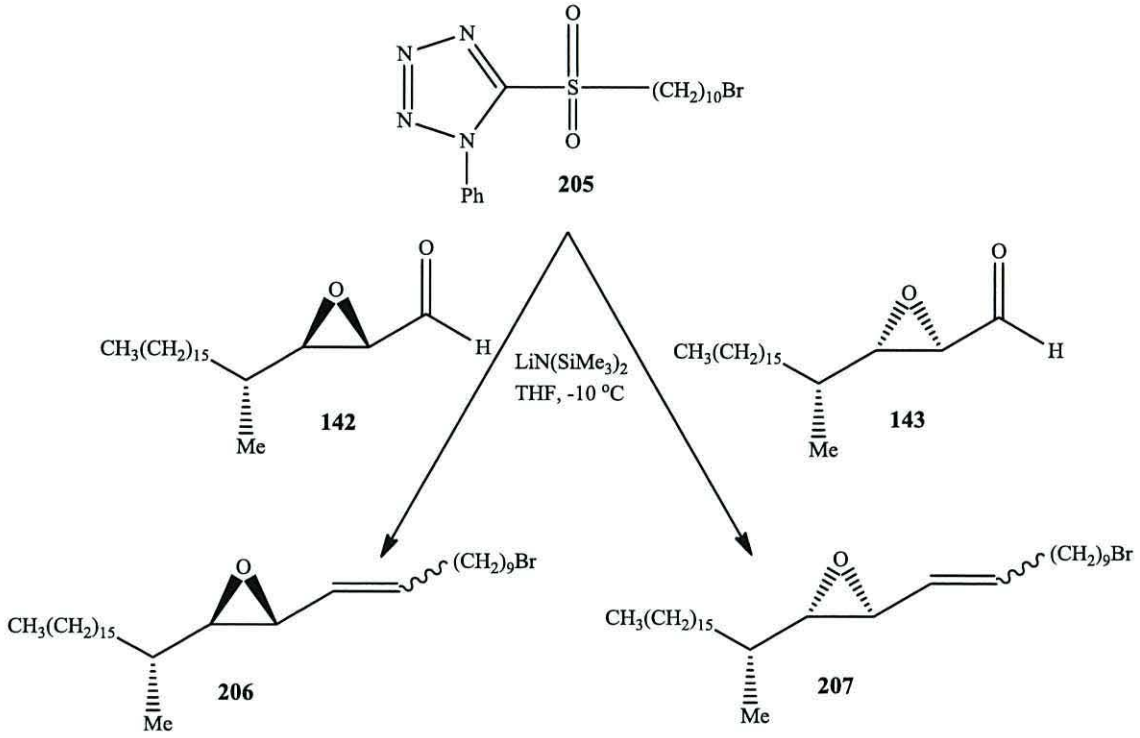
The sulfone (**205**) was prepared starting from 1,10-decanediol (**201**). The diol (**201**) was brominated using refluxing 48 % hydrobromic acid in toluene to give 10-bromodecane-1-ol (**202**),[214] followed by reaction with 1-phenyl-1*H*-tetrazole-5-thiol to give the sulfide (**203**). The subsequent oxidation of the sulfide with ammonium molybdate (VI) tetrahydrate and hydrogen peroxide gave the sulfone (**204**). Finally, the sulfone (**204**) was converted into bromo-compound (**205**) with N-bromosuccinimide and triphenylphosphine in dichloromethane. (**Scheme 46**).



Scheme 46: Preparation of bromo-sulfone (**205**)

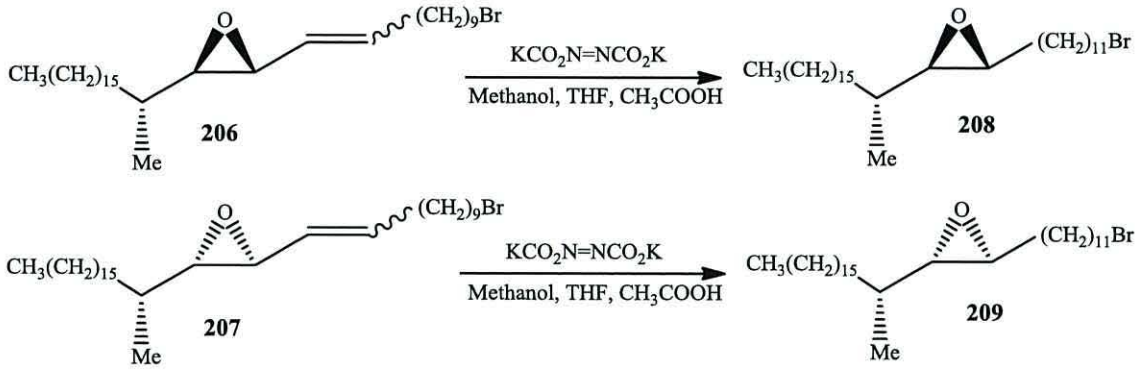
2.4.1b-The Julia olefination between aldehyde (142 / 143) and sulfone (205)

The modified Julia olefination between the aldehydes (**142**) and sulfone (**205**) in the presence of lithium bis(trimethylsilyl)amide in dry THF gave a mixture of alkenes (**206**).Compound (**207**) was prepared by the same procedure using aldehyde (**143**) and sulfone (**205**) **(Scheme 47**).



Scheme 47: The Julia reaction of (**142**) and (**143**) to produce (**206**) and (**207**)

Hydrogenation of mixtures of *E/Z*-alkene isomers of (**206**) and (**207**) using di-imide led to the saturated products (**208**) and (**209**), respectively (**Scheme 48**). Details of these reactions were again explained in **Section 2.2.8f**.

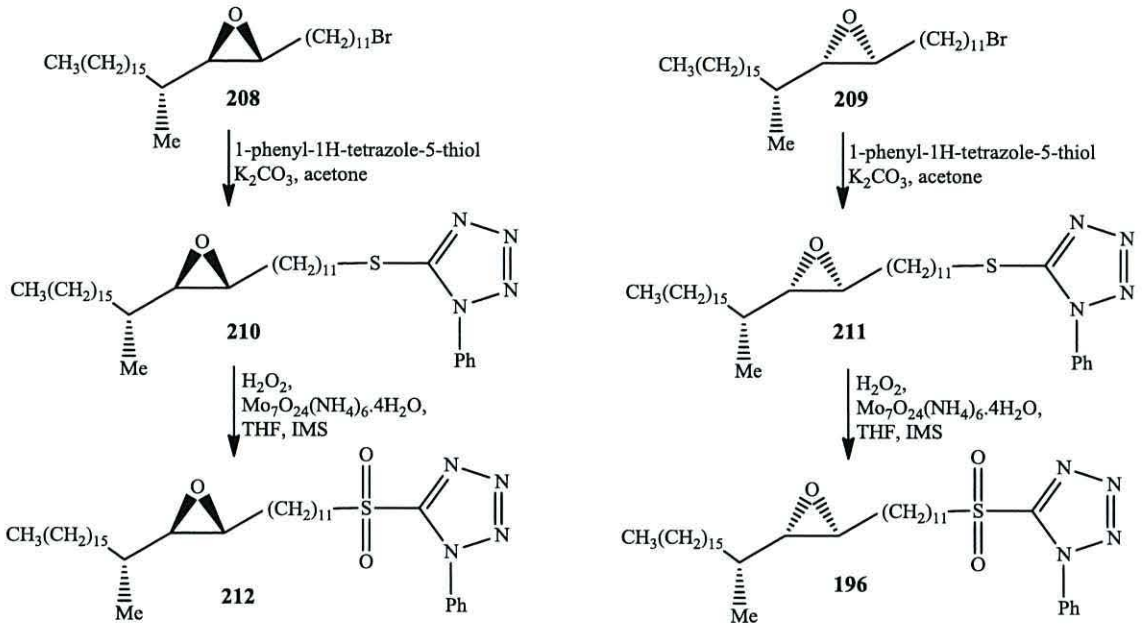


Scheme 48: Hydrogenation of (**206**) and (**207**) with dipotassium azodicarboxylate

Compounds (**208**) and (**209**) showed specific rotation of $[\alpha]_D^{24} = +11.13$ and $[\alpha]_D^{24} = -8.09$, respectively.

2.4.1c-Preparation of the (R,R) and (S,S)-epoxysulfones (212 / 196)

The bromides (**208** and **209**) were converted into the sulfides (**210** and **211**) with 1-phenyl-1*H*-tetrazole-5-thiol and potassium carbonate in acetone. Each gave a white solid (yield 91 and 71 %, respectively) (**Scheme 49**).

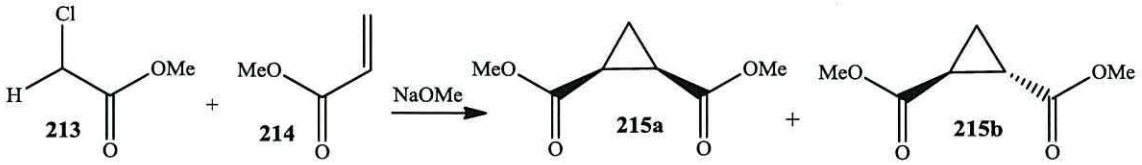


Scheme 49: Preparation of the intermediate epoxy sulfones (**212**) and (**196**)

The sulfides (**210** / **211**) were oxidized to the sulfones (**212** / **196**) and purified by column chromatography to give pure products, again as white solids (yield 90 and 84 %, respectively). (**Scheme 49**). The ^{1}H NMR of (**212**) and (**196**) as expected exhibited similar characteristics to those of the sulfones previously prepared in **Section 2.2.8h.**

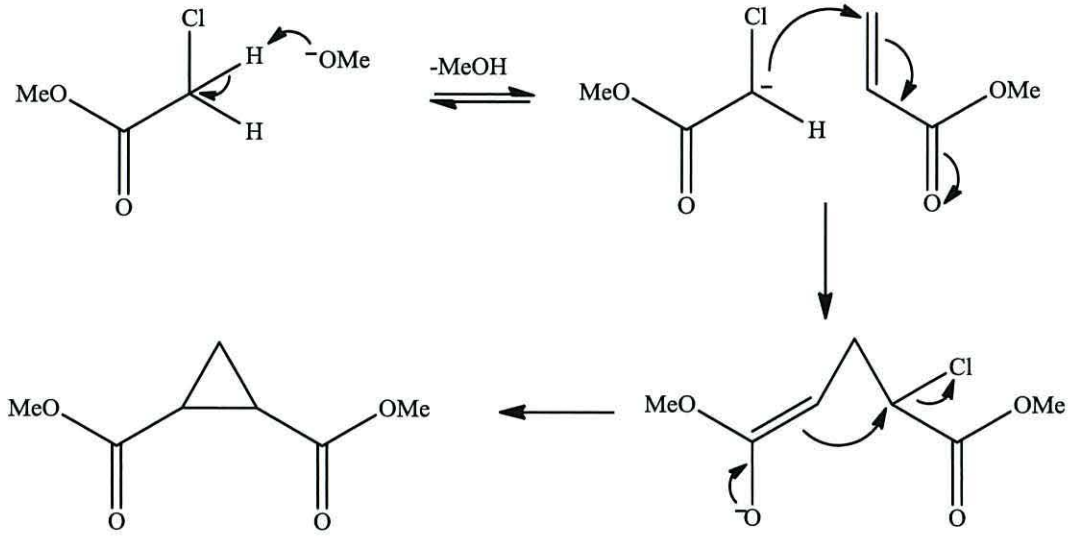
2.4.2-Preparation of the *cis*-cyclopropane

A single enantiomer of the *cis*-cyclopropane was prepared starting from methyl acrylate (**214**), methyl chloroacetate (**213**) and sodium methoxide to form a mixture *cis*-cyclopropane (**215a**) and *trans*-cyclopropane (**215b**) in a Michael Induced Ring Cyclisation.[224] It was then possible to separate the *cis*-cyclopropane-1,2-dicarboxylic acid dimethyl ester (**215a**) by vacuum distillation and flash column chromatography. (**Scheme 50**).



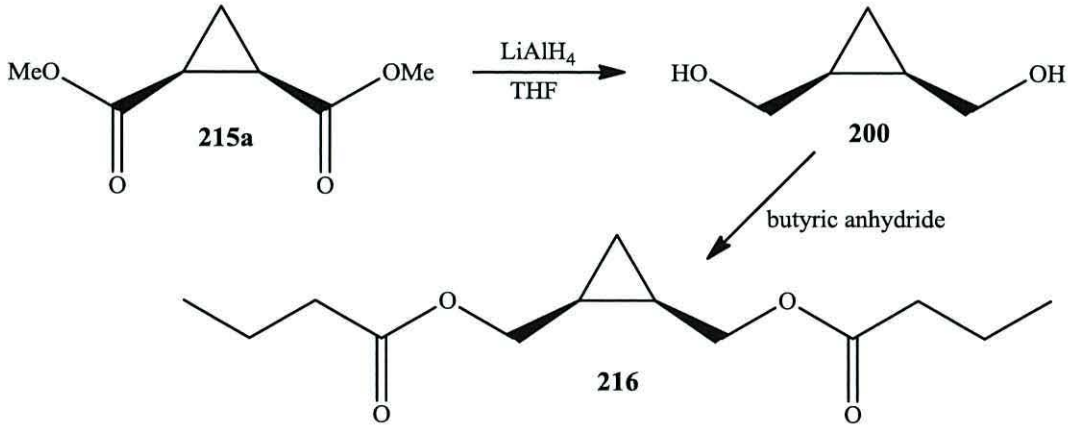
Scheme 50: Formation of diesters (**215a**) and (**215b**)

The cyclisation is initiated by the removal of an α-proton to the carbonyl group of methyl chloroacetate using sodium methoxide, and then the carbanion undergoes a Michael addition to the methyl acrylate, triggering the ring closure. (**Scheme 51).**



Scheme 51: The mechanism for Michael Induced Ring Cyclisation

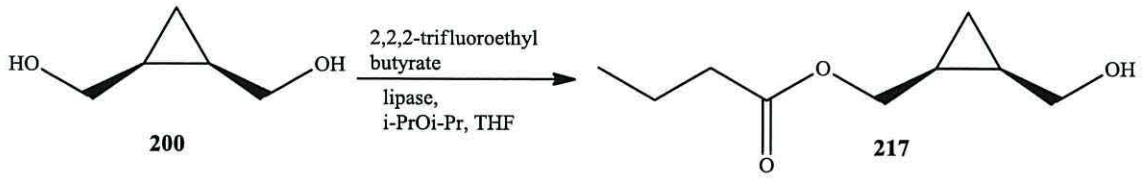
The diester (**215a**) was reduced by lithium aluminum hydride in THF to the corresponding diol (**200**). This was in turn protected with butyric anhydride to give *cis*-1,2-bis(butyryloxymethyl)cyclopropane (**216**) (**Scheme 52**).[225]



Scheme 52: Protection of the diol (**200**) as a dibutyl ester (**216**)

2.4.3-Enzymatic hydrolysis of the cyclopropyl dibutyrate

A single enantiomer of the cyclopropane unit, butyric acid (1*S*,2*R*)-*cis*-2-(hydroxymethyl)-cyclopropylmethyl ester (**217**) could be prepared directly from diol (**200**) with an isopropyl ether solution of 2,2,2-trifluoroethylbutyrate and lipase in THF as shown in (**Scheme 53**) and the optical rotation of this compound was $[\alpha]_D^{24}$ = -18.1.[226]



Scheme 53: The enzyme catalysed transesterification

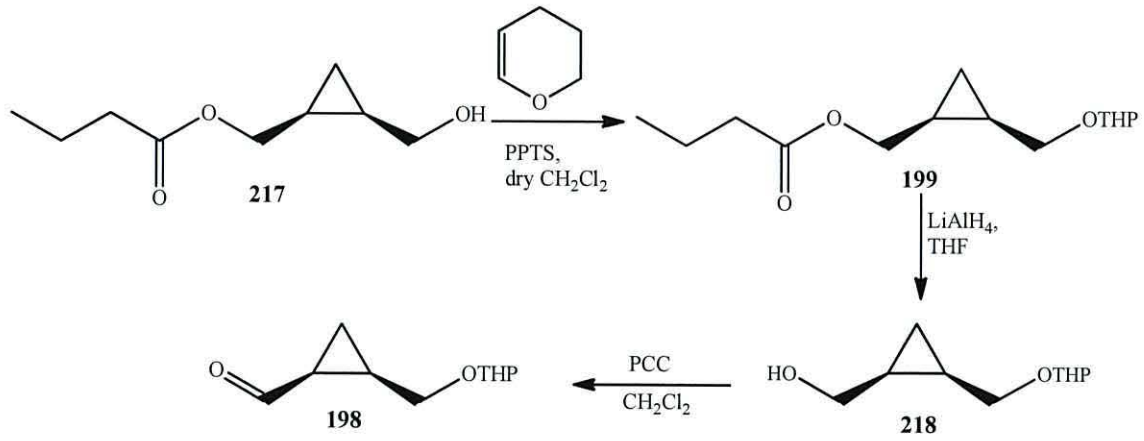
The monoester (**217**) was also characterized by its ^{1}H NMR spectra, where the splitting pattern observed for the *cis*-cyclopropane ring is in accordance with that reported in the literature. The IR spectrum showed a broad peak at 3435 cm^{-1} for OH stretch and peak at 1734 cm^{-1} for C=O stretch.

2.4.4-Preparation of *cis*-cyclopropane aldehyde for side chain extension (197)

The alcohol (**217**) was protected with 3,4-dihydro-2*H*-pyran and pyridinium-p-toluene-sulfonate as a catalyst to give the protected compound (**199**) in 84 % yield. The IR spectrum showed a peak at 1735 cm^{-1} for the C=O stretch and the disappearance of the peak for the OH stretch. The specific rotation of the compound (**199**) was $[\alpha]_D^{20} = +2.01$ (*c* 1.19, $CHCl_3$).

The cyclopropane methyl ester was reduced with $LiAlH_4$ to the corresponding alcohol (**218**). The IR spectrum of alcohol (**218**) showed a peak at 3435 cm^{-1} for OH stretch and no peak for a C=O stretch.

The alcohol (**218**) was oxidised using PCC to aldehyde (**198**) (as a pair of diastereomers because of the THP protection) (**Scheme 54**). The ^{1}H NMR spectrum of this showed a triplet at δ 9.41 (*J* 4.7 Hz) for the aldehyde proton. The protecting group protons (THP) on the ring adjacent to oxygen showed a multiplet at δ 4.59–4.58, a multiplet at δ 4.45–4.43, a doublet of doublets at δ 4.11 (*J* 2.2, 5.7 Hz) and a multiplet at δ 3.8–3.61. The ^{13}C NMR spectrum of aldehyde (**198**) showed four signals at δ 200.19 for the carbonyl carbon, δ 98.74 (acetal carbon), δ 65.16 ($O\underline{C}H_2$-) and δ 62.22 ($O\underline{C}H_2$-) for the carbons adjacent to oxygen. The specific rotation of (**198**) was $[\alpha]_D^{18} = +19.3$ (*c* 1.15, $CHCl_3$).



Scheme 54: Preparation the *cis*-cyclopropane aldehyde (**198**)

Compound (**198**) was then coupled to the previously prepared sulfone (**167**) in a modified Julia-Kocienski olefination to give a mixture of *E/Z* alkenes (**219**) in ratio 3.5:1 in 89 % yield, which was reduced by $LiAlH_4$ to give primary alcohol (**220**). Analysing the ^{1}H NMR spectrum of the product (**219**) revealed a doublet of triplets at δ 5.44 (*J* 6.6 Hz) and a multiplet at δ 5.29–5.2 for the *trans*-olefinic protons, and a doublet of triplets at δ 5.42 (*J* 7.25 Hz) and multiplet at δ 5.1–5.04 for the *cis*-olefinic protons (**See Fig. 30**).

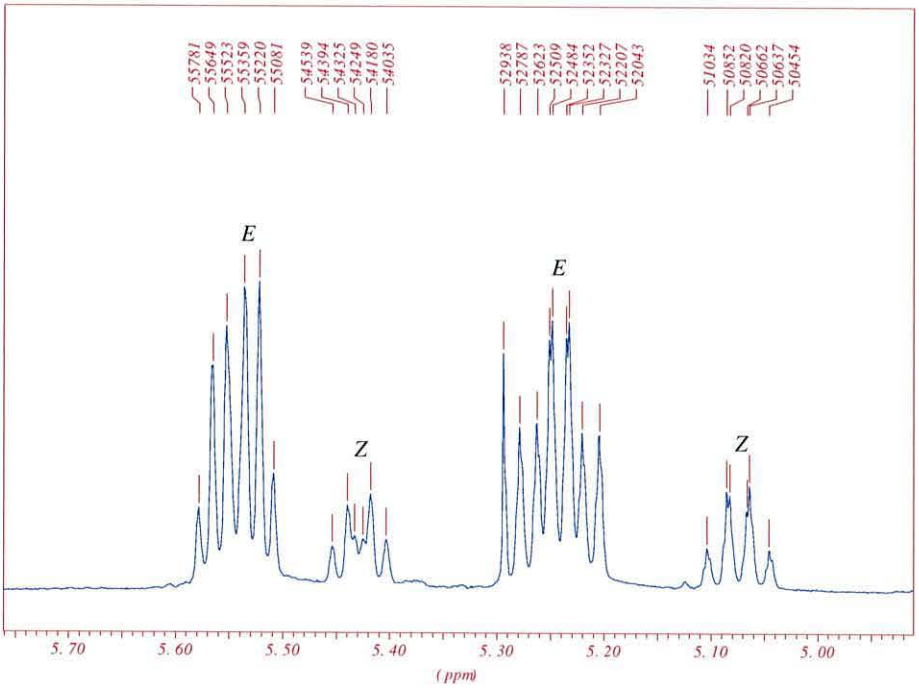
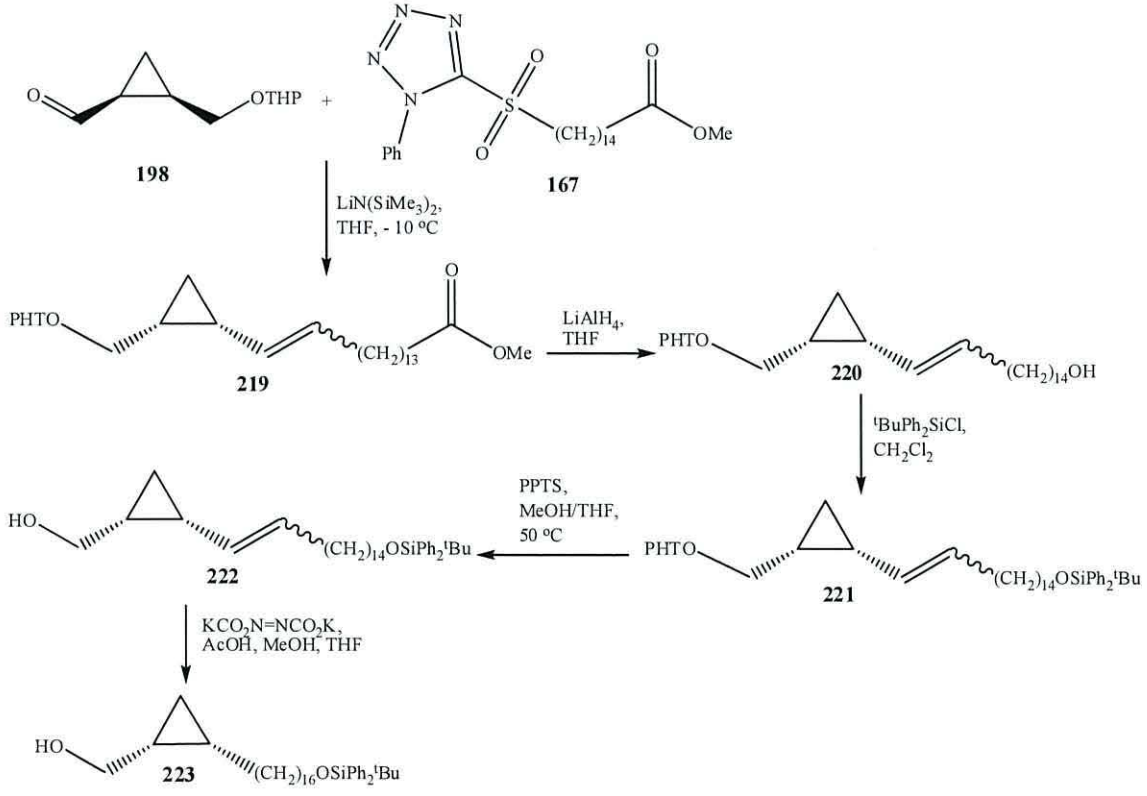


Fig 30: ^{1}H NMR of the alkene (**219**) for the olefinic region

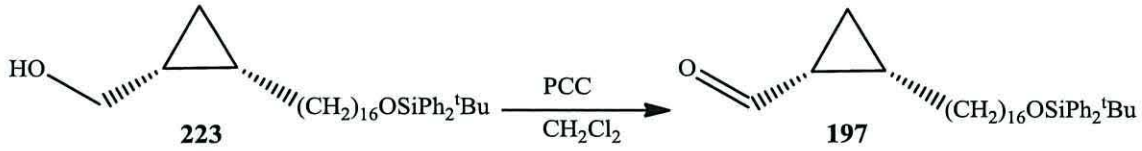
The primary alcohol (**220**) was protected as a silyl ether using *tert*-butyldiphenylchlorosilane to give the product (**221**) in 80 % yield, because the silyl

ether is stable against acids; the next step was deprotection of the tetrahydropyranyloxy (THP) group by pyridinium-*p*-toluene sulfonate in methanol giving ((1*R*,2*S*)-2-(16-(*tret*-butyldiphenylsilyloxy)-hexadecyl)cyclopropyl)methanol (**222**), followed by hydrogenation by di-imide generated from dipotassium azodicarboxylate, to give the saturated compound (**223**) as a colourless oil in 98 % yield (**Scheme 55**). The ^{1}H NMR spectrum of compound (**223**) showed no signal in the olefinic region and the silyl ether group showed two multiplets at δ 7.70–7.68 for four protons and δ 7.45–7.37 for six protons for the two phenyl groups and a nine hydrogen singlet at δ 1.06 corresponding to the *tert*-butyl group of the silyl ether. Other signals for the protons next to the oxygen group appeared as a multiplet at δ 3.68-3.64 for three protons, including a triplet at 3.67 (*J* 6.3 Hz) and a doublet of doublets at 3.59 (*J* 7.85, 11 Hz). Lastly, the cyclopropane protons showed two multiplets at δ 1.12–1.10 and δ 0.92–0.84 for two protons (-CH-), a doublet of triplets at δ 0.71 (*J* 4.45, 8.2 Hz) for the *cis* proton of the (-CH_2-) and a broad quartet at δ -0.019 (*J* 5.35 Hz) for the *trans* proton of the (-CH_2-). The ^{13}C NMR spectrum showed four signals at δ 135.54, 134.16, 129.42 and 127.52 for the carbons of the phenyl group and two signals at δ 63.99 and δ 63.29 for the carbons next to the oxygen. The IR showed a broad peak at 3368 cm^{-1} for O-H stretch.



Scheme 55: Preparation of saturated alcohol (**223**)

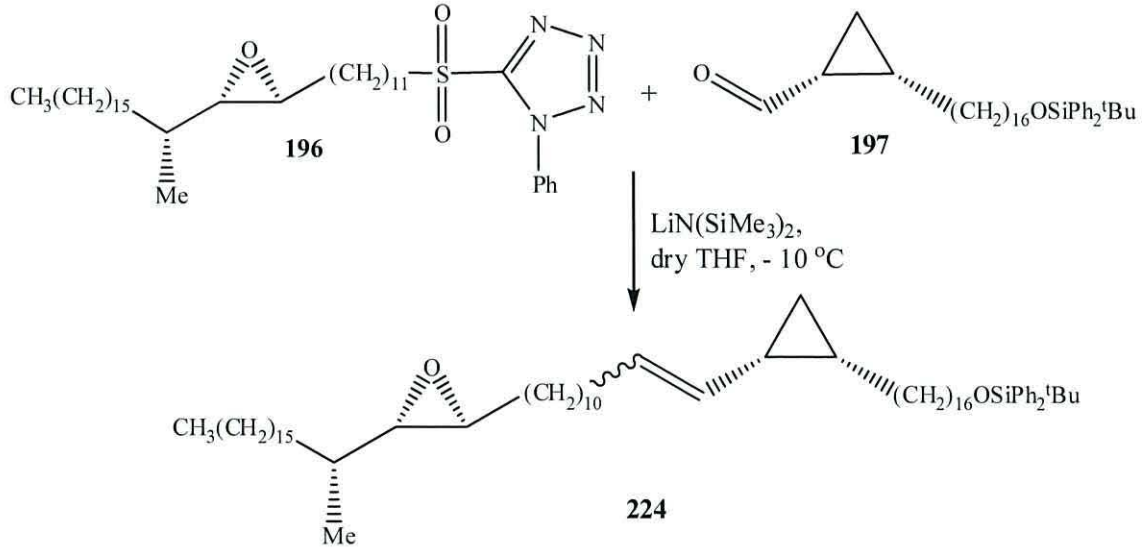
Oxidation of ((1*R*,2*S*)-2-(16-(*tert*-butyldiphenylsilyloxy)hexadecyl)cyclopropyl) methanol (**223**) with PCC in CH_2Cl_2 gave (1*S*,2*R*)-2-(16-(*tert*-butyldiphenylsilyloxy) hexadecyl)cyclopropanecarbaldehyde (**197**) in excellent yield (98 %) as a colourless oil (**Scheme 56**). The proton NMR spectrum of the aldehyde (**197**) showed a doublet at δ 9.35 (*J* 5.65 Hz) for the aldehyde proton, and a broad multiplet at δ 1.63–1.24 (including a broad singlet at 1.26) for the long chain protons.



Scheme 56: Preparation of aldehyde (**197**)

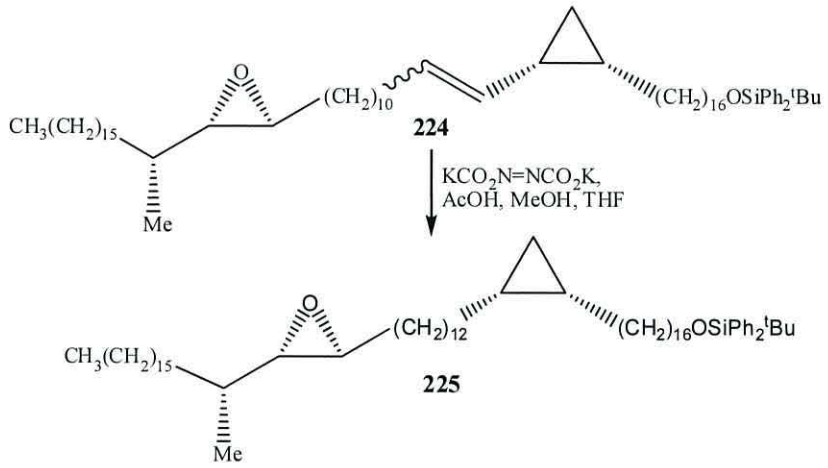
2.4.5-Final Julia reaction and hydrogenation for the preparation of *cis*-cyclopropane (*S,S*)-epoxy-meromycolaldehyde (194)

A modified Julia-Kocienski olefination was carried out in a similar fashion to that previously discussed. Reaction between aldehyde (**197**) and 5-(11-((2*S*,3*S*)-3-((*R*)-octadecan-2-yl)oxiran-2-yl)undecylsulfonyl)-1-phenyl-1*H*-tetrazole (**196**) using lithium bis(trimethylsilyl)amide as a base gave the alkene product (**224**) as a mixture of *E/Z* isomers (**Scheme 57**).



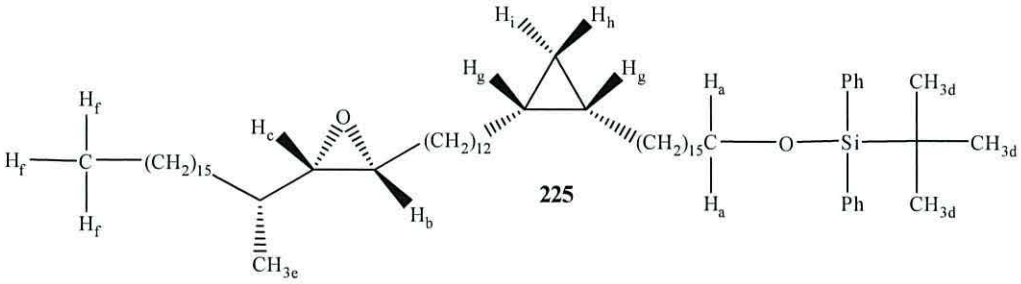
Scheme 57: Final coupling reaction between (**196**) and (**197**)

The alkene (**224**) was hydrogenated with di-imide generated from dipotassium azodicarboxylate to give saturated product (**225**) in a yield of 80 % (**Scheme 58**).



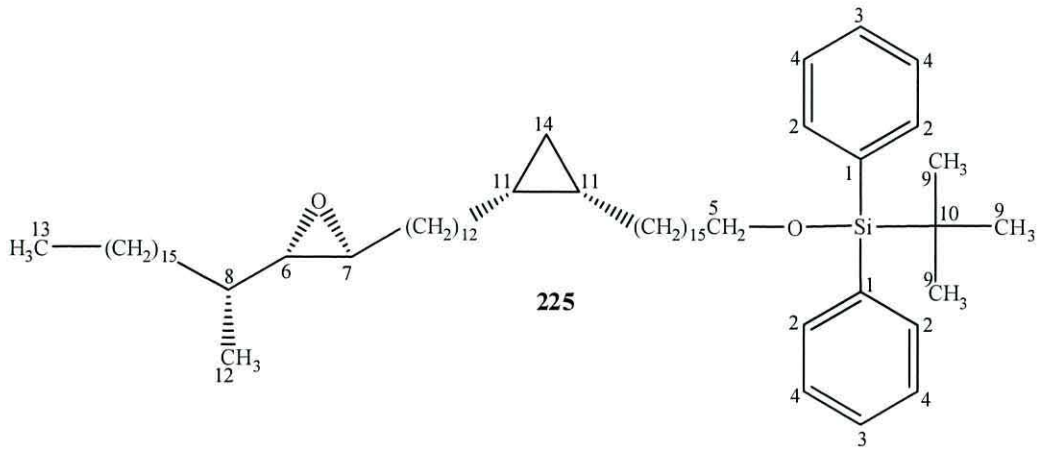
Scheme 58: Hydrogenation of (**224**) with dipotassium azodicarboxylate

The ^{1}H NMR and ^{13}C NMR of (**225**) spectra were analysed to contribute to the confirmation of its structure (**Table 5 and Table 6**).



Proton	δ	Multiplicity	integration	J (Hz)
H_a	3.65	t	2	6.6
H_b	2.72	dt	1	2.2, 5.5
H_c	2.41	dd	1	1.85, 6.9
H_d	1.05	s	9	-
H_e	1.0	d	3	6.0
H_f	0.89	t	3	6.65
H_g	0.67-0.64	m	2	-
H_h	0.56	dt	1	4.1, 8.2
H_i	-0.32	q	1	5.05

Table 5: ^{1}H NMR data analysis of (**225**)



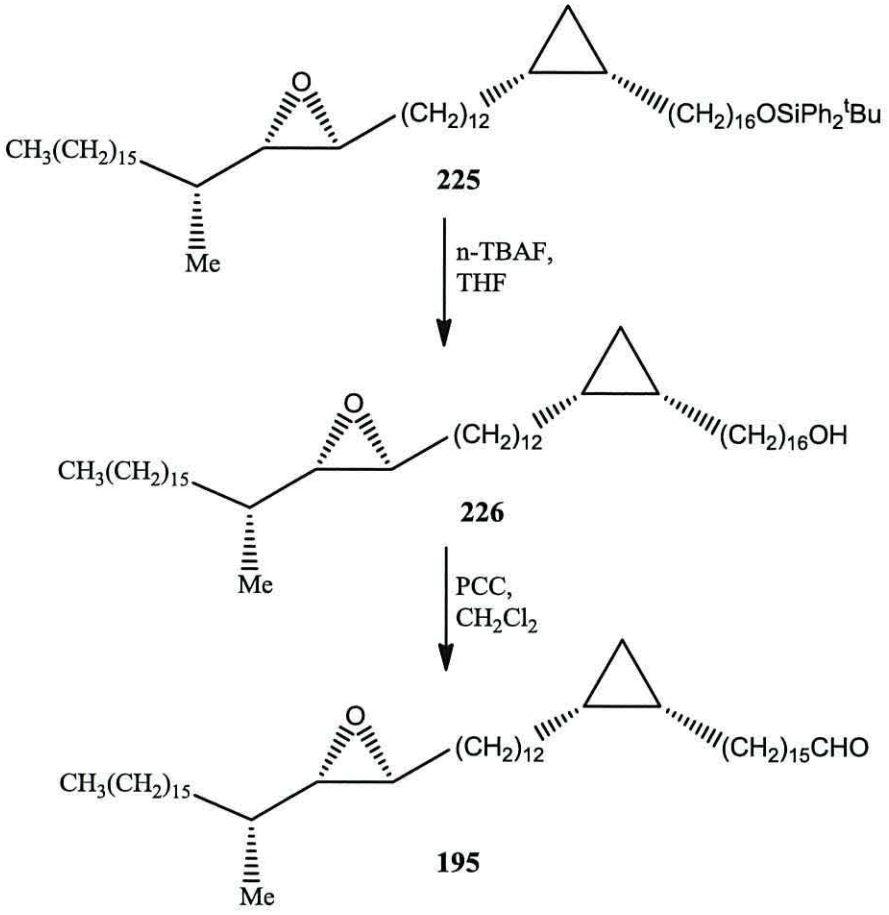
225

Carbon	δ	Carbon	δ
C_1	135.56	C_8	36.04
C_2	134.2	C_9	25.76
C_3	129.44	C_{10}	19.21
C_4	127.53	C_{11}	17.29
C_5	64.02	C_{12}	15.77
C_6	63.86	C_{13}	14.11
C_7	58.87	C_{14}	10.91

Table 6: The ^{13}C NMR data analysis of (**225**)

2.4.6-Deprotection and oxidation

The *tert*-butyldiphenylsilyl group of the compound (**225**) was removed using tetra-n-butylammonium fluoride to give the corresponding primary alcohol (**226**) (**Scheme 59**). The protected silyl ether group disappeared in the ^1H NMR spectrum of (**226**) which showed a triplet at δ 3.64 (*J* 6.6 Hz) for the two protons adjacent to the hydroxyl group. The ^{13}C NMR spectrum showed a signal at δ 63.89 for carbon next to the hydroxyl group and two signals at δ 63.09 and δ 58.9 for the two carbons of the epoxide ring. The IR included a broad peak at 3418 cm^{-1}.



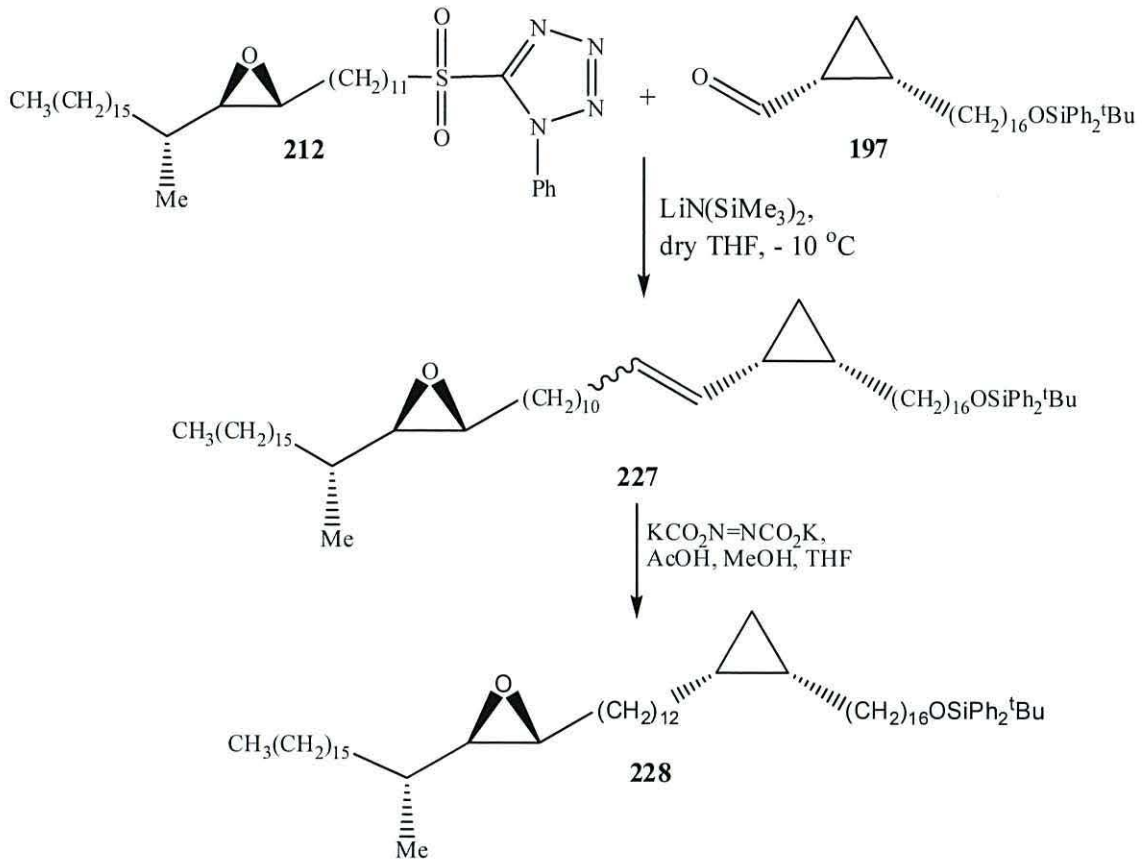
Scheme 59: Preparation of *cis*-cyclopropane (*S,S*)-epoxy-meromycolaldehyde (**195**)

Oxidation of the alcohol (**226**) with PCC gave the aldehyde (**195**) in yield 76 % (**Scheme 59**). The IR spectrum of this showed a peak at 1731 cm^{-1} for the C=O stretching and C-H stretching at 2922 and 2851 cm^{-1}. The ^{1}H NMR spectrum of (**195**) showed a triplet at δ 9.77 (*J* 1.55 Hz) for the aldehyde proton and a signal was seen in the ^{13}C NMR spectrum at δ 202.94 for the aldehyde carbon. All this confirmed that the aldehyde had formed.

2.4.7-Final Julia reaction and hydrogenation for the preparation of *cis*-cyclopropane (*R,R*)-epoxy-meromycolaldehyde (194)

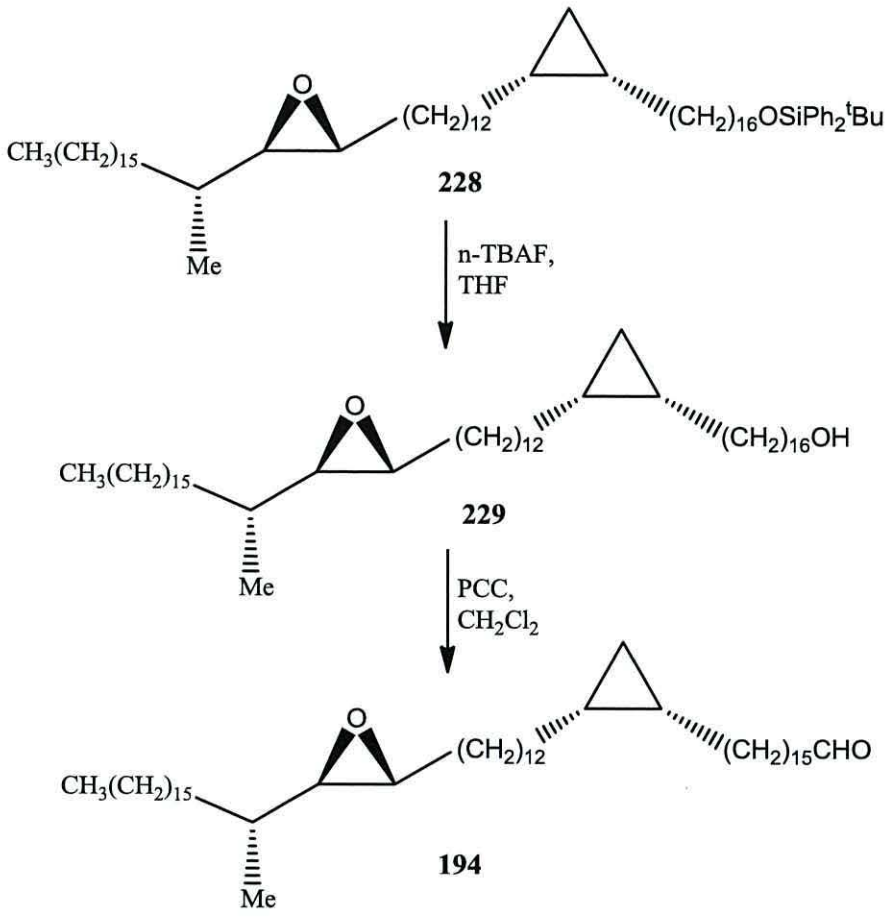
The sulfone (**212**) was then coupled to the previously prepared aldehyde (**197**) using lithium bis(trimethylsilyl)amide as base to give a mixture of alkenes (**227**), followed by hydrogenation using dipotassium azodicarboxylate to give the saturated compound (**228**) in a yield of 86 % (**Scheme 60**). The ^{1}H NMR spectrum of (**228**) showed no signals in the olefinic region, a multiplet at δ 0.67–0.62 for two protons, a broad doublet of triplets at δ 0.56 (*J* 4.1, 8.2 Hz) and a broad quartet at δ -0.32 (*J* 5.05 Hz) for the cyclopropane ring protons. The specific rotation of (**228**) was measured at $[\alpha]_D^{20} = +$

1.96 (*c* 1.02, $CHCl_3$), compared to that of compound (**225**) which was $[\alpha]_D^{20}$ = - 4.94 (*c* 0.85, $CHCl_3$).



Scheme 60: Final coupling between (**212**) and (**197**) and hydrogenation

Deprotection of the *tert*-butyldiphenylsilyl group was done using n-TBAF to give the corresponding alcohol (**229**), which was then oxidized using PCC to the aldehyde (**194**) in a yield of 80 % (**Scheme 61**).



Scheme 61: Preparation of the aldehyde (**194**)

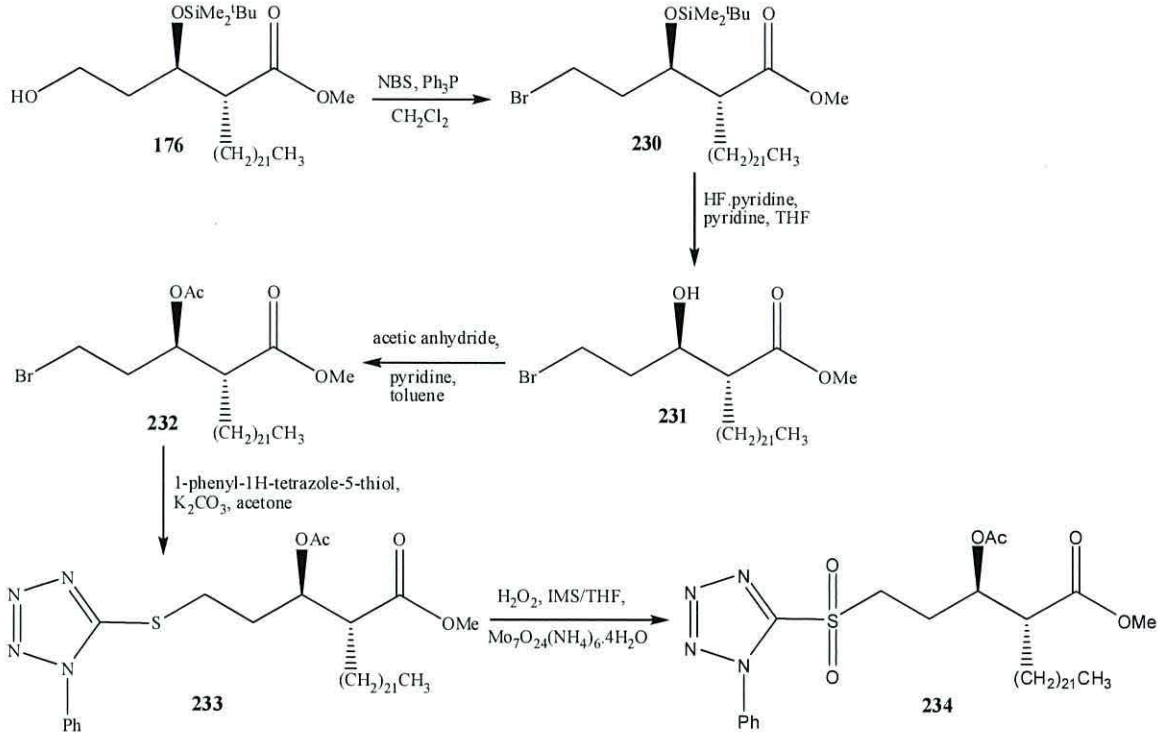
The ^{1}H NMR spectrum was used to confirm the completion of the above reactions. The protected silyl ether group disappeared in the NMR spectrum of (**229**) which showed a triplet at δ 3.64 (*J* 6.6 Hz) for the two protons adjacent to the hydroxyl group. The IR spectrum of (**229**) showed a peak at 3418 cm^{-1} to confirm the formation of the O-H bond. Oxidation of (**229**) to the aldehyde (**194**) led to a one proton triplet at δ 9.77 (*J* 1.9 Hz) for the aldehyde proton and the ^{13}C NMR spectrum showed a signal at δ 202.95 for the aldehyde carbon. The IR spectrum showed a peak at 1731 cm^{-1} for C=O stretching.

2.5-Preparation of the mycolic motif

2.5.1-Preparation of intermediate sulfone (234)

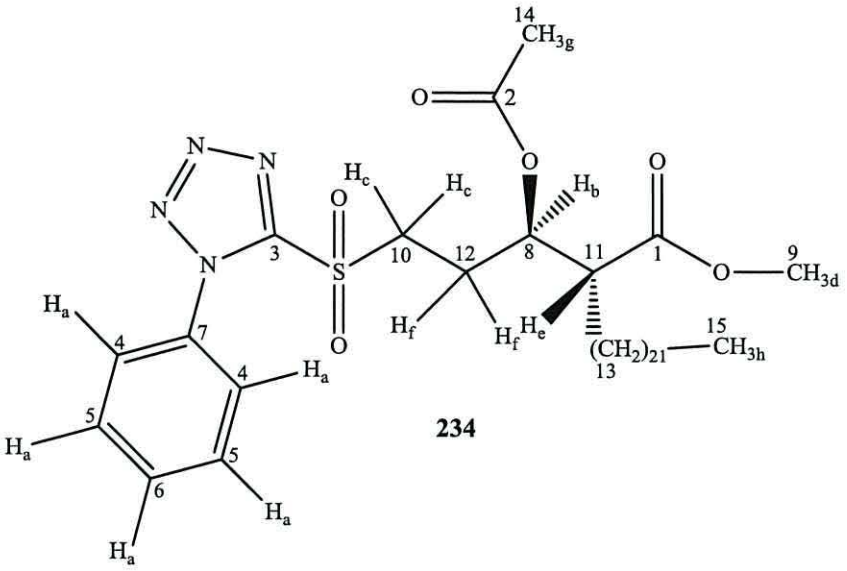
The primary alcohol (**176**) was prepared as discussed in **Section 2.3.4**. The alcohol (**176**) was brominated with N-bromosuccinimide and triphenylphospine yielding the bromide (**230**). This reaction involved changing the silyl protecting group at this stage to an acetyl group. This was because the mycolic acid contained an epoxide ring in structure, and the epoxide ring may be sensitive to the acid.

Consequently, the silyl ether was removed using hydrofluoric acid in pyridine complex to give the secondary alcohol (**231**) in 77 % yield. The IR spectrum of (**231**) included a broad peak at 3429 cm^{-1} for the OH stretch. Protection of the secondary alcohol as an acetoxy group by using acetic anhydride and anhydrous pyridine in dry toluene gave methyl ester (**232**) in an excellent yield of 95 %. The sulfide (**233**) was prepared following the same procedure as previously discussed, by reaction with 1-phenyl-1*H*-tetrazole-5-thiol in the presence of potassium carbonate and was then oxidized using ammonium molybdate (VI) tetrahydrate to give the desired sulfone intermediate (**234**) (**Scheme 62**).



Scheme 62: Preparation of sulfone (**234**)

The ^{1}H NMR spectrum of (**176**) was compared with the brominated product (**230**). The most significant change was the shift to a higher field of the multiplet at δ 4.29–4.25 for (–C<u>H</u>$_2$OH) to the multiplet at δ 3.46–3.42 for (–C<u>H</u>$_2$Br). The optical rotation of the bromo-compound (**230**), secondary alcohol (**231**), methyl ester (**232**), sulfide (**233**) and sulfone (**234**) were measured at + 7.83, + 13.8, + 26.07, + 13.84 and + 22.15, respectively. The ^{1}H NMR and ^{13}C NMR spectra of sulfone (**234**) were analysed to contribute to the confirmation of its structure (**Table 7**).



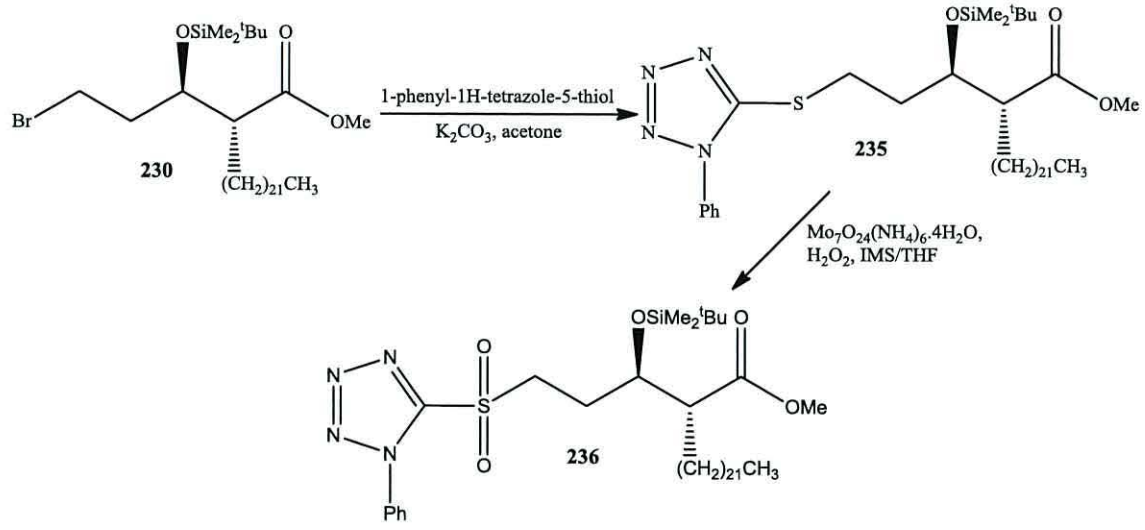
234

Proton	δ	Multiplicity	Integration	J (Hz)	Carbon	δ
H_a	7.70-7.57	m	5	-	C_1	172.67
H_b	5.23	dt	1	3.15, 7.9	C_2	170.15
H_c	3.84-3.73	m	2	-	C_3	153.13
H_d	3.7	s	3	-	C_4	132.91
H_e	2.66	ddd	1	4.1, 6.95, 10.7	C_5	131.5
H_f	2.41-2.34	m	1	-	C_6	129.74
H_f	2.27-2.20	m	1	-	C_7	124.98
H_g	2.09	s	3	-	C_8	71.68
H_h	0.88	t	3	6.6	C_9	52.52
-	-	-	-	-	C_{10}	51.87
-	-	-	-	-	C_{11}	49.16
-	-	-	-	-	C_{12}	31.89
-	-	-	-	-	C_{13}	29.7-22.7
-	-	-	-	-	C_{14}	20.84
-	-	-	-	-	C_{15}	14.09

Table 7: ^{1}H NMR and ^{13}C NMR data analysis of intermediate corenomycolate sulfone (**234**)

2.5.2-Preparation of intermediate sulfone (236)

Another method was used to prepare the intermediate sulfone moiety (**236**) containing the *tert*-butyldimethylsilyl protecting group. The bromo-compound (**230**) was converted into sulfide (**235**) using 1-phenyl-1*H*-tetrazole-5-thiol, and was then oxidized with hydrogen peroxide to give a white solid sulfone (**236**) in 90 % yield (**Scheme 63**).



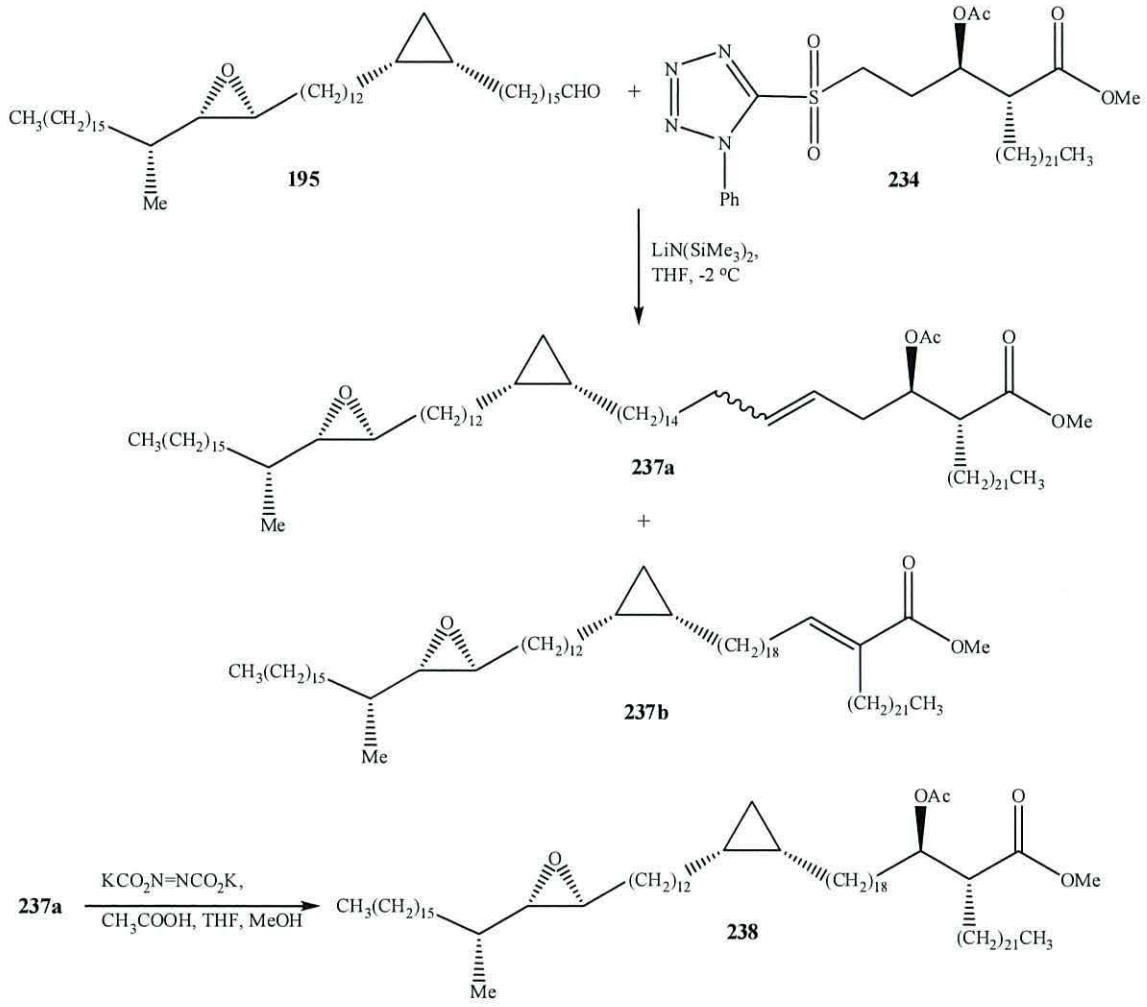
Scheme 63: Preparation the intermediate sulfone **(236)**

The characteristic sulfide protons of (**235**) in the ^{1}H NMR spectrum showed as two multiplets at δ 3.49–3.43 and 3.40–3.34 for the two protons next to the sulfanyl group (-C<u>H</u>$_2$S-). The sulfone (**236**) showed a multiplet at δ 3.81–3.77 for the two protons next to the sulfonyl group (-C<u>H</u>$_2$SO$_2$).

2.6-The synthesis of *cis*-cyclopropane (*S,S*)-epoxy-mycolic acid (105)

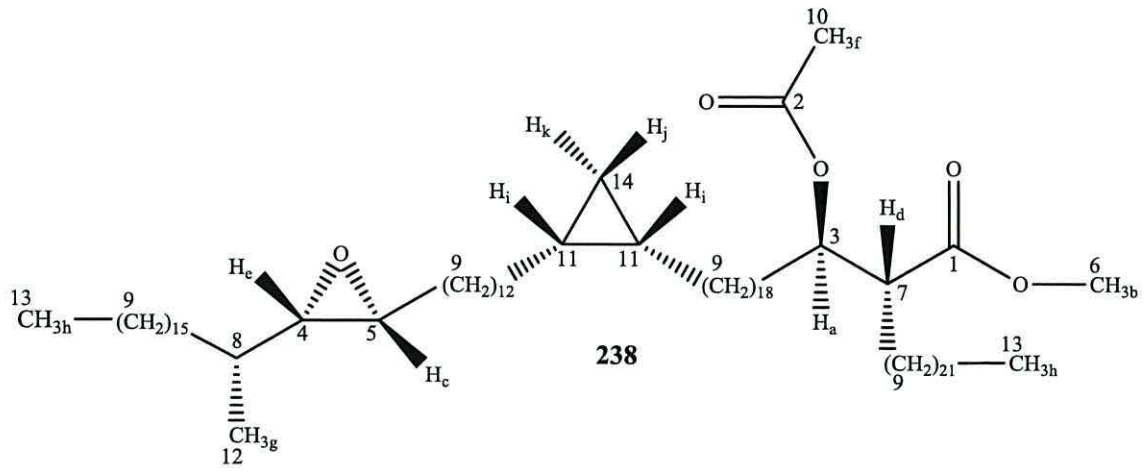
2.6.1-The final coupling reaction

A modified Julia reaction was carried out between (**195**) and (**234**) in the presence of lithium bis(trimethylsilyl)amide as a base to give the corresponding alkene (**237a**) in 25 % yield. The alkene (**237a**) was hydrogenated with di-imide generated from dipotassium azodicarboxylate and acetic acid, THF and methanol to give the saturated protected mycolic acid (**238**) (**Scheme 64**).



Scheme 64: Final coupling and hydrogenation to produce methyl mycolate (**238**)

^{1}H NMR, ^{13}C NMR, IR, optical rotation and mass spectrometry were used to confirm the structure of (**238**). The specific rotation of (**238**) was measured as $[\alpha]_D^{22} = -9.59$ (*c* 0.57, $CHCl_3$) and the IR spectrum included a peak at 1746 cm^{-1} for C=O stretch. The mass spectrum of the protected *cis*-cyclopropane (*S,S*)-epoxy-mycolic acid (**238**) showed an ion at $(M + Na)^+$: 1232.1819 while $C_{81}H_{156}NaO_5$ requires: 1232.1845. In addition selected ^{1}H NMR and ^{13}C NMR data analysis can be seen in **Table 8**.



