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Synthesis of Glycerol and Glucose Monomycolates

Sahb, Mohaned Mohammed

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Synthesis of Glycerol and Glucose Monomycolates

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by

Mohaned Mohammed Sahb

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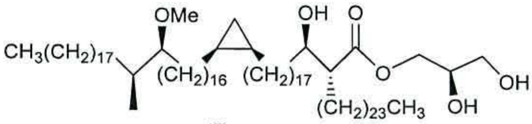
Abbreviations and acronyms

AIDS	Aquired immunodeficiency syndrome
APCs	Antigen-presenting cells
b	Broad
BCG	Bacillus Calmette-Guérin
m-CPBA	*m*-Chloroperbenzoic acid
D	Doublet
DHP	2,3-Dihydro-2*H*-pyran
ECDI	1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride
EI	Electron Impact
ELISA	Enzyme-linked immunosorbent assay
GC	Gas Chromatography
GMM	Glucose monomycolate
GroMM	Glycerol monomycolate
HIV	Human immunodeficiency virus
HMPA	Hexamethylphosphorotriamide
HPLC	High Performance Liquid Chromatography
Hz	Hertz
IMS	Industrial Methylated Spirit
IR	Infra-Red
LDA	Lithium *N,N*-diisopropylamide
m	Multiplet
MA	Mycolic acid
MALDI-TOF	Matrix Assisted Laser Desorption Ionization Time-Of-Flight
MCP	Monocyte Chemotatic Protien
MDR-TB	Multiple Drug Resistant tuberculosis
mol eq.	Molar equivalents
m.p.	Melting Point
MS	Mass Spectroscopy
NMR	Nuclear Magnetic Resonance
OPD	*o*-Phenolindiamine
PAS	*p*-Aminosalicyclic acid
PBS	Phosphate Buffered Saline

PCC	Pyridinium Chlorochromate
ppm	Parts per million
PPTS	Pyridinium *p*-toluenesulfonate
PTSA	*p*-Toluenesulfonic acid monohydrate
Pv	Pivaloyl
q	Quartet
R_f	Retardation factor
R.T.	Room temperature
S	Singlet
SAM	*S*-Adenosyl-*L*-methoine
sat.	Saturated
t	Triplet
T-cells	T Lymphocites
TB	Tuberculosis
TBAF	Tetra-n-Butylammonium fluoride
TBAI	Tetra-n-Butylammonium iodide
TBDMSCl	*tert*-butyldimethylsilyl chloride
TBDPSCl	*tert*-butyldiphenylsilyl chloridc
TDM	Trehalose dimycolate
THF	Tetrahydrofuran
THP	Tetrahydropyranyl
TLC	Thin-Layer Chromatography
TMM	Trehalose monomycolate
TNF	Tumor necrosis factor
p-TsCl	*para*-Toluene sulfonyl chloride
p-TsOH	*para*-Toluene sulfonic acid
WHO	World health organization
XDR-TB	Extensively Drug Resistant tuberculosis

Abstract

This thesis consists of three parts. The first part involved the synthesis of glycerol monomycolates (GroMM) (**I**) - (**IV**).



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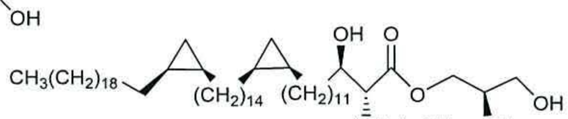

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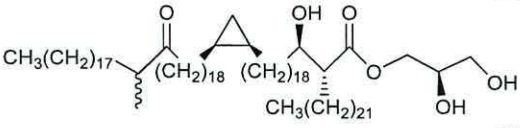

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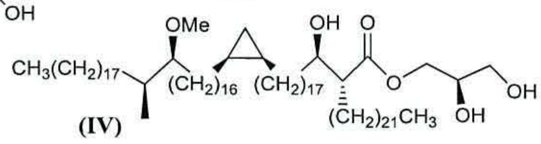
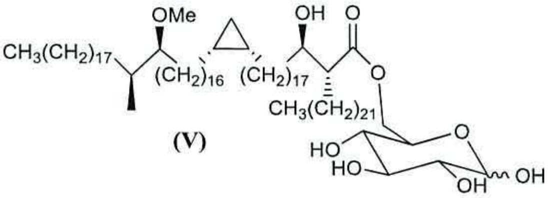


(IV)

The second part was to synthesise glucose monomycolates (GMM) (**V**) - (**X**), consisting of a mycolic acid attached to the 6-position of glucose and present in numerous bacterial species including *Mycobacterium*, *Rhodococcus* and *Nocardia*.



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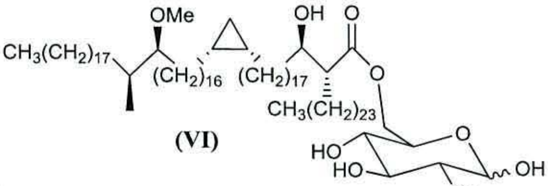

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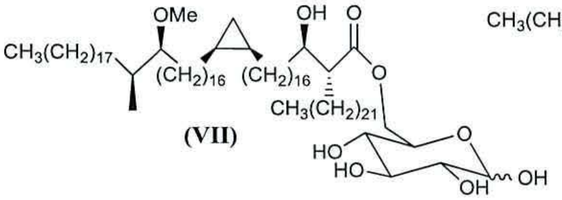

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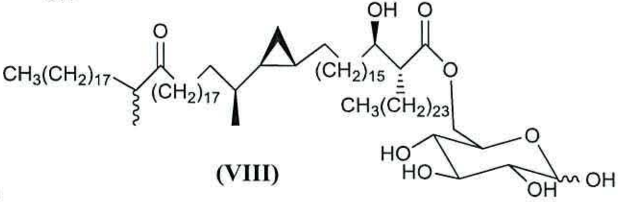

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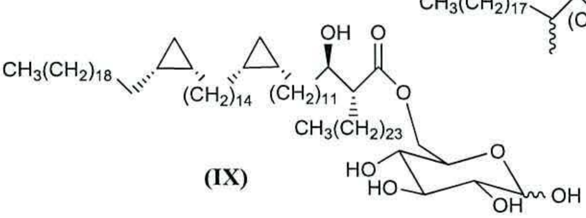

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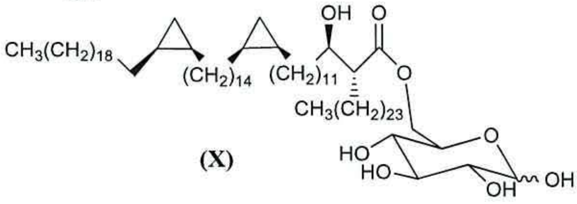
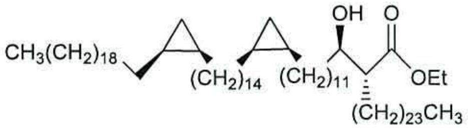


(X)

The third part comprised the development of a new method for the synthesis of the *α*-alkyl-*β*-hydroxy fragment of mycolic acids; the route involved fewer steps and used *L*-malic as starting material. This route was applied to the synthesis of **(XI)** and **(XII)**.



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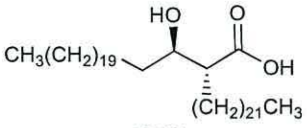


(XII)

1. Introduction

1.1 Tuberculosis (TB)

Tuberculosis (TB) is a disease which has an ancient history.[1, 2] It became a public health problem three centuries ago, when cities became overcrowded and there was a lack of health care facilities. This led people to study the disease with the aim of finding a cure for it, with early studies starting in the 17th century.[3] TB was identified as an infectious disease at the start of the 19th century and was given the name tuberculosis at this time. TB was of widespread public concern; one in six deaths was due to it until the 20th century. The World Health Organisation (WHO) estimates that roughly one third of the world's population is currently infected with TB.[4]

The principle causative agent of infection is *Mycobacterium tuberculosis*. The origin of tuberculosis is not clearly understood, but it is supposed to have originated in cattle and then transferred to humans. It is also believed that a relationship exists between TB and eating the meat of infected cattle with *Mycobacterium bovis*.[5, 6]

However, there were no real advances in the knowledge of TB until Hermann Heinrich and Robert Koch gave their presentation on the tubercle bacillus in 1882.[7] In 1890, Koch isolated a substance from the tuberculosis bacillus, which he called tuberculin, and later, he proceeded to inject himself with the tuberculin. His body developed a fever, but he never developed TB.[8] Koch was awarded the Nobel Prize in Physiology and Medicine in 1905 for his work with tuberculosis.

In 1950, the highest rate of tuberculosis infection was recorded. Tuberculosis remains an immense global problem. It is estimated that there were 9.2 million new cases of TB in 2006, which is an increase from 9.1 million in 2005, with an estimated 1.7 million deaths in 2006 alone.[9] Over one-third of the world's population now has the TB bacterium in their bodies. In developing countries more than 2 million people die each year from the disease.[10] **Figure 1** shows a map of the estimated number of new TB cases.[9]

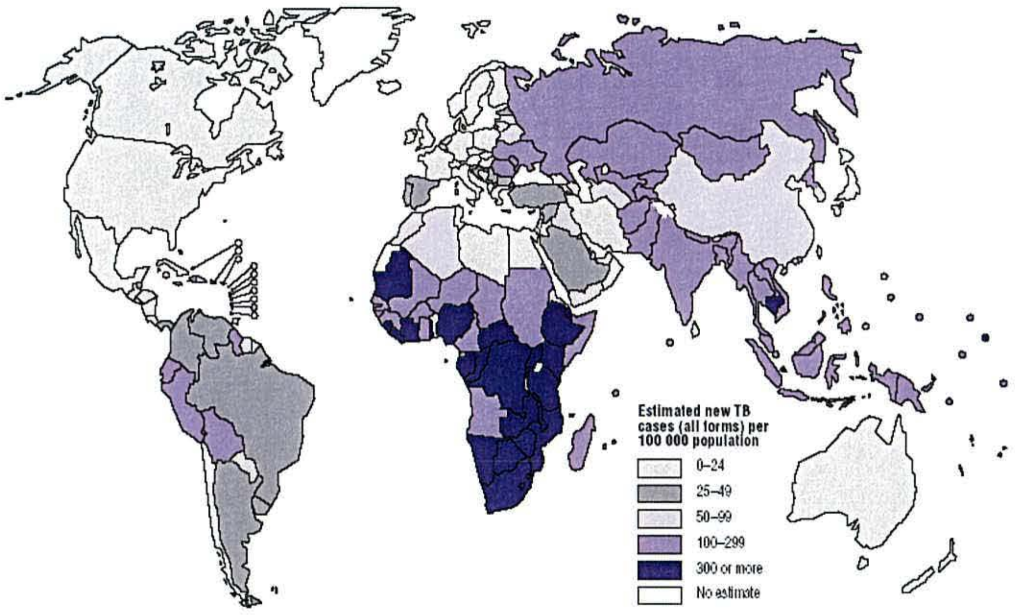


Figure 1: Map of the estimated number of new TB cases per 100,000 populations [9]

The continuing occurrence of TB, caused by *M. Tuberculosis*, is partly due to the evolution of strains highly resistant to drug treatment and a higher incidence of human immunodeficiency virus (HIV) co-infection.[11, 12] TB remains globally uncontrolled, particularly in developing countries, owing to co-infection with HIV or environmental factors such as malnutrition and poverty.[13] The incidence of TB has decreased continuously in advanced countries since the 1940s. However, the rate of decrease has dropped steadily since the 1980s, owing partially to HIV co-infection and the appearance of drug resistant *M. Tuberculosis*.[14]

1.2 HIV/TB co-infection

The continuing occurrence of TB, caused by *M. Tuberculosis*, is partly due to the evolution of strains highly resistant to drug treatment and a higher incidence of human immunodeficiency virus (HIV) co-infection.[11, 12] TB remains globally uncontrolled, particularly in developing countries, owing to co-infection with HIV or environmental factors such as malnutrition and poverty.[13] The incidence of TB has decreased continuously in advanced countries since the 1940s. However; the rate of decrease has dropped steadily since the 1980s, owing partially to HIV co-infection and the appearance of drug resistant *M. Tuberculosis*.

Because HIV weakens the immune system, it has been established that a person suffering from HIV and infected with *M. tuberculosis* is approximately 30 times as likely to die from TB as a person who is infected with *M. tuberculosis,* but who is HIV-negative.[15]

1.3 Diagnosis of TB

Diagnosis of TB is carried out definitively by microscopic examination and microbiological culture of body fluids and other clinical samples such as sputum or pus, for the identification of *M. tuberculosis*.[16] The diagnosis of TB requires not less than four weeks which must include a medical history coupled with the aforementioned physical and microbiological examinations. Diagnosis can also be made by chest X-rays, scans and tuberculin skin test. It is quite difficult to culture the slow-growing organism in the laboratory.[16] On the other hand the diagnosis for any individuals with HIV/AIDS infection, the time frame to get a result from culture could be fatal – the life expectancy of someone with active TB and HIV being three to four weeks. Therefore, studies have shown that late diagnosis of TB infection is estimated to be the cause of up to 85% of deaths of AIDS patients. Currently, TB diagnosis in HIV burdened populations poses one of the most challenging hurdles towards regaining control of the TB epidemic.[17, 18]

1.4 Prevention and treatment of TB

Since Koch's work, a reliable vaccine was not developed until 1921, when Albert Calmette and Camelle Guérin developed a vaccine from *M. Bovis* called Bacillus Calmette-Guérin (BCG).[19] The acceptance of the vaccine was slow because it uses live TB bacteria. In 1930, there was a case in Germany where 240 infants under 10 days old were vaccinated with contaminated vaccine and almost all developed TB, with 76 recorded deaths.[20]

The treatment of TB is difficult and involves long courses of multiple antibiotics.[21, 22] The first drug proved to be effective against TB was streptomycin which was isolated from *Streptomyces griseus* in the 1940s. [23] This was followed by the discovery of isoniazid in the 1950s. Subsequently, both drugs were used together in a multi-drug therapy.[24] Rifampicin, a natural compound extracted from *Streptomyces mediterranei* became available in the 1970s. These treatments soon became inadequate with the growing problem of antibiotic resistance, coupled with the emergence of Multi–Drug Resistant Tuberculosis (MDR-TB) and Extensively-Drug Resistant Tuberculosis (XDR-TB).[21, 22] Therefore' first-line drugs used in the treatment of TB have been isoniazid, pyranzinamide, ethambul and rifampicin.[24, 25]

1.5 *Mycobacterium Tuberculosis*

TB is mainly caused by *M. tuberculosis* which can be seen below (**Figure 2**).[26]

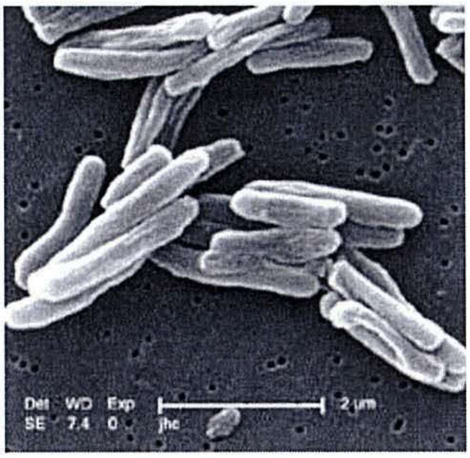


Figure 2: Scanning Electron Micrograph of M. Tuberculosis [26]

A lot more has been understood about *M. Tuberculosis* since its complete genome sequence was elucidated in 1998.[18] *M. Tuberculosis* has a complex envelope around its outside, which is of unusually low permeability to hydrophilic molecules, which contributes to its resistance to host defence mechanisms.[27, 28] Because of these unusual characteristics, knowledge of the cell envelope is crucial.

1.6 The cell envelope of *M. tuberculosis*

The mycobacterial cell envelope comprises three structural features, the plasma membrane, the cell wall and the 'capsule', as can be seen in **Figure 3.**

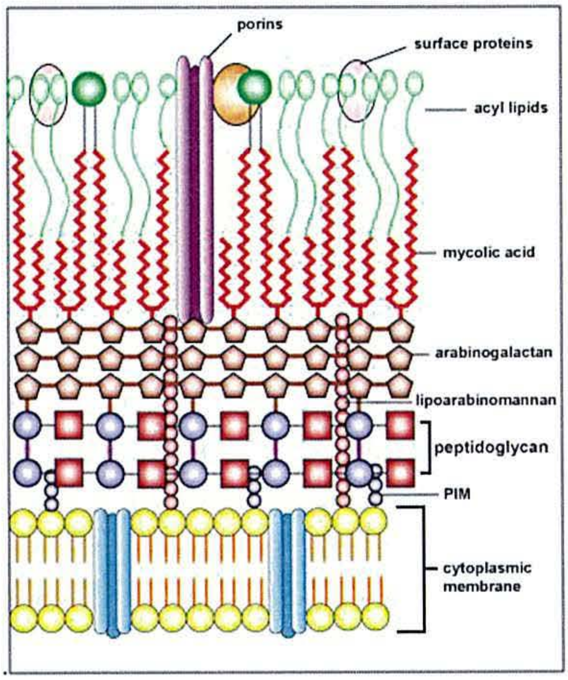


Figure 3: The cell wall of M. Tuberculosis [29]

The capsule-like layer consists of carbohydrates and proteins containing small amounts of lipids.[30] The cell wall is composed of three major parts: peptidoglycan, arabinogalatan and mycolic acid.[31] The peptidoglycan comprises alternating *N*-acetylglucosamine (NAG) and *N*-acetylmuramic (NAM) saccharides.[32] The arabinogalactan is a complex heteropoly-saccharide, composed of arabinan multi-branched chains.[33, 34] Mycolic acids are high molecular weight long chain fatty acids alkylated in the α-position and hydroxylated in the β-position.[34] Mycolic acids are the main constituent of the cell wall, and they occur along with a large number of different lipids, for example several different lipids with multi-methyl branched fatty acids. 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.[35-37] Among the most interesting and potentially useful glycolipids found in *M. tuberculosis* are the mycolic acids, cord factors, glucose monomycolates (GMM) and glycerol monomycolates (GroMM).

1.7 Mycolic acids

Mycolic acids have been reported since early in the last century.[38, 39] Minnikin *et al.* used 2D-TLC for the separation of different fractions of mycolic acids from human *Tubercle bacilli*, and used different analytical methods like I.R. spectroscopy, proton and carbon NMR spectroscopy and mass spectrometry to show that mycolic acids have a cyclopropane ring in different stereochemistries, and also suggested the main structure of mycolic acids.[40, 41] In further studies, Minnikin proposed the structure of the cell wall.[40, 42, 43]

The mycolic acids have two key moieties, the main one called the meromycolate and the other called the mycolic motif. The mycolic motif is essentially common for every mycolic acid and has the hydroxyl group and α-alkyl branch in the (*R,R*) configuration (**Figure 4**).

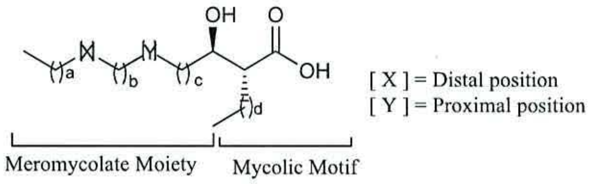


Figure 4: The general structure of a mycolic acid

The meromycolate part is different from one mycolic acid to another, and it can contain different groups in both the distal and proximal positions. These groups may be a cyclopropane ring, a methoxy group, a carbonyl group, a methyl group, oxirane ring or a double bond. The classification of mycolic acids depends on the groups present. Due to the possible variations in the functional groups that could be present in a certain mycolic acid, Watanabe *et al.* proposed a broad classification method.[44] This splits the mycolic acids into 3 types: Type 1, Type 2 and Type 3. Type 1 mycolic acids have a cyclopropane ring in the proximal position (which can be either *cis* or *trans*), Type 2 mycolic acids have a *trans* double bond in the proximal position, while a Type 3 mycolic acid would contain a *cis* double bond in the proximal position (**Figure 5**).[44]

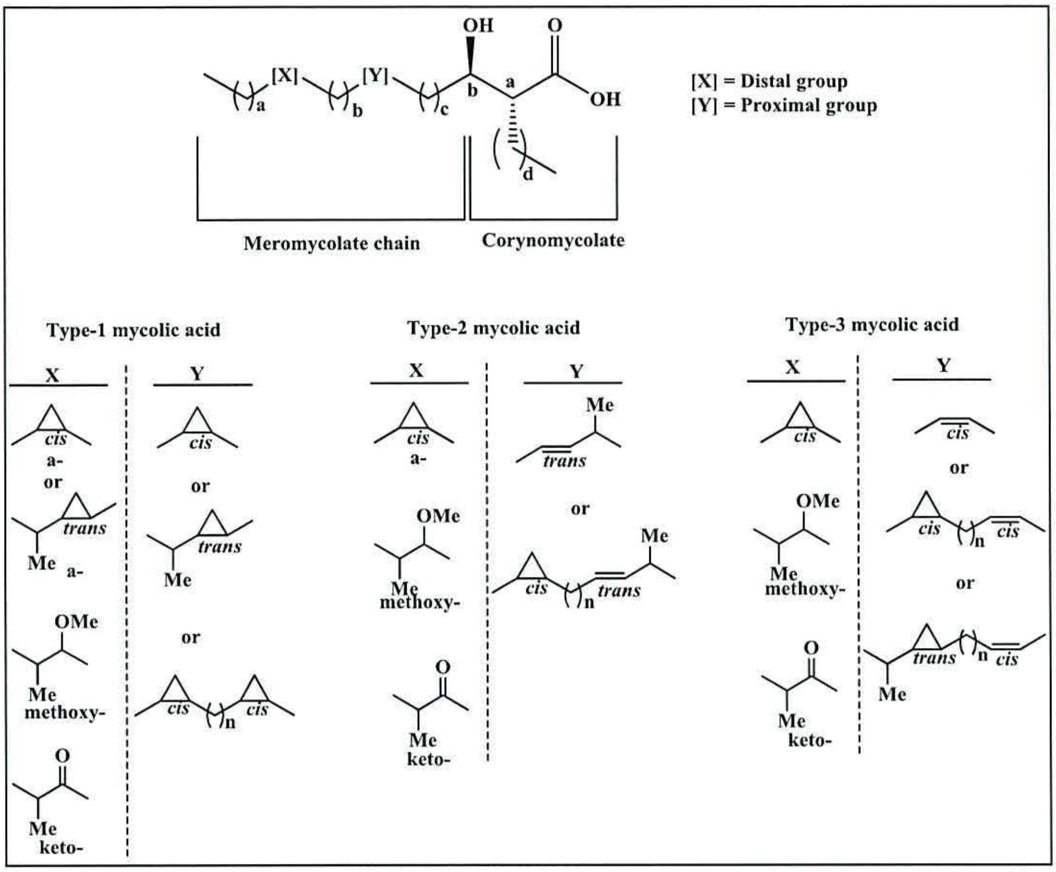
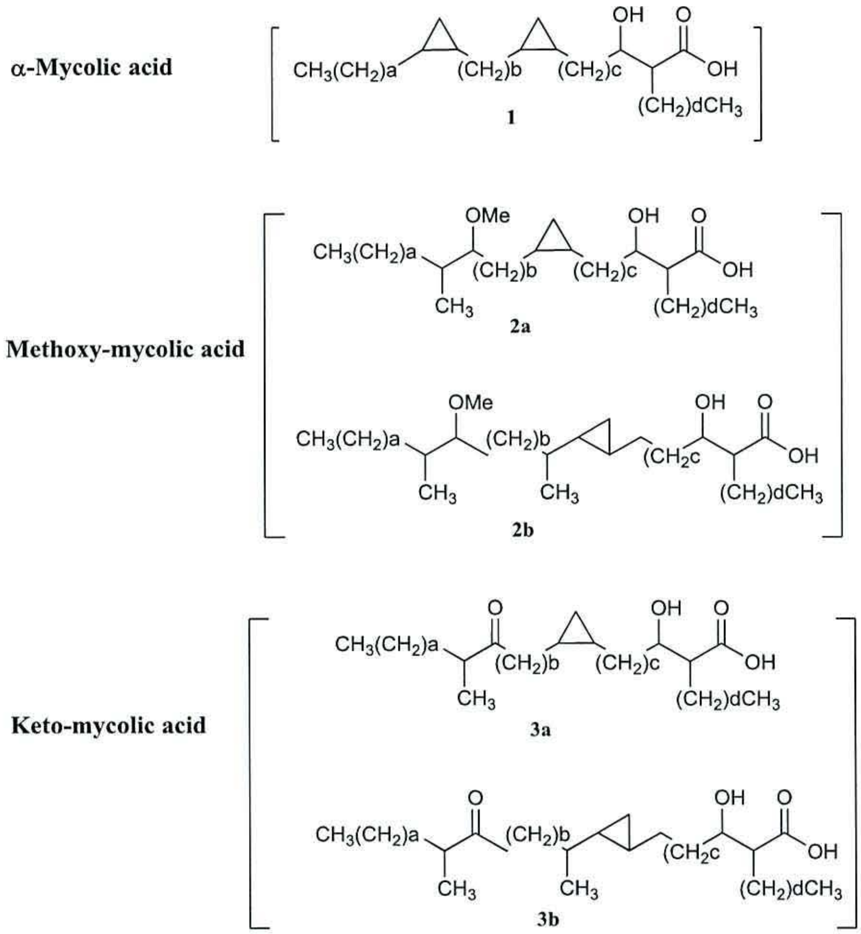


Figure 5: Generalized structures of major mycobacterial mycolic acids and functional group [44]

In addition, based on the nature of the functional groups present in the meromycolate chains, mycolic acids from *M. tuberculosis* are categorized into three major groups: α-mycolic acid (**1**) with no oxygen-containing intra-chain groups, methoxy-mycolic acid (**2**) in which the distal group has a methoxy group and keto-mycolic acid (**3**) in which the distal group has a carbonyl group. Methoxy-mycolic acids and keto-mycolic acids have methyl branches next to the oxygenated functional group and natural mixtures have both *cis*-cyclopropane and α-methyl *trans*-cyclopropane rings. Watanabe *et al.* [44, 45] have studied the nature and the location of the groups in the meromycolate chains of mycolic acids from representative mycobacteria by extensive NMR spectroscopic and mass spectrometric analyses (**Scheme 1**).



a = 15, 17, 18, 19, b = 10, 14, 16
c = 11, 15, 17, 19, 21, d = 21, 23

Scheme 1: Major types of mycolic acids from M. tuberculosis

1.7.1 Chain length

The length chain represented in the different homologues of 500 types of mycolic acids in all kinds of mycobacteria has been recognised; [46] thus for *M. smegmatis* about 100 structural isomers are found in the mixture of α-mycolates.[47]

Laval *et al.*[48] used MALDI-TOF mass spectrometry to provide a rapid and highly sensitive technique for the analysis of mycolic acids and other lipids and used this to analyse the length of the total carbon chain of the major types of mycolic acid of different mycobacteria.

The main chains of oxygenated (methoxy and keto) mycolic acids from slow growers were four to six carbon atoms longer than the corresponding α-mycolic acids, whereas rapid growers produced oxygenated homologues possessing the same chain length as their α-mycolic acids.[49,]

Mycolic acids occur within all mycobacteria, in varying combinations of functional type and chain length, and there are more than 50 reported species of mycobacteria. Consequently 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 mycobacterium.[50]

In addition, Watanabe *et al.* used MALDI spectrometry to study the mycolic acids 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.[45, 48]

1.7.2 Stereochemistry of mycolic acids

The two stereocentres 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, regardless of the groups in the meromycolate chain.[51-53] The (*R*,*R*)-configuration was confirmed first for the corynomycolic acids (**Figure 6**). 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.[51, 52, 54]

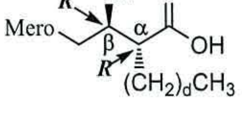
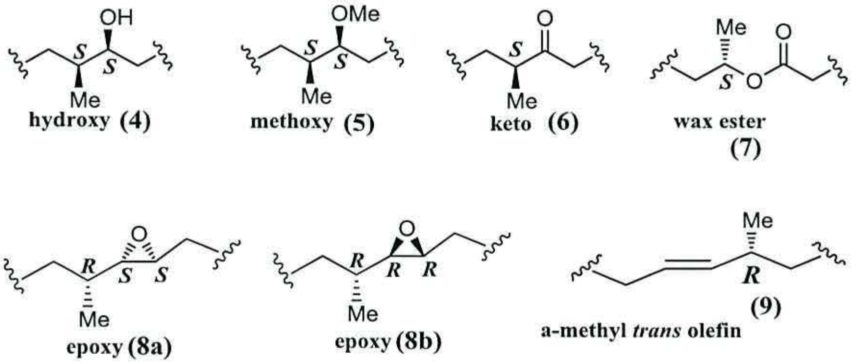


Figure 6: The (R,R)- configuration for all mycolic acids

The configuration at these two chiral centres is thought to play a vital role in T cell recognition,[55] and the generation of an immune response by the host organism against pathogenic mycobacteria.[55, 56]

The latest stereochemical studies on different mycobacteria suggest that the methyl branch adjacent to the hydroxy (**4**), methoxy (**5**) and keto (**6**) groups in the mycolic acids is in the *S*-configuration. The hydroxyl (**4**) and the methoxy (**5**) at the distal group in the mycolic acids are also thought to be in the *S*-configuration (**Scheme 2**).[41, 57, 58]



Scheme 2: The strereochemistry of chiral centres of the mycolic acids

Other reports identify an *R*-stereochemistry for the three stereocentres of the α-methyl-*trans*-epoxy unit in related mycolic acids (**8a, 8b**) However, Baird *et al.*[59] suggested that the epoxy fragment is *R,S,S* as in (**8a**) rather than *R,R,R* as in (**8b**).[58, 60] Furthermore, the methyl branch next to the *trans*-alkene unit (**9**), present in mycolic acids is in the *R*-configuration (**Scheme 2**).[60-62]

1.8 The serodiagnosis for TB

Natural mycolic acids mixtures and a number of single synthetic compounds of mycolic acids have been shown to have strong effects on the immune system and used on an ELISA (Enzyme-linked immunosorbent assay) plate assay for the serodiagnosis for TB.[63-65] ELISA assays were carried out to study the recognition of the antigens by lipid antibodies present in the serum of patients known to be infected with tuberculosis, in order to determine the value of these compounds for the detection TB. In ELISA, the protocol used for testing of these compounds was as follows: Coating the plate with the antigens (e.g. mycolic acid or cord factor) followed by the TB serum containing antibodies is layered over the antigen coating. Then the excess antibodies are washed away leaving only the antibodies which have bound onto the antigens. Then, a secondary antibody is added to the well, which binds to the primary antibody and which also contains an enzyme. This is followed by washing to remove any excess secondary antibody. The amount of antibodies binding to the antigen is determined by the intensity of the produced colour which measured as absorbance which infers the level of antibodies in the serum sample.[66, 67]

The diagnosis with ELISA assays still was not adequately accurate as a diagnostic indicator.[68] Therefore; Verschoor *et al.*, has employed a modern antibody detection technology where antibody binding is detected in real time for increasing the sensitivity of the diagnosis reaction.[69, 70]

In addition a number of cord factors have been being tested to determine their biological activities and application to serodiagnosis of TB.[71-74] Beukes *et al* in 2011 reported a range of ELISA tests conducted on a natural *M.tb* mycolic acid, and unique synthetic mycolic acids (namely α-, keto-, hydroxyl- and methoxy-mycolic acids) which represent the three main functional classes in *M.tb*. The test results confirmed that free mycolic acids were recognized by antibodies and that the individual synthetic mycolic acids showed varying antigenic activities against human TB sera, which reflected the difference in their functional groups and stereochemistries. The oxygenated mycolic acids were found to be more antigenic than the α-mycolic acid. Therefore, the methoxy-mycolic acid was found to be the most antigenic, followed by the hydroxyl-, keto- and finally α-mycolic acids having shown the least recognition.[75]

In contrast to mycolic acids, natural cord factors which contain complex mixtures of isomers,[44, 76] give a higher selectivity and sensitivity. Nonetheless, the analysis with natural TDM is complicated because many combinations of mycolic acids can be bonded to the trehalose, thereby increasing the number of possible structures which are believed to contribute to the variation of immune related effects.[77-79]

Cord factors are also known to stimulate the immune system to produce a range of regulatory immune cells such as chemokines (e.g. MCP-1) and cytokines (e.g. TNF-α).[80] For instance, the synthetic TMM and TDM species of α- and methoxy mycolic acids recently prepared by Baird *et al.* [65] analysed for cytokine and chemokine responses in order to determine their effects on the immune system

The TNF- alpha cytokine activity for the α-TDM was found to be three times higher than the commercially procured natural *M.tb*-TDM sample while the activities of the three other synthetic cord factors (methoxy-TDM, α-TMM and methoxy-TMM) were lower than the natural *M.tb*-TDM. The chemokine MCP-1 production for the α-TDM was twice more than the production for commercial sample, whereas the responses for the other synthetic cord factors were virtually the same as the commercial *M.tb*-TDM sample.[65]

1.9 Mycolic acid synthesis

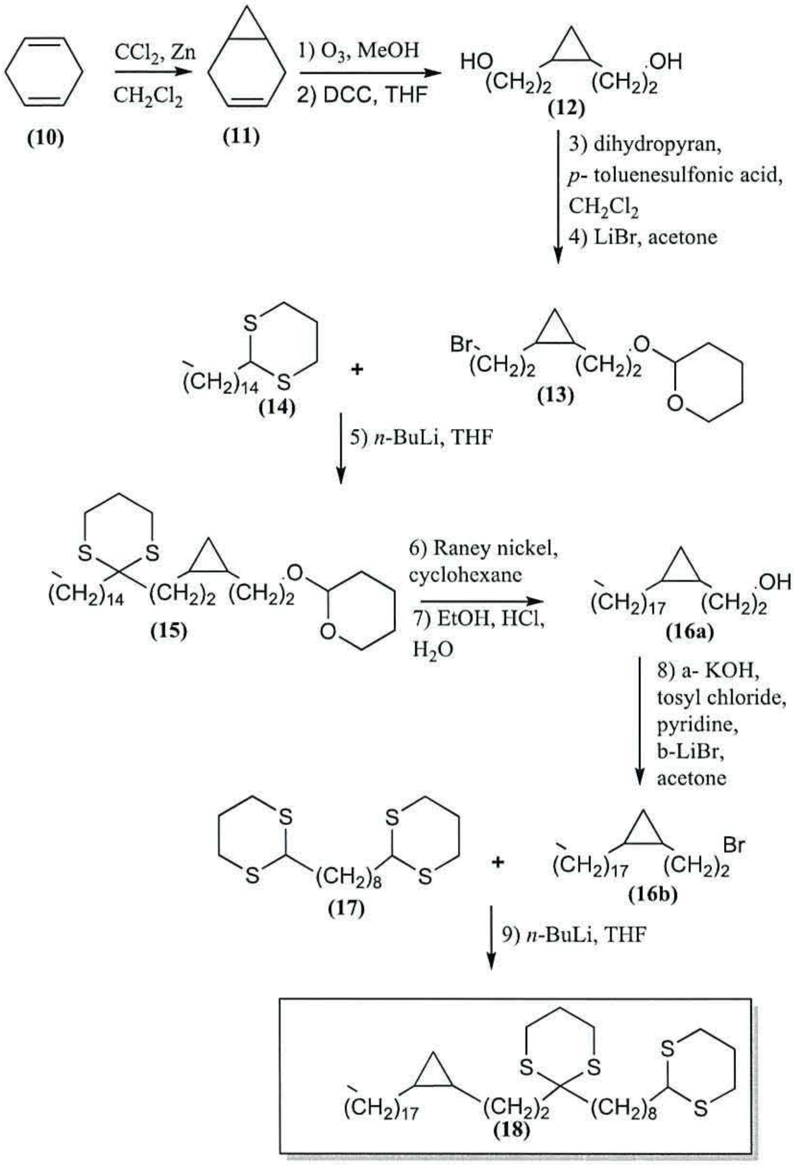
One application of synthetic mycolic acids is in the detection of TB causing *mycobacteria*. The pattern of individual mycolic acids is exclusive to one mycobacterium; therefore if the structure of these mycolic acids were to be known, an enantiomerically pure synthetic compound may be produced and compared to a sample of the mycolic acids obtained from the *mycobacteria* infecting the patient.[81, 82]

1.9.1 Synthesis of the meromycolate chain

Mycolic acids have been of interest to a variety of research groups, mainly from a synthetic point of view.[83] Synthesizing single enantiomers of mycolic acid can help in the determination of the stereochemistries of naturally occurring mycolic acids. Preparing mycolic acids with known chiral centres in the meromycolate chain can also help to provide a further understanding of the biosynthesis of mycolic acids. It has previously been shown that the length of the meromycolate chain and the functionalities present play an important role in the functions of the cell wall, so a better understanding of the effects of the mycolic acid content may lead to a new approach in the therapy of mycobacterial disease.[84]

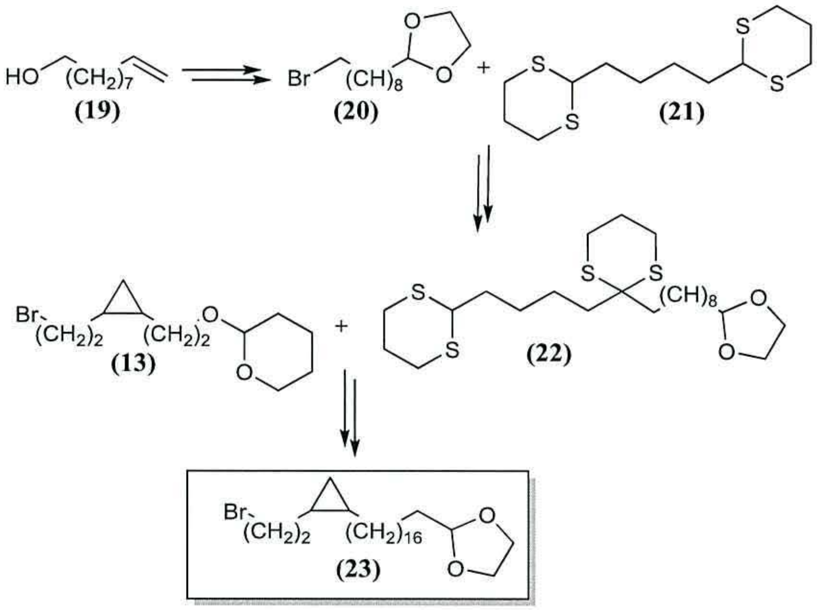
One of the first syntheses of mycolic acid was conducted by Gensler *et al.* in 1977.[85] They cyclopropanated 1,4-cyclohexadiene **(10)** giving noracarene **(11)**; ozonolysis, followed by a reduction of **(11)** gave *cis*-1,2-cyclopropanediol **(12)**.[86] Protection of the diol **(12)** with a tetrahydropyranyl group, followed by bromination of the resultant alcohol gave **(13)**. Chain extension of the bromide **(13)** was carried out using 2-pentadecyl-1,3-dithiane **(14)**, which was obtained *via* alkylation of the lithio derivative of 1,3-dithiane with pentadecyl bromide, giving the 2,2-disubstituted dithane **(15)**. Desulfurization of **(15)**,[87] followed by hydrolysis, then bromination gave the corresponding bromide **(16)**. Further chain extension using the *bis*-dithiane **(17)** gave

(18), which Gensler *et al.* considered the first major component of their target meromycolate, which they called the "Methyl End" (**Scheme 3**).[85]



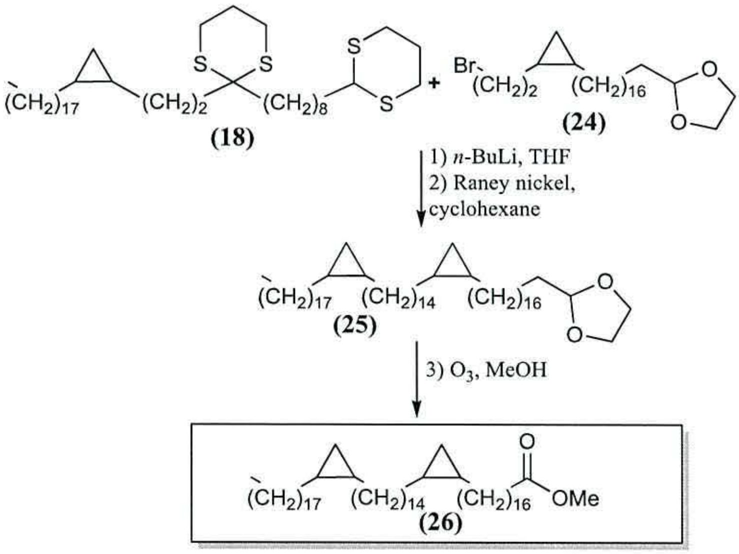
Scheme 3: Gensler et al.'s "Methyl End" Synthesis

The second major constituent of Gensler *et al.*'s meromycolate, called the "Carboxyl End", was prepared starting with the ozonoylsis of 10-undecenol **(19)**; conversion of the corresponding alcohol into the acetal was then carried out, followed by bromination to give **(20)**. Chain extension by six carbons gave **(22)**, and then coupling of this to the previously prepared bromide **(13)**, followed by desulfurization, deprotection of the tetrahydropyranyl group and bromination gave **(23)**. The acetal **(23)** was Gensler *et al.*'s "Carboxyl End" intermediate (**Scheme 4**).[85]



Scheme 4: Gensler et al.'s "Carboxyl End"

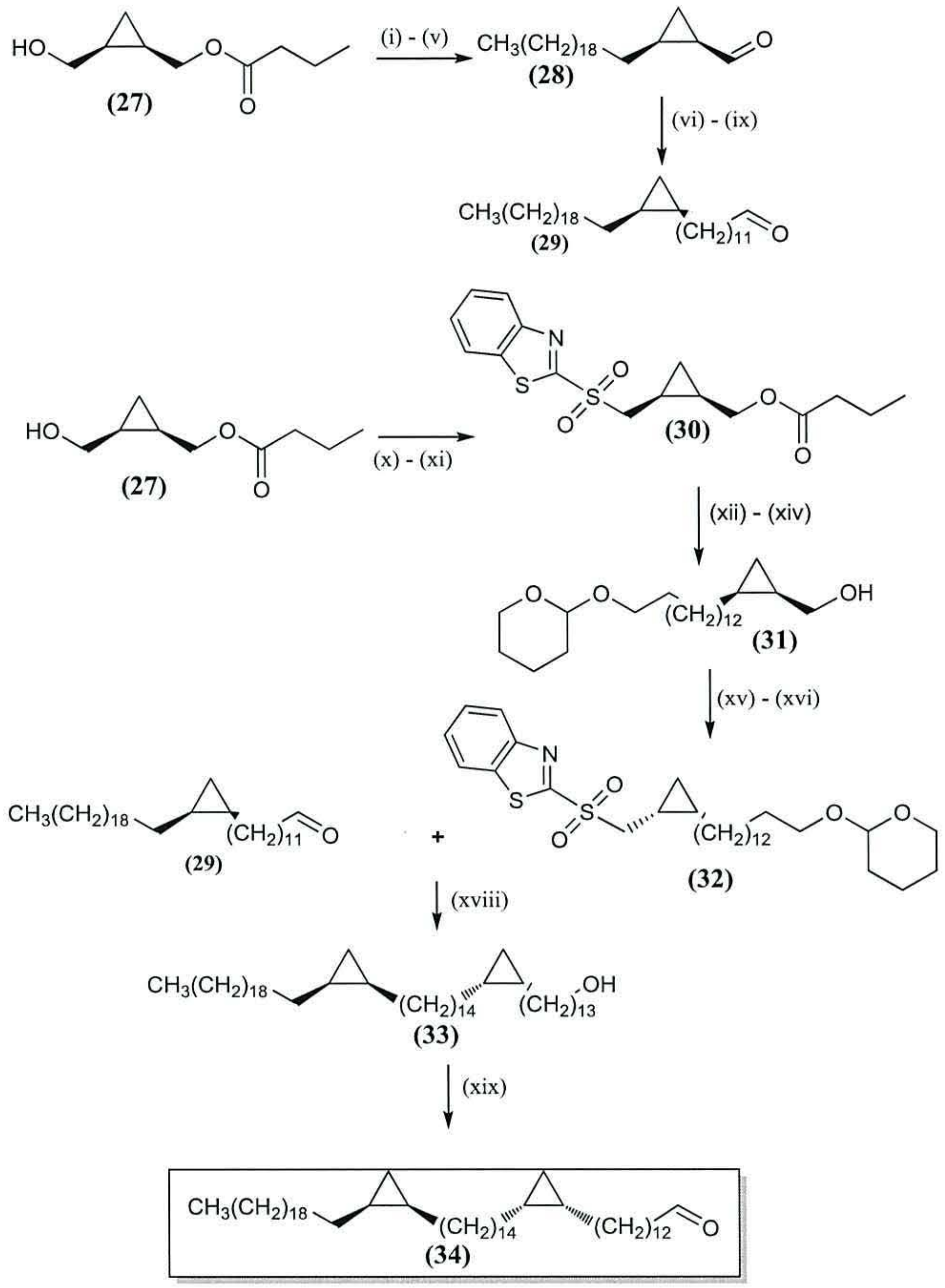
Coupling of the lithio derivative of *bis*-dithiane intermediate **(18)** with alkyl bromide **(24)**, followed by desulfurisation gave the expected product **(25)**. Ozonolysis of **(25)** gave the corresponding hydroxyether meromycolate ester, and a direct base-catalyzed ester interchange yielded the methyl meromycolate **(26)** **(Scheme 5)**.[85, 88]



Scheme 5: Gensler et al.'s "Methyl Meromycolate"

The first enantiomerically pure meromycolic acid was prepared by Baird *et al.* in 2000, where they set about preparing single enantiomers of cyclopropane intermediates, then successfully coupled the intermediates together with no loss of stereochemistry.[89] Coxon *et al.* had discussed the synthesis of single enantiomers of cyclopropane intermediates in 1999, and Baird *et al.* exploited this to obtain the first enantiomerically pure meromycolic acid. The aldehyde (**28**) was prepared from the anhydride of cyclopropane-*cis*-1,2- dicarboxylic acid.[90] A Wittig reaction of this with nonadecyltriphenylphosphonium bromide and *n*-butyl lithium, and reduction with lithium aluminium hydride, led to the alcohol as mixture of *Z*- and *E*-isomers. Saturation of the alkene was achieved with di-imide, prepared *in situ* by reaction between hydrazine, sodium periodate and acetic acid.

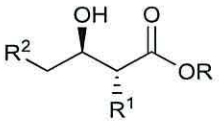
Chain extension with a second Wittig reaction gave the aldehyde (**29**). Another important feature of this approach is the coupling reaction, which was used to link the different units in several stages, securing the final desired stereochemistry. First of all, the Julia reagent sulfone (**32**) was prepared and reacted with the aldehyde (**29**) to give a mixture of *E*- and *Z*-alkene. The subsequent deprotection of the alcohol group and the reduction of the derived compound with di-imide give the alcohol (**33**), The alcohol (**33**) was oxidised under phase-transfer conditions to the corresponding meromycolic acid (**34**) (**Scheme 6**).



Scheme 6: Baird et al.'s synthesis of an enantiomerically pure meromycolic acid: (i) PCC/CH_2Cl_2; (ii) $Me(CH_2)_{18}{}^{+}PPh3Br^{-}$, BuLi; (iii) $LiAlH_4$/THF;(iv) $NH_2NH_2/CuSO_4/CH_3COOH$/i-PrOH/$NaIO_4$; (v) PCC/CH_2Cl_2; (vi) $MeOOC(CH_2)_{11}$ $+PPh_3Br$–, BuLi; (vii) $LiAlH_4$/THF; (viii) $NH_2NH_2/CuSO_4$/ CH_3COOH/i-PrOH/$NaIO_4$; (ix) PCC/CH_2Cl_2; (x) Benzthiazole, DEAD, PPh_3; (xi) MCPBA; (xii) $THPO(CH_2)_{12}/NaN(SiMe_3)_2$/THF; (xiii) K_2CO_3/MeOH; (xiv) $NH_2NH_2/CuSO_4$/ CH_3COOH/i-PrOH/$NaIO_4$; (xv) Benzthiazole, DEAD, PPh_3; (xvi) MCPBA; (xvii) $LiAlH_4$/THF; (xviii) NH_2NH_2/ $CuSO_4/CH_3COOH$/i-PrOH/$NaIO_4$; (xix) NaOMe, MeOH, THF.[91]

1.9.2 Synthesis of the α-alkyl-β-hydroxy ester unit

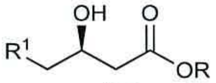
The stereochemistry of mycolic acids has been explained in **Section 1.7.2.** There are many methods reported for the preparation of (2*R*,3*R*)-α-alkyl-β-hydroxy esters with different starting materials and different catalysts (**Figure** 7).[51-53]



R = H or protecting group
R^1, R^2 = alkyl chain

Figure 7: Structure of (2R,3R)-α-alkyl-β-hydroxy esters

The first step is normally to prepare (3*R*)-β-hydroxy esters **(35)** (**Figure 8**) with the correct stereochemistry. This is followed by insertion of the alkyl chain.



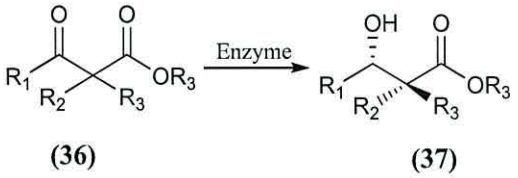
(35)

R = H or protecting group
R^1= protecting group

Figure 8: (3R)-β-hydroxy esters

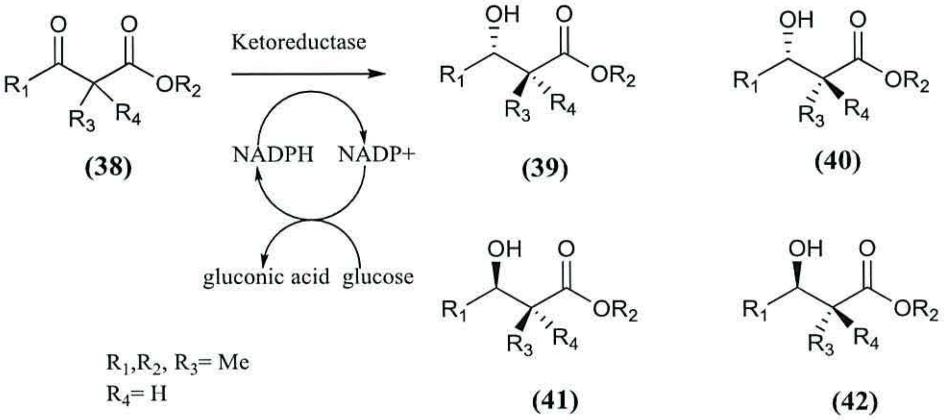
1.9.2.1 Enzyme catalysed reduction of β-ketoesters

The starting materials in the enzyme catalysed reduction method are the β-ketoester (**36**) and several different enzymes, all of which are relatively inexpensive; therefore this method is preferred in this kind of synthesis. For example, Rodríguez *et al.* used *Escherichia coli* expressing enzymes from Baker's yeast. Attempts at obtaining exclusively the (2*R*,3*R*) configuration of the *α*-alkyl-*β*-hydroxy ester, however; the reduction with bakers' yeast does not always afford alcohols with (2*R*,3*R*) configurations in satisfactory enantiomer excess (**Scheme** 7).[92]



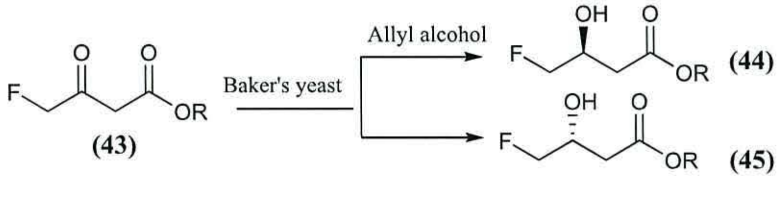
Scheme 7: The syn configuration of α-alkyl-β-hydroxy ester

Another approach was the use of NADPH as a catalyst for converting β-ketoester (**38**) into β-hydroxy ester. This method, however, produced a mixture of stereoisomers (**39**), (**40**), (**41**) and (**42**) (**Scheme 8**).[93, 94]



Scheme 8: Reduction reaction using NADPH -dependent ketoreductases

Other attempts to reduce the keto ester (**43**) involved using additives in order to control the stereochemistry of the product (**44**) and (**45**) (**Scheme 9**).[95, 96]



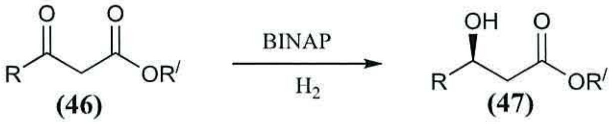
Scheme 9: The use of different additives

Increase in the amount of added allyl alcohol results in an increase in the amount of *R*-alcohol in all β-keto esters. Nakamura *et al.* [96] supposed that this effect is due to a difference in inhibition to *S*- and *R*-enantiomer-producing-enzymes. Allyl alcohol may inhibit the *S*-enantiomer-producing enzyme more efficiently than the *R*-enantiomer - producing-enzyme. This effect is also applicable to the reduction of **(43)** to produce **(44)**.[88]

1.9.2.2 Non-enzymatic methods for preparing the β-hydroxy ester

1.9.2.2.1 The use of BINAP

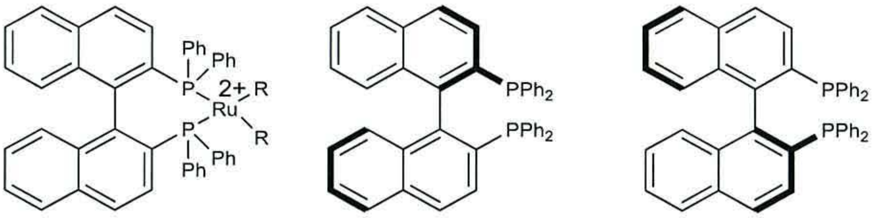
A favoured method is the use of a ruthenium-biarylbisphosphine catalyst (BINAP) to reduce β-keto esters **(46)** to β-hydroxy esters **(47)**, or to reduce the α-alkyl-β-keto ester to an α-alkyl-β-hydroxyl ester with the right stereochemistry (**Scheme 10**).[97] This method presented the opportunity to obtain this type of compound with different stereochemistry, in fewer steps.



Scheme 10: Preparation of β-hydroxy ester (47)

1.9.2.2.2 BINAP complex overview

Transition metals have been used extensively as catalysts in organic synthesis. Knowles and Horner individually reported using BINAP as a catalyst for the reduction of di-keto compounds.[98, 99] The complex Ru-BINAP **(48)** (**Scheme 11**), as a catalyst for hydrogenation reactions, gives a product with asymmetric induction. The stereochemistry depends on the complex used in the reaction, with (*R*)-BINAP **(49)** giving the (*R*)-β-hydroxy ester and (*S*)-BINAP **(50)** giving the (*S*)-β-hydroxy ester.[100, 101]

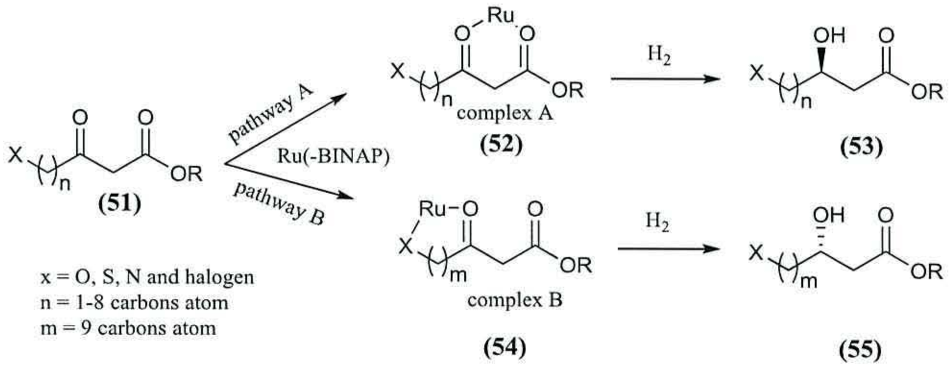


(48) *R*-BINAP **(49)** *S*-BINAP **(50)**

Scheme 11: The stereochemistry of BINAP complexes

1.9.2.2.3 Reduction of a β-diketo ester

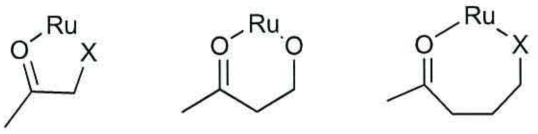
David *et al.* reported that the structure of a ketoester affects the stereochemistry of the product **(53)** and **(55)** in the reduction process. The enantioselectivity will decrease if the alkyl chain in the C-4 position is greater than nine carbons long.[97] In addition, if the β-keto ester contains other functional groups, it can also affect the stereochemistry of the product by coordinating with the catalyst to give the final *R* or *S* stereochemistry. **Scheme 12** clearly demonstrates that the same catalyst can give two products, depending on the presence and position of groups on the substrate.[102] The formation of complex A, which is a product of the coordination of the ruthenium Ru-BINAP to the two carbonyls in the ester, hinders the formation of complex B, which is the pathway that leads to *S* isomers.[102, 103]



(51) **(52)** **(53)** **(54)** **(55)**

Scheme 12: Effect of different groups on the stereochemistry of the product

If the ruthenium in the BINAP complex forms a five-(**56**), six- (**57**) or seven-(**58**) membered chelated ring (**Scheme 13**), this leads to pathway B, which gives *S* isomers. The X in the complexes (**56**), (**57**) and (**58**) could be oxygen, sulfur, nitrogen or a halogen.



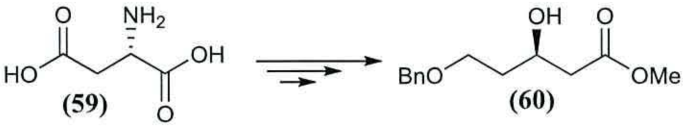
(56) **(57)** **(58)**

Scheme 13: Ruthenium complex (pathway B) [102]

The method of preparation of the catalyst plays an important role in the stereochemical outcome of the reaction. However, although this reaction has been used in industry, the difficult and the expensive conditions required limit its use in the laboratory.

1.9.2.2.4 The aspartic acid route

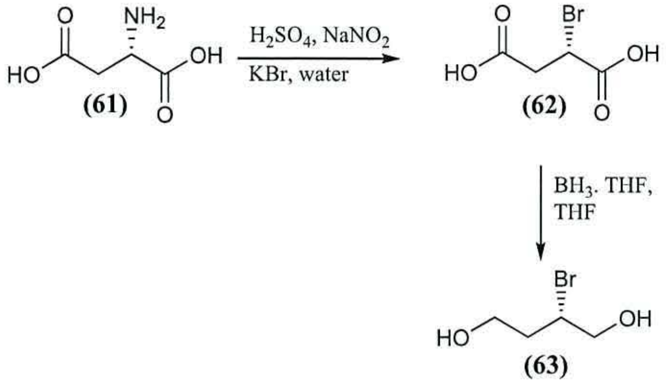
L-Aspartic acid (**59**) was used for preparation of the β-hydroxy ester (**60**) in the *R*-configuration (**Scheme 14**).



(59) **(60)**

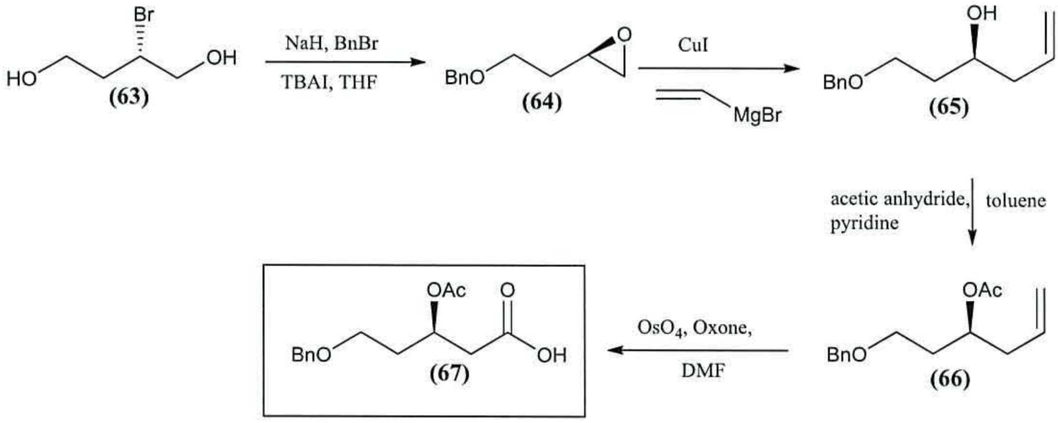
Scheme 14: The preparation of β-hydroxy ester (60)

The first step was the conversion of the α-amino acid (**61**) into α-bromosuccinic acid (**62**) with retention of configuration, by the use of KBr and $NaNO_2$ in H_2SO_4. The concentration of sulphuric acid was critical. If this was too high, the salt precipitated making the reaction impossible to complete. This is followed by reduction of the acid to the diol (**63**) using borane tetrahydrofuran (**Scheme 15**).[104] The borane is a mild reducing reagent which was able to reduce the two carboxylic acids into diols, while leaving the bromine group unchanged.



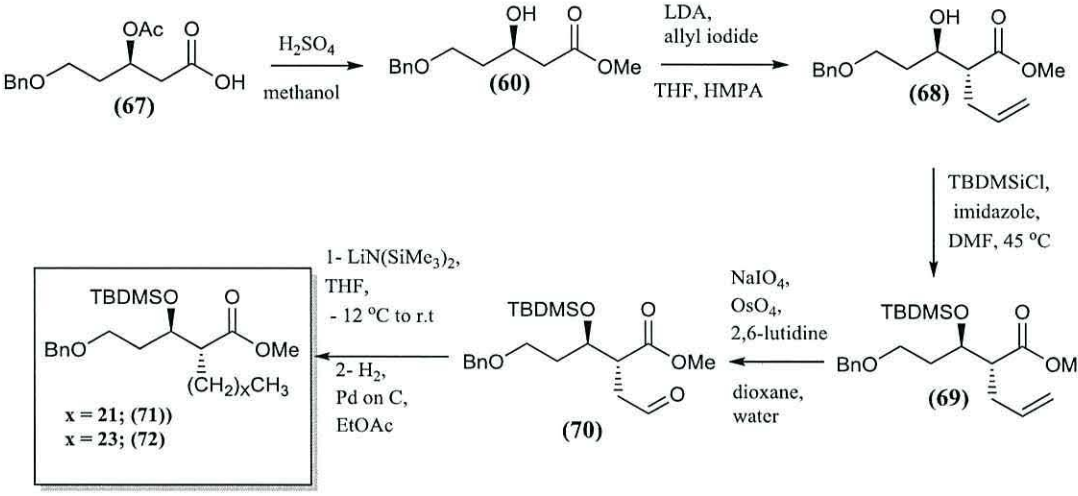
Scheme 15: Preparation of diol (63) [104]

The next step is cyclization to form the oxirane (**64**) with simultaneous protection of the other hydroxyl with a benzyl protecting group. This is followed by a Grignard reaction to extend compound (**65**) by two carbon atoms. The next step is protection of the secondary alcohol with an acetyl group using acetic anhydride and pyridine as catalyst in dry toluene to form compound (**66**). This is followed by oxidative cleavage of the alkene (**66**) to form carboxylic acid (**67**) (**Scheme 16**).[105]



Scheme 16: Preparation of carboxylic acid (67) [105]

The next step is deprotection of the secondary hydroxyl and protection of the carboxylic acid group in one step by refluxing the acid (**67**) in methanol, which acts as both reactant and solvent to form the ester (**60**). The next few steps concerned the insertion of the alkyl chain in the α-position with respect to the carboxylic acid group. This was done using a Fräter reaction for the insertion of an allyl group in α-position to give alkene (**68**). The secondary alcohol (**68**) was protected with a *tert*-butyldimethylsilyl group to give the product (**69**), followed by oxidative cleavage of olefin (**69**) with OsO_4–$NaIO_4$ and 2,6-lutidine in dioxane-water to give the aldehyde (**70**). Coupling of the aldehyde with either a C-20 or C-22 sulfone, via the modified Julia reaction, followed by hydrogenation of the alkene led to the formation of the mycolic motif (**71**) and (**72**) respectively (**Scheme 17**).[105, 106]



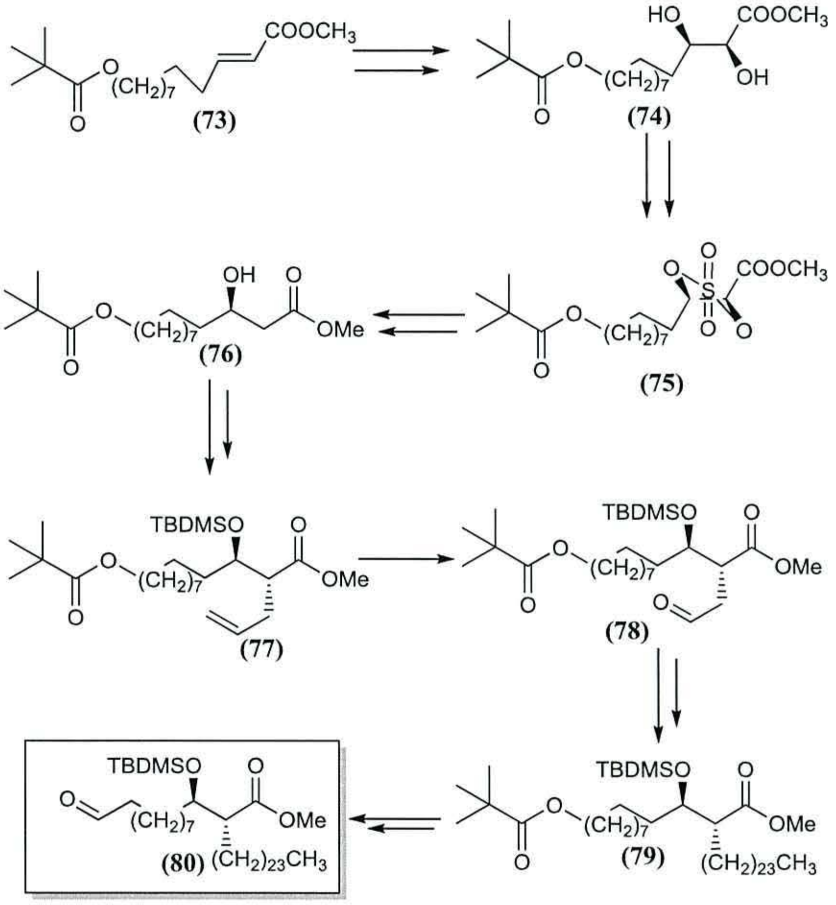
Scheme 17: Preparation of the mycolic motif with two different chain lengths

1.9.2.2.5 A *cis*-dihydroxylation route for synthesis of the α-alkyl β-hydroxy unit

Another method for the preparation of the α-alkyl β-hydroxy unit **(80)** has been reported by Baird *et al.* The *cis*-olefin ester (**73**) was transformed into the diol (**74**) using a Sharpless dihydroxylation.[107] The diol was converted into the sulfate (**75**) and then regioselectively reduced and hydrolysed to give the β-hydroxy ester (**76**).[108] The point of this strategy is that the generation of the aldehyde **(78)** permits the introduction of any chain length desired at the α-position, via a modified Julia-Kocienski olefination.[109, 110]

The alkene (**77**) was converted into the aldehyde (**78**) and coupled with the sulphone (**79**) to give an unsaturated intermediate. Finally, reduction of this alkene gave a desired long chain at the α-position (**80**) (**Scheme 18**).

Baird *et al.* suggested that one would be able to add a labelled group at this position; this would possibly help gain a further understanding of the biological behaviour of mycolic acids.

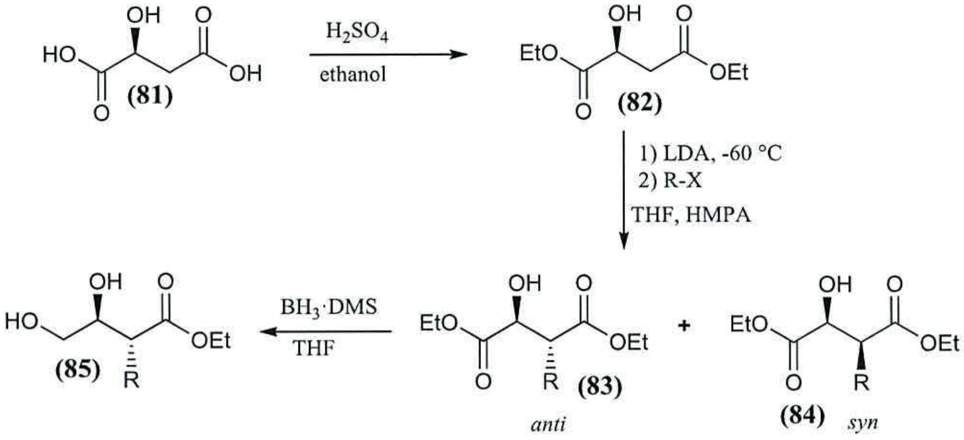


Scheme 18: Baird et al.'s improved synthesis of the α-alkyl β-hydroxy unit

Baird *et al.* have contributed greatly to the area of the complete synthesis of mycolic acids, and they have published several routes to gain enantiomerically pure mycolic acids.[91, 111-114]

1.9.2.2.6 The malic acid route for synthesis of the α-alkyl β-hydroxy unit

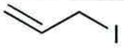
Khan *et al.* have prepared the β-hydroxy ester unit using *L*-malic acid (**81**),[115] which was esterified to give diethyl (*S*)-malate (**82**), as shown in **Scheme 19.**[116]



Scheme 19: The malic acid route for synthesis of the α-alkyl β-hydroxy unit

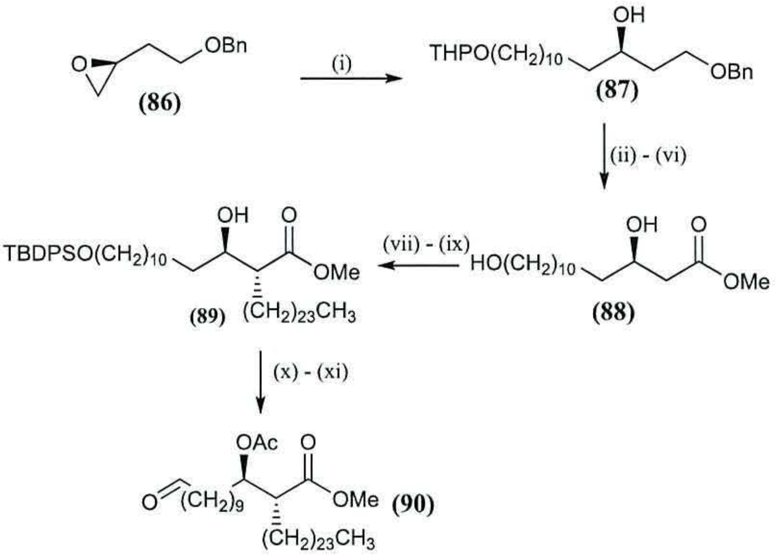
Diethyl (*S*)-malate (**82**) was alkylated with allylic halides and their saturated counterparts (**Table 1**) in THF by using conditions A: LDA (2.5 equiv.), (**82**) (1 equiv.), R–X (1.5 equiv.), or B: addition of HMPA (HMPA/THF 1:1), and then diester **(83)** was selectively reduced by treatment with $BH_3 \cdot DMS$ to give diol **(85)**.[117]

Table 1: The Fráter–Seebach alkylation using long-chain alkyl halides [115]

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

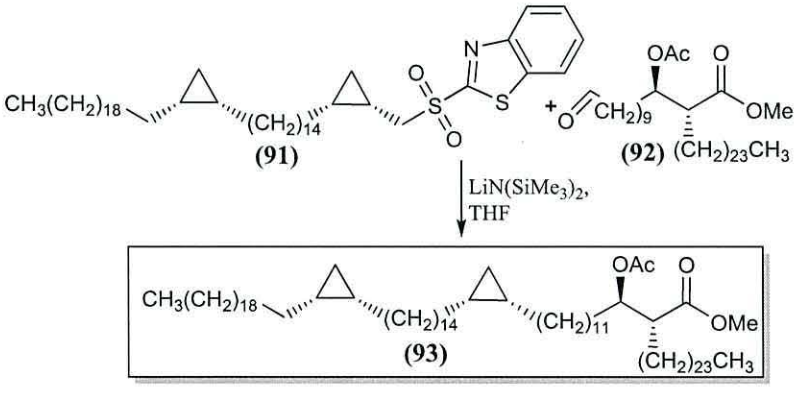
1.10 First synthesis of a single enantiomer of an α-mycolic acid

A single enantiomer of α-mycolic acid, containing two *cis*-cyclopropane rings has been synthesized successfully by Baird *et al.*[91] who prepared a corynomycolate aldehyde, so that they could then couple this to a meromycolate sulfone using a modified Julia-Kocienski olefination.[91] The corynomycolate was prepared by a Grignard ring opening of the epoxide **(86)** with 9-bromononan-1-ol tetrahydropyranyl ether to give the secondary alcohol **(87)**. This was then transformed into **(88)**; an alkylation of **(88)** and protection of the primary alcohol then gave the *tert*-butyldiphenylsilyl ether giving **(89)**. Protection of the secondary alcohol with acetate, then deprotection of the *tert*-butyldiphenylsilyl ether and oxidation yielded the corynomycolate aldehyde **(90)** (**Scheme 20**).[91]



Scheme 20: Baird et al.'s corynomycolate aldehyde: (i) $BrMg(CH_2)_9OTHP$, Cu ; (ii) imidazole, DMF; (iii) imidazole, DMF, $ButSiMe_2Cl$; (iv) H2, Pd/C, MeOH (v) $NaIO_4$, RuCl3, H_2O, CH3CN, H_2O, CCl_2; (vi) MeOH, H_2SO_4; (vii) MeOH, H_2SO_4; (viii) $ButPh_2SiCl$, DMAP, Et_3N; (ix) LDA, $CH_3(CH_2)_{23}I$, HMPA; (x) Ac_2O, pyridine; (xi) PCC/CH_2Cl_2

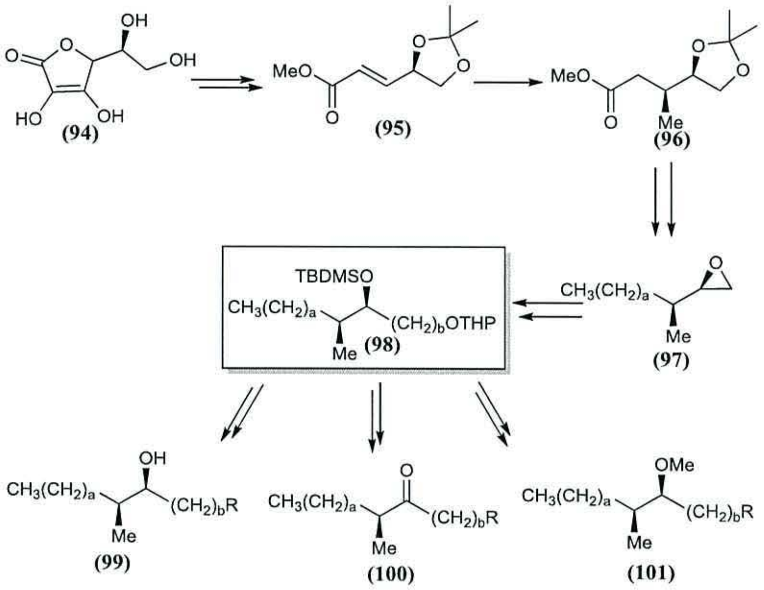
Using similar procedures to those for their first meromycolic acid; Baird *et al.* prepared the meromycolate sulfone (**91**). Coupling of this to the corynomycolate aldehyde (**92**) (**Scheme 21**), and mild hydrogenation with potassium azodicarboxylate and acetic acid in THF gave the first enantiomerically pure α-mycolic acid.[91]



Scheme 21: Baird et al.'s first α-mycolic acid [91]

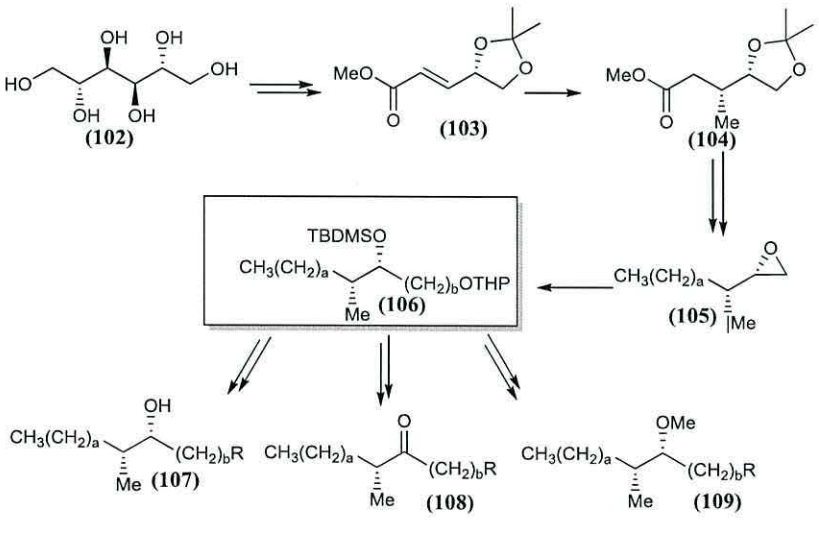
1.11 Synthesis of oxygenated mycolates

Baird *et al.* later discussed the synthesis of *S,S*-oxygenated mycolates, obtainable from *L*-ascorbic acid **(94)**.[105, 118] The procedure reported is a multi-stage synthesis to achieve the key intermediate **(98)**, from which it is possible to prepare hydroxy **(99)**, keto **(100)** and methoxy **(101)** mycolic acids (**Scheme 22**).