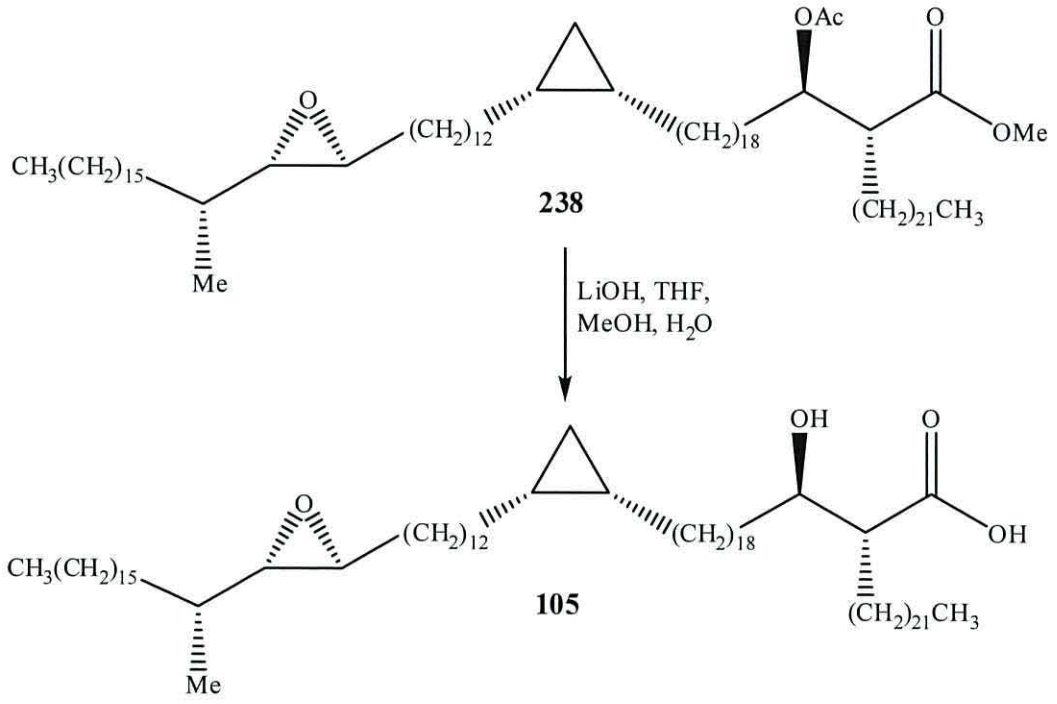
238

Proton	δ	Multiplicity	Integration	J (Hz)	Carbon	δ
H_a	5.08	dt	1	3.8, 7.55	C_1	173.64
H_b	3.68	s	3	-	C_2	170.32
H_c	2.72	dt	1	2.2, 5.65	C_3	74.09
H_d	2.62	ddd	1	4.1, 6.6, 10.7	C_4	63.85
H_e	2.41	dd	1	1.9, 7.25	C_5	58.86
H_f	2.03	s	3	-	C_6	51.52
H_g	1.0	d	3	5.95	C_7	49.57
H_h	0.88	t	6	6.65	C_8	36.03
H_i	0.65-0.64	m	2	-	C_9	33.77-21.0
H_j	0.56	dt	1	4.1, 8.2	C_{11}	17.29
H_k	-0.32	q	1	5.05	C_{12}	15.76
-	-	-	-	-	C_{13}	14.10
-	-	-	-	-	C_{14}	10.9

Table 8: ^{1}H NMR and ^{13}C NMR data analysis of compound (**238**)

2.6.2-The hydrolysis of mycolic acid methyl ester

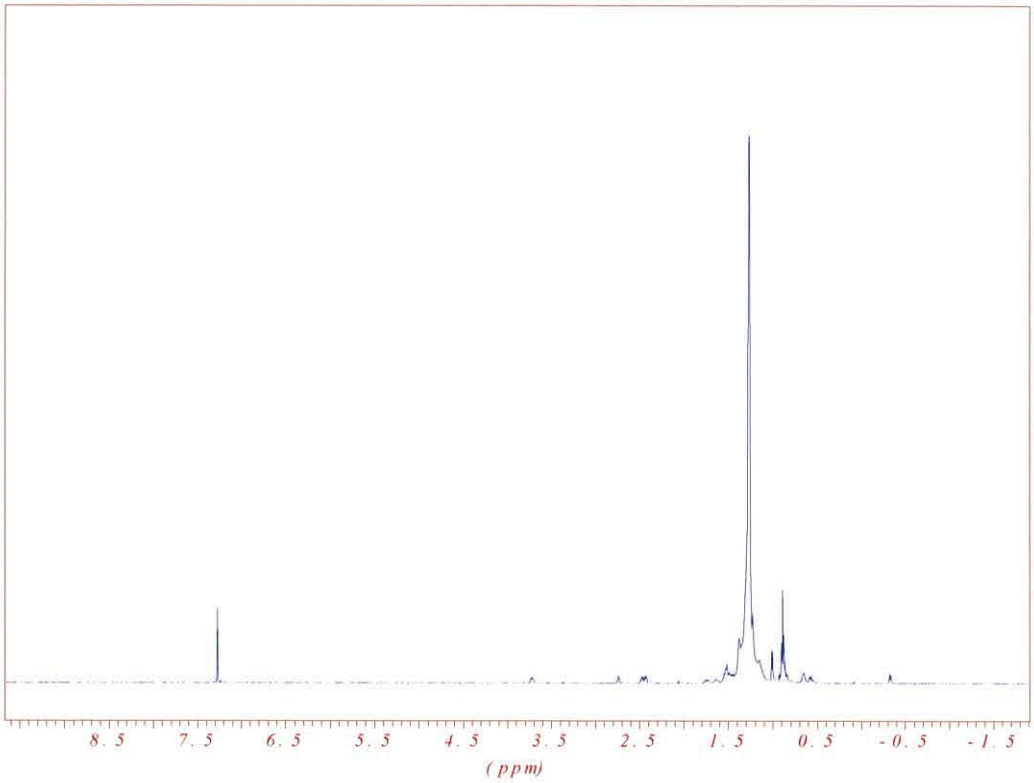
The hydrolysis of the mycolic acid methyl ester (**238**) to give free acid was necessary for biological testing. The methyl ester and acetate groups were deprotected using an excess of lithium hydroxide monohydrate in THF, methanol and water and gave free mycolic acid (**105**) (**Scheme 65**). The optical rotation of (**105**) was recorded as $[\alpha]_D^{24} = -8.44$, (*c* 0.9, $CHCl_3$). The IR spectrum showed a broad peak at 3375 cm^{-1} for the O-H stretching and the mass spectrum showed a molecular ion $(M + Na)^+$ at 1176.154, [$C_{78}H_{152}NaO_4$ requires: 1176.1583].



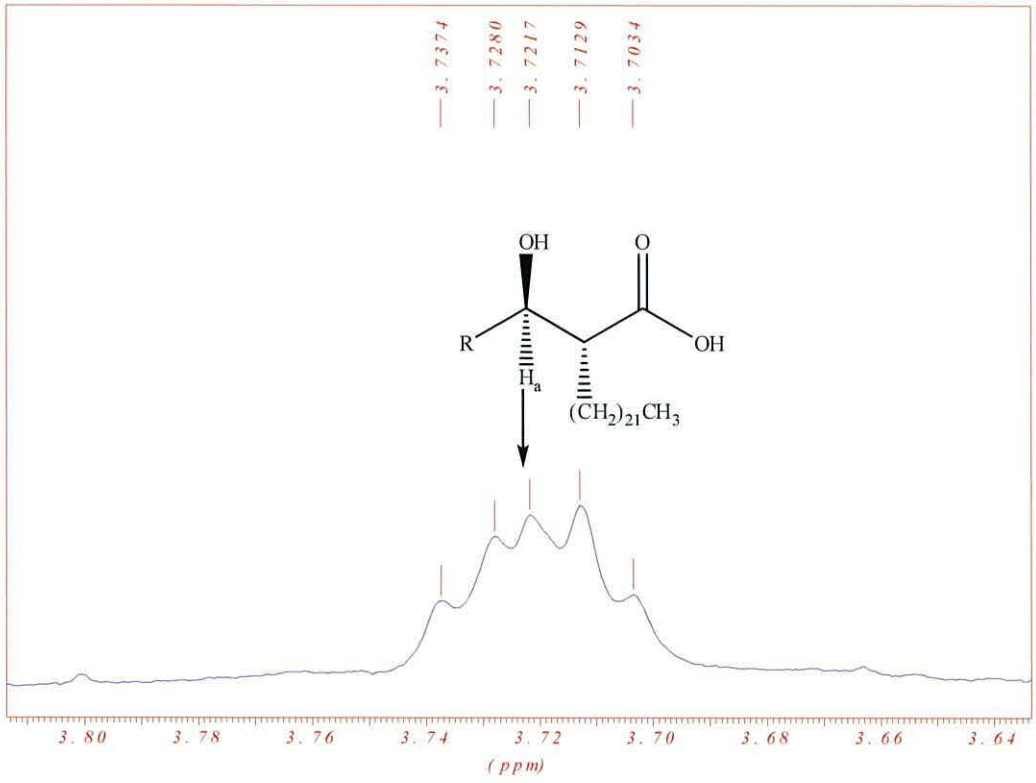
Scheme 65: The hydrolysis of mycolic acid methyl ester (**238**) to yield mycolic acid (**105**)

Expansions of the various regions of the ^1H NMR spectrum of *cis*-cyclopropane (*S,S*)-epoxy-mycolic acid (**105**) are shown in **Figure 31b-e**.

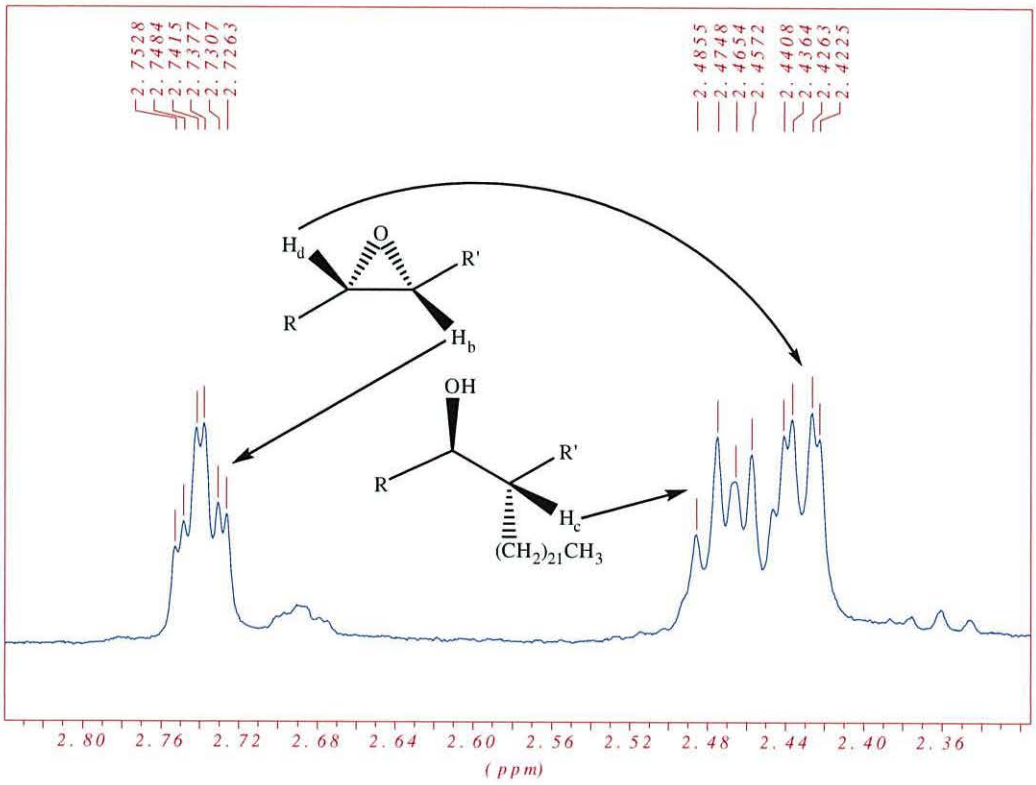
(**a**)


8.5
7.5
6.5
5.5
4.5
3.5
2.5
1.5
0.5
-0.5
-1.5
(ppm)

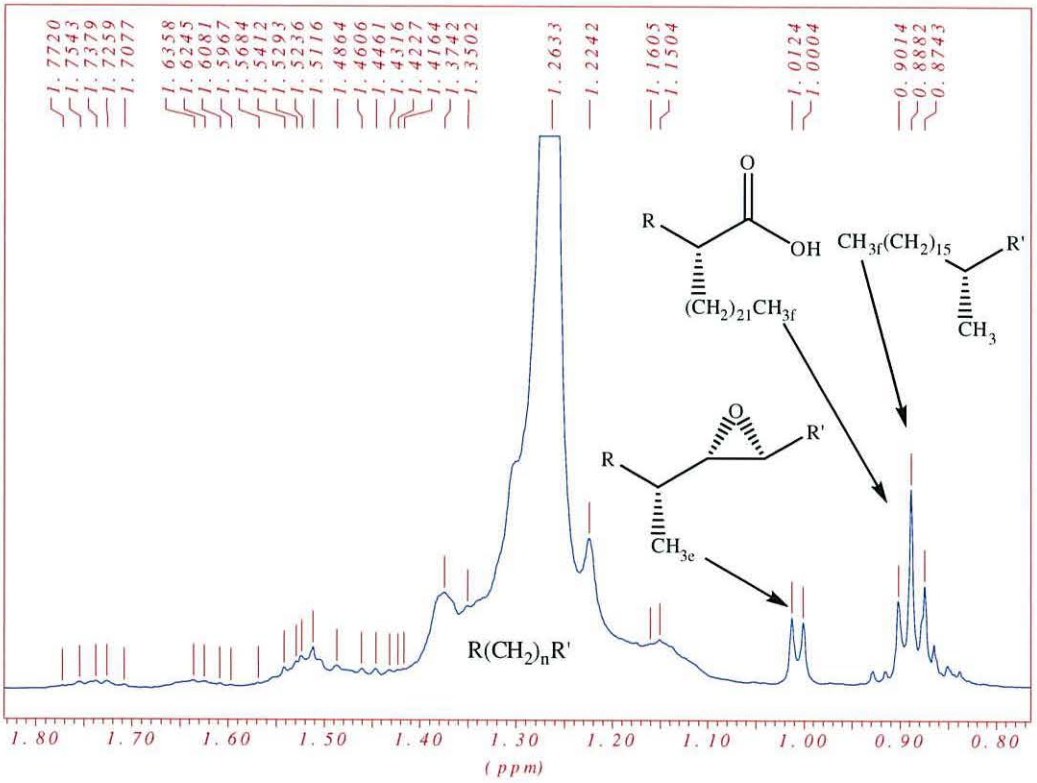
(**b**)


3.7374
3.7280
3.7217
3.7129
3.7034
OH
O
R
OH
Ha
(CH2)21CH3
3.80
3.78
3.76
3.74
3.72
3.70
3.68
3.66
3.64
(ppm)

(**c**)


2.7528
2.7484
2.7415
2.7377
2.7307
2.7263
2.4855
2.4748
2.4654
2.4572
2.4408
2.4364
2.4263
2.4225
H_d
O
R'
R
H_b
OH
R'
R
H_c
(CH_2)_21CH_3
2.80 2.76 2.72 2.68 2.64 2.60 2.56 2.52 2.48 2.44 2.40 2.36
(ppm)

(**d**)


1.7720
1.7543
1.7379
1.7259
1.7077
1.6358
1.6245
1.6081
1.5967
1.5684
1.5412
1.5293
1.5236
1.5116
1.4864
1.4606
1.4461
1.4316
1.4227
1.4164
1.3742
1.3502
1.2633
1.2242
1.1605
1.1504
1.0124
1.0004
0.9014
0.8882
0.8743
O
R
OH
(CH_2)_21CH_3f
CH_3f(CH_2)_15
R'
CH_3
O
R'
R
CH_3e
R(CH_2)_nR'
1.80 1.70 1.60 1.50 1.40 1.30 1.20 1.10 1.00 0.90 0.80
(ppm)

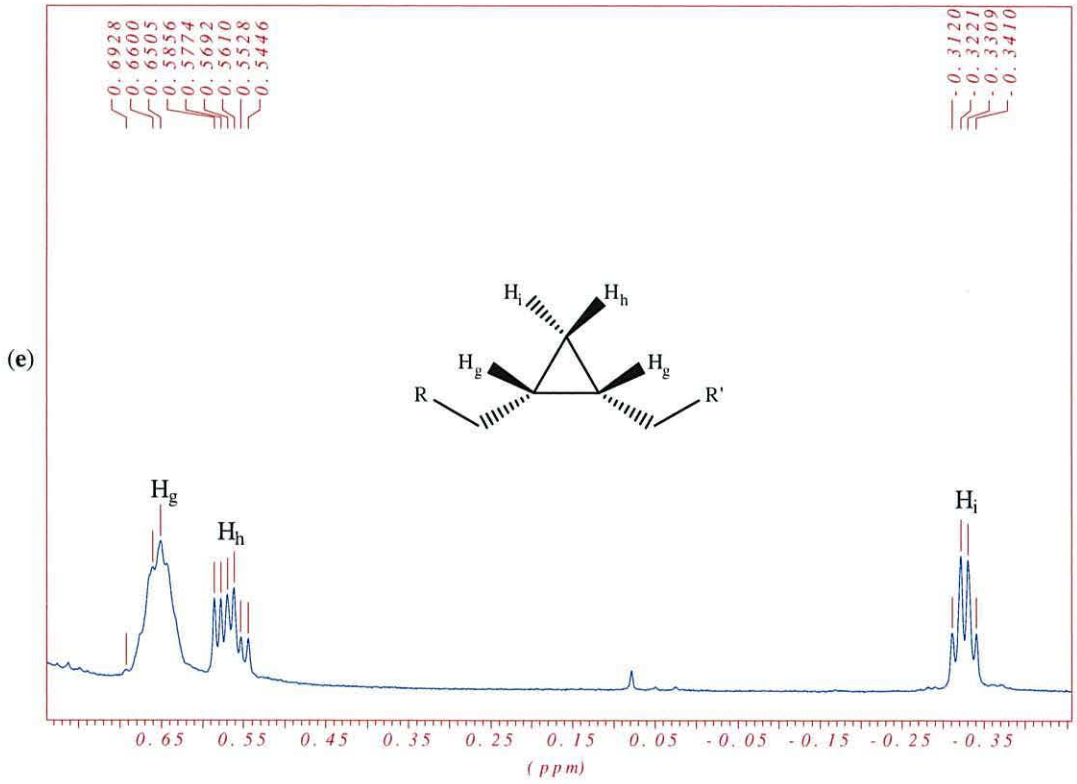


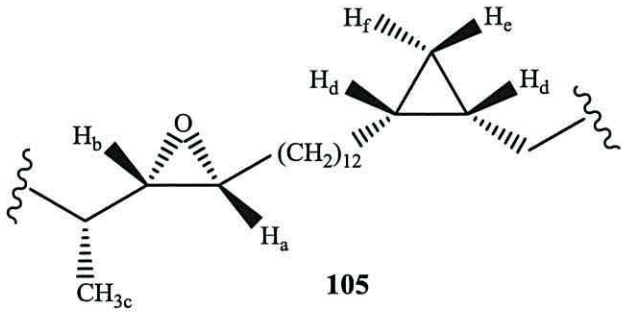
Fig. 31a-e: ^{1}H NMR spectrum of mycolic acid (**105**)

Proton H_a is shown in **Figure 31b** to give a multiplet at δ 3.73–3.70 for the proton next to the hydroxyl group in the ^{1}H NMR spectrum of (**105**). The two signals in **Figure 31c** show a doublet of triplet at δ 2.73 for H_b, a doublet of doublets at δ 2.43 for H_d and a multiplet at δ 2.48–2.44 for H_c.

Other signals appeared as a broad multiplet at δ 1.63–1.22 including a broad singlet at 1.26 for the long chain, a doublet at δ 1.0 for CH_{3e} and a triplet at δ 0.88 for two methyl groups CH_{3f} (**Fig. 31d**). The cyclopropane protons included a multiplet at δ 0.69–0.65 for H_g, a doublet of triplets at δ 0.56 for H_h and a quartet at δ -0.32 for H_i (**See Fig. 31e**).

A comparison of the proton NMR spectrum of the synthetic of *cis*-cyclopropane epoxymycolic acid (**105**) with the proton of natural *cis*-cyclopropane epoxy-mycolic acid isolated from *M. smegmatis* is shown in **Table 9**.[147] Compound (**105**) showed a doublet of triplets at δ 2.73 for H_a and a doublet of doublets at δ 2.43 for H_b the epoxide ring. The cyclopropane hydrogens appeared a multiplet at δ 0.69–0.65 for H_d, a doublet of triplets at δ 0.56 for H_e, a broad quartet at δ -0.32 for H_f and a doublet at δ 1.0 for CH_{3c} adjacent of epoxide ring. (**SeeTable 9).** Natural *cis*-cyclopropane epoxy-mycolic acid present in *M. smegmatis* showed a broad triplet at δ 2.73 for H_a and a multiplet at δ 2.42 for H_b and the cyclopropane protons showed three multiplets at δ 0.65, 0.57 and

-0.32 for H_d, H_e, H_f, respectively and a doublet at δ 1.01 for CH_{3c} adjacent of epoxide ring.



105

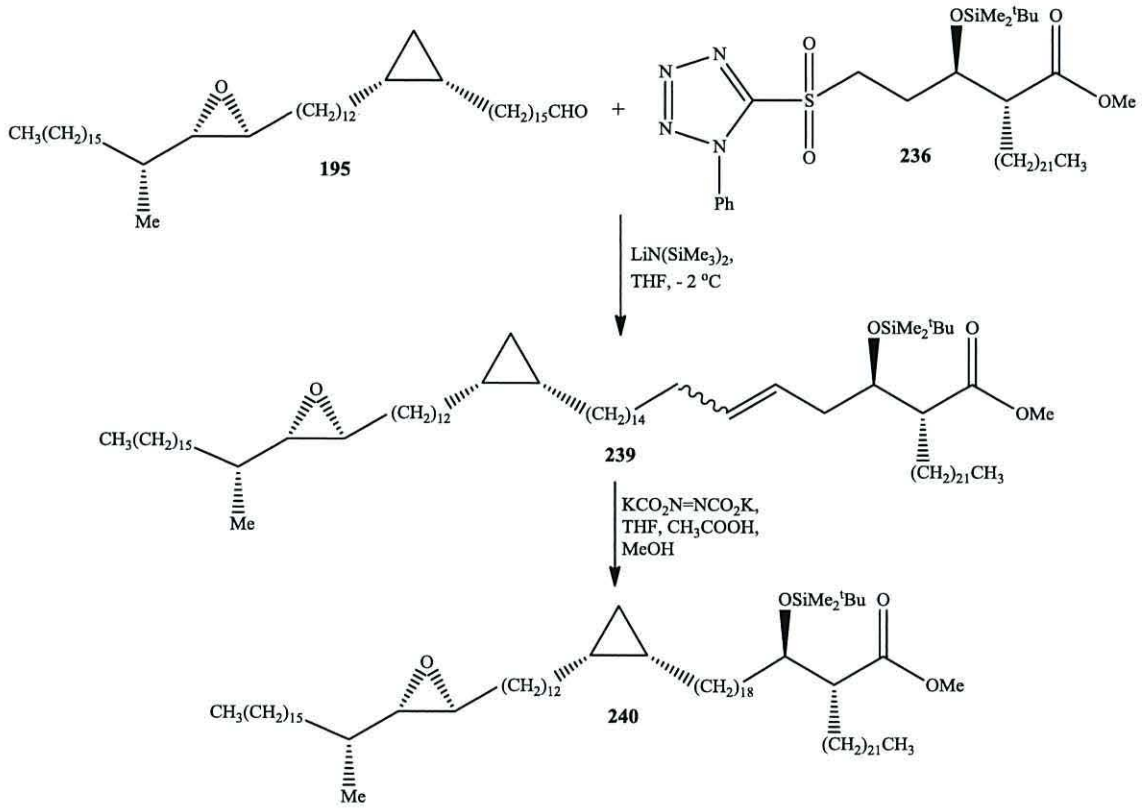
^{1}H NMR	Natural *cis*-cyclopropane epoxy mycolic acid[147]	Synthetic *cis*-cyclopropane (*S,S*)-epoxy mycolic acid (**105**)
H_a	2.73	2.73
H_b	2.42	2.43
H_c	1.01	1.0
H_d	0.65	0.69-0.65
H_e	0.57	0.56
H_f	-0.32	-0.32

Table 9: A comparison of ^{1}H NMR spectra of the synthetic of *cis*-cyclopropane epoxy mycolic acid (**105**) with a natural sample isolated from *M. smegmatis*.

The above method for the synthesis of *cis*-cyclopropane (*S,S*)-epoxy-mycolic acid gave a low yield because of the presence of the acetyl group, therefore the α-proton was acidic and easy to lose, followed by 1,2-elimination of acetyl group leading to (**237b**) as a by-product. There was a triplet at δ 6.43 for the alkene proton which confirmed the elimination of the acetyl protecting group.

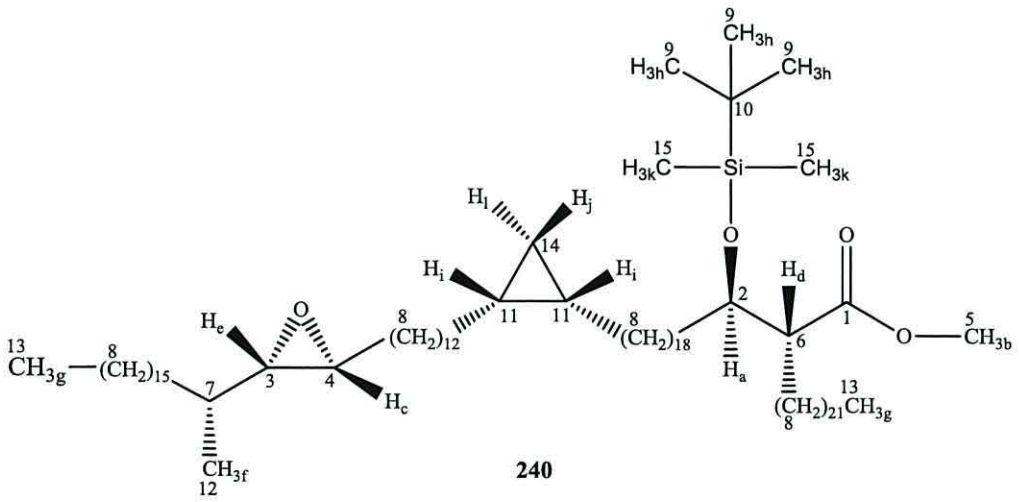
2.6.3-The Julia reaction between the meromycolate moiety (195) and corynomycolate (236)

The *cis*-cyclopropane (*S,S*)-epoxy-mycolic acid was also prepared from (**195**) and ester (**236**) in the presence of lithium bis(trimethylsilyl)amide as a base, giving the corresponding alkene (**239**) as a mixture of *E* and *Z*-isomers in ratio 2:1 with 68 % yield. Hydrogenation was carried out with dipotassium azodicarboxylate and acetic acid, THF and MeOH to give the protected ester (**240**) in 92 % yield (**Scheme 66**).



Scheme 66: The Julia reaction between (**195**) and (**236**) and hydrogenation to produce (**240**)

The synthesis of protected *cis*-cyclopropane (*S,S*)-epoxy-mycolic acid (**240**) was confirmed using ^{1}H NMR, ^{13}C NMR, mass spectrometry, specific rotation and IR. The mass spectrum gave a molecular ion [$(M + Na)^{+}$: 1305.2684, $C_{85}H_{168}NaO_4Si$ requires: 1304.2604] and the specific rotation of this compound (**240**) was $[\alpha]_D^{24} = -6.95$ (*c* 1.15, $CHCl_3$). The ^{1}H and ^{13}C NMR spectrum of (**240**) were analysed to confirm its structure (**Table 10**).



240

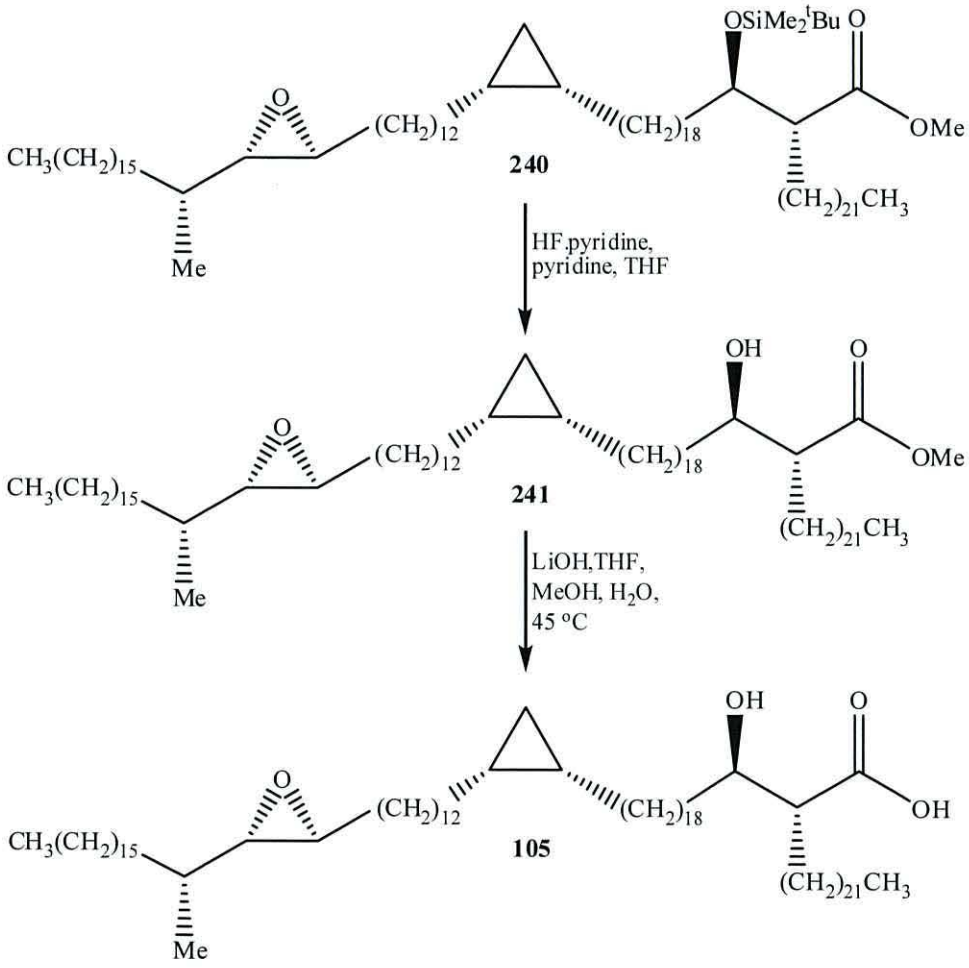
Proton	δ	Multiplicity	Integration	J (Hz)	Carbon	δ
H_a	3.92-3.89	m	1	-	C_1	175.13
H_b	3.66	s	3	-	C_2	73.21
H_c	2.72	dt	1	2.2, 5.35	C_3	63.83
H_d	2.53	ddd	1	3.75, 7.25, 11	C_4	58.85
H_e	2.41	dd	1	2.2, 7.25	C_5	51.55
H_f	1.0	d	3	5.95	C_6	51.19
H_g	0.88	t	6	6.95	C_7	36.05
H_h	0.86	s	9	-	C_8	33.79-22.6
H_i	0.66-0.64	m	2	-	C_9	25.75
H_j	0.56	dt	1	4.05, 8.15	C_{10}	17.96
H_k	0.05, 0.02	s	2 × CH_3	-	C_{11}	17.29
H_l	-0.32	q	1	5.05	C_{12}	15.77
-	-	-	-	-	C_{13}	14.11
-	-	-	-	-	C_{14}	10.91
-	-	-	-	-	C_{15}	-4.37, -4.94

Table 10: Selected ^{1}H and ^{13}C NMR data for (**240**)

This approach was better than previous method because the α-proton was more stable in presence of the silyl protecting group. In the previous method the acidic α-proton is not stable due to the presence of the acetyl group (good leaving group) on the β-position and as a result, a low yield was obtained.

2.6.4-Desilylation and hydrolysis of protected mycolic acid methyl ester

Deprotection of the *tert*-butyldimethylsilyl group of the compound (**240**) was carried out to give the *cis*-cyclopropane (*S,S*)-epoxy mycolic acid methyl ester (**241**) using hydrofluoric acid in pyridine. The mycolic acid methyl ester was then hydrolysed using lithium hydroxide monohydrate in THF, methanol and water to give the free mycolic acid (**105**) (**Scheme 67**) in 76 % yield.



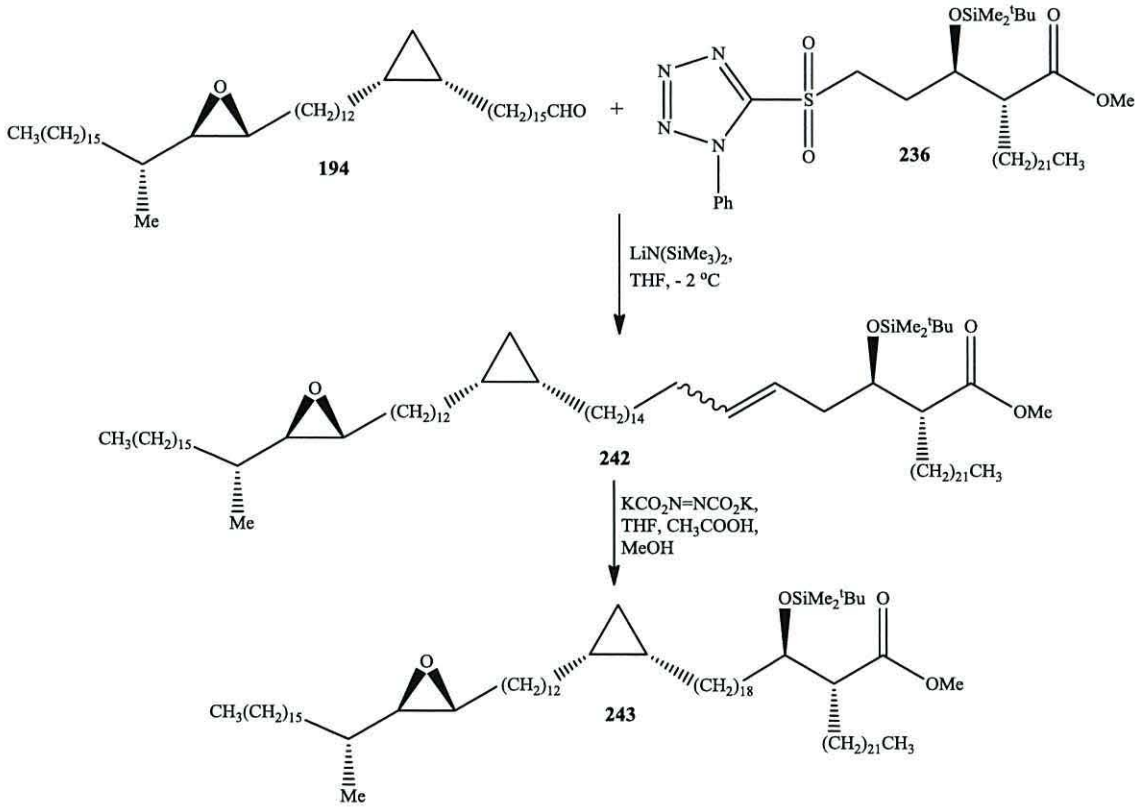
Scheme 67: Desilylation of (**240**) and hydrolysis to give mycolic acid (**105**)

This was identical by ^{1}H NMR, ^{13}C NMR, optical rotation, IR and mass spectrometry to the free mycolic acid (**105**) discussed in **Section 2.6.2, Fig. 31.**

The *cis*-cyclopropane (*S,S*)-epoxy mycolic acid methyl ester (**241**) was characterized using ^{1}H NMR, ^{13}C NMR, IR, mass spectrometry and optical rotation. The IR spectrum showed a broad peak at 3472 cm^{-1} for the OH stretch. The ^{1}H NMR spectrum of (**241**) showed no signals for the *tert*-butyldimethylsilyl group and a singlet at δ 3.71 for the (-$OC\underline{H}_3$). The proton adjacent to secondary alcohol showed as a multiplet at δ 3.67–3.65. The ^{13}C NMR showed a signals at δ 72.29 for (-$\underline{C}HOH$) and δ 51.5 for (-$O\underline{C}H_3$).

2.7-The synthesis of *cis*-cyclopropane (*R,R*)-epoxy-mycolic acid (106)

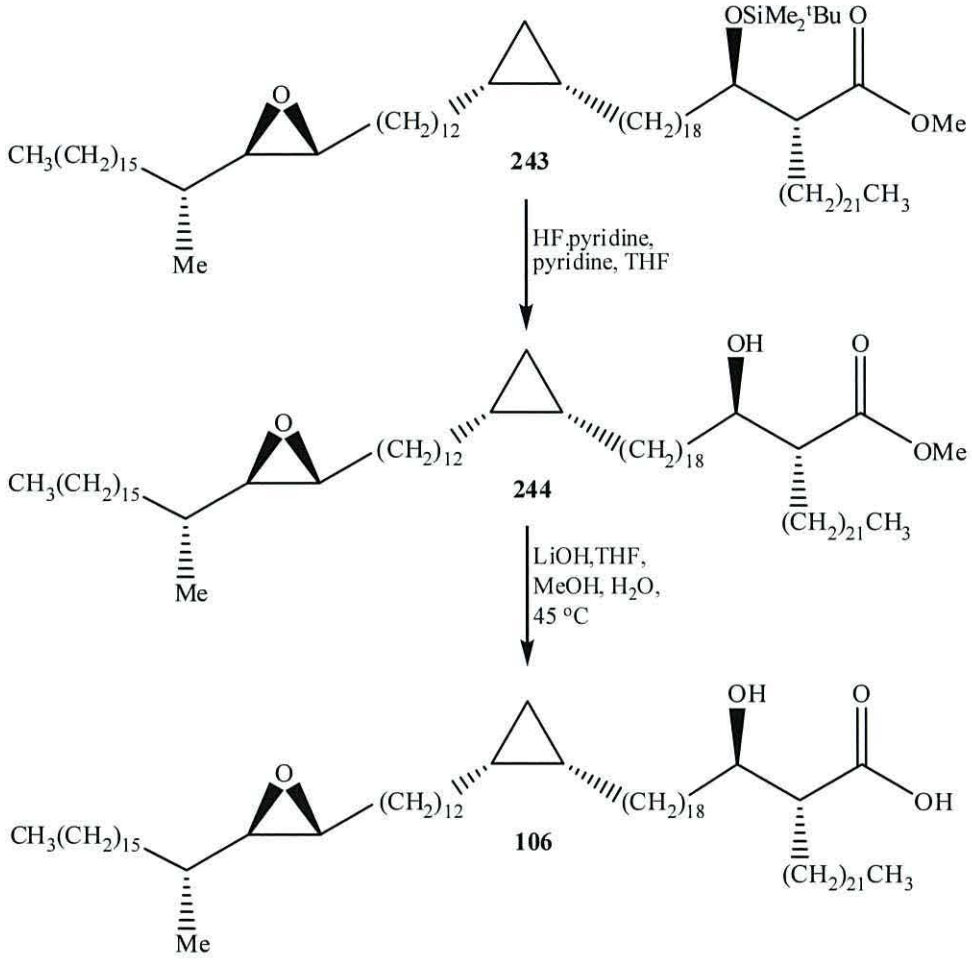
The Julia olefination was then carried out, in a similar manner to that previously discussed, using *cis*-cyclopropane (*R,R*)-epoxy meromycolaldehyde (**194**) and sulfone (**236**) with lithium bis(trimethylsilyl)amide and gave the alkene (**242**) as a mixture *E* and *Z*-isomers in 70 % yield. Hydrogenation was carried out by addition of dipotassium azodicarboxylate with acetic acid, MeOH and THF to give the protected compound ester (**243**) in 82 % yield (**Scheme 68**).



Scheme 68: Final coupling of (**194**) and (**236**) and hydrogenation to yield protected methyl mycolate (**243**)

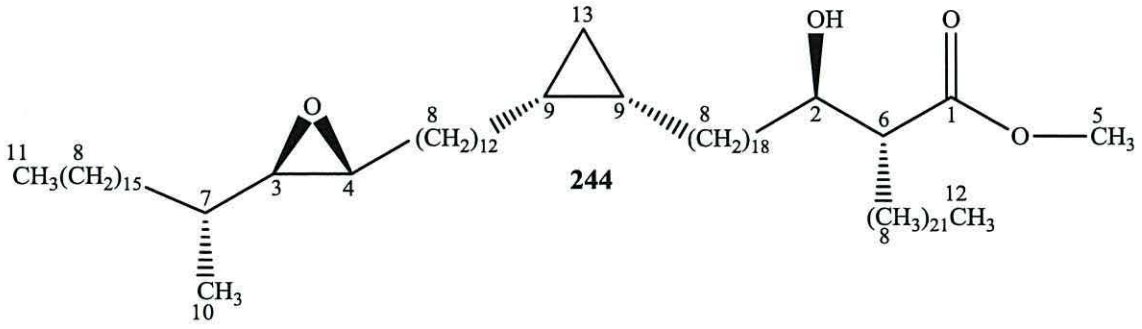
The completion of the hydrogenation was confirmed by ^{1}H and ^{13}C NMR spectra. The NMR spectra of compound (**243**) and were similar to those of compound (**240**) discussed in **Table 11**, just showing small differences in the chemical shift.

The silyl group protecting was removed with HF.pyridine in pyridine and THF to give *cis*-cyclopropane (*R,R*)-epoxy mycolic acid methyl ester (**244**). The mycolic acid methyl ester was then hydrolysed using lithium hydroxide monohydrate in THF, methanol and water to give a white solid (**106**) (**Scheme 69**) in 66 % yield. The NMR spectra of (**244**) showed no signals for the *tert*-butyldimethylsilyl group and ^{1}H NMR included a singlet at δ 3.71 for methyl protons of the ester. The optical rotation of (**244**) was measured as $[\alpha]_D^{21} = +5.15$ (*c* 0.73, $CHCl_3$) and IR showed a broad peak at 3346 cm^{-1} for OH stretch.



Scheme 69: Synthesis of free mycolic acid (**106**) from (**243**)

The ^{13}C NMR spectra of *cis*-cyclopropane (*R,R*)-epoxy mycolic acid methyl ester (**244**) was analysed as shown in **Table 11**.

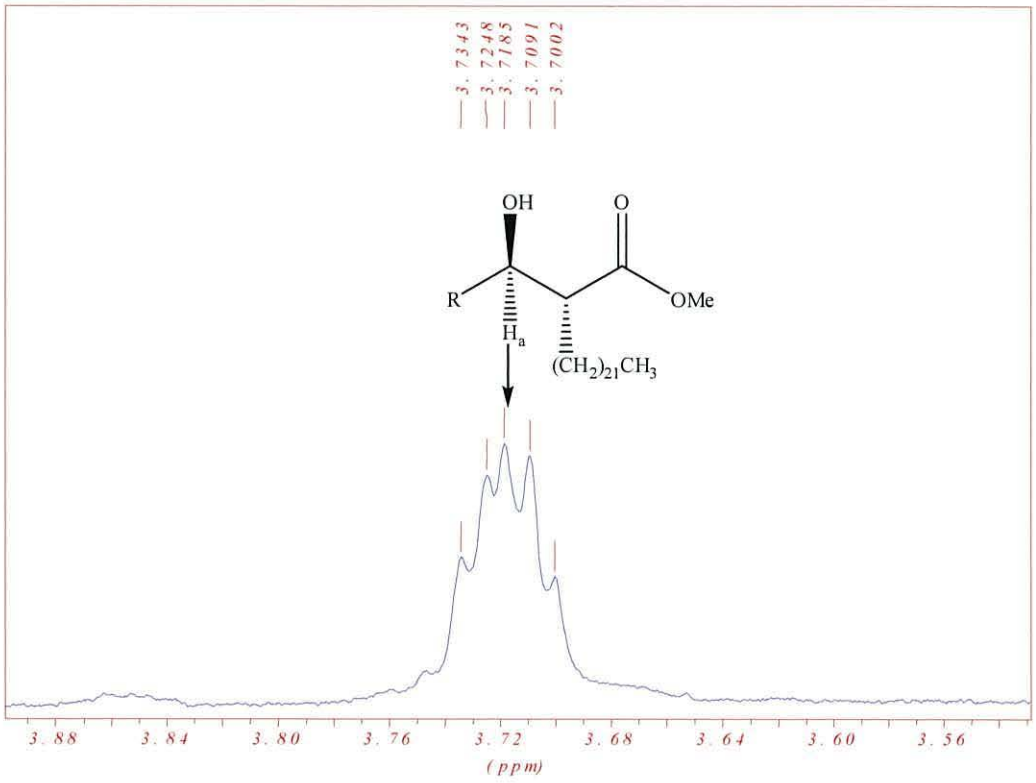


Carbon	δ	Carbon	δ
C_1	176.22	C_8	35.68-22.67
C_2	72.28	C_9	15.94
C_3	63.8	C_{10}	15.76
C_4	57.46	C_{11}	14.18
C_5	51.48	C_{12}	14.1
C_6	50.93	C_{13}	10.9
C_7	35.83	-	-

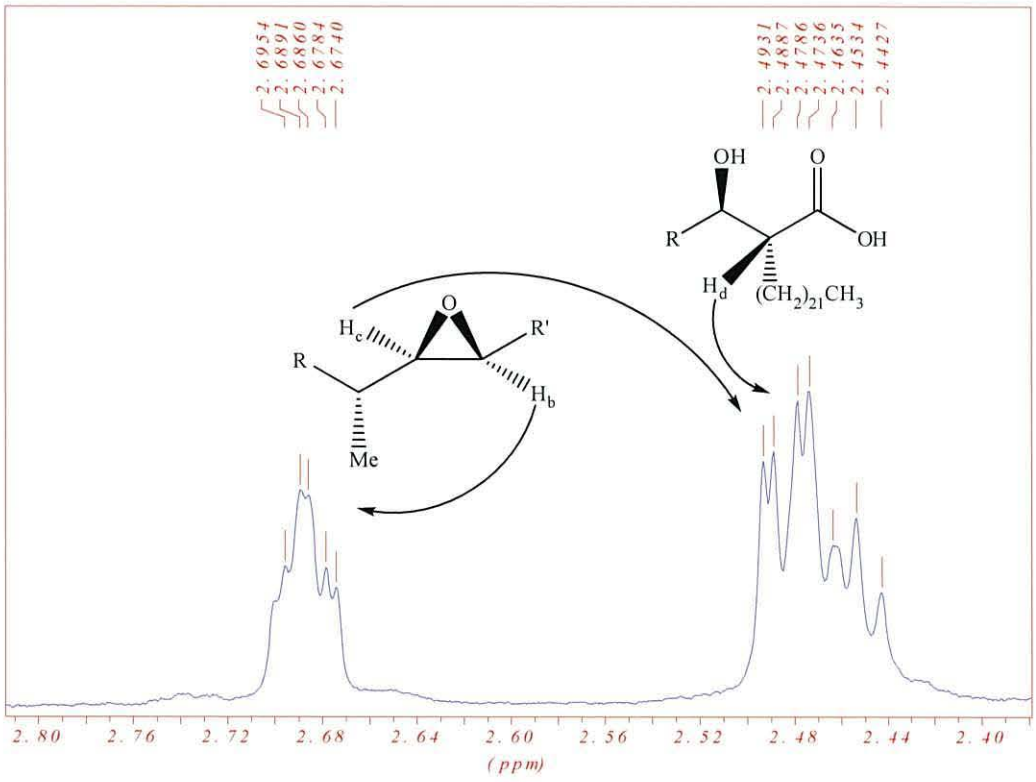
Table 11: The ^{13}C NMR data of free mycolic acid (**244**)

The ^1H NMR, ^{13}C NMR, IR, specific rotation and mass spectrometry were used to confirm the structure of the free mycolic acid (**106**). The optical rotation of (**106**) was recorded as $[\alpha]_D^{22} = +12.5$, ($_C$ 0.6, CH_3Cl), and the IR spectrum included a broad peak at 3349 cm^{-1} for the O-H stretch. In addition, ^1H NMR expansions of *cis*-cyclopropane (*R,R*)-epoxy-mycolic acid (**106**) can be seen in **Fig. 32a-d**. Proton H_a is shown in **Figure 32a** to give a multiplet at δ 3.73–3.70 for the proton next to the hydroxyl group in the ^1H NMR spectrum of (**106**). The two signals in **Figure 32b** show a doublet of triplet at δ 2.68 for H_b, a multiplet at δ 2.49-2.44 for two protons (H_c, H_d) including a double doublets at δ 2.48 for H_c. Other signals appeared as a broad multiplet at δ 1.75–1.22 including a broad singlet at 1.26 for the long chain, a doublet at δ 0.92 for CH_{3e} and a triplet at δ 0.88 for two methyl groups (CH_{3f}) (**Fig. 32c**). The four *cis*-cyclopropane protons appeared asa multiplet at δ 0.66–0.64 for H_g, a doublet of triplets at δ 0.56 for H_h and a quartet at δ -0.32 for H_i (**See Fig. 32d**).

(a)


3.7343
3.7248
3.7185
3.7091
3.7002
OH
O
R
OMe
H_a
$(CH_2)_{21}CH_3$
3.88
3.84
3.80
3.76
3.72
3.68
3.64
3.60
3.56
(ppm)

(b)


2.6954
2.6891
2.6860
2.6784
2.6740
2.4931
2.4887
2.4786
2.4736
2.4635
2.4534
2.4427
OH
O
R
OH
H_d
$(CH_2)_{21}CH_3$
O
H_c
R'
R
H_b
Me
2.80
2.76
2.72
2.68
2.64
2.60
2.56
2.52
2.48
2.44
2.40
(ppm)

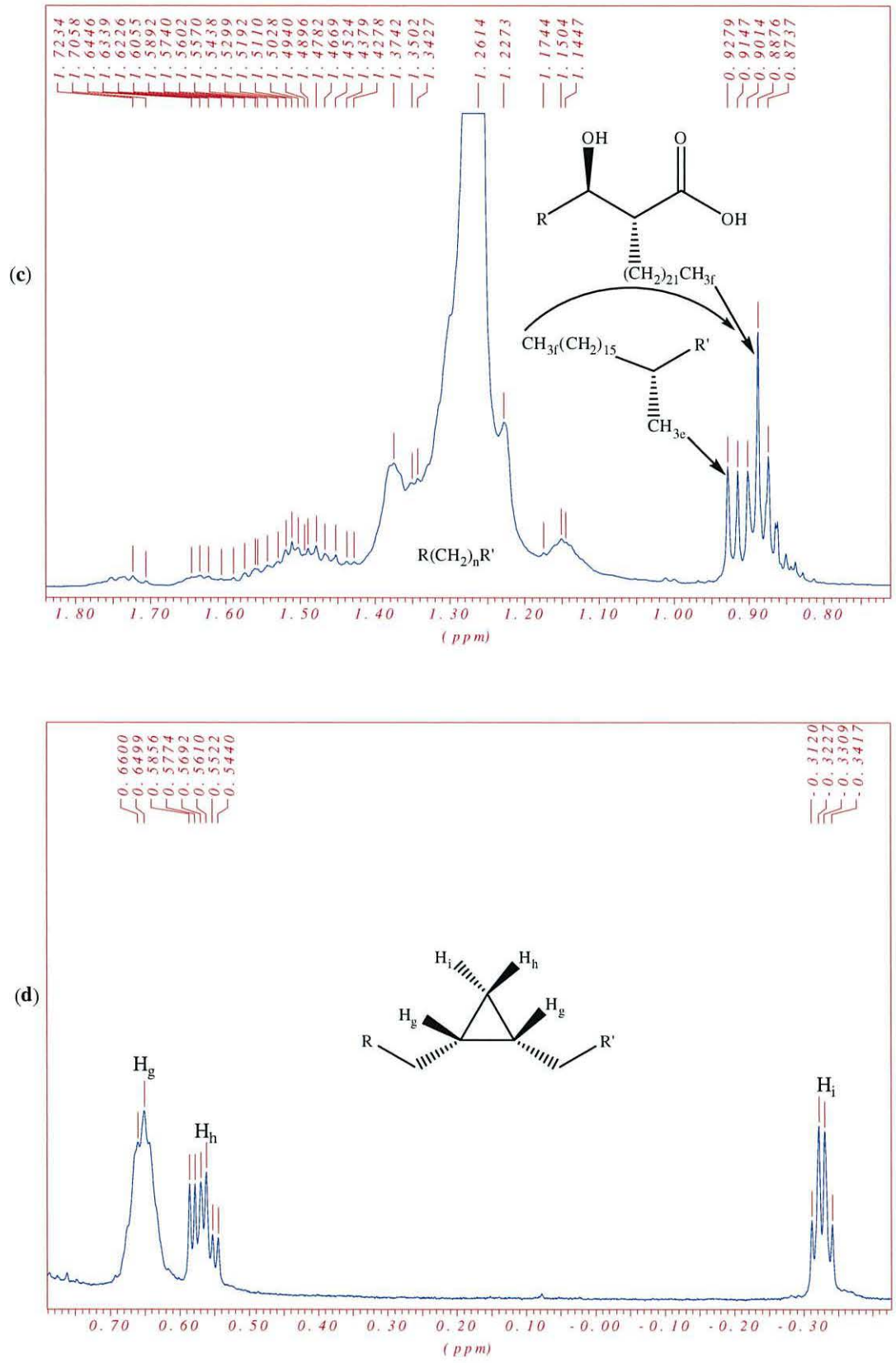


Fig. 32a-d: ^{1}H NMR spectrum of free mycolic acid (**106**)

2.8-Synthesis of (*E/Z*) (*R,R*) and (*S,S*)-epoxy-alkene mycolic acids (207) (208)

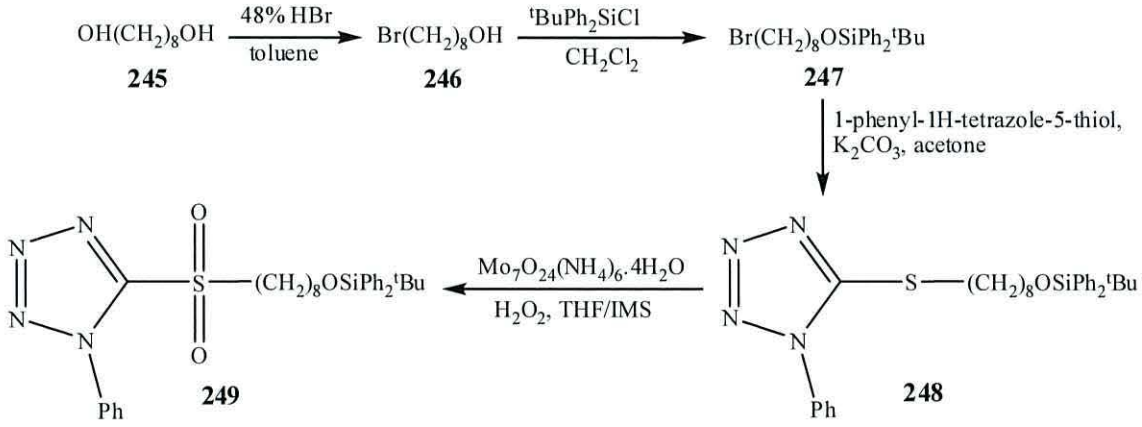
Using this method two mycolic acids, (*E/Z*) (*R,R*)-epoxy-alkene mycolic acid and (*E/Z*) (*S,S*)-epoxy-alkene mycolic acid were prepared.

2.8.1-Preparation of (*S,S*) and (*R,R*)-epoxy meromycolaldehydes (261) (262)

The (*S,S*)-epoxy meromycolaldehyde (**261**) and (*R,R*)-epoxy meromycolaldehyde (**262**) were prepared starting from D-mannitol (**109**).

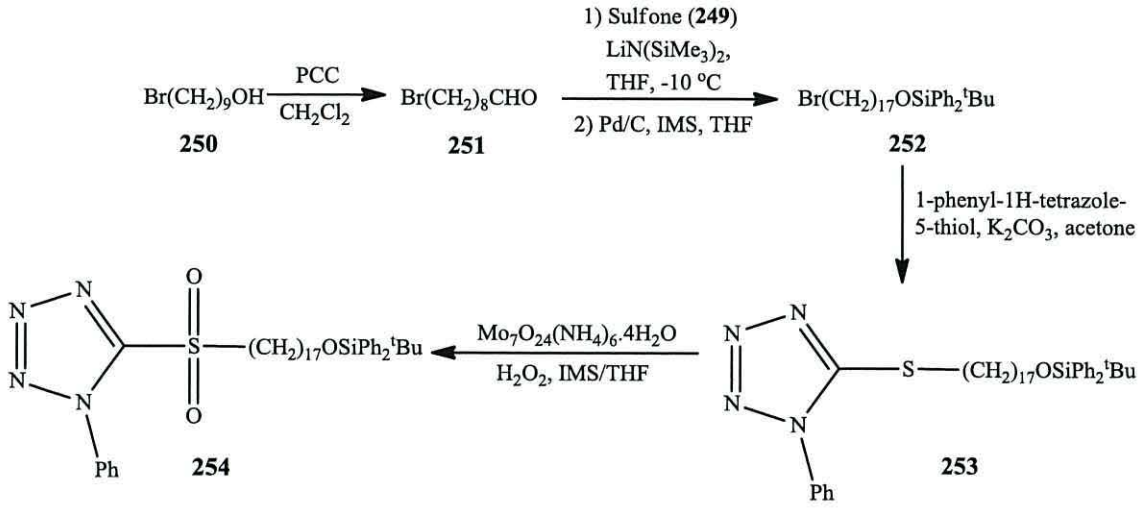
2.8.1a-Preparation of C_{17} sulfone (254)

To obtain the required chain, it was necessary to prepare a seventeen carbon unit. The sulfone (**249**) was prepared from 1,8-octanediol (**245**). The diol (**245**) was brominated using 48 % HBr to give 8-bromo-octan-1-ol (**246**), which was then protected with *tert*-butyldiphenylsilylchloride. This was further converted into the sulfide (**248**) as before, and then oxidized with hydrogen peroxide to give the sulfone (**249**) (**Scheme 70**).



Scheme 70: Preparation of sulfone (**249**)

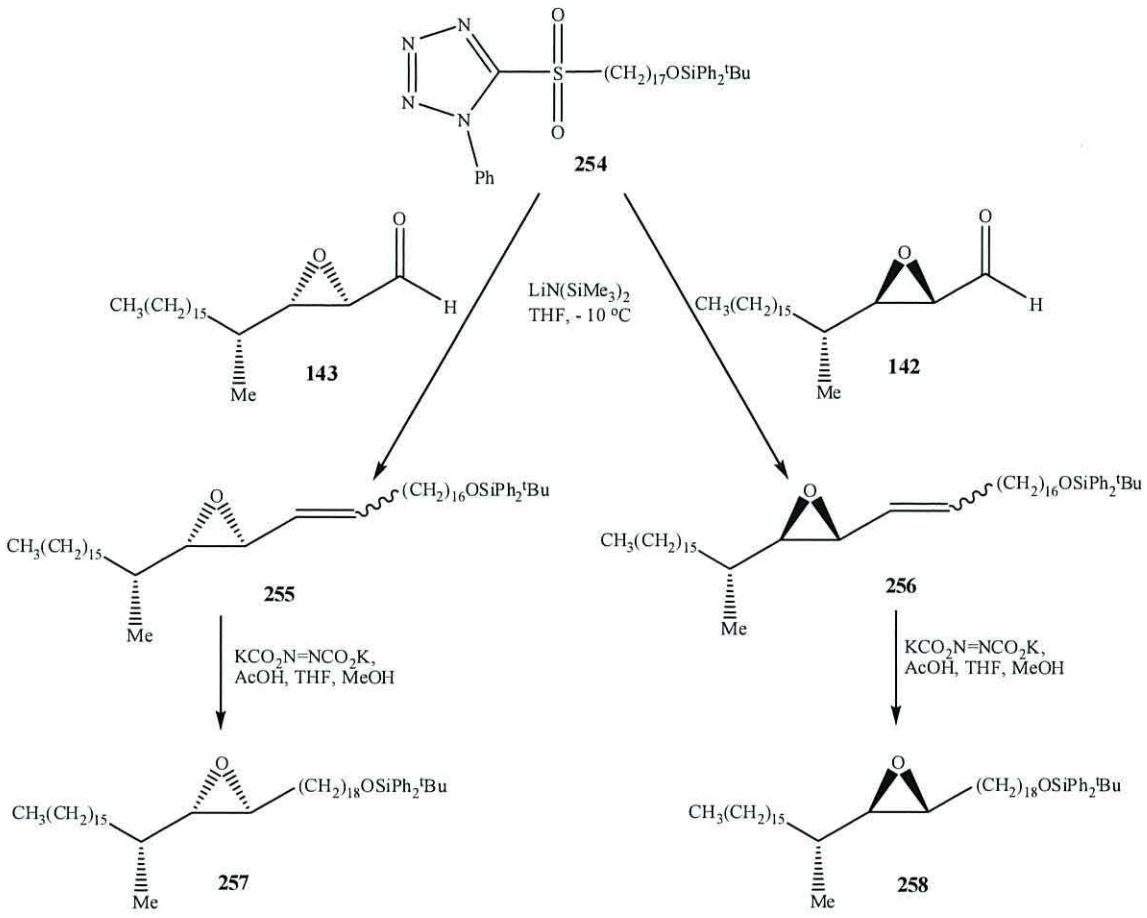
Oxidation of bromo-alcohol (**250**) to the corresponding aldehyde (**251**) was achieved using PCC. The aldehyde (**251**) was then coupled with the sulfone (**249**) and base in a modified Julia-Kocienski reaction to give the alkene, followed by hydrogenation of the alkene with a palladium catalyst to give the product (**252**), completing the formation of the seventeen carbon chain. The bromo-compound (**252**) was converted into the sulfone (**254**) (**Scheme 71**), using the same procedure as discussed before.



Scheme 71: Preparation of C_{17} sulfone (**254**)

2.8.1b-The coupling reaction

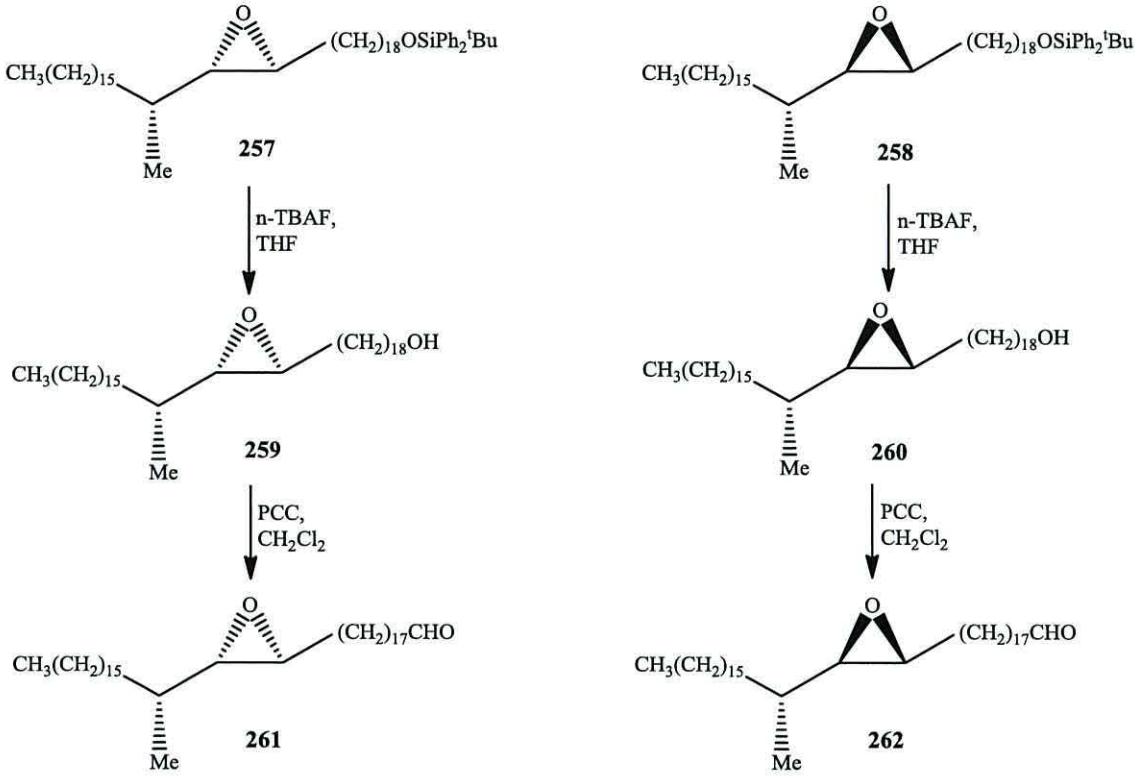
The sulfone (**254**) was coupled to the previously prepared aldehydes (**143**) and (**142**) in a modified Julia reaction to give a mixture of *E/Z* isomers of the alkenes (**255**, **256**) in the presence lithium bis(trimethylsilyl)amide as base, followed by hydrogenation using dipotassium azodicarboxylate to give the saturated compounds (**257**, **258**) in 91 and 96 % yield, respectively (**Scheme 72**). The ^{1}H NMR spectra of (**257**) or (**258**) included double doublets at δ 7.69, δ 7.68 for the four aromatic protons and a multiplet at δ 7.44–7.35, δ 7.44–7.36 for the six aromatic protons, a triplet at δ 3.67, δ 3.66 for proton next to the silyl group, a doublet of triplets at δ 2.73, δ 2.67, and a doublet of doublets at δ 2.42, δ 2.47 for the two protons of the epoxide ring, respectively. The ^{13}C NMR spectra of (**257**) or (**258**) included signals at δ 63.83, δ 63.8 and δ 58.84, δ 57.46, respectively for the carbons of the epoxide ring. Compounds (**257**) and (**258**) showed opposite specific rotations $[\alpha]_D^{20} = -7.18$, $[\alpha]_D^{17} = +5.39$, respectively.



Scheme 72: The coupling and hydrogenation

Deprotection of the *tert*-butyldiphenylsilyl groups was done using n-TBAF in dry THF to give the corresponding alcohols (**259** / **260**), which were then oxidised with pyridinium chlorochromate to give the aldehydes (**261**) and (**262**) (**Scheme 73**). The IR spectra of (**259**) and (**260**) each showed a peak at 3440 cm^{-1} to confirm of the O-H bond and the NMR spectra showed no signals for the *tert*-butyldiphenylsilyl group. The ^{1}H NMR spectra did showa triplet at δ 3.64 for ($-CH_2OH$).

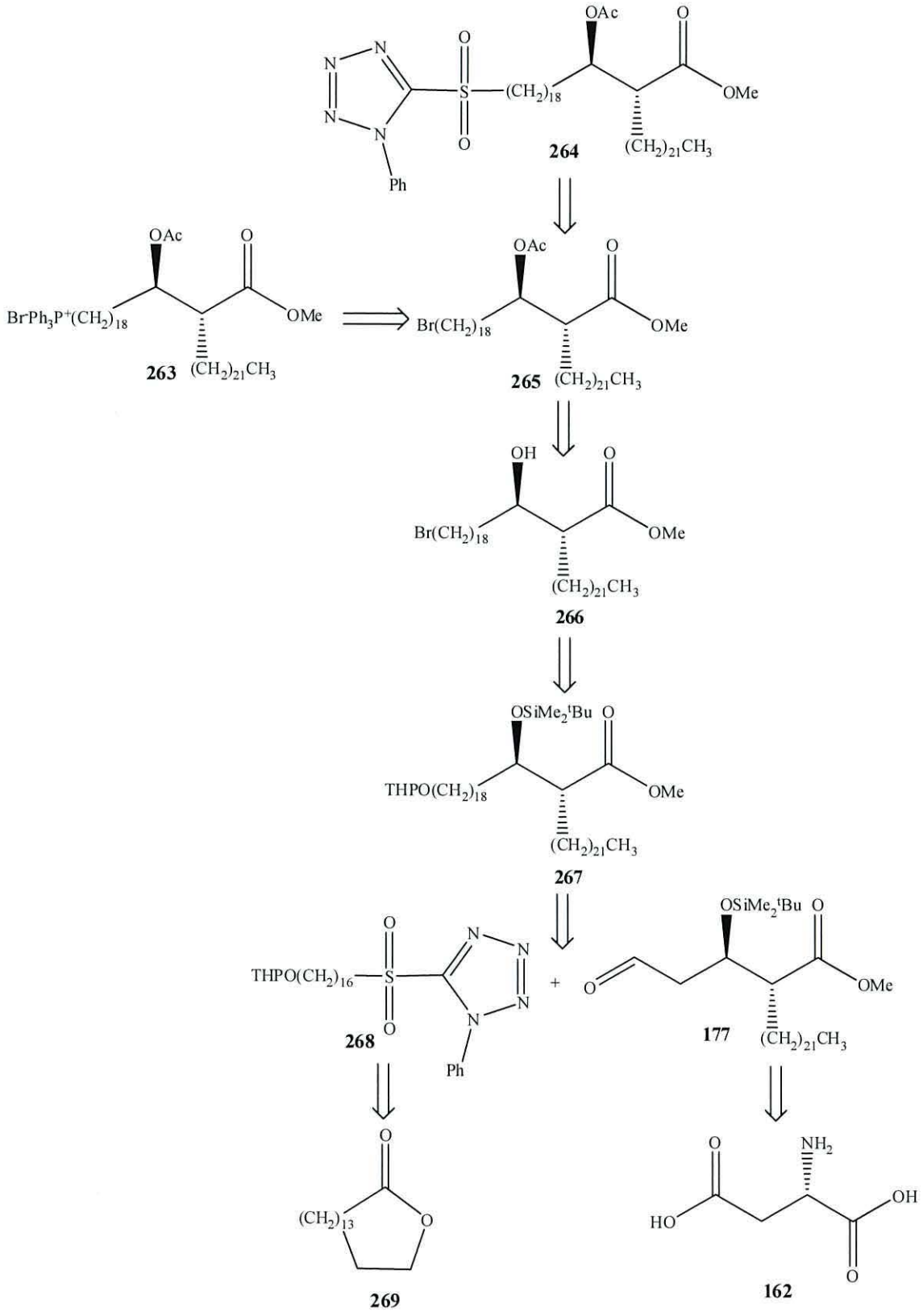
The aldehydes (**261**) or (**262**) were characterised via ^{1}H NMR, ^{13}C NMR, IR and optical rotation. The IR spectrum showed a peak at 1728 cm^{-1} for the C=O stretch and the specific rotation of the aldehydes (**261**) and (**262**) were measured as $[\alpha]_D^{22} = -26.02$ and $[\alpha]_D^{20} = +7.96$, respectively. The ^{1}H NMR spectrum of (**261**) or (**262**) included triplets at δ 9.77 for the aldehyde proton and signals at δ 202.88 and δ 202.93, respectively, for the carbon of the aldehyde.



Scheme 73: Preparation of the intermediate meromycolaldehydes (**261**, **262**)

2.8.2-Synthesis of the intermediate mycolates (263, 264)

The intermediate mycolates (**263**), (**264**) could be obtained from 16-hexadecanolide (**269**) and L-aspartic acid (**162**). These reactions are summarized in **Scheme 74**.

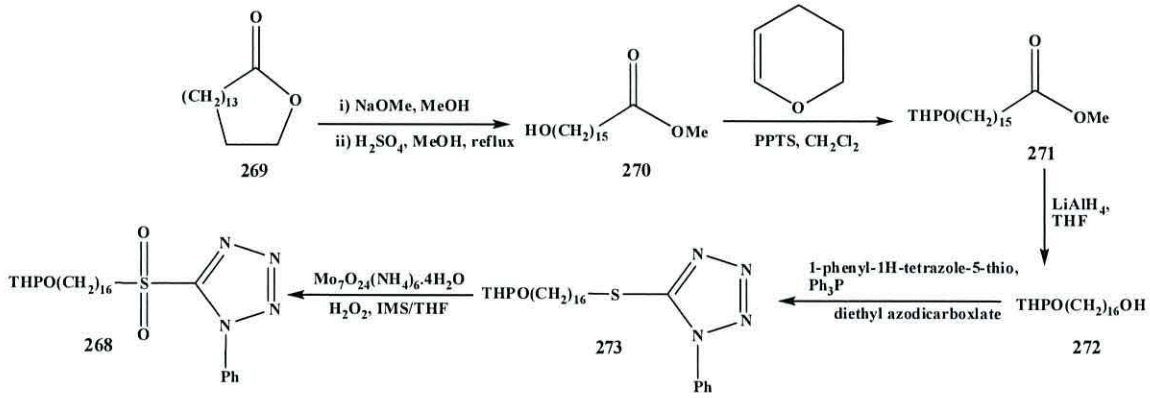


Scheme 74: Retrosynthesis of the intermediate mycolates (**263**) and (**264**)

2.8.2a-Preparation of C_{16} sulfone (268)

The sixteen carbon chain sulfone (**268**) had to be prepared, and was then used in the final coupling reaction for synthesis of the moiety. 16-Hexadecanolide (**269**) was reacted with sodium methoxide in methanol to give the product (**270**). The alcohol (**270**) was protected with 3,4-dihydro-2*H*-pyran in dichloromethane to give the ester (**271**), followed by reduction with $LiAlH_4$ in THF to lead to the protected alcohol (**272**). The NMR spectrum of (**272**) included a triplet at δ 3.62 for proton (-C<u>H</u>$_2$OH) and a signal at δ 62.96 for carbon (-<u>C</u>H$_2$OH). The IR showed a peak at 3438 cm^{-1} for the O-H stretch.

The alcohol was treated with triphenylphosphine and 1-phenyl-1*H*-tetrazole-5-thiol in the presence of diethyl azodicarboxylate in dry THF to form sulfide (**273**). Finally, the sulfide was oxidized using hydrogen peroxide to the corresponding sulfone (**268**) in 78 % yield as shown in **Scheme 75**.



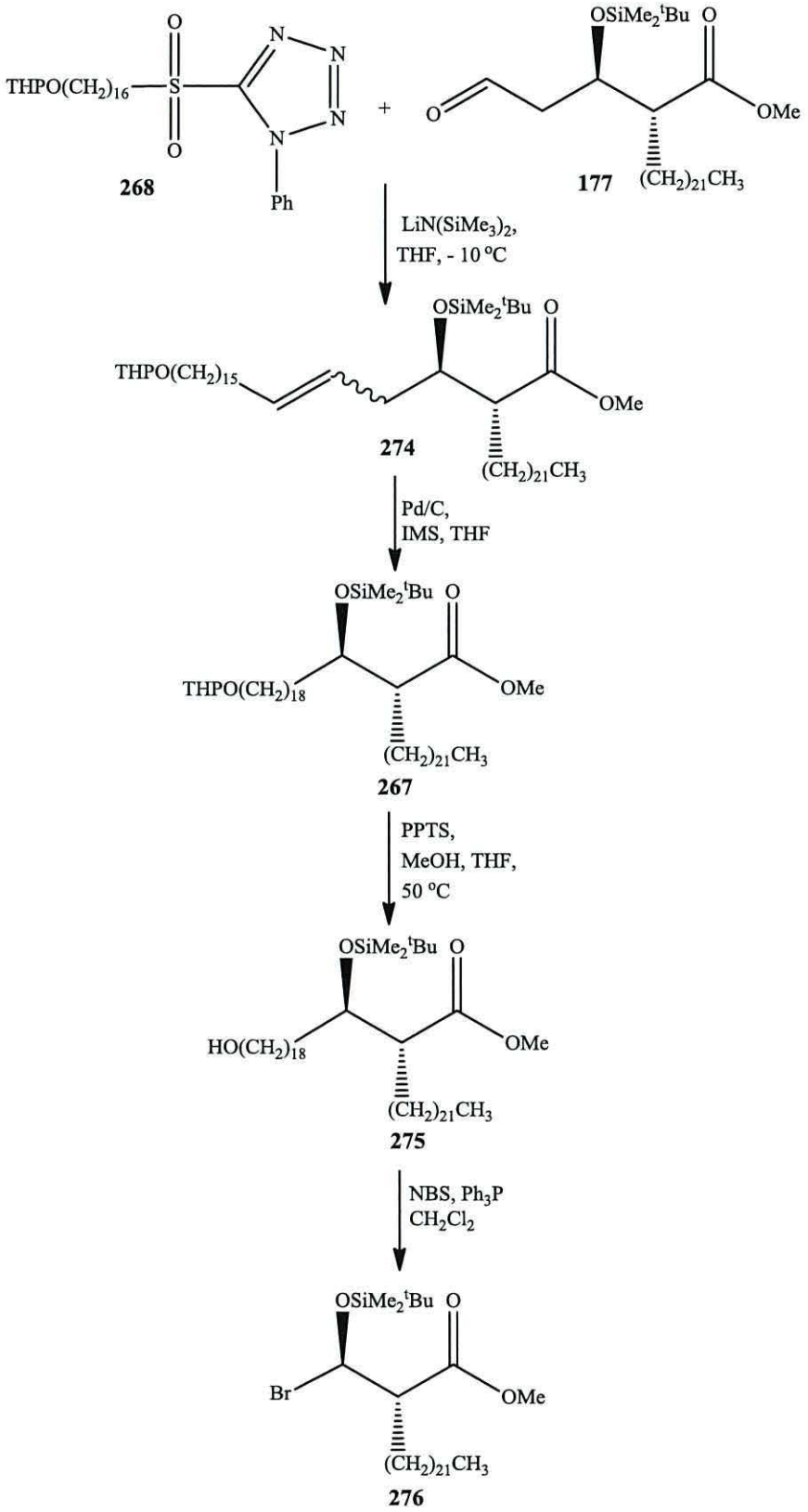
Scheme 75: Preparation of the sulfone (**268**)

The proton NMR spectrum of (**268**) showed the protecting group protons on the ring adjacent to oxygen including a broad triplet at δ 4.57 (*J* 2.5 Hz) for the acetal proton (OCHO), a multiplet at δ 3.89–3.84, and another multiplet at δ 3.75–3.70 for three protons including two protons (-C<u>H</u>$_2$SO$_2$-). The proton on the chain next to the oxygen appeared as a multiplet at δ 3.51–3.47 and a doublet of triplet at δ 3.37 (*J* 6.65, 9.45 Hz). The ^{13}C NMR spectrum of (**268**) showed a signal at δ 98.78 for the acetal carbon (O<u>C</u>HO) and two signals at δ 67.64, δ 62.28 for the carbons bonded to oxygen.

2.8.2b-Coupling using the Julia reaction and bromination

The sixteen carbon chain sulfone (**268**) was coupled to the previously prepared corynomycolate aldehyde (**177**) in a Julia olefination, followed by hydrogenation using

palladium on carbon and hydrogen to give the saturated product (**267**) in 98 % yield (**Scheme76**). The NMR spectra were as expected and the specific rotation was measured $[\alpha]_D^{19} = -4.36$ (*c* 1.03, $CHCl_3$).

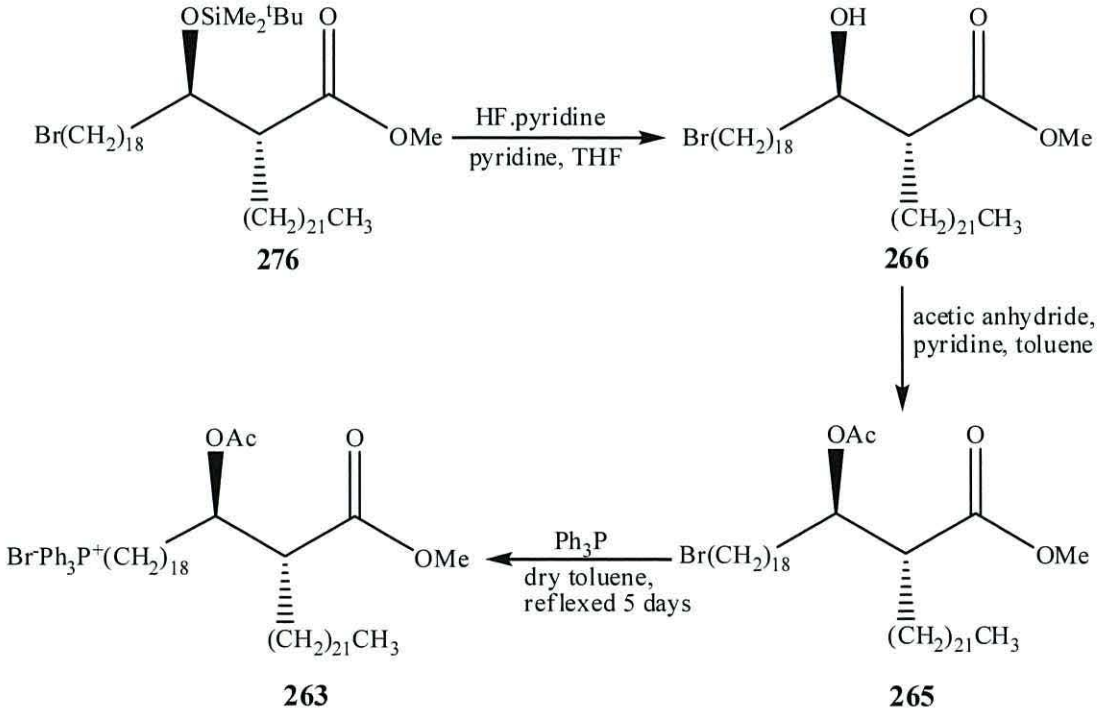


Scheme 76: The Julia reaction of (**268**) and (**177**) leading to intermediate (**276**) and bromination

Deprotection of the tetrahydropyran (THP) protecting group using pyridinum-p-toluene sulfonate gave the primary alcohol (**275**). The ^{1}H NMR spectrum of (**275**) showed no signals for the THP group, but there was a broad triplet at δ 3.64 for $C\underline{H}_2OH$ and a multiplet at δ 3.92–3.89 for the C<u>H</u> next to the oxygen of the protected silyl group. The ^{13}C NMR included signals at δ 73.21 for the carbon next to the silyl protecting group and δ 63.08 for the $\underline{C}H_2OH$. The IR included a peak at 3356 cm^{-1} for the O-H bond.
The primary alcohol (**275**) was brominated with NBS in CH_2Cl_2 to give the bromo ester (**276**) (**Scheme 76**). The NMR spectra of (**276**) showed a triplet at δ 3.41 (*J* 6.9 Hz) for the proton ($C\underline{H}_2Br$) and a signal at δ 33.98 for the carbon ($\underline{C}H_2Br$).

2.8.2c-Preparation of the phosphonium salts (263 / 277)

There are two ways to prepare the phosphonium salt (**263**). In the first method the silyl ether group was removed using HF.pyridine complex to give the corresponding secondary alcohol (**266**), and was then reacted with acetic anhydride to give the protected compound, acetate (**265**). The protected bromo-compound (**265**) was treated with triphenylphosphine in dry toluene and refluxed for five days to lead to the phosphonium salt (**263**) (**Scheme 77**).

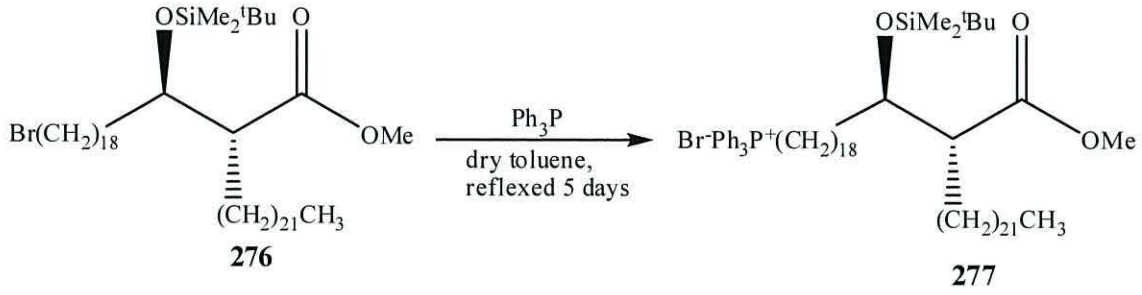


Scheme 77: Preparation of the phosphonium salt (**263**) from (**276**)

The ^{1}H NMR, ^{13}C NMR, IR, specific rotation and mass spectrometry were used to confirm the structure of phosphonium salt (**263**). The ^{1}H NMR spectrum of (**263**)

included three multiplets at δ 7.81–7.61, δ 7.52–7.40 for 15 protons of three phenyl groups and δ 5.07-5.04 for proton next acetyl group and a broad singlet at δ 3.69 for ($-C\underline{H}_2P^+Ph_3Br^-$). The ^{13}C NMR was showed two signals at δ 173.53, δ 170.21 for the carbonyl carbons and the other signals were as expected.

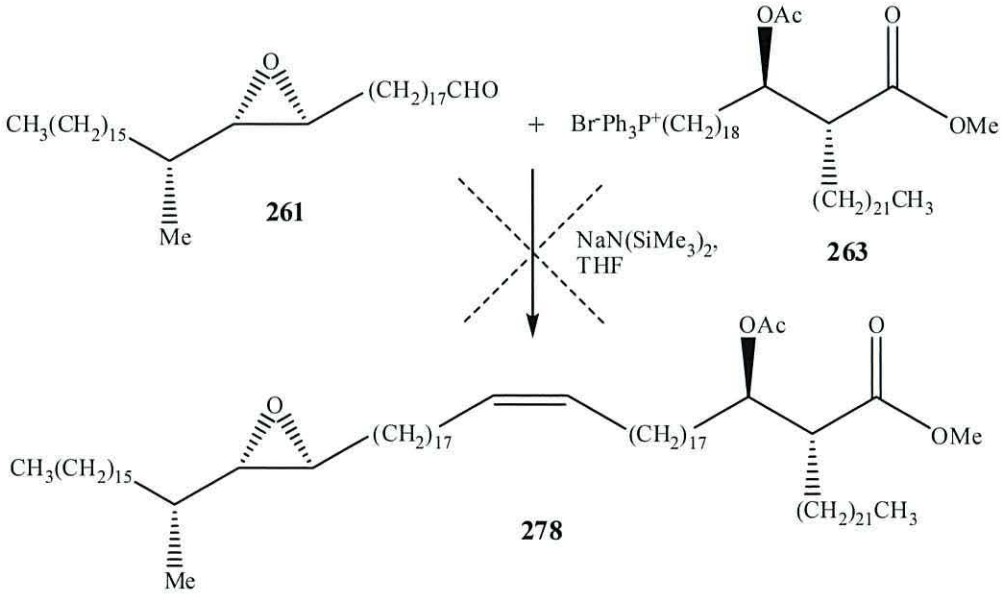
In the second method, the bromo-compound (**276**) was converted into phosphonium salt (**277**) (**Scheme 78**) using triphenylphosphine in dry toluene and refluxed for five days.



Scheme 78: Preparation of phosphonium salt (**277**)

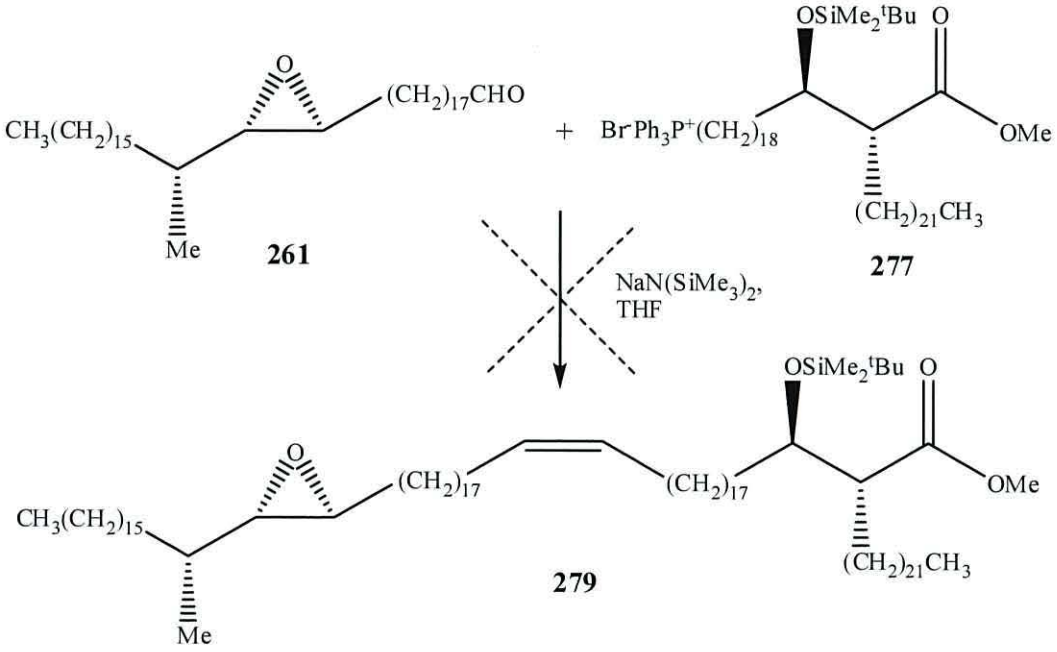
2.8.3-An attempt to synthesise the *cis*-alkene epoxy mycolic acids (278, 279)

A *cis*-alkene epoxy mycolic acid was found in *M. smegmatis*[227] and, to synthesise this compound, aldehyde (**261**) was coupled with phosphonium salt (**263**) using sodium bis(trimethylsilyl)amide as base in dry THF (**Scheme 79**). Unfortunately, the reaction did not work. The NMR spectrum showed no signals for the product, just those for the starting material.



Scheme 79: An attempt to synthesise *cis*-alkene epoxy mycolic acid methyl ester (**278**)

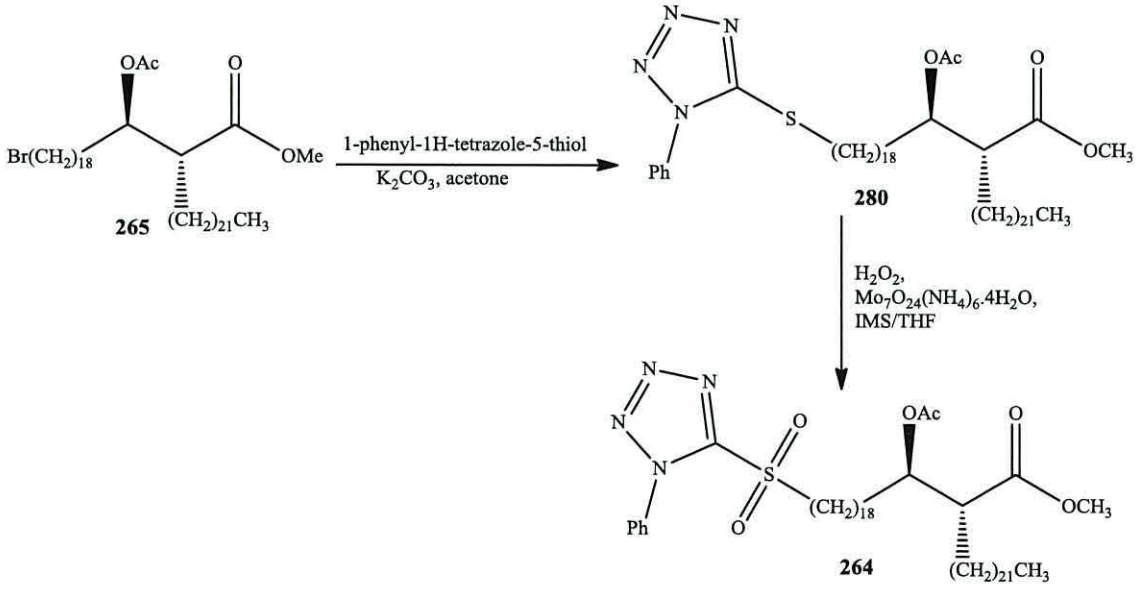
In another attempt phosphonium salt (**277**) was reacted with meromycolaldehyde (**261**) in the presence of sodium bis(trimethylsilyl)amide in dry THF (**Scheme 80**). This reaction also did not work.



Scheme 80: Another attempt to synthesise *cis*-epoxy mycolic acid (**279**)

2.8.4-Preparation of intermediate sulfone (264)

The bromo-compound (**265**) was converted into sulfide (**280**), followed by oxidation of the sulfide to the sulfone (**264**) (**Scheme 81**). The structure and stereochemistry of the sulfone (**264**) were confirmed using ^{1}H NMR, ^{13}C NMR, infra-red, optical rotation and mass spectrometry.

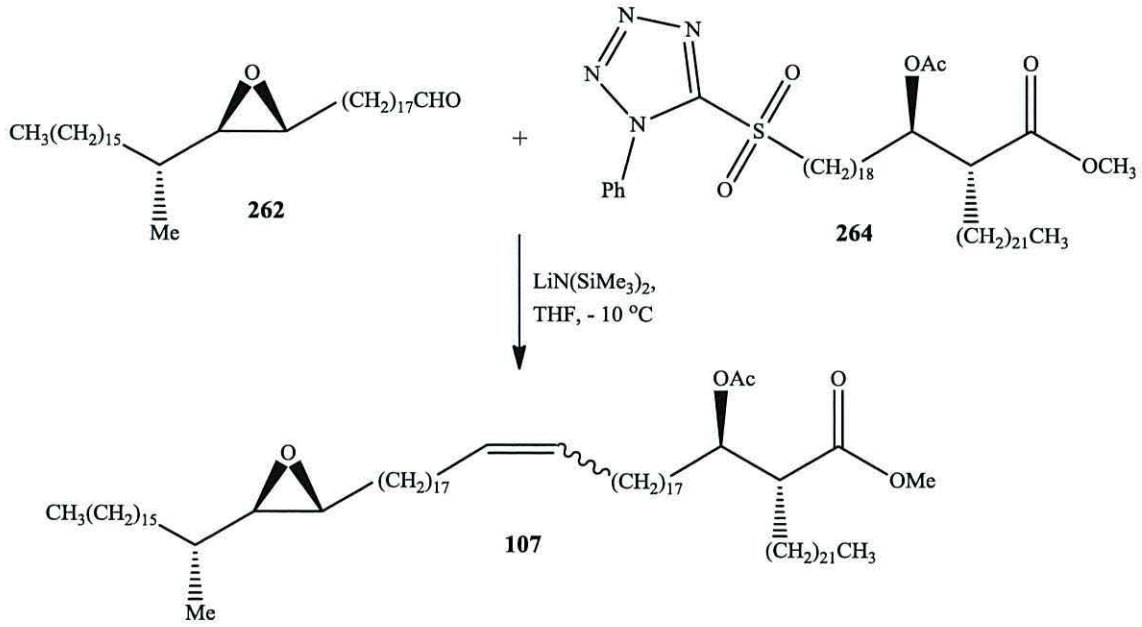


Scheme 81: Preparation of the sulfone (**264**)

2.8.5-The synthesis of (*E/Z*) (*R,R*)-epoxy-alkene mycolic acid methyl ester (107)

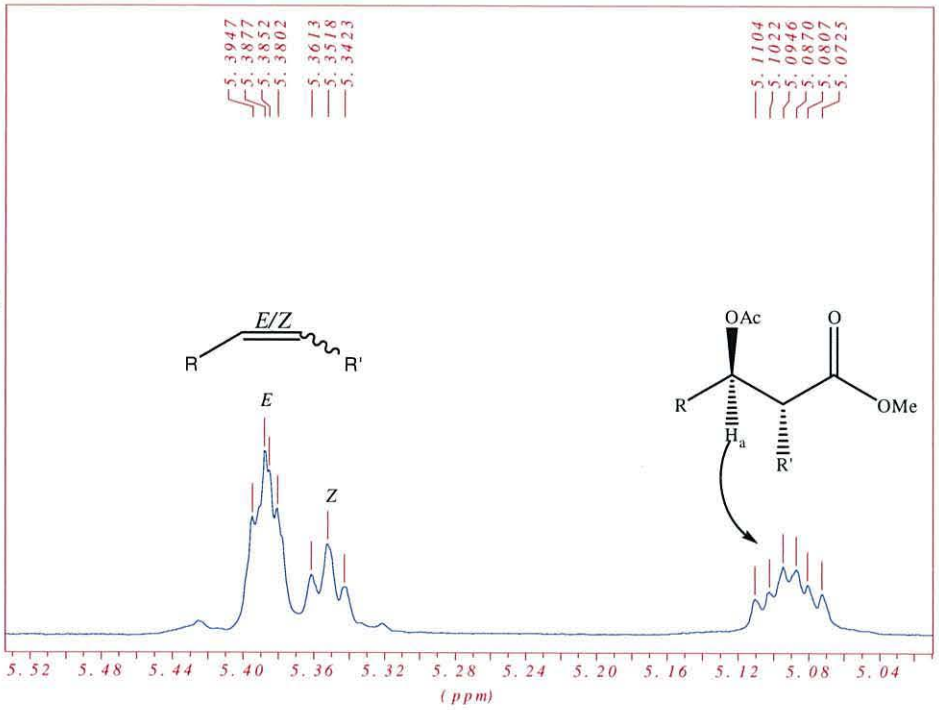
The final coupling reaction between meromycolaldehyde (**262**) and the sulfone (**264**) using lithium bis(trimethylsilyl)amide gave alkene (**107**) in 45 % yield. The product was a mixture of *E* and *Z* stereoisomers in ratio 2:1 (**Scheme 82**).

The ^{1}H NMR spectrum of (**107**) showed three multiplets at δ 5.39–5.38 for the protons of the *E*-alkene, δ 5.36–5.34 for the protons of the *Z*-alkene, and δ 5.11–5.07 for the proton (H_a) next to the acetyl group (**Fig. 33a**). The three signals in **Figure 33b** show a doublet of triplets at δ 2.72 for H_b, a doublet of doublets at δ 2.41 for H_d and a doublet of doublets of doublets at δ 2.62 for H_c. The ^{13}C NMR included two signals at δ 130.34 and 129.87 for the olefinic carbons (**Fig. 33c**).

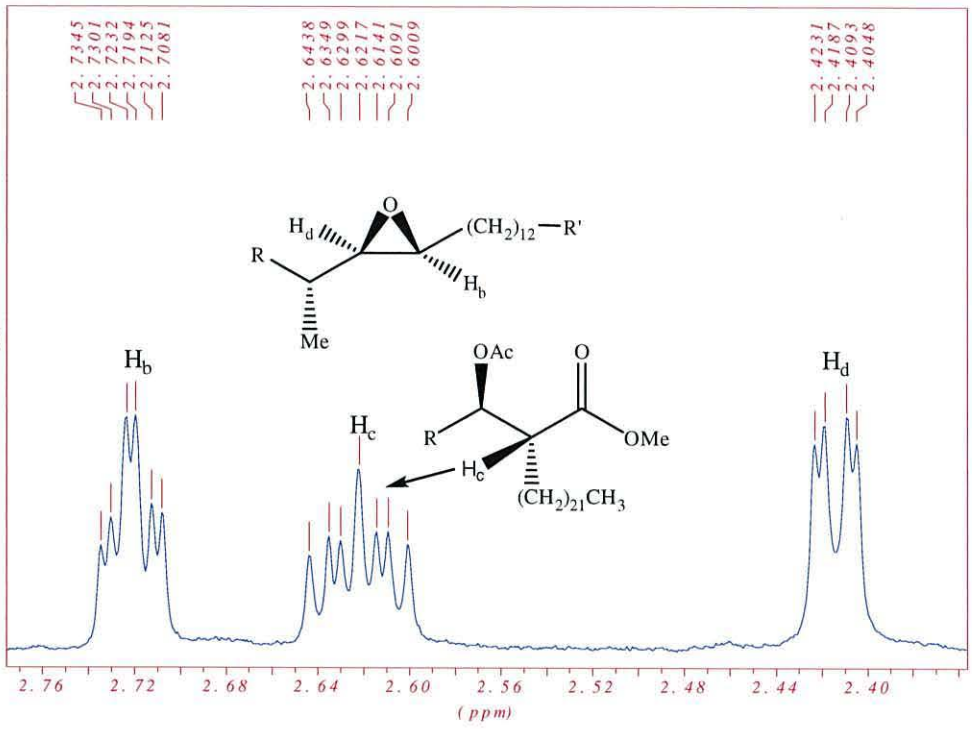


Scheme 82: Final coupling of (**262**) and (**264**) to form (*R,R*)-epoxy-alkene mycolic acid methyl ester (**107**)

(a)


5.3947
5.3877
5.3852
5.3802
5.3613
5.3518
5.3423
5.1104
5.1022
5.0946
5.0870
5.0807
5.0725
E/Z
R
R'
E
Z
OAc
O
OMe
Ha
5.52 5.48 5.44 5.40 5.36 5.32 5.28 5.24 5.20 5.16 5.12 5.08 5.04
(ppm)

(b)


2.7345
2.7301
2.7232
2.7194
2.7125
2.7081
2.6438
2.6349
2.6299
2.6217
2.6141
2.6091
2.6009
2.4231
2.4187
2.4093
2.4048
Hd
(CH2)12—R'
Hb
Me
Hb
Hc
OAc
O
OMe
(CH2)21CH3
Hd
2.76 2.72 2.68 2.64 2.60 2.56 2.52 2.48 2.44 2.40
(ppm)

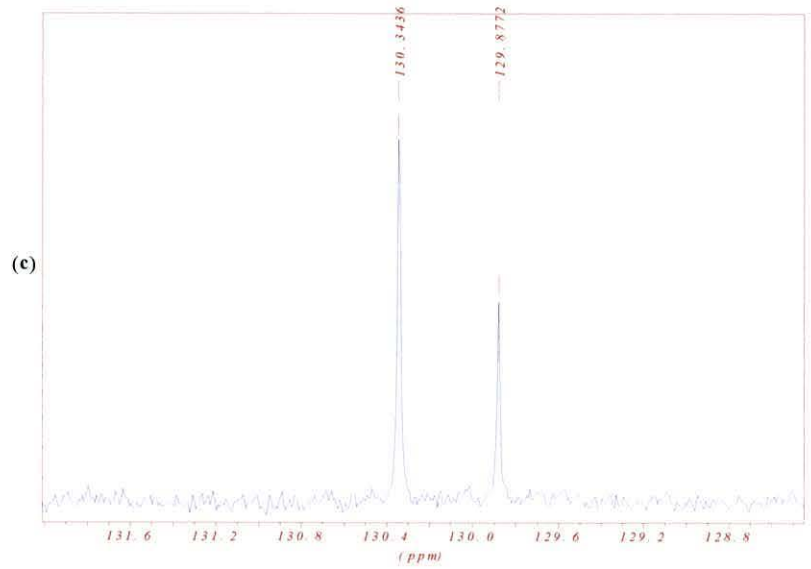
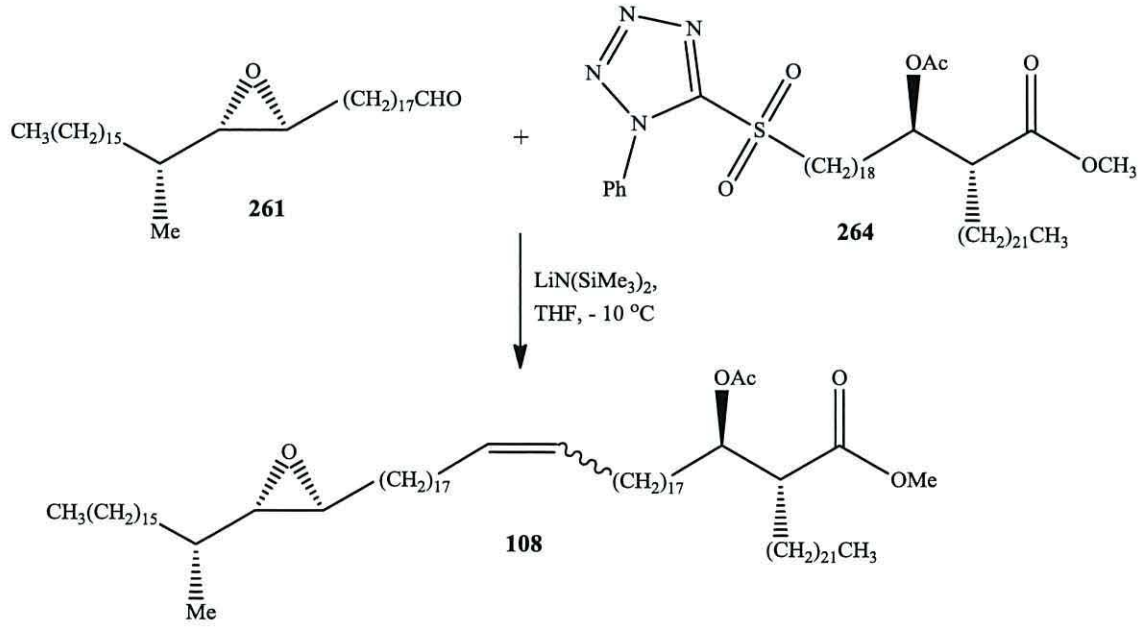


Fig 33a-c: ^{1}H and ^{13}C NMR spectrum of (**107**)

2.8.6-The synthesis of (*E/Z*) (*S,S*)-epoxy-alkene mycolic acid methyl ester (108)

Using the same method as discussed before, the aldehyde (**261**) was coupled with the sulfone (**264**) using lithium bis(trimethylsilyl)amide to give *E/Z* alkene (**108**) in 40 % yield in ratio 2:1 (**Scheme 83**).

The ^{1}H NMR spectrum included a broad triplet at δ 5.38 for protons of the *E*-isomer and a broad triplet at δ 5.35 for protons of the *Z*-isomer (**Fig. 34a**). The ^{13}C NMR spectrum showed two signals at δ 130.35 for the carbon of the *E*-alkene and δ 129.88 for the carbon of the *Z*-alkene (**Fig. 34b**).



Scheme 83: Final coupling of (**261**) and (**264**) to form (*S,S*)-epoxy-alkene mycolic acid methyl ester (**108**)

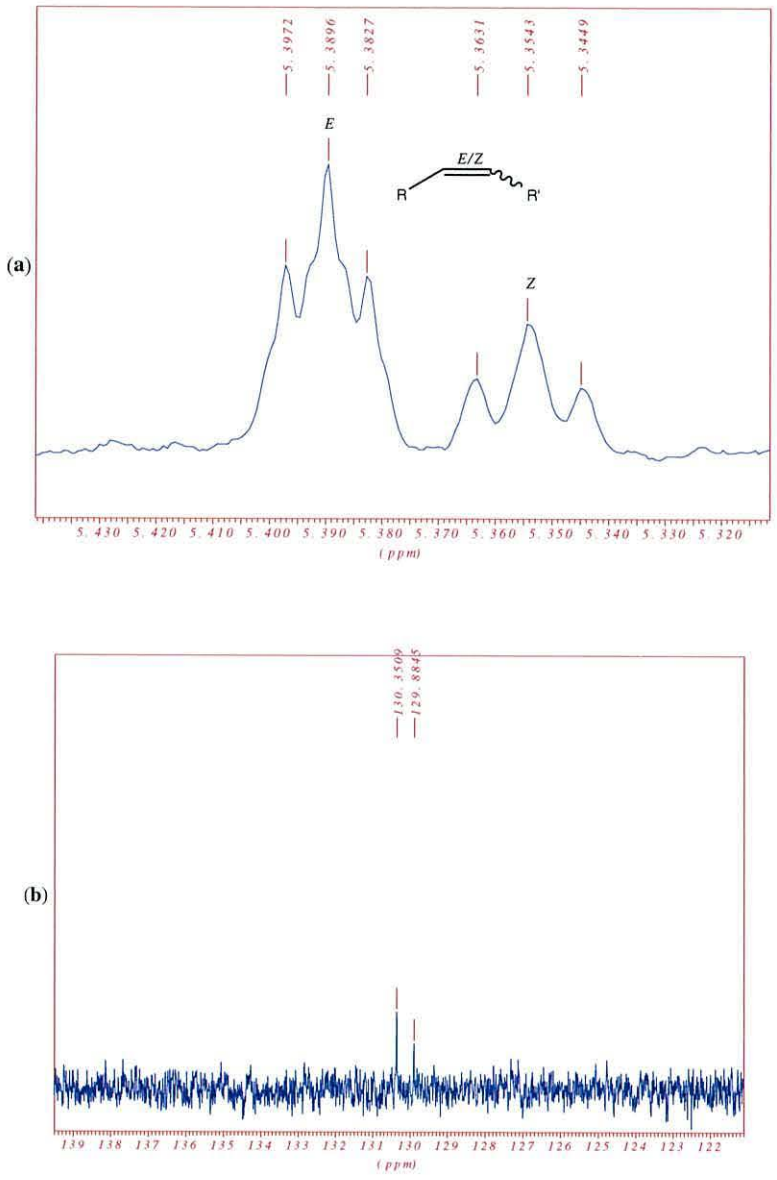


Fig. 34a,b: ^{1}H and ^{13}C NMR spectrum of (**108**)

2.8.7-An attempt to separate the (*E/Z*)-alkene-epoxy mycolic acid by silver ion TLC

Fatty acids (usually as methyl esters) can be separated according to the number and configuration of the double bonds. A mixture containing stearic acid methyl ester and oleic acid methyl ester was separated using this method.[228] This was carried out by immersing a TLC sheet in a freshly prepared 10 % silver nitrate solution in acetonitrile and removing immediately and then allowing to dry at r.t. in dark. Using this for TLC separated the mixture giving two spots. However, this method was not successful in the separation of the (*E/Z*)-mixture of alkene-epoxy mycolic acid. This may be due to the absence of a methyl branch adjacent to double bond.[223]

3-Conclusions

According to literature, some mycolic acids prepared before this project began include the following:

1-Single enantiomers of mycobacterial ketomycolic acids containing *cis*-cyclopropanes.[185]

2-Single enantiomers of the major methoxymycolic acid of *Mycobacterium tuberculosis*.[182]

3-A single enantiomer of a major α-mycolic acid of *M. tuberculosis*.[181]

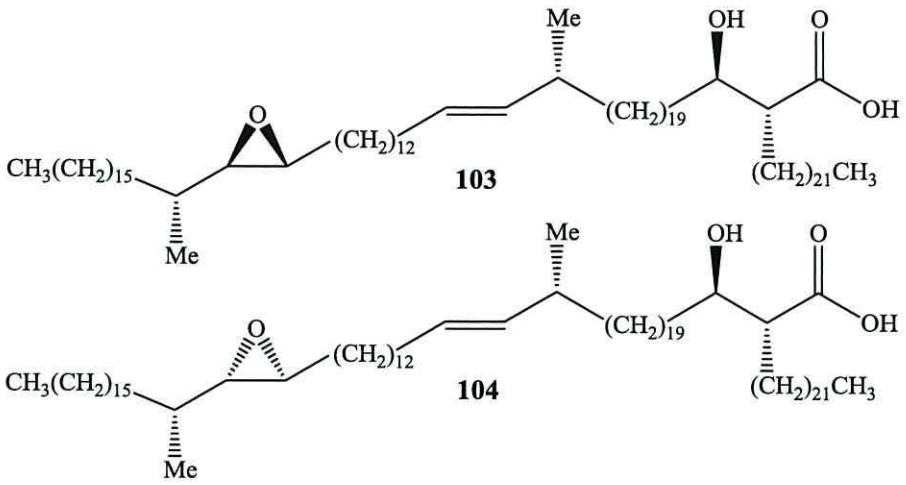
4-Single enantiomers of ketomycolic acids.[183]

None of the above compounds are in the class of epoxy-mycolic acids. This work reports the first syntheses of epoxy-mycolic acids.

The aim of this work was the total synthesis of epoxy-mycolic acids present in *M. fortuitum* and *M. smegmatis* and other mycobacteria as well as the comparison of the synthetic epoxy mycolic acids with samples of the natural mycolic acid isolated from *M. smegmatis*. The following compounds were successfully synthesised:

1- (*R,R*)-epoxy sulfone (**101**)
2- (*S,S*)-epoxy sulfone (**102**)
3- (*R,R*)-epoxy-*trans*-alkene-mycolic acid (**103**)
4- (*S,S*)-epoxy-*trans*-alkene-mycolic acid (**104**)
5- *Cis*-cyclopropane (*S,S*)-epoxy mycolic acid (**105**)
6- *Cis*-cyclopropane (*R,R*)-epoxy mycolic acid (**106**)
7- (*E/Z*)-alkene (*R,R*)-epoxy mycolic acid (**107**)
8- (*E/Z*)-alkene (*S,S*)-epoxy mycolic acid (**108**)

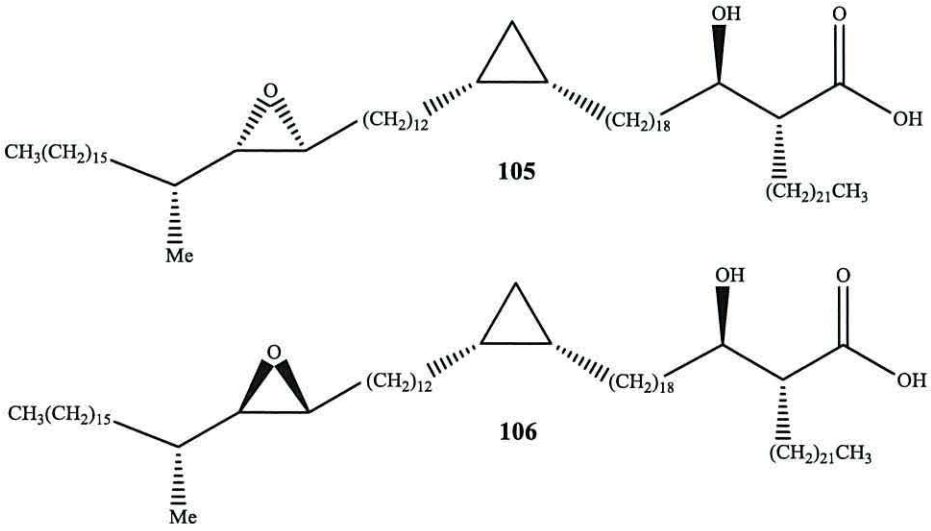
The two synthetic stereoisomeric (*R,R*)-epoxy-*trans*-alkene-mycolic acid (**103**) and (*S,S*)-epoxy-*trans*-alkene-mycolic acid (**104**) and a natural epoxy mycolic acid were characterized by mass spectra. The mass spectrum of (**103**) and (**104**) showed a molecular ion $[M + Na]^+$: 1204.1843 and $[M + Na]^+$: 1204.1912 respectively, while the major compound of the natural mycolic acid was reported to show a molecular ion at $[M + Na]^+$: 1204.19 (**Fig. 26**).



The close agreement between the ^{1}H and ^{13}C NMR spectra obtained for (**104**) and that reported by Minnikin[117] and Yuan[147] for natural epoxy-mycolic acid suggests that the stereochemistry of the epoxide ring in the natural compounds is that of (**104**). Moreover, the stereochemistry of the natural mycolic acid was also proved to be as in compound (**104**) through comparison with a report by French researchers.[136]

The synthesis of these molecules will be important for the identification of their exact structures and the stereochemistry of natural mycolic acids as well as in studying their biochemical properties. For example, they will be examined as antigens to antibodies generated by animals and man on exposure to *M. fortuitum* or *M. smegmatis*.

Two synthetic stereoisomeric *cis*-cyclopropane (*S,S*)-epoxy mycolic acid (**105**) and *cis*-cycopropane (*R,R*)-epoxy mycolic acid (**106**) were also prepared and compared by proton NMR spectroscopy with natural *cis*-cyclopropane epoxy mycolic acids isolated from *M. smegmatis*. This confirmed that the structure of (**105**) was the same natural mycolic acid.



Preliminary ELISA assays of the binding of (**106**) to antibodies in serum from TB endemic countries and from UK were carried out by Dr Alison Jones (School of Chemistry, Bangor University). As can be seen from the graph of the average absorbance (**Fig. 35**) for each set of sera there is no difference between the WHO TB positve and WHO TB negative sera; mycolic acid (**106**) is from *M. smegmatis* not *M. tuberculosis*, therefore it is not recognised. For the TB negative sera from the UK there is a higher response; it is too early to know, but this may be due to there being antibodies in the UK population to the epoxy-mycolic acid, which are not present in the overseas (WHO) samples.

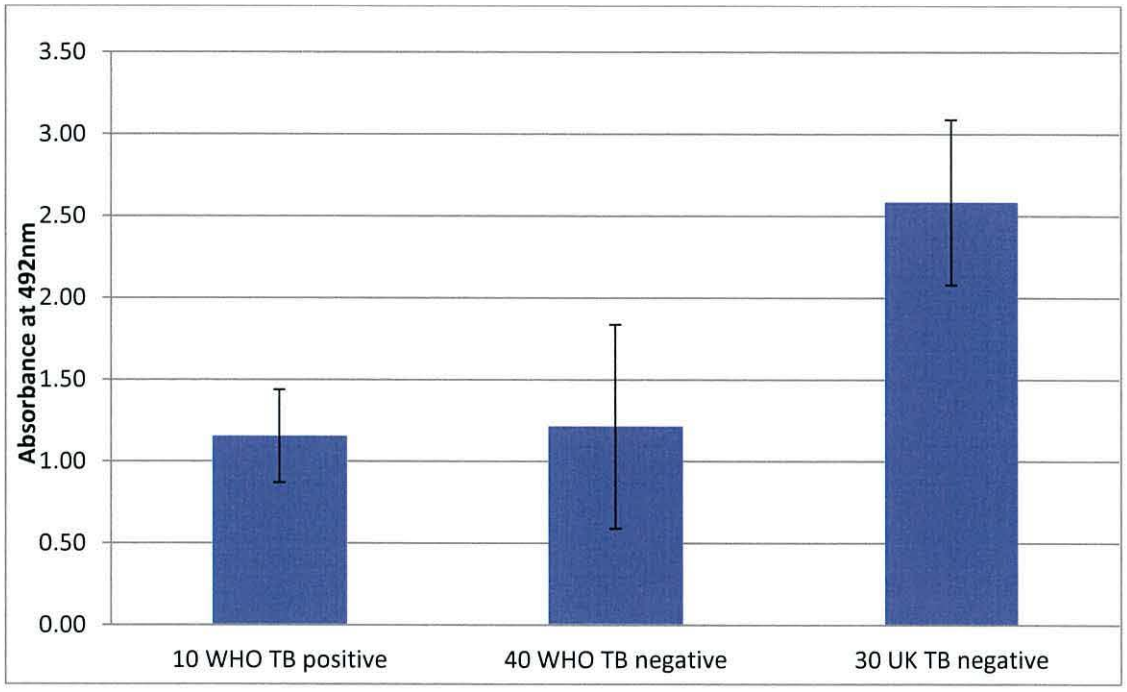
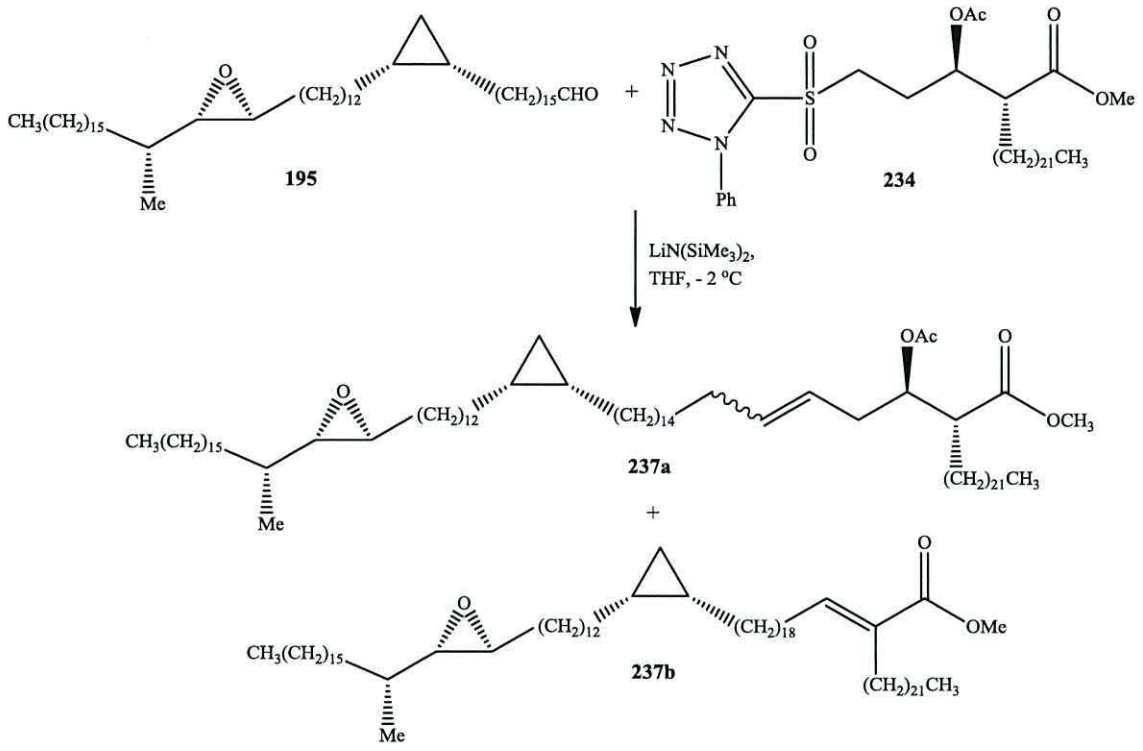
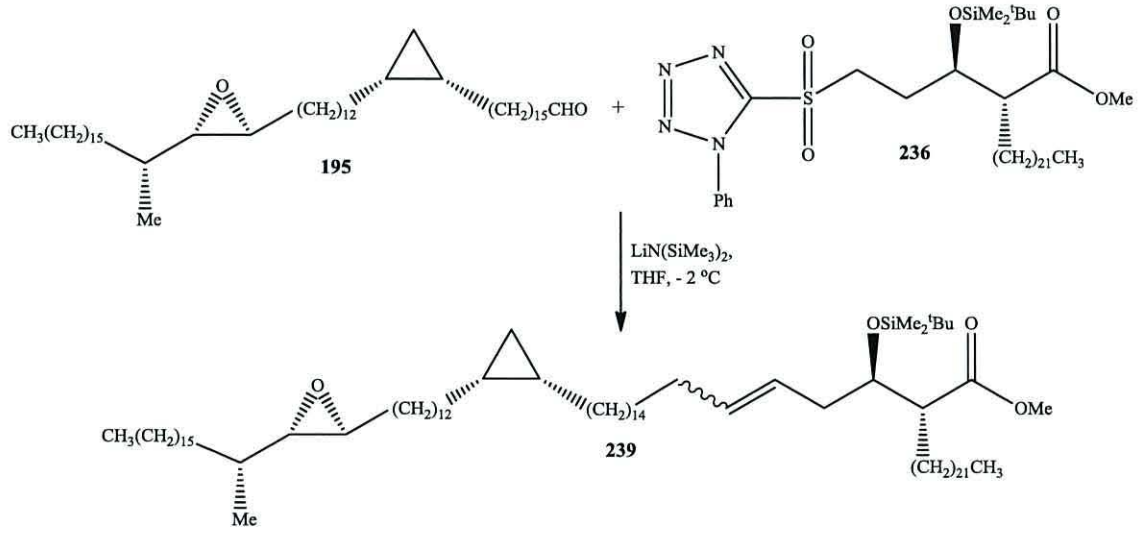


Fig. 35: Average absorbances for different sets of sera to (**106**)

These compounds were prepared by two methods. The first method gave a low yield because of the presence of the acetyl group, therefore the α-proton was acidic and easy to lose, followed by 1,2-elimination of acetyl group leading to (**237b**) as a by-product.

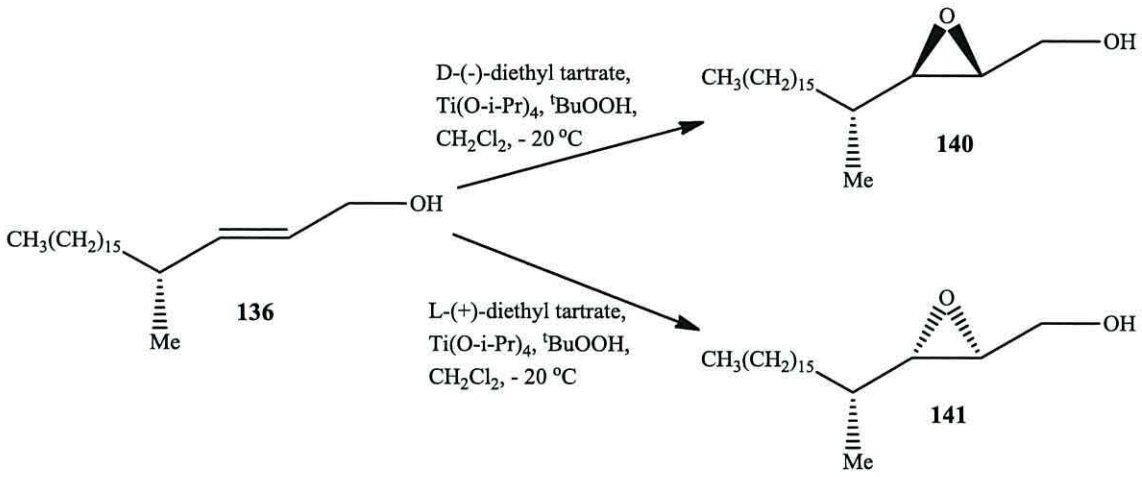


The second method gave a higher yield because the hydroxyl group in the β-position was protected with a silyl group.



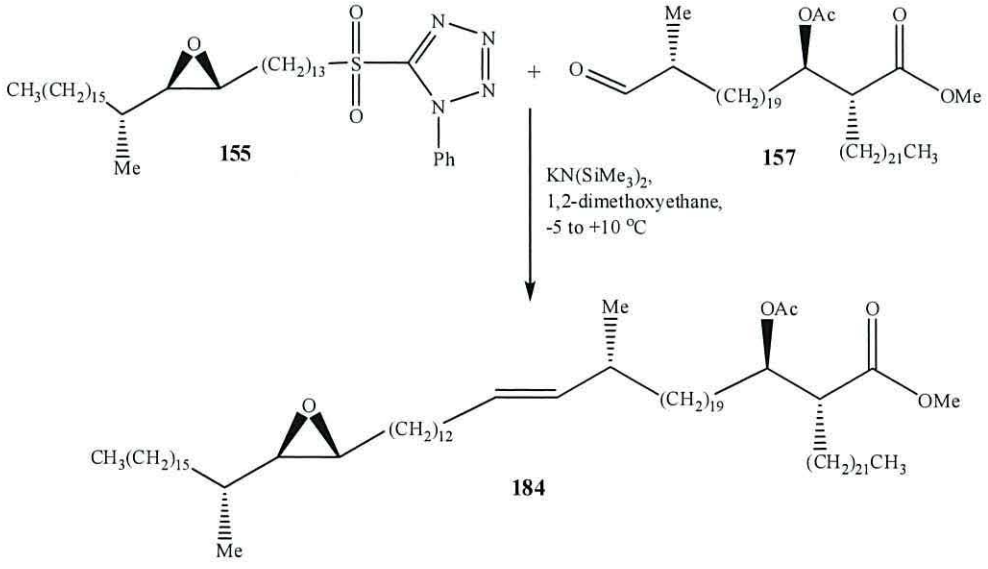
The modified Julia-Kocienski olefination was found to be the best for chain extension in the synthesis of mycolic acids. This involved coupling between a sulfone and an aldehyde using lithium bis(trimethylsilyl)amide in THF to give an alkene, followed by hydrogenation to complete the chain extension. Hydrogen gas was used for hydrogenation at atmospheric pressure for products that did not contain an epoxide ring or cyclopropane ring and di-imide was used for hydrogenation of intermediates that include an epoxide ring or cyclopropane ring. D-Mannitol was used as starting material

in the preparation of such intermediates. The epoxy sulfone was prepared by the Sharpless epoxidation. The asymmetric epoxidation was attempted with both D (-) and L (+) tartrate in order to understand the properties of the catalyst used in the synthesis of the two diastereoisomers (**140** / **141**).

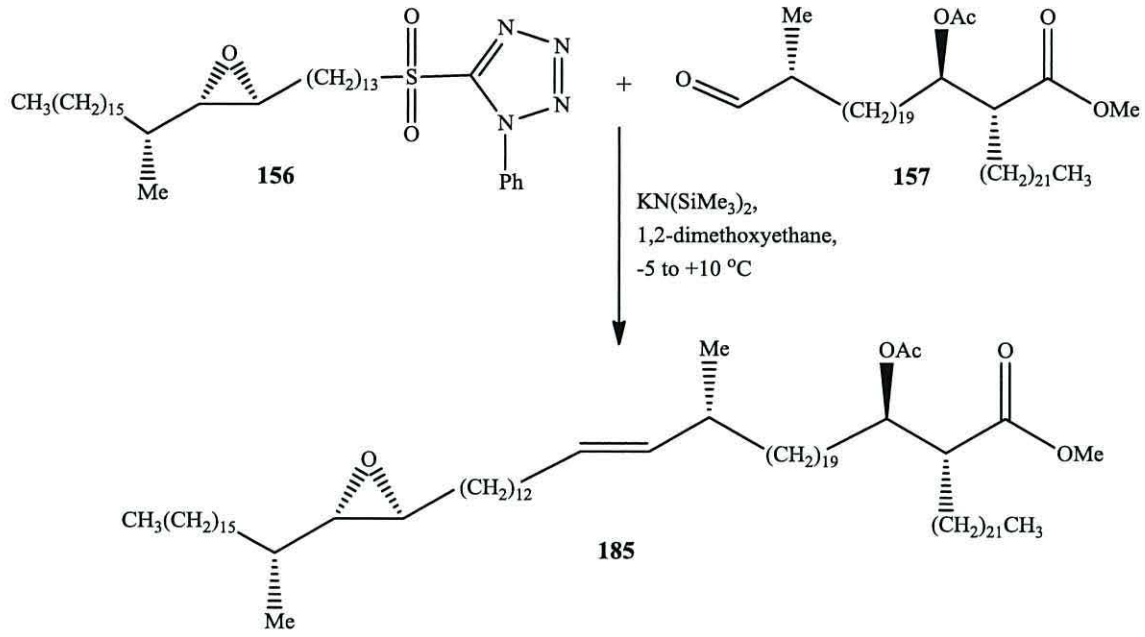


The secondary hydroxyl group in the mycolate motif part was protected with a *tert*-butyldimethylsilyl group which was again deprotected using HF.pyridine, followed reprotection with an acetyl group as (**157**) before coupling with the meromycolate moiety. The mycolate motif protected with the silyl group gave a higher yield than that protected with the acetyl group. The α-methyl-*trans*-alkene was prepared from a stereoselective coupling reaction between the aldehyde (**157**) and sulfones (**155**) or (**156**).

1-

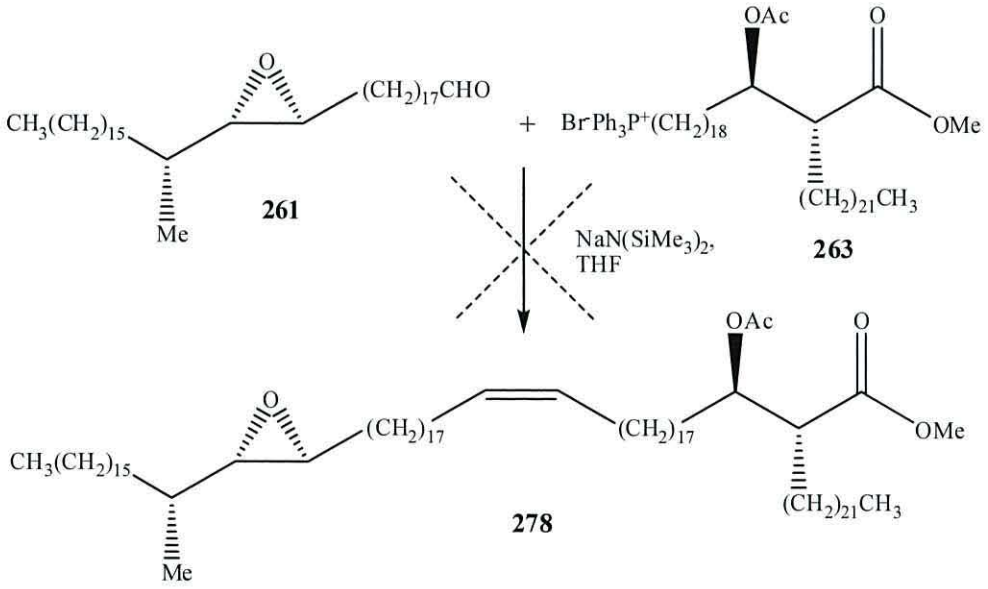


2-

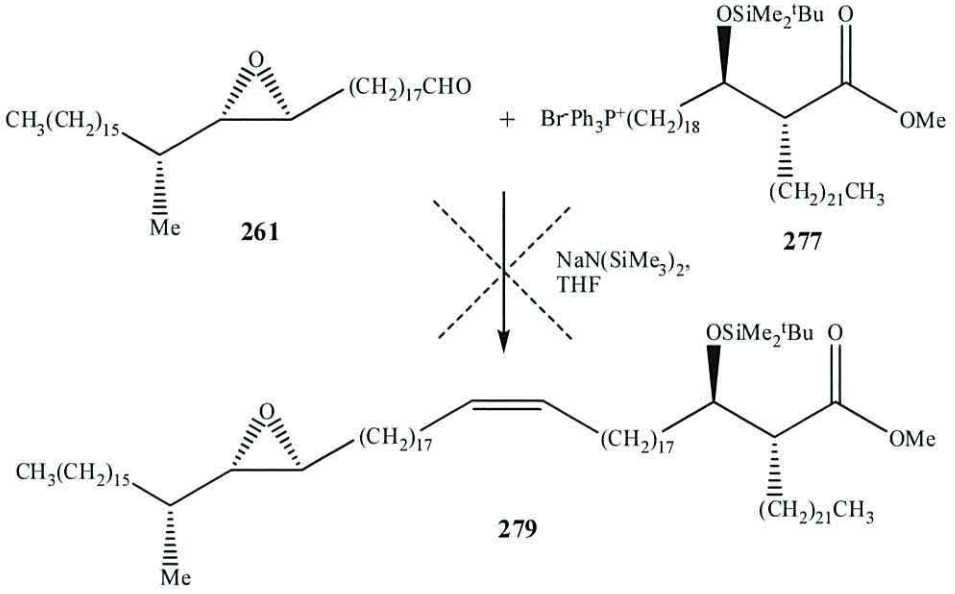


An attempt was made to synthesise *cis*-alkene containing epoxy mycolic acids by coupling the aldehyde (**261**) with phosphonium salts (**263**) or (**277**) using sodium bis(trimethylsilyl)amide for a Wittig reaction. Unfortunately the reaction did not work.