Scheme 22: Baird et al.'s preparation of S,S-oxygenated mycolates

They also reported the preparation of *R,R*-oxygenated mycolates in a similar fashion, however starting from *D*-mannitol **(102)** (**Scheme 23**).[105, 118]



Scheme 23: Baird et al.'s preparation of R, R-oxygenated mycolate

1.12 Biosynthesis of mycolic acids

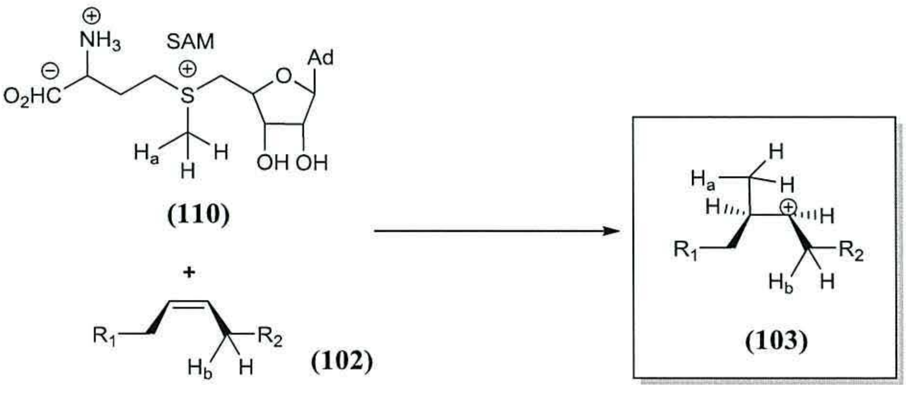
The biosynthesis of mycolic acids can be illustrated in four steps:

(a) Synthesis of C24-C26 straight chain saturated fatty acids to provide C1 and C2 atoms and an α-alkyl chain;

(b) Synthesis of the backbone of meromycolic acids of C40-C60;

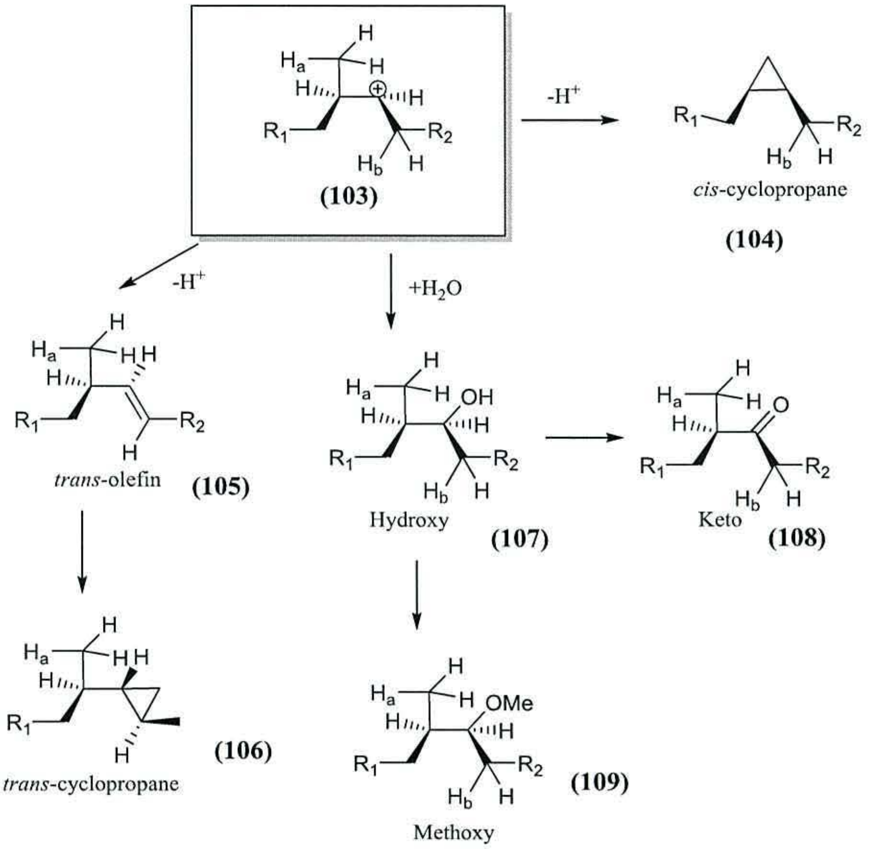
(c) Modification of meromycolate chain to introduce functional groups other than β-hydroxy; and
(d) The final condensation step to produce mycolic acids. Many enzymes involved in catalysing different steps in the biosynthesis of these molecules are targets to develop mechanism based antituberculosis drugs.[119, 120]

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).[121] Methylation of a *cis*-alkene (**102**), using SAM (**101**), gives the carbocation intermediate (**103**) involved in functionalization of the meromycolate chain and futher reaction to yield the various substituents found in mycobacterial mycolic acids (**Scheme 24**).



Scheme 24: Formation of carbocation intermediate (103)

The carbenium ion can be deprotonated to form a *cis*-cyclopropane (**104**). The removal of a proton from the methyene group in the α-position yields the α-methyl-*trans*-olefin unit (**105**). The *trans*-olefin could be the substrate for a second SAM-dependent methylation to form the α-methyl-*trans*-cyclopropane unit (**106**) seen in the oxygenated mycolates of *M. tuberculosis*. If the carbocation intermediate (**103**) undergoes a hydration reaction, the hydroxy-mycolate (**107**) is formed, precursor to methoxy- (**109**) and keto-mycolate unit (**108**) as shown in **Scheme 25**.[111, 121]



Schemem 25: The insertion of the non-oxygenated functional groups in mycolic acids [121]

It is interesting that in *M.Tb*, the gene which is required for the biosynthesis of *trans*-cyclopropyl mycolates is active in the production of both keto and methoxy *trans*-cyclopropyl mycolates.[122] This is significant 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. It is important to note that this enzymatic route has a significant implication. Thus, the stereochemistry of at least one carbon for all the functional groups present in the meromycolic chain has always to be in the same configuration.

1.13 The folding of mycolic acids

It has been revealed that the antibodies formed by animals infected with TB were able to distinguish between different types of mycolic acids.[123, 124] Experiments with T cells gave more complex results, however, regarding recognition of the fine structure of the lipid moiety in cord factor.[125, 126] In particular Grant *et al.*[127] suggested a possible reason for the stronger recognition of oxygenated mycolic acids by T cell receptors. They suggested that keto- and methoxy-mycolic acids fold in a way that allows the three polar functions of the lipid chain to be in close proximity and to form an epitope, which is well recognized by this kind of receptor (**Figure 9**).

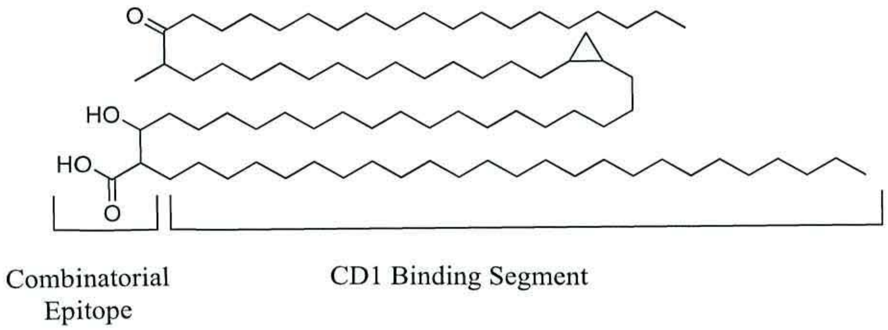


Figure 9: A possible folded conformation for keto-mycolic acid

Hasegawa *et al.*,[128-130] also proposed that mycolic acids could exist in folded conformations, although this was in a completely different context, namely, when they are arranged in monolayer films. These conformations are thought to be particularly stable in keto-mycolic acids due to strong molecular interactive forces. 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.[130]

1.14 Cord factors

Cord factor is a "free lipid" consisting of trehalose which is esterified at the 6 or at the 6-6' position primary alcohol groups with mycolic acids (**Figure 10**).[131] The cord factors 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.[132]

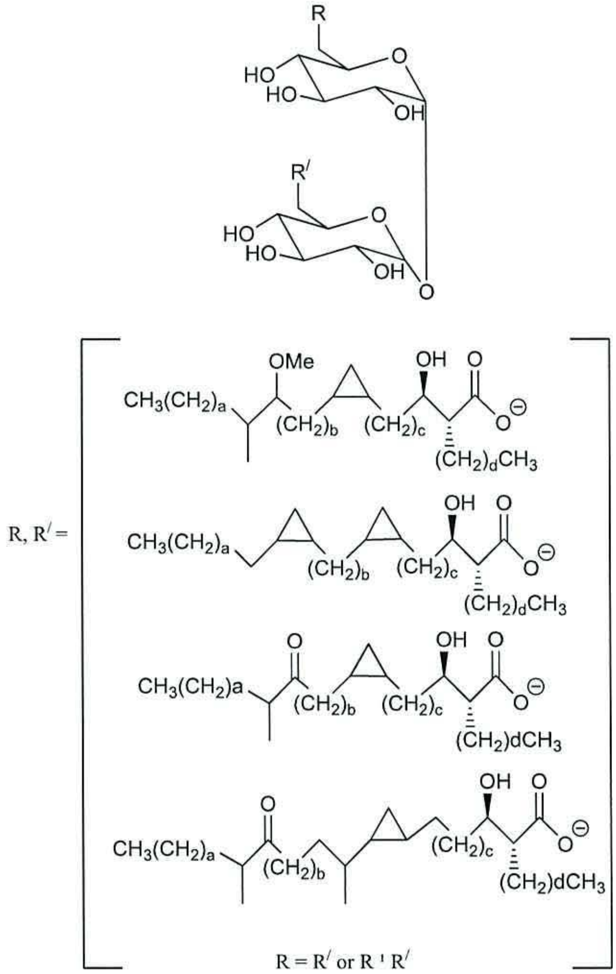


Figure 10: An example of a trehalose, "Cord factor" from M. tuberculosis

Cord factors consist of two classes, namely trehalose monomycolates (TMM) and trehalose dimycolate (TDM). Each of these derivatives consists of a non-reducing, disaccharide core, trehalose. The trehalose contains two glucose molecules linked by an α,α-1,1-glycosidic linkage. In TDM, the trehalose is esterified with two mycolic acids at the 6,6'-positions corresponding to both primary alcohol groups. In the TMM, the trehalose is esterified with one mycolic acid at the 6-position corresponding to one primary alcohol group.

Natural diesters of trehalose have been isolated from different bacteria since the 1960s. Vilkas *et al.* isolated an asymmetrically substituted trehalose from *M. fortuitum*. Ioneda *et al* described a glycolipid isolated from *Corynebacterium diphtheria* in1963 and proved that the substance was a trehalose diester containing similar proportions of corynomycolic ($C_{32}H_{64}O_3$) and corynomycolenic acid ($C_{32}H_{62}O_3$).[133, 134] Toubiana *et al.* in 1979 isolated and characterised all types of mycolic acid in a single strain of *M. tuberculosis Brevanne* and found molecular peak ion by mass spectrometry.[135]

1.14.1 Techniques of analysis used for cord factors

Bloch *et al.*, has identified the classical structural identification of cord factors from *M. tuberculosis* in 1955 using flash column chromatography. Later, a development technique allowed studies of protected glycolipids by mass spectrometry. The spectrum usually showed a peak for both trehalose monoester and diester. The main fragmentation affects the glycosidic bond of the disaccharide. Recently, TDM/TMM analysis using matrix-assisted laser desorption/ionization time-off light (MALDI-TOF) has been used for characterisation and identification. This technique is characterised by its giving rapid analysis with minor preparation, not only for TMMs and TDMs, but also for mycolic acids.[136]

1.14.2 Biological Properties of Cord factors

In vivo and *in vitro* tests have shown that TDM injected in a mitochondrial suspension induced respiration inhibition, loss of respiratory control and inhibition of electron transport.[137, 138] Rastogi *et al.*,[139] reported that cord factor coated *B. subtilis* caused inhibition of the immigration of blood leuckocytes, while a control group were unaffected.

The toxic effect of cord factor has been the subject of numerous studies. Numata *et al.*[140] reported toxic effects in mice, with the majority of animals treated with TDM under three different protocols dying after only a few days. An earlier discovery using electron microscopy proposed that cord factor weakened the peripheral part of the internal membrane. Durand *et al.* confirmed that the nature of the sugar residue is a major determinant of their activity.[141] Kato *et al* studied the toxicity of TMM by injecting different concentrations in mineral oil into mice.[142]

Since a natural cord factor containing just one or two different mycolic acids would be extremely hard, if not impossible, to obtain, a synthetic cord factor with a completely defined structure is required for testing its biological properties.[142]

1.15 CD1 proteins

Mycobacteria with their peptide and lipid antigens are phagocytosed through receptor mediated recognition. Typical receptors for the uptake of *M. Tuberculosis* include the mannose receptor (MR), and complement receptor 3 (CR3).

The CD1 proteins are nonpolymorphic cell-surface glycoproteins related in structure and evolutionary origin to the major histocompatibility complex (MHC)-encoded antigen-presenting molecules. There are five non-polymorphic CD1 proteins, CD1a, CD1b, CD1c, and CD1d, expressed at the surface of antigen-presenting cells (APCs), while CD1e is never found at the cell surface but is a complex in the processing of antigens inside the presenting cells (PCs).[143-145,146] CD1b is endocytosed together with mycobacteria at the plasma membrane. The majority of known foreign ligands for group 1 CD1 molecules are mycobacterial in origin, including dideoxy-mycobactin, mycolic acid, lipoarabinomannan, glucose monomycolate (GMM), glycerol monomycolate, diacylatedsulfoglycolipid, phosphatidylinositol mannoside, and mannosyl phosphomycoketide.[147]

1.15.1 Antigen presentation in tuberculosis (CD1 proteins)

APCs play a vital role in directing the immune response upon infection with pathogens, such as *M. Tuberculosis*. The production of proflammatory cytokines represents a significant step in controlling *M. Tuberculosis* infection because it is these cytokines that are of paramount importance in driving the production of IFN-γ.[148] Human T cells proliferate or produce interferon-γ in response to several types of mycobacterial lipid antigens presented by group 1 CD1 proteins during latent or active tuberculosis infection, suggesting a function in host response to mycobacteria.[148] Proteins and the antigen–protein complexes mediate T cells which respond in the human host by presenting GMM and other antigenic mycobacterial glycolipids by the CD1 family.[149, 150]

1.16 Glucose monomycolate (GMM)

Glucose monomycolate is a glycolipid consisting of mycolic acid attached to the 6-position of glucose which is present in numerous bacterial species including *Mycobacterium, Rhodococcus* and *Nocardia* (**Figure 11**).[151, 152]

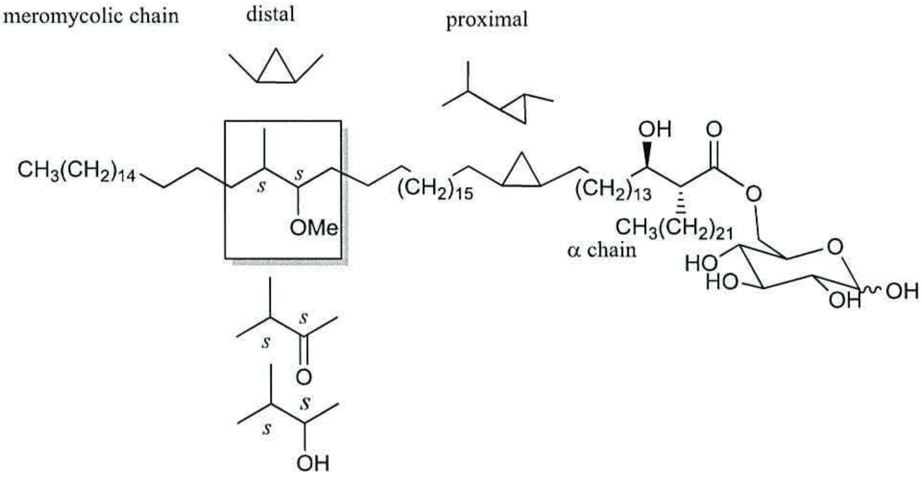
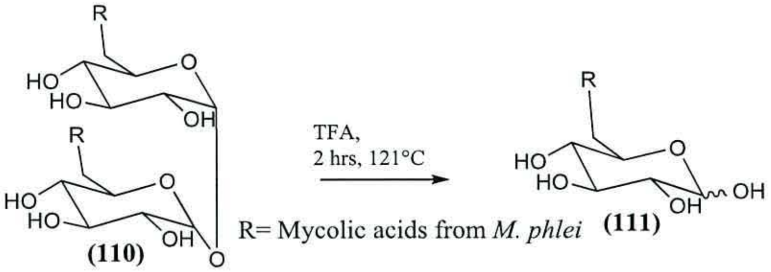


Figure 11: Glucose monomycolate (GMM, 6-O-mycoloyl-D-glucose)[153]

1.16.1 History of GMM

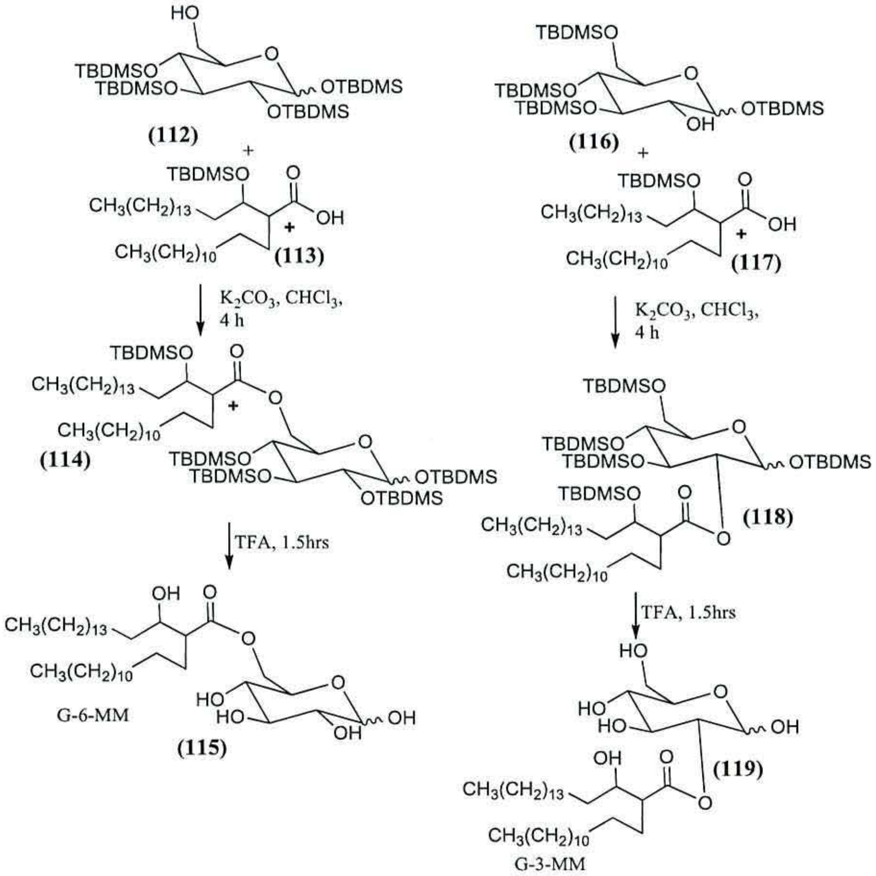
GMM is a mycobacterial antigen isolated from various species of mycobacteria, including *M. tuberculosis*.[151, 152] GMM can induce a memory T cell response by acting as protein antigen.[152] GMM and other antigenic mycobacterial glycolipids are presented to T cells by the CD1 family of proteins and the antigen–protein complexes mediate T cells response in the human host.[154] However, depending on the species, the meromycolic chain carries variable functionalities in the proximal and distal positions, which are characteristic of the species.[153] The structure of GMM from *M. tuberculosis* is shown in **Figure 11**.

Tiny amounts of GMM have been isolated and characterized from mycobacterial cultures and natural sources after long and tedious purifications. Reports pertaining to the synthetic preparation of GMMs are scattered in the literature.[155, 156] Branch *et al.* has described the synthesis of GMM (**111**) from *M. phlei,* in a process which involved acidic hydrolysis of trehalose dimycolate (**110**) by 2M trifluoroacetic acid for 2 h at 121 °C (**Scheme 26**).[155]



Scheme 26: The synthesis of GMM (111) from M. phlei by D. Branch et al[155]

Branch *et al,* proposed another strategy for the synthesis of C_{32} glucose-6-*O* -monomycolate (G-6-MM) (**115**) from 3-*tert*-butyldimethylsilylated mycolic acid (**113**) and 1,2,3,4-*tetra-O–tert*-butyldimethylsilylated glucose (**112**) as described in **Scheme 26.**[156] In addition, a glucose-3-*O*-monomycolate (G-3-MM) (**119**) was synthesized using a similar method (**Scheme 27**). These GMM structures were used in T cell assays.[156]



Scheme 27: Synthesis of G-3-MM and G-6-MM [55]

The crystal structure of human CD1b and CD1a in a complex with specific GMM glycolipids illustrates the binding of a natural bacterial lipid Ag to CD1b and shows how its novel structural features fit this molecule for its role in the immune response to intracellular bacteria.[157]

1.16.2 Techniques of analysis used for GMM

Nguyen *et al.,* [158] extracted the GMMs from *M. phlei* or *N. farcinica* and their purity was confirmed by TLC and electrospray ionization mass spectrometric analysis as in **Figure 12**.

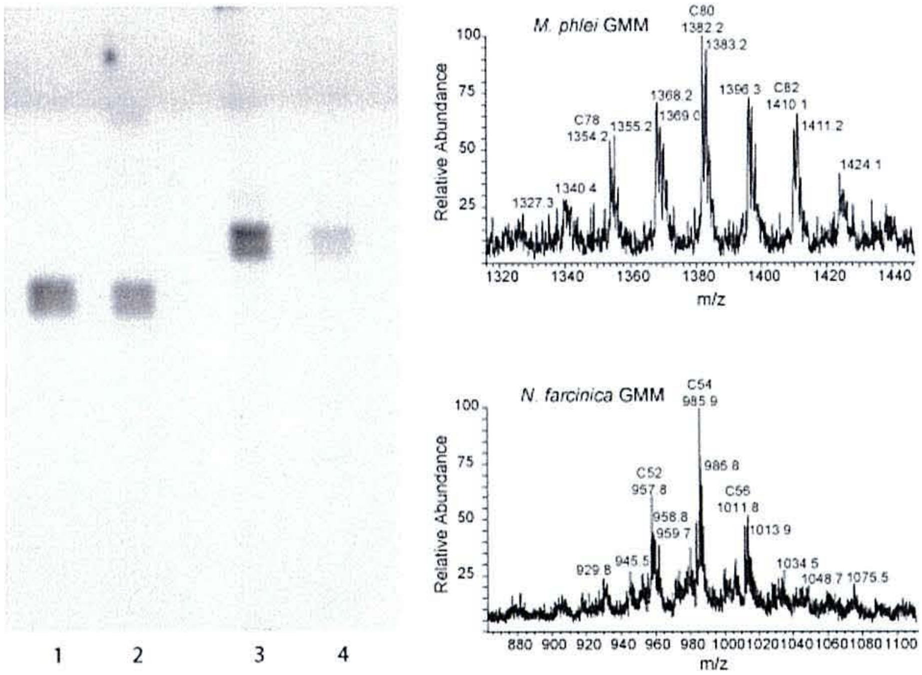


Figure 12: TLC and mass spectrometric analysis of M. phlei and N. farcinica GMM. Ten micrograms of GMM was applied to a silica TLC plate. The plate was developed in 60:16:2 chloroform:methanol:water (v:v), sprayed with 3% cupric acetate in 8% phosphoric acid, dried, and baked for 1 h at 140 ◦C. Lane 1: N. farcinica GMM standard; lane 2: purified N. farcinica GMM; lane 3: M. phlei GMM standard; lane 4: purified M. phlei GMM. Positive mode spectra were collected by electrospray ionization mass spectrometry[1584]

Prandi *et al.* [153] on the other hand has synthesized GMM from mycolic acids obtained from *M. tuberculosis* H37Rv and used NMR spectrometry and MALDI-TOF spectroscopic for identification. The MALDI-TOF spectrum for synthetic GMM (**Figure 13**) gave a complex pattern, as expected from the heterogeneity of the mycolic acid mixture.[153]

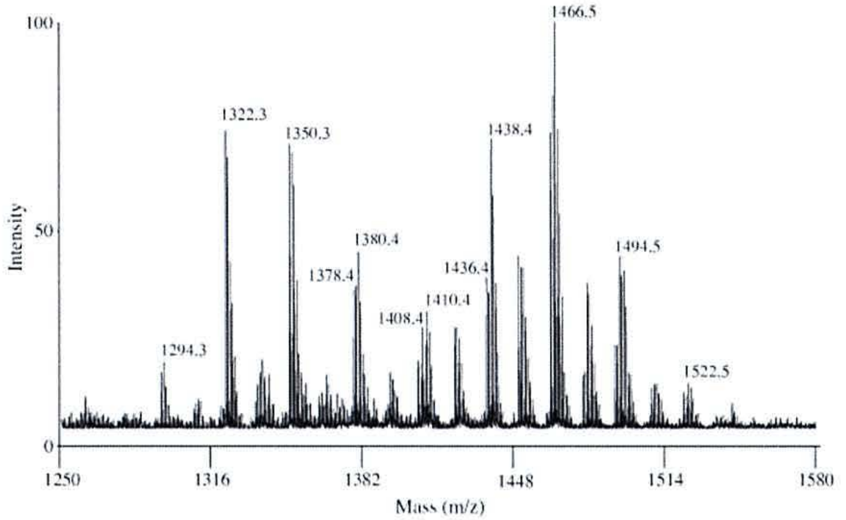


Figure 13: MALDI-TOF mass spectrum in positive ion mode of semi-synthetic GMM [153]

1.16.3 Synthesis of GMM from natural compounds

The synthesis of GMM (**125**) involves protecting the hydroxyl group of D-glucose (**120**). It has been found that protection D-glucose with several protecting groups, gave a mixture of α- and β-isomers (**Figure 14**).

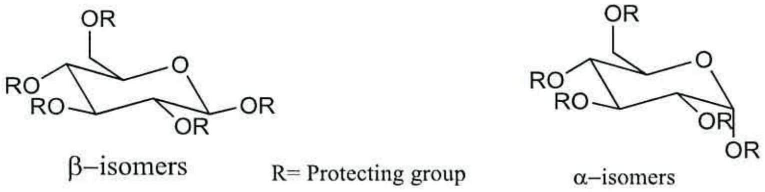
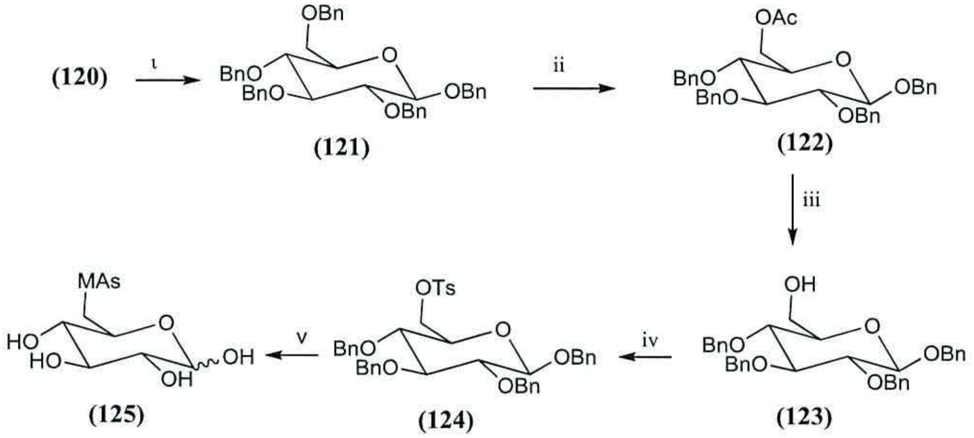


Figure 14: α- and β- glucose isomers

The protection with the benzyl group formed only the β-isomer; the reaction was done by adding NaH and benzyl bromide to give (**121**),[159] and then converting the primary benzyl into acetate (**122**), then into a hydroxyl group (**123**). The resulting product (**123**) was converted into a good leaving group, tosylate (**124**). Finally (**124**) was coupled with mycolic acids obtained from *M. tuberculosis* H37Rv using dry cesium hydrogen carbonate in a mixture of THF and DMF. The hydrolysis of the benzyl groups was done using palladium hydroxide on charcoal (**Scheme 28**).[153]



Scheme 28: The synthesis of GMM: (i) NaH, BnBr, DMF; (ii) HOAc–Ac$_2$O (5:1), ZnCl$_2$ (5 equiv); (iii) NaOMe–MeOH; (iv) tosyl chloride, DMAP, CH$_2$Cl$_2$; (v) CsHCO$_3$, THF–DMF 5:1

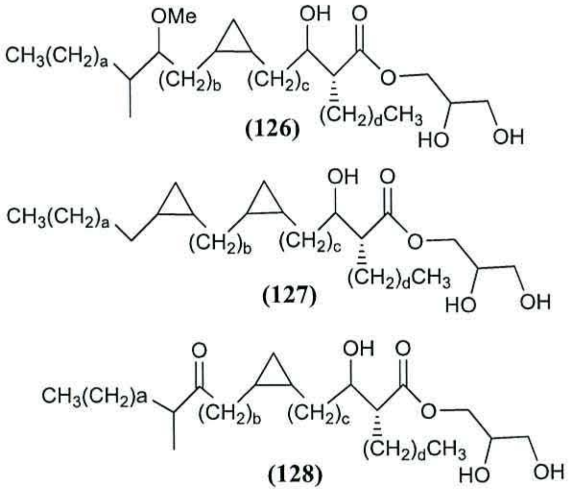
1.16.4 The biological properties of GMM

Many health problems worldwide are caused by mycobacterial infections and it has been proposed that anti lipid antibodies may contribute to protection against mycobacterial infection.[160]

GMMs have the capacity to produce T cell proliferative responses in a number of species including humans,[161] mice,[162] guinea pigs,[163] and cattle.[158] Nguyen *et al.* have described cell-mediated and humoral immune responses in cattle upon vaccination with GMM as the only antigen; as a result a T cell response was produced but no antibody responses, while the vaccine comprising a pure protein as the only antigen generated both T cell and antibody responses. However, in humans and cattle,[164, 165] Nguyen *et al* assumed that a conjugate of GMM with a protein may provide T cell help for B cells to produce antibodies against surface exposed glycolipids on mycobacteria.[158]

1.17 Glycerol Ester of fatty Acids (GroMM)

GroMM is a mycolic acid esterified with glycerol at one of the primary hydroxyl groups. Glycerol esters of fatty acids include methoxy glycerol monomycolate **(126)**, alpha glycerol monomycolate **(127)**, and keto glycerol monomycolate **(128)**, which are depicted in **Scheme 29**.[166, 167]



Scheme 29: Glycerol esters of fatty acids

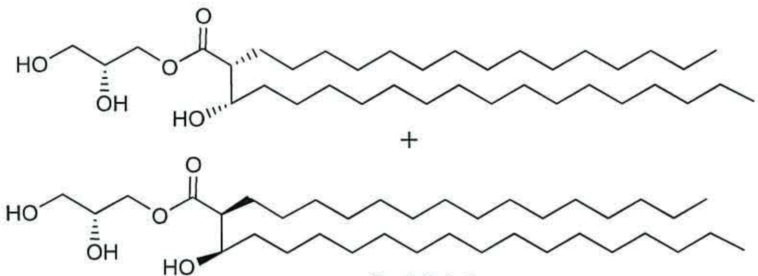
1.17.1 History of GroMM

Mycolic acids are found either in free form or esterified to GroMM, GMM, TDM, and TMM, *etc.*[168] Isolation of monoacylglycerol containing hydroxy acids was achieved after fractionation of the diethyl ether – soluble lipids of *Norcardia asteroids* by column and thin layer chromatography. The isolated lipid, which was liquid, liberated only glycerol into the aqueous phase, and had (C_{50} - C_{56}) mycolic acids as the sole constituent fatty acids.[169, 170]

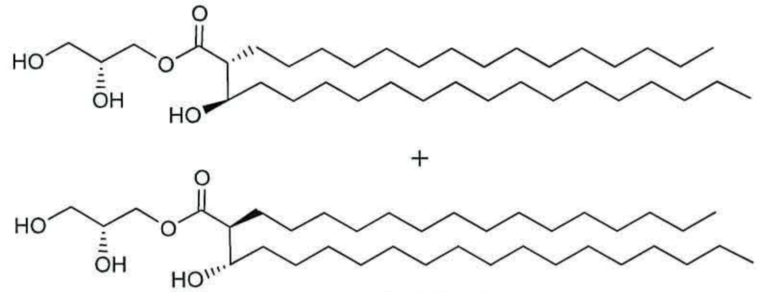
The separation by thin-layer chromatography (Ag-TLC) of per-*O*-benzoyl derivatives from 1-monomycoloyl glycerols extracted from *R. Lentifragmentus* has been done by reversed-phase high-performance liquid chromatography (RP-HPLC) of the homologous components of the corresponding species.[170, 171]

Andersen *et al.* identified GroMM *in vitro* as the most immunopotentiating compound among a number of different lipids isolated from the mycobacterial cell wall.[172] Hattori *et al.* identified GroMM as a specific immune target in human individuals with latent, but not active, tuberculosis, but the *in vivo* response to GroMM and the relevance of it to latent infection remain poorly understood. Hattori *et al.* immunized guinea pigs with bacillus Calmette–Guerin (BCG) expressing high levels of GroMM and then monitored skin reactions at the site of inoculation with GroMM-containing liposome. The host responses to GroMM produced by dormant mycobacteria contribute to their long-term survival in the host.[166]

In addition, Nordly *et al.* has synthesised a simple C_{32} lipid acid with a stereochemistry corresponding to alternative configuration of the corynomycolic acid compared to the natural GroMM compound. The structures of GroMM-6 are shown in **Figure 15**. On the other hand, GroMM-1 consists of a glycerol head group linked via an ester bond to a hydrophobic lipid acid displaying two saturated alkyl chains (C_{14} and C_{15}, respectively).[173]



GroMM-1



GroMM-6

Figure 15: Comparison of the two possible relative configurations of GroMM analogues, exemplified by GroMM -1 (alternative configuration) and GroMM -6 (native configuration). The alternative compounds display (2R,3S) and (2S,3R) configurations in a 1 : 1 ratio in the lipid acid moieties while the native compounds contain lipid acid moieties displaying (2R,3R) and (2S,3S) configurations. The depicted alkyl chain length is C_{14}/C_{15}.[173]

1.17.2 Techniques of analysis used for GroMM

GroMM has been extracted from *M. bovis* by Layre et *al.* and the identification of this novel antigen is supported by ^{1}H NMR analysis for the structure of *M. bovis* BCG GroMM as shown in **Figure 16.** [167]

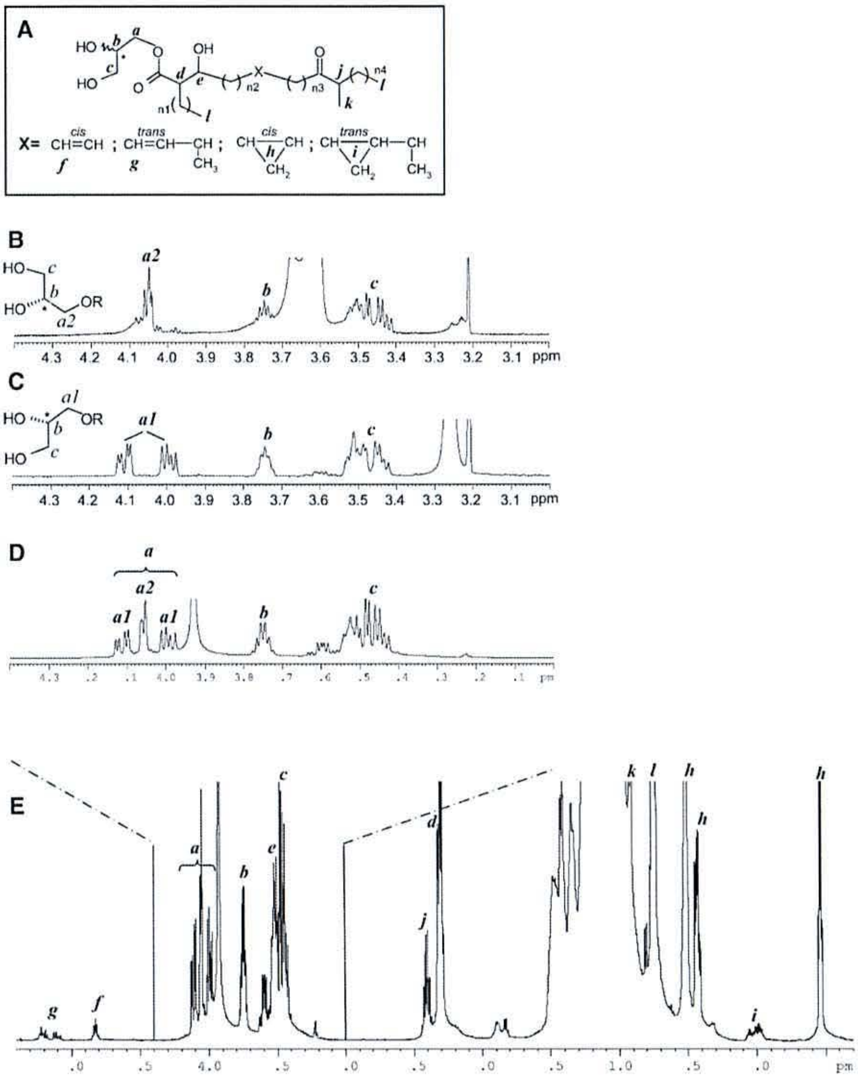
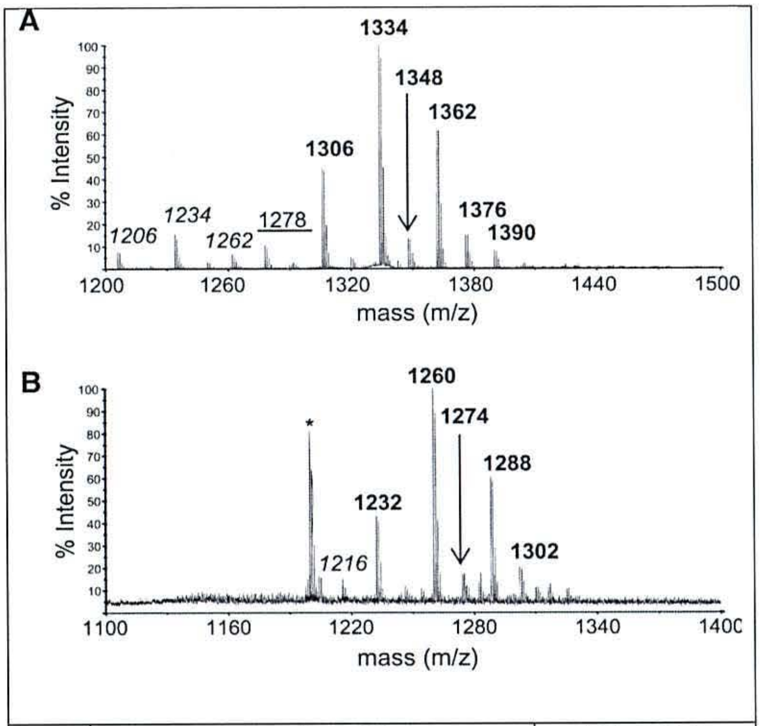


Figure 16: ^{1}H NMR Analysis of GroMM. The structure of M. bovis BCG GroMM is presented in (A). Specific GroMM protons signals are annotated a to k, and are assigned on the structure of the major forms of M. bovis BCG GroMM, esterified by ketomycolic acids. n_1 = 19, 21, or 23; n_2 and n_4 = 15, 17, or 19; n_3 = 12 to 17, (B and C) ^{1}H NMR analysis of synthetic (R)-1-O-mycoloylglycerol (B) and (S)-1-O-mycoloyl-glycerol (C). R corresponds to mycolic acid, (D and E) ^{1}H NMR analysis of M. bovis BCG GroMM). [167]

In addition, the positive mode MALDI mass spectrum for *M. bovis* BCG GroMM showed a pattern of peaks corresponding to cationized sodiated molecular ions $(M+Na)^+$ (**Figure 17**).[167]