1-

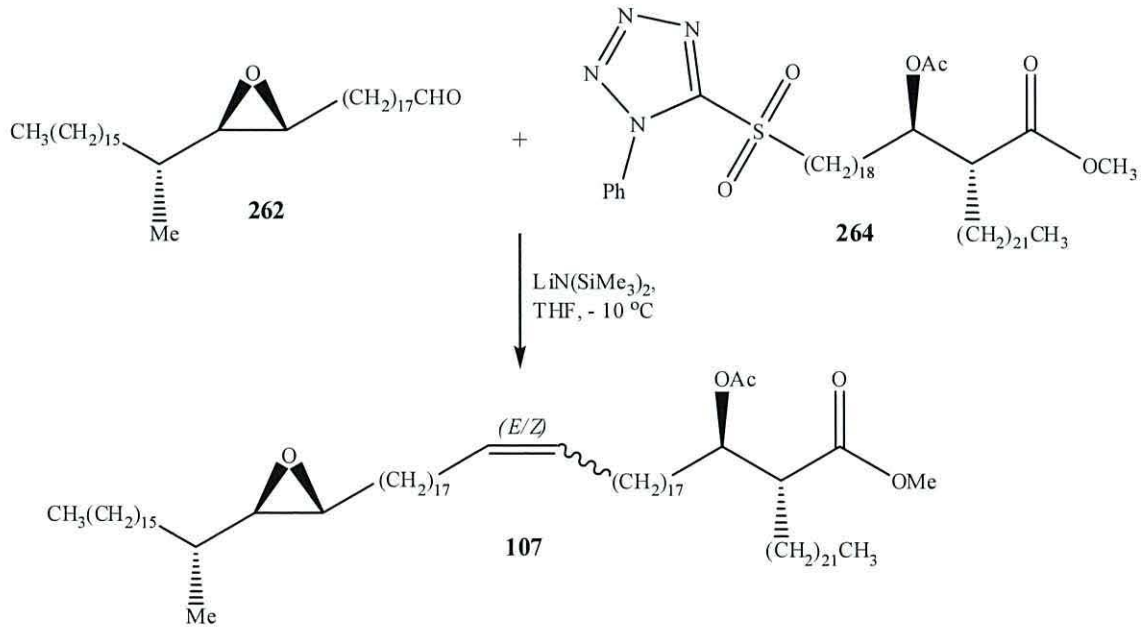


2-

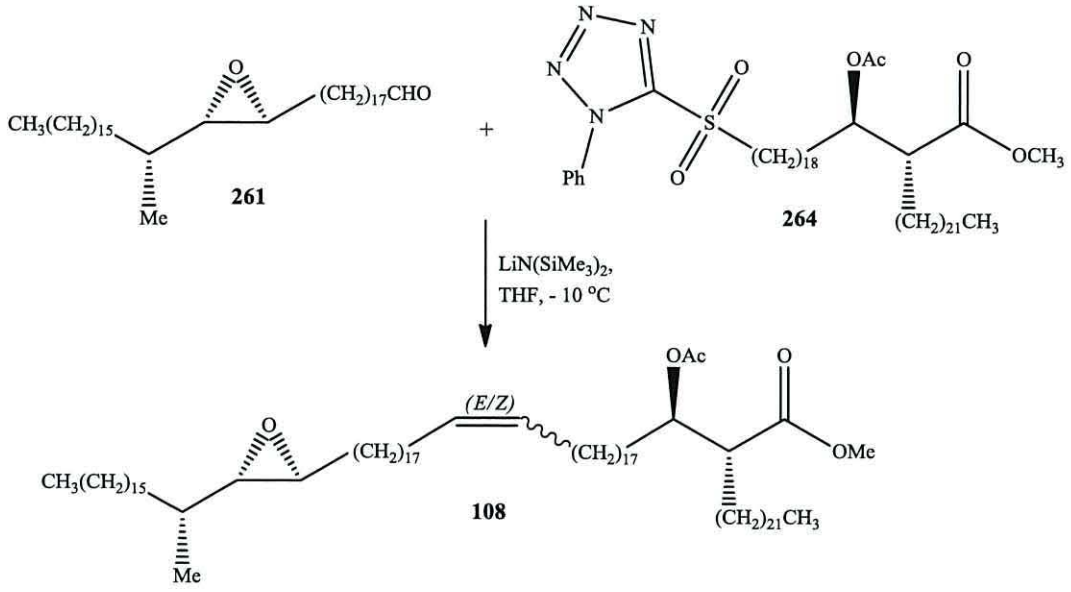


Another method of synthesis of (*E/Z*) (*R,R*)-epoxy-alkene mycolic acid (**107**) and (*E/Z*) (*S,S*)-epoxy-alkene mycolic acid (**108**) used lithium bis(trimethylsilyl)amide for a Julia reaction.

1-



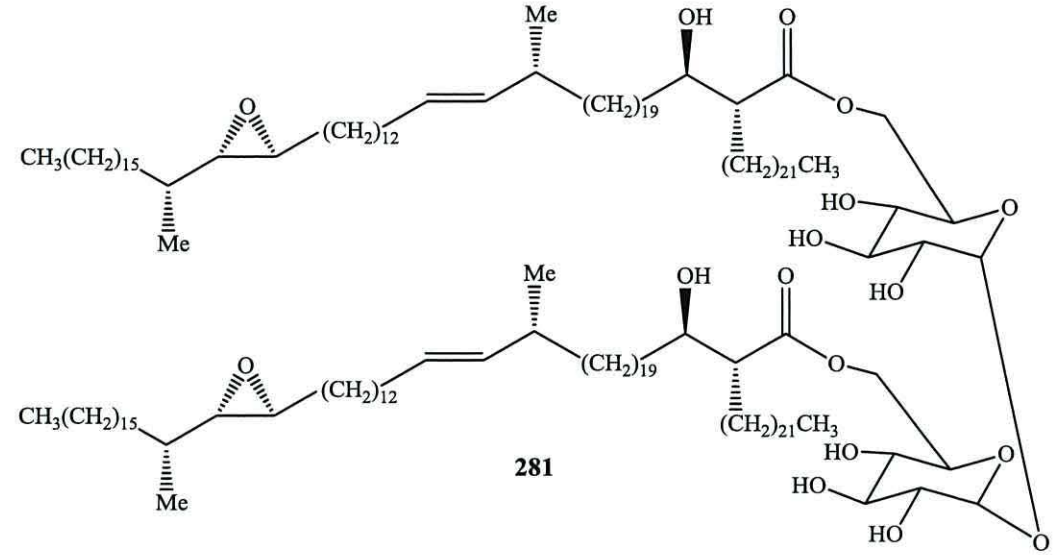
2-



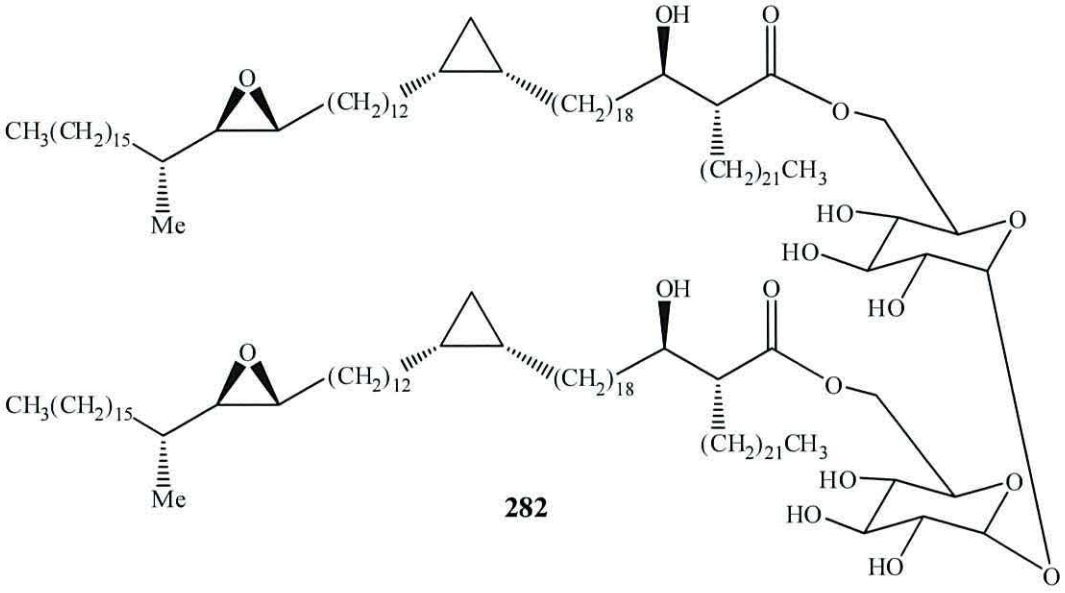
A mixture of isomers was obtained in each case. An attempt was made to separate the (*E/Z*)-alkene-epoxy mycolic acid by silver ion TLC. This method was not successful. This could be as a result of the functional groups such as the epoxide ring contained in the mycolic acid.

Finally, other areas suggested for future work could be in the preparation of other new epoxy-mycolic acids followed by testing so as to determine the physical and biological properties of mycolic acids in general. It is anticipated that this would help in the development of the existing methods for detecting mycobacterial infection and may offer possibilities for the treatment of asthma. Moreover, the biological properties of the synthetic epoxy mycolic acids are being determined in order to establish firmly whether the stereochemistry is critical to the biochemical effects in the natural material. The preparation of different synthetic analogues could also help in the determination of the role of each particular feature of the acids in the regulation mechanisms of the drug permeability of the cell envelope.

These epoxy-mycolic acids could also be used to prepare the cord factors by esterification of a trehalose sugar using DMAP and 4-DMAP. The next step deprotection of silyl group in two steps first using tetrabutylammonium fluoride to remove sugar silyl and then HF-pyridine complex to remove mycolic acid protection, releasing the free cord factor. Target molecules would be (**281**) and (**282**).



281



282

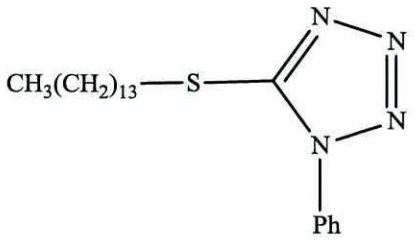
4-Experimental

4.1-General considerations

Chemicals used were obtained from commercial suppliers or prepared from them by the methods described. Solvents which were required to be dry, e.g. ether, tetrahydrofuran were dried over sodium wire and benzophenone under nitrogen, while dichloromethane and HMPA were dried over calcium hydride. Toluene was dried over sodium wire. Petrol used was of boiling point 40-60 °C. Reactions carried out under inert conditions were under a slow stream of nitrogen. Those carried out at low temperatures were cooled using a bath of methylated spirits and liquid nitrogen. All reagents and solvents used were of reagent grade unless otherwise stated. Silica gel (Merek 7736) and silica gel plates used for column chromatography and thin layer chromatography were obtained from Aldrich; separated components were detected using variously UV light, I_2 and phosphomolybdic acid solution in IMS followed by charring. Anhydrous magnesium sulfate was used to dry organic solutions. GLC was carried out on a Perkin-Elmer Model 8410 on a capillary column (15 m x 0.53 mm). Infra-red (IR) spectra were carried out on a Perkin-Elmer 1600 F.T.I.R. spectrometer as liquid films or KBr disc (solid). Melting points were measured using a Gallenkamp melting point apparatus. NMR spectra were carried out on a Bruker AC250 or Advance 500 spectrometer; for ^{1}H spectra the machine was ran at 500MHz, for ^{13}C spectra the machine was ran at 125MHz, (+ = CH_2, - = CH, CH_3). Chemical shifts for ^{1}H / ^{13}C are quoted in δ relative to chloroform (δ 7.27 ppm), and $CDCl_3$ (δ 77.0 ppm). $[\alpha]_D$ values were recorded in $CHCl_3$ on a POLAAR 2001 optical activity polarimeter. Mass spectra were recorded on a Bruker Microtof. MALDI mass spectrometry values are given plus sodium to an accuracy of 2 d.p., EI mass spectrometry values given plus hydrogen to an accuracy of 4 d.p.

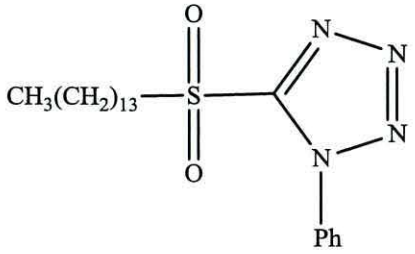
4.2-Experiments

Experiment 1: 5-Tetradecylsulfanyl-1-phenyl-1*H*-tetrazole (123)



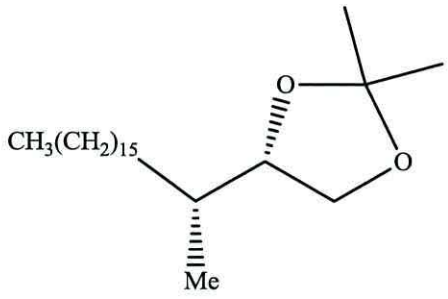
1-Bromotetradecane (25 g, 90 mmol) was added with vigorous stirring to 1-phenyl-1*H*-tetrazole-5-thiol (16 g, 90 mmol) and anhydrous potassium carbonate (25 g, 180 mmol) in acetone (250 ml).[182] The mixture was refluxed for 2.5 hours when TLC showed no starting material was left. The inorganic salts were filtered off and washed with acetone, the solution was evaporated to a small bulk and the residue extracted between dichloromethane (200 ml) and water (300 ml). The aqueous layer was extracted with dichloromethane (2 × 80 ml). The combined organic phases were washed with water (300 ml), dried and evaporated to give a solid. This was dissolved in acetone (50 ml) and diluted with methanol (100 ml) and left at ambient for 1 hour and then at 0 °C for 30 min. The crystals were filtered off and washed with cold acetone/methanol (1:2) to yield a white solid, 5-tetradecylsulfanyl-1-phenyl-1*H*-tetrazole (**123**) (28.12 g, 86 %), m.p.: 39-41 °C [Found $(M + H)^+$: 375.257, $C_{21}H_{35}N_4S$ requires: 375.2577], which showed δ_H (500MHz, $CDCl_3$): 7.6-7.51 (5H, m), 3.39 (2H, t, *J* 7.25 Hz), 1.81 (2H, pent., *J* 7.55 Hz), 1.44 (2H, pent., *J* 6.65 Hz) 1.31-1.22 (20H, m, including br s at 1.25), 0.88 (3H, t, *J* 6.6 Hz); δ_C (125MHz, $CDCl_3$): 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}: 2924, 2853, 1598, 1500, 1464, 1411, 1386, 1243, 1086, 1074, 1014 cm^{-1}.

Experiment 2: 5-(Tetradecane-1-sulfonyl)-1-phenyl-1*H*-tetrazoe (124)



A solution of ammonium molybdate (VI) tetrahydrate (41.1 g, 33.3 mmol) in ice cold H_2O_2 (35 % w/w, 96 ml) was added to a stirred solution of 5-tetradecyl-sulfanyl-1-phenyl-1*H*-tetrazole (28 g, 74 mmol) in THF (296 ml) and IMS (593 ml) at 12 °C and stirred at 15–20 °C for 2 hours.[182] A further solution of ammonium molybdate (VI) tetrahydrate (16.5 g, 13.3 mmol) in ice cold H_2O_2 (35 % w/w, 42 ml) was added at r.t., stirred for 18 hours, then poured into 3 L of water and extracted with dichloromethane (3 × 350 ml). The combined organic phases were washed with water (2 × 300 ml), dried and evaporated. The residue was dissolved in methanol (300 ml) and left at ambient for 1 hour and then at 0 °C for 1 hour. A white solid crystallized; this was filtered and washed with cold methanol to give 5-(tetradecane-1-sulfonyl)-1-phenyl-1*H*-tetrazole (**124**) (26.7 g, 88 %), m.p.: 60-61 °C [Found $(M + H)^+$: 407.2324, $C_{21}H_{35}N_4O_2S$ requires: 407.2475], which showed δ_H (500MHz, $CDCl_3$): 7.72-7.69 (2H, m), 7.65-7.58 (3H, m), 3.73 (2H, distorted t, *J* 7.85 Hz), 1.95 (2H, pent., *J* 7.55 Hz), 1.5 (2H, pent., *J* 6.6 Hz), 1.36-1.26 (20H, br m, including br s at 1.26), 0.9 (3H, t, *J* 6.6 Hz); δ_C (125MHz, $CDCl_3$): 153.5, 133.1, 131.4, 129.7, 125.1, 56.0, 31.9, 29.63, 29.60, 29.5, 29.4, 29.3, 29.2, 28.9, 28.1, 22.7, 21.9, 14.1; ν_{max}: 2924, 2854, 1596, 1498, 1464, 1343, 1153 cm^{-1}.

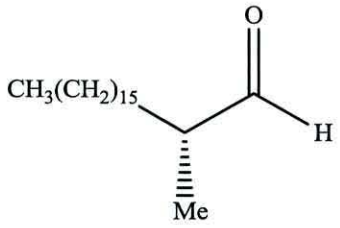
Experiment 3: (*S*)-2,2-Dimethyl-4-((*R*)-1-methylheptadecyl)-[1,3]dioxolane (133)



Lithium bis(trimethylsilyl)amide (50 ml, 52 mmol, 1.06 M) was added dropwise with stirring to (*R*)-3-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-butyraldehyde (**115**) (5.42 g, 31.5 mmol) and 5-(tetradecane-1-sulfonyl)-1-phenyl-1*H*-tetrazole (**124**) (16.6 g, 40 mmol) in dry tetrahydrofuran (250 ml) under nitrogen at -2 °C.[182] The mixture was allowed to reach room temperature and stirred for 16 hours then quenched with water (100 ml) and petrol/ether (1:1, 2 × 50 ml). The combined organic layers were washed with sat. aq. sodium chloride (2 × 100 ml), dried and evaporated to give a thick oil. Chromatography on silica gel eluting with petrol/ether (20:1) gave an oil, (*S*)-2,2-dimethyl-4-((*E/Z*)-(*R*)-1-methylheptadec-3-enyl)-[1,3]dioxolane (**132**) (7.42 g, 67 %) as a mixture of two

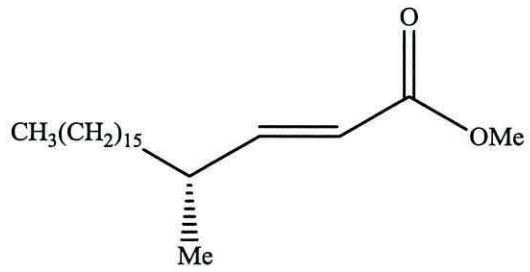
isomers in ratio 2:1. Palladium on charcoal (10 %, 0.5 g) was added to a stirred solution of the alkenes (6.4 g, 18.15 mmol) in ethanol (100 ml). The mixture was stirred under hydrogen at atmospheric pressure. When no more hydrogen was absorbed it was filtered through celite and washed with warm ethyl acetate (100 ml). The clear colourless filtrate was evaporated at 14 mm Hg to give an oil, (*S*)-2,2-dimethyl-4-((*R*)-1-methylheptadecyl)-[1,3]dioxolane (**133**) (5.9 g, 92 %), $[\alpha]_D^{22} = +17.6$ (*c* 0.625, $CHCl_3$) [Found $(M + H)^+$: 355.3582, $C_{23}H_{47}O_2$ requires: 355.3571], which showed δ_H (500MHz, $CDCl_3$): 3.99 (1H, t, *J* 6.6 Hz), 3.86 (1H, br q, *J* 6.9 Hz), 3.59 (1H, br t, *J* 7.55 Hz), 1.56-1.51 (1H, m), 1.4 (3H, s), 1.35 (3H, s), 1.31-1.26 (30H, br s), 0.96 (3H, d, *J* 6.6 Hz), 0.88 (3H, t, *J* 6.3 Hz); δ_C (125MHZ, $CDCl_3$): 108.5, 80.4, 67.8, 36.5, 32.7, 31.9, 29.86, 29.7, 29.65, 29.6, 29.4, 26.97, 26.6, 25.5, 22.7, 15.6, 14.1; ν_{max}: 2984, 2923, 2854, 1466, 1378, 1214, 1161, 1066 cm^{-1}.

Experiment 4: (*R*)-2-Methyl-octadecanal (134)



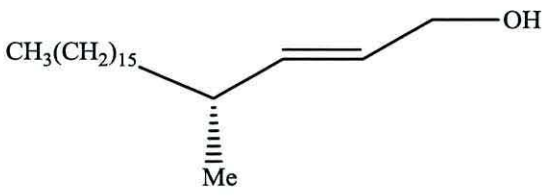
Periodic acid (11.5 g, 50 mmol) was added to a stirred solution of (*S*)-2,2-dimethyl-4-((*R*)-1-methylheptadecyl)-[1,3]dioxolane (**133**) (9 g, 25.4 mmol) in dry ether (250 ml) under nitrogen at room temperature. The mixture was stirred for 16 hours, when TLC showed no starting material. The precipitate was filtered through a bed of celite and washed with ether. The solvent was evaporated to give a residue. The crude product was purified by column chromatography eluting with petrol/ether (10:1) to give a colourless oil, (*R*)-2-methyl-octadecanal (**134**) (5.4 g, 77 %), $[\alpha]_D^{25} = -11.4$ (*c* 1.07, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 9.6 (1H, d, *J* 1.9 Hz), 2.34-2.27 (1H, m), 1.71-1.64 (1H, m), 1.34-1.25 (29H, br s), 1.08 (3H, d, *J* 7.25 Hz), 0.87 (3H, t, *J* 6.95 Hz); δ_C (125MHz, $CDCl_3$): 205.3, 46.3, 31.9, 30.5, 29.69, 29.66, 29.63, 29.57, 29.46, 29.36, 26.9, 22.68, 14.1, 13.3; ν_{max}: 2925, 2853, 2699, 1730, 1465.5, 1376 cm^{-1}.

Experiment 5: (*E*)-(*R*)-4-Methyl-eicos-2-enoic acid methyl ester (135)



Methyl (triphenylphosphoranylidene) acetate (6 g, 18.2 mmol) was added in portions to a stirred solution of aldehyde (**134**) (4.69 g, 16.6 mmol) in toluene (75 ml) at 10 °C.[229] The mixture was allowed to reach room temperature and stirred for 24 hours when TLC showed no starting material. The solvent was evaporated and the residue was refluxed with petrol/ether (1:1, 150 ml) for 10 min. The precipitate was washed with petrol/ether (10:1, 150 ml). The solvent was evaporated to give a residue. Chromatography on silica gel eluting with petrol/ethyl acetate (20:1) gave a colourless oil, (*E*)-(*R*)-4-methyl-eicos-2-enoic acid methyl ester (**135**) (3.64 g, 65 %) [Found $(M + H)^+$: 339.3269, $C_{22}H_{43}O_2$ requires: 339.3258], $[\alpha]_D^{24} = -18.6$ (*c* 1.5, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 6.86 (1H, dd, *J* 7.9,15.45 Hz), 5.77 (1H, dd, *J* 0.95, 15.75 Hz), 3.72 (3H, s), 2.28 (1H, sept., *J* 6.95 Hz), 1.39-1.25 (30H, m, including br s at 1.25), 1.03 (3H, d, *J* 6.65 Hz), 0.87 (3H, t, *J* 6.9 Hz); δ_C (125MHz, $CDCl_3$): 167.3, 155.0, 119.1, 51.3, 36.5, 36.0, 31.9, 29.7, 29.63, 29.61, 29.5, 29.3, 27.2, 22.7, 19.4, 14.1; ν_{max}: 2924, 2853, 2360, 1730, 1656, 1465, 1270, 1173, 1038 cm^{-1}.

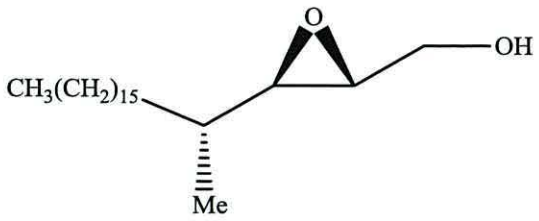
Experiment 6: (*E*)-(*R*)-4-Methyl-eicos-2-en-1-ol (136)



A solution of DIBAL-H (64.79 ml, 64.79 mmol, 1M in hexane) was added to a stirred solution of (*E*)-(*R*)-4-methyl-eicos-2-enoic acid methyl ester (**135**) (8.47 g, 25.9 mmol) in dry dichloromethane (250 ml) at - 60 °C under nitrogen.[229] The mixture was stirred overnight at room temperature and then quenched by adding sat. aq. ammonium chloride (30 ml) at - 30 °C. The mixture was allowed to reach room temperature and stirred for 0.5 hour. Subsequently, hydrochloric acid (5 %) was added until it became a clear solution. The aqueous layer was extracted with dichloromethane (3 × 150 ml) and

the combined organic layer was dried. Evaporation of the solvent yielded a pale yellow oil, which was purified by column chromatography eluting with petrol/ether (5:2) to give a white solid, (*E*)-(*R*)-4-methyl-eicos-2-en-1-ol (**136**) (7.36 g, 95 %), m.p.: 34-35 °C [Found $(M)^+$: 310.3496, $C_{21}H_{42}O$ requires: 310.323], $[\alpha]_D^{26}$ = - 15 (*c* 1.4, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 5.62-5.57 (2H, m), 4.09 (2H, d, *J* 5.05 Hz), 2.17-2.07 (1H, m), 1.31-1.26 (31H, br s), 0.98 (3H, d, *J* 6.9 Hz), 0.88 (3H, t, *J* 6.95 Hz); δ_C (125MHz, $CDCl_3$): 139.4, 127.0, 63.9, 36.8, 36.3, 31.9, 29.8, 29.69, 29.67, 29.64, 29.3, 27.3, 22.7, 20.3, 14.1; ν_{max}: 3448, 2954, 2924, 2854, 1644, 1462, 1377 cm^{-1}.

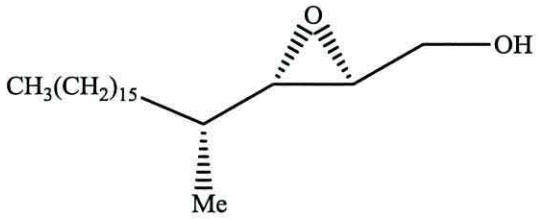
Experiment 7: [(2*R*,3*R*)-3-((*R*)-1-Methylheptadecyl)-oxiranyl]-methanol (140)



Titanium tetraisopropoxide in dry dichloromethane (3.4 M, 0.28 ml, 0.96 mmol) was added to a stirred solution of D-(-)-diethyl tartrate (0.23 ml, 1.13 mmol) in dry dichloromethane (100 ml) under nitrogen at - 20 °C in the presence of 4 A molecular sieves (0.5 g).[229] The mixture was stirred at - 20 °C for 0.5 hour then *tert*-butyl hydroperoxide in dry dichloromethane (3.3 M, 4.8 ml, 16 mmol) was added dropwise and the mixture was stirred for another 0.5 hour. To this solution, (*E*)-(*R*)-4-methyl-eicos-2-en-1-ol (**136**) (2.5 g, 8.06 mmol) in dry dichloromethane (10 ml), was added dropwise. After stirring at the same temperature for 4.5 hours, the reaction was left at - 20 °C overnight in the freezer, the quenched with water (10 ml) and allowed to reach room temperature. After the mixture had been stirred for 50 min, a solution of sodium hydroxide (30 %) in sat. aq. sodium chloride (6 ml) was added. After stirring the mixture for a further 0.5 hour, the phases were separated and the aqueous layer was extracted with dichloromethane (3 × 15 ml). The combined organic layer were dried and evaporated to give a thick oil, which was purified by column chromatography eluting with petrol/ether (5:2) to give [(2*R*,3*R*)-3-((*R*)-1-methyl-heptadecyl)-oxiranyl]-methanol (**140**) (1.74 g, 67 %), m.p.: 35-37 °C [Found $(M + H)^+$: 327.3274, $C_{21}H_{43}O_2$ requires: 327.3258], $[\alpha]_D^{28}$ = + 18.13 (*c* 0.8, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 3.9 (1H, ddd, *J* 2.5, 5.56, 12.6 Hz), 3.6 (1H, ddd, *J* 4.45, 6.95, 12 Hz), 2.94-2.92 (1H, m), 2.76 (1H, dd, *J* 2.2, 7.25 Hz), 1.53-1.48 (1H, m), 1.43-1.25 (31H, br m, including br s at

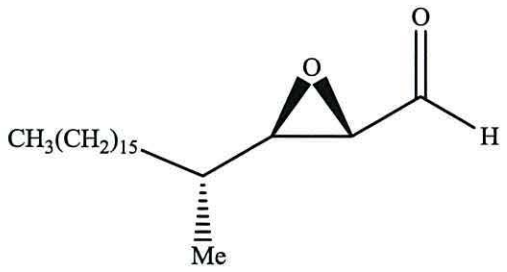
1.25), 0.92 (3H, d, *J* 6.95 Hz), 0.88 (3H, t, *J* 6.6 Hz); δ_C (125MHz, $CDCl_3$): 61.9, 60.6, 57.0, 35.3, 34.5, 31.9, 29.9, 29.7, 29.63, 29.6, 29.3, 26.8, 22.7, 15.8, 14.1; ν_{max}: 3431, 2922, 2852, 1384 cm^{-1}.

Experiment 8: [(2*S*,3*S*)-3-((*R*)-1-Methylheptadecyl)-oxiranyl]-methanol (141)



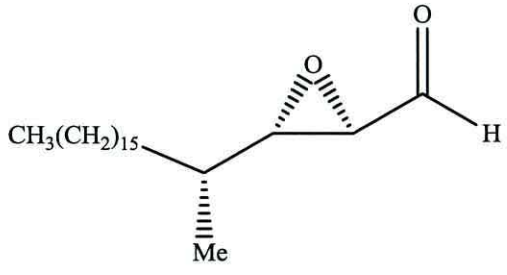
Titanium tetra isopropoxide in dry dichloromethane (3.4 M, 0.28 ml, 0.96 mmol) was added to a stirred solution of L-(+)-diethyl tartrate (0.23 ml, 1.113 mmol) in dry dichloromethane (70 ml) under nitrogen at - 20 °C in the presence of 4 A molecular sieves (0.5 g).[229] The mixture was stirred at - 20 °C for 0.5 hour then *tert*-butyl hydroperoxide in dry dichloromethane (3.3 M, 4.8 ml, 16 mmol) was added dropwise and the mixture was stirred for another 0.5 hour. To this solution, (*E*)-(*R*)-4-methyl-eicos-2-en-1-ol (**136**) (2.5 g, 8.06 mmol) in dry dichloromethane (10 ml), was added dropwise. After stirring at the same temperature for 4.5 hours, the reaction was left at - 20 °C overnight in the freezer, the quenched with water (10 ml) and allowed to reach room temperature. After the mixture had been stirred for 50 min, a solution of sodium hydroxide (30 %) in sat. aq. sodium chloride (6 ml) was added. After stirring the mixture for a further 0.5 hour, the phases were separated and the aqueous layer was extracted with dichloromethane (3 × 15 ml). The combined organic layer were dried and evaporated to give a thick oil, which was purified by column chromatography eluting with petrol/ether (5:2) to give a white solid [(2*S*,3*S*)-3-((*R*)-1-methylheptadecyl)-oxiranyl]-methanol (**141**) (1.99 g, 75 %), m.p.: 48-49 °C [Found $(M + H)^+$: 327.3258, $C_{21}H_{43}O_2$ requires: 327.3402], $[\alpha]_D^{22}$ = - 21.16 (*c* 1.56, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 3.88 (1H, ddd, *J* 2.2, 5.65, 12.6 Hz), 3.58-3.53 (1H, m), 2.94 (1H, br pent., *J* 2.5 Hz), 2.68 (1H, dd, *J* 2.55, 7.25 Hz), 2.59 (1H, br t, *J* 6.3 Hz), 1.38-1.19 (31H, br m, including br s at 1.23), 0.98 (3H, d, *J* 6.6 Hz), 0.85 (3H, t, *J* 6.6 Hz); δ_C (125MHz, $CDCl_3$): 61.8, 60.6, 58.5, 35.4, 33.6, 31.9, 29.8, 29.6, 29.59, 29.58, 29.5, 29.3, 27.1, 22.6, 17.1, 14.0 ; ν_{max}: 3430, 2918, 2849, 1463, 1384, 1071 cm^{-1}.

Experiment 9: (2*S*,3*R*)-3-((*R*)-1-Methylheptadecyl)-oxirane-2-carbaldehyde (142)



[(2*R*,3*R*)-3-((*R*)-1-Methyl-heptadecyl)-oxiranyl]-methanol (**140**) (1.23 g, 3.76 mmol) in dichloromethane (15 ml) was added to a stirred suspension of pyridinium chlorochromate (2 g, 7.53 mmol) in dichloromethane (100 ml) at room temperature.[230] The mixture was stirred vigorously and refluxed for 3 hours (without heating), when TLC showed no starting material was left. It was poured in ether (100 ml) and filtered through a pad of celite, then washed well with ether and the filtrate was evaporated to give a residue. Chromatography on silica gel eluting with petrol/ether (5:2) gave (2*S*,3*R*)-3-((*R*)-1-methyl-heptadecyl)-oxirane-2-carbaldehyde (**142**) (0.72 g, 60 %), m.p.: 40-42 °C, $\{[\alpha]_D^{28} = -63.49$ (*c* 1.06, $CHCl_3)\}$, which showed δ_H (500MHz, $CDCl_3$): 9.02 (1H, d, *J* 6.3 Hz), 3.14 (1H, dd, *J* 1.85, 6.3 Hz), 3.05 (1H, dd, *J* 1.85, 6.6 Hz), 1.55-1.48 (1H, m), 1.43-1.22 (30H, br m, including br s at 1.26), 0.95 (3H, d, *J* 6.9 Hz), 0.88 (3H, t, *J* 6.6 Hz); δ_C (125MHz, $CDCl_3$): 198.6, 61.3, 57.9, 35.3, 34.4, 31.9, 29.74, 29.68, 29.64, 29.6, 29.5, 29.3, 26.8, 22.7, 15.6, 14.1; ν_{max}: 2918, 2851, 1741, 1384 cm^{-1}, and recovered starting material (0.25 g).

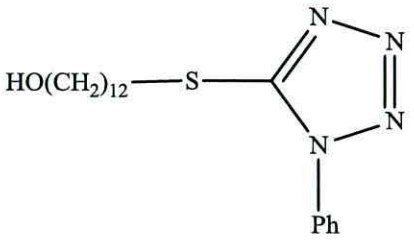
Experiment 10: (2*R*,3*S*)-3-((*R*)-1-Methylheptadecyl)-oxirane-2-carbaldehyde (143)



The procedure used in Experiment 9 was repeated in orderto oxidise the [(2*S*,3*S*)-3-((*R*)-1-methyl-heptadecyl)-oxiranyl]-methanol (**141**) (1.96 g, 6 mmol) using pyridinium chlorochromate (3.88 g, 18 mmol) in dichloromethane (100 ml). The crude product was purified by column chromatography eluting with petrol/ethyl acetate (6:2) to give a white solid, (2*R*,3*S*)-3-((*R*)-1-methyl-heptadecyl)-oxirane-2-carbaldehyde (**143**) (1.15 g,

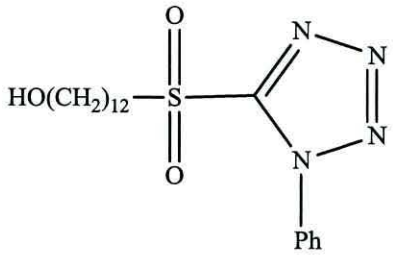
59 %), and recovered starting material (0.55 g). The aldehyde m.p.: 31-32 °C, $[\alpha]_D^{20} = +$ 53.49 (*c* 1.22, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 9.02 (1H, d, *J* 6.3 Hz), 3.19 (1H, dd, *J* 1.9, 6.3 Hz), 3.02 (1H, dd, *J* 1.9, 6.95 Hz), 1.51-1.46 (1H, br pent., *J* 6.6 Hz), 1.41-1.22 (30H, br m), 1.06 (3H, d, *J* 6.6 Hz), 0.88 (3H, t, *J* 6.65 Hz); δ_C (125MHz, $CDCl_3$): 198.5, 61.2, 59.0, 35.3, 33.3, 31.9, 29.7, 29.68, 29.64, 29.62, 29.58, 29.47, 29.35, 27.0, 22.7, 16.9, 14.1; ν_{max}: 2954, 2924, 2854, 1734, 1462, 1377 cm^{-1}.

Experiment 11: 12-(1-Phenyl-1*H*-tetrazole-5-ylsulfanyl)-dodecan-1-ol (146)



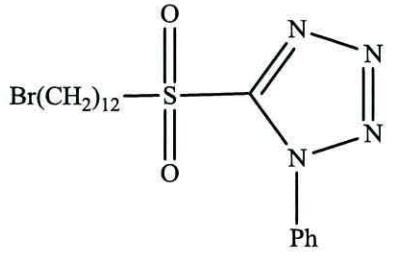
12-Bromododecane-1-ol (19 g, 69 mmol) was added with vigorous stirring to 1-phenyl-1*H*-tetrazole-5-thiol (12.3 g, 69 mmol) and anhydrous potassium carbonate (19 g, 138 mmol) in acetone (250 ml). The mixture was refluxed for 2.5 hours, when TLC showed no starting material was left. The inorganic salts were filtered off and washed with acetone, the solution was evaporated to a small bulk and the residue extracted between dichloromethane (200 ml) and water (300 ml). The aqueous layer was extracted with dichloromethane (2 × 80 ml). The combined organic phases were washed with water (300 ml), dried and evaporated to give a white solid. This was dissolved in acetone (50 ml), diluted with methanol (100 ml) and left at ambient for 1 hour and then at 0 °C for 30 min. The crystals were filtered to yield a white solid (**146**) (19.3 g, 77 %), m.p.: 62-64 °C, which showed δ_H (500MHz, $CDCl_3$): 7.58-7.5 (5H, br m), 3.62 (2H, t, *J* 6.6 Hz), 3.38 (2H, t, *J* 7.6 Hz), 1.8 (2H, pent., *J* 7.6 Hz), 1.6 (1H, s), 1.55 (2H, pent., *J* 6.9 Hz), 1.42 (2H, pent, *J* 6.6 Hz), 1.31-1.21 (14H, br m); δ_C (125 MHZ, $CDCl_3$): 154.5, 133.7, 130.0, 129.7, 123.8, 62.9, 33.3, 32.7, 29.5, 29.4, 29.3, 29.0, 28.9, 28.6, 25.7; ν_{max}: 3435, 2926, 2854, 1597, 1499, 1463, 1387, 1278, 1242, 1073, 1054, 1015, 760 cm^{-1}.

Experiment 12: 12-(1-Phenyl-1*H*-tetrazole-5-sulfonyl)-dodecan-1-ol (147)



A solution of ammonium molybdate (VI) tetrahydrate (30 g, 23.9 mmol) in ice cold H_2O_2 (27 % w/w, 70 ml) was added to a stirred solution of 12-(1-phenyl-1*H*-tetrazole-5-ylsulfanyl)-dodecan-1-ol (**146**) (19.3 g, 53 mmol) in IMS (500 ml) at 12 °C and stirred at room temperature for 2 hours. A further solution of ammonium molybdate (VI) tetrahydrate (12 g, 9.7 mmol) in ice cold H_2O_2 (27 % w/w, 30 ml) was added and the mixture was stirred at room temperature for 18 hours, then poured into 2.5 L of water and extracted with dichloromethane (3 × 400 ml). The combined organic phases were washed with water (2 × 300 ml), dried and evaporated. The residue was dissolved in methanol (200 ml) and left for 1 hour. A white solid crystallized, 12-(phenyl-1*H*-tetrazole-5-sulfonyl)-dodecan-1-ol (**147**) (20.3 g, 95 %), m.p.: 56-58 °C [Found $(M + Na)^+$: 417.1931, $C_{19}H_{30}N_4NaO_3S$ requires: 417.1826], which showed δ_H (500MHz, $CDCl_3$): 7.69-7.66 (2H, m), 7.62-7.56 (3H, m), 3.71 (2H, t, *J* 8.15 Hz), 3.6 (2H, t, *J* 6.6 Hz), 1.96-1.89 (2H, m), 1.62 (1H, s), 1.54 (2H, pent., *J* 6.6 Hz), 1.47 (2H, pent., *J* 6.95 Hz), 1.34-1.17 (14H, m); δ_C (125MHz, $CDCl_3$): 153.4, 132.9, 131.3, 129.6, 125.0, 62.8, 55.9, 32.7, 29.4, 29.33, 29.28, 29.0, 28.8, 28.0, 25.6, 21.8; ν_{max}: 3420, 2926, 2854, 1595, 1497, 1463, 1341, 1152, 1047, 1015 cm^{-1}.

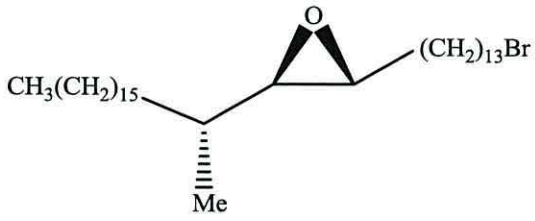
Experiment 13: 5-(12-Bromododecane-1-sulfonyl)-1-phenyl-1*H*-tetrazole (148)



N-Bromosuccinimide (5.86 g, 32.9 mmol) was added in portions over 20 min to a stirred solution of 12-(1-phenyl-1*H*-tetrazole-5-sulfonyl)-dodecan-1-ol (**147**) (10 g, 25.3 mmol) and triphenylphosphine (8.3 g, 31.6 mmol) in dichloromethane (200 ml) at 0 °C. The mixture was stirred at room temperature for 2 hours, when TLC showed no starting

material, then quenched with sat. aq. sodium metabisulphite (60 ml). The aqueous layer was re-extracted with dichloromethane (2 × 100 ml). The combined organic layers were washed with water, dried over $MgSO_4$ and evaporated to give a residue. This was treated with ether (200 ml), refluxed for 30 min and the triphenylphosphonium oxide filtered off and washed with ether. The filtrate was evaporated and the residue purified by chromatography on silica gel eluting with petrol/ethyl acetate (5:1) to give 5-(12-bromo-dodecane-1-sulfonyl)-1-phenyl-1*H*-tetrazole (**148**) (8.5 g, 72 %), m.p.: 63-65 °C [Found $(M + Na)^+$: 479.1087, $C_{19}H_{29}BrN_4NaO_2S$ requires: 479.0899], which showed δ_H (500MHz, $CDCl_3$): 7.7-7.68 (2H, m), 7.64-7.57 (3H, m), 3.72 (2H, t, *J* 7.9 Hz), 3.4(2H, t, *J* 6.95 Hz), 1.98-1.91 (2H, m), 1.85 (2H, pent., *J* 6.95Hz), 1.49 (2H, pent., *J* 6.9 Hz), 1.42 (2H, pent., *J* 6.95 Hz), 1.35-1.24 (12H, br m, including br s at 1.27); δ_C (125MHz, $CDCl_3$): 153.4, 133.0, 131.4, 129.6, 125.0, 55.9, 34.0, 32.7, 29.3, 29.29, 29.1, 28.8, 28.7, 28.1, 28.0, 21.9; ν_{max}: 2925, 2854, 1595, 1497, 1462, 1342,1152, 1046 cm^{-1}.

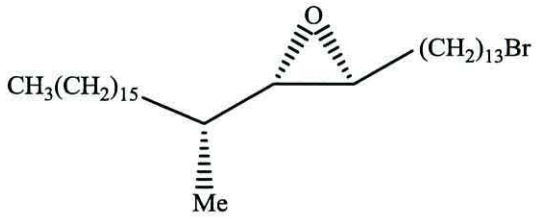
Experiment 14: (2*R*,3*R*)-2-(13-Bromotridecyl)-3-(*R*)-1-methylheptadecyl)-oxirane (151)



Lithium bis(trimethylsilyl)amide (3.6 ml, 3.84 mmol,1.06 M) was added dropwise with stirring to (2*S*,3*R*)-3-((*R*)-1-methyl-heptadecyl)-oxirane-2-carbaldehyde (**142**) (0.64 g, 1.97 mmol) and 5-(12-bromo-dodecane-1-sulfonyl)-1-phenyl-1*H*-tetrazole (1.2 g, 2.56 mmol) in dry tetrahydrofuran (42 ml) under nitrogen at - 10 °C. The mixture was allowed to reach room temperature and stirred for 1 hour, when TLC showed no starting material, then quenched with water (7 ml) and petrol/ether (1:1, 10 ml). The aqueous layer was re-extracted with petrol/ether (1:1, 2 × 20 ml). The combined organic layers were washed sat. aq. sodium chloride (2 × 20 ml), dried and evaporated to give a thick oil. Chromatography on silica gel eluting with petrol/ether (20:1) gave a white solid, (*E/Z*) (2*R*,3*R*)-2-(13-bromotridec-1-enyl)-3-((*R*)-1-methylheptadecyl)-oxirane (**149**) (0.87 g, 79 %) as a mixture of isomers in ratio 1.6:1. Dipotassium azodicarboxylate (2 g, 10.3 mmol) was added to a stirred solution of alkene (0.69 g, 1.24 mmol) in THF (10 ml) and methanol (5 ml) at 0-5 °C under nitrogen. A solution of glacial acetic acid (2 ml) in THF (4 ml) was added dropwise in small portion and the mixture was stirred

overnight at r.t. Dipotassium azodicarboxylate (1.5 g, 7.7 mmol) and glacial acetic acid (2 ml) were added and stirred for 48 hours. This mixture was slowly added to sat. aq. $NaHCO_3$ (20 ml)and extracted with petrol/ether (1:1, 3 × 30 ml) and the combined organic layers were washed with water (25 ml) and the solvent was evaporated. The procedure was repeated. The crude product was purified by column chromatography eluting with petrol/ether (10:0.5) to give a white solid, (2*R*,3*R*)-2-(13-bromotridecyl)-3-(*R*)-1-methylheptadecyl)-oxirane (**151**) (0.64 g, 93 %), m.p.: 42-44 °C [Found $(M + Na)^+$: 579.4006, $C_{33}H_{65}NaBrO$ requires: 579.4111], $[\alpha]_D^{26}$ = + 10.4 (*c* 1.1, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 3.41 (2H, t, *J* 6.95 Hz), 2.67 (1H, dt, *J* 2.25, 5.7 Hz), 2.46 (1H, dd, *J* 2.2, 7.25 Hz), 1.86 (2H, pent., *J* 6.9 Hz), 1.49-1.26 (53H, br m, including br s at 1.26), 0.92 (3H, d, *J* 6.6 Hz), 0.88 (3H, t, *J* 6.65 Hz); δ_C (125MHz, $CDCl_3$): 63.8, 57.5, 35.8, 34.6, 34.0, 32.8, 32.2, 31.9, 29.9, 29.69, 29.65, 29.61, 29.58, 29.55, 29.53, 29.43, 29.35, 28.8, 28.2, 26.9, 26.1, 22.7, 16.0, 14.1; ν_{max}: 2923, 2853, 1462, 1377, 1116 cm^{-1}.

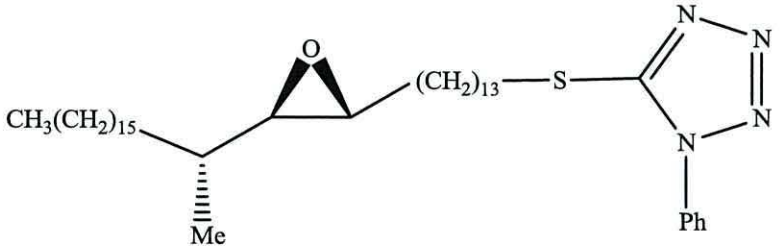
Experiment 15: (2*S*,3*S*)-2-(13-Bromotridecyl)-3-((*R*)-1-methylheptadecyl)-oxirane (152)



The procedure used in Experiment 14 was repeated in order to couple the (2*R*,3*S*)-3-((*R*)-1-methylheptadecyl)-oxirane-2-carbaldehyde (**143**) (0.95 g, 2.9 mmol) with 5-(12-bromododecane-1-sulfonyl)-1-phenyl-1*H*-tetrazole (1.74 g, 3.8 mmol) using lithium bis(trimethylsilyl)amide (5.4 ml, 5.77 mmol, 1.06 M) in dry THF under nitrogen at -10 °C. The crude product was purified by column chromatography on silica gel eluting with petrol/ethyl acetate (20:1) to give a white solid, (*E/Z*)-(2*S*,3*S*)-2-(13-bromo-tridec-1-enyl)-3-((*R*)-1-methyl heptadecyl)-oxirane (**150**) (1.16 g, 71 %), as a mixture of two isomers in ratio 1.6:1. Hydrogenation was carried out with dipotassium azodicarboxylate as before and the crude product was purified via column chromatography eluting with petrol/ether (20:1) to give a white solid, (2*S*,3*S*)-2-(13-bromotridecyl)-3-(*R*)-1-methyl heptadecyl)-oxirane (**152**) (0.64 g, 93 %), m.p.: 42-44 °C [Found $(M + Na)^+$: 579.3633, $C_{33}H_{65}NaBrO$ requires: 579.4111], $[\alpha]_D^{26}$ = - 13.13 (*c*

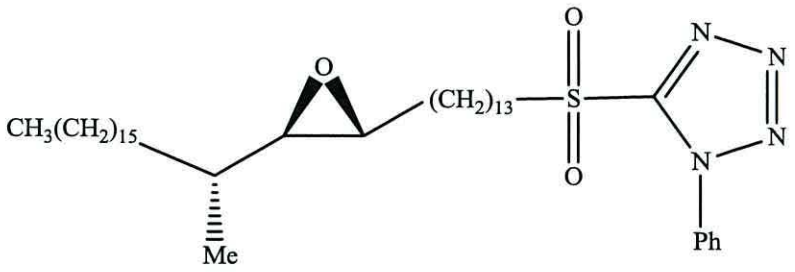
1.2, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 3.41 (2H, t, *J* 6.95 Hz), 2.71 (1H, dt, *J* 2.2, 5.35 Hz), 2.41 (1H, dd, *J* 1.9, 6.95 Hz), 1.85 (2H, pent., *J* 6.9 Hz), 1.54-1.22 (53H, br m, including br s at 1.26), 1.0 (3H, d, *J* 5.95 Hz), 0.88 (3H, t, *J* 6.6 Hz); δ_C (125MHz, $CDCl_3$): 63.83, 58.84, 36.0, 34.0, 33.77, 32.84, 32.3, 31.9, 29.9, 29.66, 29.63, 29.6, 29.56, 29.54, 29.53, 29.49, 29.43, 29.36, 28.77, 28.2, 27.2, 26.1, 22.7, 22.6, 17.3, 14.1; ν_{max}: 2919, 2850, 1462, 1377, 1116 cm^{-1}.

Experiment 16: 5-(13-[(2*R*,3*R*)-3-((*R*)-1-Methylheptadecyl)-oxiranyl]-tridecylsulfanyl)-1-phenyl- 1*H*-tetrazole (153)



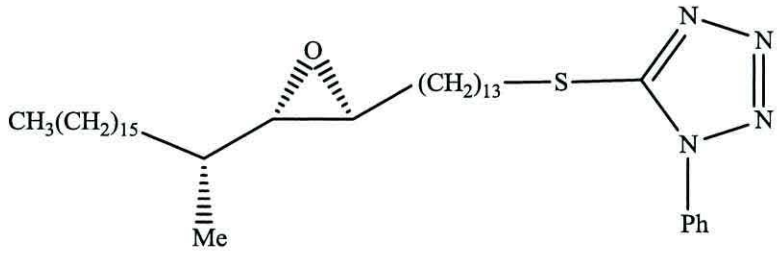
(2*R*,3*R*)-2-(13-Bromotridecyl)-3-((*R*)-1-methylheptadecyl)-oxirane (**151**) (0.53 g,0.95 mmol) was added with vigorous stirring to 1-phenyl-1*H*-tetrazole-5-thiol (0.16 g, 0.95 mmol) and anhydrous potassium carbonate (0.26 g, 1.9 mmol) in acetone (25 ml). The mixture was refluxed for 2.5 hours when TLC showed no starting material was left. The inorganic salts were filtered off and washed with acetone then the solution was evaporated to a small bulk and the residue extracted between dichloromethane (25 ml) and water (30 ml). The aqueous layer was extracted with dichloromethane (2 × 15 ml). The combined organic phases were washed with water (25 ml), dried over $MgSO_4$ and evaporated to give a solid, which was purified by chromatography on silica gel eluting with petrol/ethyl acetate (10:1) to give a white solid, 5-(13-[(2*R*,3*R*)-3-((*R*)-1-methylheptadecyl)-oxiranyl]-tridecylsulfanyl)-1-phenyl-1*H*-tetrazole (**153**) (0.52 g, 84 %), m.p.: 40-42 °C [Found $(M + H)^+$: 655.5342, $C_{40}H_{71}N_4OS$ requires: 655.5343], $[\alpha]_D^{20} = +6.85$ (*c* 1.07, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 7.6-7.51 (5H, m), 3.39 (2H, t, *J* 7.6 Hz), 2.66 (1H, dt, *J* 2.2, 5.65 Hz), 2.45 (1H, dd, *J* 2.25, 7.25 Hz), 1.81 (2H, pent., *J* 7.25 Hz), 1.57-1.22 (53H, br m, including br s at 1.25), 0.91 (3H, d, *J* 6.6 Hz), 0.88 (3H, t, *J* 6.6 Hz); δ_C (125MHz, $CDCl_3$): 154.5, 133.8, 130, 129.7, 123.8, 63.8, 57.4, 35.8, 34.6, 33.3, 32.2, 31.9, 29.9, 29.7, 29.64, 29.6, 29.58, 29.55, 29.52, 29.5, 29.4, 29.3, 29.1, 29.0, 28.6, 26.9, 26.1, 22.7, 15.9, 14.1; ν_{max}: 2922, 2854, 1501, 1462, 1377 cm^{-1}.

Experiment 17: 5-(13-[(2*R*,3*R*)-3-((*R*)-1-Methylheptadecyl)-oxiranyl]-tridecane-1-sulfonyl)-1-phenyl-1*H*-tetrazole (155)



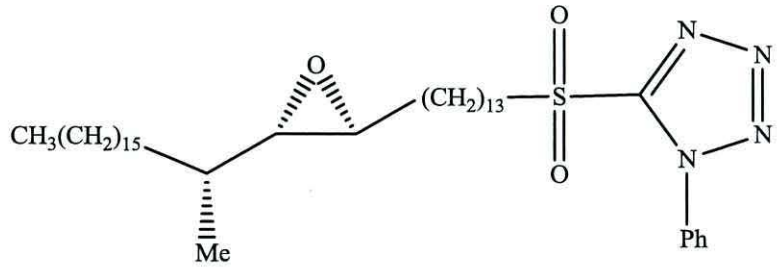
A solution of ammonium molybdate (VI) tetrahydrate (0.4 g, 0.322 mmol) in ice cold H_2O_2 (35 % w/w, 1 ml) was added to a stirred solution of 5-(13-[(2*R*,3*R*)-3-((*R*)-1-methyl-heptadecyl)-oxiranyl]-tridecylsulfanyl)-1-phenyl-1*H*-tetrazole (**153**) (0.47 g, 0.717 mmol) in THF (10 ml) and IMS (15 ml) at 12 °C and stirred at r.t. for 2 hours. A further solution of ammonium molybdate (VI) tetrahydrate (0.4 g, 0.322 mmol) in ice cold H_2O_2 (35 %, w/w, 1 ml) was added and the mixture was stirred at r.t. 18 hours, then poured into water (50 ml) and extracted with dichloromethane (3 × 25 ml). The combined organic phases were washed with water (2 × 15 ml), dried and evaporated to give a solid, which was purified by chromatography on silica gel eluting with petrol/ethyl acetate (10:1) to give a white solid, 5-(13-[(2*R*,3*R*)-3-((*R*)-1-methylheptadecyl)-oxiranyl]-tridecane-1-sulfonyl)-1-phenyl-1*H*-tetrazole (**155**) (0.34 g, 69 %), m.p.: 41-42 °C [Found $(M)^+$: 687.524, $C_{40}H_{70}N_4O_3S$ requires: 687.5241], $[\alpha]_D^{20}$ = + 6.25 (*c* 1.3, $CHCl_3$); δ_H (500MHz, $CDCl_3$): 7.7-7.68 (2H, m), 7.64-7.57 (3H, m), 3.72 (2H, t, *J* 7.9 Hz), 2.66 (1H, dt, *J* 1.9, 5.65 Hz), 2.45 (1H, dd, *J* 2.25,7.25 Hz), 1.98-1.91 (2H, m), 1.58-1.25 (53H, br m, including br s at 1.25), 0.91 (3H, d, *J* 6.6 Hz), 0.87 (3H, t, *J* 6.6Hz); δ_C (125MHz, $CDCl_3$): 153.5, 133.0, 131.4, 129.6, 125.0, 63.74, 57.41, 56.0, 35.8, 34.6, 32.2, 31.9, 29.9, 29.7, 29.6, 29.57, 29.55, 29.52, 29.5, 29.47, 29.4, 29.3, 29.1, 28.8, 28.1, 26.8, 26.1, 22.6, 21.9, 15.9, 14.1; ν_{max}: 2921, 2853, 1500, 1463, 1377, 1344, 1153, 1049 cm^{-1}.

Experiment 18: 5-(13-[(2*S*,3*S*)-3-((*R*)-1-Methylheptadecyl)-oxiranyl]-tridecylsulfanyl)-1-phenyl-1*H*-tetrazole (154)



The procedure used in Experiment 16 was repeated using (2*S*,3*S*)-2-(13-bromotridecyl)-3-((*R*)-1-methylheptadecyl)-oxirane (**152**) (0.8 g, 1.43 mmol), 1-phenyl-1*H*-tetrazole-5-thiol (0.25 g, 1.43 mmol), and anhydrous potassium carbonate (0.39 g, 2.86 mmol) in acetone (50 ml). The crude product was purified by column chromatography on silica gel eluting with petrol/ethyl acetate (10:1) to give a white solid, 5-(13-[(2*S*,3*S*)-3-((*R*)-1-methylheptadecyl)-oxiranyl]-tridecylsulfanyl)-1-phenyl-1*H*-tetrazole (**154**) (0.77 g, 82 %), m.p.: 41–43 °C [Found $(M + H)^+$: 655.5341, $C_{40}H_{71}N_4OS$ requires: 655.5343], $[\alpha]_D^{20}$ = - 12.47 (*c* 1.09, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 7.60-7.52 (5H, m), 3.40 (2H, t, *J* 7.55 Hz), 2.71 (1H, dt, *J* 2.2, 5.65 Hz), 2.41 (1H, dd, *J* 2.2, 7.25 Hz), 1.82 (2H, br pent., *J* 7.55 Hz), 1.54-1.26 (53H, br m, including br s at 1.26), 1.0 (3H, d, *J* 6.3 Hz), 0.88 (3H, t, *J* 6.65 Hz); δ_C (125MHz, $CDCl_3$): 154.5, 133.8, 130.03, 129.74, 123.85, 63.83, 58.84, 36.03, 33.8, 33.4, 32.3, 31.9, 29.9, 29.69, 29.66, 29.64, 29.63, 29.59, 29.56, 29.53, 29.49, 29.4, 29.3, 29.1, 29.0, 28.6, 27.2, 26.1, 22.7, 17.3, 14.1; ν_{max}: 2954, 2924, 2854, 1501, 1462, 1377 cm^{-1}.

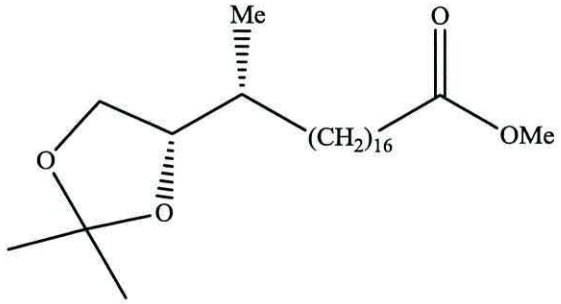
Experiment 19: 5-(13-[(2*S*,3*S*)-3-((*R*)-1-Methylheptadecyl)-oxiranyl]-tridecane-1-sulfonyl)-1-phenyl-1*H*-tetrazole (156)



The procedure used in Experiment 17 was repeated using the 5-(13-[(2*S*,3*S*)-3-((*R*)-1-methylheptadecyl)-oxiranyl]-tridecylsulfanyl)-1-phenyl-1*H*-tetrazole (**154**) (0.78 g, 1.19 mmol), ammonium molybdate (VI) tetrahydrate (0.6 g, 0.53 mmol) in H_2O_2 (35 %, w/w,

1.5 ml), THF (15 ml) and IMS (25 ml) and further ammonium molybdate (VI) tetrahydrate (0.6 g, 0.53mmol) in H_2O_2 (35 %, w/w, 1.5 ml). The crude product was purified by column chromatography eluting with petrol/ethyl acetate (5:1) to give a white solid, 5-(13-[(2*S*,3*S*)-3-((*R*)-1-methylheptadecyl)-oxiranyl]-tridecane-1-sulfonyl)-1-phenyl-1*H*-tetrazole (**156**) (0.7 g, 67 %), m.p.: 45-47 °C [Found $(M + Na)^+$: 709.5018, $C_{40}H_{70}N_4NaO_3S$ requires: 709.5061], $[\alpha]_D^{20}$ = - 8.45 (*c* 0.97, $CHCl_3$); δ_H (500MHz, $CDCl_3$): 7.72-7.69 (2H, m), 7.66-7.59 (3H, m), 3.73 (2H, t, *J* 7.9 Hz), 2.72 (1H, dt, *J* 2.55, 5.7 Hz), 2.41 (1H, dd, *J* 2.2, 6.95 Hz), 1.99-1.93 (2H, m), 1.56-1.26 (53H, br m, including br s at 1.26), 1.0 (3H, d, *J* 6.3 Hz), 0.88 (3H, t, *J* 6.6 Hz); δ_C (500MHz, $CDCl_3$): 153.5, 133.1, 131.4, 129.7, 125.0, 63.8, 58.8, 56.0, 36.0, 33.76, 32.3, 31.9, 29.9, 29.7, 29.64, 29.63, 29.57, 29.55, 29.53, 29.49, 29.44, 29.34, 29.2, 28.9, 28.1, 27.2, 26.1, 22.7, 21.9, 17.3, 14.1; ν_{max}: 2918, 2851, 1498, 1464, 1344, 1153 cm^{-1}.

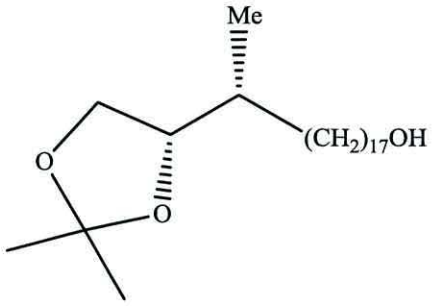
Experiment 20: (*R*)-18-((*S*)-2,2-Dimethyl-[1,3]dioxolan-4-yl)-nonadecanoic acid methyl ester (169)



Lithium bis(trimethylsilyl)amide (27.8 ml, 29.47 mmol.1.06 M) was added dropwise with stirring to (*R*)-3-((S)-2,2-dimethyl-[1,3]dioxolan-4-yl)-butyraldehyde (**115**) (3 g, 17.44 mmol) and 15-(1-phenyl-1*H*-tetrazole-5-sulfonyl)-pentadecanoic acid methyl ester (167) (10.5, 22.67 mmol) in dry THF (150 ml) under nitrogen at - 10 °C. The mixture was allowed to reach r.t. and stirred for 1 hour, when TLC showed no starting material, then quenched with water (80 ml) and petrol/ethyl acetate (1:1, 80 ml). The aqueous layer was re-extracted with petrol/ethyl acetate (1:1, 2 × 50 ml). The combined organic layers were washed with sat. aq. sodium chloride (2 × 80 ml), dried and evaporated to give a thick oil. Chromatography on silica gel eluting with petrol/ethyl acetate (20:1) gave a thick oil, (*E,Z*)-(*R*)-18-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-nonadec-15-enoic acid methyl ester (**168**) (4.4 g, 61 %) as a mixture of two isomers in ratio 2:1. Palladium 10 % on carbon (1 g) was added to a stirred solution of the alkene (4.4 g, 10.71 mmol) in ethanol (100 ml) and THF (20 ml). Hydrogenation under an

atmosphere of hydrogen was carried out for 2 hours. The solution was filtered on a bed of celite and the solvent was evaporated to give a white solid, (*R*)-18-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-nonadecanoic acid methyl ester (**169**) (4.15, 93 %), m.p.: 36-38 °C [Found $(M + Na)^+$: 435.338, $C_{25}H_{48}NaO_4$ requires: 435.3445], $[\alpha]_D^{20} = +11.33$ (*c* 1.5, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 4.0 (1H, br q, *J* 6.3 Hz), 3.86 (1H, br q, *J* 7.25 Hz), 3.66 (3H, s), 3.59 (1H, br t, *J* 7.55 Hz), 2.30 (2H, t, *J* 7.55 Hz), 1.61 (1H, pent., *J* 7.25 Hz), 1.57-1.53 (2H, m), 1.40 (3H, s), 1.35 (3H, s), 1.28-1.21 (27H, br s), 1.10-1.06 (1H, m), 0.96 (3H, d, *J* 6.6 Hz); δ_C (125MHz, $CDCl_3$): 174.3, 108.5, 80.4, 67.8, 51.4, 36.5, 34.1, 32.7, 29.8, 29.64, 29.62, 29.59, 29.57, 29.4, 29.2, 29.1, 27.0, 26.6, 25.5, 24.9, 15.6; ν_{max}: 2922, 2853, 1736, 1463, 1377, 1213, 1169, 1051 cm^{-1}.