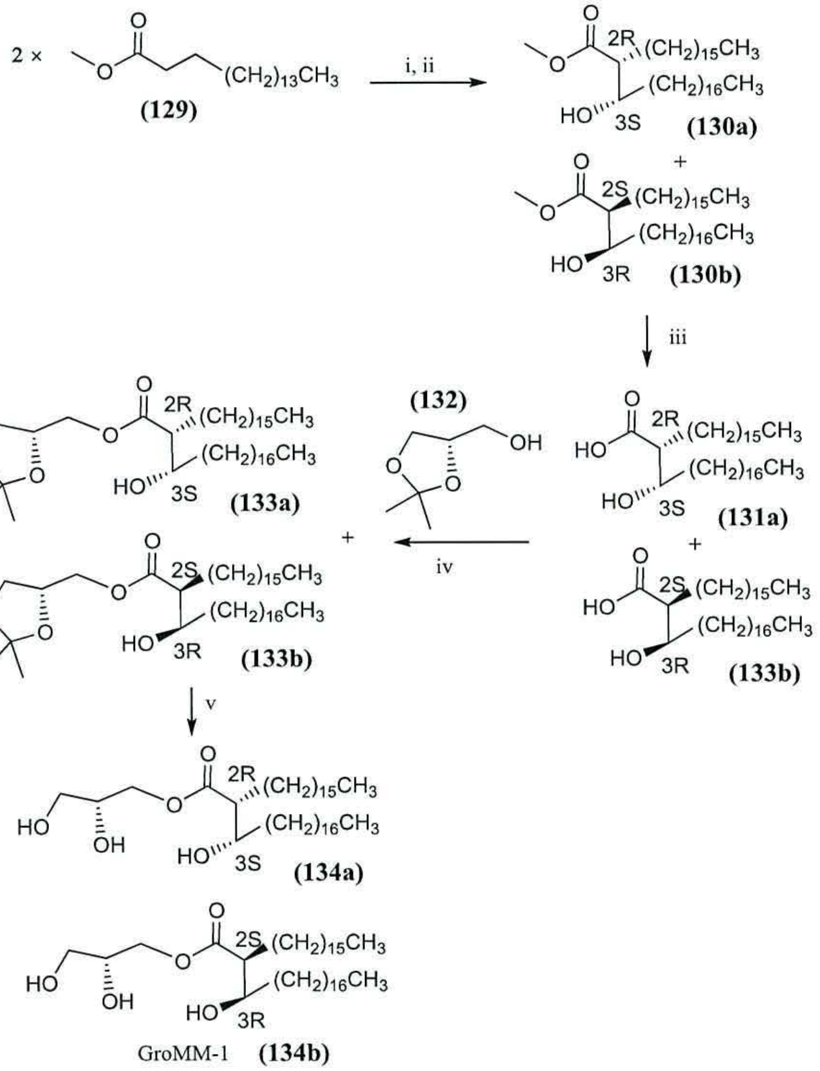
C

Mycolic acids		[GroMM, $Na^+]^+$
Type	[RCOOH, $Na^+]^+$	
α-	1132	1206
	1160	1234
	1188	1262
	1216	1290
Methoxy-	1204	1278
	1246	1320
	1248	1322
	1276	1350
	1304	1378
	1332	1406
Keto-	1232	1306
	1260	1334
	1274	1348
	1288	1362
	1302	1376
	1316	1390

Figure 17: Positive Mode MALDI-TOF MS Analysis of M. bovis BCG GroMM (A, B, C) [167]

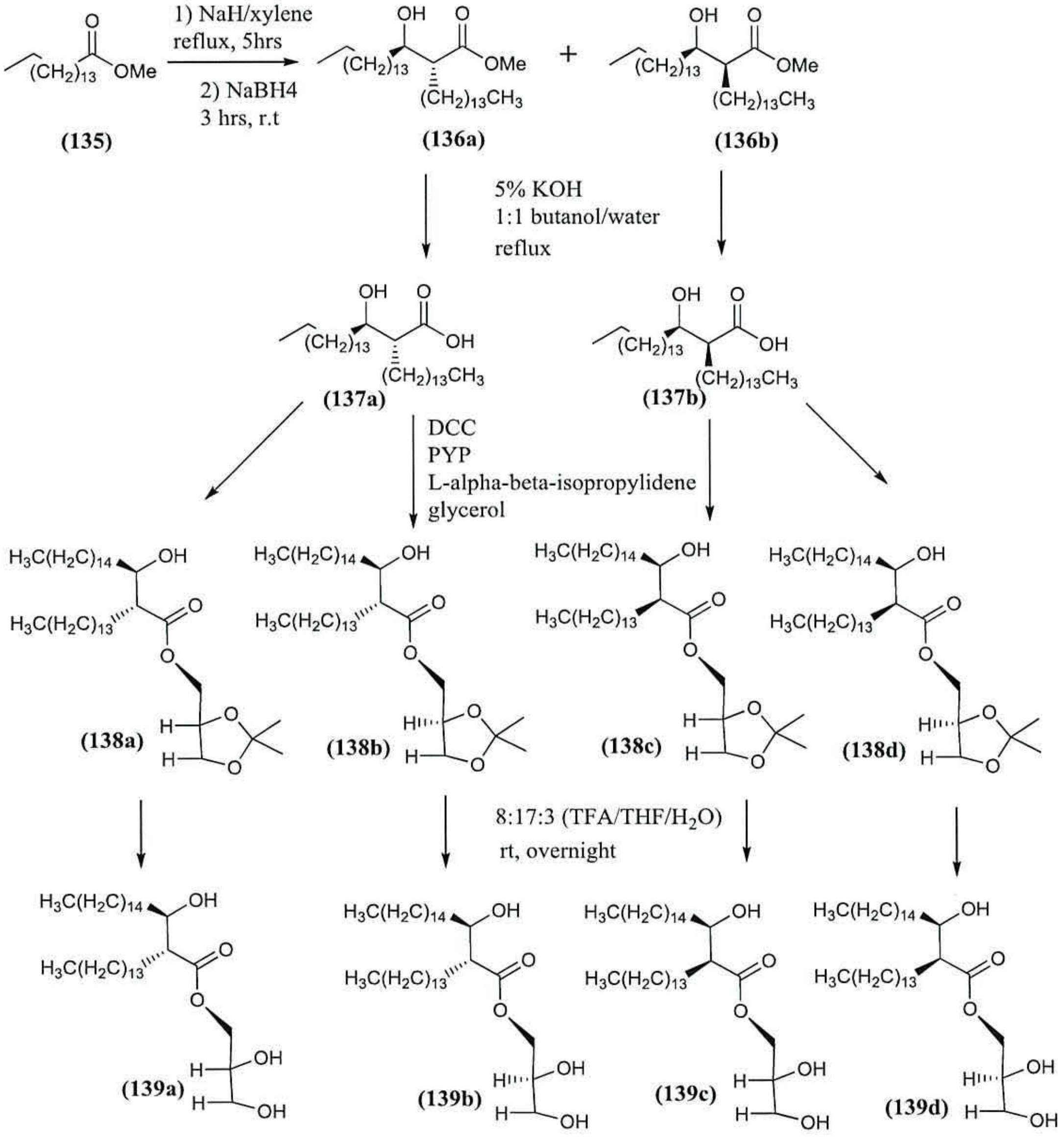
1.17.3 Synthesis of glycerol esters

Nordly *et al.* has described a synthetic analogue of the mycobacterial GroMM referred to as MMG-1. It was synthesized and combined with the cationic surfactant *N,N*-dimethyl-N-octadecyloctadecan-1-aminium salt (DDA) (**Scheme 30**). The purpose of the study was to provide a thorough pharmaceutical characterization of the resulting DDA/GroMM-1 binary system and to evaluate how incorporation of GroMM-1 affected the adjuvant activity of DDA liposomes.[174]



Scheme 30: (A) Molecular structure of dimethyldioctadecylammonium (DDA), (B) Synthesis of GroMM: (i) NaH, dry xylene, reflux, 4 h; (ii) $NaBH_4$, $CHCl_3$–MeOH, 1 h; (iii) 5% KOH, EtOH, 75 °C, 3.5 h; (iv) DCC, HOAt, DMAP, dry CH_2Cl_2, room temperature, 16 h; (v) TFA–THF–H_2O (8:17:3), room temperature, 3.5 h. MMG-1 consists of two diastereomers with (2R, 3S, 20R)- and (2S, 3R, 20R) configuration, respectively.[174]

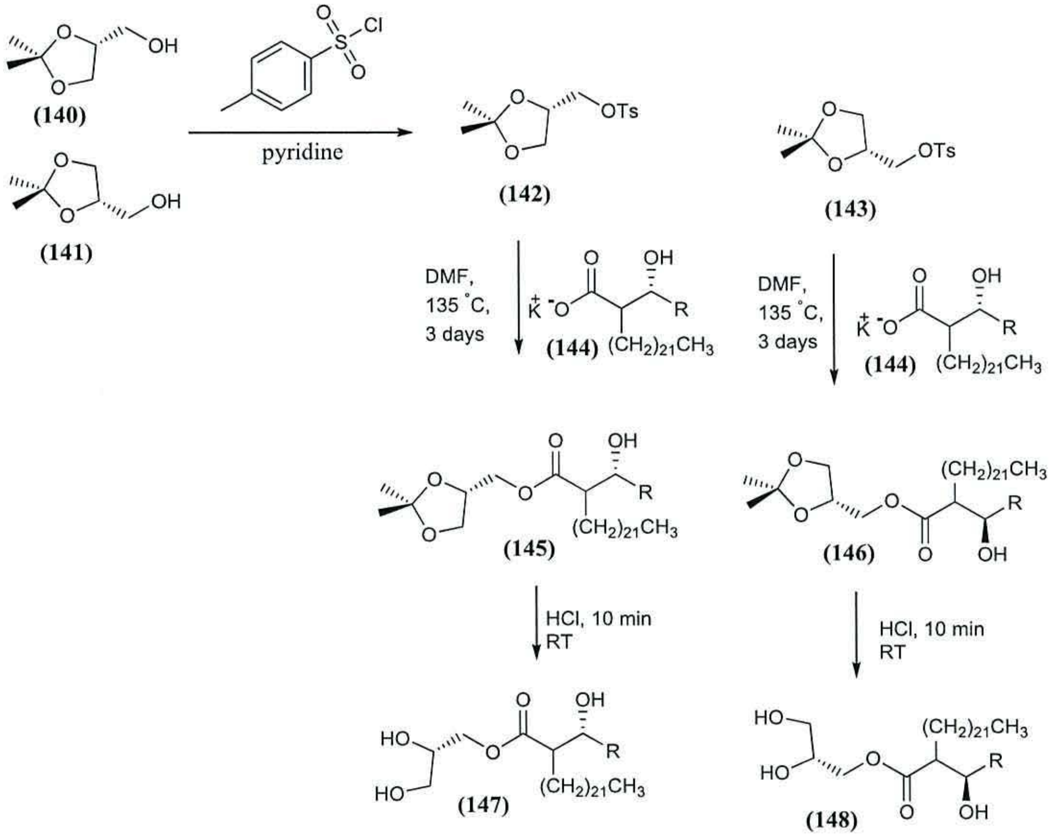
In addition, the isomeric C_{32} corynomycolate esters of glycerol **(139a – 139d)** were synthesised by the method of Datta *et al.* (**Scheme 31**).[175] In this method, a protected glycerol was coupled to the C_{32} corynomycolate silyl-protected motif using dicyclohexylcarbodiimide (DCC) and 4-pyrrolidinopyridine (PYP). Flash column chromatography of the mixture gave **(138a – 138d)**. Trifluoroacetic acid, tetrahydrofuran and water 8:17:3 was used for deprotection, giving *erythro* and *threo* diastereoisomers of glycerol coupled products **(139a – 139d) (Scheme 31).**[175]



Scheme 31: Synthesis isomeric C_{32} corynomycolate esters of glycerol

1.17.4 The first synthesis of GroMM with a natural mycolic acid mixture

Martine *et al.* synthesised GroMM with a natural mycolic acid mixture using mycolates isolated from *M. tuberculosis* H37Rv.[167] The synthesis started using (*R*)-isopropylidene-glycerol **(140)** and (*S*)-isopropylidene-glycerol **(141)**, activated by *p*-toluene-sulfonyl to give **(142)** or **(143)** **(Figure 16)**.[176] The esterification of glycerol by mycolic acids was realized by heating **(142)** or **(143)** in the presence of potassium mycolates **(109)**.[167] Both isopropylidene-mycoloyl-glycerol isomers were then deprotected under acidic conditions giving synthetic (*R*) or (*S*)-GroMM **(147)** and **(148)** respectively (**Scheme 32**).



Scheme 32: The first synthesis of GroMM with natural mycolic acid, [166, 167]*Martine et al concluded that GroMM synthesised by using mycolates isolated from M. tuberculosis H37Rv is composed of the two stereoisomers in equal proportions. Using (R) - or (S) - GroMM to stimulate T cells, they found that the R isomer was more active than the S one.*[167]

1.17.5 The biological properties of GroMM.

Glycerol esters of complex mixtures of natural mycolic acids have strong effects in the immune system.[177] Many mycobacterial components are proven to be strong adjuvants. The identification of GroMM lipid species formed by mycobacteria infected hosts, as well as the analysis of the host response directed toward them, will provide important new insights into host-microbe interactions intuberculosis. These latent mycobacteria are unlikely to produce cell wall lipids at a level comparable with that for actively replicating microbes, but a recent study has identified GroMM as a mycobacterial lipid species potentially associated with latent infection.[178]

GroMM was presented by *M. tuberculosis*-infected dendritic cells, demonstrating that the antigen is available for presentation during natural infection WHO published the results of BCG vaccine which showed that GroMM stimulated T cells from vaccinated or latently infected healthy donors but not cells from patients with active tuberculosis, suggesting that GroMM-specific T cells are primed during infection and their detection correlates with lack of clinical active disease.[179] The policies regarding this vaccine stated that several factors must be considered including the environmental and immunological characteristics of the population studied.[180]

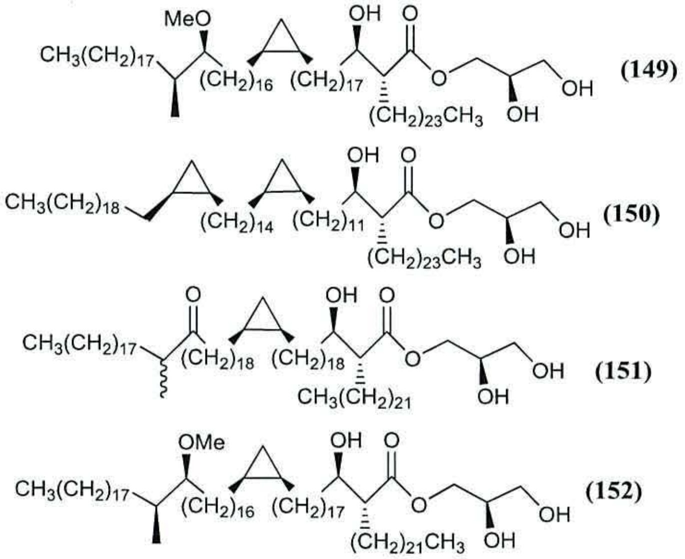
Mycobacteria have long been known to exert a number of immunomodulatory effects and are a good source of adjuvant preparations. However; in order to induce a sufficient protective immune response *Mycobacterium tuberculosis* requires a cell-mediated immune response, and a suitable adjuvant for a subunit vaccine directed against TB.[175] TB in man has been struggled by vaccination with reduced strain of *Mycobacterium bovis*. The *M. bovis* BCG vaccine developed more than decades ago. However; it does not always provide satisfactory resistance in every population. Bhowruth *et al.* separated a lipid extract of *M. bovis* BCG Copenhagen into its components, showing four lipids fractions. One of those fractions was a C_{70-90} GroMM; this component induced high levels of IL-12 (250 pico gram/ml) and TNF-α (3500 pico gram/ml).

GroMM has been implicated as a specific immune target in human individuals with latent, but not active, tuberculosis, but the in *vivo* response to GroMM and the relevance of it to latent infection remain poorly understood. Hattori *et al.* immunized guinea pigs with bacillus Calmette–Guerin (BCG) expressing high levels of GroMM and then, monitored skin reactions at the site of challenge with GroMM-containing liposome.[177a] GroMM has been observed to have adjuvant activity in murine models,[181, 182] with the fine structure of the mycolate components being of importance for its proinflammatory activity.[183] The synthesis of GroMM using single synthetic mycolic acids will result in a rich knowledge of the nature of the cell wall of *M. tuberculosis,* and hence give a better understanding of their effects in the immune system.[177] The identification of the simple GroMM lipid, play an essential role in directing the immune response upon infection with pathogens, such as *Mycobacterium tuberculosis*. This activity was shown by a synthetic analogue of GroMM with shorter fatty acids.[177] The development of such a novel class of synthetic lipids is suitable for vaccines for use in humans.

2. Results and Discussion

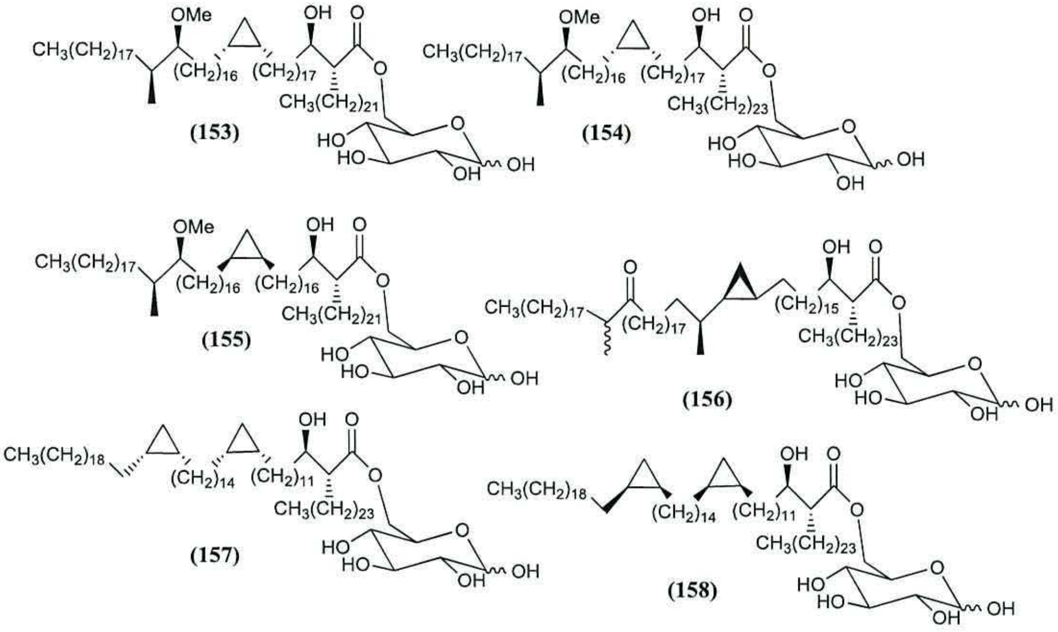
2.1 Aims of the project

This project consists of three parts. The aim of first part was to synthesise GroMM, using single synthetic mycolic acids. The glycerol esters of fatty acids synthesised were **(149)** - **(152)** (**Scheme 33**).



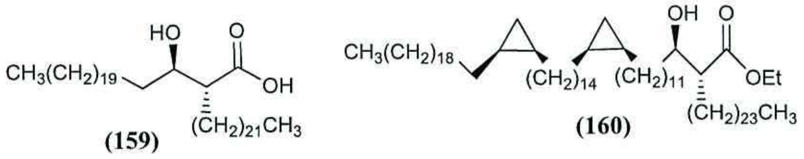
Scheme 33: The glycerol monomycolates synthesised

The second part was to synthesise GMM, again with single synthetic mycolic acids. This part consisted of the synthesis of glucose monomycolates **(153)** - **(158)** as shown in **Scheme 34**.



Scheme 34: The glucose monomycolates synthesised

The third aim was to develop a new approach the α-alkyl-β-hydroxy ester unit of mycolic acids. This was then applied to the synthesis of mycolic acids (**159**) and (**160**) (**Scheme 35**).



Scheme 35: The mycolic acids (159) and (160) synthesised using the new method

2.2 Synthesis of glycerol esters

The methods used to prepare the glycerol esters are shown in **Figure 18**.

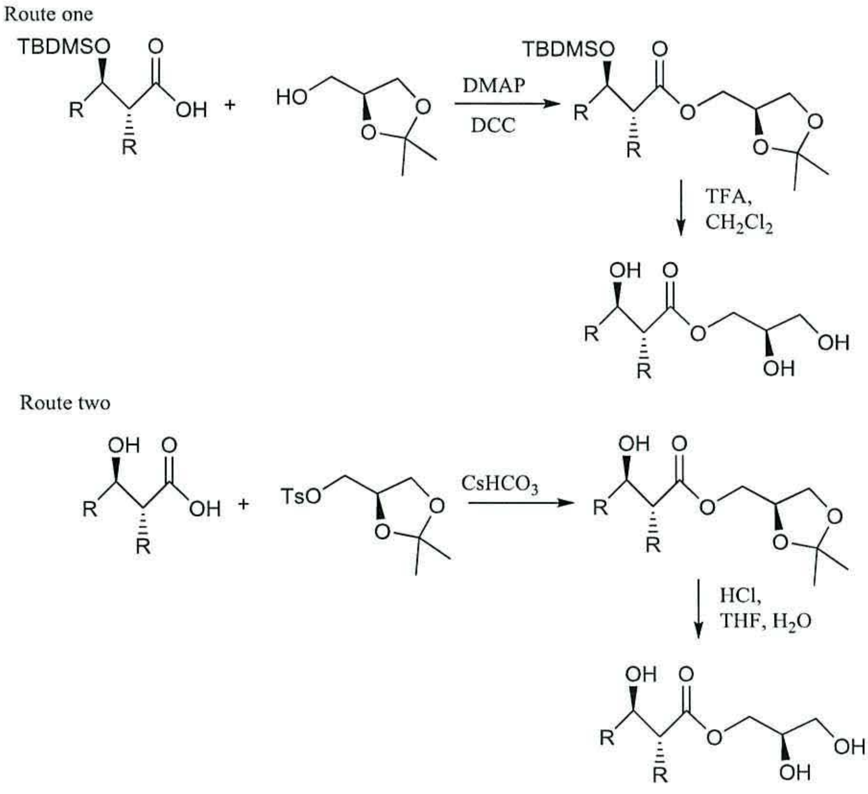
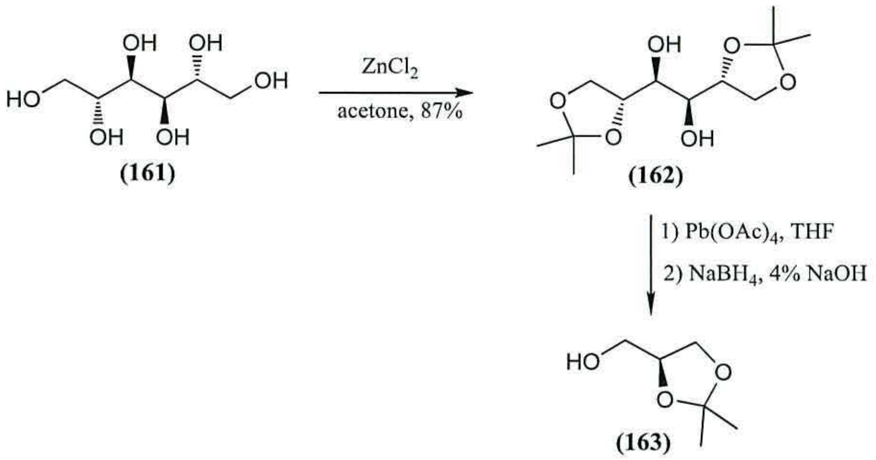


Figure 18: The proposed preparation of glycerol ester [184]

2.3.1 Route one

2.3.1.1 Preparation of the (*S*)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanol unit

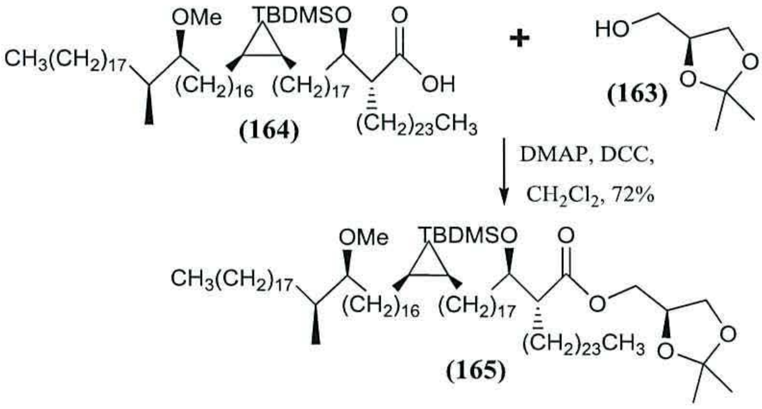
D-Mannitol (**161**) was protected using acetone in the presence of anhydrous zinc chloride to give the diol (**162**) in 87% yield.[184, 185] The diol (**162**) was cleaved with dry lead tetra-acetate in dry THF at 10 °C. The mixture was stirred for 30 min in an ice bath and then a solution of $NaBH_4$ in 4% NaOH was added to give alcohol (**163**) in 96% yield, which gave spectra identical to those in the literature (**Scheme 36**).[184]



Scheme 36: Preparation of the (S)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanol[184]

2.3.1.2 Synthesis of a *cis*-cyclopropane methoxy mycolic acid glycerol ester with a C-23 α-chain

The protected methoxy mycolic acid **(164)**,[188] supplied by Dr. Al Dulayymi, was coupled with alcohol **(163)** using DMAP and DCC to give the protected mycolic acid glycerol ester **(165)** (**Scheme 37**).

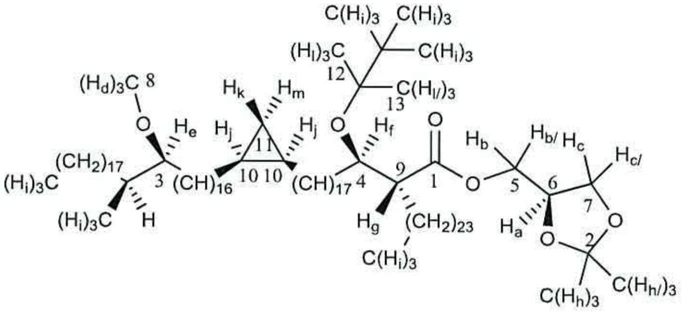


Scheme 37: Coupling reaction to synthesise cis-cyclopropane methoxymycolic acid glycerol ester

The ^{1}H and ^{13}C NMR data for the protected *cis*-cyclopropane methoxymycolic acid glycerol ester **(165)** are given in **Table 2**. The MALDI of **(165)** also confirmed that the coupling of the two compounds **(163)** and **(164)** was successful as the value obtained was $(M+Na)^+$: 1505.5, which is in agreement with the expected mass for

(165) ($C_{97}H_{192}NaO_6Si$ requires: 1504.4). In addition the specific rotation of **(165)** was $[\alpha]_D^{24}$ - 6.7 (*c* 1.4, $CHCl_3$).

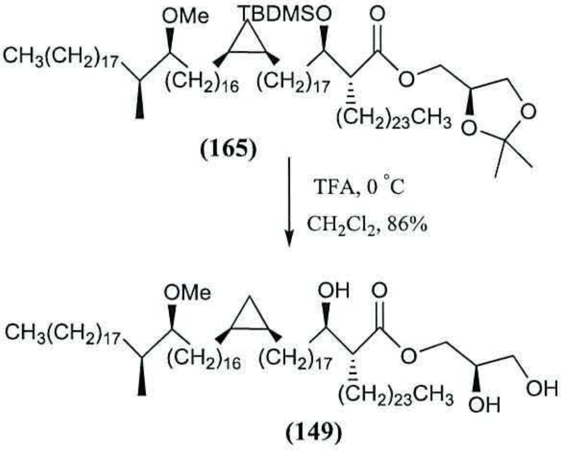
Table 2: Selected ^1H and ^{13}C NMR data of (165)



Proton	δ	Multiplicity	*J* (Hz)	Carbon	δ
H_a	4.33–4.26	m		1	174.7
H_b	4.11	dd	2.4, 5.4	2	109.8
Hc	4.06	dd	6.4, 8.4	3	85.6
$H_{b/}$	3.94–3.87	m		4	73.7
$H_{c/}$	3.75	dd	6.0, 8.4	5	66.8
CH_{3d}	3.34	s		6	64.4
H_e	3.24–3.15	m		7	57.9
H_f	2.98–2.92	m		8	55.9
H_g	2.55	ddd	3.8, 6.9, 10.8	9	51.6
CH_{3h}	1.43	s		10	18.1
$CH_{3h/}$	1.36	s		11	11.1
CH_{3i}	0.91–0.83	m		12	-4.2
H_j	0.69–0.61	m		13	-4.7
H_k	0.56	dt	3.7, 7.6		
CH_{3l}	0.04	s			
$CH_{3l/}$	0.01	s			
H_k	-0.34	q	5.2		

2.3.2.3 Deprotection of (165)

Deprotection of the *tert*-butyldimethylsilyl group and dioxolanyl groups of (**165**) was achieved using trifluoroacetic acid in dichloromethane, to give the *cis*-cyclopropane methoxymycolic acid glycerol ester (**149**) (**Scheme 38**).



Scheme 38: Deprotection of cis-cyclopropane methoxymycolic acid glycerol ester (165)

The successful deprotection was confirmed by the ^{1}H NMR spectrum of the product which showed that all signals for the protecting group had disappeared. The ^{1}H NMR spectrum of ester (**149**) showed five signals in region at δ 4.50-3.60 belonging to the glycerol ester (**Figure 19a**). The signals between δ 0.66 to-0.34 corresponded to the four protons of the *cis*-cyclopropane (**Figure 19c**) and those at δ 3.77-2.70 included a three hydrogen singlet at δ 3.48 for the methoxy group, and the singlet at δ 3.19 for the β-proton and δ 2.75 for the α proton (**Figure 19b**). The spectrum showed two doublets of doublets at δ 4.25 (*J* 3.3, 9.9 Hz) and 4.12 (*J* 4.3, 9.9 Hz) respectively for CH_2 adjacent to the carboxylic group, a multiplet at δ 4.76 belonged to the CH on the glycerol group, while the CH_2 on the glycerol group appeared as two doublets of doublets at δ 3.47 (*J* 3.3, 9.9 Hz) and 3.40 (*J* 4.3, 9.9 Hz) respectively. The MALDI of (**149**) was found to be correct, $(M+Na)^+$: 1350.4 for $C_{88}H_{174}O_6Na$ (requires: 1350.3). The specific rotation of (**149**) was $[\alpha]_D^{23}$ – 8.4 (*c* 1.1, $CHCl_3$).

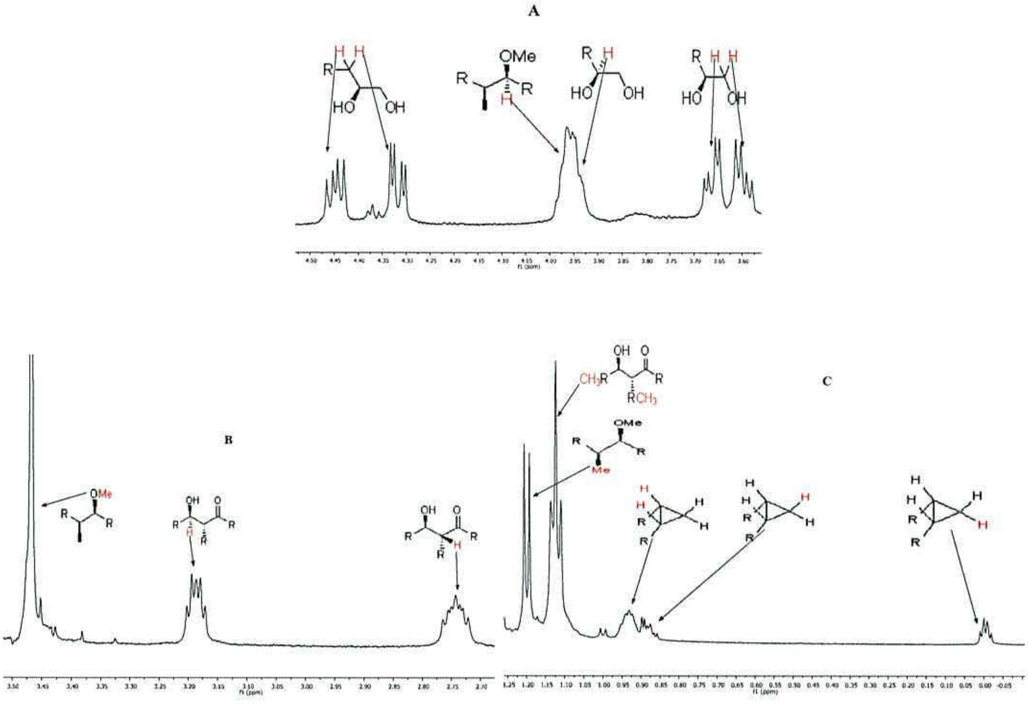


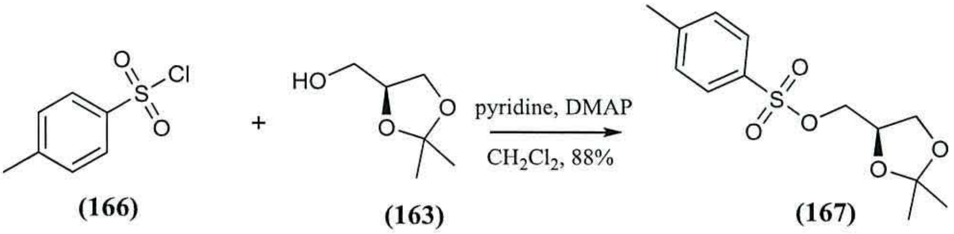
Figure 19: Selected ^1H NMR data for ester (149)

2.4 Route two

This method avoids the need to protect the hydroxyl group of the mycolic acid.

2.4.1 (*R*)-(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl 4-methyl-benzenesulfonate

For the synthesis of glycerol monomycolate from unprotected mycolic acid, it was necessary to convert the hydroxyl group in (**163**) into a good leaving group such as to *p*-toluenesulfonate (tosylate). The alcohol (**163**) was dissolved in THF and tosyl chloride was added in the presence of pyridine and DMAP at – 0 °C. The mixture was stirred at room temperature for 18 h to give (**167**) in 88% yield (**Scheme 39**).



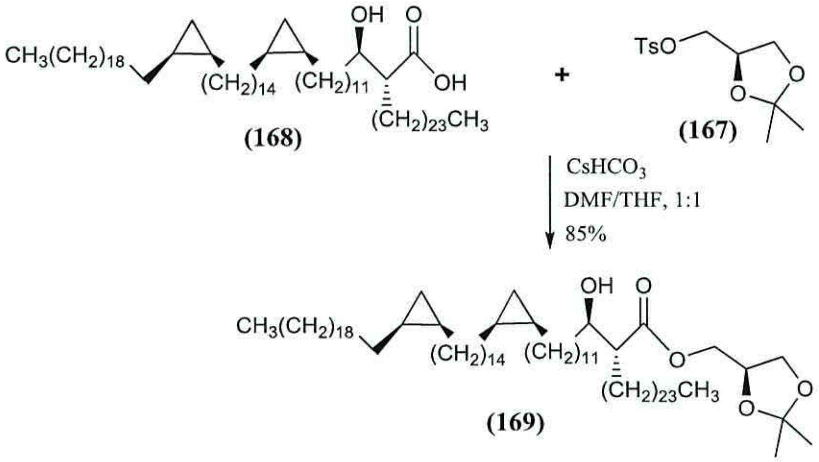
Scheme 39: Preparation of (R)-(2,2-dimethyl-1,3-dioxolan-4-yl)methyl 4-methylbenzenesulfonate

Compound (**167**) gave spectra identical to those in the literature. The optical rotation of this tosylate was determined to be $[\alpha]_D^{23} - 12$ (*c* 0.9, $CHCl_3$) (*lit.*[184] $[\alpha]_D^{24} - 8.8$).

2.4.2 Synthesis of α-mycolic acid-glycerol ester

2.4.2.1 The coupling reaction

Reaction of the *cis*-cyclopropane *α*-mycolic acid (**168**),[189] supplied by Dr. Al Dulayymi, with tosylate (**167**), using $CsHCO_3$ in dry DMF/THF, gave the protected *cis*-cyclopropane α-mycolic acid glycerol ester (**169**) in 89% yield (**Scheme 40**).



Scheme 40: The coupling reaction

The ^1H NMR spectrum of ester (**169**) showed a multiplet at δ 4.23 which belongs to the CH on the dioxolanyl group, two doublets of doublets at δ 4.10 (*J* 3.3, 9.9 Hz) and 4.06 (*J* 4.3, 9.9 Hz) respectively for CH_2 adjacent to the carboxylic group, while the CH_2 on the dioxolanyl group appeared as two doublets of doublets at δ 3.97 (*J* 3.3, 9.9 Hz) and 3.65 (*J* 4.3, 9.9 Hz). More data of the protected *cis*-cyclopropane α-mycolic acid glycerol ester (**169**) can be seen in **Figure 20** and **Table 3**. The MALDI mass spectrum of the protected *cis*-cyclopropane α-mycolic acid glycerol ester (**169**) gave an ion at ($M+Na^+$): 1274.2 [$C_{84}H_{162}O_5Na$ requires: 1274.2]. The specific rotation was $[\alpha]_D^{20}$ - 4.7 (*c* 1.2, $CHCl_3$).