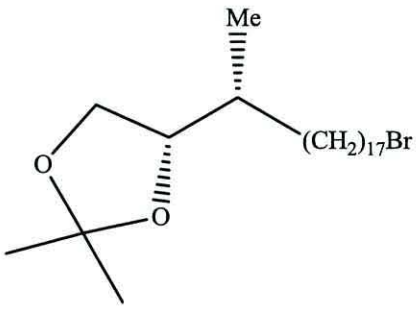
Experiment 21: (*R*)-18-((*S*)-2,2-Dimethyl-[1,3]dioxolan-4-yl)-nonadecan-1-ol (170)



(*R*)-18-((*S*)-2,2-Dimethyl-[1,3]dioxolan-4-yl)-nonadecanoic acid methyl ester (**169**) (4 g, 9.69 mmol) in THF (30 ml) was added dropwise over 15 min to a suspension of lithium aluminium hydride (0.56 g, 14.54 mmol) in THF (100 ml) at r.t. The mixture was refluxed for 1 hour, when TLC showed that no starting material was left, then cooled to r.t. and quenched carefully with freshly prepared sat. aq. sodium sulfate decahydrate (10 ml) until a white precipitate was formed, followed by the addition of magnesium sulphate (10 g). The mixture was stirred vigorously for 10 min and then filtered through a pad of celite and washed well with THF (100 ml). The combined organic layers were evaporated to give a residue, which was purified by chromatography on silica gel eluting with petrol/ethyl acetate (2:1) to give a white solid, (*R*)-18-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-nonadecan-1-ol (3.4 g, 91 %), m.p.: 56-57 °C [Found $(M + H)^+$: 385.3678, $C_{24}H_{49}O_3$ requires : 385.3678], $[\alpha]_D^{18} = +17.57$ (*c* 1.1, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 3.99 (1H, dd, *J* 3.6, 7.85 Hz), 3.86 (1H, br q, *J* 6.9 Hz), 3.63 (2H, t, *J* 6.6 Hz), 3.59 (1H, t, *J* 7.85 Hz), 2.1 (1H, s), 1.59-1.51 (4H, m), 1.39 (3H, s), 1.34 (3H, s), 1.32-1.21 (28H, br m, including br s at 1.25), 1.11-1.03

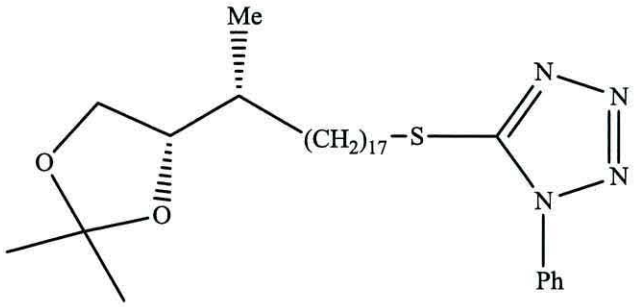
(1H, m), 0.95 (3H, d, *J* 6.9 Hz); δ_C (125MHz, $CDCl_3$): 108.5, 80.4, 67.8, 63.0, 36.5, 32.78, 32.68, 29.8, 29.64, 29.63, 29.61, 29.58, 29.4, 27.0, 26.6, 25.7, 25.5, 15.6; ν_{max}: 3448, 2961, 2923, 2852, 1465, 1377, 1155, 1056 cm^{-1}.

Experiment 22: (*S*)-4-((*R*)-18-Bromo-1-methyloctadecyl)-2,2-dimethyl-[1,3]dioxolane (171)



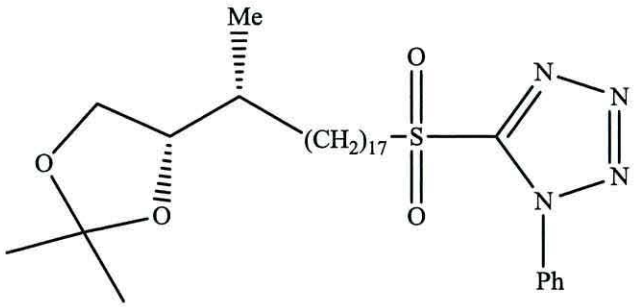
N-Bromosuccinimide (2.04 g, 11.51 mmol) was added in portions over 15 mins to a stirred solution of (*R*)-18-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-nonadecan-1-ol (**170**) (3.4 g, 8.85 mmol), triphenyl phosphine (2.89 g, 11.04 mmol) and sodium hydrogen carbonate (0.2 g) in dichloromethane (70 ml) at 0 °C. The mixture was stirred at r.t. for 1 hour, when TLC indicated completion of the reaction, then quenched with sat. aq. sodium meta-bisulfite (50 ml). The aqueous layer was re-extracted with dichloromethane (2 x 50 ml) and the combined organic extracts washed with water (50 ml), dried and evaporated to give a residue. This was refluxed for 30 mins with ether (150 ml) and then filtered and washed with petrol/ethyl acetate (5:1, 50 ml). The filtrate was evaporated and the resultant residue purified via column chromatography eluting with petrol/ethyl acetate (5:1) to give a colourless oil, (*S*)-4-((*R*)-18-bromo-1-methyloctadecyl)-2,2-dimethyl-[1,3]dioxolane (3.8 g, 96 %) [Found $(M)^+$: 447.2831, $C_{24}H_{47}BrO_2$ requires: 447.2832], $[\alpha]_D^{18}$ = + 16.45 (*c* 1.1, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 3.98 (1H, dd, *J* 5.95, 7.55 Hz), 3.85 (1H, q, *J* 7.25 Hz), 3.58 (1H, t, *J* 7.6 Hz), 3.38 (2H, t, *J* 6.9 Hz), 1.84 (2H, pent., *J* 6.95 Hz), 1.59-1.51 (1H, m), 1.44-1.40 (2H, m), 1.38 (3H, s), 1.33 (3H, s), 1.31-1.2 (27H, br m, including br s at 1.25), 1.0-1.03 (1H, m), 0.95 (3H, d, *J* 6.6 Hz); δ_C (125MHz, $CDCl_3$): 108.4, 80.4, 67.8, 36.5, 33.7, 32.84, 32.77, 29.83, 29.63, 29.57, 29.5, 29.4, 28.74, 28.2, 27.0, 26.6, 25.5, 15.6; ν_{max}: 2985, 2921, 2851, 1470, 1377, 1369, 1250, 1216, 1160, 1056 cm^{-1}.

Experiments 23: 5-[(*R*)-18-((*S*)-2,2-Dimethyl-[1,3]dioxolan-4-yl)nonadecyl sulfanyl]-1-phenyl-1*H*-tetrazole (172)



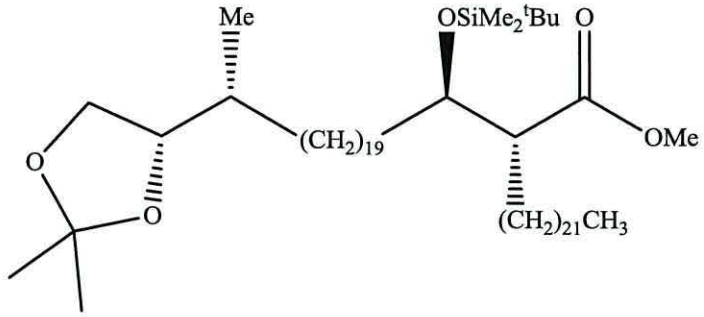
(*S*)-4-((*R*)-18-Bromo-1-methyloctadecyl)-2,2-dimethyl-[1,3]dioxolane (**171**) (3.8 g, 8.49 mmol) was added with vigorous stirring to 1-phenyl-1*H*-tetrazole-5-thiol (1.51 g, 8.49 mmol) and anhydrous potassium carbonate (2.34 g, 16.98 mmol) in acetone (100 ml). The mixture was refluxed for 2.5 hours, when TLC showed no starting material was left. The inorganic salts were filtered off and washed with acetone, the solution was evaporated to a small bulk and the residue extracted between CH_2Cl_2 (100 ml) and water (120 ml), dried and evaporated to give residue, which was purified by chromatography on silica gel eluting with petrol/ethyl acetate (8:2) to give a yellow oil, 5-[(*R*)-18-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)nonadecylsulfanyl]-1-phenyl-1*H*tetrazole (4.2 g, 90 %) [Found $(M + H)^+$: 545.3860, $C_{31}H_{53}N_4O_2S$ requires: 545.3884], $[\alpha]_D^{20} = +12.55$ (*c* 1.46, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 7.58-7.49 (5H, m), 3.98 (1H, dd, *J* 6.3, 7.45 Hz), 3.85 (1H, q, *J* 6.95 Hz), 3.58 (1H, t, *J* 7.55 Hz), 3.37 (2H, t, *J* 7.25 Hz), 1.80 (2H, pent., *J* 7.25 Hz), 1.58-1.50 (1H, m), 1.45-1.41 (2H, m), 1.38 (3H, s), 1.30 (3H, s), 1.29-1.2 (27H, br m, including br s at 1.24), 1.11-1.02 (1H, m), 0.94 (3H, d, *J* 6.6 Hz); δ_C (125MHz, $CDCl_3$): 154.4, 133.8, 129.94, 129.7, 123.8, 108.4, 80.33, 67.74, 36.43, 33.4, 32.72, 29.8, 29.59, 29.55, 29.53, 29.46, 29.4, 29.1, 28.95, 28.6, 26.9, 26.6, 25.5, 15.5; ν_{max}: 2983, 2924, 2853, 1598, 1500, 1464, 1379, 1245, 1064 cm^{-1}.

Experiment 24: 5-[(*R*)-18-((*S*)-2,2-Dimethyl-[1,3]dioxolan-4-yl)nonadecanesulfonyl]-1-phenyl-1*H*-tetrazole (159)



A solution of ammonium molybdate (IV) tetrahydrate (4 g, 3.30 mmol) in ice cold H_2O_2 (35% w/w,10ml) was added to a stirred solution of 5-[(*R*)-18-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)nonadecylesulfanyl]-1-phenyl-1*H*-tetrazole (**172**) (4 g, 7.34 mmol) in IMS (100 ml) and THF (10 ml) at 12 °C and stirred at r.t. for 2 hours. A further solution of ammonium molybdate (IV) tetrahydrate (4 g, 3.30 mmol) in ice cold H_2O_2 (35 % w/w, 10 ml) was added and the mixture was stirred at r.t. for 18 hours, then poured into water (120 ml) and extracted with CH_2Cl_2 (3 × 100 ml). The combined organic phases were washed with water (2 × 100 ml), dried and evaporated to give a thick oil, which was purified by chromatography on silica gel eluting with petrol/ethyl acetate (8:2) to give a white solid, 5-[(*R*)-18-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)nonadecanesulfonyl]-phenyl-1*H*-tetrazole (3.9 g, 92 %), m.p.: 52-54 °C [Found $(M + Na)^+$: 599.3032, $C_{31}H_{52}N_4NaO_4S$ requires: 599.3601], $[\alpha]_D^{20} = +12.45$ (*c* 1.1, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 7.70-7.68 (2H, m), 7.64-7.58 (3H, m), 4.0 (1H, dd, *J* 6.3, 7.9 Hz), 3.87 (1H, q, *J* 6.95 Hz), 3.73 (2H, t, *J* 7.85 Hz), 3.60 (1H, t, *J* 7.85 Hz), 1.98-1.92 (2H, m), 1.58-1.54 (1H, m), 1.52-1.47 (2H, m), 1.40 (3H, s), 1.35 (3H, s), 1.33-1.22 (27H, br m, including br s at 1.26), 1.11-1.07 (1H, m), 0.96 (3H, d, *J* 6.65 Hz); δ_C (125MHz, $CDCl_3$): 153.6, 133.2, 131.4, 129.7, 125.1, 108.5, 80.4, 67.8, 56.1, 36.5, 32.8, 29.9, 29.64, 29.61, 29.6, 29.5, 29.4, 29.2, 28.9, 28.1, 27.0, 26.6, 25.5, 22.0, 15.6; ν_{max}: 2921, 2853, 1460, 1377, 1149, 1066 cm^{-1}.

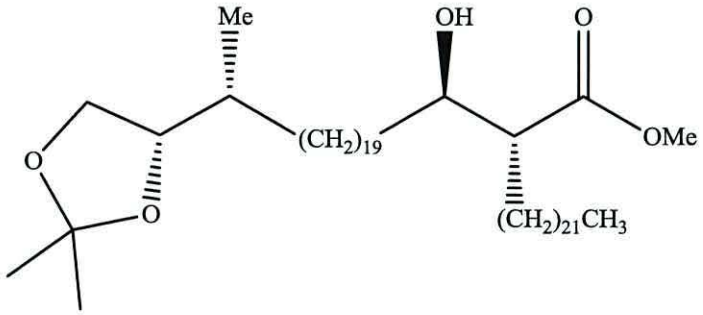
Experiment 25: (2*R*,3*R*,23*R*)-3-(*tert*-Butyldimethylsilanyloxy)-23-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-2-docosyltetracosanoic acid methyl ester (182)



Lithium bis(trimethylsilyl)amide (9 ml, 9.6 mmol, 1.06 M) was added dropwise with stirring to (*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilanyloxy)-3-oxopropyl]-tetracosanoic acid methyl ester (**160**) (3.5 g, 6.15 mmol) and 5-[(*R*)-18-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)nonadecanesulfonyl]-1-phenyl-1*H*-tetrazole (**159**) (4.26 g, 7.38 mmol) in dry THF (50 ml) under nitrogen at -10 °C. The mixture was allowed to reach r.t. and stirred for 2 hours, then quenched with water (50 ml) and petrol/ethyl acetate (1:1, 50 ml). The aqueous layer was re-extracted with petrol/ethyl acetate (1:1, 2 × 50 ml). The combined organic layers were washed with sat. aq. sodium chloride (2 × 50 ml), dried and evaporated to give a thick oil. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a colourless oil, (*E/Z*)-(2*R*,3*R*,23*R*)-3-(*tert*-butyldimethyl-silanyloxy)-23-((*S*)-2,2-dimethyl-[1.3]dioxolan-4-yl)-2-docosyl-tetracos-5-enoic acid methyl ester (**178**) (4.4 g, 89 %) as a mixture of two isomers in ratio 2:1. Palladium on carbon (10 %, 1.5 g) was added to a stirred solution of the alkenes (4 g, 4.04 mmol) in IMS (30 ml) and THF (10 ml). Hydrogenation was carried out as before for 2 hours. The solution was filtered through a bed of celite and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a colourless oil, (2*R*,3*R*,23*R*)-3-(*tert*-butyldimethylsilanyloxy)-23-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-2-docosyl-tetracosanoic acid methyl ester (**182**) (3.53 g, 95 %) [Found $(M + Na)^+$: 943.8484, $C_{58}H_{116}NaO_5Si$ requires: 943.880, $[\alpha]_D^{20} = +4.41$ (*c* 1.62, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 4.0 (1H, dd, *J* 6.3, 7.55 Hz), 3.92-3.90 (1H, m), 3.87 (1H, br q, *J* 6.95 Hz), 3.65 (3H, s), 3.60 (1H, br t, *J* 7.9 Hz), 2.52 (1H, ddd, *J* 3.75, 7.25, 11.0 Hz), 1.59-1.53 (2H, m), 1.49-1.42 (2H, m), 1.40 (3H, s), 1.35 (3H, s), 1.29-1.22 (77H, br m, including br s at 1.26), 0.96 (3H, d, *J* 6.65 Hz), 0.88 (3H, t, *J* 7.25 Hz), 0.86 (9H, s), 0.047 (3H, s), 0.023 (3H, s); δ_C (125MHz, $CDCl_3$): 175.1, 108.5, 80.4, 73.2, 67.8, 51.91,

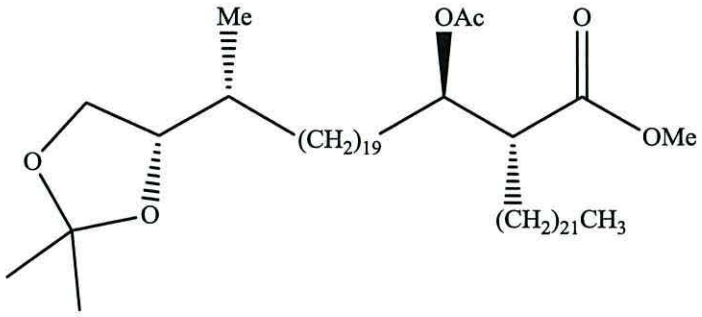
51.56, 36.5, 33.7, 32.73, 31.92, 29.9, 29.82, 29.69, 29.67, 29.65, 29.61, 29.57, 29.55, 29.43, 29.35, 27.8, 27.5, 27.0, 26.6, 25.75, 25.5, 23.7, 22.7, 18.0, 15.6, 14.1, -4.37, -4.9; ν_{max}: 2924, 2853, 1740, 1464, 1377, 1368, 1253, 1165, 1067 cm^{-1}.

Experiment 26: (2*R*,3*R*,23*R*)-23-((*S*)-2,2-Dimethyl-[1,3]dioxolan-4-yl)-2-docosyl-3-hydroxytetracosanoic acid methyl ester (183)



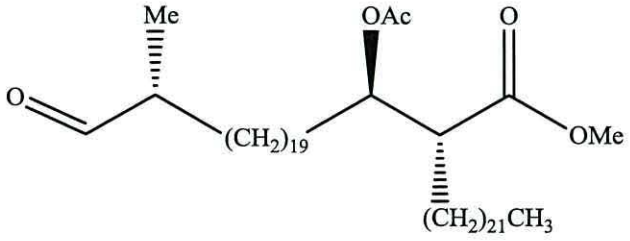
(2*R*,3*R*,23*R*)-3-(*tert*-Butyldimethylsilanyloxy)-23-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-2-docosyl-tetracosanoic acid methyl ester (**182**) (3.85 g, 4.17 mmol) was dissolved in dry THF (50 ml) in a dry polyethylene vial under nitrogen at r.t. and stirred. Pyridine (1.5 ml) and hydrogen fluoride-pyridine complex (4 ml) were added and the mixture was stirred for 17 hours at 40 °C, when TLC showed the reaction was complete. The reaction was diluted with petrol/ethyl acetate (1:1, 70 ml), and neutralized with sat. aq. $NaHCO_3$ until no more carbon dioxide was liberated. The aqueous layer was re-extracted with petrol/ethyl acetate (1:1, 2 × 50 ml). The combined organic layers were washed with brine and dried. The solvent was evaporated and the crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a white solid, (2*R*,3*R*,23*R*)-23-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-2-docosyl-3-hydroxy tetracosanoic acid methyl ester (**183**) (2.7 g, 80 %), m.p.: 56-58 °C [Found $(M + Na)^+$: 829.7531, $C_{52}H_{102}NaO_5$ requires: 829.7619], $[\alpha]_D^{25}$ = + 13.27 (*c* 1.07, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 4.0 (1H, dd, *J* 6.3, 7.85 Hz), 3.87 (1H, br q, *J* 7.25 Hz), 3.71 (3H, s), 3.65 (1H, br s), 3.60 (1H, br t, *J* 7.55 Hz), 2.43 (1H, dt, *J* 5.35, 10.4 Hz), 1.73-1.67 (1H, m), 1.60-1.53 (2H, m), 1.47-1.43 (2H, m), 1.40 (3H, s), 1.35 (3H, s), 1.31-1.21 (77H, br m), 0.96 (3H, d, *J* 6.65 Hz), 0.88 (3H, t, *J* 6.6 Hz); δ_C (125MHz, $CDCl_3$): 176.2, 108.5, 80.4, 72.3, 67.8, 51.5, 50.93, 36.5, 35.7, 32.7, 31.9, 29.9, 29.69, 29.66, 29.62, 29.59, 29.55, 29.53, 29.49, 29.41, 29.35, 27.4, 26.97, 26.6, 25.7, 25.5, 22.7, 15.6, 14.1; ν_{max}: 3369, 2953, 2922, 2853, 1709, 1461, 1377, 1188, 1164 cm^{-1}.

Experiment 27: (2*R*,3*R*,23*R*)-3-Acetoxy-23((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-2-docosyltetracosanoic acid methyl ester (158)



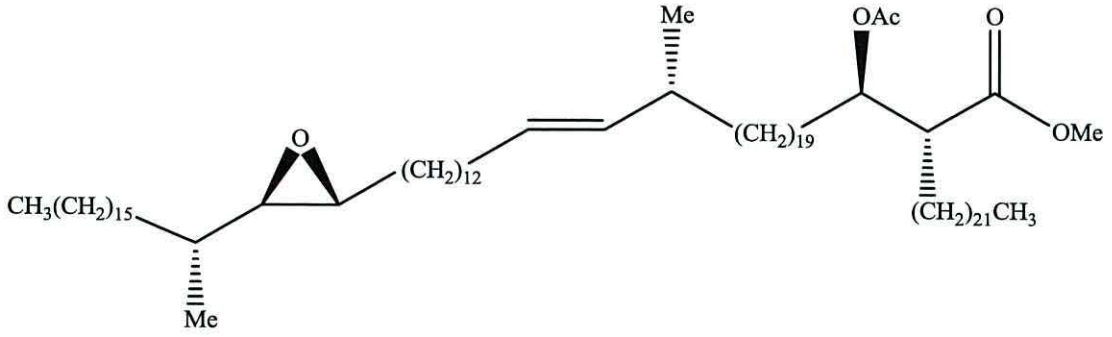
A mixture of acetic anhydride (10 ml) and anhydrous pyridine (10 ml) was added to stirred solution of the alcohol (**183**) (2.6 g, 3.22 mmol) in dry toluene (35 ml) at r.t. and the mixture was stirred for 18 hours, then diluted with toluene (10 ml) and the solvent evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give a white solid, (2*R*,3*R*,23*R*)-3-acetoxy-23((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-2-docosyl-tetracosanoic acid methyl ester (**158**) (2.72 g, 98 %), m.p.: 51-52 °C [Found $(M + Na)^+$: 871.5758, $C_{54}H_{104}NaO_6$ requires: 871.7725], $[\alpha]_D^{25} = +15.40$ (*c* 1.1, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 5.08 (1H, br dq, *J* 4.1, 8.2 Hz), 4.0 (1H, dd, *J* 6.3, 7.85 Hz), 3.87 (1H, br q, *J* 7.57 Hz), 3.68 (3H, s), 3.60 (1H, br t, *J* 7.6 Hz), 2.61 (1H, ddd, *J* 4.4, 6.9, 11 Hz), 2.03 (3H, s), 1.64-1.21 (80H, br m, including br s at 1.25), 1.40 (3H, s), 1.35 (3H, s), 1.11-1.04 (1H, m), 0.96 (3H, d, *J* 6.6 Hz), 0.88 (3H, t, *J* 6.6Hz); δ_C (125MHz, $CDCl_3$): 173.6, 170.3, 108.5, 80.4, 74.1, 67.81, 51.5, 49.6, 36.5, 32.73, 31.92, 31.7, 29.86, 29.69, 29.64, 29.55, 29.45, 29.42, 29.38, 29.34, 28.1, 27.5, 26.97, 26.6, 25.5, 24.98, 22.7, 21.0, 15.6, 14.1; ν_{max}: 2953, 2923, 2854, 1748, 1462, 1377, 1235, 1161 cm^{-1}.

Experiment 28: (2*R*,3*R*,23*R*)-3-Acetoxy-2-docosyl-23-methyl-24-oxo-tetracosanoic acid methyl ester (157)



Periodic acid (1.8 g, 7.94 mmol) was added to a stirred solution of (2*R*,3*R*,23*R*)-3-acetoxy-23-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-2-docosyl-tetracosanoic acid methyl ester (**158**) (2.7 g, 3.17 mmol) in dry ether (70 ml) at r.t. under nitrogen. The mixture was stirred for 16 hours, 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 (7:1) to give a white solid, (2*R*,3*R*,23*R*)-3-acetoxy-2-docosyl-23-methyl-24-oxo-tetracosanoic acid methyl ester (**157**) (1.55 g, 64 %), m.p.: 41-43 °C, $[\alpha]_D^{25} = +4.90$ (*c* 1.02, $CHCl_3$), which showed δ_H (500MHz, CDCl3): 9.61 (1H, d, *J* 2.2 Hz), 5.08 (1H, br dt, *J* 3.75, 7.85 Hz), 3.68 (3H, s), 2.61 (1H, ddd, *J* 4.45, 6.95, 11.05 Hz), 2.37-2.29 (1H, m), 2.03 (3H, s), 1.72-1.21 (80H, br m, including br s at 1.25), 1.09 (3H, d, *J* 6.95 Hz), 0.88 (3H, t, *J* 6.6 Hz); δ_C (125MHz, CDCl3): 205.4, 173.6, 170.3, 74.1, 51.5, 49.6, 46.3, 31.9, 31.7, 30.53, 29.72, 29.4, 29.39, 29.34, 28.1, 27.5, 26.93, 25.0, 22.7, 21.0, 14.1, 13.3; ν_{max}: 2923, 2852, 1745, 1465, 1371, 1236, 1165. 1022 cm^{-1}.

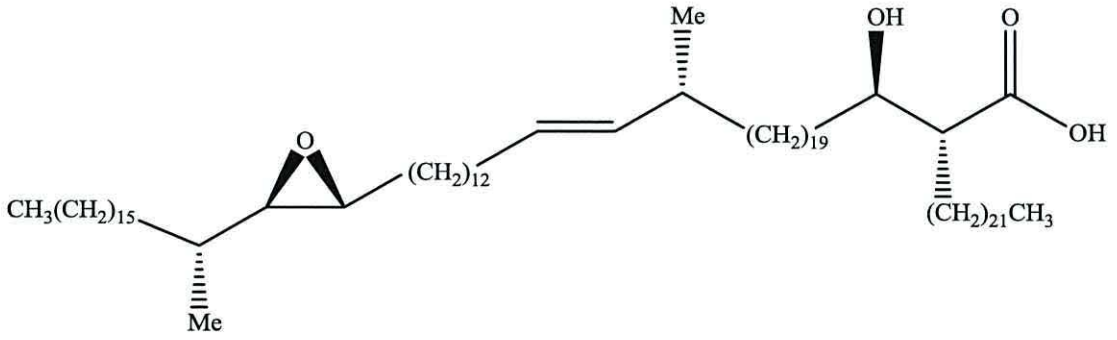
Experiment 29: (*E*)-(2*R*,3*R*,23*R*)-3-Acetoxy-23-methyl-37-[(2*R*,3*R*)-3-((*R*)-1-methyl-heptadecyl)-oxiranyl]-2-docosylheptatriacont-24-enoic acid methyl ester (184)



Potassium bis(trimethylsilyl)amide (0.2 g, 0.13 mmol, 0.5 M) was added dropwise with stirring to aldehyde (**157**) (50 mg, 0.064 mmol) and sulfone (**155**) (53 mg, 0.077 mmol) in dry 1,2-dimethoxy ethane (7 ml) under nitrogen at - 5 °C. The mixture was allowed to reach + 10 °C, when TLC showed no starting material, then quenched with water (5 ml) and petrol/ethyl acetate (20:1, 20 ml) added and extracted. The aqueous layer was re-extracted with petrol/ethyl acetate (20:1, 2 × 15 ml). The combined organic layer was washed with sat. aq. sodium chloride (2 × 15 ml), dried and evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1)

to give a white solid, (*E*)-(2*R*,3*R*,23*R*)-3-acetoxy-23-methyl-37-[(2*R*,3*R*)-3-((*R*)-1-methyl-heptadecyl)-oxiranyl]-2-docosyl-heptatriacont-24-enoic acid methyl ester (**184**) (26 mg, 32 %), m.p.: 38-39 °C [Found $(M + Na)^+$: 1260.2157, $C_{83}H1_{60}NaO_5$ requires: 1260.2158], $[\alpha]_D^{18} = +7.15$ (*c* 1.02, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 5.33 (1H, dt, *J* 6.6, 15.45 Hz), 5.24 (1H, dd, *J* 7.55, 15.45 Hz), 5.09 (1H, br dq, *J* 3.8, 7.9 Hz), 3.68 (3H, s), 2.66 (1H, dt, *J* 2.2, 5.65 Hz), 2.62 (1H, ddd, *J* 4.4, 6.95, 11.05 Hz), 2.46 (1H, dd, *J* 2.2, 7.25 Hz), 2.03 (3H, s), 1.99-1.94 (2H, m), 1.69-1.13 (134H, br m, including br s at 1.26), 0.94 (3H, d, *J* 6.95 Hz), 0.92 (3H, d, *J* 6.6 Hz), 0.88 (6H, t, *J* 6.95 Hz); δ_C (125MHz, $CDCl_3$): 173.6, 170.3, 136.5, 128.4, 74.1, 63.8, 57.5, 51.5, 49.6, 37.24, 36.7, 35.83, 34.62, 32.6, 32.2, 31.9, 31.7, 30.3, 29.93, 29.8, 29.69, 29.62, 29.56, 29.52, 29.46, 29.39, 29.35, 29.1, 28.1, 27.5, 27.4, 26.9, 26.1, 25.0, 22.7, 21.0, 20.9, 16.0, 14.1; ν_{max}: 2920, 2851, 1744, 1468, 1236 cm^{-1}.

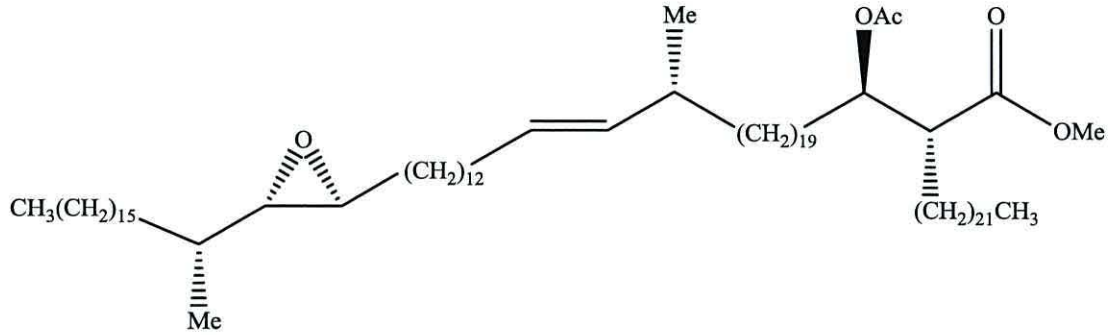
Experiment 30: (*E*)-(2*R*,3*R*,23*R*)-3-Hydroxy-23-methyl-37-[(2*R*,3*R*)-3-((*R*)-1-methy-lheptadecyl)-oxiranyl]-2-docosylheptatriacont-24-enoic acid (103)



Lithium hydroxide monohydrate (20 mg, 0.483 mmol) was added to a stirred solution of the acetyl protected methyl ester (**184**) (20 mg, 0.016 mmol) in THF (4 ml), methanol (0.5 ml) and water (0.5 ml) at r.t. The mixture was stirred at 45 °C for overnight, when TLC showed a small amount of starting material was left. It was cooled to r.t. and a mixture of petrol/ethyl acetate (7:2, 5 ml) was added. The mixture was acidified to pH 1 with potassium hydrogen sulphate. Further petrol/ethyl acetate (7:2, 10 ml) was added and extracted. The aqueous layer was re-extracted with petrol/ethyl acetate (7:2, 2 × 10 ml). The solvent was dried and evaporated. The crude product was purified by column chromatography on silica eluting with petrol/ethyl acetate (7:2) to give a white solid, (*E*)-(2*R*,3*R*,23*R*)-3-hydroxy-23-methyl-37-[(2*R*,3*R*)-3-((*R*)-1-methyl-heptadecyl) oxiranyl]-2-docosyl-heptatriacont-24-enoic acid (**103**) (10 mg, 52 %), m.p: 54-55 °C [Found $(M + Na)^+$: 1204.1843, $C_{80}H_{156}NaO_4$ requires: 1204.1896], $[\alpha]_D^{20} = +6.02$ (*c*

0.52, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 5.33 (1H, dt, *J* 6.6, 15.1 Hz), 5.24 (1H, dd, *J* 7.55, 15.15 Hz), 3.77-3.69 (1H, m), 2.69-2.67 (1H, m), 2.49-2.45 (2H, m), 2.05-2.02 (2H, m), 1.96 (2H, q, *J* 6.6 Hz), 1.79-1.03 (134H, br m, including br s at 1.26), 0.94 (3H, d, *J* 6.65 Hz), 0.92 (3H, d, *J* 6.6 Hz), 0.88 (6H, t, *J* 6.1 Hz); δ_C (125MHz, $CDCl_3$): 178.2, 136.45, 128.41, 72.1, 63.91, 57.6, 50.63, 37.2, 36.7, 35.8, 35.6, 34.6, 32.6, 32.2, 31.9, 29.9, 29.8, 29.65, 29.59, 29.57, 29.52, 29.5, 29.44, 29.41, 29.35, 29.2, 29.1, 27.3, 26.9, 26.1, 25.7, 22.7, 21.0, 15.9, 14.1; ν_{max}: 3392, 2924, 2853, 1748, 1464, 1070 cm^{-1}.

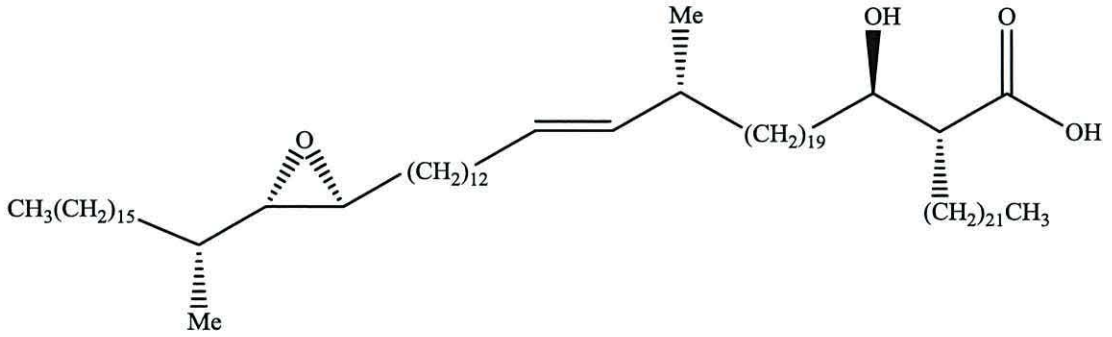
Experiment 31: (*E*)-(2*R*,3*R*,23*R*)-3-Acetoxy-23-methyl-37-[(2*S*,3*S*)-3-((*R*)-1-methyl-heptadecyl)-oxiranyl]-2-docosylheptatriacont-24-enoic acid methyl ester (185)



Potassium bis(trimethylsilyl)amide (0.35 g, 0.176 mmol, 0.5 M) was added dropwise with stirring to aldehyde (**157**) (88 mg, 0.113 mmol) and sulfone (**156**) (93 mg, 0.135 mmol) in dry 1,2-dimethoxy ethane (7 ml) under nitrogen at - 5 °C. The mixture was allowed to reach + 10 °C, when TLC showed no starting material, then quenched with water (5 ml) and petrol/ethyl acetate (20:1, 20 ml) added and extracted. The aqueous layer was re-extracted with petrol/ethyl acetate (20:1, 2 × 15 ml). The combined organic layer was washed with sat. aq. sodium chloride (2 × 15 ml), dried and evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a white solid, (*E*)-(2*R*,3*R*,23*R*)-3-acetoxy-23-methyl-37-[(2*S*,3*S*)-3-((*R*)-1-methylheptadecyl)-oxiranyl]-2-docosylheptatriacont-24-enoic acid methyl ester (**185**) (37 mg, 26 %), m.p.: 34-35 °C [Found $(M + Na)^+$: 1260.2128, $C_{83}H_{160}NaO_5$ requires: 1260.2158], $[\alpha]_D^{18}$ = - 2 (*c* 1.0, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 5.33 (1H, br dq, *J* 6.65, 15.45 Hz), 5.24 (1H, dd, *J* 7.6, 15.45 Hz), 5.11-5.07 (1H, m), 3.68 (3H, s), 2.71 (1H, dt, *J* 2.2, 5.35 Hz), 2.62 (1H, ddd, *J* 4.1, 6.6, 10.7 Hz), 2.40 (1H, dd, *J* 5.05, 7.25 Hz), 2.03 (3H, s), 1.99-1.94 (2H, m), 1.69-1.08 (134H, br m, including br s at 1.26), 1.00 (3H, d, *J* 6.0 Hz), 0.94 (3H, d, *J* 6.95 Hz), 0.89 (6H, t, *J* 6.6 Hz); δ_C

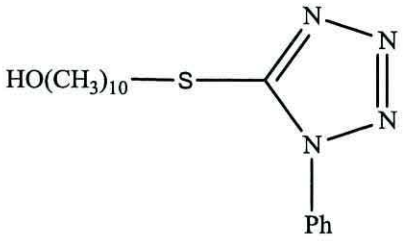
(125MHz, $CDCl_3$): 173.6, 170.3, 136.5, 128.4, 74.1, 63.8, 58.8, 51.5, 49.6, 37.2, 36.7,36.0, 33.8, 32.6, 32.3, 31.9, 31.7, 29.9, 29.8, 29.69, 29.66, 29.65, 29.58, 29.55, 29.53, 29.5, 29.46, 29.43, 29.39, 29.35, 29.1, 28.1, 27.5, 27.3, 27.2, 26.1, 25.0, 22.7, 21.0, 20.9, 17.3, 14.1; ν_{max}: 2919, 2851, 1737, 1470, 1238 cm^{-1}.

Experiment 32: (*E*)-(2*R*,3*R*,23*R*)-3-Hydroxy-23-methyl-37-[(2*S*,3*S*)-3-((*R*)-1-methyl-heptadecyl)-oxiranyl]-2-docosylheptatriacont-24-enoic acid (104)



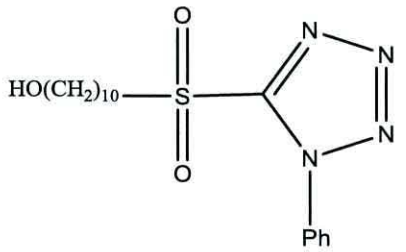
Lithium hydroxide monohydrate (31.8 mg, 0.759 mmol) was added to a stirred solution of the acetyl protected methyl ester (**185**) (31.4 mg, 0.0253 mmol) in THF (4 ml), methanol (0.5 ml) and water (0.5 ml) at r.t. The mixture was stirred at 45 °C for overnight, when TLC showed a small amount of starting material was left. It was cooled to r.t. and a mixture of petrol/ethyl acetate (7:2, 5 ml) was added. The mixture was acidified to pH 1 with potassium hydrogen sulphate. Further petrol/ethyl acetate (7:2, 10 ml) was added and extracted. The aqueous layer was re-extracted with petrol/ethyl acetate (7:2, 2 × 10 ml). The solvent was dried and evaporated. The crude product was crystallized from petrol/ethyl acetate (10:1, 15 ml) and left 30 min and then at 0 °C for 15 min. The crystals were filtered off to give a white solid, (*E*)-(2*R*,3*R*,23*R*)-3-hydroxy-23-methyl-37-[(2*S*,3*S*)-3-((*R*)-1-methylheptadecyl)-oxiranyl]-2-docosylheptatriacont-24-enoic acid (**104**) (21.5, 72 %), m.p.: 71-73 °C [Found $(M + Na)^+$: 1204.1912, $C_{80}H_{156}NaO_4$ requires: 1204.1896], $[\alpha]_D^{20}$ = - 5.49, (c 0.74, $CHCl_3$), δ_H (500MHz, $CDCl_3$): 5.33 (1H, td, *J* 6.3, 15.15 Hz), 5.24 (1H, dd, *J* 7.55, 15.1 Hz), 3.74-3.70 (1H, m), 2.73 (1H, dt, *J* 2.2, 5.35 Hz), 2.49-2.45 (1H, m), 2.43 (1H, dd, *J* 2.2, 7.25 Hz), 2.11-2.01 (2H, m), 1.96 (2H, q, *J* 6.65 Hz), 1.78-1.06 (134H, br m, including br s at 1.26), 1.0 (3H, d, *J* 6.3 Hz), 0.94 (3H, d, *J* 6.65 Hz), 0.89 (6H, t, *J* 6.6 Hz); δ_C (125MHz, $CDCl_3$): 178.7, 136.46, 128.41, 72.1, 63.99, 59.0, 50.7, 37.2, 36.7, 36.0, 35.5, 33.8, 32.6, 32.2, 31.9, 29.9, 29.8, 29.7, 29.6, 29.53, 29.5, 29.42, 29.36, 29.1, 27.3, 27.2, 26.1, 25.7, 22.7, 21.0, 17.3, 14.1; ν_{max}: 3368, 2922, 2851, 1686, 1463, 1048 cm^{-1}.

Experiment 33: 10-(1-phenyl-1*H*-tetrazol-5-ylsulfanyl)-decan-1-ol (203)



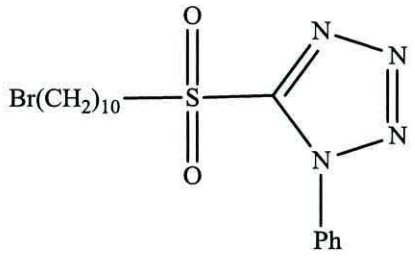
The procedure used in Experiment 1 was repeated in order to convert the 10-bromo-decan-1-ol (45.9 g, 192.05 mmol) using 1-phenyl-1*H*-tetrazole-5-thiol (34.2 g, 192.05 mmol) and anhydrous potassium carbonate (53.08 g, 384.1 mmol) in acetone (400 ml) into a white solid (60 g, 93 %), m.p.: 36-38 °C, which showed δ_H (500MHz, $CDCl_3$): 7.59-7.5 (5H, m), 3.62 (2H, t, *J* 6.6 Hz), 3.38 (2H, t, *J* 7.25 Hz), 1.8 (2H, pent., *J* 7.55 Hz), 1.58-1.52 (3H, m), 1.43 (2H, pent., *J* 6.65 Hz), 1.33-1.24 (10H, m); δ_C: 154.47, 133.71, 130.0, 129.7, 123.79, 62.92, 33.3, 32.7, 29.36, 29.27, 29.22, 29.0, 28.88, 28.51, 25.64; ν_{max}: 3391, 2925, 2854, 1498, 1387, 1241, 1051 cm^{-1}.

Experiment 34: 10-(1-Phenyl-1*H*-tetrazole-5-sulfonyl)-decan-1-ol (204)



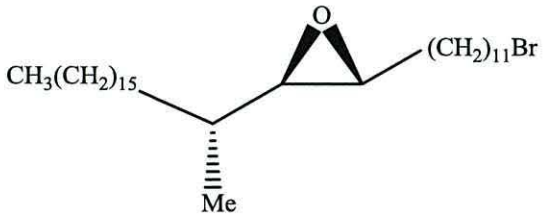
The procedure used in Experiment 2 was repeated using the 10-(1-phenyl-1*H*-tetrazole-5-ylsulfanyl)-decan-1-ol (**203**) (60 g, 179.3 mmol) in IMS (600 ml) and THF (200 ml), ammonium molybdate (VI) tetrahydrate (99.64 g, 80.68 mmol) in ice cold H_2O_2 (35 % w/w, 200 ml), and further solution of ammonium molybdate (VI) tetrahydrate (40 g, 32.38 mmol) in ice cold H_2O_2 (35 % w/w, 100 ml). The crude product was purified by column chromatography eluting with petrol/ethyl acetate (5:1) to give a white solid, 10-(1-phenyl-1*H*-tetrazole-5-sulfonyl)-decan-1-ol (50 g, 76 %), m.p.: 52-54 °C, which showed δ_H (500MHz, $CDCl_3$): 7.70-7.68 (2H, m), 7.65-7.58 (3H, m), 3.73 (2H, t, *J* 7.6 Hz), 3.63 (2H, t, *J* 6.65 Hz), 1.98-1.92 (2H, m), 1.59-1.46 (5H, m), 1.36-1.25 (10H, m); δ_C (125MHz, $CDCl_3$): 153.45, 133.01, 131.42, 129.68, 125.03, 62.96, 55.96, 32.7, 29.31, 29.25, 29.0, 28.78, 28.04, 25.63, 21.88; ν_{max}: 3400, 2928, 2855, 1497, 1462,1341, 1152 cm^{-1}.

Experiment 35: 5-(10-Bromodecylsulfonyl)-1-phenyl-1*H*-tetrazole (205)



The procedure used in Experiment 13 was repeated in order to convert the 10-(1-phenyl-1*H*-tetrazole-5-sulfonyl)-decan-1-ol (**204**) (15 g, 40.92 mmol) using NBS (9.47 g, 53.20 mmol) and PPh_3 (13.41 g, 51.16 mmol) in CH_2Cl_2 (200 ml) into a pale yellow solid,5-(10-bromodecylsulfonyl)-1-phenyl-1*H*-tetrazole (15.5 g, 88 %), which showed δ_H (500MHz, $CDCl_3$): 7.68-7.66 (2H, m), 7.62-7.55 (3H, m), 3.7(2H, t, *J* 7.85 Hz), 3.38 (2H, t, *J* 6.9 Hz), 1.96-1.89 (2H, m), 1.83 (2H, pent., *J* 6.95 Hz), 1.47 (2H, pent., *J* 6.95 Hz),1.41-1.37 (2H, m), 1.34-1.28 (8H, m); δ_C (125MHz, $CDCl_3$): 153.43, 132.98, 131.38, 129.64, 125.01, 55.91, 33.94, 32.69, 29.14, 28.96, 28.75, 28.57, 28.02, 21.86; ν_{max}: 2921, 2852, 1637, 1494, 1355, 1150 cm^{-1}.

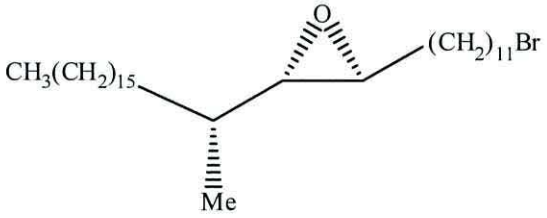
Experiment 36: (2*R*,3*R*)-2-(11-Bromoundecyl)-3-((*R*)-octadecan-2-yl)oxirane (208)



Lithium bis(trimethylsilyl)amide (4.1 ml, 4.43 mmol, 1.06 M) was added dropwise with stirring to (2*S*,3*R*)-3-((*R*)-1-methyl-heptadecyl)-oxirane-2-carbaldehyde (**142**) (0.8 g, 2.4 mmol) and 5-(10-bromo-decylsulfonyl)-1-phenyl-1*H*-tetrazole (**205**) (1.27 g, 2.95 mmol) in dry tetrahydrofuran (36 ml) under nitrogen at - 10 °C. The mixture was allowed to reach room temperature and stirred for 1 hour, when TLC showed no starting material, then quenched with water (7 ml) and petrol/ethyl acetate (1:1, 10 ml). The aqueous layer was re-extracted with petrol/ethyl acetate (1:1, 2 × 30 ml). The combined organic layers were washed with sat. aq. sodium chloride (2 × 25 ml), dried and evaporated to give a thick oil. The crude product was purified by column chromatography on silica gel eluting with petrol/ethyl acetate (20:1) to give (2*R*,3*R*)-2-((*E*/*Z*)-11-bromoundec-1-enyl)-3-((*R*)-octadecan-2-yl)oxirane (**206**) (0.8 g, 61 %). Hydrogenation of the (2*R*,3*R*)-2-((*E*/*Z*)-11-bromoundec-1-enyl)-3-((*R*)-octadecan-2-

yl)oxirane (0.8 g, 1.51 mmol) was carried out using dipotassium azodicarboxylate (2.5 g, 12.87 mmol) in THF (15 ml), methanol (5 ml) and a solution of glacial acetic acid (2 ml) in THF (4 ml). The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a white solid, (2*R*,3*R*)-2-(11-bromoundecyl)-3-((*R*)-octadecan-2-yl)oxirane (**208**) (0.78 g, 97 %), m.p.: 42-44 °C [Found $(M + Na)^+$: 551.3782, $C_{31}H_{61}BrNaO$ requires: 551.3798], $[\alpha]_D^{24} = + 11.13$ (*c* 1.15, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 3.41 (2H, t, *J* 6.6 Hz), 2.66 (1H, dt, *J* 2.2, 5.65 Hz), 2.46 (1H, dd, *J* 2.25, 6.95 Hz), 1.85 (2H, pent., *J* 6.95 Hz), 1.60-1.22 (49H, br m, including br.s at 1.26), 0.92 (3H, d, *J* 6.6 Hz), 0.88 (3H, t, *J* 6.95 Hz); δ_C (125MHz, $CDCl_3$): 63.77, 57.41, 35.81, 34.61, 33.9, 32.84, 32.21, 31.92, 29.93, 29.69, 29.64, 29.61, 29.51, 29.47, 29.42, 29.39, 29.34, 28.75, 28.17, 26.89, 26.13, 22.67, 15.93, 14.08; ν_{max}: 2921, 2852, 1464, 1376 cm^{-1}.

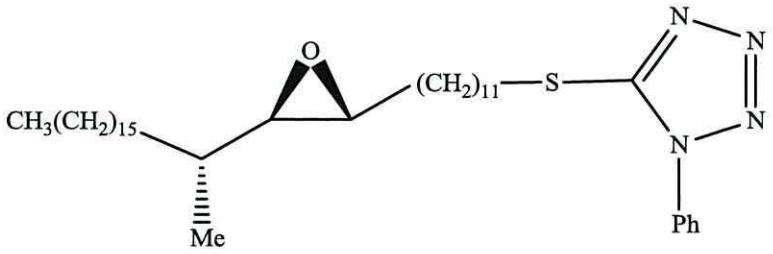
Experiment 37:(2*S*,3*S*)-2-(11-Bromoundecyl)-3-((*R*)-octadecan-2-yl)oxirane (209)



The procedure used in Experiment 36 was repeated in order to couple the (2*R*,3*S*)-3-((*R*)-1-methylheptadecyl)-oxirane-2-carbaldehyde (**143**) (1 g, 3 mmol) and 5-(10-bromo-decylsulfonyl)-1-phenyl-1*H*-tetrazole (**205**) (1.58 g, 3.69 mmol) using lithium bis(trimethylsilyl)amide (5.23 ml, 5.54 mmol, 1.06 M) in dry THF (40 ml) under nitrogen at - 10 °C. The crude product was purified by column chromatography on silica gel eluting with petrol/ethyl acetate (20:1) to give (2*S*,3*S*)-2-((*E*/*Z*)-11-bromoundec-1-enyl)-3-((*R*)-octadecan-2-yl)oxirane (**207**) (1.03 g, 63 %). Hydrogenation was carried out with dipotassium azodicarboxylate (2.5 g, 12.87 mmol) as before and the crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a white solid, (2*S*,3*S*)-2-(11-bromoundecyl)-3-((*R*)-octadecan-2-yl)oxirane (**209**) (1.0 g, 96 %), m.p.: 44-46 °C [Found $(M + Na)^+$: 551.3806, $C_{31}H_{61}BrNaO$ requires: 551.3798], $[\alpha]_D^{24} = - 8.09$ (*c* 1.05, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 3.40 (2H, t, *J* 6.95 Hz), 2.71 (1H, dt, *J* 2.2, 5.35 Hz), 2.41 (1H, dd, *J* 1.9, 6.95 Hz), 1.88-1.82 (3H, m), 1.54-1.24 (48H, br m, including br.s at 1.26), 0.99 (3H, d, *J* 6.25 Hz), 0.88 (3H, t, *J* 6.95 Hz); δ_C (125MHz, $CDCl_3$): 63.8, 58.78, 35.99, 33.87, 33.77, 32.83, 32.23,

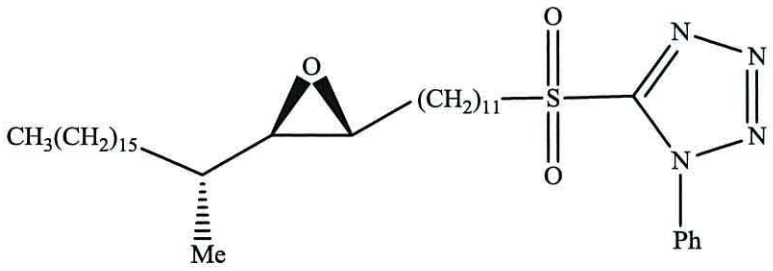
31.9, 29.85, 29.68, 29.65, 29.63, 29.61, 29.58, 29.52, 29.47, 29.39, 29.33, 28.74,28.16, 27.19, 26.05, 22.65, 17.22, 14.16, 14.06; ν_{max}: 2924, 2853, 1465, 1376 cm^{-1}.

Experiment 38: 5-(11-((2*R*,3*R*)-3-((*R*)-Octadecan-2-yl)oxiran-2-yl)undecylthio)-1-phenyl-1*H*-tetrazole (210)



The procedure used in Experiment 1 was repeated using (2*R*,3*R*)-2-(11-bromoundecyl)-3-((*R*)-octadecan-2-yl)oxirane (**208**) (0.78 g, 1.47 mmol), 1-phenyl-1*H*-tetrazole-5-thiol (0.28 g, 1.61 mmol) and anhydrous potassium carbonate (0.4 g, 2.94 mmol) in acetone (35 ml) to give a white solid, 5-(11-((2*R*,3*R*)-3-((*R*)-octadecan-2-yl)oxiran-2-yl)undecylthio)-1-phenyl-1*H*-tetrazole (**210**) (0.84 g, 91 %), m.p.: 30-32 °C [Found (M + H)$^+$: 627.5037, $C_{38}H_{67}N_4OS$ requires: 627.503], $[\alpha]_D^{21} = +8.94$ (*c* 1.23, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 7.60-7.53 (5H, m), 3.40 (2H, t, *J* 7.25 Hz), 2.66 (1H, dt, *J* 2.25, 5.7 Hz), 2.46 (1H, dd, *J* 2.2, 7.25 Hz), 1.85 (1H, pent., *J* 7.6 Hz), 1.59-1.22 (50H, m, including br.s at 1.26), 0.91 (3H, d, *J* 6.65 Hz), 0.88 (3H, t, *J* 6.6 Hz); δ_C (125MHz, $CDCl_3$):154.49, 133.84, 130.02, 129.73, 123.87, 63.77, 57.42, 35.82, 34.61, 33.40, 32.21, 31.91, 29.93, 29.68, 29.64, 29.61, 29.51, 29.47, 29.42, 29.39, 29.34, 29.10, 29.01, 28.63, 26.89, 26.13, 22.67, 15.95, 14.08; ν_{max}: 2923, 2852, 1500, 1466, 1385, 1243, 1073 cm^{-1}.

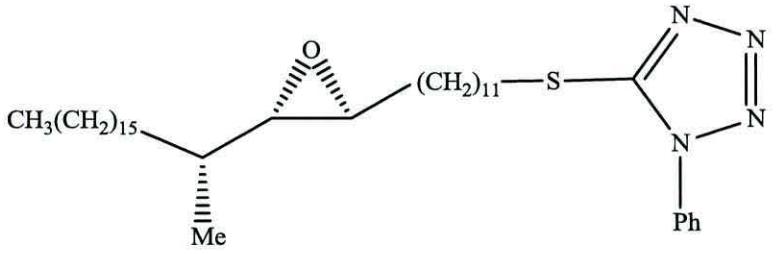
Experiment 39:5-(11-((2*R*,3*R*)-3-((*R*)-Octadecan-2-yl)oxiran-2-yl)undecylsulfonyl)-1-phen yl-1*H*-tetrazole (212)



The procedure used in Experiment 2 was repeated using 5-(11-((2*R*,3*R*)-3-((*R*)-octadecan-2-yl)oxiran-2-yl)undecylthio)-1-1*H*-tetrazole (**210**) (0.79 g, 1.25 mmol),

ammonium molybdate (VI) tetrahydrate (0.7 g, 0.566 mmol) in ice cold H_2O_2 (35 % w/w, 3 ml) in IMS (25 ml) and THF (5 ml), and a further solution of ammonium molybdate (VI) tetrahydrate (0.3 g, 0.24 mmol) in ice cold H_2O_2 (35 % w/w, 2 ml). The crude product was purified by column chromatography eluting with petrol/ethyl acetate (5:1) to give a white solid, 5-(11-((2*R*,3*R*)-3-((*R*)-octadecan-2-yl)oxiran-2-yl)undecylsulfonyl)-1-phenyl-1*H*-tetrazole (**212**) (0.75 g, 90 %), m.p.: 43-45 °C [Found $(M + H)^+$: 659.4939, $C_{38}H_{67}N_4O_3S$ requires: 659.4928], $[\alpha]_D^{21} = + 4.37$ (*c* 0.96, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 7.71-7.69 (2H, m), 7.65-7.58 (3H, m), 3.73 (2H, t, *J* 8.2 Hz), 2.66 (1H, dt, *J* 2.2, 5.65 Hz), 2.46 (1H, dd, *J* 2.2, 7.25 Hz), 1.99-1.93 (2H, m), 1.57-1.22 (49H, m, including br.s at 1.26), 0.92 (3H, d, *J* 6.6 Hz), 0.88 (3H, t, *J* 6.65 Hz); δ_C (125MHz, $CDCl_3$): 153.56, 133.12, 131.42, 129.69, 125.09, 63.77, 57.42, 56.06, 35.83, 34.61, 32.21, 31.92, 29.93, 29.68, 29.64, 29.61, 29.49, 29.41, 29.39, 29.34, 29.15, 28.87, 28.13, 26.89, 26.13, 22.67, 21.95, 15.95, 14.08; ν_{max}: 2923, 2853, 1463, 1342, 1152 cm^{-1}.

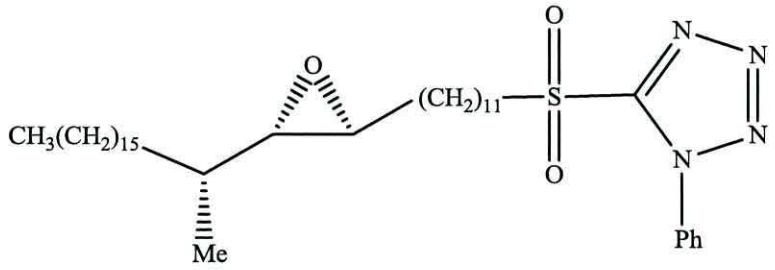
Experiment 40:5-(11-((2*S*,3*S*)-3-((*R*)-Octadecan-2-yl)oxiran-2-yl)undecylthio)-1-phenyl-1*H*-tetrazole (211)



The procedure used in Experiment 1 was repeated in order to convert (2*S*,3*S*)-2-(11-bromoundecyl)-3-((*R*)-octadecan-2-yl)oxirane (**209**) (1 g, 1.88 mmol) using 1-phenyl-1*H*-tetrazole-5-thiol (0.37 g, 2.07 mmol) and anhydrous potassium carbonate (0.52 g, 3.77 mmol) in acetone (35 ml) into a white solid, 5-(11-((2*S*,3*S*)-3-((*R*)-octadecan-2-yl)oxiran-2-yl)undecylthio)-1-phenyl-1*H*-tetrazole (**211**) (0.84 g, 71 %), m.p.: 39-40 °C [Found $(M + H)^+$: 627.5033, $C_{38}H_{67}N_4OS$ requires: 627.503], $[\alpha]_D^{21} = - 4.84$ (*c* 0.99, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 7.60-7.53 (5H, m), 3.39 (2H, t, *J* 7.25 Hz), 2.71 (1H, dt, *J* 2.2, 5.65 Hz), 2.41(1H, dd, *J* 2.2, 7.25 Hz), 1.82 (1H, pent., *J* 7.25 Hz), 1.54-1.22 (50H, m, including br.s at 1.26), 0.99 (3H, d, *J* 6.3 Hz), 0.88 (3H, t, *J* 6.6 Hz); δ_C (125MHz, $CDCl_3$): 154.47, 133.83, 130.0, 129.73, 123.85, 63.78, 58.76, 35.99, 33.76, 33.39, 32.24, 31.90, 29.85, 29.68, 29.65, 29.63, 29.58, 29.52, 29.48, 29.40,

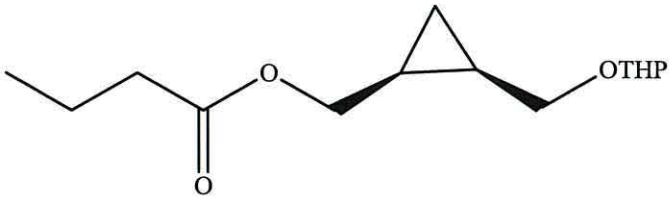
29.33, 29.10, 29.01, 28.63, 27.19, 26.06, 22.66, 17.22, 14.07; ν_{max}: 2923, 2852, 1500, 1466, 1385, 1243, 1073 cm^{-1}.

Experiment 41:5-(11-((2*S*,3*S*)-3-((*R*)-Octadecan-2-yl)oxiran-2-yl) undecylsulfonyl)-1-phenyl-1*H*-tetrazole (196)



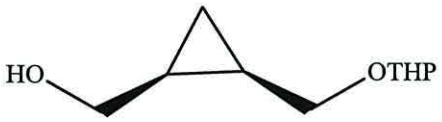
The procedure used in Experiment 2 was repeated using 5-(11-((2*S*,3*S*)-3-((*R*)-octadecan-2-yl)oxiran-2-yl)undecylthio)-1-phenyl-1*H*-tetrazole (**211**) (0.79 g, 1.25 mmol), ammonium molybdate (VI) tetrahydrate (0.7 g, 0.566 mmol) in ice cold H_2O_2 (35 % w/w, 3 ml) in IMS (25 ml) and THF (5 ml) and a further solution of ammonium molybdate (VI) tetrahydrate (0.3 g, 0.24 mmol) in ice cold H_2O_2 (35 % w/w, 2 ml). The crude product was purified by column chromatography eluting with petrol/ethyl acetate (5:1) to give a white solid (**196**) (0.7 g, 84 %), m.p.: 42-44 °C [Found $(M + H)^+$: 659.4907, $C_{38}H_{67}N_4O_3S$ requires: 659.4928], $[\alpha]_D^{20}$ = -14.17 (*c* 0.79, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 7.71-7.69 (2H, m), 7.63-7.58 (3H, m), 3.73 (2H, t, *J* 7.9 Hz), 2.71 (1H, dt, *J* 2.2, 5.7 Hz), 2.41 (1H, dd, *J* 2.2, 6.95 Hz), 1.99-1.92 (2H, m), 1.58-1.22 (49H, m, including br.s at 1.26), 1.0 (3H, d, *J* 6.3 Hz), 0.88 (3H, t, *J* 6.65 Hz); δ_C (125MHz, $CDCl_3$): 153.56, 133.11, 131.40, 129.68, 125.08, 63.78, 58.75, 56.05, 35.97, 33.76, 32.24, 31.90, 29.85, 29.68, 29.63, 29.58, 29.49, 29.45, 29.43, 29.39, 29.33, 29.15, 28.88, 28.13, 27.19, 26.06, 22.66, 21.94, 17.21, 14.07; ν_{max}: 2923, 2853, 1463, 1342, 1152 cm^{-1}.

Experiment 42: ((1*S*,2*R*)-2-(Tetrahydro-2*H*-pyran-2-yloxy)methyl)cyclopropyl butyrate (199)



3,4-Dihydro-2*H*-pyran (7.81 g, 92.9 mmol) and pyridinium-p-toluene–sulfonate (0.58 g, 2.32 mmol) were added to a stirred solution of ((1*S*,2*R*)-2-(hydroxymethyl)cyclopropyl)methyl butyrate (**217**) (8 g, 46.4 mmol) in dry CH_2Cl_2 (150 ml) under nitrogen at r.t. The reaction was stirred at r.t. for 3 hours and worked up with sat. aq. $NaHCO_3$ (20 ml). The mixture was extracted and dried. The solvent was evaporated and the crude product was purified by column chromatography on silica gel eluting with petrol/ethyl acetate (10:1) to give colourless oil, ((*1S,2R*)-2-(tetrahydro-2*H*-pyran-2-yloxy)methyl)cyclopropyl butyrate (**199**) (10 g, 84 %) [Found $(M + Na)^+$: 279.1594, $C_{14}H_{24}NaO_4$ requires: 279.1567], $[\alpha]_D^{20} = +2.01$ (*c* 1.19, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 4.61 (1H, t, *J* 3.8 Hz), 4.53 (1H, t, *J* 4.1 Hz), 4.16 (1H, td, *J* 2.2, 3.45 Hz), 4.14 (1H, dd, *J* 2.2, 7.6 Hz), 4.04-3.97 (2H, m), 3.85-3.75 (3H, m), 3.61 (1H, br dd, *J* 7.55, 10.7 Hz), 3.51-3.43 (3H, m), 3.32 (1H, br dd, *J* 7.6, 10.75 Hz), 2.26 (2H, t, *J* 7.25 Hz), 2.25 (2H, t, *J* 7.55 Hz), 1.82-1.77 (2H, m), 1.70-1.46 (14H, m), 1.28-1.22 (4H, m), 0.91 (6H, t, *J* 7.25 Hz), 0.85-0.79 (2H, m), 0.28 (1H, q, *J* 5.35 Hz), 0.25 (1H, q, *J* 5.4 Hz); δ_C (125MHz, $CDCl_3$): 173.6, 173.56, 98.67, 98.21, 94.47, 67.08, 66.98, 64.49, 64.45, 62.21, 61.88, 36.13, 30.6, 30.57, 30.51, 25.38, 25.33, 19.61, 19.52, 19.29, 18.34, 15.61, 15.34, 14.42, 14.23, 13.54, 8.52, 8.3; ν_{max}: 2942, 2874, 1735, 1458, 1374, 1259, 1183, 1026 cm^{-1}.