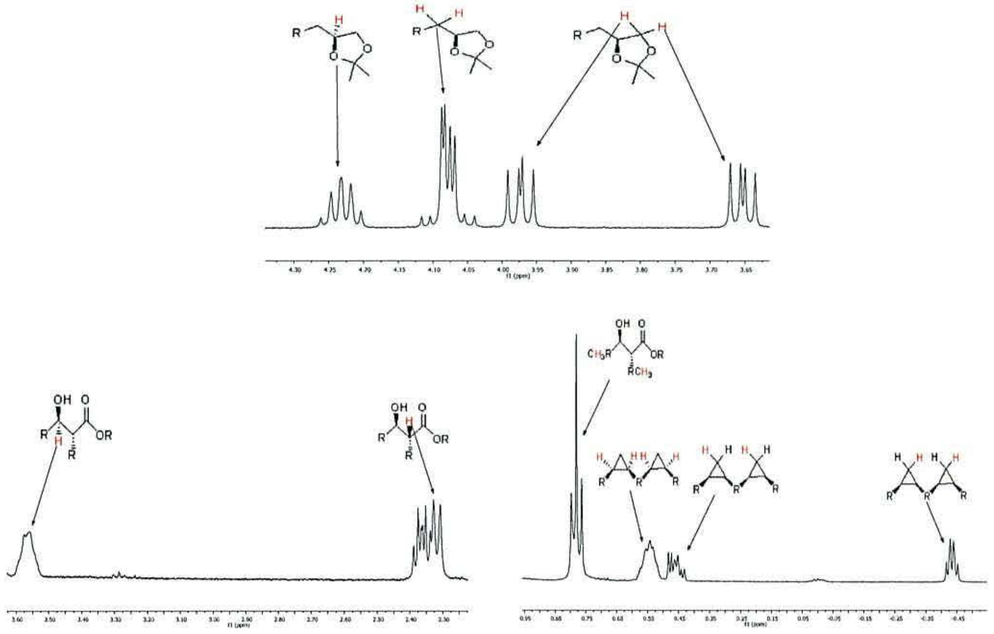
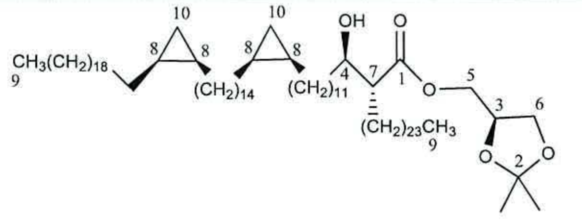


*Figure 20: *

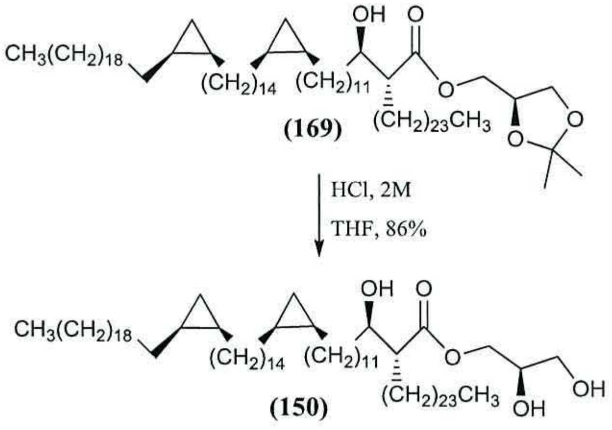
Figure 20: ^{1}H NMR data for protected cis-cyclopropane α-mycolic acid glycerol ester (169)

Table 3: ^{13}C NMR data of protected cis-cyclopropane α mycolic acid glycerol e ster (169)

	
Carbon	δ
1	175.3
2	109.8
3	73.7
4	72.3
5	66.3
6	64.4
7	51.3
8	15.7
9	14.1
10	10.9

2.4.2.2 The hydrolysis reaction

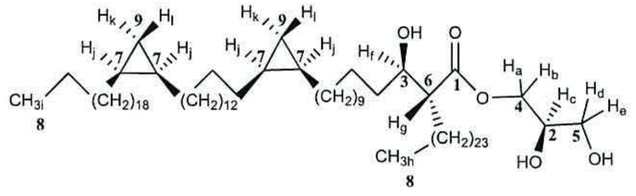
The hydrolysis of the *cis*-cyclopropane α-mycolic acid glycerol ester (**169**) is very important because it might hydrolyse the ester group in addition to the dioxolanyl group. The dioxolanyl group was deprotected using hydrochloric acid in THF and water at 45 °C for 18 h to give a semi-solid (**150**) (**Scheme 41**) in 86% yield.



Scheme 41: The hydrolysis reaction

The ^{1}H NMR spectrum of ester (**150**) showed two doublets of doublets at δ 4.10, (*J* 3.3, 9.9 Hz) and 4.06 (*J* 4.3, 9.9 Hz) for CH_2 adjacent to the carboxylic group, a multiplet at δ 4.23 belongs to the CH on the glycerol group, while the CH_2 on the glycerol group appeared as two doublets of doublets at δ 3.97 (*J* 3.3, 9.9 Hz) and 3.65 (*J* 4.3, 9.9 Hz). More data of ester (**150**) was analysed as shown in **Table 4**. The MALDI of the final *cis*-cyclopropane α-mycolic acid glycerol ester (**150**) was found to be correct, $(M+Na)^+$: 1234.9 for $C_{81}H_{158}NaO_5$ (requires: 1234.2). In addition the specific rotation of (**150**) was $[\alpha]_D^{22} - 6.5$ (*c* 0.9, $CHCl_3$).

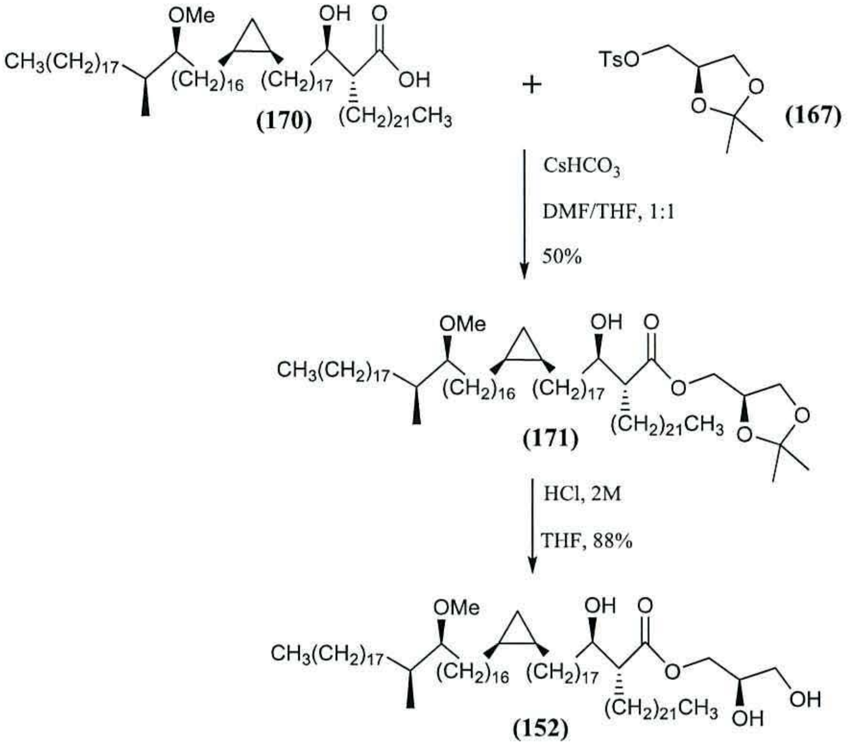
Table 4: Selected ^1H and ^{13}C NMR of cis-cyclopropane α-mycolic acid glycerol ester (150)



Proton	δ	Multiplicity	*J* (Hz)	Carbon	δ
H_a, H_b	4.30 – 4.26	m		1	175.6
H_c	4.01 – 3.95	m		2	73.1
H_d, H_e, H_f	3.76 – 3.61	m		3	70.1
H_g	2.54 – 2.44	m		4	65.4
$CH_{3h,i}$	0.90	t	6.8	5	63.4
CH_{3j}	0.71 – 0.63	m		6	52.3
CH_{3k}	0.56	dt	3.7, 7.6	7	15.9
CH_{3l}	-0.31	q	5.2	8	14.3
		m		9	11.1

2.4.3 Synthesis of a *cis*-cyclopropane methoxy mycolic acid glycerol ester with a C-21 α-chain

The *cis*-cyclopropane methoxy mycolic acid glycerol ester with C 21 α-chain (**152**) was prepared by a similar method to that used to prepare the α-mycolic acid glycerol ester (**150**). The target was obtained by coupling of the *cis*-cyclopropane methoxy mycolic acid (**170**), supplied by Dr. Al Dulayymi, with tosylate (**167**) using $CsHCO_3$ to give (**171**) in 50% yield (**Scheme 42**).

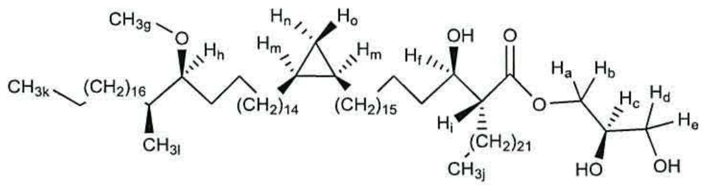


Scheme42: Synthesis of cis-cyclopropane methoxy mycolic acid glycerol ester (152)

The protected *cis*-cyclopropane methoxy mycolic acid glycerol ester (**171**) was hydrolysed using hydrochloric acid in THF and water to give a semi-solid (**152**) (**Scheme 6**) in 88% yield. The ^{1}H NMR spectrum of ester (**152**) showed five signals in region at δ 4.40-3.60, a multiplet at δ 4.26 belongs to the CH_2 adjacent to the carboxylic group, another at δ 3.95 to the CH on the glycerol group, a doublet of doublets at δ 3.62 (*J* 5.5, 11.4 Hz) to one proton of the CH_2 of the glycerol; the second proton appeared as a multiplet at δ 3.70. The signals between δ 0.66 to -0.34 correspond to the four protons of the *cis*-cyclopropane ring, a multiplet at δ 3.48 for the methoxy group, and the singlet at δ 3.19 for the β proton and δ 2.46 for the α-proton; more NMR data are shown in **Table 5**.

Table 5: Selected ^1H and ^{13}C NMR data of cis-cyclopropane methoxy mycolic acid glycerol ester

(152)

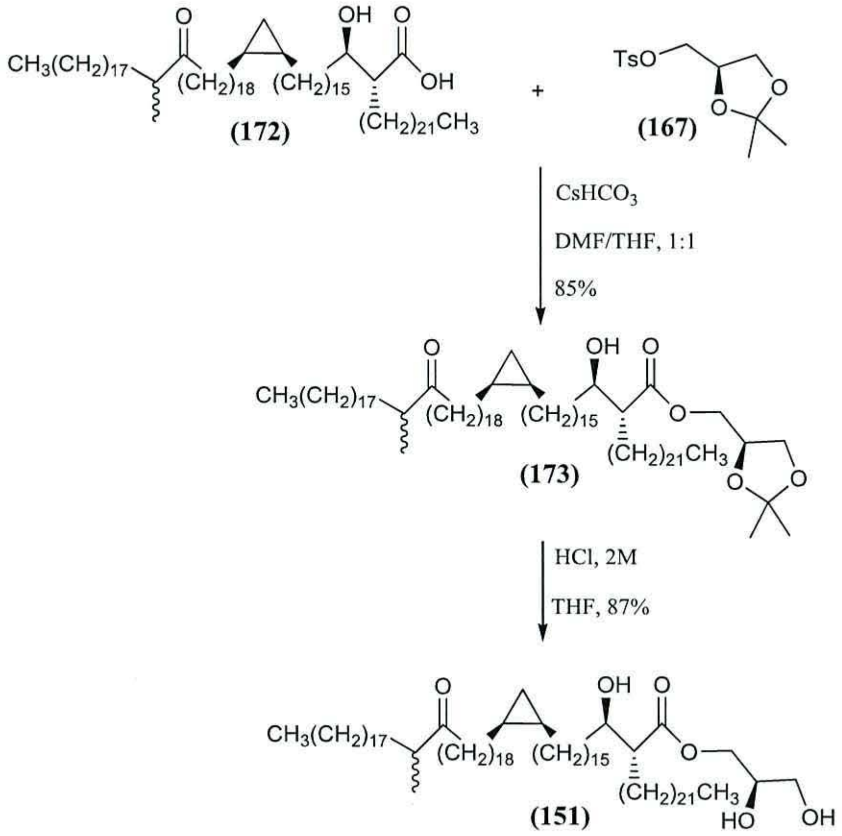


Proton	δ	Multiplicity	*J* (Hz)	Carbon	δ
H_a, H_b	4.30 – 4.22	m		1	175.6
H_c	3.99 – 3.92	m		2	85.6
H_d, H_f	3.74 – 3.67	m		3	73.1
H_e	3.62	ddd	5.5, 11.4	4	70.1
CH_{3g}	3.34	s		5	65.4
H_h	2.98 – 2.92	m		6	63.4
H_i	2.50 – 2.42	m		7	57.9
$CH_{3j,\ k}$	0.88	t	6.9	8	52.3
CH_{3l}	0.84	d	6.9	9	15.9
H_m	0.69 – 0.60	m		10	15.0
H_n	0.55	dt	3.9, 8.4	11	14.3
H_o	-0.34	q	5.1	12	11.1

2.4.4 Synthesis of cis-cyclopropane ketomycolic acid glycerol ester

The *cis*-cyclopropane ketomycolic acid glycerol ester (**151**) was prepared by a similar method to that used to prepare the α-mycolic acid glycerol ester. The target was again obtained by coupling of the *cis*-cyclopropane ketomycolic acid (**172**), supplied by Dr.

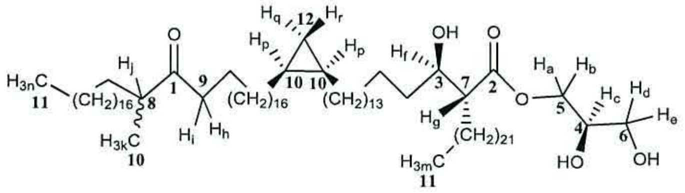
Al Dulayymi with tosylate (**167**) using $CsHCO_3$ to give (**173**) in 85% yield (**Scheme 43**).



Scheme 43: Synthesis of cis-cyclopropane ket mycolic acid glycerol ester (151)

The ester (**173**) was hydrolysed using hydrochloric acid in THF and water to give a semi-solid (**151**) (**Scheme 43**) in 87% yield. The ^1H NMR spectrum of ester (**151**) showed two doublets of doublets at δ 4.30 (*J* 4.1, 11.8 Hz) and 4.25 (*J* 6.7, 11.8 Hz) for CH_2 adjacent to the carboxylic group. A multiplet at δ 3.98 belongs to the CH on the glycerol group, a doublet of doublets at δ 3.65 (*J* 4.6, 10.5 Hz) belongs to one proton of CH_2 on the glycerol group, the second proton appeared as a multiplet at δ 3.73; more data of ester (**151**) was analysed as shown in **Table 6**.

Table 6: Selected ^1H and ^{13}C NMR data of cis-cyclopropane keto- mycolic acid glycerol ester (151)



Proton	δ	Multiplicity	*J* (Hz)	Carbon	δ
Ha,	4.30	dd	4.1, 11.8	1	215.3
Hb	4.25	dd	6.7, 11.8	2	175.4
H_c	4.03 – 3.94	m		3	72.9
$H_{d,\,f}$	3.77 – 3.70	m		4	69.7
H_e	3.65	dd	4.6, 10.5	5	65.2
$H_{g,\,h,\,i,\,j}$	2.57 – 2.39	m		6	63.3
$CH3_k$	1.07	d	6.9	7	52.2
$CH3_{m,\,n}$	0.90	t	6.8	8	46.3
H_p	0.66	m		9	41.1
H_q	0.58	dt	3.7, 7.8	10	16.3
H_r	-0.34	q	5.2	11	15.8
				12	14.1
				13	10.9

In addition the molecular rotation for GroMM was measured by converting the specific rotation into a molecular rotation, M_D. M_D is calculated from the measured specific rotation (α_D) and the molecular weight of the sample.[186, 187]

Where:

$$M_D = \alpha_D \times (\text{Mol. Wt.}/100)$$

However; this method is not accurate in small molecules as the effect on molecular rotation will be considerably different to the complete compound. Because of the chiral centre in these compounds is present in the centre of two very long chains, the molecular rotation for a particular absolute stereochemistry is largely independent of the chain length and so may provide an incremental value that can be used to predict M_D, and therefore, α_D for a molecule containing several such chiral groups.

Baird *et al.*, was calculated the optical rotation for **(164a)** and **(168)** and found - 1.1 and + 2.0 respectively.[188, 189] In addition Baird's group calculated the optical rotations for **(170)** and **(172)** at - 1.1 and + 4.1 respectively. In addition the optical rotation for synthesized GroMM **(149)** - **(152)** were measured in this work.The molecular rotation of the synthesized GroMM **(149)** - **(152)** and the mycolic acids **(164a)**, **(168)**, **(170)**, and **(172)** were calculated using the above equation as explained in **Table 7**.

Table 7: The optical and the molecular rotation for the mycolic acids and the synthesized GroMMs (149) - (152)

MAs. No	α_D	M_D	GroMMs.No	α_D	M_D	M_D Differences
(164a)	- 1.1	- 14	**(149)**	- 8.4	- 116	- 102
(168)	+ 2.0	+ 23	**(150)**	- 6.5	- 79	- 102
(170)	- 1.1	- 14	**(151)**	- 9.3	- 123	- 109
(172)	+ 4.9	+ 61	**(152)**	- 12	- 159	- 220

It is clear from **Table 7** that the molecular rotation values of the GroMM **(149)** - **(152)** have higher negative values than the molecular rotation values of the mycolic acids **(164a)**, **(168)**, **(170)** and **(172)**, and all the differences between GroMMs and the mycolic acids molecular rotation are negative values. This effect accounts for the effect of the added glycerol molecule of these mycolic acids. It is apparent thast the differences between the M_D values of the GroMM **(149)** - **(151)** with their parent mycolic acid have changed by approximately the same magnitude between - 102 and -109. The difference for the GroMM **(152)** is of a much higher magnitude than that of the others which might be due to the fact that the keto-mycolic acid **(172)** used in this synthesis was a mixture of diastereoisomers.

2.4.5 Biological data for synthetic GroMM

Many mycobacterial components are demonstrated to be strong adjuvants. As an example, natural TDM is able to stimulate the immune system so it will produce a range of chemokines (MCP-1, IL-8) and cytokines (IFN-γ, TNF-α, IL-12, IL-10, IL-4).[190]

Another component which induced high levels of IL-12 (250 pico gram/ml), TNF-α (3500 pico gram/ml), and IL-6 (5000 pico gram/ml) is the lipid extract of *M. bovis* BCG Copenhagen. This lipid extract was separated into its four lipids components and one of those lipids was a C_{70-90} GroMM. This component is proven to be strong adjuvant.[191]

On other hand Bhowruth *et al* synthesised and tested the immune activity of a simplified version of C_{32} GroMM compounds (**Figure 15, page 56**). The targeted molecule of GroMM was identified by ELISA assays and a variety of cytokines and chemokines were tested as IFN-γ and IL-6 signals. The results of this study strongly support the use of synthetic C_{32} GroMM as a potent adjuvant with excellent potential. This study also emphasises the importance of optimization of adjuvant dose or adjuvant ratio in particulate delivery platforms containing co-adjuvants. These results also show the potential for dimethyl dioctadecyl ammonium bromide (DDA) vesicles as a platform for the delivery of lipid based co-adjuvants.[192]

In this thesis, biological assays were carried out in Brussels to study the recognition of the GroMMs **(149)** - **(152)** (**Figure 21**). The TNF-α signal was measured for these synthetic GroMMs by stimulating mouse RAW 264.7 cells. The selected GroMMs samples were suspended at a concentration of 10μg/well of each compound in isopropanol and sonicated in a bath sonicator for 5 min. The suspension was incubated at 60 °C for 10 min. and sonication repeated. The resulting solution was layered onto tissue culture in 24-well cultures cell plates at the indicated concentrations and incubated at 37 °C. TNF-α production was measured for these synthetic GroMMs and compared to that of a commercial sample of *M. tuberculosis* TDM (Commercial mix), control, synthetic TDM, and finally Zymosan and Curdan (which are insoluble largely polysaccharide fractions of yeast cell walls and induce the experimental sterile inflammation cytokines). The results indicated a significant differences in mean as compared to the standards.

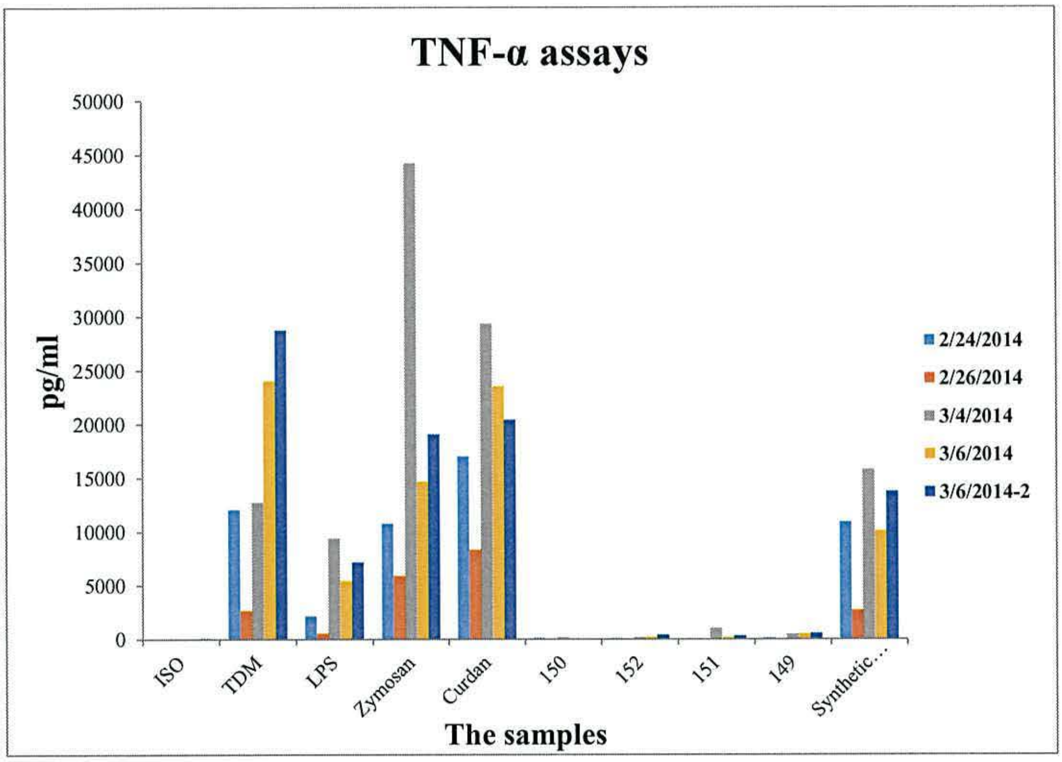


Figure21: TNF-α assays for TDM (Commercial mix), control, synthetic TDM, finally Zymosan and Curdan compare to the synthetic GroMM (149) - (152)

The data show a clear increase in cytokines TNF-α production for commercial mix TDM samples, Zymosan and Curdan compared with the control. The Commercial mix TDM samples and Curdan were proved to induce similar levels of cytokines, but results were significantly higher when dealing with Zymosan, On the other hand the results of the synthetic GroMM (**149**) - (**152**) were very low; this was not expected and will be the target for the future work to increase the level of TNF-α production. In addition the low results of the synthetic GroMM (**149**) - (**152**) might belong to the wrong selection of cytokines TNF-α production because the cytokines INF-γ production preferred as a signal for GroMMs than the cytokines TNF-α signals.[192]

Another possible reason might be the cause for the lack of activity belonged to the stereochemistry of the chiral centre of the hydroxyl group in Glycerol unit. In addition the choice of the different mycolic acids might be another possible reason to give better results.

2.5 Synthesis of glucose esters

The synthesis of glucose monomycolates (GMM) was achieved in the same way as discussed previously (**Figure 22**).[193]

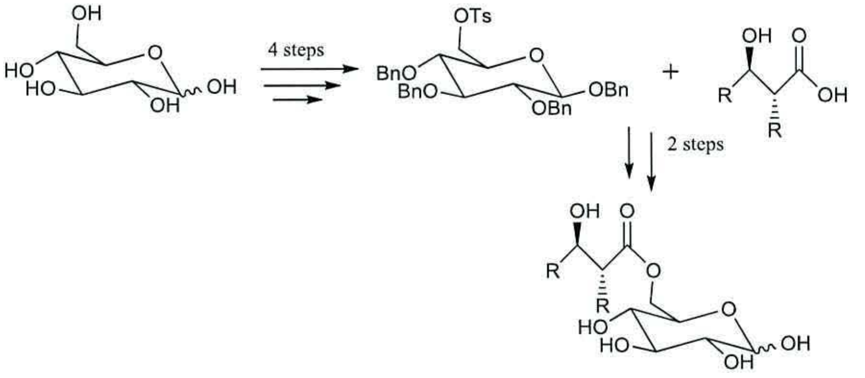
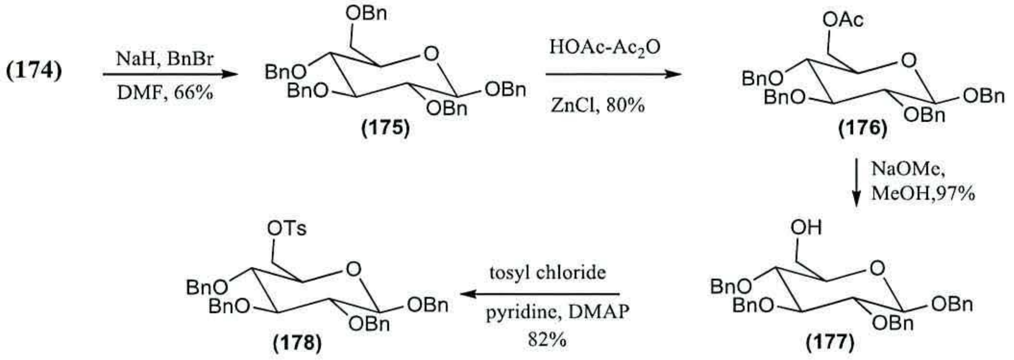


Figure 22: The proposed preparation of glucose monomycolates.[193]

2.5.1 Preparation of ((2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5,6-tetrakis(benzyloxy)-tetrahydro-2H-pyran-2-yl)methyl 4-methylbenzenesulfonate

This was carried out by the literature method. *D*-Glucose was first protected as (**175**) employing an excess of NaH and BnBr in DMF in 66% yield.[194] Selective debenzylation–acetolysis of the benzyl protecting group at C-6 of (**175**) with $ZnCl_2$ in 1:1 HOAc–Ac_2O gave the intermediate (**176**). Deprotection of (**176**) using sodium methoxide and methanol led to the alcohol (**177**). Finally, the alcohol was tosylated with tosyl chloride and pyridine in the presence of DMAP, to yield the tosylate (**178**) in 82% yield (**Scheme 44**). The overall yield was 42%, while the overall yield reported by Lu *et al.* was 47%.[193]

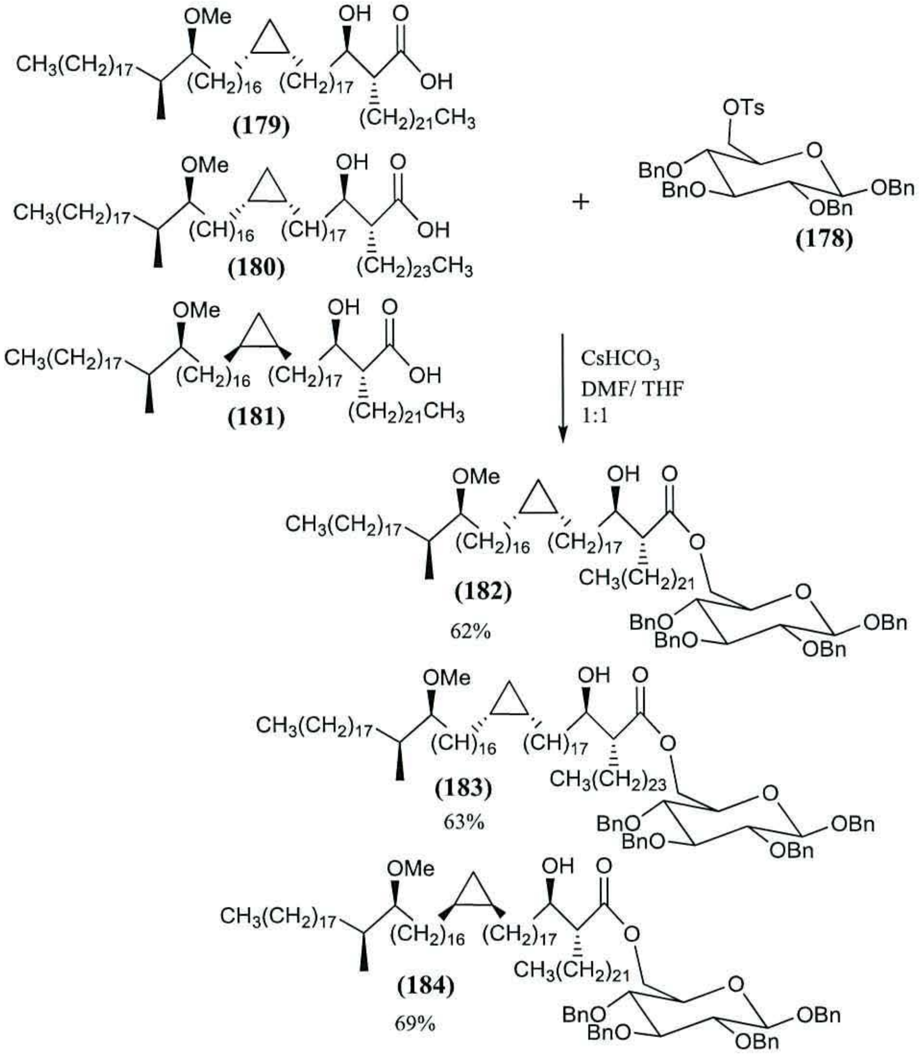


Scheme 44: Preparation of tosylate (178)

The ^{1}H and ^{13}C NMR spectra for (**178**) were the same as those reported in the literature and the specific rotation was -3.3 (c = 1.0, $CHCl_3$) whereas the literature value was -3.4 (c = 1.1, $CHCl_3$).[193]

2.5.2 Synthesis of *cis*-cyclopropane methoxy mycolic acid glucose ester

The synthesis of *cis*-cyclopropane methoxy mycolic acid glucose esters was undertaken with three types of methoxy mycolic acid **(179) - (181)**,[188] supplied by Dr. Al Dulayymi. The mycolic acids were coupled with tosylate (**178**) using $CsHCO_3$ in dry DMF/THF to form esters (**182**), (**183**), and (**184**) (**Scheme 45**).



Scheme 45: Coupling reactions to synthesise cis-cyclopropane methoxy mycolic acid glucose esters

The ^{1}H NMR spectra of compounds **(182)**, **(183)** and **(184)** showed a complex multiplet at δ 7.32 – 7.15 for the twenty aromatic protons. The four benzylic CH_2 resonances appeared between δ 4.90 – 4.50 ppm and gave a series of doublets at δ 4.88 (*J* 11.0 Hz), 4.87 (*J* 10.8 Hz), 4.84 (*J* 12.4 Hz), 4.81 (*J* 11.5 Hz), 4.71 (*J* 11.0 Hz), 4.63 (*J* 10.8 Hz), 4.56 (*J* 12.4 Hz) and 4.82 (*J* 11.5 Hz) ppm, while the α-proton in the β-anomeric compound (H1), and one proton from the CH_2 next to the ester group (H10) appeared as a multiplet at δ 4.44 – 4.48 ppm (**Figure 23**).

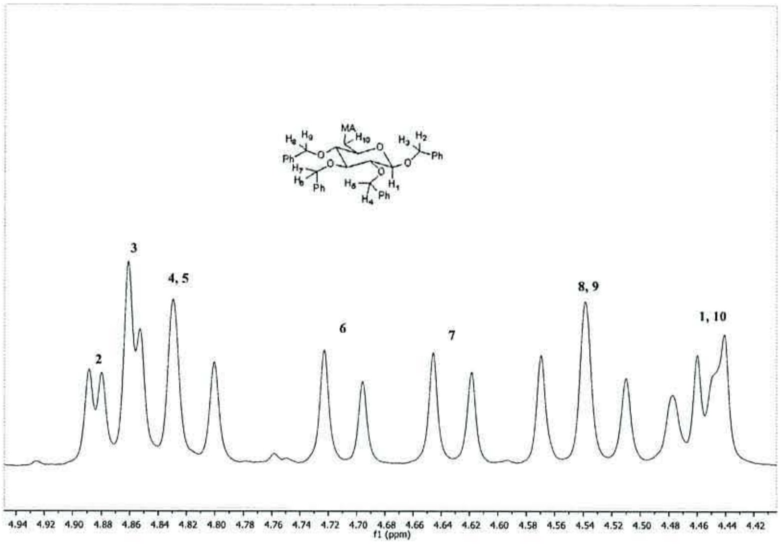
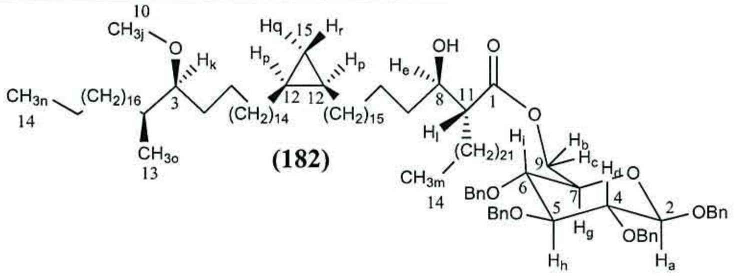


Figure 23: The CH_2 protons for the benzyl protecting group for (182)

In each case, the four protons of the *cis*-cyclopropane ring appeared between δ 0.65 to -0.41. The rest of the signals could be seen at δ 3.77-1.71, which included a three hydrogen singlet at δ 3.26 for the methoxy group; more ^{1}H NMR and ^{13}C NMR data can be seen in **Table 8**. The MALDI of (**182**), (**183**), and (**184**) also confirmed that the coupling of the three mycolic acids (**179**) - (**181**) and with tosylate **(178)** was successful as the values were obtained were ($M+Na^+$): 1770.8, 1798.9 and 1798.9, which was in agreement with the expected masses for of (**182**), (**183**), and (**184**). In addition the specific rotations of of (**182**), (**183**), and (**184**) were $[\alpha]_D^{22}$ + 14 (c 1.1, $CHCl_3$), $[\alpha]_D^{22}$ + 11 (c 1.3, $CHCl_3$), $[\alpha]_D^{24}$ + 16 (*c* 1.2, $CHCl_3$); more detail of the ^{1}H NMR and ^{13}C NMR data can be seen in **Table 8**.

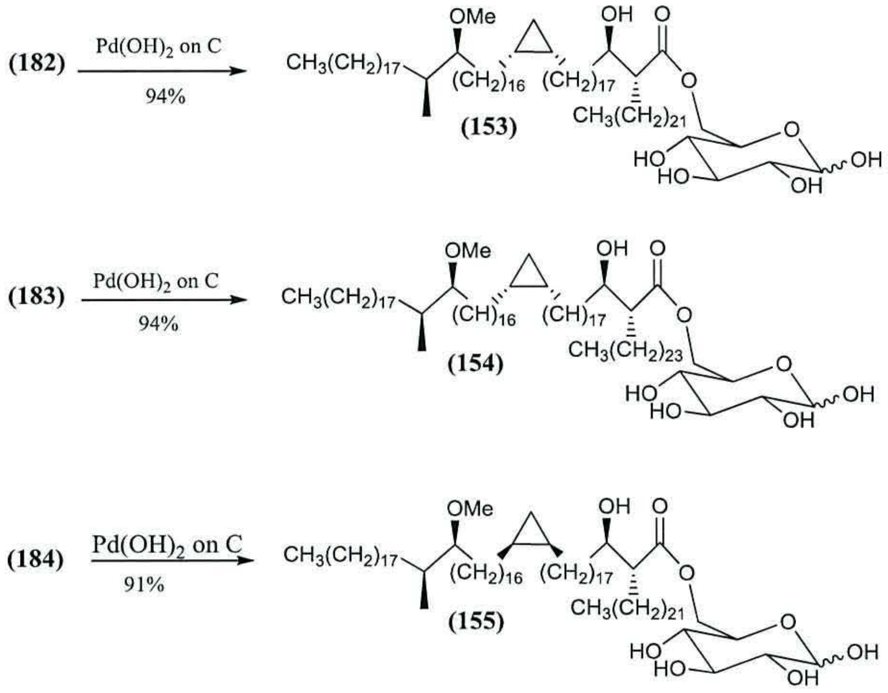
Table 8: Selected ^1H and ^{13}C NMR data for (182)



(182)

Proton	δ	Multiplicity	J (Hz)	Carbon	δ
H_a	4.88	d	3.6	1	175.4
H_b	4.49 – 4.43	m		2	102.5
H_c	4.14	dd	4.6, 11.7	3	85.6
$H_{d, e}$	3.65 – 3.53	m		4	84.7
$H_{g, h, i}$	3.48 – 3.38	m		5	82.4
$CH_{3\,j}$	3.26	s		6	78.0
H_k	2.92 – 2.85	m		7	73.0
H_l	2.44 – 2.33	m		8	72.5
$CH_{3m, n}$	0.81	t	6.8	9	63.0
CH_{3o}	0.77	d	6.9	10	57.9
H_p	0.65 – 0.52	m		11	51.5
H_q	0.48	dt	3.8, 7.6	12	15.9
Hr	-0.41	q	5.2	13	15.0
				14	14.3
				15	11.1

Compounds **(182)**, **(183)**, and **(184)** were debenzylated using hydrogen and palladium hydroxide on charcoal as a catalyst in CH_2Cl_2/CH_3OH to give a mixture of *cis*-cyclopropane methoxymycolic acid α- and β-glucose esters **(153)**, **(154)**, and **(155)** (**Scheme 46**). The products showed the same ratio of two isomers in each case as reported in the literature.[30]

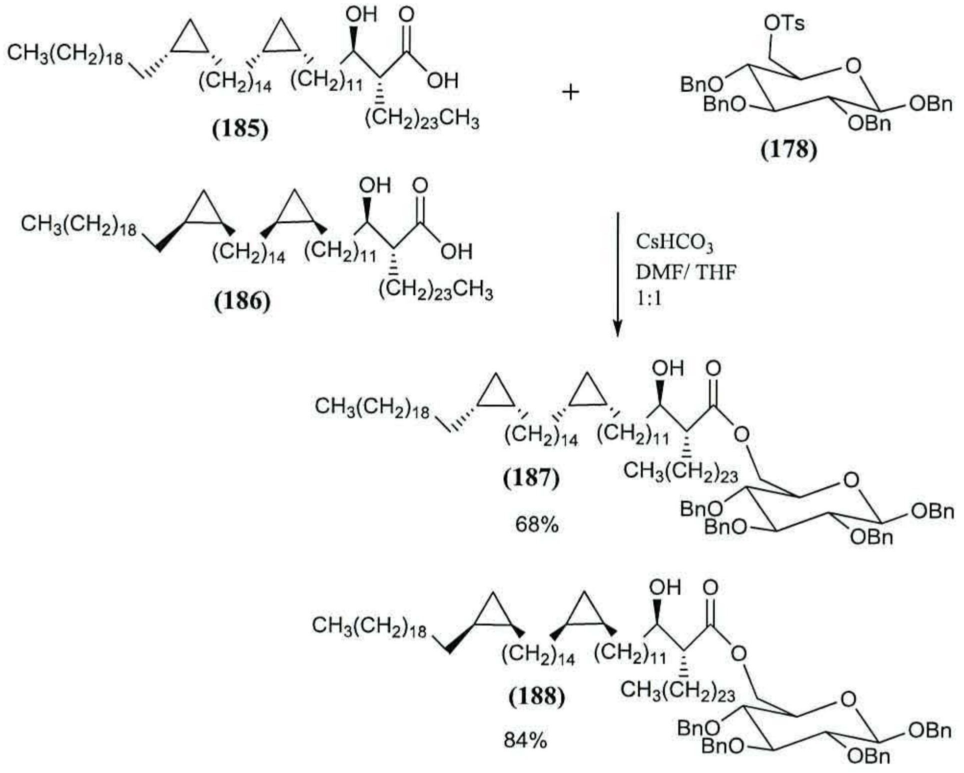


Scheme 46: Debenzylation to synthesise cis-cyclopropane methoxy mycolic acid-glucose esters

The ^1H and ^{13}C NMR spectra showed that there were no signals in the aromatic region, which proved that the debenzylation had been completed. The ^1H NMR spectrum showed a broad singlet at δ 5.09 for the α-proton in the beta anomeric compound and a doublet at δ 4.44 (*J* 7.8 Hz) for the β-proton in the alpha anomeric compound. The MALDI of the final *cis*-cyclopropane methoxy mycolic acid glucose esters **(153)**, **(154)**, and **(155)** gave the correct masses, $(M+Na)^+$: 1410.7, (requires: 1410.30), 1438.8, (requires: 1438.33) and 1410.3 (requires: 1410.3) respectively. The optical rotations of these esters were $[\alpha]_D^{22}$ + 35 (c 1.2, $CHCl_3$), $[\alpha]_D^{22}$ + 32 (c 1.3, $CHCl_3$), and $[\alpha]_D^{22}$ + 37.9 (c 1.2, $CHCl_3$) respectively.