Experiment 43: ((1*S*,2*R*)-2-((Tetrahydro-2*H*-pyran-2-yloxy)methyl)cyclopropyl) methanol (218)



((1*S*,2*R*)-2-((Tetrahydro-2*H*-pyran-2-yloxy)methyl)cyclopropyl butyrate (**199**) (10 g, 39.01 mmol) in tetrahydrofuran (50 ml) was added dropwise over 15 min to a suspension of lithium aluminum hydride (2.28 g, 58.51 mmol) in tetrahydrofuran (200 ml) at room temperature. The mixture was refluxed for 1 hour, when TLC showed no starting material was left then cooled to room temperature and quenched carefully with freshly prepared sat. aq. sodium sulfate decahydrate (20 ml) until a white precipitate was formed, followed by the addition of magnesium sulphate (5 g). The mixture was stirred vigorously for 10 min then filtered through a pad of celite and washed well with tetrahydrofuran (2 × 100 ml). The combined organic layers were evaporated to give a residue, which was purified by chromatography on silica eluting with petrol/ethyl

acetate (5:1 then 5:2) to give a colourless oil, ((1*S*,2*R*)-2-((tetrahydro-2*H*-pyran-2-yloxy) methyl)cyclopropyl) methanol (**218**) (6.7 g, 92 %) [Found $(M + Na)^+$: 209.1262, $C_{10}H_{18}NaO_3$ requires: 209.1172], $[\alpha]_D^{20}$ = + 17.73 (*c* 1.19, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 4.64-2.62 (2H, m), 4.18 (1H, dd, *J* 5.7, 11.05 Hz), 3.93-3.85 (4H, m), 3.82-3.78 (1H, m), 3.53-3.46 (2H, m), 3.37 (1H, t, *J* 11 Hz), 3.24 (1H, dd, *J* 10.4, 11.95 Hz), 3.19 (1H, dd, *J* 10.7, 12.3 Hz), 3.09 (1H, t, *J* 10.75 Hz), 1.82-1.75 (2H,m), 1.74-1.67 (2H. m), 1.6-1.46 (9H, m), 1.37-1.20 (5H, m), 0.8-0.75 (2H, m), 0.19-0.14 (2H, m); δ_C (125MHz, $CDCl_3$): 98.8, 98.23, 67.91, 67.65, 62.86, 62.63, 62.51, 62.14, 30.51, 30.38, 25.24, 25.14, 19.59, 19.18, 18.4, 18.37, 14.92, 14.49, 8.09, 8.05; ν_{max}: 3435, 2924, 2854, 1456, 1377, 1118, 1021 cm^{-1}.

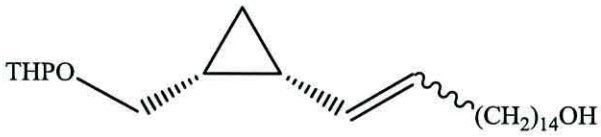
Experiment 44: (1*S*,2*R*)-2-((Tetrahydro-2*H*-pyran-2-yloxy)methyl) cyclopropanecarbaldehyde (198)

The procedure used in Experiment 9 was repeated in order to oxidise the (1*S*,2*R*)-2-((tetrahydro-2*H*-pyran-2-yloxy)methyl)cyclopropyl)methanol (**218**) (6.48 g, 34.79 mmol) using PCC (18.7 g, 86.97 mmol) in dichloromethane (200 ml). The crude product was purified by column chromatography on silica gel eluting with petrol/ethyl acetate (1:1) to give a colourless oil, (1*S*,2*R*)-2-((tetrahydro-2*H*-pyran-2-yloxy)methyl)cyclopropanecarbaldehyde (**198**) (3.5 g, 54 %), $[\alpha]_D^{18}$ = + 19.3 (*c* 1.15, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 9.41(1H, t, *J* 4.7Hz), 9.38 (1H, t, *J* 5.05 Hz), 4.59-4.58 (1H, m), 4.45-4.43 (1H, m), 4.11 (1H, dd, *J* 2.2, 5.7 Hz), 3.83-3.76 (1H, m), 3.75-3.72 (1H, m), 3.71-3.561 (1H, m), 3.48-3.42 (2H, m), 3.35-3.30 (1H, m), 2.01-1.91 (2H, m), 1.83-1.68 (3H, m), 1.65-1.60 (2H, m), 1.53-1.46 (8H, m), 1.30-1.24 (2H, m),1.23-1.17 (2H, m), 0.54-0.49 (1H, m), 0.17-0.15 (1H, m); δ_C (125MHz, $CDCl_3$): 200.19, 200.12, 98.74, 98.08, 65.16, 65.08, 62.22, 61.69, 30.35, 30.25, 26.84, 26.78, 25.15, 23.54, 22.97, 19.37, 18.97, 13.93, 12.02, 11.96; ν_{max}: 2944, 2871, 1704, 1371, 1201, 1172, 1120, 1061 cm^{-1}.

Experiment 45: (*E/Z*)-16-((1*R*,2*R*)-2-((Tetrahydro-2*H*-pyran-2-yloxy)methyl) cyclopropyl)hexadec-15-enoate (219)

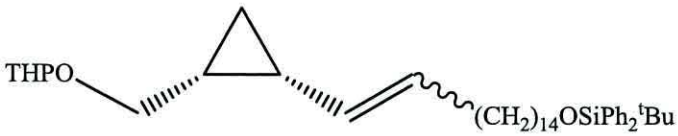
Lithium bis(trimethylsilyl)amide (18 g, 28.72 mmol, 1.06 M) was added dropwise with stirring to (1*R*,2*R*)-2-((tetrahydro-2*H*-pyran-2-yloxy)methyl)cyclopropanecarbaldehyde (**198**) (2.94 g, 15.95 mmol) and 15-(1-phenyl-1*H*-tetrazole-5-sulfonyl)-pentadecanoic acid methyl ester (**167**) (8.88 g, 20.74 mmol) in dry THF (100 ml) under nitrogen at - 10 °C. The mixture was allowed to reach r.t. and stirred for 1.5 hour, when TLC no starting material, then quenched with sat. aq. NH_4Cl (10 ml) and petrol/ethyl acetate (1:1, 50 ml). The aqueous layer was re-extracted with petro/ethyl acetate (1:1, 2 × 50 ml), dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (7:1) to give a colourless oil, (*E/Z*)-16-((1*R*,2*R*)-2-((tetrahydro-2*H*-pyran-2-yloxy)methyl)cyclopropyl)hexadec-15-enoate (**219**) (6 g, 89 %) as a mixture of isomers in ratio 3.5:1.

Experiment 46: *(E/Z)*-16-((1*R*,2*R*)-2-((Tetrahydro-2*H*-pyran-2-yloxy) methyl)cyclopropyl) hexadec-15-en-1-ol (220)



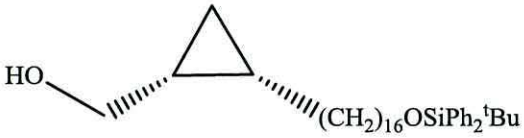
The procedure used in Experiment 21 was repeated in order to reduce the (*E/Z*)-16-((1*R*,2*R*)-2-((tetrahydro-2*H*-pyran-2-yloxy)methyl)cyclopropyl)hexadec-15-enoate (**219**) (6 g, 14.19 mmol) using lithium aluminum hydride (0.8 g, 21.28 mmol) in tetrahydrofuran (150 ml). The crude product was purified by chromatography on silica gel eluting with petrol/ethyl acetate (2:1) to give a colourless oil, *(E/Z)*-16-((1*R*,2*R*)-2-((tetrahydro-2*H*-pyran-2-yloxy)methyl)cyclopropyl)hexadec-15-en-1-ol (**220**) (4.5 g, 80 %).

Experiment 47: *tert*-Butyldiphenyl-((*E/Z*)-16-((1*R*,2*R*)-2-((tetrahydro-2*H*-pyran-2-yloxy)methyl)cyclopropyl)hexadec-15-enyloxy)silane (221)



Triethylamine (1.38 ml, 13.68 mmol) was added dropwise at 0 °C to a solution of *(E/Z)*-16-((1*R*,2*R*)-2-((tetrahydro-2*H*-pyran-2-yloxy)methyl)cyclopropyl)hexadec-15-en-1-ol (**220**) (4.5 g, 11.40 mmol) in dry dichloromethane (50 ml) under nitrogen. After stirring for 10 min, *tert*-butyl diphenylchlorosilane (3.44 g, 12.54 mmol) was added followed by the addition of 4-dimethylaminopyridine (29 mg). The reaction mixture was then stirred for 5 hours at r.t. followed by quenching with water (10 ml). The aqueous layer was extracted with dichloromethane (3 × 30 ml) and the combined organic layers were dried and concentrated to give the crude product. This was purified by column chromatography eluting with petrol/ethyl acetate (5:1) to give colourless oil, *tert*-butyldiphenyl-((*E/Z*)-16-((1*R*,2*R*)-2-((tetrahydro-2*H*-pyran-2-yloxy)methyl) cyclopropyl)hexadec-15-enyloxy)silane (**221**) (6.87 g, 95%).

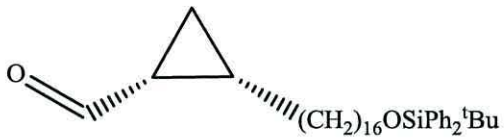
Experiment 48: ((1*R*,2*S*)-2-(16-(*tert*-Butyldiphenylsilyloxy)hexadecyl)cyclopropyl) methanol (223)



A solution of pyridinium-*p*-toluene sulfonate (1.36 g, 5.42 mmol) in methanol (10 ml) was added to a stirred solution of *tert*-butyldiphenyl ((*E/Z*)-16-((1*R*,2*R*)-2-((tetrahydro-2*H*-pyran-2-yloxy)methyl)cyclopropyl)hexadec-15-enyloxy)silane (**221**) (6.87 g, 10.85 mmol) in THF (40 ml) and stirred at 50 °C overnight. TLC showed that the reaction was almost complete. Sat. aq. $NaHCO_3$ (10 ml) and water (25 ml) were added and extracted with ethyl acetate (3×40 ml). The combined organic layers were dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (6:1) to give a colourless oil, ((1*R*,2*R*)-2-((*E/Z*)-16-(*tert*-butyldiphenylsilyloxy)hexadec-1-enyl)cyclopropyl)methanol (**222**) (4.88 g, 82 %). Hydrogenation was carried out with dipotassium azodicarboxylate as before and the crude product was purified by column chromatography eluting with petrol/ethyl acetate

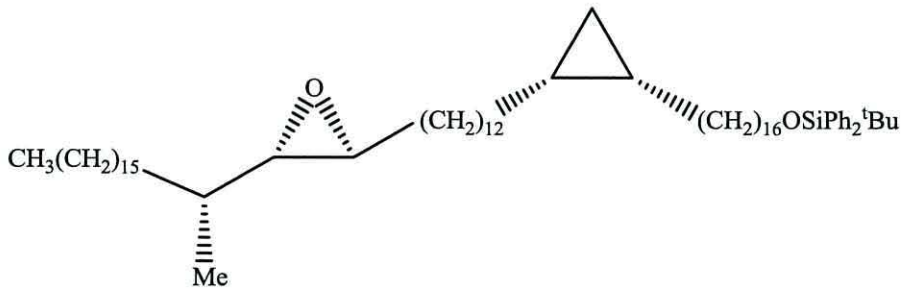
(2:1) to give a colourless oil, ((1*R*,2*S*)-2-(16-(*tert*-butyldiphenylsilyloxy)hexadecyl)cyclopropyl)methanol (**223**) (4.8 g, 98 %) [Found (M + Na)$^+$: 573.4099, $C_{36}H_{58}NaO_2Si$ requires: 573.4098], $[\alpha]_D^{22}$ = + 7.47 (*c* 1.07, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 7.70-7.68 (4H, m), 7.45-7.37 (6H, m), 3.68-3.64 (3H, including a triplet resonated at δ 3.67 (*J* 6.3 Hz)), 3.59 (1H, dd, *J* 7.85, 11 Hz), 1.57 (2H, pent., *J* 6.95 Hz), 1.49-1.23 (29H, m), 1.12-1.10 (1H, m), 1.06 (9H, s), 0.92-0.84 (1H, m), 0.71 (1H, dt, *J* 4.45, 8.2 Hz), -0.019 (1H, br q, *J* 5.35 Hz); δ_C (125MHz, $CDCl_3$): 135.54, 134.16, 129.42, 127.52, 63.99, 63.29, 32.56, 30.15, 29.67, 29.65, 29.63, 29.60, 29.55, 29.36, 28.53, 26.84, 25.74, 19.18, 18.13, 16.13, 9.45; ν_{max}: 3368, 3072, 2924, 2853, 1746, 1593, 1463, 1428, 1111, 1030 cm^{-1}.

Experiment 49: (1*S*,2*R*)-2-(16-(tert-butyldiphenylsilyloxy)hexadecyl) cyclopropanecarbaldehyde (197)



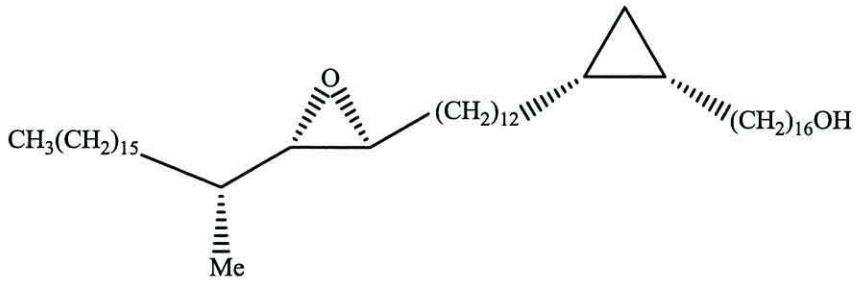
The procedure used in Experiment 9 was used repeated in order to oxidise the ((1*R*,2*S*)-2-(16-(*tert*-butyldiphenylsilyloxy)hexadecyl)cyclopropyl)methanol (**223**) (1.2 g, 2.17 mmol) using PCC (1.17 g, 5.44 mmol) in dichloromethane (30 ml). The crude product was purified by column chromatography on silica eluting with petrol/ethyl acetate (1:1) to give a colourless oil, (1*S*,2*R*)-2-(16-(*tert*-butyldiphenylsilyloxy)hexadecyl) cyclopropanecarbaldehyde (**197**)(1.18 g, 98 %), $[\alpha]_D^{23}$ = + 48.64 (*c* 1.11, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 9.35 (1H, d, *J* 5.65 Hz), 7.69-7.67 (4H, m), 7.44-7.36 (6H, m), 3.66 (2H, t, *J* 6.6 Hz), 1.89-1.84 (1H, m), 1.63-1.24 (33H, m, including br s at 1.26), 1.05 (9H, s); δ_C (125MHz, $CDCl_3$): 201.80, 135.56, 134.19, 129.43, 127.53, 64.01, 32.57, 29.96, 29.66, 29.63, 29.61, 29.53, 29.37, 29.24, 28.21, 27.80, 26.86, 25.76, 24.77, 19.21, 14.73; ν_{max}: 3072, 2925, 2854, 1704, 1463, 1428, 1111 cm^{-1}.

Experiment 50: *tert*-Butyl(16-((1*S*,2*R*)-2-(12-((2*S*,3*S*)-3-((*R*)-octadecan-2-yl)-oxiran-2-yl)dodecyl)cyclopropyl)hexadecyloxy)diphenylsilane (225)



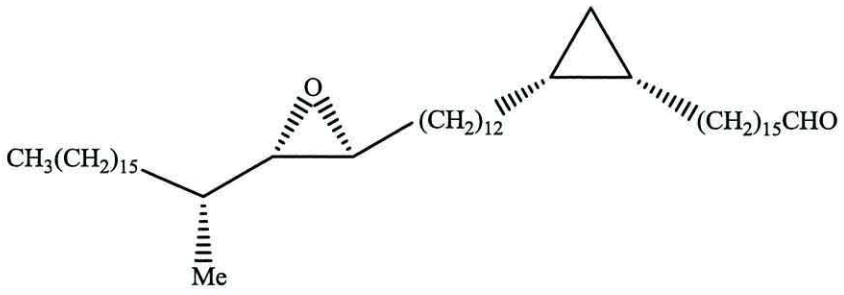
Lithium bis(trimethylsilyl)amide (1.54 g, 1.63 mmol, 1.06 M) was added dropwise with stirring to (1*S*,2*R*)-2-((tetrahydro-2*H*-pyran-2-yloxy)methyl)cyclopropanecarbaldehyde (**197**) (0.5 g, 0.91 mmol) and 5-(11-((2*S*,3*S*)-3-((*R*)-octadecan-2-yl)oxiran-2-yl)undecylsulfonyl)-1-phenyl-1*H*-tetrazole (**196**) (0.72 g, 1.09 mmol) in dry THF (30 ml) under nitrogen at - 10 °C. The mixture was allowed to reach r.t. and stirred for 1.5 hour, when TLC no starting material, then quenched with NH_4Cl (5 ml) and petrol/ethyl acetate (1:1, 20 ml). The aqueous layer was re-extracted with petrol/ethyl acetate (1:1, 2 × 20 ml), dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a colourless oil, *tert*-butyl-[16-((*S*)-2-{(*E/Z*)-12-[(2*S*,3*S*)-3-((*R*)-1-methyl-heptadecyl)-oxiranyl]dodec-1-enyl}-cyclopropyl)-hexadecyloxy]-diphenylsilane (**224**) (0.8 g, 89 %). Hydrogenation was carried out with potassium azodicarboxylate as before and crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a white solid, *tert*-butyl-(16-((1*S*,2*R*)-2-(12-((2*S*,3*S*)-3-((*R*)-octadecan-2-yl)-oxiran-2-yl)dodecyl)cyclopropyl)hexadecyloxy)diphenylsilane (**225**) (0.64 g, 80 %) [Found $(M + Na)^+$: 1005.8743, $C_{67}H_{118}NaO_2Si$ requires: 1005.8793], $[\alpha]_D^{20}$ = - 4.94 (*c* 0.85, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 7.68 (4H, dd, *J* 1.25, 7.9 Hz), 7.43-7.36 (6H, m), 3.65 (2H, t, *J* 6.6 Hz), 2.72 (1H, dt, *J* 2.2, 5.65 Hz), 2.41(1H, dd, *J* 1.85, 6.9 Hz), 1.60-1.22 (85H, br m, including br s at 1.25), 1.05 (9H, s), 1.0 (3H, d, *J* 6.0 Hz), 0.89 (3H, t, *J* 6.65 Hz), 0.67-0.64 (2H, m), 0.56 (1H, br dt, *J* 4.1, 8.2 Hz), -0.32 (1H, q, *J* 5.05 Hz); δ_C (125MHz, $CDCl_3$):135.56, 134.2, 129.44, 127.53, 64.02, 63.86, 58.87, 36.04, 33.77, 32.58, 32.26, 31.92, 30.22, 29.86, 29.74, 29.69, 29.63, 29.6, 29.58, 29.5, 29.38, 29.36, 28.72, 27.21, 26.86, 26.08, 25.76, 22.68, 19.21, 17.29, 15.77, 14.11, 10.91; ν_{max}: 3072, 2924, 2853, 1464, 1111 cm^{-1}.

Experiment 51: 16-((1*S*,2*R*)-2-{12-[(2*S*,3*S*)-3-((*R*)-1-Methylheptadecyl)-oxiranyl]-dodecyl}-cyclopropyl)-hexadecan-1-ol (226)



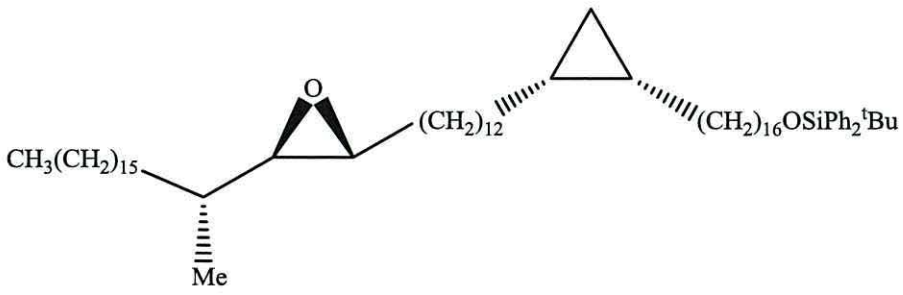
Tetra-n-butyl ammonium fluoride (0.97 ml, 0.97 mmol, 1M sol. in THF) was added to a stirred solution of *tert*-butyl-[16-((1*S*,2*R*)-2-{12-[(2*S*,3*S*)-3-((*R*)-1-methylheptadecyl)-oxiranyl]-dodecyl}-cyclopropyl)-hexadecyloxy]-diphenylsilane (**225**) (0.64 g, 0.65 mmol) in dry THF (30 ml) at 0 °C under nitrogen. The mixture was allowed to reach r.t. and stirred overnight, when TLC showed no starting material. The mixture was cooled to 5 °C and quenched with sat. aq. NH_4Cl (5 ml) and the product was extracted with ethyl acetate (3 × 50 ml), then washed with brine (20 ml), dried over $MgSO_4$ and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (6:1) to give a white solid, 16-((1*S*,2*R*)-2-{12-[(2*S*,3*S*)-3-((*R*)-1-methylheptadecyl)-oxiranyl]-dodecyl}-cyclopropyl)-hexadecan-1-ol (**226**) (0.44 g, 91 %), m.p: 60-63 °C [Found $(M + H)^+$: 745.7781, $C_{51}H_{101}O_2$ requires: 745.7796], $[\alpha]_D^{25}$ = - 6.63 (*c* 1.07, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 3.64 (2H, t, *J* 6.6 Hz), 2.72 (1H, dt, *J* 2.2, 5.35 Hz), 2.41 (1H, dd, *J* 2.2, 7.25 Hz), 1.60-1.23 (86H, br m, including br s at 1.26), 1.0 (3H, d, *J* 5.95 Hz), 0.88 (3H, t, *J* 6.95 Hz), 0.69-0.64 (2H, m), 0.56 (1H, dt, *J* 4.1, 8.2 Hz), -0.32 (1H, q, *J* 5.05 Hz); δ_C (125MHz, $CDCl_3$): 63.89, 63.09, 58.9, 36.03, 33.77, 32.8, 32.25, 31.92, 30.21, 29.85, 29.72, 29.69, 29.63, 29.60, 29.58, 29.5, 29.42, 29.35, 28.71, 27.2, 26.54, 26.07, 25.72, 22.68, 17.3, 15.76, 14.11, 10.89; ν_{max}: 3418, 2925, 2854, 1464, 1112 cm^{-1}.

Experiment 52: 16-((1*S*,2*R*)-2-{12-[(2*S*,3*S*)-3-((*R*)-1-Methylheptadecyl)-oxiranyl]-dodcyl}-cyclopropyl)-hexadecanal (195)



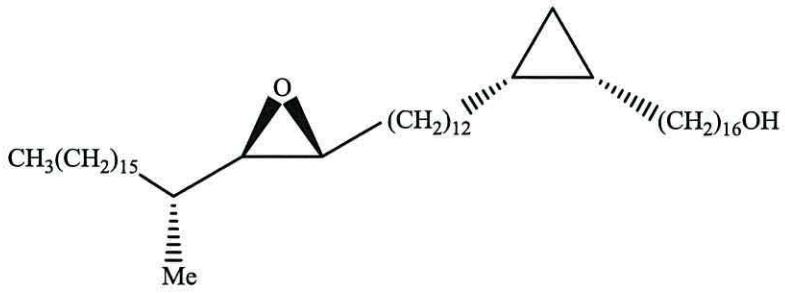
16-((1*S*,2*R*)-2-{12-[(2*S*,3*S*)-3-((*R*)-1-Methylheptadecyl)-oxiranyl]-dodecyl}-cyclopropyl)-hexadecan-1-ol (**226**) (0.44 g, 0.59 mmol) in dichloromethane (10 ml) was added to a stirred suspension of pyridinium chlorochromate (0.3 g, 1.47 mmol) in dichloromethane (30 ml) at room temperature. The mixture was stirred vigorously at r.t. for 2 hours, when TLC showed no starting material was left. It was poured into petrol/ethyl acetate (10:1, 30 ml) and filtered through a pad of silica and celite then washed well with petrol/ethyl acetate and the filtrate was evaporated to give a residue. The crude product was purified by column chromatography on silica gel eluting with petrol/ethyl acetate (8:1) to give a white solid, 16-((1*S*,2*R*)-2-{12-[(2*S*,3*S*)-3-((*R*)-1-methylheptadecyl)-oxiranyl]-dodcyl}-cyclopropyl)-hexadecanal (**195**) (0.33 g, 76 %), m.p.: 49-51 °C, $[\alpha]_D^{21}$ = - 11.4 (*c* 0.7, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 9.77 (1H, t, *J* 1.55 Hz), 2.72 (1H, dt, *J* 1.9, 5.35 Hz), 2.44-2.40 (2H, m), 1.66-1.22 (84H, br m including br s at 1.26), 1.0 (3H, d, *J* 6 Hz), 0.88 (3H, t, *J* 6.6 Hz), 0.69-0.64 (2H, m), 0.56 (1H, dt, *J* 4.1, 8.15 Hz), -0.32 (1H, q, *J* 5.05 Hz); δ_C (125MHz, $CDCl_3$): 202.94, 63.86, 58.87, 43.91, 36.04, 33.77, 32.25, 31.92, 30.22, 29.86, 29.73, 29.69, 29.66, 29.63, 29.58, 29.5, 29.42, 29.35, 29.16, 28.71, 27.21, 26.54, 26.07, 22.68, 22.08, 17.29, 15.76, 14.11, 10.9; ν_{max}: 2922, 2851, 1731, 1463, 1372, 1231, 1115 cm^{-1}.

Experiment 53: *tert*-Butyl(16-((1*S*,2*R*)-2-(12-((2*R*,3*R*)-3-((*R*)-octadecan-2-yl)-oxiran-2-yl)dodecyl)cyclopropyl)hexadecyloxy)diphenylsilane (228)



The procedure used in Experiment 50 was repeated in order to couple (1*S*,2*R*)-2-((tetrahydro-2*H*-pyran-2-yloxy)methyl)cyclopropanecarbaldehyde (**197**) (0.5 g, 0.91 mmol) and 5-(11-((2*R*,3*R*)-3-((*R*)-octadecan-2-yl)oxiran-2-yl)undecylsulfonyl)-1-phenyl-1*H*-tetrazole (**212**) (0.72 g, 1.09 mmol) using lithium bis(trimethylsilyl)amide (1.54 g, 1.63 mmol, 1.06 M) in dry THF (30 ml) under nitrogen at - 10 °C. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a colourless oil, *tert*-butyl-[16-((*S*)-2-{(*E/Z*)-12-[(2*R*,3*R*)-3-((*R*)-1-methylheptadecyl)-oxiranyl]dodec-1-enyl}-cyclopropyl)-hexadecyloxy]-diphenylsilane (**227**) (0.88 g, 89 %). Hydrogenation was carried out with potassium azodicarboxylate as before and crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a white solid, *tert*-butyl (16-((1*S*,2*R*)-2-(12-((2*R*,3*R*)-3-((*R*)-octadecan-2-yl)-oxiran-2-yl)dodecyl)cyclopropyl)hexadecyloxy)diphenylsilane (**228**) (0.76 g, 86 %) [Found $(M + Na)^+$: 1005.8773, $C_{67}H_{118}NaO_2Si$ requires: 1005.8793], $[\alpha]_D^{20} = +1.96$ (*c* 1.02, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 7.67 (4H, dd, *J* 1.6, 7.9 Hz), 7.43-7.36 (6H, m), 3.65 (2H, t, *J* 6.6 Hz), 2.67 (1H, dt, *J* 2.2, 5.35 Hz), 2.47 (1H, dd, *J* 2.55, 7.25 Hz), 1.89-1.81 (1H, m), 1.58-1.22 (84H, br m, including br s at 1.26), 1.05 (9H, s), 0.92 (3H, d, *J* 6.6 Hz), 0.89 (3H, t, *J* 6.65 Hz), 0.67-0.62 (2H, m), 0.56 (1H, br dt, *J* 4.1, 8.2 Hz), -0.32 (1H, q, *J* 5.05 Hz); δ_C (125MHz, $CDCl_3$): 135.56, 134.19, 129.43, 127.53, 64.01, 63.81, 57.47, 35.82, 34.6, 32.57, 32.2, 31.91, 30.21, 29.93, 29.7, 29.69, 29.64, 29.61, 29.55, 29.44, 29.37, 29.35, 29.11, 28.71, 26.88, 26.86, 26.13, 25.76, 23.9, 22.67, 19.2, 15.94, 15.76, 14.1, 10.9; ν_{max}: 3072, 2924, 2853, 1464, 1111 cm^{-1}.

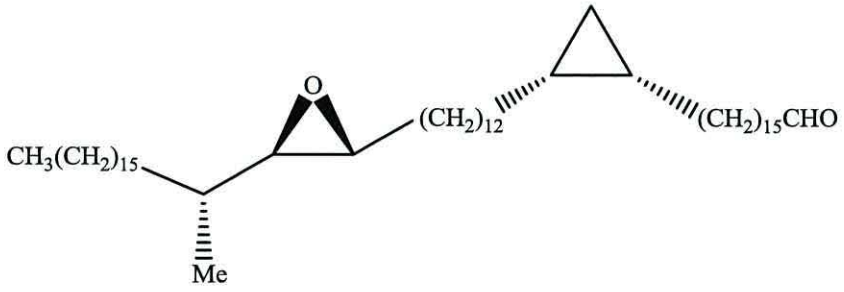
Experiment 54: 16-((1*S*,2*R*)-2-{12-[(2*R*,3*R*)-3-((*R*)-1-Methylheptadecyl)-oxiranyl]-dodecyl}-cyclopropyl)-hexadecan-1-ol (229)



n-TBAF (1.15 ml, 1.158 mmol) was added to a stirred solution of *tert*-butyl-[16-((1S,2*R*)-2-{12-[(2*R*,3*R*)-3-((*R*)-1-methylheptadecyl)-oxiranyl]-dodecyl}-cyclopropyl)-

hexadecyloxy]-diphenylsilane (**228**) (0.76 g, 0.77 mmol) in dry THF (30 ml) at 0 °C under nitrogen. The mixture was allowed to reach r.t. and stirred overnight, when TLC showed no starting material. The mixture was cooled to 5 °C and quenched with sat. aq. NH_4Cl (5 ml) and the product was extracted with ethyl acetate (3 × 50 ml), then washed with brine (20 ml), dried over $MgSO_4$ and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (6:1) to give a white solid, 16-((1*S*,2*R*)-2-{12-[(2*R*,3*R*)-3-((*R*)-1-methylheptadecyl)-oxiranyl]-dodecyl}-cyclopropyl)-hexadecan-1-ol (**229**) (0.52, 91 %), m.p.: 55-57 °C [Found $(M + Na)^+$: 767.7632, $C_{51}H_{100}NaO_2$ requires: 767.7616], $[\alpha]_D^{25} = + 5.27$ (*c* 1.08, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 3.64 (2H, t, *J* 6.65 Hz), 2.67 (1H, dt, *J* 2.2, 5.7 Hz), 2.46 (1H, dd, *J* 2.2, 7.25 Hz), 1.59-1.22 (86H, br m, including br s at 1.26), 0.92 (3H, d, *J* 6.6 Hz), 0.89 (3H, t, *J* 6.6 Hz), 0.69-0.61 (2H, m), 0.56 (1H, dt, *J* 4.1, 7.85 Hz), -0.32 (1H, q, *J* 5.05 Hz); δ_C (125MHz, $CDCl_3$): 63.82, 63.09, 57.48, 35.82, 34.6, 32.8, 32.21, 31.92, 30.21, 29.93, 29.72, 29.69, 29.61, 29.55, 29.44, 29.42, 29.35, 28.71, 26.88, 26.54, 26.14, 25.73, 22.67, 15.94, 15.77, 14.1, 10.9; ν_{max}: 3418, 2925, 2854, 1464, 1112 cm^{-1}.

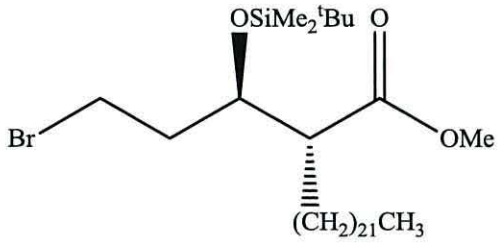
Experiment 55: 16-((1*S*,2*R*)-2-{12-[(2*R*,3*R*)-3-((*R*)-1-Methylheptadecyl)-oxiranyl]-dodcyl}-cyclopropyl)-hexadecanal (194)



The procedure used in Experiment 9 was repeated in order to oxidise the 16-((1*S*,2*R*)-2-{12-[(2*R*,3*R*)-3-((*R*)-1-methylheptadecyl)-oxiranyl]-dodecyl}cyclopropyl)hexadecan-1-ol (**229**) (0.36 g, 0.483 mmol) using PCC (0.26 g, 1.207 mmol) in dichloromethane (30 ml). The crude product was purified by column chromatography on silica eluting with petrol/ethyl acetate (8:1) to give a white solid, 16-((1*S*,2*R*)-2-{12-[(2*R*,3*R*)-3-((*R*)-1-methylheptadecyl)-oxiranyl]-dodcyl}cyclopropyl)hexadecanal (**194**) (0.28 g, 80 %), m.p.: 36-38 °C, $[\alpha]_D^{20} = + 8.13$ (*c* 1.07, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 9.77 (1H, t, *J* 1.9 Hz), 2.67 (1H, dt, *J* 2.25, 5.4 Hz), 2.46 (1H, dd, *J* 2.2, 7.25 Hz), 2.42 (2H,dt, *J* 1.6, 7.25 Hz), 1.66-1.22 (83H, br m, including br s at 1.26), 0.92 (3H, d, *J* 6.9

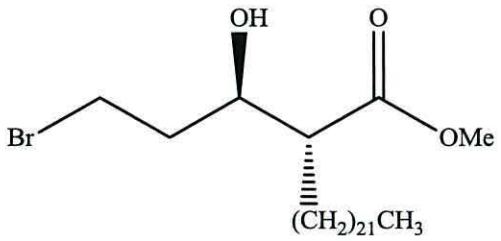
Hz), 0.88 (3H, t, *J* 6.95 Hz), 0.67-0.64 (2H, m), 0.56 (1H, dt, *J* 4.1, 8.2 Hz), -0.32 (1H, q, *J* 5.05 Hz); δ_C (125MHz, $CDCl_3$): 202.95, 63.81, 57.47, 43.91, 35.83, 34.61, 32.21, 31.92, 30.21, 29.93, 29.73, 29.69, 29.65, 29.61, 29.58, 29.56, 29.44, 29.42, 29.35, 29.16, 28.71, 26.89, 26.54, 26.14, 22.68, 22.08, 15.95, 15.76, 14.11, 10.91; ν_{max}: 2923, 2853, 1731, 1464, 1373, 1234, 1115 cm^{-1}.

Experiment 56: (*R*)-2-[(*R*)-3-Bromo-1-(*tert*-butyldimethylsilanyloxy)-propyl]-tetracosanoic acid methyl ester (230)



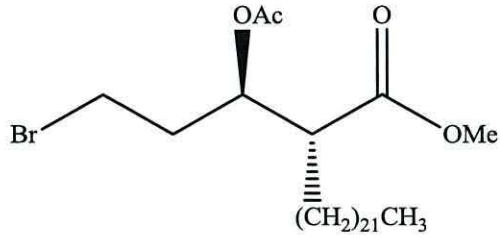
The procedure used in Experiment 13 was repeated in order to convert (*R*)-2-[(*R*)-1-(*tert*-butyl-dimethyl-silanyloxy)-3-hydroxy-propyl]-tetracosanoic acid methyl ester (**176**) (3 g, 5.25 mmol), NBS (1.21 g, 6.83 mmol) and triphenylphosphine (1.72 g, 6.56 mmol) in dichloromethane (70 ml) at 0 °C into a thick oil, (*R*)-2-[(*R*)-3-bromo-1-(*tert*-butyl-dimethylsilanyloxy)-propyl]-tetracosanoic acid methyl ester (**230**) (2 g, 60 %) [Found $(M + Na)^+$: 655.4085, $C_{34}H_{69}BrNaO_3Si$ requires: 655.4092], $[\alpha]_D^{22}$ = + 7.83 (*c* 1.06, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 4.10-4.07 (1H, m), 3.68 (3H, s), 3.46-3.42 (2H, m), 2.54 (1H, ddd, *J* 3.8, 5.65, 9.45 Hz), 2.09-2.02 (1H, m), 1.97 (1H, dddd, *J* 3.45, 7.85, 11.35, 15.45 Hz), 1.67-1.22 (42H, br m, including br s at 1.26), 0.9-0.86 (12H, m, including singlet resonated at 0.88 for the ^{t}Bu and a triplet resonated at 0.89 with *J* 5.65 Hz), 0.11 (3H, s), 0.08 (3H, s); δ_C (125MHz, $CDCl_3$): 174.08, 71.30, 51.62, 51.49, 36.81, 31.92, 29.69, 29.64, 29.61, 29.58, 29.55, 29.42, 29.35, 28.0, 26.47, 25.71, 22.67, 17.95, 14.11, 14.04, -4.56, -4.79; ν_{max}: 2924, 2854, 1741, 1463, 1254, 1072 cm^{-1}.

Experiment 57: (*R*)-2-((*R*)-3-Bromo-1-hydroxy-propyl]-tetracosanoic acid methyl ester (231)



The procedure used in Experiment 26 was repeated using (*R*)-2-[(*R*)-3-bromo-1-(*tert*-butyl-dimethyl-silanyloxy)-propyl]-tetracosanoic acid methyl ester (**230**) (2 g, 3.155 mmol), pyridine (0.2 ml) and hydrogen fluoride-pyridine complex (1.5 ml) dissolved in dry THF (20 ml). The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1 then 5:1) to give a white solid, (*R*)-2-((*R*)-3-bromo-1-hydroxy-propyl]-tetracosanoic acid methyl ester (**231**) (1.26 g, 77 %), m.p.: 59-61 °C [Found $(M + Na)^+$: 541.3209, $C_{28}H_{55}BrNaO_3$ requires: 541.3227], $[\alpha]_D^{23} = +13.8$ (*c* 1.0, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 3.91 (1H, br s), 3.72 (3H, s), 3.59-3.55 (2H, m), 2.69 (1H, d, *J* 7.55 Hz), 2.45 (1H, dt, *J* 5.35, 8.8 Hz), 2.05-1.91 (2H, m), 1.73-1.69 (1H, m), 1.66-1.59 (1H, m), 1.38-1.22 (40H, br m, including br s at 1.26), 0.88 (3H, t, *J* 6.65 Hz); δ_C (125MHz, $CDCl_3$): 175.92, 70.04, 51.71, 50.61, 38.42, 31.91, 30.14, 29.69, 29.64, 29.60, 29.53, 29.50, 29.44, 29.37, 29.34, 27.22, 22.67, 14.11; ν_{max}: 3429, 2918, 2850, 1728, 1473 cm^{-1}.

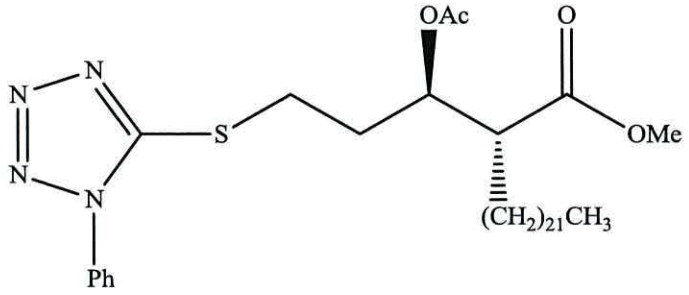
Experiment 58: (*R*)-2-((*R*)-1-Acetoxy-3-bromopropyl]-tetracosanoic acid methyl ester (232)



The procedure used in Experiment 27 was repeated using the secondary alcohol (**231**) (1.2 g, 2.309 mmol), acetic anhydride (5 ml) and anhydrous pyridine (5 ml) in dry toluene (15 ml). The crude product was purified by column chromatography eluting with petrol/ethyl acetate (8:1) to give a white solid, (*R*)-2-((*R*)-1-acetoxy-3-bromo-propyl]-tetracosanoic acid methyl ester (**232**) (1.24 g, 95 %), m.p.: 50-52 °C [Found (M

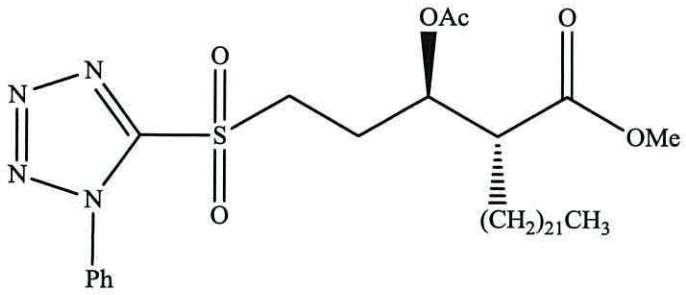
+ Na)$^+$: 585.3296, $C_{30}H_{57}BrNaO_4$ requires: 583.3332], $[\alpha]_D^{23}$ = + 26.07 (*c* 0.56, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 5.22 (1H, ddd, *J* 4.4, 6.6, 8.2 Hz), 3.70 (3H, s), 3.40-3.31 (2H, m), 2.66 (1H, ddd, *J* 4.1, 6.3, 10.4 Hz), 2.21-2.15 (2H, m), 2.06 (3H, s), 1.70-1.63 (1H, m), 1.49-1.45 (1H, m), 1.38-1.21 (40H, br m, including br s at 1.25), 0.88 (3H, t, *J* 6.6Hz); δ_C (125MHz, $CDCl_3$): 172.97, 170.15, 72.4, 51.74, 49.24, 35.19, 31.91, 29.69, 29.65, 29.61, 29.53, 29.42, 29.35, 28.24, 27.81, 27.43, 22.67, 20.89, 14.11; ν_{max}: 2919, 2851, 1744, 1473, 1381, 1234, 1028 cm^{-1}.

Experiment 59: (*R*)-2-[(*R*)-1-Acetoxy-3-(1-phenyl-1*H*-tetrazol-5-ylsulfanyl)-propyl]-tetracosanoic acid methyl ester (233)



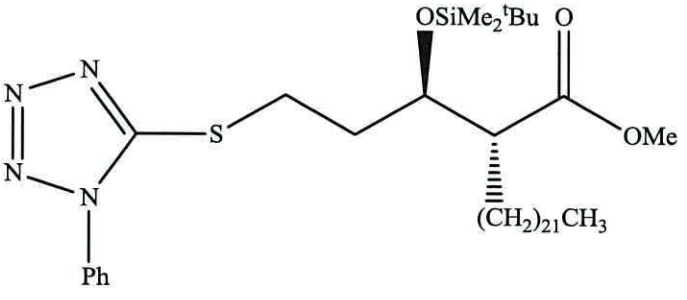
The procedure used in Experiment 1 was repeated using (*R*)-2-((*R*)-1-acetoxy-3-bromo-propyl]-tetracosanoic acid methyl ester (**232**) (1.21 g, 2.154 mmol), 1-phenyl-1*H*-tetrazole-5-thiol (0.42 g, 2.369 mmol) and anhydrous potassium carbonate (0.59 g, 4.3 mmol) in acetone (30 ml). The crude product was purified by chromatography on silica gel eluting with petrol/ethyl acetate (5:1) to give a white solid, (*R*)-2-[(*R*)-1-acetoxy-3-(1-phenyl-1*H*-tetrazol-5-ylsulfanyl)-propyl]-tetracosanoic acid methyl ester (**233**) (0.52 g, 84 %), m.p.: 50-52 °C [Found (M + H)$^+$: 659.4538, $C_{37}H_{63}N_4O_4S$ requires: 659.4565], $[\alpha]_D^{24}$ = + 13.84 (*c* 0.52, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 7.62-7.52 (5H, m), 5.24 (1H, ddd, *J* 3.15, 7.25, 9.8 Hz), 3.68 (3H, s), 3.47 (1H, ddd, *J* 5, 8.15, 13.55 Hz), 3.29 (1H, dt, *J* 7.9, 13.9 Hz), 2.67 (1H, ddd, *J* 4.1, 6.95, 10.7 Hz), 2.28-2.22 (1H, m), 2.16-2.09 (1H, m), 2.06 (3H, s), 1.68-1.24 (42H, br m, including br s at 1.25), 0.88 (3H, t, *J* 6.9 Hz); δ_C (125MHz, $CDCl_3$): 173.11, 170.42, 153.94, 133.60, 130.13, 129.8, 123.79, 72.57, 51.72, 49.36, 31.9, 31.58, 29.68, 29.65, 29.63, 29.6, 29.53, 29.41, 29.38, 29.34, 29.24, 28.01, 27.37, 22.67, 20.96, 14.1; ν_{max}: 2922, 2852, 1743, 1500, 1465, 1233 cm^{-1}.

Experiment 60: (*R*)-2-[(*R*)-1-Acetoxy-3-(1-phenyl-1*H*-tetrazole-5-sulfonyl)-propyl]-tetracosanoic acid methyl ester (234)



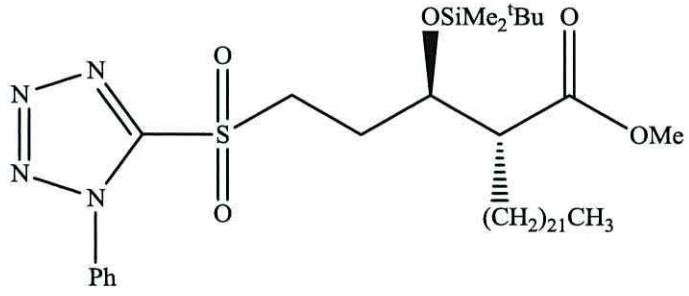
The procedure used in Experiment 2 was repeated using (*R*)-2-[(*R*)-1-acetoxy-3-(1-phenyl-1*H*-tetrazole-5-ylsulfany]-propyl]-tetracosanoic acid methyl ester (**233**) (1.38 g, 2.09 mmol), ammonium molybdate (VI) tetrahydrate (1.16 g, 0.942 mmol) in ice cold H_2O_2 (35 % w/w, 2.5 ml), IMS (15 ml) and THF (15 ml), and a further solution of ammonium molybdate (VI) tetrahydrate (0.6 g, 0.487 mmol) in ice cold H_2O_2 (35 % w/w, 2 ml) to give a crude product. This was purified by column chromatography eluting with petrol/ethyl acetate (5:1) to give a white solid, (*R*)-2-[(*R*)-1-acetoxy-3-(1-phenyl-1*H*-tetrazole-5-sulfonyl)-propyl]-tetracosanoic acid methyl ester(**234**) (1.15 g, 80 %), m.p.: 80-82 °C [Found $(M + Na)^+$: 713.4282, $C_{37}H_{62}N_4NaO_6S$ requires: 713.4282], $[\alpha]_D^{20} = +22.15$ (*c* 0.79, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 7.70-7.57 (5H, m), 5.23 (1H, dt, *J* 3.15, 7.9 Hz), 3.84-3.73 (2H, m), 3.7 (3H, s), 2.66 (1H, ddd, *J* 4.1, 6.95, 10.7 Hz), 2.41-2.34 (1H, m), 2.27-2.20 (1H, m), 2.09 (3H, s), 1.69-1.60 (2H, m), 1.51-1.21 (40H, br m, including br s at 1.25), 0.88 (3H, t, *J* 6.6 Hz); δ_C (125MHz, $CDCl_3$): 172.67, 170.15, 153.13, 132.91, 131.50, 129.74, 124.98, 71.68, 52.52, 51.87, 49.16, 31.89, 29.67, 29.64, 29.63, 29.58, 29.50, 29.38, 29.34, 27.89, 27.28, 24.88, 22.66, 20.84, 14.09; ν_{max}: 2918, 2850, 1737, 1468, 1375, 1337,1228, 1167 cm^{-1}.

Experiment 61: (*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-3-(1-phenyl-1*H*-tetrazole-5-ylsulfanyl)-propyl]-tetracosanoic acid methyl ester (235)



The procedure used in Experiment 1 was repeated using (*R*)-2-((*R*)-3-bromo-1-(*tert*-butyl-dimethylsilanyloxy)-propyl]-tetracosanoic acid methyl ester (**230**) (0.7 g, 1.10 mmol), 1-phenyl-1*H*-tetrazole-5-thiol (0.21 g, 1.21 mmol) and anhydrous potassium carbonate (0.3 g, 2.2 mmol) in acetone (30 ml) to give a colourless oil, (*R*)-2-[(*R*)-1-(*tert*-butyl-dimethyl-silanyloxy)-3-(1-phenyl-1*H*-tetrazole-5-ylsulfanyl)-propyl]-tetracosanoic acid methyl ester (**235**) (0.76 g, 95 %) [Found $(M + Na)^+$: 753.517, $C_{41}H_{74}N_4NaO_3SSi$ requires: 753.5143], $[\alpha]_D^{22}$ = - 9.87 (*c* 0.82, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 7.61-7.51 (5H, m), 4.07 (1H, dt, *J* 4.1, 6.3 Hz), 3.66 (3H, s), 3.49-3.43 (1H, m), 3.40-3.34 (1H, m), 2.59 (1H, ddd, *J* 4.75, 6.95, 11.35 Hz), 2.14-2.07 (1H, m), 2.01-1.93 (1H, m), 1.59-1.21 (42H, br m, including br s at 1.25), 0.89-0.87 (12H, m, including a singlet resonating at 0.88 for ^{t}Bu and a triplet at 0.87 with *J* 4.1 Hz), 0.07 (3H, s), 0.05 (3H, s); δ_C (125MHz, $CDCl_3$)): 174.39, 154.15, 133.71, 130.07, 129.76, 123.81, 72.0, 51.5, 51.46, 33.12, 31.92, 29.69, 29.64, 29.63, 29.56, 29.45, 29.35, 28.56, 27.84, 27.1, 25.71, 22.67, 17.92, 14.11, -4.42, -4.91; ν_{max}: 2924, 2853, 1738, 1504, 1466, 1254, 1085 cm^{-1}.

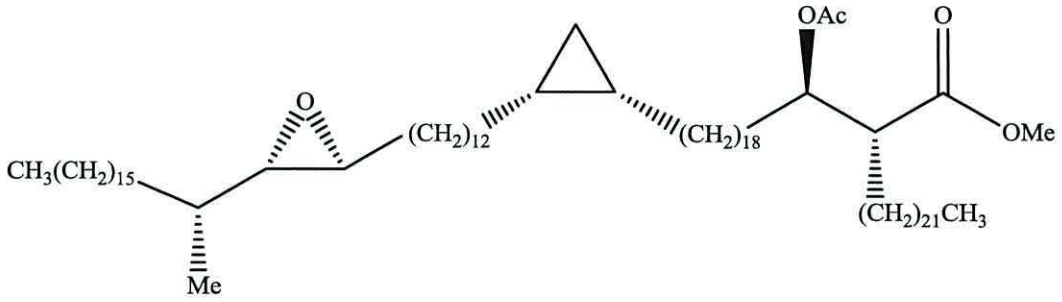
Experiment 62: (*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-3-(1-phenyl-1*H*-tetrazole-5-sulfonyl)-propyl]-tetracosanoic acid methyl ester (236)



The procedure used in Experiment 2 was repeated using the (*R*)-2-[(*R*)-1-(*tert*-butyl-dimethylsilanyloxy)-3-(1-phenyl-1*H*-tetrazole-5-ylsulfanyl)-propyl]-tetracosanoic acid methyl ester (**235**) (0.75 g, 1.02 mmol), ammonium molybdate (VI) tetrahydrate (0.57 g, 0.461 mmol) in ice cold H_2O_2 (35 % w/w, 1.5 ml) in IMS (15 ml) and THF (2 ml) and a further solution of ammonium molybdate (VI) tetrahydrate (0.3 g, 0.218 mmol) in ice cold H_2O_2 (35 % w/w, 1 ml). The crude product was purified by column chromatography eluting with petrol/ethyl acetate (6:1) to give a white solid, (*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilanyloxy)-3-(1-phenyl-1*H*-tetrazole-5-sulfonyl)-propyl]-tetracosanoic acid methyl ester (**236**) (0.71 g, 90 %), m.p: 65-67 °C [Found $(M + Na)^+$:

785.5025, $C_{41}H_{74}N_4NaO_5SSi$ requires: 785.5041], $[\alpha]_D^{20}$ = - 9.63 (*c* 0.82, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 7.71-7.7 (2H, m), 7.65-7.59 (3H, m), 4.16-4.12 (1H, m), 3.81-3.77 (2H, m), 3.68 (3H, s), 2.52 (1H, ddd, *J* 3.75, 7.55, 11.35 Hz), 2.23-2.07 (2H, m), 1.61-1.22 (42H, br m, including br s at 1.25), 0.89-087 (12H, m, including a singlet resonated at 0.88 for the ^{t}Bu and a triplet resonated at 0.87 with *J* 3.15 Hz), 0.10 (3H, s), 0.06 (3H, s); δ_C (125MHz, $CDCl_3$): 174.08, 153.36, 133.01, 131.45, 129.72, 125.0, 123.81, 70.92, 51.74, 51.58, 51.33, 31.91, 29.69, 29.64, 29.61, 29.54, 29.49, 29.4, 29.35, 27.63, 27.39, 26.17, 25.71, 25.64, 22.67, 17.87, 14.11, -4.51, -5.06; ν_{max}: 2925, 2854, 1739, 1463, 1498, 1343, 1154, 1080 cm^{-1}.

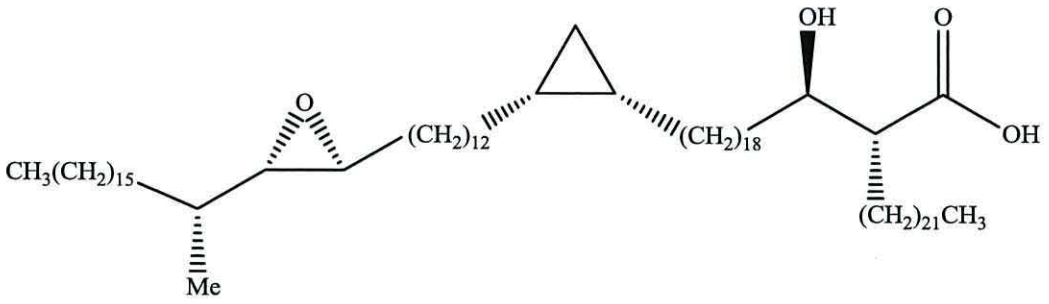
Experiment 63: (*R*)-2-[(*R*)-Acetoxy-19-((1*S*,2*R*)-2-{12-[(2*S*,3*S*)-3-((*R*)-1-methyl-heptadecyl)-oxiranyl]-dodecyl}-cyclopropyl)-nonadecyl]-tetracosanoic acid methyl ester (238)



Lithium bis(trimethylsilyl)amide (0.45 ml, 0.403 mmol, 1.06 M) was added dropwise with stirring to 16-((1*S*,2*R*)-2-{12-[(2*S*,3*S*)-3-((*R*)-1-methylheptadecyl)-oxiranyl]-dodecyl}-cyclopropyl)-hexadecanal (**195**) (0.2 g, 0.269 mmol) and (*R*)-2-[(*R*)-1-acetoxy-3-(1-phenyl-1*H*-tetrazole-5-sulfonyl)-propyl]-tetracosanoic acid methyl ester (**234**) (0.22 g, 0.322 mmol) in dry tetrahydrofuran (15 ml) under nitrogen at - 2 °C. The mixture was allowed to reach room temperature and stirred for 1 hour, when TLC no starting material, then cooled to 0 °C and quenched with sat. aq. ammonium chloride (10 ml). The aqueous layer was extracted with petrol/ethyl acetate (1:1, 3 × 20 ml). The combined organic extracts were dried and evaporated to give a thick oil which was purified by chromatography on silica gel eluting with petrol/ethyl acetate (20:1) to give a white solid, (*R*)-2-[(*E*/*Z*)-(*R*)-acetoxy-19-((1*S*,2*R*)-2-{12-[(2*S*,3*S*)-3-((*R*)-1-methylheptadecyl)-oxiranyl]-dodecyl}-cyclopropyl)-nonadec-3-enyl]-tetracosanoic acid methyl ester (**237**) (80 mg, 25 %). Hydrogenation was carried out with dipotassium azodicarboxylate (3 g, 15.44 mmol), which was added to a stirred solution of (*R*)-2-

[(*E/Z*)-(*R*)-acetoxy-19-((1*S*,2*R*)-2-{12-[(2*S*,3*S*)-3-((*R*)-1-methylheptadecyl)-oxiranyl]-dodecyl}-cyclopropyl)-nonadec-3-enyl]-tetracosanoic acid methyl ester (**237**) (80 mg, 0.066 mmol) in THF (10 ml), methanol (5 ml) and a solution of glacial acetic acid (2 ml) in THF (4 ml). The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a white solid, (*R*)-2-[(*R*)-acetoxy-19-((1*S*,2*R*)-2-{12-[(2*S*,3*S*)-3-((*R*)-1-methyl-heptadecyl)-oxiranyl]-dodecyl}-cyclopropyl)-nonadecyl]-tetracosanoic acid methyl ester (**238**) (50 mg, 62 %), m.p.: 34-35 °C [Found $(M + Na)^+$: 1232.1819, $C_{81}H_{156}NaO_5$ requires: 1232.1845], $[\alpha]_D^{22} = -9.59$ (*c* 0.57, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 5.08 (1H, dt, *J* 3.8, 7.55 Hz), 3.68 (3H, s), 2.72 (1H, dt, *J* 2.2, 5.65 Hz), 2.62 (1H, ddd, *J* 4.1, 6.6, 10.7 Hz), 2.41 (1H, dd, *J* 1.9, 7.25 Hz), 2.03 (3H, s), 1.65-1.22 (133H, br m), 1.0 (3H, d, *J* 5.95 Hz), 0.88 (6H, t, *J* 6.65 Hz), 0.65-0.64 (2H, m), 0.56 (1H, dt, *J* 4.1, 8.2 Hz), -0.32 (1H, q, *J* 5.05 Hz); δ_C (125MHz, $CDCl_3$): 173.64, 170.32, 74.09, 63.85, 58.86, 51.52, 49.57, 36.03, 33.77, 32.25, 31.92, 31.7, 30.22, 29.86, 29.69, 29.58, 29.55, 29.50, 29.46, 29.43, 29.39, 29.36, 28.71, 28.1, 27.46, 27.21, 26.08, 24.97, 22.68, 21.01, 17.29, 15.76, 14.10, 10.9; ν_{max}: 2923, 2853, 1746, 1464, 1377, 1239, 1024 cm^{-1}.

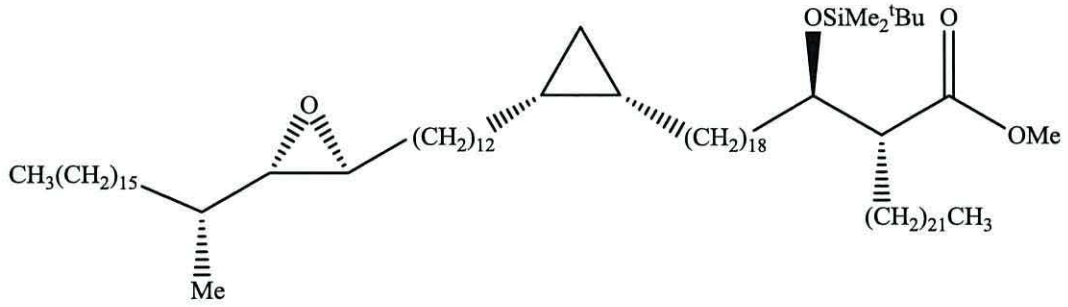
Experiment 64: (*R*)-2-[(*R*)-Hydroxy-19-((1*S*,2*R*)-2-{12-[(2*S*,3*S*)-3-((*R*)-1-methyl-heptadecyl)-oxiranyl]-dodecyl}-cyclopropyl)-nonadecyl]-tetracosanoic acid (105)



Lithium hydroxide monohydrate (31 mg, 0.741 mmol) was added to a stirred solution of (*R*)-2-[(*R*)-acetoxy-19-((1*S*,2*R*)-2-{12-[(2*S*,3*S*)-3-((*R*)-1-methylheptadecyl)-oxiranyl]-dodecyl}-cyclopropyl)-nonadecyl]-tetracosanoic acid methyl ester (**238**) (30 mg, 0.0247 mmol) in THF (4 ml), methanol (0.5 ml) and water (0.5 ml) at r.t. The mixture was stirred at 45 °C for 18 hours, when TLC showed a small amount of starting material was left. It was cooled to r.t. and a mixture of petrol/ethyl acetate (7:1, 5 ml) was added. The mixture was acidified to pH 1 with addition of dropwise potassium hydrogen sulphate. Further petrol/ethyl acetate (7:2, 10 ml) was added and extracted. The aqueous layer was re-extracted with petrol/ethyl acetate (7:2, 3×15 ml). The solvent was dried and

evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (7:2) to give a white solid, (*R*)-2-[(*R*)-hydroxy-19-((1S,2*R*)-2-{12-[(2*S*,3*S*)-3-((*R*)-1-methylheptadecyl)-oxiranyl]-dodecyl}-cyclopropyl)-nonadecyl]-tetracosanoic acid (**105**) (20 mg, 71 %), m.p.: 60-63 °C [Found $(M + Na)^+$: 1176.154, $C_{78}H_{152}NaO_4$ requires: 1176.1583], $[\alpha]_D^{24}$ = - 8.44, (*c* 0.9, $CHCl_3$), which showed δ_H (500MHz, CDCl3): 3.73-3.70 (1H, m), 2.73 (1H, dt, *J* 2.2, 5.65 Hz), 2.48-2.44 (1H, m), 2.43 (1H, dd, *J* 2.2, 7.25 Hz), 1.77-1.70 (1H, m), 1.63-1.22 (134H, br m, including br s at 1.26), 1.0 (3H, d, *J* 6 Hz), 0.88 (6H, t, *J* 6.6 Hz), 0.69-0.65 (2H, m), 0.56 (1H, dt, *J* 4.1, 8.2 Hz), -0.32 (1H, q, *J* 5.05 Hz); δ_C (125MHz, $CDCl_3$): 179.03, 72.11, 63.99, 59.02, 50.73, 36.03, 35.52, 33.76, 32.23, 31.92, 30.21, 29.86, 29.70, 29.58, 29.5, 29.42, 29.36, 28.71, 27.32, 27.21, 26.07, 25.71, 22.69, 17.3, 15.77, 14.11, 10.9; ν_{max}: 3375, 2917, 2849, 1683, 1465, 1381, 1266, 1070 cm^{-1}.

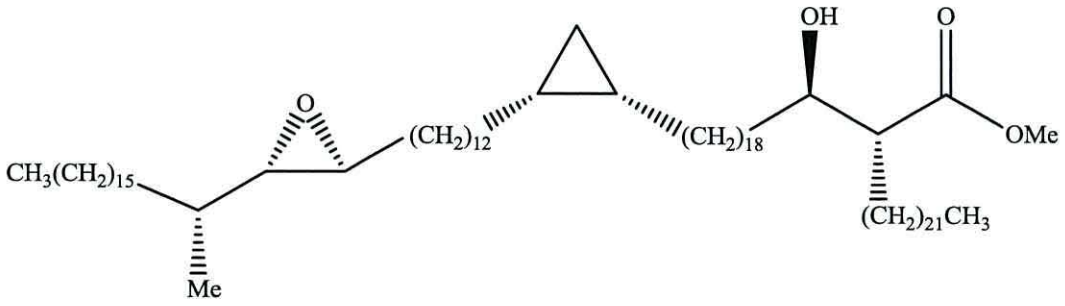
Experiment 65: (*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-19-((1*S*,2*R*)-2-{12-[(2*S*,3*S*)-3-((*R*)-1-methylheptadecyl)-oxiranyl]-dodecyl}-cyclopropyl)-nonadecyl]-tetracosanoic acid methyl ester (240)



The procedure used in Experiment 63 was repeated in order to couple 16-((1*S*,2*R*)-2-{12-[(2*S*,3*S*)-3-((*R*)-1-methylheptadecyl)-oxiranyl]-dodecyl}-cyclopropyl)-hexadecanal (**195**) (0.12 g, 0.16 mmol) and (*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilanyloxy)-3-(1-phenyl-1*H*-tetrazole-5-sulfonyl)-propyl]-tetracosanoic acid methyl ester (**236**) (0.14 g, 0.193 mmol) using lithium bis(trimethylsilyl)amide (0.21ml, 0.271 mmol, 1.06 M) in dry tetrahydrofuran (10 ml) under nitrogen at - 2 °C. The crude product was purified by chromatography on silica gel eluting with petrol/ethyl acetate (20:1) to give (*R*)-2-[(*E/Z*)-(*R*)-1-(*tert*-butyldimethylsilanyloxy)-19-((1*S*,2*R*)-2-{12-[(2*S*,3*S*)-3-((*R*)-1-methylheptadecyl)-oxiranyl]-dodecyl}-cyclopropyl)-nonadec-3-enyl]-tetracosanoic acid methyl ester (**239**) (0.14 g, 68 %). Hydrogenation was carried out with dipotassium azodicarboxylate (2 g, 10.3 mmol) which was added to a stirred solution of (*R*)-2-[(*E/Z*)-(*R*)-1-(*tert*-butyldimethylsilanyloxy)-19-((1*S*,2*R*)-2-{12-[(2*S*,3*S*)-3-((*R*)-1-

methyl-heptadecyl)-oxiranyl]-dodecyl}-cyclopropyl)-nonadec-3-enyl]-tetracosanoic acid methyl ester (**239**) (0.14 g, 0.109 mmol) in THF (10 ml), methanol (5ml) and solution of glacial acetic acid (2 ml) in THF (4 ml). The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a semi-solid,(*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilanyloxy)-19-((1*S*,2*R*)-2-{12-[(2*S*,3*S*)-3-((*R*)-1-methylheptadecyl)-oxiranyl]-dodecyl}-cyclopropyl)-nonadecyl]-tetracosanoic acid methyl ester (**240**) (0.13, 92 %) [Found $(M + Na)^+$: 1305.2684, $C_{85}H_{168}NaO_4Si$ requires: 1304.2604], $[\alpha]_D^{24}$ = - 6.95 (*c* 1.15, $CHCl_3$); δ_H (500MHz, $CDCl_3$): 3.92-3.89 (1H, m), 3.66 (3H, s), 2.72 (1H, dt, *J* 2.2, 5.35 Hz), 2.53 (1H, ddd, *J* 3.75, 7.25, 11Hz), 2.41 (1H, dd, *J* 2.2, 7.25 Hz), 1.70-1.22 (133H, br m, including br s at 1.26), 1.0 (3H, d, *J* 5.95 Hz), 0.88 (6H, t, *J* 6.95 Hz), 0.86 (9H, s), 0.66-0.64 (2H, m), 0.56 (1H, dt, *J* 4.05, 8.15 Hz), 0.05 (3H, s), 0.02 (3H, s), -0.32 (1H, q, *J* 5.05 Hz); δ_C (125MHz, $CDCl_3$): 175.13, 73.21, 63.83, 58.85, 51.55, 51.19, 36.05, 33.79, 33.66, 32.27, 31.93, 30.23, 29.87, 29.83, 29.71, 29.67, 29.59, 29.51, 29.44, 29.37, 28.72, 27.82, 27.50, 27.22, 26.09, 25.75, 23.67, 22.69, 22.61, 17.96, 17.29, 15.77, 14.11, 10.91, -4.37, -4.94 ; ν_{max}: 2924, 2853, 1741, 1464, 1377, 1258, 1170 cm^{-1}.

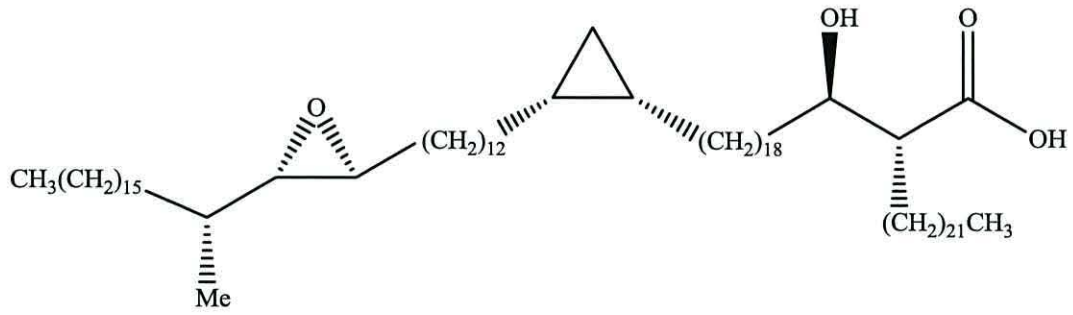
Experiment 66: (*R*)-2-[(*R*)-1-Hydroxy-19-((1*S*,2*R*)-2-{12-[(2*S*,3*S*)-3-((*R*)-1-methyl-heptadecyl)-oxiranyl]-dodecyl}-cyclopropyl)-nonadecyl]-tetracosanoic acid methyl ester (241)



The procedure in Experiment 26 was repeated using (*R*)-2-[(*R*)-1-(*tert*-butyl-dimethyl-silanyloxy)-19-((1*S*,2*R*)-2-{12-[(2*S*,3*S*)-3-((*R*)-1-methylheptadecyl)-oxiranyl]-dodecyl}-cyclopropyl)-nonadecyl]-tetracosanoic acid methyl ester (**240**) (0.18 g, 0.14 mmol), pyridine (0.15 ml) and HF.Pyridine (1 ml) dissolved in dry THF (4 ml). The crude product was purified by column chromatography eluting with petrol/ethyl acetate (9:1) to give a white solid, (*R*)-2-[(*R*)-1-hydroxy-19-((1*S*,2*R*)-2-{12-[(2*S*,3*S*)-3-((*R*)-1-methylheptadecyl)-oxiranyl]-dodecyl}-cyclopropyl)-nonadecyl]-tetracosanoic acid methyl ester (**241**) (90 mg, 56 %), m.p.: 47-49 °C [Found $(M + Na)^+$: 1190.1755,

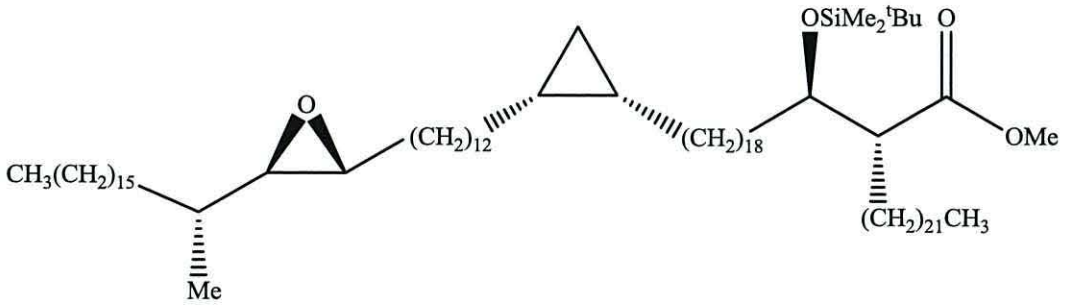
$C_{79}H_{154}NaO_4$ requires: 1190.1739], $[\alpha]_D^{21}$ = - 2.03 (*c* 0.54, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 3.71 (3H, s), 3.67-3.65 (1H, m), 2.72 (1H, dt, *J* 2.2, 5.35 Hz), 2.47-2.40 (2H, m, including dd at 2.41, *J* 1.9, 6.95 Hz), 1.74-1.22 (134H, br m, including br s at 1.26), 1.0 (3H, d, *J* 6.3 Hz), 0.88 (6H, t, *J* 6.95 Hz), 0.69-0.64 (2H, m), 0.56 (1H, dt, *J* 4.1, 7.9 Hz), -0.32 (1H, q, *J* 5.05 Hz); δ_C (125MHz, $CDCl_3$): 176.23, 72.29, 63.86, 58.86, 51.5, 50.92, 36.04, 35.69, 33.77, 32.26, 31.92, 30.22, 29.86, 29.74, 29.69, 29.63, 29.6, 29.58, 29.53, 29.49, 29.42, 29.36, 28.71, 27.41, 27.21, 26.08, 25.71, 22.68, 17.29, 15.76, 14.11, 10.9; ν_{max}: 3472, 2924, 2853, 1727, 1464, 1377, 1166 cm^{-1}.

Experiment 67: (*R*)-2-[(*R*)-Hydroxy-19-((1*S*,2*R*)-2-{12-[(2*S*,3*S*)-3-((*R*)-1-methyl-heptadecyl)-oxiranyl]-dodecyl}-cyclopropyl)-nonadecyl]-tetracosanoic acid (105)



The procedure used in Experiment 30 was repeated in order to hydrolyse (*R*)-2-[(*R*)-acetoxy-19-((1*S*,2*R*)-2-{12-[(2*S*,3*S*)-3-((*R*)-1-methylheptadecyl)-oxiranyl]-dodecyl}-cyclopropyl)-nonadecyl]-tetracosanoic acid methyl ester (**241**) (80 mg, 0.068 mmol) using lithium hydroxide monohydrate (43 mg, 1.02 mmol) in THF (4 ml), methanol (0.5 ml) and water (0.5 ml). The crude product was purified by column chromatography eluting with petrol/ethyl acetate (7:2) to give a white solid, (*R*)-2-[(*R*)-hydroxy-19-((1*S*,2*R*)-2-{12-[(2*S*,3*S*)-3-((*R*)-1-methyl-heptadecyl)-oxiranyl]-dodecyl}-cyclopropyl)-nonadecyl]-tetracosanoic acid (**105**) (60 mg, 76 %), m.p.: 60-63 °C [Found $(M + Na)^+$: 1176.154, $C_{78}H_{152}NaO_4$ requires: 1176.1583], $[\alpha]_D^{24}$ = - 8.44, (c 0.9, $CHCl_3$), which showed δ_H: 3.73-3.70 (1H, m), 2.73 (1H, dt, *J* 2.2, 5.65 Hz), 2.48-2.44 (1H, m), 2.43 (1H, dd, *J* 2.2, 7.25Hz), 1.77-1.70 (1H, m), 1.63-1.22 (134 H, br m, including br s at 1.26), 1.0 (3H, d, *J* 6 Hz), 0.88 (6H, t, *J* 6.6Hz), 0.69-0.65 (2H, m), 0.56 (1H, dt, *J* 4.1, 8.2 Hz), -0.32 (1H, q, *J* 5.05 Hz); δ_C: 179.03, 72.11, 63.99, 59.02, 50.73, 36.03, 35.52, 33.76, 32.23, 31.92, 30.21, 29.86, 29.70, 29.58, 29.5, 29.42, 29.36, 28.71, 27.32, 27.21, 26.07, 25.71, 22.69, 17.3, 15.77, 14.11, 10.9; ν_{max}: 3375, 2917, 2849, 1683, 1465, 1381, 1266, 1070 cm^{-1}.

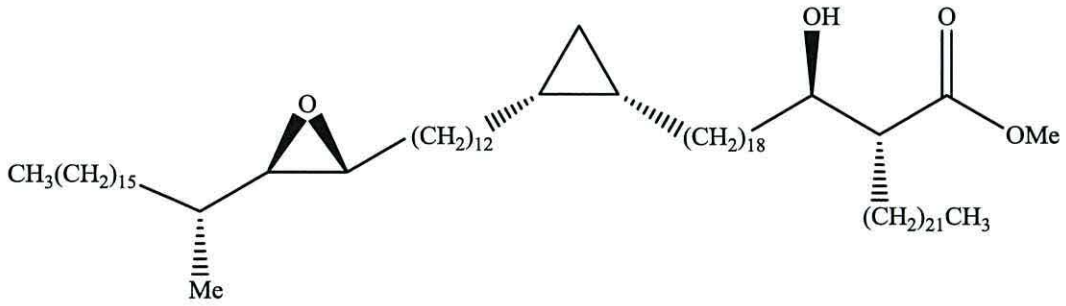
Experiment 68: (*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-19-((1*S*,2*R*)-2-{12-[(2*R*,3*R*)-3-((*R*)-1-methylheptadecyl)-oxiranyl]-dodecyl}-cyclopropyl)-nonadecyl]-tetracosanoic acid methyl ester (243)



The procedure used in Experiment 63 was repeated in order to couple 16-((1*S*,2*R*)-2-{12-[(2*R*,3*R*)-3-((*R*)-1-methylheptadecyl)-oxiranyl]-dodecyl}-cyclopropyl)-hexadecanal (**194**) (0.15 g, 0.201 mmol) and (*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilanyloxy)-3-(1-phyenyl-1*H*-tetrazole-5-sulfonyl)-propyl]-tetracosanoic acid methyl ester (**236**) (0.184 g, 0.242 mmol) using lithium bis(trimethylsilyl)amide (0.34 ml, 0.363 mmol, 1.06 M) in dry tetrahydrofuran (13 ml) under nitrogen at - 2 °C. The crude product was purified by chromatography on silica eluting with petrol/ethyl acetate (20:1) to give (*R*)-2-[(*E/Z*)-(*R*)-1-(*tert*-butyldimethylsilanyloxy)-19-((1*S*,2*R*)-2-{12-[(2*R*,3*R*)-3-((*R*)-1-methylheptadecyl)-oxiranyl]-dodecyl}-cyclopropyl)-nonadec-3-enyl]-tetracosanoic acid methyl ester (**242**) (0.18 g, 70 %). Hydrogenation was carried out by addition of dipotassium azodicarboxylate (3 g, 15.44 mmol) to a stirred a solution of (*R*)-2-[(*E/Z*)-(*R*)-1-(*tert*-butyl-dimethyl-silanyloxy)-19-((1*S*,2*R*)-2-{12-[(2*R*,3*R*)-3-((*R*)-1-methyl-heptadecyl)-oxiranyl]-dodecyl}-cyclopropyl)-nonadec-3-enyl]-tetracosanoic acid methyl ester (**242**) (0.17 g, 0.132 mmol) in THF (10 ml), methanol (5 ml) and solution of glacial acetic acid (2 ml) in THF (4 ml). The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a semi solid (**243**) (0.14 g, 82 %) [Found $(M + Na)^+$: 1304.2559, $C_{85}H_{168}NaO_4Si$ requires: 1304.2604], $[\alpha]_D^{22} = +$ 2.98 (*c* 0.77, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 3.9 (1H, dt, *J* 4.7, 7.25 Hz), 3.65 (3H, s), 2.66 (1H, dt, *J* 2.2, 5.65 Hz), 2.52 (1H, ddd, *J* 3.8, 7.25, 11.05 Hz), 2.45 (1H, dd, *J* 2.2, 7.25 Hz), 1.58-1.21 (133H, br m, including br s at 1.25), 0.91 (3H, d, *J* 6.6 Hz), 0.88 (6H, t, *J* 6.6 Hz), 0.86 (9H, s), 0.65-0.63 (2H, m), 0.55 (1H, dt, *J* 3.8, 7.85 Hz), 0.04 (3H, s), 0.019 (3H, s), -0.33 (1H, q, *J* 5.05 Hz); δ_C (125MHz, $CDCl_3$): 175.11, 73.21, 63.78, 57.44, 51.54, 51.18, 34.63, 33.66, 32.21, 31.92, 30.22, 29.94, 29.82, 29.7, 29.62, 29.57, 29.44, 29.36, 28.72, 27.81, 27.49, 26.89, 26.14, 25.74, 23.65, 22.68,

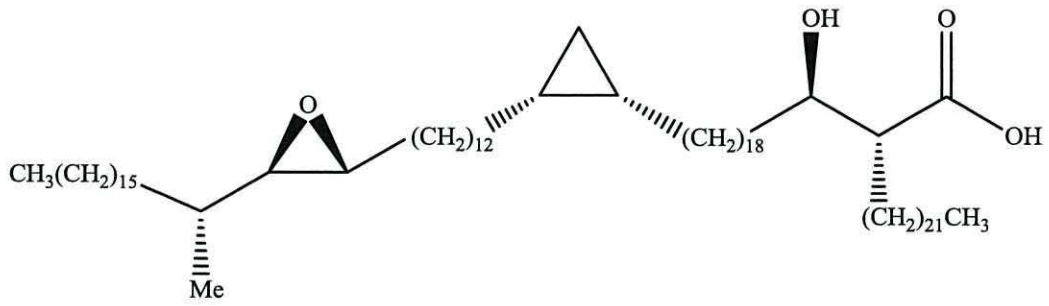
17.95, 15.95, 15.76, 14.1, 10.9, -4.38, -4.95; ν_{max}: 2924, 2853, 1746, 1470, 1258, 1170 cm^{-1}.

Experiment 69: (*R*)-2-[(*R*)-1-Hydroxy-19-((1*S*,2*R*)-2-{12-[(2*R*,3*R*)-3-((*R*)-1-methyl-heptadecyl)-oxiranyl]-dodecyl}-cyclopropyl)-nonadecyl]-tetracosanoic acid methyl ester (244)



The procedure used in Experiment 26 was repeated using (*R*)-2-[(*R*)-1-(*tert*-butyl-dimethyl-silanyloxy)-19-((1*S*,2*R*)-2-{12-[(2*R*,3*R*)-3-((*R*)-1-methylheptadecyl)-oxiranyl]-dodecyl}-cyclopropyl)-nonadecyl]-tetracosanoic acid methyl ester (**243**) (0.1 g, 0.0779 mmol), pyridine (0.05 ml) and hydrogen fluoride-pyridine complex (0.5 ml) dissolved in dry THF (4 ml). The crude product was purified by column chromatography eluting with petrol/ethyl acetate (9:1) to give a white solid, (*R*)-2-[(*R*)-1-hydroxy-19-((1*S*,2*R*)-2-{12-[(2*R*,3*R*)-3-((*R*)-1-methyl-heptadecyl)-oxiranyl]-dodecyl}-cyclopropyl)-nonadecyl]-tetracosanoic acid methyl ester (**244**) (61 mg, 66 %), m.p.: 40-41 °C [Found $(M + Na)^+$: 1190.1736, $C_{79}H_{154}O_4$ requires: 1190.1739], $[\alpha]_D^{21} =$ + 5.15 (*c* 0.73, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 3.71 (3H, s), 3.67-3.64 (1H, m), 2.66 (1H, dt, *J* 2.2, 5.35 Hz), 2.47-2.42 (2H, m), 1.74-1.22 (134H, br m, including br s at 1.25), 0.91 (3H, d, *J* 6.65 Hz), 0.88 (6H, t, *J* 6.95 Hz), 0.67-0.63 (2H, m), 0.56 (1H, dt, *J* 4.1, 8.2 Hz), -0.32 (1H, q, *J* 5.4 Hz); δ_C (125MHz): 176.22, 72.28, 63.8, 57.46, 51.48, 50.93, 35.83, 35.68, 34.61, 32.21, 31.92, 30.21, 29.69, 29.65, 29.62, 29.59, 29.55, 29.53, 29.35, 28.71, 27.4, 26.89, 26.14, 25.71, 22.67, 15.94, 15.76, 14.18, 14.1, 10.9; ν_{max}: 3346, 2924, 2853, 1725, 1463, 1381, 1169 cm^{-1}.

Experiment 70: (*R*)-2-[(*R*)-1-Hydroxy-19-((1*S*,2*R*)-2-{12-[(2*R*,3*R*)-3-((*R*)-1-methyl-heptadecyl)-oxiranyl]-dodecyl}-cyclopropyl)-nonadecyl]-tetracosanoic acid (106)



The procedure used in Experiment 30 was repeated in order to hydrolyse the (*R*)-2-[(*R*)-acetoxy-19-((1*S*,2*R*)-2-{12-[(2*R*,3*R*)-3-((*R*)-1-methyl-heptadecyl)-oxiranyl]-dodecyl}-cyclopropyl)-nonadecyl]-tetracosanoic acid methyl ester (**244**) (60 mg, 0.051 mmol) using lithium hydroxide monohydrate (32 mg, 0.769 mmol) in THF (4 ml), methanol (0.5 ml) and water (0.5 ml) at r.t. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (7:2) to give a white solid, (*R*)-2-[(*R*)-hydroxy-19-((1*S*,2*R*)-2-{12-[(2*R*,3*R*)-3-((*R*)-1-methyl-heptadecyl)-oxiranyl]-dodecyl}-cyclopropyl)-nonadecyl]-tetracosanoic acid methyl ester (**106**) (39 mg, 66 %), m.p.: 65-66 °C [Found $(M + Na)^+$: 1176.154, $C_{78}H_{152}NaO_4$ requires: 1176.1583], $[\alpha]_D^{22} = +12.5$, ($_C$ 0.6, CH_3Cl), which showed δ_H (500MHz, $CDCl_3$): 3.73-3.70 (1H, m), 2.68 (1H, dt, *J* 2.2, 6 Hz), 2.49-2.44 (2H, m, including dd at 2.48, *J* 2.2, 7.25 Hz), 1.75-1.22 (135H, br m, including br s at 1.26), 0.92 (3H, d, *J* 6.6 Hz), 0.88 (6H, t, *J* 6.9 Hz), 0.66-0.64 (2H, m), 0.56 (1H, dt, *J* 4.1, 8.2 Hz), -0.32 (1H, q, *J* 5.13 Hz); δ_C (125MHz, $CDCl_3$): 178.97, 72.11, 63.92, 57.6, 50.73, 35.81, 35.53, 34.6, 32.18, 31.92, 30.21, 29.93, 29.7, 29.65, 29.62, 29.57, 29.5, 29.44, 29.42, 29.36, 28.71, 27.32, 26.88, 26.13, 25.71, 22.68, 15.93, 15.77, 14.11, 10.9; ν_{max}: 3349, 2921, 2851, 1684, 1465, 1219 cm^{-1}.

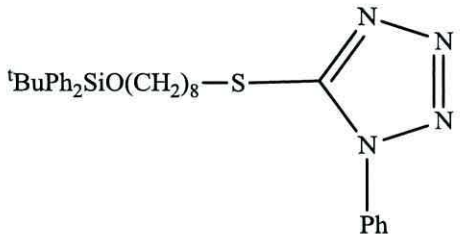
Experiment 71: (8-Bromo-octyloxy)-*tert*-butyldiphenylsilane (247)

$^tBuPh_2SiO(CH_2)_8Br$

Triethylamine (8 ml, 57.4 mmol) was added dropwise at 0 °C to a solution of bromo-octan-1-ol (**246**) (10 g, 47.8 mmol) in dry dichloromethane (100 ml) under nitrogen. After stirring for 10 min, *tert*-butyldiphenylchlorosilane (14.5 g, 52.6 mmol) was added followed by the addition of 4-dimethylaminopyridine (65 mg). The reaction mixture was then stirred for 16 hours at r.t. followed by quenching with water (25 ml). The aqueous layer was extracted with dichloromethane (3 × 20 ml) and the combined

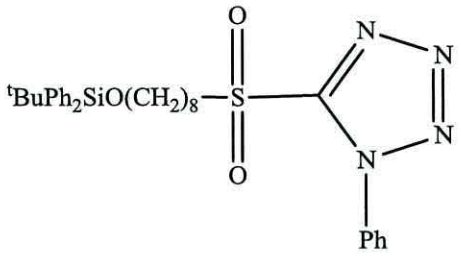
organic layers were dried and concentrated to give the crude product. This was purified by column chromatography eluting with petrol and ethyl acetate (5:1) to give a colourless oil, (8-bromo-octyloxy)-*tert*-butyldiphenylsilane (**247**) (21 g, 98 %), [Found $(M + Na)^+$: 469.1548, $C_{24}H_{35}BrNaOSi$ requires: 469.1533], which showed δ_H (500Mz, $CDCl_3$): 7.83-7.82 (1H, m), 7.74 (3H, dd, *J* 1.55, 7.9 Hz), 7.51-7.42 (6H, m), 3.73 (2H, t, *J* 6.6 Hz), 3.44 (2H, t, *J* 6.9 Hz), 1.90 (2H, pent., *J* 6.9 Hz), 1.63 (2H, pent., *J* 6.6 Hz), 1.47-1.33 (8H, m), 1.13 (9H, s); δ_C: 135.53, 135.16, 134.12, 132.46, 130.19, 129.46, 127.82, 127.54, 63.89, 33.86, 32.77, 32.47, 29.1, 28.67, 28.06, 26.87, 26.46, 25.63, 19.19; ν_{max}: 3071, 2928, 2855, 1589, 1471, 1427, 1389, 1361, 1258, 1109 cm^{-1}.

Experiment 72: 5-[8-(*tert*-Butyldiphenylsilanyloxy)-octylsulfanyl)]-1-phenyl-1*H*-tetrazole (248)



The procedure used in Experiment 1 was repeated using (8-bromo-octyloxy)-*tert*-butyldiphenylsilane (**247**) (21 g, 46.92 mmol), 1-phenyl-1*H*-tetrazole-5-thiol (8.36 g, 46.92 mmol) and anhydrous potassium carbonate (12.97 g, 93.85 mmol) in acetone (300 ml). The crude product was purified by column chromatography eluting with petrol/ethyl acetate (10:1, then 5:1) to give a colourless oil (**248**) (22 g, 86 %), [Found $(M + Na)^+$: 567.2597, $C_{31}H_{40}NaN_4OSSi$ requires: 567.2584], which showed δ_H (500MHz, $CDCl_3$): 7.77-7.75 (1H, m), 7.70 (3H, dd, *J* 1.55, 7.85 Hz), 7.71-7.51 (4H, m), 7.44-7.37 (7H, m), 3.68 (2H, t, *J* 6.3 Hz), 3.41 (2H, t, *J* 7.25 Hz), 1.83 (2H, pent., *J* 7.25 Hz), 1.58 (2H, pent., *J* 6.65 Hz), 1.47-1.31 (8H, m), 1.08 (9H, s); δc (125MHz, $CDCl_3$): 154.4, 135.46, 135.28, 134.73, 134.03, 133.65, 129.95, 129.65, 129.45, 129.41, 127.56, 127.48, 123.74, 63.80, 33.27, 32.39, 29.04, 28.96, 28.88, 28.47, 26.79, 26.49, 25.57, 19.12, 14.11; ν_{max} : 3069, 2929, 2855, 1596, 1499, 1461, 1427, 1387, 1243, 1110 cm^{-1}.

Experiment 73: 5-[8-(*tert*-Butyldiphenylsilanyloxy)-octane-1-sulfonyl]-1-phenyl-1*H*-tetrazole (249)



The procedure used in Experiment 2 was repeated using 5-[8-(*tert*-butyldiphenylsilanyloxy)-octylsulfanyl)]-1-phenyl-1*H*-tetrazole (**248**) (21.6 g, 39.6 mmol), ammonium molybdate (VI) tetrahydrate (22 g, 22 mmol) in ice cold H_2O_2 (35 % w/w, 52 ml) in THF (100 ml) and IMS (350 ml) and a further solution of ammonium molybdate (VI) tetrahydrate (11 g, 8.92 mmol) in ice cold H_2O_2 (35 % w/w, 30 ml). The crude product was purified column chromatography eluting with petrol/ethyl acetate (1:1) to give a colourless oil, 5-[8-(*tert*-butyldiphenylsilanyloxy)-octane-1-sulfonyl]-1-phenyl-1*H*-tetrazole (**249**) (22 g, 96 %), [Found $(M + Na)^+$: 599.2511, $C_{31}H_{40}N_4NaO_3SSi$ requires: 599.2483], which showed δ_H (500MHz, $CDCl_3$): 7.75-7.69 (5H, m), 7.66-7.58 (4H, m), 7.45-7.37 (6H, m), 3.74 (2H, br t, *J* 7.9 Hz), 3.68 (2H, t, *J* 6.6 Hz), 1.96 (2H, pent., *J* 7.9 Hz), 1.57 (2H, pent., *J* 6.65 Hz), 1.49 (2H, pent., *J* 6.9 Hz), 1.40-1.30 (6H, m), 1.08 (9H, s); δc (500MHz, $CDCl_3$): 153.44, 135.51, 134.73, 134.04, 132.99, 131.37, 129.63, 129.53, 129.45, 127.62, 127.52, 125.01, 63.78, 55.92, 32.35, 28.85, 28.78, 28.01, 26.82, 26.50, 25.52, 21.87, 19.16, 14.13; ν_{max}: 3069, 2930, 2856, 1497, 1462, 1427, 1341, 1152, 1110 cm^{-1}.

Experiment 74: 9-Bromo-nonanal (251)

$Br(CH_2)_8CHO$

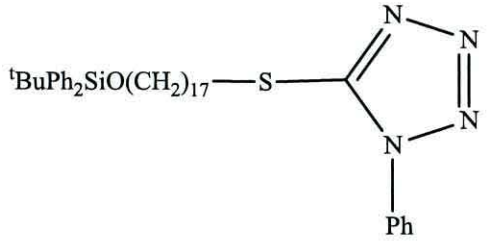
The procedure used in Experiment 9 was repeated in order to oxidise the 9-bromo-nonan-1-ol (**250**) (10 g, 44.84 mmol) in using PCC (19.33 g, 89.68 mmol) in dichloromethane (250 ml). The crude product was purified by column chromatography eluting with petrol/ethyl acetate (5:1) to give 9-bromo-nonanal (**251**) (8.1 g, 81 %), which showed δ_H (500MHz, $CDCl_3$): 9.73 (1H, br t, *J* 1.9 Hz), 3.38 (2H, t, *J* 6.6 Hz), 2.40 (2H, dq, *J* 1.9 Hz), 1.82 (2H, pent., *J* 6.95 Hz), 1.63-1.57 (2H, m), 1.43-1.37 (2H, m), 1.35-1.26 (6H, m), δ_C (125MHz, $CDCl_3$): 202.75, 43.75, 33.85, 32.64, 29.05, 28.91, 28.82, 28.42, 27.96, 24.53, 21.9, 14.1, ν_{max}: 2937, 2858, 2719, 1727, 1463, 1249 cm^{-1}.

Experiment 75: (17-Bromoheptadecyloxy)-*tert*-butyldiphenylsilane (252)

$^{t}BuPh_2SiO(CH_2)_{17}Br$

The procedure used in Experiment 3 order to couple the 9-bromononanal (**251**) (7 g, 31.67 mmol) and 5-[*tert*-butyldiphenylsilanyloxy)-octane-1-sulfonyl]-1-phenyl-1*H*-tetrazole (**249**) (21.92 g, 20.09 mmol) using lithium bis(trimethylsilyl)amide (46.6 ml, 49.41 mmol, 1.06 M) in dry tetrahydrofuran (200 ml) under nitrogen at - 10 °C. The crude product was purified by column chromatography on silica gel eluting with petrol/ethyl acetate (20:1) to give a thick oil, (17-bromoheptadec-8-enyloxy)-*tert*-butyldiphenylsilane (14.7 g, 81 %). Palladium on charcoal (10 %, 0.5 g) and hydrogen were added to a stirred solution of the alkenes (14.76 g, 25.81 mmol) in IMS (100 ml) and THF (20 ml) to give oil. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a colourless oil, (17-bromoheptadecyloxy)-*tert*-butyldiphenylsilane (**252**) (14.7 g, 99 %) [Found $(M + Na)^+$: 597.2895, $C_{33}H_{53}BrNaOSi$ requires: 595.2941], which showed δ_H (500MHz, $CDCl_3$): 7.53 (4H, dd, *J* 1.3, 7.9 Hz), 7.28-7.21 (6H, m), 3.51 (2H, t, *J* 6.6 Hz), 3.25 (2H, t, *J* 6.65 Hz), 1.71 (2H, pent., *J* 6.95 Hz), 1.42 (2H, pent., *J* 6.6 Hz), 1.29-1.08 (26H, br m), 0.91 (9H, s); δc (125MHz, $CDCl_3$): 135.56, 134.18, 129.44, 127.53, 64.0, 41.34, 33.96, 32.83, 32.58, 29.67, 29.62, 29.54, 29.44, 29.38, 28.77, 28.18, 26.87, 25.76, 22.61, 19.2; ν_{max}: 3070, 2926, 2854, 1464, 1427, 1389, 1111 cm^{-1}.

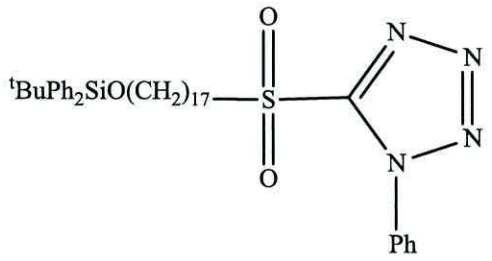
Experiment 76: 5-[17-(*tert*-Butyldiphenylsilanyloxy)-heptadecylsulfanyl]-4-phenyl-4*H*-[1,2,3]triazole (253)



The procedure used in Experiment 1 was repeated using (17-bromoheptadecyloxy)-*tert*-butyl-diphenylsilane (**252**) (14.7 g, 25.62 mmol), 1-phenyl-1*H*-tetrazole-5-thiol (4.56 g, 25.62 mmol) and anhydrous potassium carbonate (7 g, 51.24 mmol) in acetone (250 ml) to give a colourless oil, 5-[17-(*tert*-butyldiphenylsilanyloxy)-heptadecylsulfanyl]-4-phenyl-4*H*-[1,2,3]triazole (**253**) (15.87 g, 92 %) [Found $(M + Na)^+$: 693.4015, $C_{40}H_{58}N_4NaOSSi$ requires: 693.3993], which showed δ_H (500MHz, $CDCl_3$): 7.69-7.67

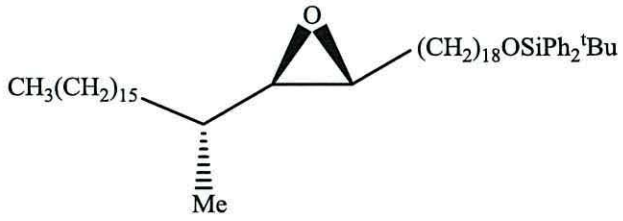
(4H, m), 7.61-7.52 (5H, m), 7.44-7.37 (6H, m), 3.66 (2H, t, *J* 6.3 Hz), 3.4 (2H, t, *J* 7.55Hz), 1.83 (2H, pent., *J* 7.55 Hz), 1.67 (1H, br s), 1.57 (2H, pent., *J* 6.6 Hz), 1.45 (2H, pent., *J* 6.65 Hz), 1.34-1.22 (23H, br m, including br s at 1.26), 1.06 (9H, s); δc (125MHz, $CDCl_3$): 154.48, 135.53, 134.16, 133.74, 130.0, 129.71, 129.42, 127.51, 123.82, 63.99, 33.35, 32.56, 29.64, 29.59, 29.52, 29.41, 29.35, 29.04, 29.0, 28.61, 26.84, 25.74, 19.18; ν_{max}: 3069, 2926, 2854, 1500, 1427, 1388,1100 cm^{-1}.

Experiment 77: 5-[17-(*tert*-Butyldiphenylsilanyloxy)-heptadecane-1-sulfonyl]-1-phenyl-1*H*-tetrazole (254)



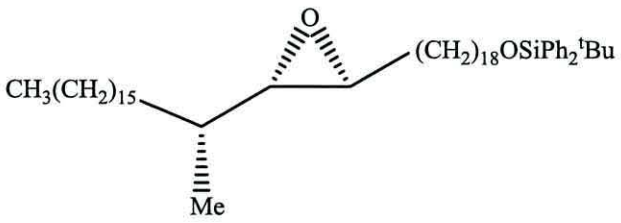
The procedure used in Experiment 2 was repeated using5-[17-(*tert*-butyldiphenylsilanyloxy)-heptadecylsulfanyl]-4-phenyl-4*H*-[1,2,3]triazole (**253**) (15.9 g, 23.69 mmol), ammonium molybdate (VI) tetrahydrate (13.17 g, 10.66 mmol) in ice cold H_2O_2 (35 % w/w, 36 ml) in IMS (250 ml) and THF (60 ml) and a further solution of ammonium molybdate (VI) tetrahydrate (10 g, 8.09 mmol) in ice cold H_2O_2 (35 % w/w, 20 ml). The crude product was purified by column chromatography eluting with petrol/ethyl acetate (6:1) to give a colourless oil,5-[17-(*tert*-butyldiphenylsilanyloxy)-heptadecane-1-sulfonyl]-1-phenyl-1*H*-tetrazole (**254**) (15.2 g, 91 %) [Found $(M + Na)^+$: 725.3898, $C_{40}H_{58}N_4NaO_3SSi$ requires: 725.3891], which showed δ_H (500MHz, $CDCl_3$): 7.76-7.68 (5H, m), 7.66-7.59 (4H, m), 7.44-7.37 (6H, m), 3.74 (2H, br t, *J* 8.2 Hz), 3.67 (2H, t, *J* 6.6Hz), 1.99-1.93 (2H, m), 1.57 (2H, pent., *J* 6.6 Hz), 1.53-1.47 (2H, m), 1.35-1.23 (24H, br m, including br s at 1.26), 1.06 (9H, s); δc (125MHz, $CDCl_3$): 153.47, 135.53, 134.16, 133.03, 131.39, 129.65, 129.41, 127.51, 125.03, 63.98, 55.98, 32.55, 29.63, 29.58, 29.57, 29.53, 29.42, 29.34, 29.15, 28.86, 28.10, 26.84, 25.73, 21.90, 19.18, 14.16; ν_{max}: 3071, 2926, 2854, 1498, 1463, 1428, 1343, 1152, 1111 cm^{-1}.

Experiment 78: *tert*-Butyl-[18-((2*R*,3*R*)-3-hexadecyloxiranyl)-octadecyloxy)-diphenyl-silane (258)



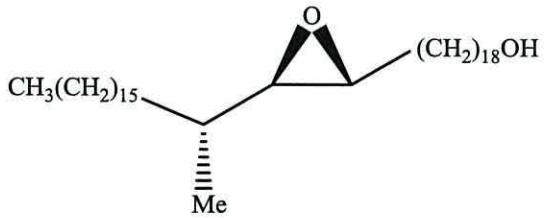
The procedure used in Experiment 14 was repeated in order to couple (2*S*,3*R*)-3-((*R*)-1-methylheptadecyl)-oxirane-2-carbaldehyde (**142**) (0.92 g, 2.8 mmol) and 5-[17-(*tert*-butyldiphenylsilanyloxy)-heptadecane-1-sulfonyl)-1-phenyl-1-*H*-tetrazole (**254**) (2.39 g, 3.4 mmol) using lithium bis(trimethylsilyl)amide (5.6 ml, 5.1 mmol, 1.06 M) in dry tetrahydrofuran (50 ml) under nitrogen at - 10 °C. The crude product was purified by column chromatography on silica eluting with petrol/ether (20:1) to give a colourless oil, *tert*-butyl-{(*E/Z*)-18-[(*R*)-3-((*R*)-1-methyl-heptadecyl)-oxiranyl)-octadec-17-enyloxy}-diphenylsilane (**256**) (1.37 g, 60 %) in ratio 1.2:1. Hydrogenation was carried out with dipotassium azodicarboxylate (2 g, 10.3 mmol), which was added to a stirred solution of alkene (1.37 g, 1.7 mmol) in THF (15 ml), methanol (5 ml) and a solution of glacial acetic acid (2 ml) in THF (4 ml). The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a colourless oil, *tert*-butyl-[18-((2*R*,3*R*)-3-hexadecyl-oxiranyl)-octadecyloxy)-diphenylsilane (**258**) (1.18 g, 96 %) [Found $(M + Na)^+$: 825.6881, $C_{54}H_{94}NaO_2Si$ requires: 825.6915], $[\alpha]_D^{17} = 5.39$ (*c* 1.02, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 7.68 (4H, dd, *J* 1.6, 7.9 Hz), 7.44-7.36 (6H, m), 3.66 (2H, t, *J* 6.3 Hz), 2.67 (1H, dt, *J* 2.2, 6 Hz), 2.47 (1H, dd, *J* 1.9, 6.95 Hz), 1.59-1.26 (65H, br m), 1.06 (9H, s), 0.92 (3H, d, *J* 6.6 Hz), 0.89 (3H, t, *J* 6.95 Hz) ; δ_C (125MHz, $CDCl_3$): 135.56, 134.21, 129.43, 127.53, 64.02, 63.8, 57.46, 35.83, 34.62, 32.59, 32.21, 31.92, 29.93, 29.69, 29.67, 29.65, 29.61, 29.56, 29.44, 29.38, 29.35, 26.89, 26.87, 26.14, 25.76, 22.68, 19.21, 15.95, 14.1; ν_{max}: 3071, 2924, 2854, 1464, 1428, 1111 cm^{-1}.

Experiment 79: *tert*-Butyl-[18-((2*S*,3*S*)-3-hexadecyloxiranyl)-octadecyloxy)-diphenylsilane (257)



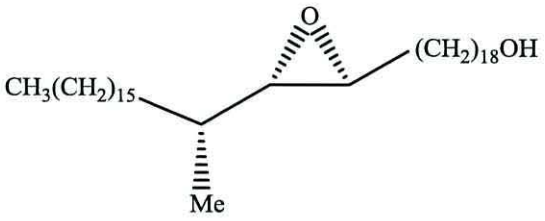
The procedure used in Experiment 14 was repeated in order to couple (2*R*,3*S*)-3-((*R*)-1-methyl-heptadecyl)-oxirane-2-carbaldehyde (**143**) (1 g, 3 mmol) and 5-[17-(*tert*-butyldiphenyl-silanyloxy)-heptadecane-1-sulfonyl)-1-phenyl-1-*H*-tetrazole (**254**) (2.81 g, 4 mmol) using lithium bis(trimethylsilyl)amide (5.6 ml, 6 mmol,1.06 M) dry tetrahydrofuran (50 ml) under nitrogen at - 10 °C. The crude product was purified by column chromatography on silica eluting with petrol/ether (20:1) to give a white solid, *tert*-butyl-{(*E/Z*)-18-[(*S*)-3-((*R*)-1-methyl-heptadecyl)-oxiranyl)-octadec-17-enyloxy}-diphenylsilane (**255**) (1.25 g, 50 %) in ratio 1.2:1. Hydrogenation of the alkene (1.18 g, 2.04 mmol) using dipotassium azodicarboxylate (2 g, 10.3 mmol) in THF (15 ml), methanol (5 ml) and a solution of glacial acetic acid (2 ml) in THF (4 ml) were carried out as before. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a colourless oil, *tert*-butyl-[18-((2*S*,3*S*)-3-hexadecyl-oxiranyl)-octadecyloxy)-diphenylsilane (**257**) (1.08 g, 91 %) [Found $(M + Na)^+$: 825.6915, $C_{54}H_{94}NaO_2Si$ requires: 825.6915], $[\alpha]_D^{20} = -7.18$ (*c* 1.1, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 7.69 (4H, dd, *J* 1.25, 7.9 Hz), 7.44-7.35 (6H, m), 3.67 (2H, t, *J* 6.65 Hz), 2.73 (1H, dt, *J* 2.2, 5.7 Hz), 2.42 (1H, dd, *J* 2.2, 7.25 Hz), 1.60-1.24 (65H, br m, including br s at 1.27), 1.06 (9H, s), 1.01 (3H, d, *J* 6.3 Hz), 0.9 (3H, t, *J* 7.0 Hz); δ_C (125MHz, $CDCl_3$): 135.56, 134.20, 129.43, 127.53, 64.01, 63.83, 58.84, 36.03, 33.78, 32.59, 32.26, 31.92, 29.86, 29.70, 29.66, 29.63, 29.60, 29.58, 29.50, 29.39, 29.36, 27.21, 26.87, 26.08, 25.77, 22.68, 19.21, 17.29,14.11; ν_{max}: 3071, 2925, 2854, 1464, 1428, 1389, 1111 cm^{-1}.

Experiment 80: 18-[(2*R*,3*R*)-3-((*R*)-1-Methylheptadecyl)-oxiranyl]-octadecan-1-ol (260)



The procedure used in Experiment 51 was repeated using *tert*-butyl-[18-((2*R*,3*R*)-3-hexadecyloxiranyl)-octadecyloxy]-diphenylsilane (**258**) (0.94 g, 1.17 mmol) and n-TBAF (1.75 ml, 1.75 mmol) in dry THF (50 ml) at 0 °C under nitrogen. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (6:1) to give a white solid (**260**) (0.52 g, 79 %), m.p: 64-66 °C [Found $(M + H)^+$: 587.5695, $C_{38}H_{77}O_2$ requires: 565.5918], $[\alpha]_D^{20} = 0.8$ (*c* 1.0, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 3.64 (2H, t, *J* 6.6 Hz), 2.67 (1H, dt, *J* 2.2, 6.0 Hz), 2.46 (1H, dd, *J* 2.2, 7.25 Hz), 1.57-1.22 (66H, br m, including br s at 1.26), 0.91 (3H, d, *J* 6.6 Hz), 0.88 (3H, t, *J* 6.95 Hz); δ_C (125MHz, $CDCl_3$): 63.81, 63.08, 57.47, 35.82, 34.6, 32.81, 32.2, 31.92, 29.93, 29.69, 29.66, 29.64, 29.61, 29.58, 29.55, 29.42, 29.35, 26.88, 26.54, 26.13, 25.73, 22.67, 15.94, 14.10; ν_{max}: 3440, 2919, 2849, 1465, 1059 cm^{-1}.

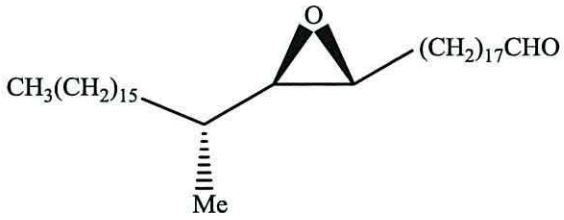
Experiment 81: 18-[(2*S*,3*S*)-3-((*R*)-1-Methylheptadecyl)-oxiranyl]-octadecan-1-ol (259)



The procedure used in Experiment 51 was repeated using *tert*-butyl-[18-((2*S*,3*S*)-3-hexadecyloxiranyl)-octadecyloxy]-diphenylsilane (**257**) (1.05 g, 1.30 mmol) and n-TBAF (1.96 ml, 1.96 mmol) in dry THF (50 ml) at 0 °C under nitrogen. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (6:1) to give a white solid (**259**) (0.61, 84 %), m.p.: 69-71 °C [Found $(M + H)^+$: 565.5898, $C_{38}H_{77}O_2$ requires: 565.5918], $[\alpha]_D^{21} = -10.5$ (*c* 0.96, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 3.64 (2H, t, *J* 6.65 Hz), 2.72 (1H, dt, *J* 2.2, 5.7 Hz), 2.41 (1H, dd, *J* 2.2, 6.95 Hz), 1.60-1.22 (66H, br m, including br s at 1.26), 1.0 (3H, d, *J* 6.3 Hz), 0.88 (3H, t, *J* 6.65 Hz), δ_C (125MHz, $CDCl_3$): 63.86, 63.11, 58.88, 36.04, 33.77, 32.82,

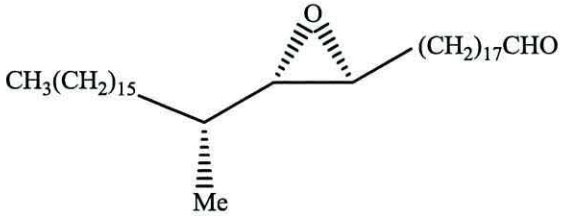
32.26, 31.92, 29.86, 29.67, 29.6, 29.58, 29.5, 29.43, 29.36, 27.21, 26.08, 25.74, 22.68, 17.3, 14.11; ν_{max}: 3440, 2919, 2849, 1465, 1059 cm^{-1}.

Experiment 82: 18-[(2*R*,3*R*)-3-((*R*)-1-Methylheptadecyl)-oxiranyl]-octadecanal (262)



The procedure used in Experiment 9 was repeated in order to oxidise 18-[(2*R*,3*R*)-3-((*R*)-1-methylheptadecyl)-oxiranyl]-octadecan-1-ol (**260**) (0.05 g, 0.088 mmol) using pyridinium chlorochromate (0.047 g, 0.22 mmol) in dichloromethane (5 ml). The crude product was purified by column chromatography on silica eluting with petrol/ethyl acetate (10:1) to give a white solid, 18-[(2*R*,3*R*)-3-((*R*)-1-methylheptadecyl)-oxiranyl]octadecanal (**262**) (0.04 g, 81 %), m.p.: 50-52 °C, $[\alpha]_D^{20}$ = + 7.96 (*c* 0.64, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 9.77 (1H, t, *J* 1.6 Hz), 2.67 (1H, dt, *J* 2.2, 5.7 Hz), 2.46 (1H, dd, *J* 2.2, 7.25 Hz), 2.42 (2H, dt, *J* 1.9, 7.25 Hz), 1.63 (4H, pent., *J* 7.25 Hz), 1.57-1.22 (59H, br m, including br s at 1.26), 0.92 (3H, d, *J* 6.6 Hz), 0.88 (3H, t, *J* 6.6 Hz); δ_C (125MHz, $CDCl_3$): 202.93, 63.8, 57.46, 43.91, 35.84, 34.62, 32.21, 31.92, 29.93, 29.69, 29.67, 29.66, 29.63, 29.56, 29.44, 29.42, 29.35, 29.16, 26.89, 26.14, 22.68, 22.08, 15.96, 14.11; ν_{max}: 2923, 2852, 1728, 1463 cm^{-1}.

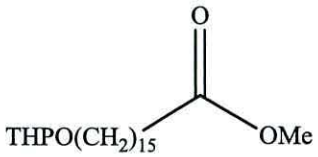
Experiment 83: 18-[(2*S*,3*S*)-3-((*R*)-1-Methylheptadecyl)-oxiranyl]-octadecanal (261)



The procedure used in Experiment 9 was repeated in order to oxidise 18-[(2*S*,3*S*)-3-((*R*)-1-methylheptadecyl)-oxiranyl]-octadeca-1-ol (**259**) (0.2 g, 0.353 mmol) using pyridinium chlorochromate (0.19 g, 0.88 mmol) in dichloromethane (20 ml). The crude product was purified by column chromatography on silica gel eluting with petrol/ethyl acetate (10:1) to give a white solid (**261**) (0.18 g, 90 %), m.p.: 58-59 °C [Found (M +

H)$^+$: 563.5761, $C_{38}H_{75}O_2$ requires: 563.5762], $[\alpha]_D^{22}$ = - 26.02 (*c* 0.83, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 9.77 (1H, t, *J* 1.85 Hz), 2.71 (1H, dt, *J* 2.2, 5.35 Hz), 2.42 (3H, dt, *J* 1.6, 7.25 Hz), 1.63 (2H, pent., *J* 7.25 Hz), 1.57-1.23 (61H, br m, including br s at 1.26), 1.0 (3H, d, *J* 6.3 Hz), 0.88 (3H, t, *J* 6.6 Hz); δ_C (125MHz, $CDCl_3$): 202.88, 63.83, 58.84, 43.90, 36.03, 33.77, 32.25, 31.92, 29.85, 29.68, 29.63, 29.59, 29.57, 29.49, 29.42, 29.34, 29.16, 27.2, 26.07, 22.67, 22.08, 17.28, 14.09; ν_{max}: 2924, 2853, 1728, 1463 cm^{-1}.

Experiment 84: 16-Tetrahydropyran-2-yloxy)-hexadecanoic acid methyl ester (271)



3,4-Dihydro-*2H*-pyran (6.17 g, 73.42 mmol) and pyridinium-*p*-toluene–sulfonate (0.46 g, 1.83 mmol) were added to a stirred solution of 16-hydroxyhexadecanoic acid methyl ester (**270**) (10.5 g, 36.71 mmol) in dry CH_2Cl_2 (150 ml) under nitrogen at r.t. The reaction was stirred at r.t. for 3 hours and works up sat. aq. $NaHCO_3$ (50 ml). The mixture was extracted and dried. The solvent was evaporated and the crude product was re-diluted in hot petrol (100 ml) and left at ambient for 30 min and then at 0 °C for 30 min. The crystals were filtered off and washed with petrol to give a white solid (**271**) (13 g, 95 %), m.p.: 34-35 °C [Found (M + Na)$^+$: 393.2984, $C_{22}H_{42}NaO_4$ requires: 393.2975], which showed δ_H (500MHz, $CDCl_3$): 4.58 (1H, dd, *J* 2,85, 4.4 Hz), 3.90-3.85 (1H, m), 3.73 (1H, dt, *J* 6.95, 9.45 Hz), 3.67 (3H, s), 3.52-3.48 (1H, m), 3.38 (1H, dt, *J* 6.95, 9.45 Hz), 2.3 (2H, t, *J* 7.55 Hz), 1.86-1.80 (1H, m), 1.74-1.69 (1H, m), 1.65-1.50 (10H, m), 1.35-1.21 (28H, m, including br s at 1.25); δc (125MHz, $CDCl_3$): 174.32, 98.83, 67.69, 62, 51.4, 34.11, 30.78, 29.75, 29.63, 29.59, 29.47, 29.43, 29.23, 29.14, 26.23, 25.50, 24.95, 19.69; ν_{max}: 2920, 2850, 1733, 1471, 1440, 1239, 1196, 1140 cm^{-1}.

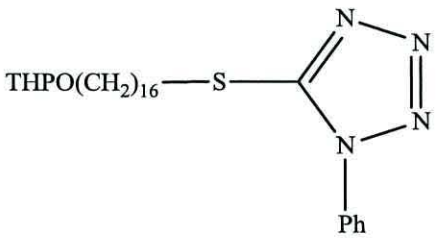
Experiment 85: 16-(Tetrahydropyran-2-yloxy)-hexadecan-1-ol (272)

$THPO(CH_2)_{16}OH$

The procedure used in Experiment 21 was repeated in order to reduce 16-(tetrahydro-pyran-2-yloxy)-hexadecanoic acid methyl ester (**271**) (13 g, 35.08 mmol) using lithium

aluminum hydride (2.05 g, 52.62 mmol) in THF (200 ml). The crude product was purified by column chromatography on silica gel eluting petrol/ethyl acetate (5:2) to give a white solid, 16-(tetrahydropyran-2-yloxy)-hexadecan-1-ol (**272**) (10.6 g, 88 %), m.p.: 55-58 °C [Found $(M + Na)^+$: 365.3024, $C_{21}H_{42}NaO_3$ requires: 365.3026], which showed δ_H (500MHz, $CDCl_3$): 4.57 (1H, br t, *J* 2.85 Hz), 3.88-3.84 (1H, m), 3.71 (1H, dt, *J* 6.95, 9.45 Hz), 3.62 (2H, t, *J* 6.95 Hz), 3.51-3.47 (1H, m), 3.37 (1H, dt, *J* 6.95, 9.45 Hz), 1.85-1.78 (1H, m), 1.73-1.67 (1H, m), 1.61-1.48 (9H, m), 1.34-1.20 (24H, br m, including br s at 1.24); δc (125MHz, $CDCl_3$): 98.77, 67.65, 62.96, 62.26, 32.76, 30.73, 29.70, 29.59, 29.55, 29.54, 29.44, 29.39, 26.19, 25.70, 25.45, 19.62; ν_{max}: 3438, 2922, 2850, 1468, 1350, 1201, 1121, 1060 cm^{-1}.

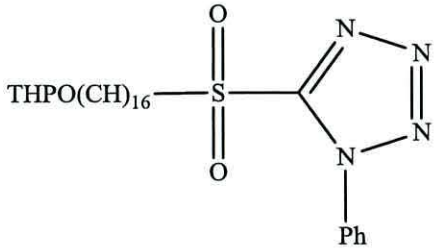
Experiment 86: 4-Phenyl-5-[16-(tetrahydropyran-2-yloxy)-hexadecylsulfanyl]-4*H*-[1,2,3]triazole (273)



16-(Tetrahydropyran-2-yloxy)-hexadecan-1-ol (**272**) (12.6 g, 36.78 mmol), triphenylphosphine (12.54 g, 47.81 mmol) and 1-phenyl-1*H*-tetrazole-5-thiol (7.86 g, 44.13 mmol) were dissolved in dry THF (200 ml) and cooled to 0 °C with stirring. Diethyl azodicarboxylate (7.68 g, 44.13 mmol) in dry THF (15 ml) was added and the mixture was allowed to reach r.t. and stirred overnight. The solvent was evaporated and the residue was stirred with petrol/ethyl acetate (1:1, 150 ml) for 30 min and filtered. The filtrate was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (1:1) to give a white solid, 4-phenyl-5-[16-(tetrahydropyran-2-yloxy)-hexadecylsulfanyl]-4*H*-[1,2,3]triazole (**273**) (17.33 g, 93 %), m.p.: 42-44 °C [Found $(M + Na)^+$: 525.3238, $C_{28}H_{46}N_4NaO_2S$ requires: 525.3234], which showed δ_H (500MHz, $CDCl_3$): 7.52-7.40 (4H, m), 7.25-7.19 (1H, m), 4.48 (1H, t, *J* 2.85 Hz), 3.80-3.75 (1H, m), 3.63 (1H, dt, *J* 6.95, 9.45 Hz), 3.42-3.38 (1H, m), 3.31-3.26 (2H, m), 1.80 (2H, pent., *J* 7.25 Hz), 1.73-1.67 (1H, m), 1.61-1.20 (32H, br m, including br s at 1.24); δc (125MHz, $CDCl_3$): 154.44, 133.73, 133.71, 133.57, 129.97, 129.68, 129.13, 128.62, 128.43, 128.37, 123.77, 123.66, 98.77, 67.62, 62.27, 33.31,

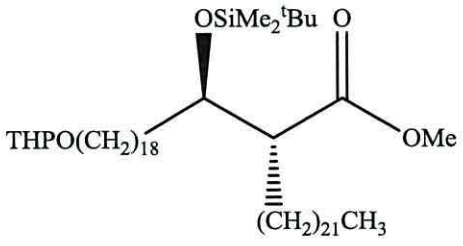
30.73, 29.69, 29.58, 29.54, 29.47, 29.42, 29.36, 29.01, 28.96, 28.57, 26.17, 25.44, 19.64; ν_{max} : 2922, 2851, 1500, 1470, 1385, 1244, 1136, 1077 cm^{-1}.

Experiment 87: 4-Phenyl-5-[16-(tetrahydropyran-2-yloxy)-hexadecane-1-sulfonyl]-4*H*-[1,2,3]triazole (268)



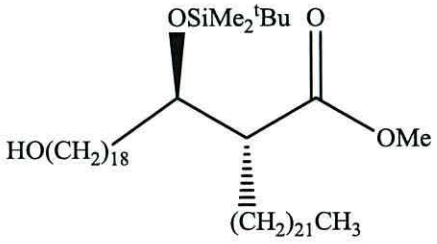
The procedure used in Experiment 2 was repeated using 4-phenyl-5-[16-(tetrahydropyran-2-yloxy)-hexadecylsulfanyl]-4*H*-[1,2,3]triazole (**273**) (17.3 g, 34.4 mmol), ammonium molybdate (VI) tetrahydrate (19.13 g, 15.48 mmol) in ice cold H_2O_2 (35 % w/w, 52 ml) in THF (100 ml) and IMS (300 ml) and a further solution of ammonium molybdate (VI) tetrahydrate (9 g, 7.28 mmol) in ice cold H_2O_2 (35 % w/w, 30 ml). The crude product was purified by column chromatography eluting with petrol/ethyl acetate (5:1) to give a colourless oil (**268**) (14.4 g, 78 %), m.p.: 50-52 °C [Found $(M + Na)^+$: 557.3221, $C_{28}H_{46}N_4NaO_4S$ requires: 557.3132], which showed δ_H (500MHz, $CDCl_3$): 7.70-7.68 (2H, m), 7.64-7.58 (3H, m), 4.57 (1H, br t, *J* 2.5 Hz), 3.89-3.84 (1H, m), 3.75-3.70 (3H, m), 3.51-3.47 (1H, m), 3.37 (1H, dt, *J* 6.65, 9.45 Hz), 1.97-1.91 (2H, m), 1.85-1.78 (1H, m), 1.73-1.68 (1H, m), 1.61-1.46 (9H, m), 1.341.25 (21H, br m, including br s at 1.25); δc (125MHz, $CDCl_3$): 153.43, 133.0, 131.38, 129.65, 125.01, 98.78, 67.64, 62.28, 55.95, 30.73, 29.7, 29.58, 29.55, 29.54, 29.50, 29.44, 29.39, 29.13, 28.84, 28.08, 26.19, 25.45, 21.89, 19.65; ν_{max}: 3104, 2917, 2851, 1731, 1599, 1496, 1343, 1136, 1082 cm^{-1}.

Experiment 88: (*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-19-(tetrahydropyran-2-yloxy)-nonadecyl]-tetracosanoic acid methyl ester (267)



The procedure used in Experiment 3 was repeated in order to couple the (*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilanyloxy)-3-oxopropyl]-tetracosanoic acid methyl ester (**177**) (2.4 g, 4.22 mmol) and 4-phenyl-5-[16(tetrahydropyran-2-yloxy)-hexadecane-1-sulfonyl]-4*H*-[1,2,3]triazole (268) (2.7 g, 5.06 mmol) using lithium bis(trimethylsilyl)amide (7.1 ml, 7.59 mmol, 1.06 M) in dry tetrahydrofuran (100 ml) under nitrogen at -10 °C. The crude product was purified by column chromatography on silica gel eluting with petrol/ethyl acetate (20:1) to give a yellow oil, (*R*)-2-[(*E/Z*)-(*R*)-1-(*tert*-butyldimethylsilanyloxy)-19-(tetrahydropyran-2-yloxy)-nonade-3-enyl]tetracosanoic acid methyl ester (**274**) (2.7 g, 73 %). Hydrogenation was carried out with alkenes (2.7 g, 3.07 mmol) in IMS (30 ml) and THF (10 ml) using palladium on charcoal (10 %, 0.5 g) and hydrogen to give oil. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a colourless oil, (*R*)-2-[(*R*)-1-(*tert*-butyldiphenylsilanyloxy)-19-(tetrahydropyran-2-yloxy)-nonadecyl]-tetracosanoic acid methyl ester (**267**) (2.66 g, 98 %) [Found $(M + Na)^+$: 901.8024, $C_{55}H_{110}NaO_5Si$ requires: 901.8015], $[\alpha]_D^{19} = -4.36$ (*c* 1.03, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 4.58 (1H, br t, *J* 2.85 Hz), 3.92-3.85 (2H, m), 3.73 (1H, dt, *J* 6.95, 9.45 Hz), 3.66 (3H, s), 3.52-3.48 (1H, m), 3.38 (1H, dt, *J* 6.95, 9.45 Hz), 2.53 (1H, ddd, *J* 3.75, 7.25, 11Hz), 1.87-1.80 (1H, m), 1.74-1.69 (1H, m), 1.53-1.22 (80H, br m, including br s at 1.25), 0.88 (3H, t, *J* 6.95 Hz), 0.86 (9H, s), 0.04 (3H, s), 0.02 (3H,s); δc (125MHz, $CDCl_3$): 175.14, 98.83, 73.21, 67.69, 62.32, 51.55, 51.21, 33.66, 31.92, 30.78, 29.82, 29.75, 29.69, 29.65, 29.61, 29.58, 29.55, 29.50, 29.43, 29.35, 27.81, 27.48,26.24, 25.75, 25.50, 23.67, 22.68, 19.69, 17.96, 14.11, -4.37, - 4.94; ν_{max}: 2924, 2854, 1741, 1464, 1255, 1034 cm^{-1}.

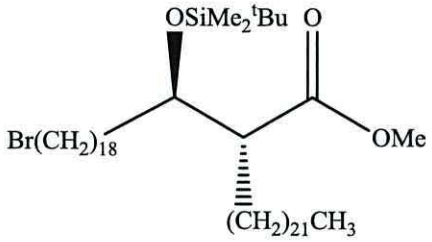
Experiment 89: (*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-19-hydroxynonadecyl] tetracosanoic acid methyl ester (275)



The procedure in Experiment 48 was repeated using (*R*)-2-[(*R*)-1-(*tert*-butyldiphenylsilanyloxy)-19-(tetrahydropyran-2-yloxy)-nonadecyl]-tetracosanoic acid methyl ester (2.61 g, 2.9 mmol) and pyridinium-*p*-toluene sulfonate (**267**) (0.37 g, 1.48 mmol) in

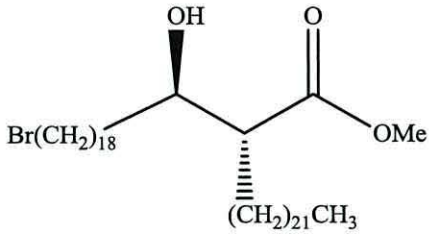
methanol (10 ml) and THF (25 ml) and stirred at 50 °C overnight. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (7:1) to give a thick oil, (*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilanyloxy)-19-hydroxynonadecyl]-tetracosanoic acid methyl ester (**275**) (1.9 g, 80 %) [Found $(M + Na)^+$: 817.7448, $C_{50}H_{102}NaO_4Si$ requires: 817.7440], $[\alpha]_D^{21} = -3.65$ (*c* 0.82, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 3.92-3.89 (1H, m), 3.65 (3H, s), 3.64 (2H, br t, *J* 7.25 Hz), 2.52 (1H, ddd, *J* 3.8, 7.25, 11.05 Hz), 1.59-1.21 (77H, br m, including br s at 1.26), 0.88 (3H, t, *J* 6.95 Hz), 0.86 (9H, s), 0.04 (3H, s), 0.02 (3H, s); δc (125MHz, $CDCl_3$): 175.14, 73.21, 63.08, 51.56, 51.21, 33.66, 32.80, 31.91, 29.81, 29.69, 29.64, 29.61, 29.59, 29.57, 29.42, 29.34, 27.81, 27.48, 25.74, 23.67, 22.67, 17.96, 14.10, -4.36, -4.94; ν_{max}: 3356, 2924, 2853, 1740, 1464, 1253, 1070 cm^{-1}.

Experiment 90: (*R*)-2-[(*R*)-19-Bromo-1-(*tert*-butyldimethylsilanyloxy)-nonadecyl]-tetracosanoic acid methyl ester (276)



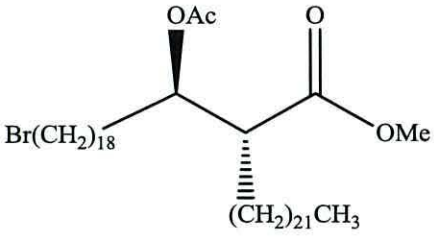
The procedure in Experiment 13 was repeated using (*R*)-2-[(*R*)-1-(*tert*-butyldimethyl-silanyloxy)-19-hydroxy-nonadecyl]-tetracosanoic acid methyl ester (**275**) (1.83 g, 2.60 mmol), N-bromosuccinimide (0.6 g, 3.38 mmol) and triphenylphosphine (0.85 g, 3.25 mmol) in dichloromethane (50 ml) at 0 °C. The crude product was purified by column chromatography on silica eluting with petrol/ethyl acetate (10:1) to give a thick oil, (*R*)-2-[(*R*)-19-bromo-1-(*tert*-butyldimethylsilanyloxy)-nonadecyl]-tetracosanoic acid methyl ester (**276**) (1.75, 78 %) [Found $(M + Na)^+$: 879.6590, $C_{50}H_{101}BrNaO_3Si$ requires: 879.6596], $[\alpha]_D^{18} = -8.14$ (*c* 0.81, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 3.92-3.89 (1H, m), 3.66 (3H, s), 3.41 (2H, t, *J* 6.9 Hz), 2.53 (1H, ddd, *J* 3.8, 6.95, 10.7 Hz), 1.86 (2H, pent., *J* 6.95 Hz), 1.60-1.22 (74H, br m, including br s at 1.26), 0.88 (3H, t, *J* 6.95 Hz), 0.86 (9H, s), 0.04 (3H, s), 0.02 (3H, s); δc (125MHz, $CDCl_3$): 175.12, 73.21, 51.57, 51.20, 33.98, 33.67, 32.85, 31.92, 29.82, 29.69, 29.65, 29.62, 29.58, 29.55, 29.44, 29.35, 28.77, 28.18, 27.82, 27.48,25.75, 23.70, 22.68, 17.97, 14.10, -4.37, -4.93; ν_{max}: 2924, 2854, 1741,1464, 1254, 1166, 1073 cm^{-1}.