2.5.3 Synthesis of *cis*-cyclopropane α-mycolic acid glucose ester

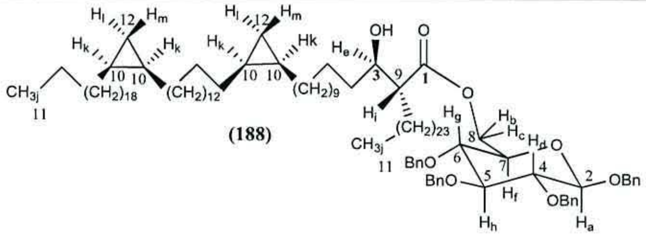
The synthesis of *cis*-cyclopropane α-mycolic acid glucose ester was undertaken with two types of methoxy mycolic acid **(185)** and **(186)**,[189, 195] supplied by Dr. Al Dulayymi. The mycolic acids were coupled with tosylate **(178)** using $CsHCO_3$ in dry DMF/THF to form the protected *cis*-cyclopropane α-mycolic acid glucose ester esters **(187)** and **(188)** (**Scheme 47**).



Scheme 47: Coupling reactions to synthesise cis cyclopropane α-mycolic acid glucose esters

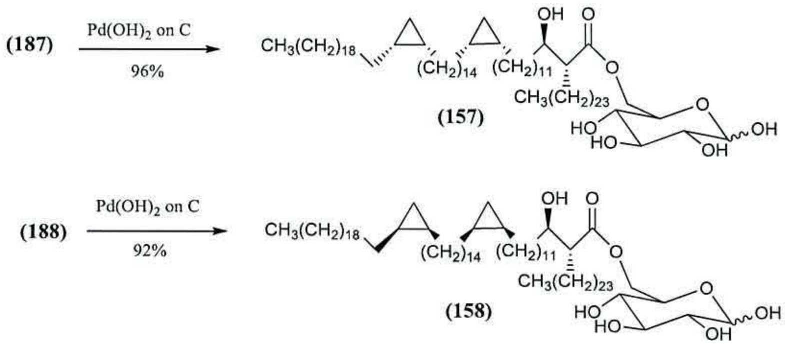
The ^{1}H NMR spectra of compounds (**187**) and (**188**) showed multiplets at δ 7.31 – 7.16 for the aromatic protons. The CH_2 for all the protecting benzyl protons appeared between 4.88 – 4.50, which showed a doublet at δ 4.88 (*J* 3.6 Hz), a singlet at δ 4.86, a multiplet at δ 4.80, a doublet at δ 4.71 (*J* 10.9 Hz), a doublet at δ 4.63 (*J* 10.9 Hz), a multiplet at δ 4.54, while the α-proton in the beta anomeric compound and one proton from the CH_2 next to the ester group appeared as a multiplet at δ 4.46, while the signals between δ 0.65 to -0.41 corresponded to the eight protons of the *cis*-cyclopropane ring. More detail of the ^{1}H NMR and ^{13}C NMR data analysis for compounds (**187**) and (**188**) can be seen in **Table 9**.

Table 9: Selected ^1H and ^{13}C NMR data for (188)



Proton	δ	Multiplicity	J (Hz)	Carbon	δ
H_a	4.88	d	3.6	1	175.4
H_b	4.49 – 4.43	m		2	102.5
H_c	4.14	dd	4.6, 11.7	3	84.7
$H_{d, e}$	3.63 – 3.55	m		4	82.4
$H_{f, g, h}$	3.49 – 3.38	m		5	78.0
H_i	2.44 – 2.34	m		6	73.0
CH_{3j}	0.81	t	6.8	7	72.5
H_k	0.62 – 0.53	m		8	63.0
H_l	0.48	dt	3.8, 7.6	9	51.5
H_m	-0.41	q	4.7	10	15.9
				11	14.3

Compounds **(187)** and **(188)** were debenzylated with hydrogen gas using palladium hydroxide on charcoal as a catalyst in CH_2Cl_2/CH_3OH as before to give glucose esters **(157)** and **(158)** (**Scheme 48**). The product in each case was again a mixture of α- and β-stereoisomers in a ratio of 1:1.

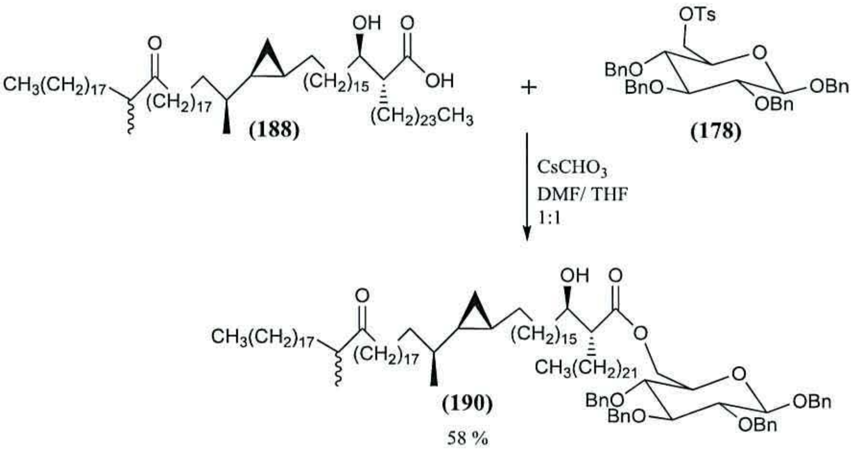


Scheme 48: Debenzylation to synthesise cis-cyclopropane α-mycolic acid glucose esters

The ^1H and ^{13}C NMR spectra showed that there were no signals in the aromatic region, which proved that the debenzylation had been completed. The ^1H NMR spectrum showed a broad singlet at δ 5.09 for the β-proton in the alpha anomeric compound, and a doublet at δ 4.44 (*J* 7.8 Hz) for the α-proton in the beta anomeric compound, in addition to all the expected signals for the *cis*-cyclopropane α-mycolic acid. The signals of the eight protons of the *cis*-cyclopropane ring between δ 0.65 to -0.41 showed that no reductive opening of the cyclopropane rings had occurred during the hydrogenolysis of the benzyl ethers.

2.5.4 Synthesis of *trans*-cyclopropane ketomycolic acid glucose ester

The synthesis of *trans*-cyclopropane keto mycolic acid glucose ester was undertaken with *trans*-cyclopropane ketomycolic acid **(189)**, which was supplied by Dr. Al Dulayymi. The mycolic acid was coupled with tosylate **(178)** using $CsHCO_3$ in dry DMF/THF to form protected *trans*-cyclopropane ketomycolic acid glucose ester **(190)** **(Scheme 49)**.



Scheme 49: Coupling reaction to synthesise trans-cyclopropane ketomycolic acid glucose ester

The ^1H NMR spectrum of compound **(190)** showed a multiplet at δ 7.31 – 7.16 for the aromatic protons. An area of the spectrum which is of particular interest is that between δ 0.45 and δ 0.05, which corresponds to the four protons directly bonded to the *trans*-cyclopropane ring. The region between δ 0.14-0.05 contains signals for three different hydrogens. This is because Hr and Hs, are non-equivalent and each has three couplings, each signal splitting to give a double doublet of doublets (**8 lines**), which leads to 16 lines. Hq should give a double double double doublet of doublets (32 lines) as it is coupled to five non-equivalent protons; however, due to overlap with the signals for Hr and Hs, Hq cannot be resolved fully at δ 0.20-0.05. Hp, represented by the broad multiplet at δ 0.46-0.38, should split to give a doublet double doublet of doublets (16 lines). However, a complex broad multiplet is observed due to the presence of four similar coupling constants leading to overlapping of peaks (**Figure 24**).

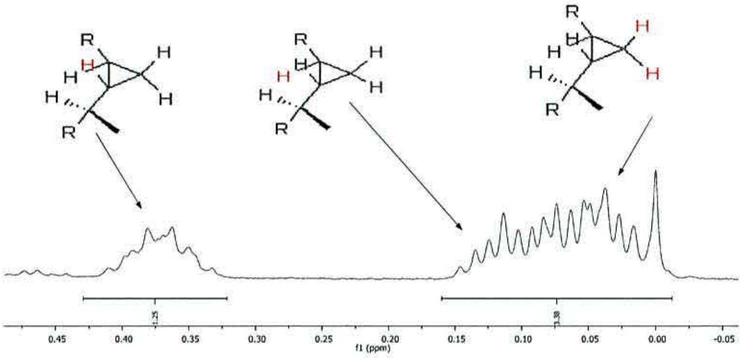
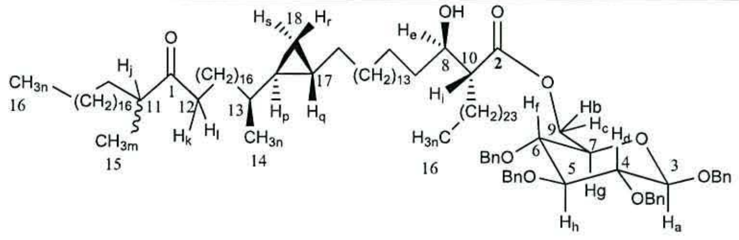


Figure 24: ^1H NMR expansions of trans-cyclopropane protons

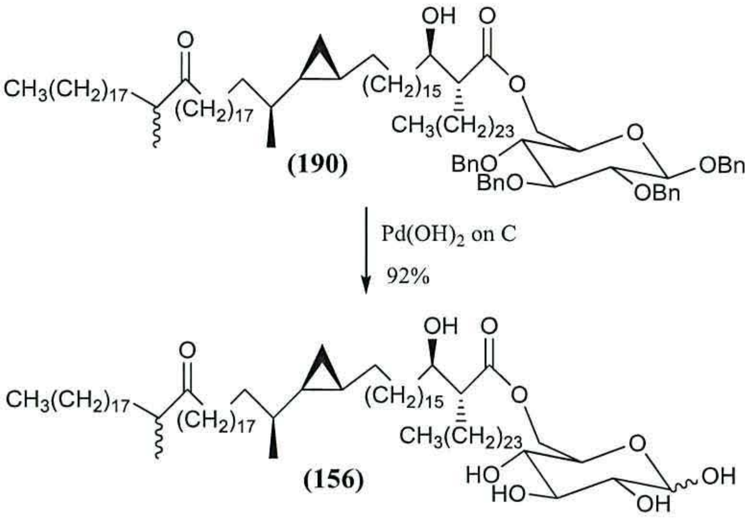
More detail of the ^1H NMR and ^{13}C NMR data analysis for compound **(190)** can be seen in **Table 10**.

Table 10: Selected ^{1}H and ^{13}C NMR data for (190)



Proton	δ	Multiplicity	*J* (Hz)	Carbon	δ
H_a	4.88	d	3.6	1	215.2
H_b	4.49 – 4.43	m		2	175.2
H_c	4.14	dd	4.6, 11.7	3	102.3
$H_{d, e}$	3.65 – 3.53	m		4	84.5
$H_{f, g, h}$	3.48 – 3.38	m		5	82.3
$H_{i, j, k, l}$	2.48 – 2.30	m		6	77.8
CH_{3m}	2.92 – 2.85	d	6.9	7	72.9
CH_{3n}	0.85 – 0.78	m		8	71.1
H_p	0.46-0.38	m		10	51.3
$H_{r, s, q}$	0.20-0.05	m		11	46.3
				12	41.1
				13	38.1
				14	19.7
				15	18.6
				16	16.4
				17	14.1
				18	10.5

The compound **(190)** was debenzylated as before to form *trans*-cyclopropane ketomycolic acid glucose ester **(156)** (**Scheme 50**), as a mixture of α- and β-stereoisomers in a ratio of 1:1



Scheme 50: Debenzylation to synthesise trans-cyclopropane ketomycolic acid glucose ester

The ^{1}H NMR spectrum showed a broad singlet at δ 5.11 for the β-proton in the alpha anomeric compound and a doublet at δ 4.46 (*J* 7.8 Hz) for α-proton in the beta anomeric compound, in addition to all the expected signals for the *trans*-cyclopropane keto mycolic acid. The MALDI of the compound **(156)** gave an ion at 1465.0 ((M+Na)$^+$ for $C_{84}H_{162}NaO_8$ requires: 1465.4). The specific rotation of the compound was $[\alpha]_D^{24}$ + 16 (*c* 1.2, $CHCl_3$).

In addition the molecular rotation M_D for GMMs was measured by same method of measuring M_D of GroMMs by using the converting equation below.[186, 187]

Where:

$$M_D = \alpha_D \times (\text{Mol. Wt.}/100$$

Baird's *et al.*, calculated the optical rotation for **(180)**, **(185)**, and **(186)** and found - 1.8, + 4.4 and + 2.0 respectively.[188, 189, 195] In addition Baird's group calculated the optical rotations for **(179)**, **(181)**, and **(188)** at - 1.1, - 1.1, and + 4.7 respectively. In addition the optical rotation for synthesized GroMM **(153)** - **(158)** were measured in this work. The molecular rotation of the synthesized GMMs **(153)** - **(158)** and the mycolic acids **(179)** - **(181)**, **(185)**, **(186)**, and **(188)** were calculated using the equation above as explained in **Table 11**.

Table 11: The optical and the molecular rotation for the mycolic acids (179), (180), (181), (188), (185), (186) and the synthesized GMMs (153) - (158)

MAs. No.	α_D	M_D	GMMs.No.	α_D	M_D	M_D Differences
(179)	- 1.1	- 14	**(153)**	+ 35	+ 490	+ 504
(180)	- 1.8	- 23	**(154)**	+ 32	+ 456	+ 479
(181)	- 1.1	- 14	**(155)**	+ 38	+ 526	+ 540
(188)	+ 4.1	+ 53	**(156)**	+ 16	+ 224	+ 171
(185)	+ 4.4	+ 50	**(157)**	+ 15	+ 199	+ 149
(186)	+ 2.0	+ 23	**(158)**	+ 14	+ 185	+ 162

A comparison of the relative M_D values of the GMMs and their parent mycolic acids (**Table 11**) show a clear relationship between the two sets of compounds. The M_D differences of the GMMs (**153**), (**154**) and (**155**) are comparable and in the region of 479-540. The M_D differences of the GMMs (**156**), (**157**) and (**158**) are also of the same relative magnitude, but differ from the previous compounds as they are between 149 and 171. This difference is surprising as it would be expected that the six compounds would give relatively similar data and there is no apparent reason why these should differ. Further work will be needed in order to elucidate the reason for this difference.

2.5.6 Tests for antibody activity

Natural mycolic acids can be used for the serodiagnosis for TB run in an ELISA plate assay though the selectivity is not high enough for application.[196] However; patients with HIV and TB still have high levels of antibodies to mycolic acids,[197] which making this method attractive in detecting TB in co-infected patients which explained in details in the Introduction (**Section 1.4 page 18**)

Yano *et al.*, has reported the application of an ELISA assay specific for antituberculosis antibodies in human serum for the clinical evaluation of tuberculosis. The group has developed a TLC method to detect specific antigens TDM and gylcolipid fractions.[198] These antigens were individually purified from *M. tuberculosis* H37Rv for antibodies in the serum of patients with tuberculosis..[199] The purified homogeneous TDM was mixed with the more hydrophilic glycolipids, reacted with patients' serum and applied to the enzyme immunoassay as an antigen.[199]

Having an early method for detection TB means not spending time in quarantine and being able to receive anti-TB therapy in more quickly, leading to a non-infectious patient within days. A specific synthetic GMM antigen could give more reliable data and better distinction between TB positive and TB negative patient sera. One purpose of making such compounds is to study the antigenic activity of GMM antigen using TB positive and TB negative serum samples with ELISA.

In this thesis, ELISA assays were carried out to study the recognition of the GMM by lipid antibodies present in the serum of patients known to be infected with tuberculosis, in order to determine the value of these compounds for the detection TB. The ELISA tests were carried out by Dr. A. Jones. In these tests the protocol used for testing of these compounds was as follows. The surface of the well was coated with GMM (antigen), with casein/PBS buffer being used to block any free non-specific binding sites. The coated surface was then treated with sera containing specific antibodies, which may or may not bind to the antigen. Excess antibodies were removed by washing the surface with casein/PBS buffer, leaving only bound antibodies on the ELISA plate. This was followed by the addition of a secondary antibody, which contains a peroxidase enzyme, is added to the wells, and this will in turn bind to any primary antibodies that are present, followed by washing to remove excess antibody. In order to visualise the results of the ELISA tes, a colour reagent (OPD/H_2O_2 solution) was added. Depending on the amount of secondary antibody present this gives a detectable colour, the absorbance of which is measured at 492 nm. In ELISA tests, the proportion of actual TB positive sera which are correctly identified by the antigen compared to the total TB positive sera is refered to as the selectivity, while the proportion of the TB negatives which are correctly identified to the total TB negative sera is refered to as the specificity.[200] ELISA tests were carried out using each of the five GMM (**153**) - (**157**) as antigens and a set of sera from TB-indigenous countries provided by the WHO and diagnosed as TB-positive or TB-negative based on a range of assays. Compounds (**154**), (**156**) and (**157**) showed a distinction between the TB+ and TB- serum, while (**153**) and (**155**) show no distinction with the average responses for both the TB+ and TB- samples being almost the same (**Figure 25**).

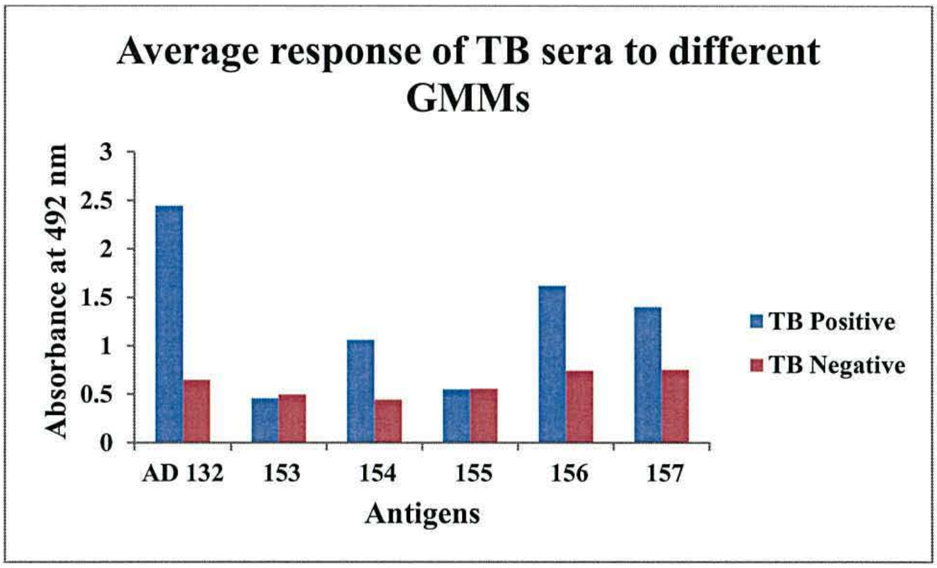


Figure 25: ELISA signals of antibody binding to both TB positive and TB negative sera for the GMM antigens in comparision with best results of TDM (AD 132)

By choosing appropriate 'cut-off' values for the absorbance for each individual GMM, the selectivity and specificity for all the synthetic compounds was also calculated; these were found to fall below or raise above the accepted minimum WHO standard of 85 % for both selectivity and specificity together (**Table 12**).[201]

Table 12: ELISA data for GMM antigens

	AD 132	**(153)**	**(154)**	**(155)**	**(156)**	**(157)**
Sensitivity (%)	89	83	44	56	67	89
Specificity (%)	78	41	84	53	93	59

Compound (**157**) showed the best sensitivity (89%) which was the same as that observed for the best antigen (AD132), and (**156**) gave the best specificity. Compound (**153**) also gave a good sensitivity (83%); however the specificity was again low, while (**154**) and (**157**) showed a high specificity of 84% and 93% respectively, though the sensitivity for these antigens was again low.

2.6 Method development for the synthesis of the mycolic motif

The main part of this project concerned the development of a new method for the synthesis of the α-alkyl-β-hydroxy fragment of mycolic acids (**Figure 26**), which is also known as the mycolic motif. Improving this synthetic method is also of major importance as the α-alkyl-β-hydroxy unit is common to all mycolic acids.

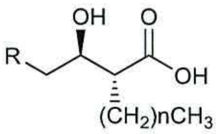


Figure 26: The α-alkyl-β-hydroxy fragment of mycolic acids

There are a lot of methods for the preparation of *α*-alkyl-*β*-hydroxy unit with the correct stereochemistry at both α- and *β*-carbon atoms.[51-53] Two basic methods have been applied to synthesise mycolic acids.[202, 203] The first one involves a direct alkylation of a long chain acid at the α-position relative to the carboxylic group (**60**); in this method Al Dulayymi *et al.* used a chiral intermediates prepared from available *D*-aspartic acid based on a method developed by Frick *et al.*[202] In the second procedure, a short chain allylation was initially carried out, followed by a further elongation achieved using a modified Julia-Kocienski reaction in order to obtain the desired carbon chain length (**Figure 27**).

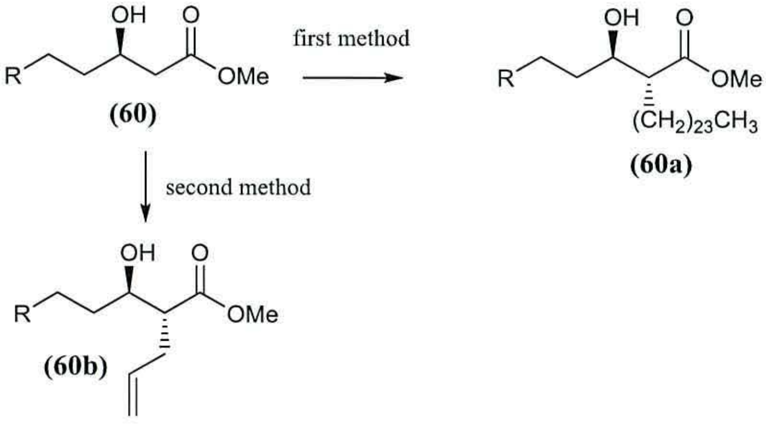


Figure 27: The two basic methods of the synthesis of the α-alkyl-β-hydroxy fragment

The second method was put forward by Toschi *et al.*[204] in which a *cis*-olefin (derived from very simple materials such as 1,12-decanediol) was the starting material (**see page 39**).

On the other hand, work was carried out by Koza *et al.*[205] in which *L*-aspartic acid (**61**) was the starting material for the synthesis of the (2-benzeneoxyethyl)oxirane intermediate (**64**) (**Figure 21**). Then a short chain Fräter allylation followed by a further Julia-Kocienski chain extension was carried out on the *α*-position.[205] The synthesis from (**61**) was achieved in 17 steps with an overall yield of 17% (**Figure 28**).

The work in this thesis involved developing alternative, more efficient, routes, starting from the cheaper starting material, *L*-Malic acid (**81**).

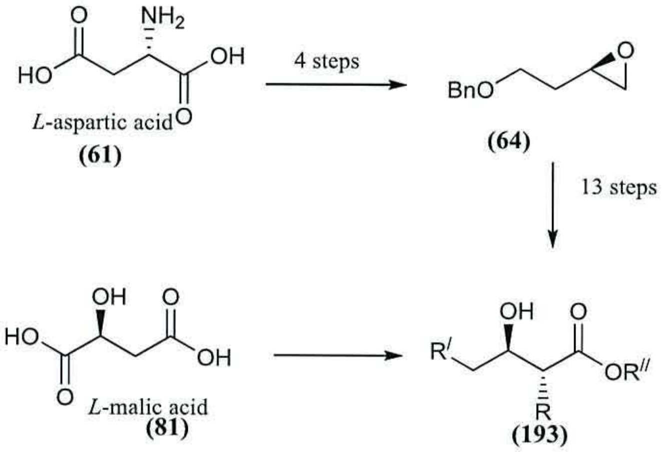


Figure 28: Alternative starting materials for the synthesis of the α-alkyl-β-hydroxy unit

2.6.1 Preparation of diethyl (*S*)-2-hydroxysuccinate (diethyl malate)

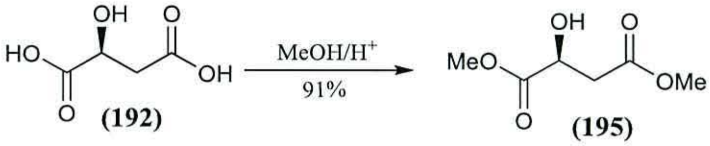
L-Malic acid (**81**) was esterified to the diester by using EtOH in the presence of concentrated H_2SO_4 at 90 °C (**Scheme 51**).

Scheme 51: Esterification of L-malic acid into diesters

The ^{1}HNR and ^{13}C NMR data of compound **(194)** were the same as those reported in literature.[206]

2.6.2 Preparation of dimethyl (*S*)-2-hydroxysuccinate (dimethyl malate)

Preparation of the dimethyl (*S*)-2-hydroxysuccinate (**195**) was achieved as described previously in **Section 2.6.1(Scheme 52**).



Scheme 52: Esterification of L-malic acid into diesterdimethyl malate

Again, the structure of compound **(195)** was confirmed by NMR spectra which were identical to those reported in the literature.[207, 208]

2.6.3 Three routes for the preparation of the α-alkyl-β-hydroxy unit originating from dialkyl malates

Three routes were investigated for the preparation of the α-alkyl-β-hydroxy unit in this work (**Figure 29**).

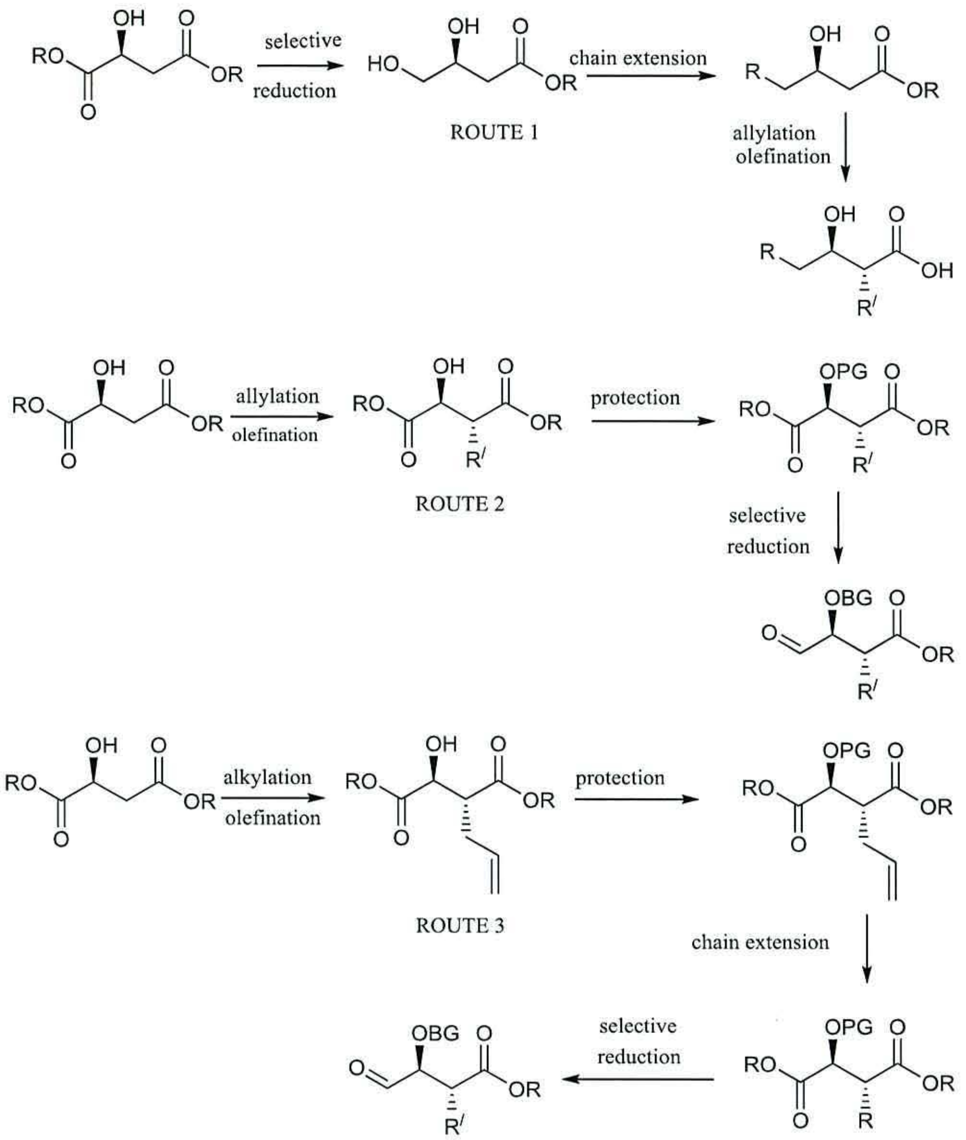
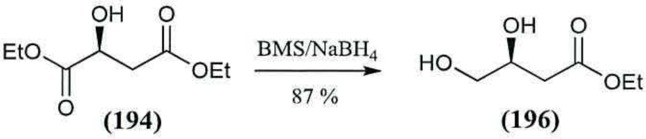


Figure 29: Routes investigatd for the preparation of the α-alkyl-β-hydroxy unit

2.6.4 Preparation α-alkyl-β-hydroxy unit from diethyl malate (route I)

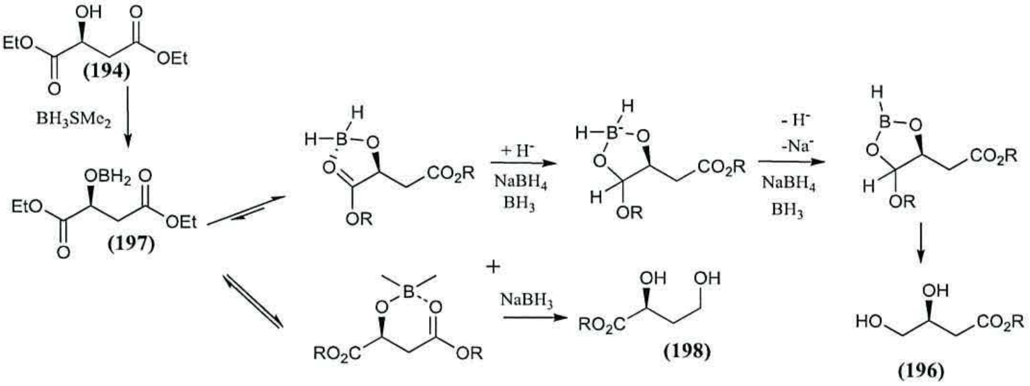
2.6.4.1 Selective reduction of diethyl malate

Selective reduction of diethyl malate has been achieved with borane dimethyl sulfide complex (BMS) and catalytic $NaBH_4$ (**Scheme 53**). The ester group adjacent to the hydroxyl group in diethyl malate (**194**) was selectively reduced to give a vicinal diol (**196**).[209-211]



Scheme 53: Selective reduction of diethyl malate

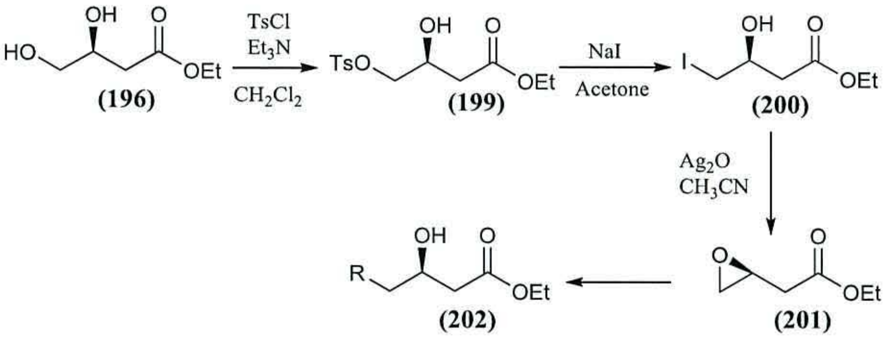
At room temperature, diethyl malate was dissolved in dry THF and the BMS was added; evolution of hydrogen gas took place immediately and ceased after 40 minutes. The selective reduction was monitored by TLC. The first product should be the oxyborane-type intermediate **(197)** (**Scheme 54**). After NaBH4 was added, TLC showed the commencement of reduction, which was completed in 1.5 hours at room temperature. The reaction was worked up through addition of EtOH. The ^{1}H NMR spectrum of compound **(196)** showed a triplet at 1.25 (J = 7.1 Hz) for just one of the terminal C<u>H</u>$_3$ group and of doublets at 3.50 (J = 11.3, 6.3 Hz) and 3.64 (J = 11.3, 2.9 Hz,), for the C<u>H</u>$_2$OH. The carbonyl appeared in the ^{13}C NMR at δ 172.6. The IR spectrum showed a peak at 3321 for the O–H stretching vibration. The optical rotation of **(196)** was $[\alpha]_D^{22}$ + 7.2 (*c* 1.1, CHCl$_3$) (*lit.* $[\alpha]_D^{22}$ + 6.2 (*c* 0.5, CHCl$_3$)).[210, 211] The mechanism of selective reduction of diethyl malate by BMS-NaBH4 system can be seen in **Scheme 54.**[210]



Scheme 54: Mechanism for selective reduction[210]

2.6.4.2 The chain extension of diethyl malate

The chain extension requires first the transformation of the diol **(196)** into the tosylate (**199**) which is then converted into the iodohydrin (**200**), then into the chiral epoxide intermediate (**201**). Then ring opening of the epoxide (**201**) was done by reacting with a Grignard reagent to achieve the desired product (**202**) as in **Scheme 55**.



Scheme 55: Rout (1) of preparation α-alkyl-β-hydroxy unit

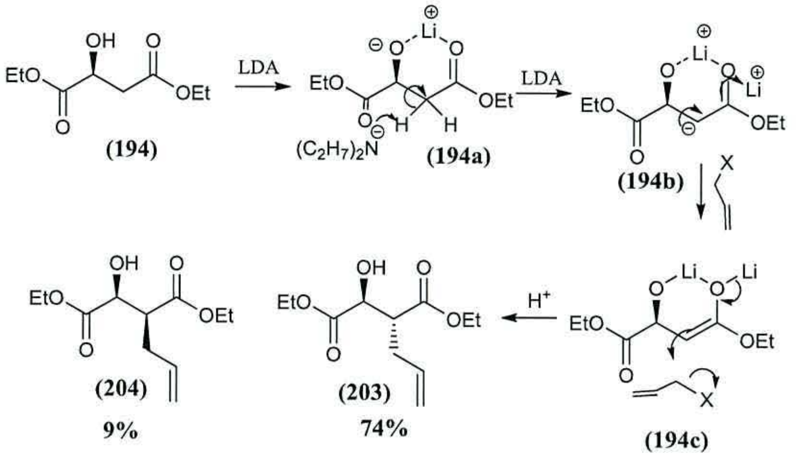
As can be seen from the scheme above, this approach requires the use of expensive materials such as silver dioxide.[212] In this work, the process was done by using K_2CO_3, MeOH instead of Ag_2O, but unfortunately the reaction failed to form (**201**). Therefore, another route was examined to prevent these difficulties

2.6.5 Preparation the α-alkyl-β-hydroxy unit from diethyl malate (route 2)

The second route for the preparation of the α-alkyl-β-hydroxy fragment of the mycolic acid using diethyl malate (**194**) is summarised in **Figure 29**.

2.6.5.1 Preparation diethyl (2*R*,3*S*)-2-allyl-3-hydroxysuccinate

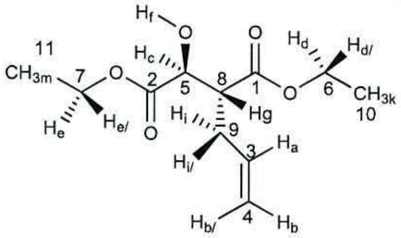
The Fräter alkylation,[213,214] was used for the stereoselective installation of the required alkyl chain at the α-position of the β-hydroxy diester (**Scheme 56**).



Scheme 56: Formation of the chelated enolate intermediate

Using the method given in the literature, [208] LDA was generated *in situ* with n-BuLi and di-isopropylamine in dry THF below 0 °C. After 45 minutes, it was cooled to – 85 °C and then reacted with diethyl (*S*)-2-hydroxysuccinate (**194**). In order to ensure the formation of the intermediates (**194a**) and (**194b**), the mixture was stirred at – 85 to – 75 °C for 2 h, then it was treated with allyl bromide (2 eq) and the temperature was gradually raised to – 20 °C. A further quantity of allyl bromide (1.5 eq) was added to the mixture which was then stirred at the same temperature 30 minutes. The reaction was slowly allowed to reach 0 °C and then worked up with sat. NH_4Cl. The product was obtained as mixture of distereoisomers (2*R*,3*S*) (**203**) and (2*S*,3*S*) (**204**) which was separated by column chromatography to give **(203)** in a good yield of 74% (Scheme 56). The success of the allylation was confirmed by the ^1H and ^{13}C NMR spectrum (**Table 13**). Further confirmation was provided by COSY NMR (**Figure 30**). The specific rotation was $[\alpha]_D^{20}$ + 10 (c 0.8, $CHCl_3$).