Experiment 91: (*R*)-2-((*R*)-19-Bromo-1-hydroxy-nonadecyl)-tetracosanoic acid methyl ester (266)



The procedure in Experiment 26 was repeated using the (*R*)-2-[(*R*)-19-bromo-1-(*tert*-butyldimethylsilanyloxy)-nonadecyl]-tetracosanoic acid methyl ester (**276**) (1.5 g, 1.74 mmol), pyridine (0.3 ml) and HF.pyridine (2.5 ml) in dry THF (20 ml). The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1, then 5:1) to give a white solid, (*R*)-2-((*R*)-19-bromo-1-hydroxynonadecyl)-tetracosanoic acid methyl ester (**266**) (1.18 g, 88 %), m.p.: 65-67 °C [Found $(M + Na)^+$: 765.5715, $C_{44}H_{87}BrNaO_3$ requires: 765.5731], $[\alpha]_D^{18}$ = + 4.21 (*c* 0.76, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 3.71 (3H, s), 3.68-3.65 (1H, br m), 3.41 (2H, t , *J* 6.95 Hz), 2.44 (1H, dt, *J* 5.35, 9.45 Hz), 1.86 (2H, pent., *J* 6.8 Hz), 1.75-1.68 (1H, br m), 1.60-1.53 (3H, m), 1.48-1.22 (71H, br m, including br s at 1.26), 0.88 (3H, t, *J* 6.9 Hz); δ_C (125MHz, $CDCl_3$): 176.23, 72.29, 51.5, 50.93, 35.7, 34.02, 32.84, 31.92, 29.69, 29.65, 29.62, 29.59, 29.56, 29.53, 29.49, 29.43, 29.42, 29.35, 28.76, 28.18, 27.41, 25.72, 22.68, 14.10; ν_{max}: 3400, 2975, 2918, 2850, 1713, 1650, 1384, 1049 cm^{-1}.

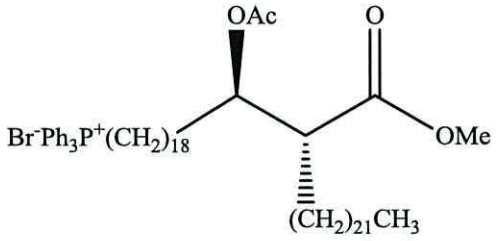
Experiment 92: (*R*)-2-((*R*)-19-Bromo-1-methoxycarbonyloxynonadecyl)-tetracosanoic acid methyl ester (265)



The procedure used in Experiment 27 was repeated using the alcohol (**266**) (1.18 g, 1.58 mmol), acetic anhydride (5 ml) and anhydrous pyridine (5 ml) in dry toluene (15 ml). The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a white solid, (*R*)-2-((*R*)-19-bromo-1-methoxycarbonyloxynonadecyl)-tetracosanoic acid methyl ester (**265**) (1.18 g, 95 %),

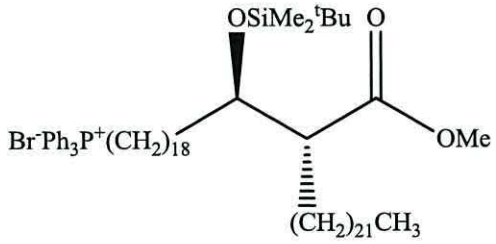
m.p.: 44-45 °C [Found $(M + Na)^+$: 807.5803, $C_{46}H_{89}BrNaO_4$ requires: 807.5836], $[\alpha]_D^{17}$= + 8.85 (*c* 0.92, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 5.8 (1H, br dq, *J* 3.75, 7.85 Hz), 3.68 (3H, s), 3.41 (2H, t, *J* 6.95 Hz), 2.61 (1H, ddd, *J* 4.1, 6.6, 10.7 Hz), 2.03 (3H, s), 1.85 (2H, pent., *J* 6.95 Hz), 1.62-1.21 (74H, br m, including br s at 1.25), 0.88 (3H, t, *J* 6.9 Hz); δ_C (125MHz, $CDCl_3$): 173.63, 170.32, 74.09, 51.51, 49.57, 34.0, 32.83, 31.91, 31.7, 29.69, 29.64, 29.61, 29.54, 29.53, 29.45, 29.42, 29.38, 29.34, 28.75, 28.17, 28.1, 27.46, 24.97, 22.67, 21.0, 14.18, 14.09; ν_{max}: 2918, 2850, 1742, 1236 cm^{-1}.

Experiment 93: (*R*)-2-[(*R*)-1-Acetoxy-19-(triphenyl-λ^5-phosphanyl)-nonadecyl]-tetracosanoic acid methyl ester bromide (263)



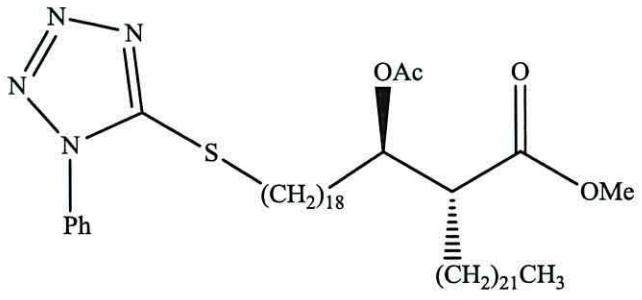
Triphenylphosphine (0.2 g, 0.279 mmol) was added to a solution of (*R*)-2-((*R*)-19-bromo-1-methoxycarbonyloxy-nonadecyl)-tetracosanoic acid methyl ester (**265**) (0.2 g, 0.254 mmol) in dry toluene (4 ml), and refluxed for 5 days. The solvent was evaporated to give a brown solid, which was washed with ether and filtered. The filtrate was evaporated to give a residue. The crude product was purified by column chromatography eluting with petrol then methanol to give a semi-solid (**263**) (0.2 g, 75 %) [Found $(M)^+$: 967.7623, $C_{64}H_{104}O_4P$ requires: 967.7667], $[\alpha]_D^{19}$= + 7.28 (*c* 1.27, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 7.81-7.61 (7H, m), 7.52-7.40 (8H, m), 5.07-5.04 (1H, m), 3.69 (2H, br s), 3.64 (3H, s), 2.59 (1H, ddd, *J* 4.4, 6.65, 10.7 Hz), 1.99 (3H, s)1.58-1.15 (77H, br m, including br s at 1.22), 0.84 (3H, t, *J* 5.7 Hz); δ_C (125MHz, $CDCl_3$): 173.53, 170.21, 134.87, 134.85, 133.55, 133.47, 132.67, 131.96, 131.88, 131.84, 131.82, 130.39, 130.29, 128.41, 128.31, 73.96, 51.39, 49.43, 31.76, 31.57, 29.53, 29.49, 29.46, 29.41, 29.39, 29.32, 29.3, 29.28, 29.23, 29.19, 29.06, 27.97, 27.3, 24.85, 22.52, 20.88, 13.97; ν_{max}: 2925, 2854, 1739, 1493, 1451, 1118, 1050 cm^{-1}.

Experiment 94: (*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-19-(triphenyl-λ^5-phosphanyl)-nonadecyl]-tetracosanoic acid methyl ester bromide (277)



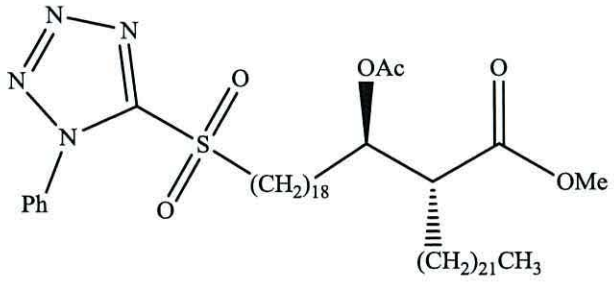
Triphenylphosphine (0.08 g, 0.307 mmol) was added to a solution of (*R*)-2-[(*R*)-19-bromo-1-(*tert*-butyldimethylsilanyloxy)-nonadecyl]-tetracosanoic acid methyl ester (**276**) (0.24 g, 0.279 mmol) in dry toluene (4 ml) and refluxed for 5 days. The solvent was evaporated to give a brown solid, which was washed with ether and filtered. The filtrate was evaporated to give a residue. The crude product was purified by column chromatography eluting with petrol then methanol to give a semi-solid (**277**) (0.15 g, 48 %) [Found $(M)^+$: 1039.8393, $C_{68}H_{116}O_3PSi$ requires: 1039.8426], $[\alpha]_D^{21} = -5.55$ (*c* 0.9, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 7.84-7.75 (3H, m), 7.70-7.62 (6H, m), 7.54-7.72 (6H, m), 3.9-3.87 (1H, m), 3.73 (2H, br s), 3.63 (3H, s), 2.50 (1H, ddd, *J* 3.5, 6.95, 10.75 Hz), 1.60-1.17 (76H, br m, including br s at 1.23), 0.86-0.82 (12H, including a singlet resonated at 0.84 for the ^{t}Bu and a triplet resonated at 0.85 with *J* 6.3 Hz), 0.024 (3H, s), 0.0006 (3H, s); δ_C (125MHz, $CDCl_3$): 175.02, 134.9, 134.88, 133.63, 133.55, 132.76, 132.02, 131.94, 131.88, 131.86, 130.43, 130.34, 128.46, 128.36, 118.66, 117.99, 73.11, 68.15, 51.49, 51.12, 33.58, 31.81, 30.39, 30.28, 29.73, 29.62, 29.59, 29.54, 29.52, 29.46, 29.34, 29.25, 29.12, 27.73, 27.36, 25.67, 23.64, 22.86, 22.58, 18.68, 17.87, 14.02, -4.45, -5.01; ν_{max}: 2925, 2855, 1735, 1493, 1451, 1118, 1050 cm^{-1}.

Experiment 95: (*R*)-2-[(*R*)-1-Acetoxy-19-(1-phenyl-1*H*-tetrazol-5-ylsulfany]-nonadecyl]-tetracosanoic acid methyl ester (280)



(*R*)-2-((*R*)-19-Bromo-1-methoxycarbonylnonadecyl)-tetracosanoic acid methyl ester (**265**) (0.2, 0.25 mmol) was added with vigorous stirring to 1-phenyl-1*H*-tetrazole-5-thiol (0.047 g, 0.25 mmol) and anhydrous potassium carbonate (0.07 g, 0.5 mmol) in acetone (6 ml). The mixture was refluxed for 5 hours, when TLC showed no starting material was left. The inorganic salts were filtered off and washed with acetone; the solution was extracted between dichloromethane (15 ml) and water (20 ml). The aqueous layer was extracted with dichloromethane (2 × 15 ml). The combined organic phases were washed with water (15 ml), dried and evaporated to give a residue. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (7:1) to give a white solid, (*R*)-2-[(*R*)-1-acetoxy-19-(1-phenyl-1*H*-tetrazol-5-ylsulfany]-nonadecyl]-tetracosanoic acid methyl ester (**280**) (0.15 g, 68 %), m.p.: 39-42 °C [Found $(M + H)^+$: 883.7062, $C_{53}H_{95}N_4O_4S$ requires: 883.7068], $[\alpha]_D^{20} = +7.34$ (*c* 1.13, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 7.61-7.52 (5H, m), 5.10-5.07 (1H, m), 3.68 (3H, s), 3.4 (2H, t, *J* 7.25 Hz), 2.62 (1H, ddd, *J* 4.45, 6.95, 11.05 Hz), 2.03 (3H, s), 1.82 (2H, pent., *J* 7.55 Hz), 1.67-1.22 (74H, br m), 0.88 (3H, t, *J* 6.6 Hz); δ_C (125MHz, $CDCl_3$): 173.64, 170.33, 130.03, 129.74, 123.86, 74.09, 51.53, 49.59, 33.38, 31.92, 31.72, 29.69, 29.62, 29.55, 29.47, 29.44, 29.39, 29.35, 29.08, 29.03, 29.64, 28.11, 27.46, 24.99, 22.68, 22.6, 14.11; ν_{max}: 2923, 2853, 1744, 1500, 1464, 1237, 1016 cm^{-1}.

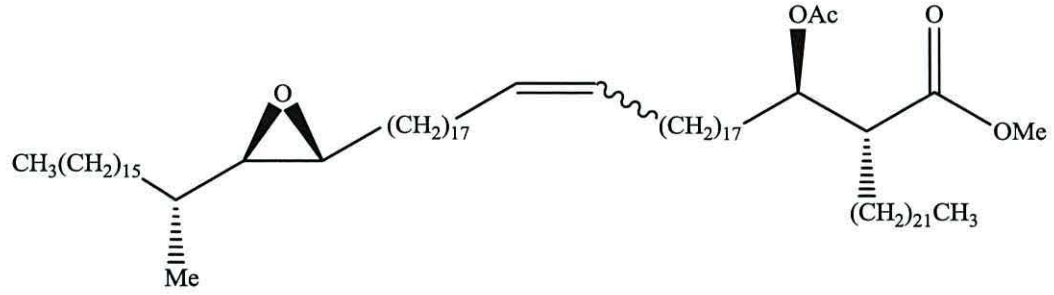
Experiment 96: (*R*)-2-[(*R*)-1-Acetoxy-19-(1-phenyl-1*H*-tetrazole-5-sulfonyl)-nonadecyl]-tetracosanoic acid methyl ester (264)



A solution of ammonium molybdate (VI) tetrahydrate (0.088 g, 0.071 mmol) in ice cold H_2O_2 (35 % w/w, 0.5 ml) was added to a stirred solution of (*R*)-2-[(*R*)-1-acetoxy-19-(1-phenyl-1*H*-tetrazol-5-ylsulfany]-nonadecyl]-tetracosanoic acid methyl ester (**280**) (0.14 g, 0.15 mmol) in IMS (5 ml) and THF (3 ml) at room temperature for 2 hours. A further solution of ammonium molybdate (VI) tetrahydrate (0.04 g, 0.049mmol) in ice cold H_2O_2 (35 % w/w, 0.2 ml) was added and the mixture was stirred at room temperature for 18 hours, then poured into water (50 ml) and extracted with dichloromethane (3 × 25

ml). The combined organic phases were washed with water (2 × 20 ml), dried over $MgSO_4$ and evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (6:1) to give a white solid, (*R*)-2-[(*R*)-1-acetoxy-19-(1-phenyl-1*H*-tetrazole-5-sulfonyl)-nonadecyl]-tetracosanoic acid methyl ester (**264**) (0.14 g, 96 %). m.p.: 53-55 °C [Found $(M + Na)^+$: 937.6798, $C_{53}H_{94}N_4NaO_6S$ requires: 937.6786], $[\alpha]_D^{18} = +5.14$ (*c* 0.7, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 7.71-7.69 (2H, m), 7.65-7.59 (3H, m), 5.10-5.07 (1H, m), 3.73 (2H, t, *J* 7.9 Hz), 3.68 (3H, s), 2.62 (1H, ddd, *J* 4.4, 6.95, 11.05 Hz), 2.03 (3H, s), 1.99-1.92 (2H, m), 1.65-1.22 (74H, br m, including br s at 1.26), 0.88 (3H, t, *J* 6.6 Hz); δ_C (125MHz, $CDCl_3$): 173.63, 170.32, 153.5, 133.06, 131.42, 129.69, 125.06, 74.08, 56.01, 51.52, 49.58, 31.91, 31.71, 29.62, 29.55, 29.45, 29.43, 29.38, 29.34, 29.18, 28.88, 28.13, 28.1, 27.46, 24.98, 22.67, 21.93, 21.01, 14.10; ν_{max}: 2923, 2852, 1742, 1462, 1344, 1235, 1152 cm^{-1}.

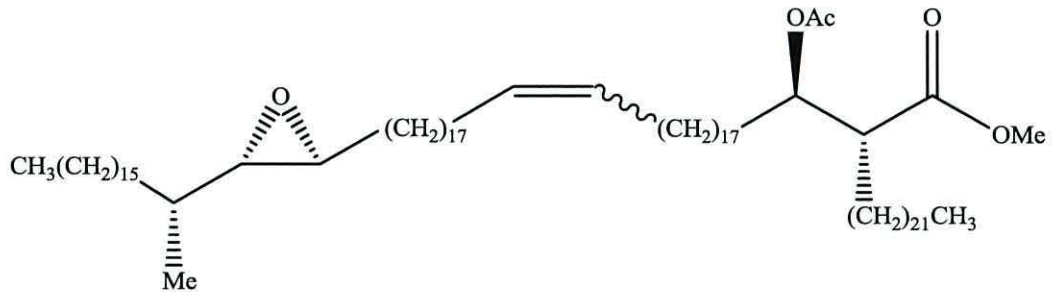
Experiment 97: (*E/Z*)-(2*R*,3*R*)-3-Acetoxy-39-[(2*R*,3*R*)-3-((*R*)-1-methylheptadecyl)-oxiranyl]-2-docosyl-nonatricont-21-enoic acid methyl ester (107)



Lithium bis(trimethylsilyl)amide (0.15 ml, 0.15 mmol, 1.06 M) was added dropwise with stirring to 18-(2*R*,3*R*)-3-((*R*)-1-methylheptadecyl)-oxiranyl)-octadecanal (**262**) (50 mg, 0.088 mmol) and (*R*)-2-[(*R*)-1-acetoxy-19-(1-phenyl-1*H*-tetrazole-5-sulfonyl)-nonadecyl]-tetracosanoic acid methyl ester (**264**) (97 mg, 0.106 mmol) in dry tetrahydrofuran (9 ml) under nitrogen at - 5 °C. The mixture was allowed to reach room temperature and stirred for 2 hours, when TLC showed no starting material, then quenched with sat. aq. NH_4Cl at 0 °C and petrol/ether (1:1, 2 ml) was added. The aqueous layer was re-extracted with petrol/ether (1:1, 2 × 15 ml). The combined organic layers were dried and evaporated to give thick oil. The crude product was purified by column chromatography on silica eluting with petrol/ethyl acetate (20:1) to give a white solid (**107**) (50 mg, 45 %) as a mixture of two isomers in ratio 2:1, which showed δ_H (500MHz, $CDCl_3$): 5.39-5.38 (1H, m), 5.35 (1H, br t, *J* 2.4 Hz), 5.09 (1H, dt, *J* 4.1, 7.9 Hz), 3.68 (3H, s), 2.72 (1H, dt, *J* 2.2, 5.65 Hz), 2.62 (1H, ddd, *J* 4.45, 6.95, 11.05 Hz),

2.41 (1H, dd, *J* 2.2, 6.9 Hz), 2.03 (3H, s), 1.99-1.95 (2H, m), 1.65-1.21 (139H, br m), 1.0 (3H, d, *J* 5.6 H), 0.87 (6H, br t, *J* 5.05 Hz); δ_C (125MHz, $CDCl_3$): 173.64, 170.32, 130.34, 129.87, 74.08, 63.8, 57.45, 51.51, 49.57, 41.34, 36.06, 35.84, 34.62, 33.7, 32.6, 32.2, 31.92, 31.7, 29.93, 29.77, 29.7, 29.56, 29.46, 29.44, 29.39, 29.36, 29.32, 29.18, 28.1, 27.65, 27.46, 27.2, 26.89, 26.14, 24.97, 22.68, 22.59, 20.43, 19.42, 15.95, 14.29, 41.1; ν_{max}: 2919, 2850, 1740, 1651, 1470, 1239 cm^{-1}.

Experiment 98: (*E/Z*)-(2*R*,3*R*)-3-Acetoxy-39-[(2*S*,3*S*)-3-((*R*)-1-methylheptadecyl)-oxiranyl]-2-docosylnonatricont-21-enoic acid methyl ester (108)



The procedure used in Experiment 97 was repeated in order to couple 18-(2*S*,3*S*)-3-((*R*)-1-methylheptadecyl)-oxiranyl)-octadecanal (**261**) (20 mg, 0.035 mmol) and (*R*)-2-[(*R*)-1-acetoxy-19-(1-phenyl-1*H*-tetrazole-5-sulfonyl)-nonadecyl]-tetracosanoic acid methyl ester (264) (38 mg, 0.042 mmol) using lithium bis(trimethylsilyl)amide (0.05 ml, 0.055 mmol, 1.06 M) in dry tetrahydrofuran (5 ml) under nitrogen at - 5 °C. The crude product was purified by column chromatography on silica gel eluting with petrol/ethyl acetate (20:1) to give a white solid (**108**) (18 mg, 40 %) as a mixture of two isomers in ratio 2:1, which showed δ_H (500MHz, $CDCl_3$): 5.08 (1H, br t, *J* 3.45 Hz), 5.35 (1H, br t, *J* 4.45 Hz), 5.08 (1H, dt, *J* 3.8, 7.85 Hz), 3.68 (3H, s), 2.71 (1H, dt, *J* 2.2, 5.65 Hz), 2.62 (1H, ddd, *J* 4.1, 6.6, 10.7 Hz), 2.41 (1H, dd, *J* 1.9, 6.95 Hz), 2.03 (3H, s), 1.98-1.95 (2H, m), 1.85-1.79 (1H, m), 1.64-1.22 (138H, br m), 1.0 (3H, d, *J* 6 Hz), 0.88 (6H, br t, *J* 6.6 Hz);δ_C (125MHz, $CDCl_3$): 171.13, 169.86, 130.35, 129.88, 72.57, 63.85, 58.86, 50.49, 49.5, 41.29, 36.03, 34.97, 33.77, 32.61, 32.26, 31.92, 29.86, 29.69, 29.65, 29.58, 29.46, 29.39, 29.35, 29.21, 28.96, 27.21, 26.08, 25.6, 22.68, 17.28, 14.19, 14.11; ν_{max}: 2920, 2851, 1742, 1653, 1470, 1238 cm^{-1}.

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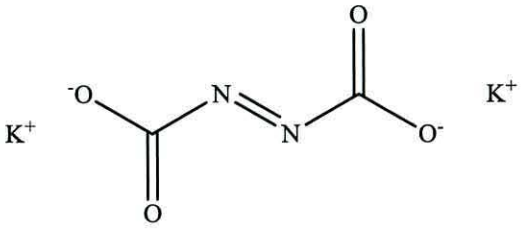
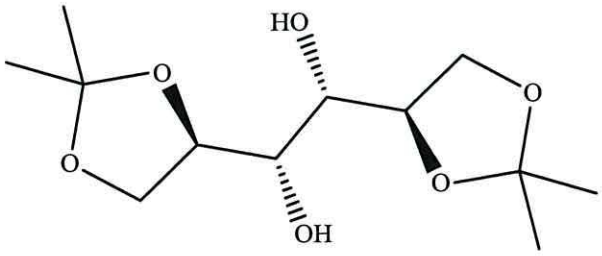
6-Appendices

Appendix 1: Preparation of di-potassium azodicarboxylate



Azodicarbonamide (7.5 g, 64 mmol) was slowly added in small portions to a vigorously stirred solution of KOH (15 g, 260 mmol) in de-ionised water (15 ml) at 0 °C on a salted ice-water bath, maintaining the temperature below 5 °C. The bright yellow solution was stirred at 0-5 °C for a further 45 min., during which time a thick bright yellow precipitate of dipotassium salt formed. The precipitate was filtered into a sintered funnel and washed with ice-cold methanol (60 ml). The yellow precipitate was dissolved in water (40 ml) on the sintered glass funnel at 18 °C. The yellow solution was sucked through the sinter by vacuum into pre-cooled (- 20 °C) methylated spirit (60 ml) giving a yellow precipitate. The yellow precipitate was again filtered through a sinter funnel and washed with cold (- 20 °C) methanol (50 ml), followed by cold (- 20 °C) petrol (50 ml). The solid was dried by vacuum and powdered with a spatula before being transferred under nitrogen into a pre-cooled round bottomed flask. The flask was sealed and stored in a freezer.[215]

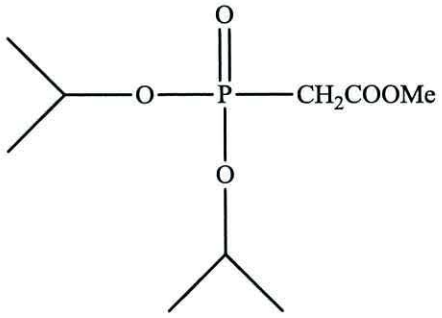
Appendix 2: Preparation 1,2:5,6-Di-*O*-isopropylidene–D-mannitol



Anhydrous zinc chloride (99.64 g, 731 mmol) was added to acetone (600 ml), and the mixture was stirred until the zinc chloride was dissolved. D-mannitol (60 g, 329 mmol) was added to the solution and the mixture was stirred for 18 hours at room temperature.

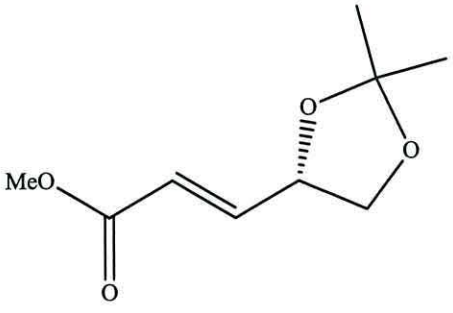
Potassium carbonate (100 g, 724 mmol) was dissolved in water (100 ml) and added to the D-mannitol solution. The resulting solution was filtered under reduced pressure and washed with dichloromethane (4 × 100 ml). The solvent was removed in *vacuo* and the resulting precipitate was re-dissolved in dichloromethane (300 ml). The solution was extracted with water (100 ml) and the organic layer was washed with brine (150 ml) and then dried over $MgSO_4$. The dichloromethane was then removed in *vacuo*. The precipitate was the re-crystallized in ethyl acetate (100 ml) and petroleum ether (500 ml) and left overnight at room temperature to give 1,2:5,6-di-*O*-isopropylidene–D-mannitol (45.7 g, 53 %)as a glassy white precipitates, which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), ν_{max} identical to the literature.[189]

Appendix 3: Preparation methyldiisopropyl phosphinylacetate



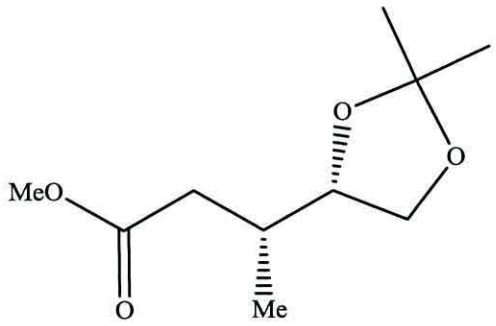
Methyl bromoacetate (100 g, 653 mmol) was added dropwise while stirring to triisopropyl phosphate (131.1 g, 507 mmol) at 143 °C. The mixture was stirred and refluxed for 2.5 hours at 143 °C. After vacuum pressure flash distillation the solution was separated to give the product oil at a boiling point of 108 °C. Yield (132.47 g, 85%).[231]

Appendix 4: (*E*)-3-((*S*)-2,2-Dimethyl-[1,3]dioxolan-4-yl)acrylic acid methyl ester (112)



A stirred solution of 1,2:5,6-di-*O*-isopropylidene-D-mannitol (44 g, 168.2 mmol) in aqueous (5 %) sodium hydrogen carbonate (300 ml) was cooled to 0 °C. Sodium *meta*-periodate (42.8 g, 200 mmol) in water (150 ml) was added dropweise and the mixture was stirred at room temperature for 1 hour. The mixture was then cooled to 0 °C once more and methyl diisopropyl phosphinylacetate (88 g, 369 mmol) was added dropwise, while maintaining the temperature. 6 M potassium carbonate solution (165.6 g) in water (400 ml), was then added and mixture was stirred for 20 hours. The mixture was then extracted with dichloromethane (2 × 600 ml), dried over $MgSO_4$, and the solvent removed *in vacuo* to yield a crude oil. The crude product was then purified by column chromatography on silica using petrol/ether (5:1) to give as a colourless oil, (*E*)-3-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl) acrylic acid methyl ester (**112**) (22.51 g, 72 %), which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), ν_{max} identical to the literature, $[\alpha]_D^{22} = +45.2$ (*c* 1.25, $CHCl_3$); lit. value $[\alpha]_D^{24} = +40.4$ (*c* 1.09, $CHCl_3$).[195,215]

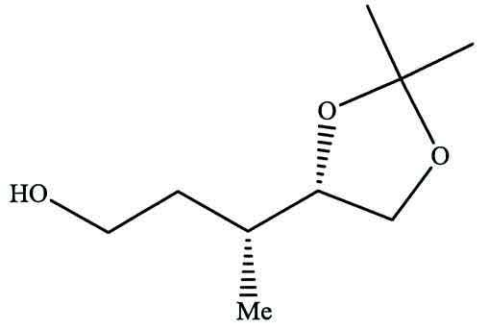
Appendix 5: (*R*)-3-[(*S*)-2,2-Dimethyl[1,3]dioxolan-4-yl)butric acid methyl ester (113)



Methyl lithium (64.5 ml, 96.7 mmol, 1.5 M) was added to a stirred solution of (*E*)-3-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl) acrylic acid methyl ester (9 g, 48.3 mmol) in dry ether (250 ml) at - 78 °C under nitrogen, stirred at this temperature for 2.5 hours, then allowed to reach - 60 °C followed by the addition of water (10 ml). After 5 min, sat. aq. ammonium chloride (60 ml) was added, where upon the temperature rose to - 40 °C. The mixture was allowed to reach 0 °C and quenched with water (100 ml). The organic layer was separated and the aqueous layer was extracted with ether (2 × 50 ml). The combined layers were washed with sat. aq. sodium chloride (2 × 50 ml) dried and evaporated to give a yellow oil, which was purified by chromatography on silica eluting with petrol/ether (8:2) to give a colourless oil, (*R*)-3-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)butric acid methyl ester (**113**) (6.65 g, 68 %), which showed δ_H (500 MHz, $CDCl_3$),

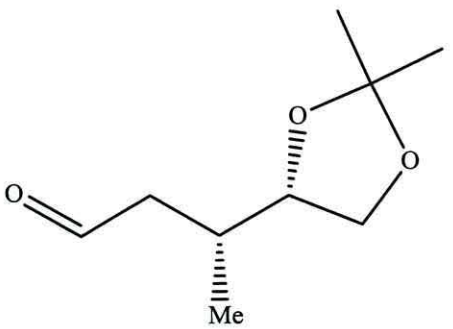
δ_C (125 MHz, $CDCl_3$), v_{max} identical to the literature, $[\alpha]_D^{25} = +7.46$ (*c* 1.2, $CHCl_3$); lit. value $[\alpha]_D^{24} = +8.6$ (*c* 1.05, $CHCl_3$).[195,215]

Appendix 6: (*R*)-3-((*S*)-2,2-Dimethyl-[1,3]dioxolan-4-yl)butane-1-ol (114)



(*R*)-3-((*S*)-2,2-dimethyl-[1,3]dioxolan-4yl)butyric acid methyl ester (15 g, 74 mmol) in tetrahydrofuran (60 ml) was added dropwise over 15 min to a suspension of lithium aluminum hydride (16.9 g, 433 mmol) in tetrahydrofuran (250 ml) at room temperature. The mixture was refluxed for 1 hour, when TLC showed no starting material was left then cooled to room temperature and quenched carefully with freshly prepared sat. aq. sodium sulphate decahydrate (30 ml) until a white precipitate was formed, the mixture was stirred vigorously for 10 min then filtered through a pad of celite and washed well with tetrahydrofuran (2 × 100 ml). The combined organic layers were evaporated to give a residue, which was purified by chromatography on silica eluting with petrol/ethyl acetate (1:1) to give (*R*)-3-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)butan-1-ol (**114**) (12.57 g, 97 %), which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), v_{max} identical to the literature, $[\alpha]_D^{25} = +19.86$ (*c* 1.24 , $CHCl_3$); lit. value $[\alpha]_D^{24} = +19.2$ (*c* 1.12, $CHCl_3$).[215,232]

Appendix 7: (*R*)-3-((*S*)-2,2-Dimethyl[1,3]dioxolan-4-yl)-butyralehyde (115)



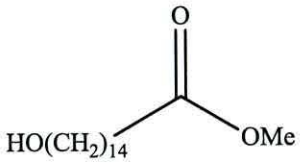
(*R*)-3-((*S*)-2,2-dimthyl-[1,3]dioxolan-4-yl)butan-1-ol (6.79 g, 39 mmol) in dichloromethane (40 ml) was added to a stirred suspension of pyridinium chlorochromate (16.8 g, 78 mmol) in dichloromethane (250 ml) at room temperature. The mixture was stirred vigorously and refluxed for 30 min (without heating), when TLC showed no starting material was left. It was cooled, poured into ether (200 ml) and filtered through a pad of silica and celite then washed well with ether and the filtered was evaporated to give a residue. Chromatography on silica eluting with petrol/ether (1:1) gave (*R*)-3-((*S*)-2,2-dimethyl [1,3]dioxolan-4-yl) butraldehyde (**115**) as a colourless oil (5.42 g, 81 %), which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), ν_{max} identical to the literature, $[\alpha]_D^{25} = +8.24$ (*c* 1.06, $CHCl_3$); lit. value $[\alpha]_D^{20} = +8.27$ (*c* 1.44, $CHCl_3$).[232]

Appendix 8: 12-Bromododecan-1-ol (145)

$HO(CH_2)_{12}Br$

1,2-Dodecandiol (25 g, 124 mmol) was dissolved in toluene (300 ml)and aqueous hydrobromic acid (30 ml, 48 % w.w.) was added. The mixture was refluxed for 22 hours monitoring the reaction with TLC. The mixture was then cooled to room temperature, the organic layer was separated and the solvent was removed. The residue, brown oil, was dissolved in dichloromethane (600 ml) and washed with sodium bicarbonate (300 ml). The aqueous layer was re-extracted with dichloromethane (3 × 150 ml). The combined organic layers were dried and the solvent evaporated to give the crude product which was purified by column chromatography eluting with petrol and ether (5:2) to give 12-bromododecan-1-ol (**145**) (22 g, 67 %), which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), ν_{max} identical to the literature.[233]

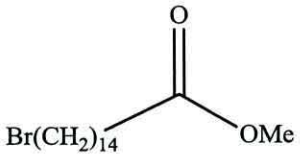
Appendix 9: 15-Hydroxy pentadecanoic acid methyl ester (164)



Sodium (4.75 g, 206.5 mmol) was added to methanol (250 ml) at 0 °C with stirring. The mixture was warmed to r.t. and stirred until all of the sodium was consumed. ω-

pentadecalactone (20 g, 83.3 mmol) was added with stirring and the solution was stirred at 80 °C for 3 hours. The reaction was quenched with aq. HCl (250 ml, 1N) and diluted with water (200 ml). The mixture was extracted with ethyl acetate (2 × 350 ml), the combined organic layers were washed with water (250 ml) and then brine (150 ml) and dried. The solvent was evaporated to give a white solid, and the product was mixture of the ester and acid, so the white solid was dissolved with methanol (150 ml) and (2 ml) of H_2SO_4 in methanol (5 ml) was added. The solution was refluxed for 90 min, cooled to r.t. and methanol was evaporated. The product was dissolved with ethyl acetate (250 ml), washed with sat. aq. $NaHCO_3$ (150 ml) and then brine (150 ml) and dried. The ethyl acetate evaporated and the crude product was purified by column chromatography eluting with petrol/ethyl acetate (2:1) to give a white solid, 15-hydroxypentadecanoic acid methyl ester (**164**)[218] (19.33 g, 85 %), m.p.: 44 - 46 °C, which showed δ_H (500 MHz, $CDCl_3$): 3.67 (3H, s), 3.64 (2H, t, *J* 6.6 Hz), 2.31 (2H, t, *J* 7.6 Hz), 1.65-1.54 (4H, m), 1.36-1.26 (21H, m); δ_C (125MHz, $CDCl_3$): 174.3, 63.1, 51.4, 34.1, 32.8, 29.6, 29.5, 29.4, 29.2, 25.7, 25.0, 24.9; ν_{max}: 3298, 2919, 2850, 1742, 1464, 1178 cm^{-1}.

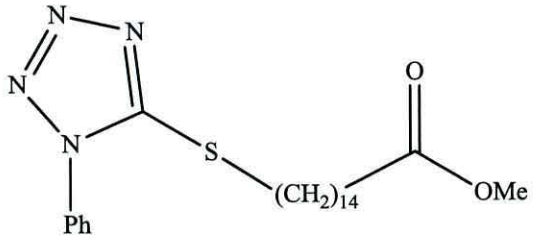
Appendix 10: 15-Bromopentadecanoic acid methyl ester (165)



Triphenylphosphine (22.9 g, 87.31 mmol) was added to a stirred solution of 15-hydroxypentadecanoic acid methyl ester (19 g, 69.85 mmol) in dichloromethane (350 ml) and then sodium bicarbonate (0.5 g) was added. The mixture was cooled to 0 °C and NBS (16.16 g, 90.8 mmol) was added portionwise over 20 min at 0 °C. Stirring was continued at r.t. for 1 hour, when TLC indicated that the reaction was complete. A saturated solution of sodium bisulfate (120 ml) was added and the mixture was extracted. The aqueous layer was re-extracted with CH_2Cl_2 (2 × 100 ml) and the combined organic layers were washed with water (100 ml). The solution was dried, the solvent was evaporated and petrol/ethyl acetate (20:1, 250 ml) was added. The mixture was refluxed for 30 min and the triphenylphosphonium oxide was filtered and washed well with a mixture of petrol/ethyl acetate (20:1, 150 ml). The solvent was evaporated

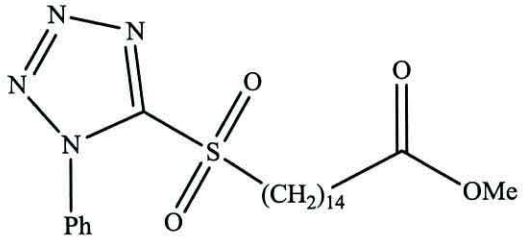
and the crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a white solid, 15-bromopentadecanoic acid methyl ester (**165**)[218] (20.62 g, 88 %), m.p.: 38-39 °C [Found $(M)^+$: 335.1568, $C_{16}H_{31}BrO_2$ requires: 335.158], which showed δ_H (500MHz, $CDCl_3$): 3.67 (3H, s), 3.41 (2H, t, *J* 6.6 Hz), 2.31 (2H, t, *J* 7.6 Hz), 1.89-1.83 (2H, quintet, *J* 7.0 Hz), 1.65-1.60 (2H, m), 1.40 (2H, m), 1.30.1.26 (18H, m); δ_C (125MHz, $CDCl_3$): 174.3, 51.4(-), 34.1(+), 34.0(+), 32.9(+), 32.59(+), 29.57(+), 29.52(+), 29.4(+), 29.3(+), 29.2(+), 28.8(+), 28.2(+), 25.0(+); ν_{max}: 2918, 2848, 1737, 1435, 1251, 1173 cm^{-1}.

Appendix 11: 15-(1-Phenyl-1*H*-tetrazole-5-ylsulfanyl)-pentadecanoic acid methyl ester (166)



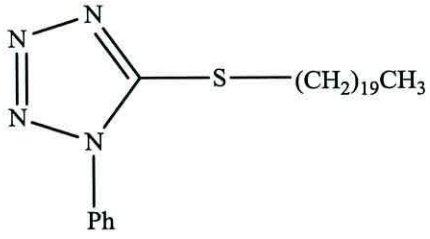
The procedure used in Experiment 1 was repeatedusing15-bromopentadecanoic acid methyl ester (20.6 g, 61.49 mmol), 1-phenyl-1*H*-tetrazole-5-thiol (10.95 g, 61.49 mmol), anhydrous potassium carbonate (16.99 g, 122.98 mmol) and acetone (250 ml). The crude product was re-crystallized from acetone (50 ml) and diluted with methanol (100 ml) to give a white solid, 15-(1-phenyl-1*H*-tetrazole-5-ylsulfanyl)-pentadecanoic acid methyl ester (**166**)[215] (24 g, 90 %), m.p.: 62-64 °C [Found $(M + Na)^+$: 455.2448, $C_{23}H_{36}N_4NaO_2S$ requires: 455.2451], which showed δ_H (500MHz, CDCl3): 7.75-7.60 (5H, m), 3.67 (3H, s), 3.39 (2H, t, *J* 7.3 Hz), 2.30 (2H, t, *J* 7.6 Hz), 1.82 (2H, quintet, *J* 7.4 Hz), 1.62 (2H, quintet, *J* 7.4 Hz), 1.47-1.41 (2H, m), 1.32-1.25 (18H, m); δ_C (125MHz, CDCl3): 174.3, 154.5, 133.8, 130.1(-), 129.8(-), 123.9(-), 51.4(-), 34.1(+), 33.4(+), 29.59(+), 29.56(+), 29.52(+), 29.4(+), 29.3(+), 29.2(+), 29.1(+), 29.1(+), 29.0(+), 28.6(+), 25.0(+) ; ν_{max}: 2916, 2850, 1742, 1499, 1472, 1250, 1171 cm^{-1}.

Appendix 12: 15-(1-Phenyl-1*H*-tetrazole-5-sulfonyl)-pentadecanoic acid methyl ester (167)



The procedure used in Experiment 2 was repeated usingthe 15-(1-phenyl-1*H*-tetrazole-5-ylsulfanyl)-pentadecanoic acid methyl ester (24 g, 55.5 mmol), ammonium molybdate (IV) tetrahydrate (30.87 g, 24.98 mmol) in H_2O_2 (35 % w/w, 70 ml) in THF (300 ml) and IMS (500 ml), and further ammonium molybdate (IV) tetrahydrate (13 g, 10.5 mmol) in H_2O_2 (35 %, w/w, 35 ml). The crude product was re-crystallized from methanol (300 ml) to give a white solid, 15-(1-phenyl-1*H*-tetrazole-5-sulfonyl)-pentadecanoic acid methyl ester (**167**)[215] (23.6 g, 91 %), m.p.: 72-73 °C [Found (M + Na)$^+$: 487.2343, $C_{23}H_{36}N_4NaO_4S$ requires: 487.2349], which showed δ_H (500MHz, $CDCl_3$): 7.71-7.68 (2H, m), 7.63-7.58 (3H, m), 3.73 (2H, distorted t, *J* 8.15 Hz), 3.67 (3H, s), 2.30 (2H, t, *J* 7.6 Hz), 1.98-1.92 (2H, m), 1.64-1.58 (2H, m), 1.52-1.46 (2H, m), 1.34-1.25 (18H, m); δ_C (125MHz, $CDCl_3$): 174.3, 153.5, 133.0, 131.4(+), 129.9(+), 129.68(+), 125.0(+), 124.98(+), 56.0(-), 51.4(+), 34.1(-), 29.53(-), 29.53 (-), 29.51(-), 29.49(-), 29.39(-), 29.2(-), 29.15(-), 28.9(-), 28.1(-), 25.0(-), 21.9(-); ν_{max}: 2918, 2948, 1729, 1497, 1464, 1343, 1255, 1199, 1157 cm^{-1}.

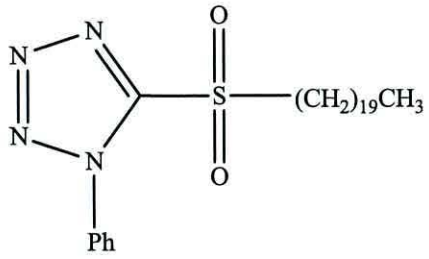
Appendix 13: 5-Icosylsulfanyl-1-phenyl-1*H*-tetrazole (174)



The procedure in Experiment 1 was repeated using 1-bromoeicosane (15 g, 41.5 mmol), 1-phenyl-1*H*-tetrazole-5-thiol (7.77 g, 43.57 mmol), anhydrous potassium carbonate (12.04 g, 87.15 mmol) and acetone (500 ml). The crude product was re-crystallised from acetone (80 ml) and methanol (170 ml) to give a white solid, 5-icosylsulfanyl-1-

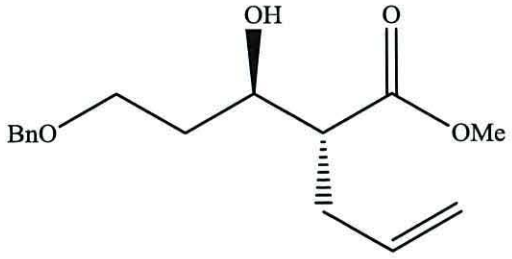
phenyl-1*H*-tetrazole (**174**) (18.85 g, 99 %), which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), ν_{max} identical to the literature.[215]

Appendix 14: 5-(Icosane-1-sulfonyl)-1-phenyl-1*H*-tetrazole (175)



The procedure used in Experiment 2 was repeated in order to oxidise the 5-icosylsulfanyl-1-phenyl-1*H*-tetrazole (18.43 g, 40.18 mmol) using ammonium molybdate (VI) tetrahydrate (22.35 g, 18.08 mmol) in 35 % H_2O_2 (50 ml) in THF (200 ml) and IMS (400 ml), and further ammonium molybdate (VI) tetrahydrate (8.5 g, 6.88 mmol) in 35 % H_2O_2 (21.5 ml). The crude product was purified by column chromatography eluting with petrol/ether (8:1) to give a white solid, 5-(icosane-1-sulfonyl)-1-phenyl-1*H*-tetrazole (**175**) (16.25 g, 84 %), which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), ν_{max} identical to the literature.[215]

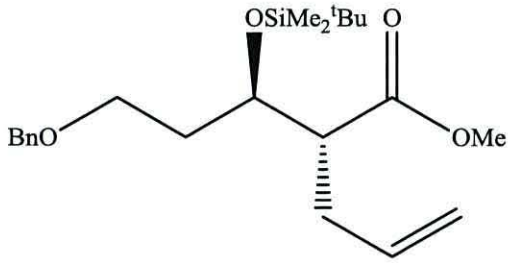
Appendix 15: (*R*)-2-((*R*)-3-Benzyloxy-1-hydroxypropyl)-pent-4-enoic acid methyl ester (176)



Diisopropylamine (7.86 g, 77.7 mmol) was dissolved in dry THF (100 ml) and cooled to - 78 °C. MeLi (54.4 ml, 81.6 mmol, 1.5M) was added and stirred at + 16 °C for 30 min., then re-cooled to - 61 °C and (*R*)-5-benzyloxy-3-hydroxypentanoic acid methyl ester (8.6 g, 36.1 mmol) in dry THF (50 ml) was added and the mixture was stirred at - 45 °C for 1 hour, - 20 °C for 40 min and then at - 20 °C to -10 °C for 20 min. It was re-cooled to - 62 °C and allyl iodide (5.0 ml, 54.2 mmol) in dry THF (20 ml) and HMPA (12.6 ml, 72.3 mmol) were added and the mixture was stirred at - 45 °C for 1 hour, - 45 °C to - 20

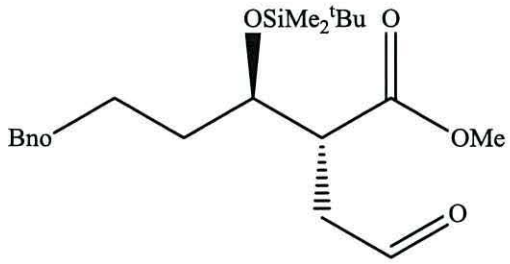
°C for 30 min. and then - 20 °C for 30 min. Further allyl iodide (0.9 ml) was added and stirred at - 20 °C to - 10 °C for 30 min. and then - 10 °C for 30 min. Sat. aq. NH_4Cl (70 ml) was added and extracted with ether/ethyl acetate (1:1, 3 x 100 ml), dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (2:1) to give a colourless oil, (*R*)-2-((*R*)-3-benzyloxy-1-hydroxypropyl)-pent-4-enoic acid methyl ester (7.64 g, 76 %), which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), v_{max} identical to the literature, $[\alpha]_D^{21} = -6.9$ (*c* 1.09, $CHCl_3$).[215]

Appendix 16: (*R*)-2-[(*R*)-3-Benzyloxy-1-(*tert*-butyldimethylsilanyloxy)-propyl]-pent-4-enoic acid methyl ester (161)



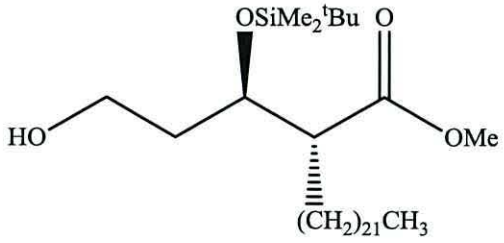
Imidazole (2.55 g, 37.63 mmol) was added to a stirred solution of (*R*)-2-((*R*)-3-benzyloxy-1-hydroxypropyl)-pent-4-enoic acid ester (4.1 g, 15.05 mmol) in dry DMF (70 ml) at r.t., followed by addition of *tert*-butyldimethylchlorosilane (2.94 g, 19.56 mmol) was added at 5-0 °C. The cooling bath was removed and the reaction mixture was stirred at 45 °C for 18 hours. TLC showed the reaction was complete and the DMF was removed by flash distillation. Water (200 ml) was added and the product was extracted with CH_2Cl_2 (3 × 150 ml). The combined organic layers were washed with water (150 ml), dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (5:1) to give a colourless oil, (*R*)-2-[(*R*)-3-benzyloxy-1-(*tert*-butyldimethylsilanyloxy)-propyl]-pent-4-enoic acid methyl ester (**161**) (4.49 g, 76 %), $\{[\alpha]_D^{26} = -17.2$ (*c* 0.93, $CHCl_3)\}$.[215]

Appendix 17: (2*R*,3*R*)-5-Benzyloxy-3-(*tert*-butyldimethylsilanyloxy)-propyl]-2-(2-oxo-ethyl)-pentanoic acid methyl ester (177)



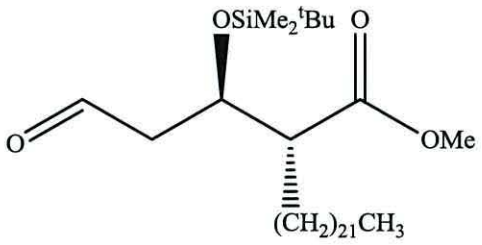
2,6-Lutidine (2.66 ml, 22.89 mmol), OsO_4 2.5 % in 2-methyl-2-propanol (2.58 ml, 0.206 mmol), and then $NaIO_4$ (9.79 g, 45.78 mmol) were added to a stirred solution of the (*R*)-2-[(*R*)-3-benzyloxy-1-(*tert*-butyldimethylsilanyloxy)-propyl]-pent-4-enoic acid methyl ester (4.49 g, 11.4 mmol) in 1,4-dioxane-water (3:1, 120 ml) at r.t. The reaction was stirred at r.t. for 2 hours, when TLC showed complete reaction. Water (200 ml) and CH_2Cl_2 (200 ml) were added and extracted. The aqueous layer was re-extracted (2 × 100 ml) and the combined organic layers were washed with brine (200 ml) and dried. The solvent was evaporated and the crude product was purified by column chromatography eluting petrol/ethyl acetate (10:3) to give a colourless oil, (2*R*,3*R*)-5-benzyloxy-3-(*tert*-butyldimethylsilanyloxy)-propyl]-2-(2-oxo-ethyl)-pentanoic acid methyl ester (**177**)[215] (4.29 g, 95 %), [Found $(M + H)^+$: 395.2244, $C_{21}H_{35}O_5Si$ requires: 395.2248], $[\alpha]_D^{24}$ = - 12.7 (*c* 0.6, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 9.80 (1H, br. s), 7.36-7.27 (5H, br m), 4.48 (1H, d, *J* 11.95 Hz), 4.45 (1H, d, *J* 11.95 Hz), 4.28-4.25 (1H, m), 3.67 (3H, s), 3.55-3.49 (2H, m), 3.23 (1H, td, *J* 4.1, 10.7 Hz), 2.97 (1H, dd, *J* 10.7, 18.25 Hz), 2.69 (1H, dd, *J* 3.5, 18.3 Hz), 1.73-1.63 (2H, m), 0.86 (9H, s), 0.07 (3H, s), 0.06 (3H, s); δ_C (125MHz, $CDCl_3$): 200.4, 172.4, 138.3, 128.3, 127.53, 127.5, 72.8, 68.8, 67.1, 66.5, 51.94, 45.24, 40.0, 33.7, 25.67, 17.9, -4.74, -4.88; ν_{max}: 2956, 2859, 1730, 1463, 1285, 1255, 1159 cm^{-1}.

Appendix 18: (*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-3-hydroxypropyl]-tetracosanoic acid methyl ester (180)



The procedure used in Experiment 20 was repeated in order to couple the (2*R*,3*R*)-5-benzyloxy-3-(*tert*-butyldimethylsilanyloxy)-2-(2-oxoethyl)-pentanoic acid methyl ester (4.2 g, 10.64 mmol) and 5-(eicosane-1-sulfonyl)-1-phenyl-1*H*-tetrazole (6.79 g, 13.83 mmol) using lithium bis(trimethylsilyl)amide (19.58 g, 20.75 mmol, 1.06 M) in dry THF (100 ml) under nitrogen at - 10 °C. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a yellow oil, (*E/Z*)-(*R*)-2-[(*R*)-3-benzyloxy-1-(*tert*-butyldimethylsilanyloxy)-propyl]-tetracos-4-enoic acid methyl (**178**) (5.19 g, 74 %) as a mixture of two isomers in ratio 2:1. Palladium 10 % on carbon (1.5 g) was added to stirred solution of the alkenes (5.19 g, 7.88 mmol) in IMS and THF (1:1, 50 ml). Hydrogenation was carried out for 3 days. The solution was filtered over a bed of celite and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (5:1) to give a white solid, (*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilanyloxy)-3-hydroxy-propyl]-tetracosanoic acid methyl ester (**180**)[215] (3.79 g, 84 %), m.p.: 35-37 °C [Found (M + H)$^+$: 571.5101, $C_{34}H_{71}O_4Si$ requires: 571.5116], $[\alpha]_D^{22}$ = - 8.3 (*c* 0.4, C_6H_6), which showed δ_H (500MHz, $CDCl_3$): 4.52 (1H, ddd, *J* 4.75, 9.8, 14.5 Hz), 4.29-4.25 (2H, m), 3.48 (3H, s), 2.30 (1H, ddd, *J* 3.15, 5, 8.15 Hz), 2.11-2.05 (1H, m), 2.01-1.88 (2H, m), 1.59-1.05 (42H, br m, including br s at 1.25), 0.88-0.85 (12H, m, including a singlet resonated at 0.88 for the ^{t}Bu and a triplet resonated at 0.87 with *J* 5.4 Hz), 0.08 (3H, s), 0.07 (3H, s); δ_C (125MHz, $CDCl_3$): 173.1, 65.9, 64.7, 50.8, 47.6, 31.9, 31.7, 29.7, 29.64, 29.61, 29.59, 29.58, 29.55, 29.3, 27.2, 26.4, 25.6, 22.7, 18.0, 14.1, -4.3, -5.1; ν_{max}: 3449, 2924, 2854, 1741, 1465, 1361, 1255, 1196, 1167, 1094 cm^{-1}.

Appendix 19: (*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-3-oxopropyl]-tetracosanoic acid methyl ester (160)



(*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-3-hydroxypropyl]-tetracosanoic acid methyl ester (3.79 g, 6.63 mmol) in CH_2Cl_2 (20 ml) was added to a stirred suspension of PCC (3.29 g, 15.26 mmol) in CH_2Cl_2 (130 ml) at r.t. The mixture was stirring vigorously and refluxed for 2 hours, when TLC showed no starting material was left. It was poured into petrol/ethyl acetate (10:1, 300 ml) and filtered through a pad of silica and celite then washed well with petrol/ethyl acetate and evaporated to give a residue, which was purified by chromatography on silica eluting with petrol/ethyl acetate (10:1) to give a colourless oil, (*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilanyloxy)-3-oxopropyl]-tetracosanoic acid methyl ester (**160**)[215] (3.52 g, 93 %), [Found $(M + Na)^+$: 591.4774, $C_{34}H_{68}NaO_4Si$ requires: 591.4779], $[\alpha]_D^{26}$ = - 5.0 (*c* 1.23, $CHCl_3$), which showed δ_H (500MHz, $CDCl_3$): 9.8 (1H, t, *J* 1.6 Hz), 4.43 (1H, br q, *J* 5.95 Hz), 3.68 (3H, s), 2.66-2.57 (3H, br m), 1.60-1.08 (42H, br m, including br s at 1.25), 0.89-0.85 (12H, m, including a singlet resonated at 0.85 for the ^{t}Bu and a triplet resonated at 0.88 with 7.25 Hz), 0.07 (3H, s), 0.06 (3H, s); δ_C (125MHz,$CDCl_3$): 201.3(-), 174.0, 68.8(-), 52.3(-), 51.5(-), 48.1(+), 31.9(+), 29.7(+), 29.66(+), 29.62(+), 29.55(+), 29.5(+), 29.4(+), 29.3(+), 27.8(+), 27.0(+), 25.6(-), 22.7(+), 17.9, 14.1(-), -4.6(-), -4.9(-); ν_{max}: 2925, 2854, 1736, 1465, 1362, 1255, 1196, 1168, 1098 cm^{-1}.

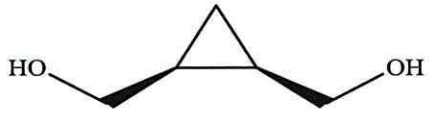
Appendix 20: 10-Bromo-decan-1-ol (202)

$Br(CH_2)_{10}OH$

1,10-Decanediol (25 g, 0.14 mmol) was dissolved in toluene (300 ml) and aqueous HBr (30 ml, 0.27 mmol, 48 % w.w.) was added then the mixture was refluxed for 18 hours. The mixture was cooled to r.t. and the organic layer was separated. The toluene was removed; the residue a brown oil was dissolved in dichloromethane (300 ml) and washed with sat. aq. $NaHCO_3$ (150 ml). The aqueous layer was re-extracted with

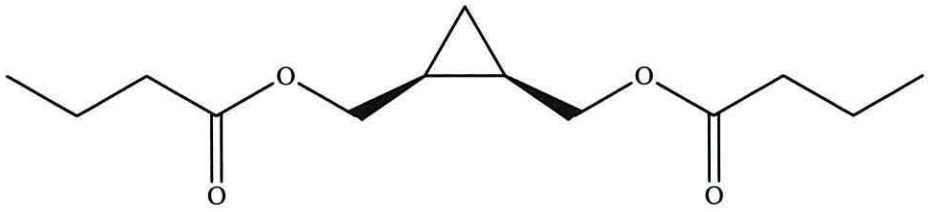
dichloromethane. The combined organic layers were dried and evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (5:2) to give a colourless oil, 10-bromo-decan-1-ol (24.52 g, 72 %), which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), ν_{max} identical to the literature.[215]

Appendix 21: (*cis*-2-Hydroxymethyl-cyclopropyl)-methanol (200)



Lithium aluminum hydride (18.68 g, 492.2 mmol) was added portionwise to a stirred THF (350 ml, HPLC grade) at - 20 °C, when vigorous evolution of hydrogen was observed. A solution of *cis*-cyclopropane-1,2-dicarboxylic acid dimethyl ester (**115a**) (25.92 g, 164.0 mmol) in THF (50 ml) was added dropwise to the above suspension at - 20 °C and then the reaction mixture was refluxed for 2 hours. When TLC analysis indicated completion of the reaction a freshly prepared solution of sat. aq. sodium sulfate (40 ml) was added at - 20 °C, when formation of a white precipitate was observed, and the reaction mixture was stirred at r.t. for 2 hours. The solution was filtered through a bed of silica and the solvent evaporated. The resulting solution was taken up in dichloromethane (100 ml) and washed with water (25 ml) and then dried. The solvent was evaporated and the crude product was purified via column chromatography eluting with petrol/ether (20:1, then 1:1) to give a colourless oil, (*cis*-2-hydroxymethyl-cyclopropyl)-methanol (12.86 g, 80 %), which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), ν_{max} identical to the literature.[226]

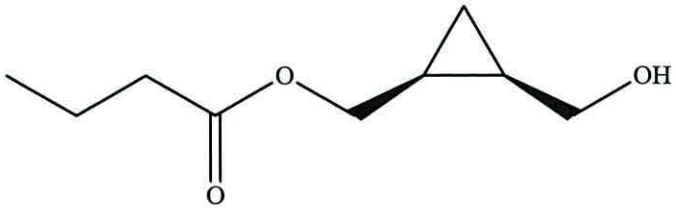
Appendix 22: Butyric acid *cis*-2-butyryloxymethylcyclopropylmethyl ester (216)



Butyric anhydride (27.3 g, 172.6 mmol, 2.2 mol eq.) was added to the (*cis*-2-hydroxymethyl-cyclopropyl)-methanol (**200**) (8 g, 78.4 mmol) and the mixture was refluxed at 120 °C for 1 hour then cooled to r.t. CH_2Cl_2 (100 ml) and sodium NaOH solution (7 g in 100 ml water) were added, then extracted. The aqueous layer was re-

extracted with CH_2Cl_2 (2 x 25 ml) and the combined organic layers were washed with aq. $NaHCO_3$ (50 ml). The solution was dried, the solvent was evaporated and excess of butyric anhydride was distilled at high vacuum. The crude product was purified by column chromatography eluting with petrol/ether (5:1 then 1:1) to give a colourless oil, butyric acid *cis*-2-butyryloxymethylcyclopropylmethyl ester (13.4 g, 71 %), which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), ν_{max} identical to the literature.[226]

Appendix 23: Butyric acid (1*S*,2*R*)–*Cis*–2–(hydroxylmethyl)cyclopropylmethyl ester (217)



An isopropyl ether solution of 2,2,2-triflouroethyl butyrate (76.6 g) was prepared by dissolving 2,2,2-trifluoroethanol (50 g) in isopropyl ether (500 ml), and then butyric anhydride (45 ml) was added. The mixture was cooled to about 4 °C whilst stirring and trimethylsilyl trifluoromethane sulfonate (2 ml) was added. An exothermic reaction occurred which raised the temperature to about 25 °C. The mixture was stirred at room temperature, over a period of 1.5 hours until GC showed the absence of butyric anhydride. 1.25 M solution of sodium hydroxide (400 ml) was added, followed by sodium hydrogen carbonate (10 g) and the mixture was stirred for 10 minutes. The aqueous phase was extracted with more isopropyl ethanol (75 ml) and the combined organic phases were washed with brine (400 mL) and dried with magnesium sulfate.

***Cis*–2–Hydroxymethylcyclopropyl)methanol** (25 g) dissolved in tetrahydrofuran (120 ml) was added to the above isopropyl ethanol solution of 2,2,2-triflouroethyl butyrate followed by lipase (24 g). The mixture was stirred at room temperature for over 24 hours until GC showed low diol content. Subsequently, the mixture was filtered through a bed of celite, washed thoroughly with isopropyl ethanol and the filtrate was evaporated to yield a yellow liquid. The liquid was dissolved in dichloromethane (400 ml) and washed with saturated sodium hydrogen carbonate (100 ml), dried and evaporated. The product was purified by chromatography on silica gel, eluting with petrol/ethyl acetate (5:2) and a clear liquid, butyric acid*(1S,2R)-2-cis-*

(hydroxymethyl)cyclopropylmethyl ester (28 g, 66 %)[227] was obtained, $[\alpha]_D^{20}$ = - 22.7 (*c* 1.5, $CHCl_3$), $[\alpha]_D^{24}$ = - 18.1 The compound also showed the following: δ_H (500MHz, $CDCl_3$): 4.39 (1H, dd, *J* 5.8, 12.1 Hz),3.70–3.83 (2H, m),3.38 (1H, dd, *J* 8.9, 11.8 Hz), 2.40 (1H, br s),2.25(2H, t, *J* 7.4 Hz),1.60 (2H, sext, *J* 7.4 Hz), 1.19-1.30 (2H, m),0.91 (3H, t, *J* 7.5 Hz),0.77-0.83 (1H, m,),0.19 (1H,q, *J* 5.5 Hz); δ_C (125MHz, $CDCl_3$): 173.9, 64.6, 62.6, 36.4, 18.6, 14.6, 13.8, 7.9; ν_{max}: 3435, 1734, 1187 cm^{-1}.

Appendix 24:8-Bromo-octan-1-ol (245)

$HO(CH_2)_8Br$

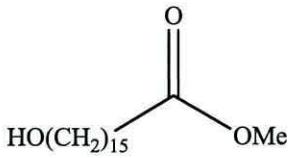
1,8-Octanediol (100 g, 683.8 mmol) was dissolved in toluene (350 ml) and aqueous hydrobromic acid (50 ml, 48 % w.w) was added. The mixture was refluxed for 18 hrs. The mixture was cooled to r.t., the organic layer was separated and the solvent was removed. The residue a brown oil was dissolved in CH_2Cl_2 (600 ml) and washed with sat. aq. Sodium bicarbonate (300 ml). The aqueous layer was re-extracted with CH_2Cl_2 (3 × 150 ml). The combined organic layers were dried and the solvent evaporated to give the crude product which was purified by column chromatography eluting petrol/ethyl acetate (10:1, then 5:1) to give 8-bromo-octan-1-ol (70.3 g, 56 %), which showed δ_H (500 MHz, $CDCl_3$), δ_C (125 MHz, $CDCl_3$), ν_{max} identical to the literature.[234]

Appendix 25: 9-Bromononan-1-ol (249)

$HO(CH_2)_9Br$

1,9-Nonanediol (25 g, 156.0 mmol) was dissolved in toluene (300 ml) and aqueous hydrobromic acid (30 ml, 48 % w.w) was added. The mixture was refluxed for 18 hours and cooled to r.t., the organic layer was separated and the solvent was removed. The residue a brown oil was dissolved in CH_2Cl_2 (600 ml) and washed with sat. aq. Sodium bicarbonate (300 ml). The aqueous layer was re-extracted with CH_2Cl_2 (3 × 150 ml). The combined organic layers were dried and the solvent evaporated to give the crude product which was purified by column chromatography eluting petrol/ethyl acetate (10:1, then 5:1) to give 9-bromo-nonan-1-ol (29.7 g, 85 %), whose NMR spectra were identical to the ones reported.[215,235]

Appendix 26: 16-Hydroxy-hexadecanoic acid methyl ester (270)



The procedure used in Appendix 9 was repeated in order to ring open 16-hexadecanolide (**268**) (10 g, 39.3 mmol) using sodium (2.5 g, 108.69 mmol) in methanol (200 ml) at 0 °C. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (5:1, then 5:2) to give a white solid, 16-hydroxy-hexadecanoic acid methyl ester[236] (10.6 g, 94 %), m.p.: 57-58 °C [Found $(M + Na)^+$: 309.2375, $C_{17}H_{34}NaO_3$ requires: 309.2400]; δ_H (500MHz, $CDCl_3$): 3.66 (3H, s), 3.63 (2H, t, *J* 6.6 Hz), 2.30 (2H, t, *J* 7.6 Hz), 1.63-1.1.53 (5H, m), 1.48-1.25 (22H, m); δ_C (125MHz, $CDCl_3$): 174.3, 63.05, 51.4, 34.39, 34.09, 32.78, 29.6, 29.58, 29.57, 29.55, 29.4, 29.22, 29.12, 25.7, 24.9; ν_{max}: 3369, 2918, 2850, 1739, 1467, 1174 cm^{-1}.