Table 13: Selected ^1H and ^{13}C NMR signals for compound (203)



Proton	δ	Multiplicity	*J* (Hz)	Carbon	δ
H_a	5.88 – 5.76	m		1	173.4
H_b, $H_{b/}$	5.22 – 5.08	m		2	171.9
$H_{c,\,d,\,d/}$	4.31 – 4.21	m		3	134.9
$H_{e,\,e}$	4.18 – 4.11	m		4	118.0
H_f	3.16	d	7.0	5	70.2
H_g	2.96	ddd	8.4, 6.7,3.1	6	61.7
H_i	2.68 – 2.58	m		7	60.8
$H_{i/}$	2.49 – 2.39	m		8	48.1
CH_{3k}	1.30	t	7.1	9	32.0
CH_{3m}	1.23	t	7.1	10	14.09
				11	14.05

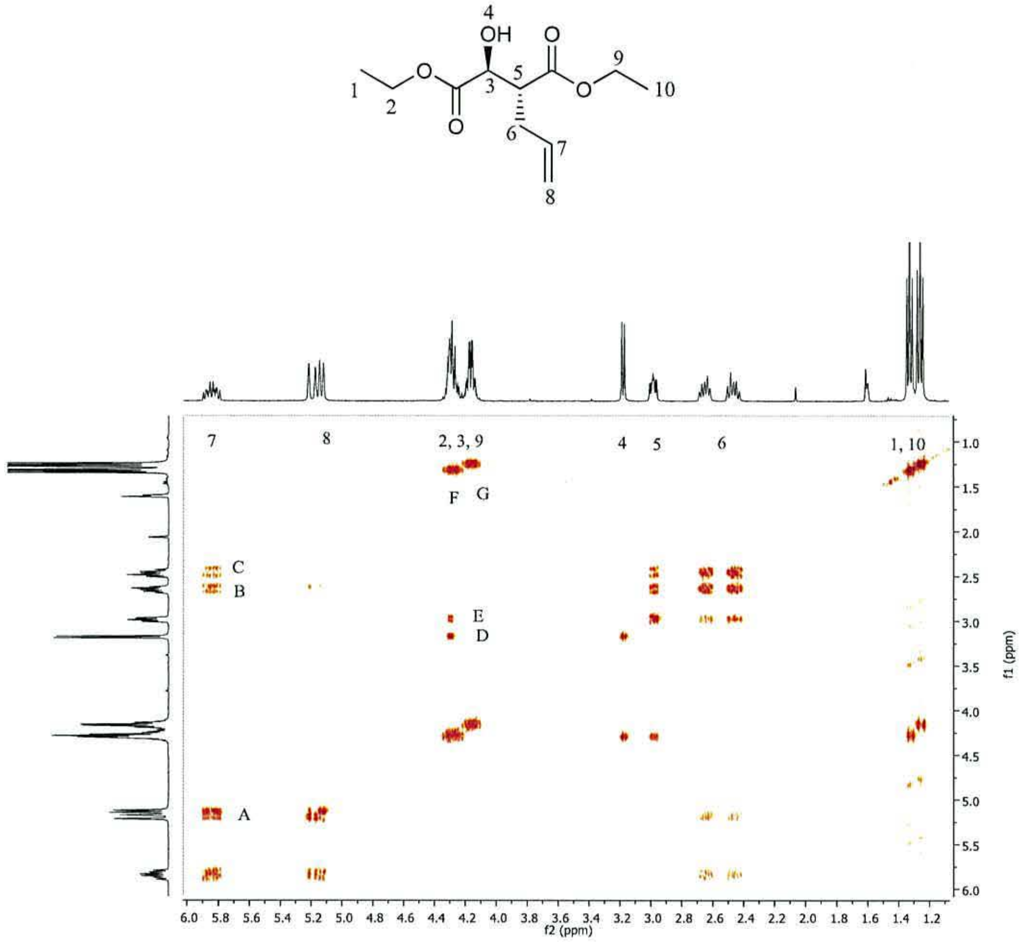
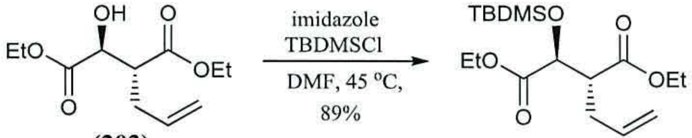


Figure 30: COSY spectrum of (2R,3S)-2-allyl-3-hydroxysuccinate

The COSY NMR spectrum of (**203**) shows peak **A** which indicates a coupling between H7 at δ 5.84 ppm and H8 at δ 5.18 ppm for the CH_2 group and the adjacent H on the alkene. Peaks **B** and **C** indicate a coupling between the H7 at δ 5.84 ppm and the H6 at δ 2.44 ppm, 2.61 ppm respectivlely. This corresponds to the coupling of the CH_2 group next to the alkene and the adjacent H on the alkene. Peak **D** indicates a coupling interaction between the H3 at δ 4.29 ppm and the H4 at δ 3.18 ppm. This corresponds to the coupling of the OH group and the H on the β position. Peak **E** points to a coupling between the H3 at δ 4.29 ppm and the H5 at δ 2.99 ppm. The peaks **F** and **G** indicate a coupling interaction between the H2, 9 at δ 4.31, 4.15 ppm and H1, 10 at δ 1.38, 1.28 ppm. This corresponds to the coupling of the CH_3 group and the adjacent CH_2 on the two ethyl groups.

2.6.5.2 Protection of the secondary alcohol

The secondary alcohol of (2*R*,3*S*)-2-allyl-3-hydroxysuccinate (**203**) was protected with a *tert*-butyldimethylsilyl group in order to complete the chain extension at the α-position. The alcohol (**203**) in DMF was treated with *tert*-butyldimethyl-chlorosilane and imidazole, and then stirred at 70 °C for 18 h to give (**205**) in 89% yield (**Scheme 57**). The protecting group protons appeared in the ^{1}H NMR spectrum as a singlet at δ 0.88 for the *tert*-butyl group and two singlets at δ 0.07 and 0.04 for the two methyl groups. The two methyl groups are not equivalent because of the nearby chiral centre. The specific rotation of the product (**205**) was $[\alpha]_D^{22}$ – 9.4 (*c* 1.2, $CHCl_3$).



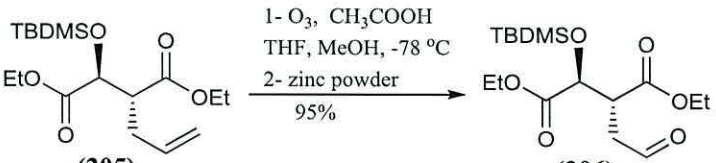
(203)

(205)

Scheme 57: Protection of the secondary alcohol

2.6.5.3 Oxidation of diethyl (2*R*,3*S*)-2-allyl-3-((*tert*-butyldimethylsilyl) oxy) succinate to aldehyde (206)

The olefin (**205**) was oxidised to the aldehyde (**206**) with ozone and acetic acid in THF: MeOH (1:1) at -78 °C for 30 min, then the reaction mixture was allowed to reach room temperature and zinc powder was added. The aldehyde (**206**) was obtained in 95% yield (**Scheme 58**). The formation of the aldehyde was confirmed by the ^{1}H NMR spectrum, which showed a broad singlet at δ 9.79 belongs to the aldehyde proton, and by the ^{13}C NMR spectrum, which showed a signal at δ 199.9 corresponding to the aldehyde carbonyl group.



(205)

(206)

Scheme 58: Oxidation of the olefin to aldehyde

2.6.5.4 The chain extension of diethyl (2*S*,3*R*)-2-((*tert*-butyldimethylsilyl)oxy)-3-(2-oxoethyl)succinate

In order to couple the aldehyde prepared in **Section 2.6.5.3** with sulfone reagents of appropriate carbon chain lengths, the modified Julia-Kocienski reaction was employed. The procedure is based on a method originally devised by M. Julia *et al.*, called the Julia olefination.[215] Work carried out by P.J. Kocienski *et al.* on the modification of this method has led it to become an important C - C bond reaction in organic synthesis.[216] The reaction goes via the deprotonation of the acidic α-proton of the phenylsulfone, acylation of the aldehyde to yield a β-alkoxysulfone, followed by a reductive elimination to give a mixture of the two alkene isomers (**Figure 31**).

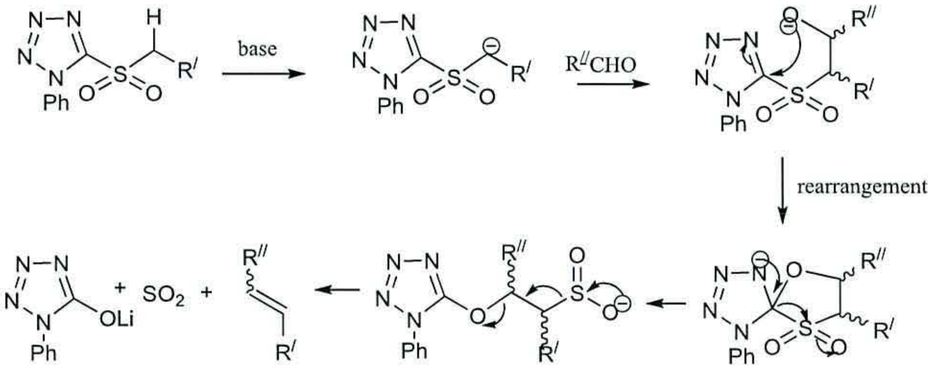
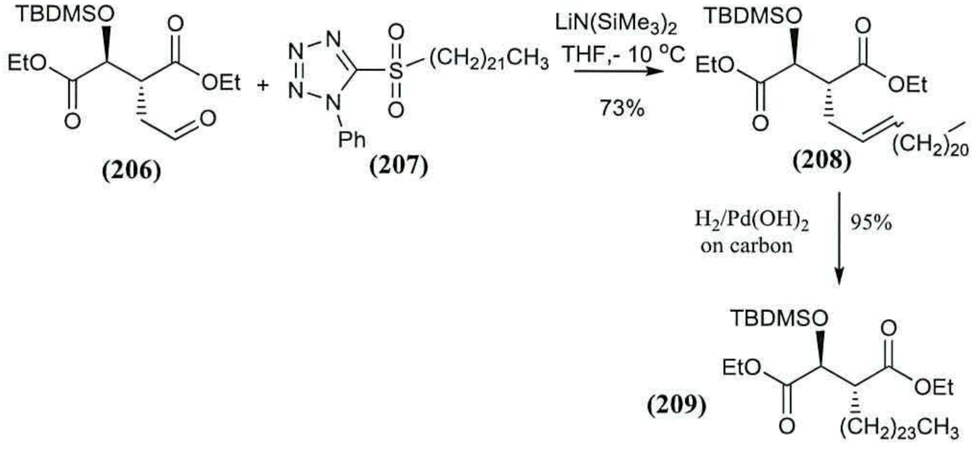


Figure 31: Modified Julia-Kocienski olefination

In this way, the sulfone (**207**) was coupled with the previously prepared aldehyde (**206**) (**Scheme 59**). A mixture of *E*- and *Z*-alkenes (**208**) in a ratio 2:1 was obtained using lithium *bis*(trimethylsilyl)amide as the base, and the yield was 73%. The ^{1}H NMR spectrum of the product showed the protons of the C–C double bond at δ 5.34 – 5.26 and δ 5.51 – 5.43, and with no signal for the aldehyde proton. The ^{13}C NMR spectrum showed signals at δ 129.9 and 129.9 ppm for the alkene carbon.



Scheme 59: The chain extension at the α-position

The alkene (**208**) was hydrogenated using hydrogen gas and palladium hydroxide on carbon as catalyst in THF and IMS (1:1). The product (**209**) gave NMR spectra similar to (**208**) but without the signals for the alkene protons.

2.6.5.5 Selective reduction of the diester

The key step for the preparation of the α-alkyl-β-hydroxy unit from diester (**209**), is the selective reduction of the ester side (**1**) of the compound (**209**) (**Figure 32**).

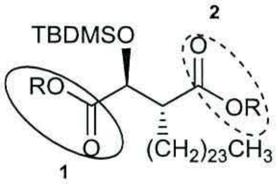
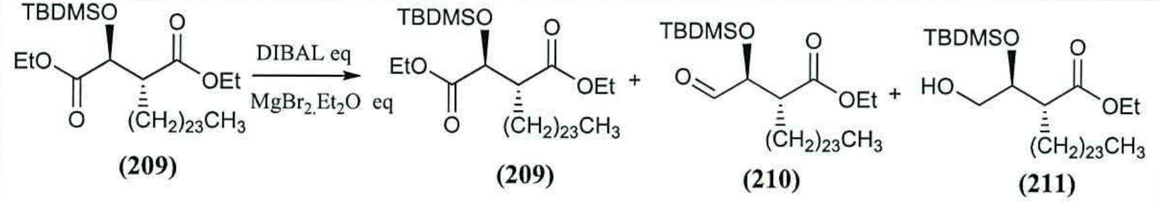


Figure 32: Selective reduction of the diester

The selective reduction of such systems without the α-chain was reported in the literature,[217, 218] and involves the use of diisobutylaluminum hydride (DIBAL-H) and magnesium bromide diethyl etherate ($MgBr_2.Et_2O$) as in entry (**1**) (**Table 16**). However; these conditions were not successful because of the solubility of the compound (**209**) due to the α-chain of this compound. A range of different temperatures and reagent equivalents were therefore investigated in the process of optimisation, which are given in **Table 14**. This part was done with help from Dr. Dr. D. Pritchard.

Table 14: The range of different conditions



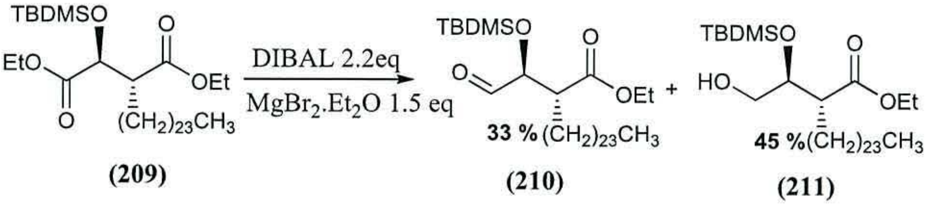
(209) **(209)** **(210)** **(211)**

Entry	Temperature	MgBr2.Et2O eq	DIBAL eq	Product
1	-78 °C	1	1.5	N.R
2	-78 to -55 °C	1	1.5	209 and 210
3	-55 to 0 °C	1	1.5	209, 210, 211
4	-55 to 0 °C	1	2	209, 210, 211
5	-55 to 0 °C	1.2	2	209, 210, 211
6	-55 to 0 °C	1.5	2.2	210, 211

Table 14 showed the entry (**6**) was the best for the selective reduction of (**209**), which gave (**210**) and (**211**) in yield 33 and 45% respectively.

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

Selective reduction of (**209**) was carried out as in entry (6) (**Table 16**). A solution of magnesium bromide diethyl etherate (1.5 eq) and 10 g of (**209**) in methylene chloride was stirred at room temperature for one hour, and then the mixture was cooled down to -55 °C. Diisobutylaluminum hydride (2.2 eq) was added dropwise over 60 min, and the mixture was then allowed to warm to 0 °C. The product was purified by column chromatography to give (**210**) and (**211**) (**Scheme 60**).



Scheme 60: Selective reduction of diester (209)

The structure of product (**210**) was verified by ^{1}H NMR, which gave a doublet at δ 9.62 (J = 1.7 Hz) corresponding to the aldehyde proton, a multiplet at δ 4.22 – 4.06 for the on the ester group, a doublet of doublets at 4.04 δ (J = 1.7, 5.2 Hz) of the proton adjacent to the aldehyde, and a doublet of doublets of doublets 2.77 (J = 2.9, 5.7, 11.2) for the α-alkyl proton. The carbon NMR gave signals at δ 203.0 and δ 172.1 ppm for the two carbonyl groups. The COSY spectrum of the product is given in **Figure 33**.

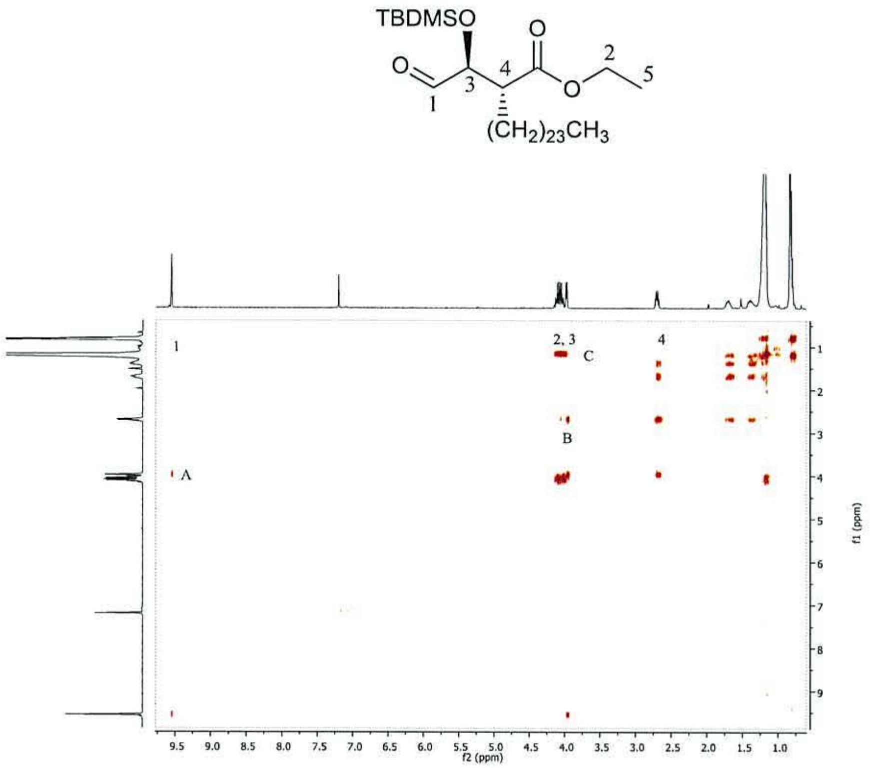


Figure 33: COSY spectrum for the product (210)

In the COSY NMR spectrum of (**210**), peak **A** which indicates a coupling between H1 at δ 9.54 ppm and H3 at δ 3.95 ppm. This corresponds to the coupling of the H on the aldehyde group and the adjacent H on the β position, while the peak **B** indicates a coupling interaction between the H3 at δ 3.95 ppm and the H4 at δ 2.69 ppm. This corresponds to the coupling of the H on the α-position and the adjacent H on the β-position. Finally, peak **C** indicates a coupling interaction between the H2 at δ 4.07 ppm and the H5 at δ 1.18 ppm. This corresponds to the coupling of the CH_3 group and the adjacent CH_2 on the ethyl group.

The ^1H NMR spectrum of coumpond (**211**) showed the expected signals, including a multiplet at δ 4.21 – 4.06 for the CH_2 adjacent to the ester croup, a multiplet at δ 3.95 – 3.84 for the proton at the β-position, a broad doublet at δ 3.61 ($J = 3.6$ Hz) for the CH_2 adjacent to the primary alcohol, and a multiplet at δ 2.74 – 2.64 for the α-proton. The ^{13}C NMR spectrum showed a signal at δ 174.7 for the carbonyl carbon of the ester, at δ 49.2 for the carbon of the α-position, and at δ 74.0 for the carbon of the β-position. The COSY spectrum of the product is given in **Figure 34**.

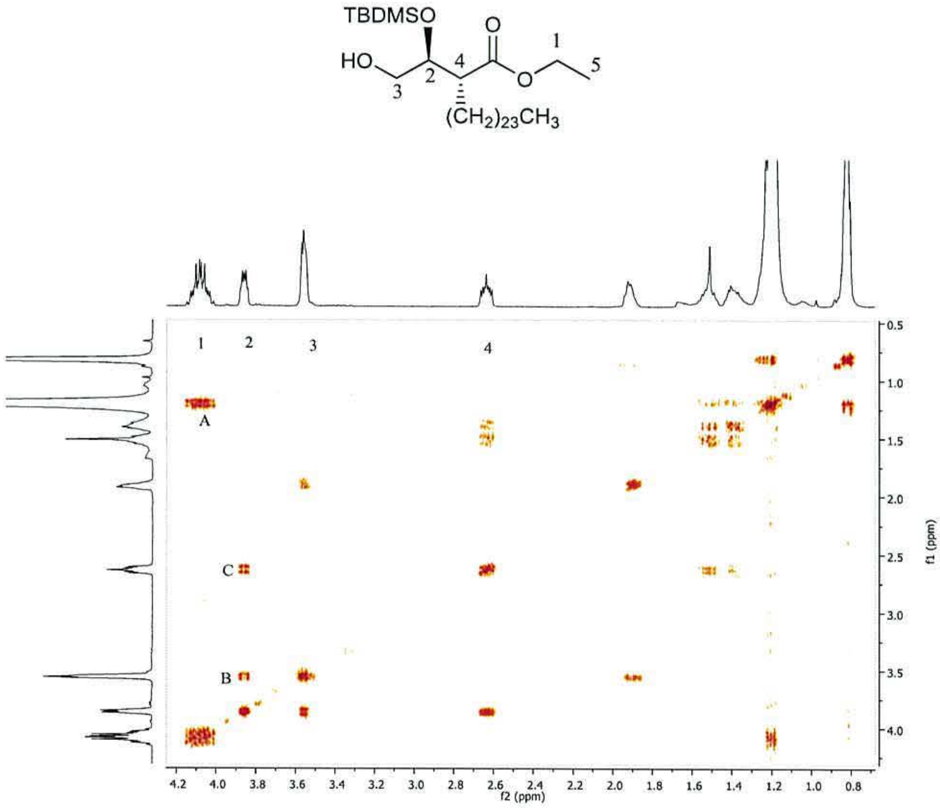
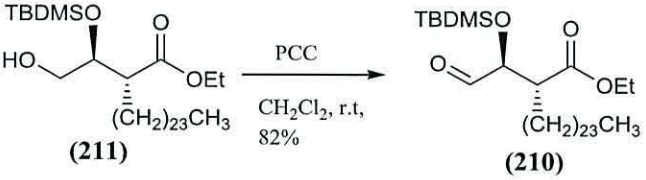


Figure 34: COSY spectrum for product (211)

In the COSY NMR spectrum of **(211)**, peak **A** which indicates a coupling between H1 at δ 4.09 ppm and H5 at δ 1.20 ppm. This corresponds to the coupling of the CH_3 group and the adjacent CH_2 on the ethyl group. Peak **B** indicates a coupling between H2 at δ 3.86 ppm and H3 at δ 3.55 ppm. This corresponds to the coupling of the CH_2 and the adjacent H on the β-position. Peak **C** indicates a coupling interaction between H2 at δ 3.86 ppm and H4 at δ 2.62 ppm. This corresponds to the coupling of the H on the α-position and the adjacent H on the β-position.

2.6.5.7 Oxidation of alcohol (211)

PCC is the reagent of choice for oxidation of primary alcohols to aldehydes, as it works under mild conditions, and will not further oxidise the aldehyde. Therefore, throughout this work, PCC was used for the oxidation of alcohol **(211)** to aldehyde **(210)** (**Scheme 61**).



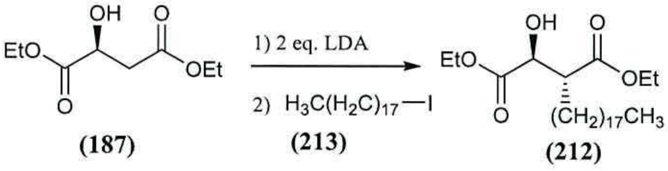
Scheme 61: oxidation of alcohol (211)

The ^{1}H NMR and the ^{13}C NMR spectra were identical to those for the same compound described in **Section 2.6.5.6**.

The route 2 of this work was achieved in fewer steps, in comparison with Koza *et al.* method (**Figure 21**).[205] This new method is shorter by 11 steps, without the need for any further chain extension required in order to couple with various functional groups of the meromycolate unit, will explain in the next two sections (**Section 2.7** and **Section 2.8**). The overall yield for the entire scheme was 40% and this was achieved in 6 steps.

2.6.6 Preparation α-alkyl-β-hydroxy unit from diethyl malate (route 3)

In this route, the possibility of the direct alkylation with the long chain alkyl iodide at the α-position relative to the carboxylic group was investigated (**Scheme 62**).



Scheme 62: direct alkylation of diethyl (S)-2-hydroxysuccinate

The range of different temperatures and equivalents of the iodide used in the process of optimisation is given in **Table 15**.

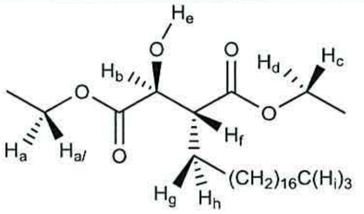
Table 15: The range of temperatures and equivalents of the iodide

Entry	Temperature	Iodide	Temperature
1	-78 °C	1.5	0%
2	-78 to -55 °C	1.5	12%
3	-55 to -33 °C	1.5	34%
4	-33 to 0 °C	1.5	10%
5	0 °C	1.5	0%
6	R. T	1.5	0%
7	-55 to -33 °C	2	34%

The best conditions for Fräter alkylation given as in entry (3) (**Table 15**) were used for the synthesis of **(212)**. The LDA was generated *in situ* with n-BuLi and di-isopropylamine in dry THF below 0 °C. After 45 minutes, it was cooled to – 55 °C and then reacted with the diethyl (*S*)-2-hydroxysuccinate **(187)**. The mixture was stirred at – 55 to – 33 °C for 2 h, and was then treated with alkyl iodide **(213)** (1.5 eq). The temperature was gradually raised to – 20 °C, and the mixture was stirred at the same temperature for 30 minutes. The reaction was slowly allowed to reach room temperature and then worked up with NH_4Cl. The product **(212)** was obtained in a yield of 34% (**Scheme 62**). The formation of this product was confirmed by the ^1H

NMR spectrum which showed a multiplet at δ 4.32 – 4.20 for the CH_2 protons next to oxygen of the ethoxy group and the proton at the β-position, and two doublet of doublets at δ 4.16 (*J* 1.9, 7.1 Hz) and 4.12 (*J* 1.8, 7.1 Hz) for the other CH_2 protons next to oxygen of the ethoxy group which are not equivalent because the different adjacent groups. A broad double triplet at δ 2.83 (*J* 3.6, 7.4 Hz) was for the α proton. More NMR data can be seen in **Table 16** and **Figure 35**. The ^{13}C NMR spectrum showed signals at δ 173.5 and 172.9 for the carbonyl carbons of the two esters, at δ 74.0 of the carbon of the β-position, at δ 49.2 of the carbon of the α -position, two signals at δ 61.82, 60.8 for the carbon of the ethane group next to the esters, and signal at δ 14.1 for the terminal carbon. The specific rotation was $[\alpha]_D^{22}$ + 12 (*c* 1.1, $CHCl_3$).

Table 16: ^{1}H NMR analysis for (212)

<table>
<tr><td colspan="5"> </td></tr>
<tr><td>H_x</td><td>δ</td><td>Multiplicity</td><td>Integration</td><td>*J* (Hz)</td></tr>
<tr><td>H_a, $H_{a/}$, H_b</td><td>4.32 – 4.20</td><td>m</td><td>3</td><td></td></tr>
<tr><td>H_c</td><td>4.16</td><td>dd</td><td>1</td><td>1.9, 7.1</td></tr>
<tr><td>H_d</td><td>4.12</td><td>dd</td><td>1</td><td>1.8, 7.1</td></tr>
<tr><td>H_e</td><td>3.19</td><td>d</td><td>1</td><td>7.6</td></tr>
<tr><td>H_f</td><td>2.83</td><td>dt</td><td>1</td><td>3.6, 7.4</td></tr>
<tr><td>H_g</td><td>1.71 – 1.60</td><td>m</td><td>1</td><td></td></tr>
<tr><td>H_h</td><td>1.71 – 1.60</td><td>m</td><td>1</td><td></td></tr>
<tr><td>H_f</td><td>0.87</td><td>t</td><td>3</td><td>6.8</td></tr>
</table>

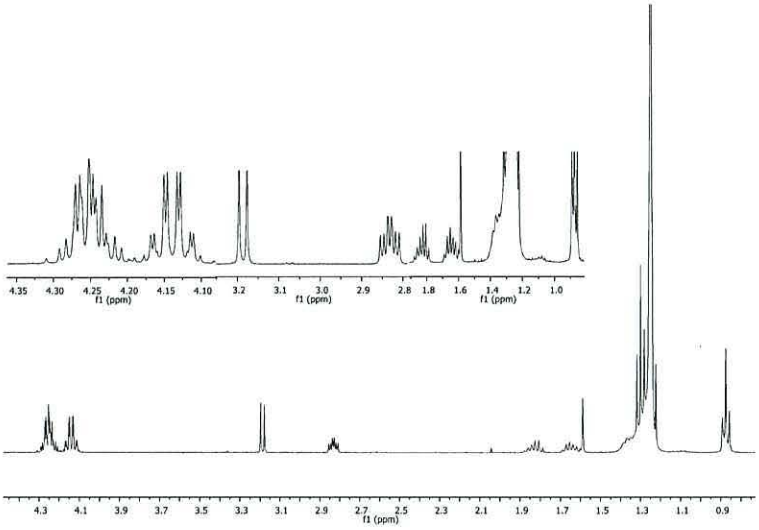


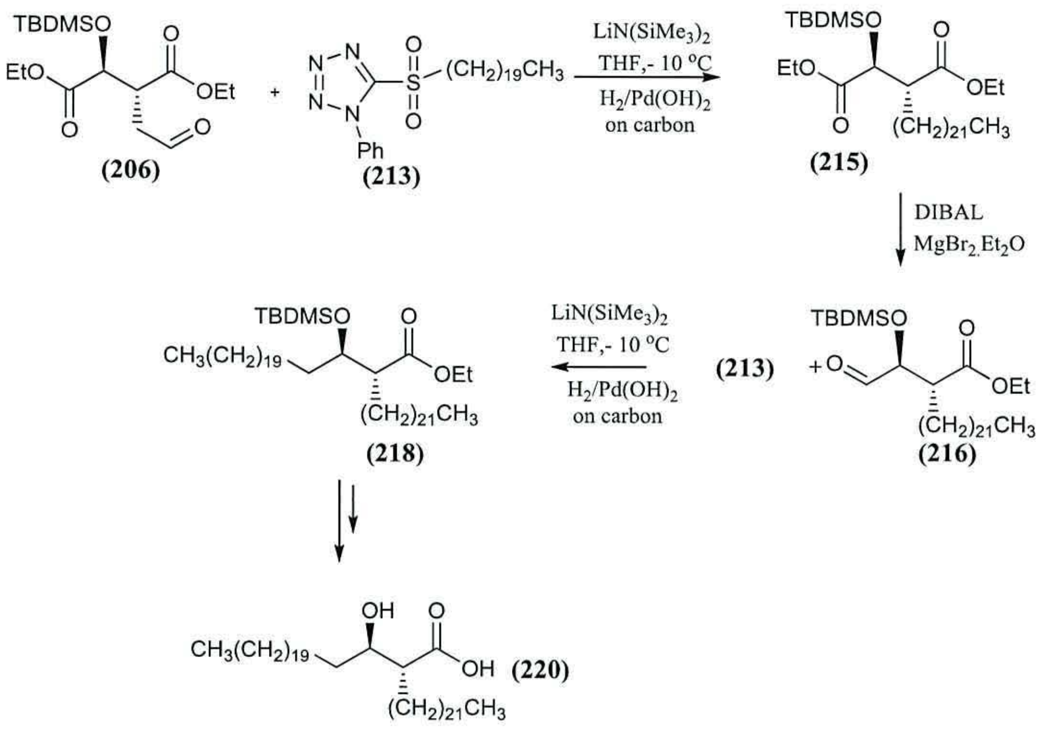
Figure 35: ^1H NMR spectrum of compound (212)

The route 3 has two major issues; the first one, the Fräter alkylation with un active long chain was not easy to be repeated 100%, while the second issue was the high expense preparation of the iodine with C_{24} chain (which is the main α-chain of the major mycolic acid in *M. Tuberculosis*). Therefore; these two issues will be our targets in future work to make the route 3 valuable to use for synthesis the mycolic acid motif.

2.7 Synthesis of model mycolic acid (220)

The main aim of this section was to confirm that the new method for the synthesis of the mycolic motif can be used to prepare different types of mycolic acid.

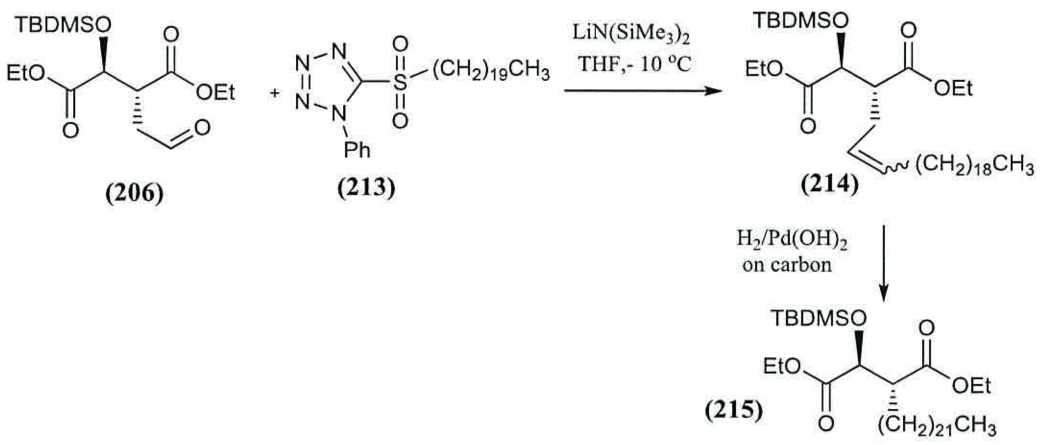
The synthesis of the first component of the model mycolic acid (**220**), the aldehyde (**206**) has been described in **Section 2.6.5.4**. The second part of the synthesis entailed the coupling of this to the sulfone (**213**) using the Julia-Kocienski olefination. The third part entailed the preparation of the aldehyde (**216**) by selective reduction of the diester (**215**) with the DIBAL and $MgBr_2.Et_2O$ and then coupling with sulfone (**213**) to obtain product (**218**). Deprotection of the silyl group and hydrolysis of the ethyl ester now completed the synthesis (**220**) (**Scheme 63**).



Scheme 63: Synthesis of model mycolic acid (220)

2.7.1 Preparation of (215)

The sulfone (**213**) and the aldehyde (**206**) were dissolved in THF, followed by addition of lithium *bis*(trimethylsilyl) amide at – 10 ℃. The mixture was allowed to reach room temperature and stirred for 2 h. The reaction afforded a mixture of the *E*/*Z* isomers of (**214**) in a ratio 2:1, with an 82% yield (**Scheme 64**). The ^{1}H NMR spectrum of (**214**) showed two multiplets at δ 5.47–5.40 and at δ 5.37–5.31 respectively for the two alkene protons. The ^{13}C NMR spectrum showed two signals at δ 132.7 and 127.5 for the olefinic carbons. This was followed by catalytic hydrogenation with palladium on carbon catalyst in THF/IMS to give the saturated product **(215)** in 91% yield (**Scheme 64**).

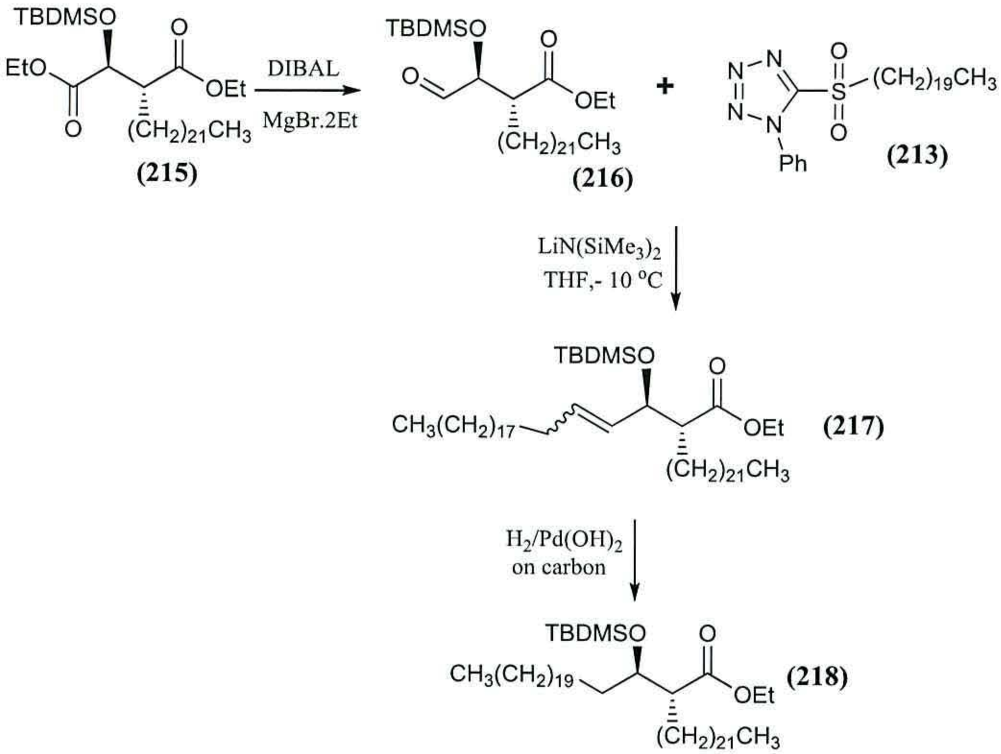


Scheme 64: Preparation of (215)

The product **(215)** gave NMR spectra similar to those for **(214)** but without the signals for the alkene protons, which proved that the hydrogenation had proceeded to completion.

2.7.2 Preparation of (218)

Following the same procedure as delineated in **Section 2.6.5.5**, the selective reduction of diester (**215**) with diisobutylaluminium hydride and magnesium bromide diethyl etherate in dry dichloromethane was undertaken to give the aldehyde (**216**) in 72% yield. The ^{1}H NMR spectrum of the product showed a doublet at δ 9.62 (J = 1.7 Hz) corresponding to the aldehyde proton. The ^{13}C NMR spectrum gave a signal at δ 203.0 corresponding to the carbonyl group for the aldehyde. The aldehyde (**216**) and 5-(icosylsulfonyl)-1-phenyl-1H-tetrazole (**213**) were coupled by the Julia reaction to give the olefin (**217**) (82%). The product was a mixture of *E*- and *Z*- isomers in a ratio 2:1 (**Scheme 65**).

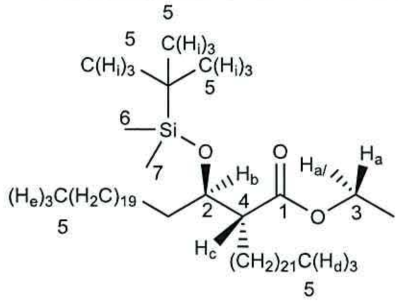


Scheme 65: Preparation of (218)

The ^{1}H NMR spectrum showed multiplets between 5.46-5.32 ppm and between 5.33-5.18 ppm with an integration of one proton each corresponding to the olefinic environment, and no signal for the aldehyde proton was evident.

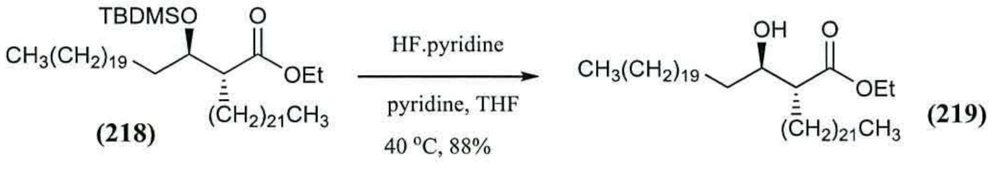
This Julia reaction was followed by catalytic hydrogenation with palladium on carbon catalyst in THF/IMS to give the saturated product (**218**) in 91% yield (**Scheme 65**). The ^{1}H NMR spectrum of the product (**218**) showed the absence of the alkene signals; ^{1}H and ^{13}C NMR data are shown in **Table 17**. The protons of the long chain section appeared as a broad multiplet at δ 1.28 – 1.20.

Table 17: NMR analysis for (218)

<table>
<tr><td colspan="6"> </td></tr>
<tr><td>Hx</td><td>δ</td><td>Multiplicity</td><td><i>J</i> (Hz)</td><td>carbon</td><td>δ</td></tr>
<tr><td>H$_a$, H$_{a/}$</td><td>4.20 – 4.04</td><td>m</td><td></td><td>1</td><td>174.8</td></tr>
<tr><td>H$_b$</td><td>3.95 – 3.86</td><td>m</td><td></td><td>2</td><td>73.4</td></tr>
<tr><td>H$_c$</td><td>2.49</td><td>ddd</td><td>10.8, 7.0, 3.8</td><td>3</td><td>60.1</td></tr>
<tr><td>CH$_{3d, e, i}$</td><td>0.93 – 0.80</td><td>m</td><td></td><td>4</td><td>51.7</td></tr>
<tr><td>SiMe</td><td>0.04</td><td>s</td><td></td><td>5</td><td>14.3</td></tr>
<tr><td>SiMe</td><td>0.02</td><td>s</td><td></td><td>6</td><td>-4.2</td></tr>
</table>

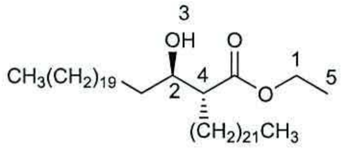
2.7.3 Desilylation of (69)

Following the method in the literature,[16] deprotection of the *tert*-butyldimethylsilyloxy group on compound **(218)** to give the corresponding secondary alcohol at the β-hydroxy of the carboxylic acid group was undertaken. This was carried out in a dry polyethylene vial and compound **(218)** was dissolved in dry THF, followed by the addition of pyridine and HF.pyridine complex. The mixture was stirred at 40 °C for 18 h to give the alcohol **(219)** in 88% yield (**Scheme 66**).



Scheme 66: Desilylation of compound (219)

The product (**219**) gave NMR spectra which were the same as the ones for the previous compound (**218**), except there were no signals corresponding to the protons of the *tert*-butyldimethylsilyl protecting group. The COSY spectrum of (**218**) showed a peak **A** which indicates a coupling between H1 at δ 4.20 ppm and H2 at δ 1.29 ppm. This corresponds to the coupling of the CH_3 group and the adjacent CH_2 on the ethyl group. Peak **B** shows a coupling between H2 at δ 3.67 ppm and H3 at δ 2.52 ppm. This corresponds to the coupling of the H on the hydroxyl group and the adjacent H on the β-position. Peak **C** indicates a coupling between H2 at δ 3.66 ppm and H4 at δ 2.42 ppm. This corresponds to the coupling of the H on the α-position and the adjacent H on the β-position (**Figures 36**).



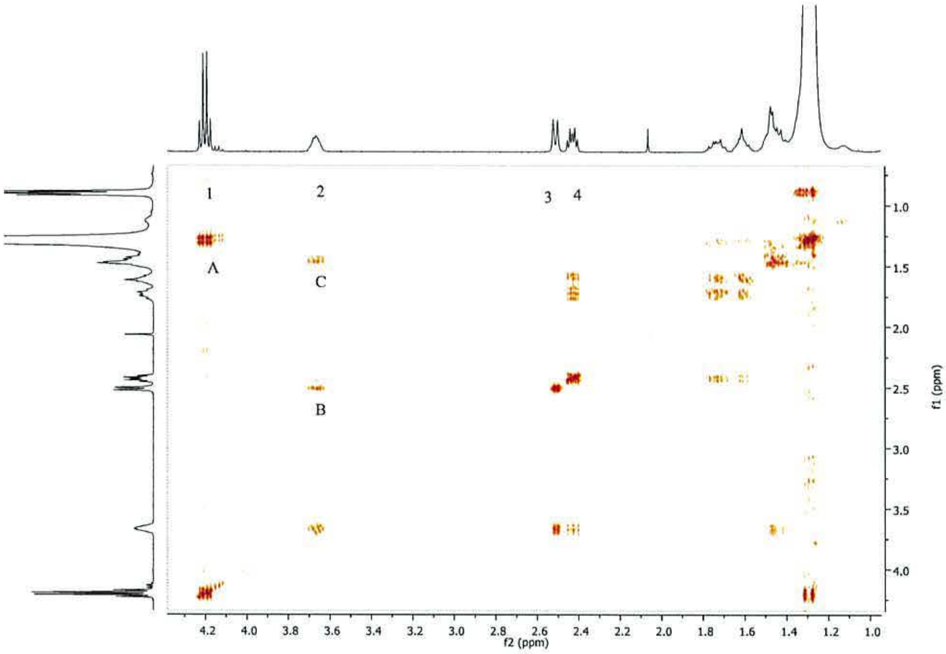
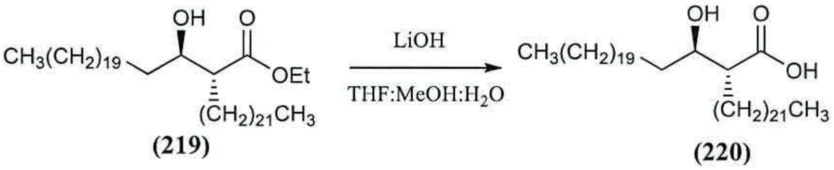


Figure 36: COSY spectrum of the product (219)

2.7.4 Preparation of (2*R*,3*R*)-2-docosyl-3-hydroxytetracosanoic acid (220)

The final step required for the preparation of the model mycolic acid (**220**), was the hydrolysis of the ethyl group. The ester (**219**) was stirred with lithium hydroxide monohydrate (15 mol. eq.) in a mixture of water, THF and MeOH (1:3:1) at 45 °C overnight (**Scheme 67**).



Scheme 67: Preparation of the mycolic acid (220)

The ^1H NMR spectrum of the final product (**220**) did not show any quartet corresponding to the CH_2 protons next to the oxygen of the ethoxy group. The acid showed two multiplets at δ 2.54 – 2.43 and 3.76 – 3.68 for the α- and β-protons respectively. The CH_2 protons of the long aliphatic chains were seen as a multiplet at δ 2.18 – 1.55, and a triplet at δ 0.88 for six protons corresponding to two terminal alkyl groups. In the ^{13}C NMR spectrum for (**220**), the carboxylic acid signal was observed at δ 174.5, followed by the carbon at the β-position which accused at δ 72.3. The α-carbon appeared at δ 50.7.

2.8 Synthesis of an α-mycolic acid using the new method to prepare the mycolic motif

Minnikin and Polgar reported that mycolic acids containing two *cis*-cyclopropanes were the major components of *M. tuberculosis.*[219] The molecule targeted in this section was the α-mycolic acid (**221**) (**Figure 32**) which is one stereoisomer of the major components of *M. tuberculosis*.

The method used to prepare the α-mycolic acid which can be summarised as shown in **Figure 37**. The strategy for the synthesis is divided into the following elements:

A. Preparation of single enantiomers of *cis*-cyclopropanes
B. Chain extension with a 19 – carbon unit
C. Chain extension with a 12 – carbon unit
D. Chain extension with a 9 – carbon unit
E. Preparation of the mycolic motif

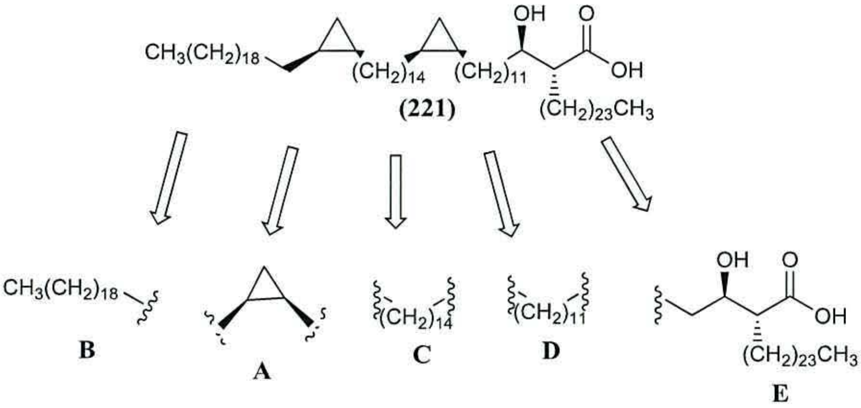


Figure 37: Synthesis of α-mycolic acid

A number of the steps of this synthesis were repeated from an earlier synthesis.[195]

2.8.1 Preparation of single enantiomers of *cis*-cyclopropanes

The single enantiomer of the *cis*-cyclopropane is a common unit in most mycolic acid types. The *cis*-cyclopropane ring in the *R,R*configuration was prepared by a standard method starting from methyl acrylate, methyl chloroacetate and sodium methoxide yielding the *cis*-diester **(222)** in a Michael Induced Ring Cyclisation (**Figure 38**).

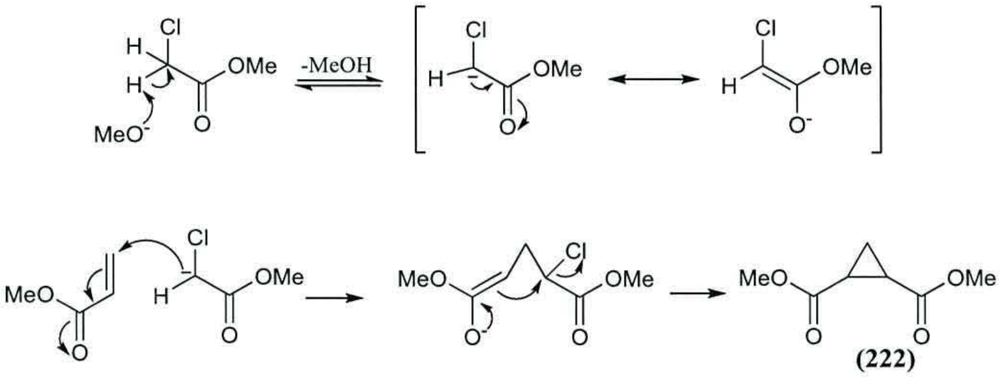
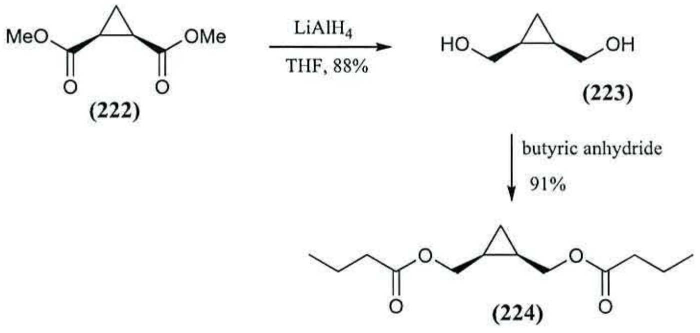


Figure 38: The mechanism of the Michael Induced Ring Cyclisation reaction

The cyclisation is initiated by the deprotonation of methyl chloroacetate using a base, and then the carbanion undergoes a Michael addition to the double bond of the methyl acrylate, to form an intermediate which then undergoes cyclisation.

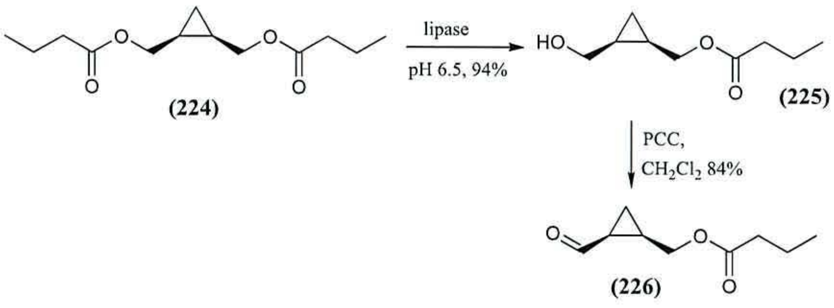
2.8.2 Reduction of the dimethyl *cis*-cyclopropane-1,2-dicarboxylate

Dimethyl *cis*-cyclopropane-1,2-dicarboxylate (**222**) was refluxed in a suspension of $LiAlH_4$ in THF for 1.5 h, and the reaction was then quenched with sodium sulfate decahydrate, to yield to diol **(223)**. The diol (**223**) was protected with butyric anhydride to give the dibutyrate (**224**) (**Scheme 68**).



Scheme 68: Preparation of the cis-cyclopropane-1,2-diylbis(methylene) dibutyrate (224)

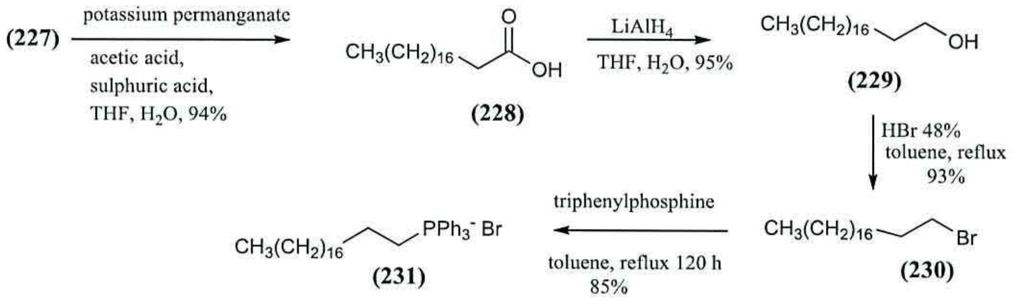
The key step in the synthesis was to prepare a monoester from the diester (**224**), This was carried out using a method reported by Grandjean *et al.*[220] The crude lipase extracted from pig pancreas (PPL) was added to the dibutyrate (**224**) at pH 6.8. When hydrolysis began, the pH was lowered due to the formation of butyric acid. An aqueous solution of sodium hydroxide was added by syringe to bring it back to pH 6.5. The completion of hydrolysis took 5 h and then work up gave the single enantiomer, ((1*R*,2*S*)-2-(hydroxymethyl*cis*-cyclopropyl) methyl butyrate (**225**) (**Scheme 69**). The ^1H NMR spectrum of (**225**) showed two doublets of doublets at δ 4.48 (*J* 5.7, 12.0 Hz) and 3.85 (*J* 5.4, 12.0 Hz) respectively, which corresponded to the two protons adjacent to the ester. The two protons adjacent to the hydroxy group appeared as doublets of doublets at δ 3.81 (*J* 9.8, 12.0 Hz) and 3.40 (*J* 9.2, 12.0 Hz), and the cyclopropane protons appeared as a multiplet at δ 1.37–1.24 (for two CH), as a doublet of triplets at δ 0.85 (for the *cis* proton of the CH_2, *J* 5.1, 8.5 Hz). The absolute stereochemistry of the cyclopropane was confirmed by its optical rotation giving a value of $[\alpha]_D^{22}$ + 23 which was close to the literature value of + 18.2.[19] Finally the alcohol (**225**) was oxidised to aldehyde (**226**) using pyridinium chlorochromate (PCC) in dichloromethane for the Wittig reaction (**Scheme 69**). The ^1H NMR spectrum of the product showed a signal at δ 9.45 due to the CHO proton.



Scheme 69: The enzymatic hydrolysis

2.8.3 Preparation of a C_{20} phosphonium salt

The phosphonium salt (**231**) was prepared starting from eicosene (**227**) using a standard method.[221] The alkene was oxidized to nonadecanoic acid (**228**) with potassium permanganate in the presence of water, acetic acid, hexadecyltrimethylammonium bromide and sulphuric acid. The nonadecanoic acid (**228**) was reduced by lithium aluminium hydride to nonadecan-1-ol (**229**) (**Scheme 70**).[221]



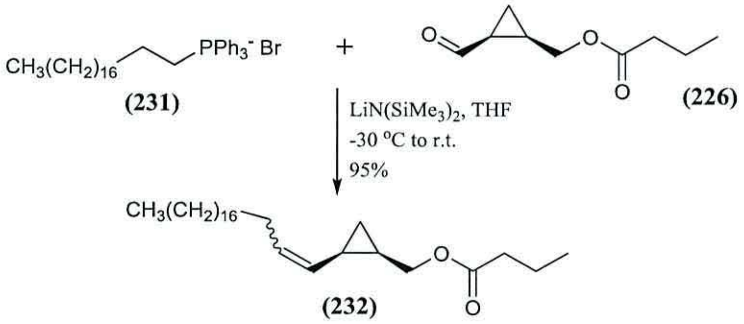
Scheme 70: Preparation of C_{20} phosphonium salt

The ^{1}H NMR spectrum of the protected compound (**229**) showed a triplet at δ 3.64 (*J* 6.6 Hz) for the protons adjacent to the hydroxy group The ^{13}C NMR spectrum showed a signal at δ 63.3 for the carbon adjacent to the hydroxy group.

The alcohol (**229**) was brominated to 1-bromononadecane (**230**) with 48% HBr refluxing in toluene (**Scheme 70**). The ^{1}H NMR spectrum of compound (**230**) showed a triplet at δ 3.40 (*J* 6.9 Hz) for the protons adjacent to the bromine. Finally the bromo-compound (**230**) was reacted with triphenylphosphine in toluene to give the phosphonium salt (**231**) (**Scheme 73**). The ^1H NMR spectrum showed a multiplet at δ 7.90 –7.65 for the phenyl group protons, and the ^{13}C NMR spectrum showed six signals in the aromatic region at δ 135.1, 135.0, 133.8, 133.7, 130.6 and 130.5. The required starting material, the phosphonium salt (**231**), was thus obtained in four steps with an overall yield of 70% (**Scheme 70**).

2.8.4 The chain extension

((1*R*,2*S*)-2-Formylcyclopropyl)methyl butyrate (**226**) and phosphonium salt (**231**) were coupled by a Wittig reaction to give the olefin (**232**) (95%). The product was a mixture of *E*- and *Z*-stereoisomers in ratio 1:4 (**Scheme 71**).



Scheme 71: The Wittig reaction

The success of the olefination was confirmed by the ^1H NMR spectrum of (**232**) which showed a doublet of triplets at δ 5.56 (*J* 14.8, 7.3 Hz) and a doublet of doublets at δ 5.21 (*J* 14.2, 7.8 Hz) for the olefinic protons in the *E*-stereoisomer. The high coupling constant (14.8 Hz) confirmed the formation of the *E*-isomer. A doublet of triplets at δ 5.47 (*J* 10.7, 7.3 Hz) and a doublet of doublets at δ 5.03 (*J* 10.7, 9.2 Hz) appeared for the olefinic protons in the *Z*-stereoisomer. The ^{13}C spectrum showed two signals at δ 132.3 and 127.3 for the *E*-isomer and two signals at δ 132.2 and 127.4 for the *Z*-isomer as shown in **Figure 39**.

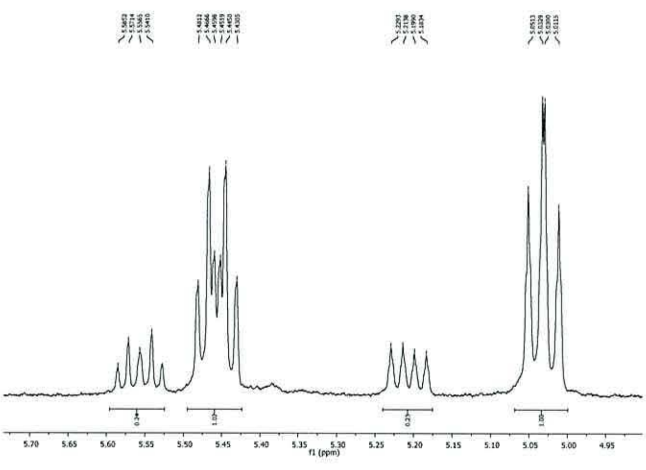
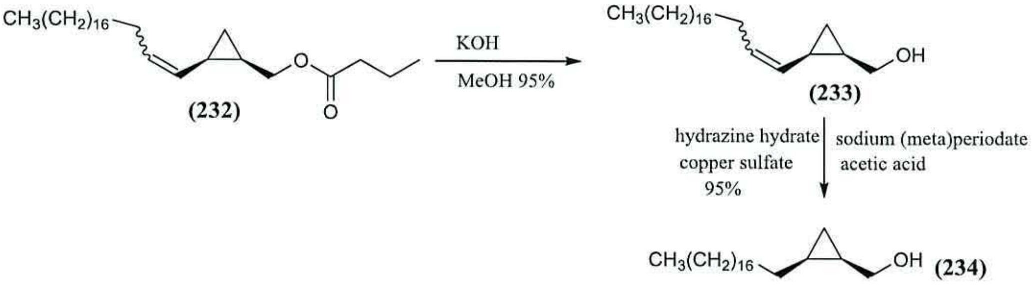


Figure 39: The ^1H NMR spectrum of the alkene (232) for the olefinic region

2.8.5 Reduction and hydrogenation of the alkene (232)

The unsaturated ester (**232**) was converted into ((1*R*,2*R*)-2-((*E/Z*)-icos-1-en-1-yl)cyclo-propyl)methanol (**233**) by KOH in THF in 95% yield (**Scheme 72**).



Scheme 72: Reduction and Hydrogenation of the alkene (232)

Hydrogenation of the unsaturated alcohol (**233)** using di–imide gave ((1R,2S)-2-henicosylcyclopropyl)methanol (**234)** in a yield of 95%. The di–imide was generated from hydrazine hydrate by oxidation with sodium (meta) periodate in the presence of glacial acetic acid and saturated aqueous copper sulfate (**Figure 40**).

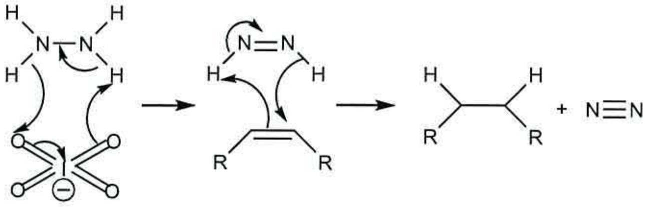


Figure 40: Mechanism for hydrogenation by di–imide

The NMR spectra of the product showed no peaks between δ 6.00 and 5.00. This suggested that the unsaturation had been removed, The ^{1}H NMR showed two doublet of doublets at δ 3.62 (*J* 7.1, 11.3 Hz) and 3.56 (*J* 8.0, 11.3 Hz) for the two protons adjacent to hydroxyl group. The two cyclopropane protons appeared as a triplet of doublets at δ 0.71 (*J* 4.6, 8.3 Hz) and a broad quartet δ - 0.06 (*J* 5.4 Hz) (**Figure 41**). The IR spectrum showed a broad peak at 3315 cm^{-1} for the O–H stretch. The synthesis of its enantiomer has been reported by Al Dulayymi *et al.*[221] and this showed identical spectroscopic properties. The specific rotation was +13, whereas in the literature the corresponding value for the enantiomer was –7.5. [221]

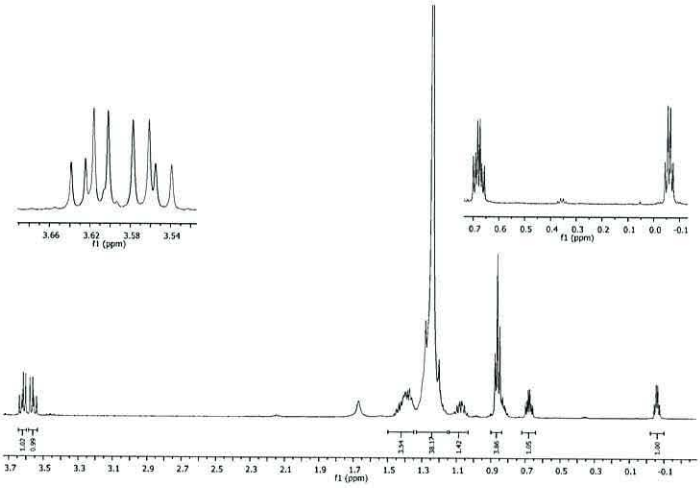
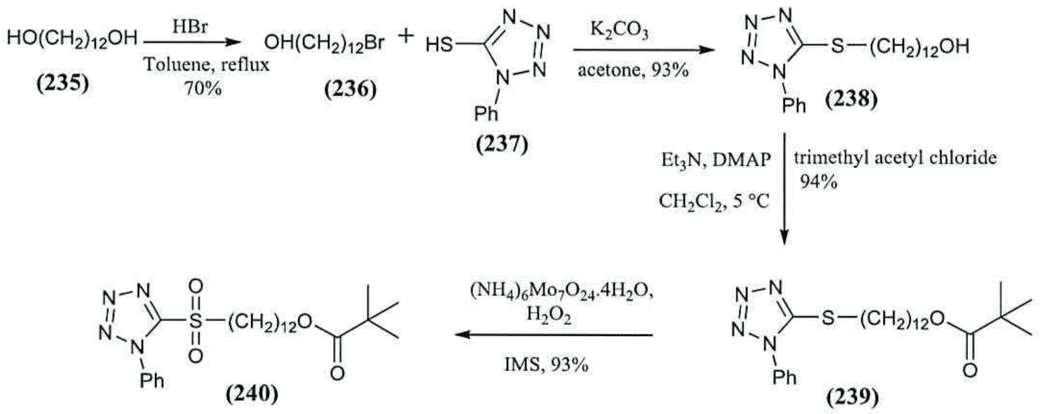


Figure 41: The ^{1}H NMR of the alcohol (234)

2.8.6 Preparation of C12 sulfone

The sulfone (**240**) with a protected hydroxyl group was prepared from commercially available dodecane-1,12-diol (**235**). The diol was refluxed with 48% HBr in toluene to give the mono-bromo alcohol (**236**). 1-Phenyl-1*H*-tetrazole-5-thiol (**237**) was then reacted with this to form 12-((1-phenyl-1*H*-tetrazol-5-yl)thio)dodecan-1-ol (**238**) (**Scheme 73**).



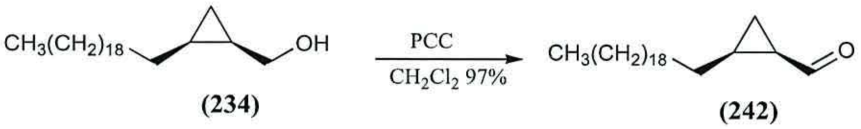
Scheme 73: Preparation of C₁₂ sulfone

The ^1H NMR spectrum of the protected compound (**238**) showed a multiplet at δ 7.63 – 7.49 for the phenyl group protons. There was also a triplet at 3.63 (*J* 6.6 Hz) for the protons adjacent to the hydroxyl group, and a triplet at δ 3.38 (*J* 7.4 Hz) for the -CH_2- bonded to the S atom. The ^{13}C NMR spectrum showed four signals at δ 133.9, 130.2, 129.9 and 124.0 for the phenyl group carbons. One signal was seen at δ 154.7 for the tetrazole ring carbon. The mono-alcohol (**238**) was protected with trimethylacetyl chloride in the presence of triethylamine and 4-dimethylamino-pyridine (DMAP) to give the sulfane (**239**). This was then oxidised to corresponding sulfone (**240**) using an excess of hydrogen peroxide and ammonium molybdate (VI) tetrahydrate (**Scheme 73**). The ^1H NMR spectrum of the protected sulfone (**240**) showed a multiplet at δ 7.63 – 7.46 for the phenyl group protons, a triplet at δ 4.02 (*J* 6.6 Hz) for the protons adjacent to the carbonyl group, and a triplet at δ 3.37 (*J* 7.4 Hz) for the CH_2 adjacent to the sulfur. The *tert*-butyl group protons appeared at δ 1.17 as a singlet. The ^{13}C NMR spectrum showed a signal at δ 178.6 for the carbonyl group and five signals in the aromatic region. The *tert*-butyl methyl carbons appeared at δ 27.2. The IR showed a peak at 1729 cm^{-1} for the C=O stretch. The sulfone (**240**) was obtained in an overall yield of 57% (**Scheme 73**).

2.8.7 The second chain extension of the cyclopropane

2.8.7.1 Oxidation of the alcohol (234) to aldehyde (242)

The alcohol (**234**) was oxidised to the corresponding aldehyde (**242**) with PCC in dichloromethane in excellent yield (97%) for a further Julia reaction (**Scheme 74**).

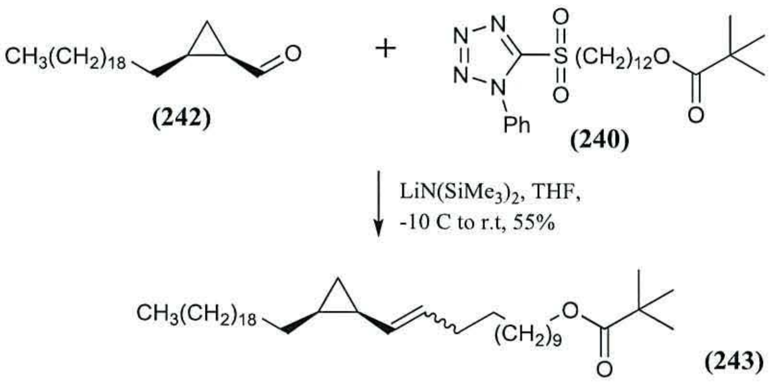


Scheme 74: Oxidation of the alcohol (234) to aldehyde (242)

The ^1H NMR spectrum of the aldehyde (**242**) showed a doublet at δ 9.3 (*J* 1.6 Hz) for the aldehyde proton. The ^{13}C NMR spectrum showed a signal at δ 202.0 for the carbonyl group of the aldehyde.

2.8.7.2 The Julia coupling reaction

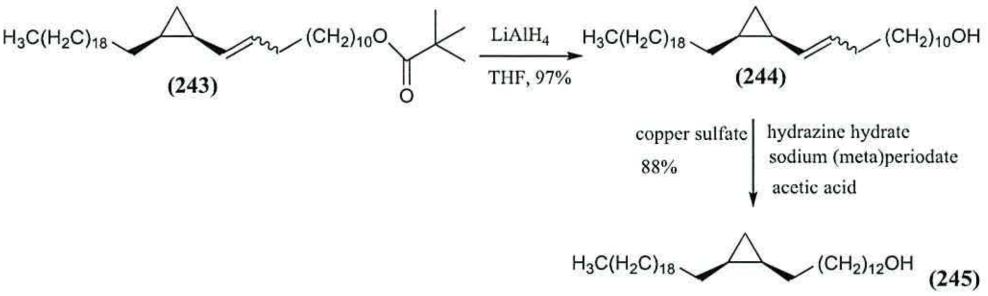
The coupling of the aldehyde (**242**) with the sulfone (**240**) was carried out by means of a modified Julia reaction in the presence of lithium *bis*(trimethylsilyl)amide in dry THF to give the olefin (**243**) as a mixture of *E*- and *Z*-isomers in a yield of 55% (**Scheme 75**).



Scheme 75: The Julia coupling reaction between (242) and (240)

2.8.7.2 Deprotection and hydrogenation

The stereoisomeric mixture of unsaturated esters (**243**) was deprotected employing a suspension $LiAlH_4$ in dry THF to obtain the primary alcohol (**244**) in 97% yield. The unsaturated alcohol (**244**) was then saturated as described in **Section 2.8.5**, to give (**245**) in a very good yield of 88% (**Scheme 76**).



Scheme 76: Deprotection and hydrogenation of the unsaturated ester (243)

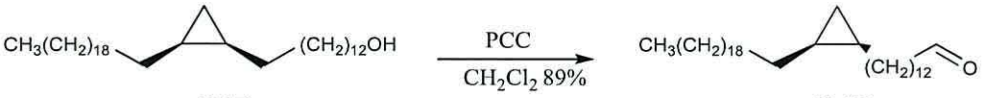
The ^1H NMR spectrum of the product compound (**245**) showed no peaks between δ 6.00 and δ 5.00 corresponding to the unsaturated olefin protons, which showed that the hydrogenation had been successful. The ester protons in the ^1H NMR spectrum disappeared and a multiplet appeared at δ 3.69 – 3.60 for the two protons adjacent to the hydroxy group. The four cyclopropane protons appeared as a 2 hydrogen multiplet between at δ 0.69 – 0.61, a one hydrogen doublet of triplets at δ 0.71 (*J* 4.4, 8.2 Hz) and a one hydrogen quartet at δ - 0.34 (*J* 5.3 Hz). The specific rotation for the compound (**245**) $[\alpha]_D^{22}$ - 2.1 (c 1.2, $CHCl_3$) was in good agreement with that in the literature, $[\alpha]_D^{22}$ - 2.0 (c 1.0, $CHCl_3$).[221]

2.8.8 Coupling of the two cyclopropane fragments

In this final series of reactions to form the meromycolate moiety, compound (**245**) was converted into an aldehyde (**246**) in order to couple it with the required sulfone in a modified Julia-Kocienski olefination reaction.

2.8.8.1 Oxidation of the alcohol (245) to aldehyde (246)

The alcohol (**245**) was oxidised to the corresponding aldehyde (**246**) with PCC in dichloromethane in excellent yield (89%) for a further Julia reaction (**Scheme 77**).



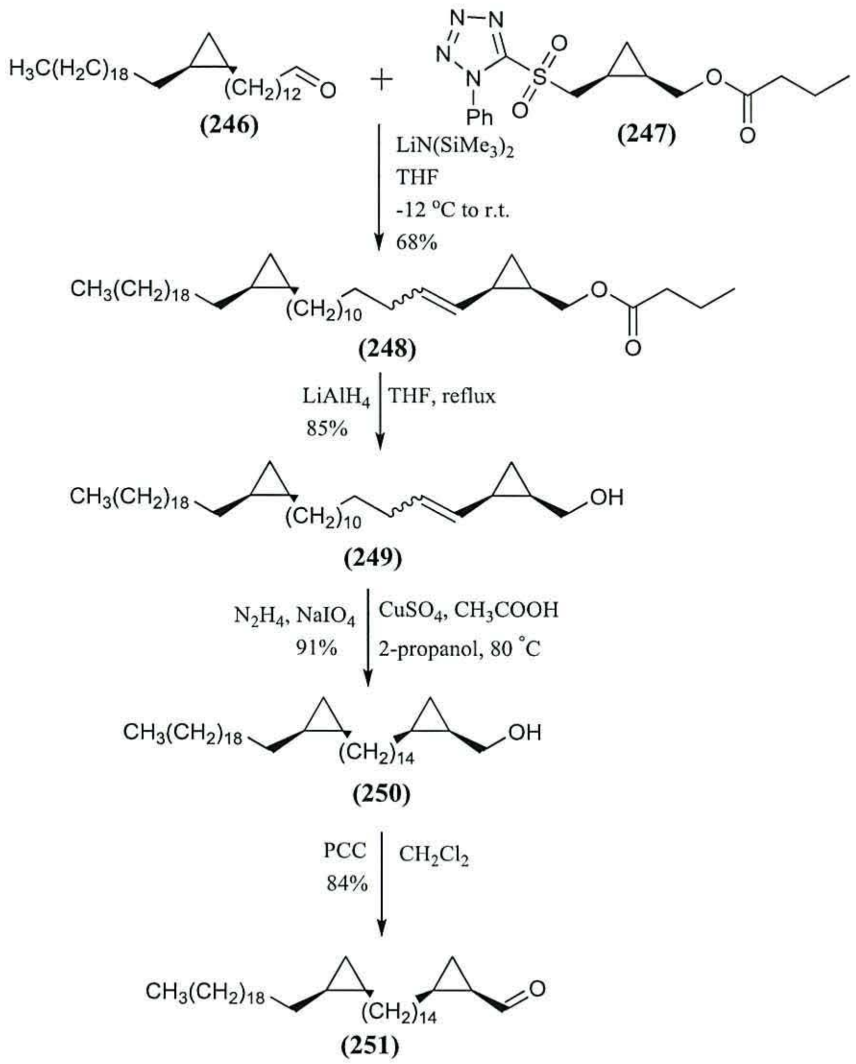
(245) (246)

Scheme 77: Oxidation of the alcohol (245) to aldehyde (246)

The aldehyde (**246**) was characterised by its ^{1}H NMR spectrum which identical to the literature.[195]

2.8.8.2 Preparation of the aldehyde (251)

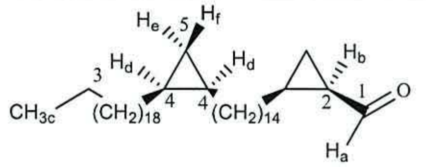
By following the procedure in **Sections 2.8.7.1**, **2.8.7.2**, and **2.8.7.3**., the aldehyde (**251**) was prepared. Through use of the Julia reaction, aldehyde (**246**) and butyrate (**247**) were coupled to give the olefinic ester (**248**) (68%). The product was a mixture of *E*- and *Z*-stereoisomers (**Scheme 78**). The ester (**248**) was then reduced using lithium aluminium hydride following the same procedure as applied to compound (**243**), to yield alcohol (**249**), which was then hydrogenated by di-imide to yield the required dicyclopropane intermediate (**250**) (**Scheme 78**).



Scheme 78: Preparation of the aldehyde (251)

Using the same procedure as given in Section **2.8.8.1**, oxidation of (**250**) with pyridinium chlorochromate (PCC) in dichloromethane gave 84% of the corresponding aldehyde (**251**) (**Scheme 79**). Selected NMR data are shown in **Table 18**

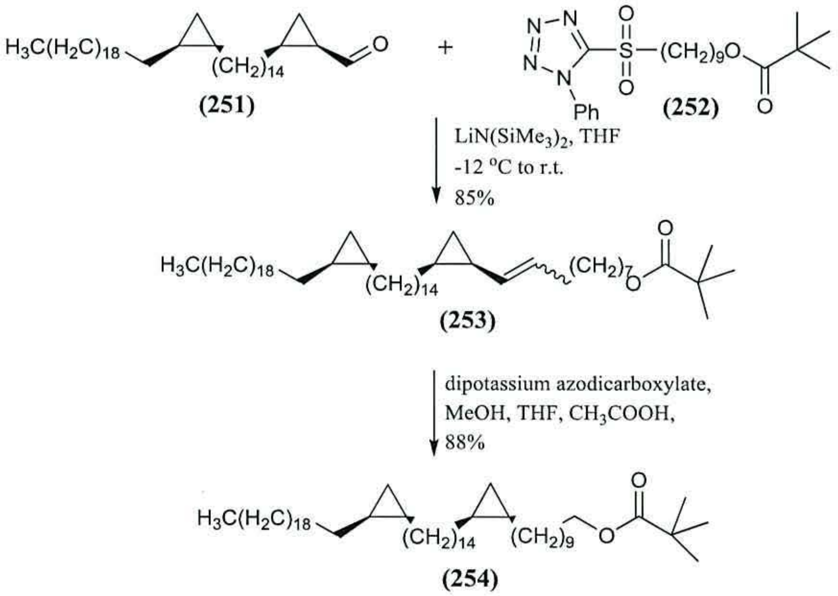
Table 18: The ^{1}H and ^{13}C NMR data of aldehyde (251)



Proton	δ	Multiplicity	J	Carbon	^{13}C NMR
H_a	9.35	d	5.6	1	201.9
H_b	1.91 – 1.81	m		2	27.9
H_c	0.88	t	6.8	3	16.0
H_d	0.69 – 0.60	m		4	15.0
H_e	0.56	br.dt	4.1, 8.2	5	11.1
H_f	-0.33	br.q	5.4		

2.8.9 The chain extension

The aldehyde (**251**) was coupled with the sulfone (**252**) to give the olefin (**253**) in 85% yield as a mixture of *E*- and *Z*-isomers (**Scheme 79**).



Scheme 79: The chain extension

The alkene (**253**) was dissolved in THF and MeOH, and treated with di-imide, in this case generated from dipotassium azodicarboxylate. A solution of acetic acid in THF was added at 5℃ to this stirred mixture and after 2 h more acetic acid solution was added and the mixture was stirred for 2 days at room temperature. The NMR spectra showed that there was still unreacted alkene; therefore the reaction was repeated to complete the hydrogenation. The saturated product (**254**) was obtained as a white solid in 88% yield (**Scheme 79**). Selected NMR data are shown in **Figure 42** and **Table 19**.

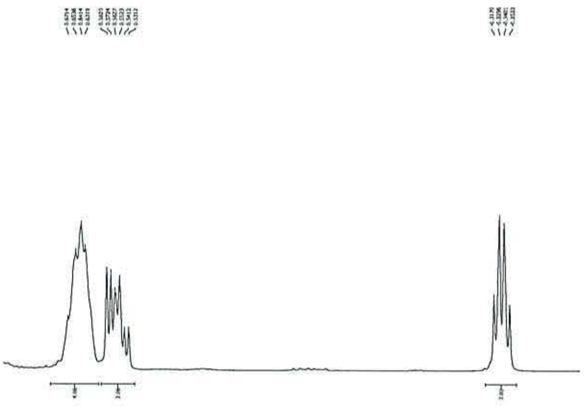
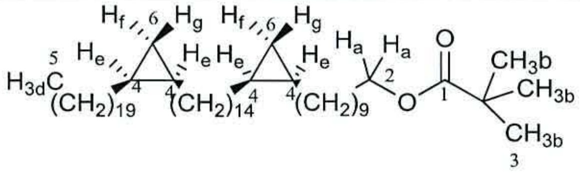


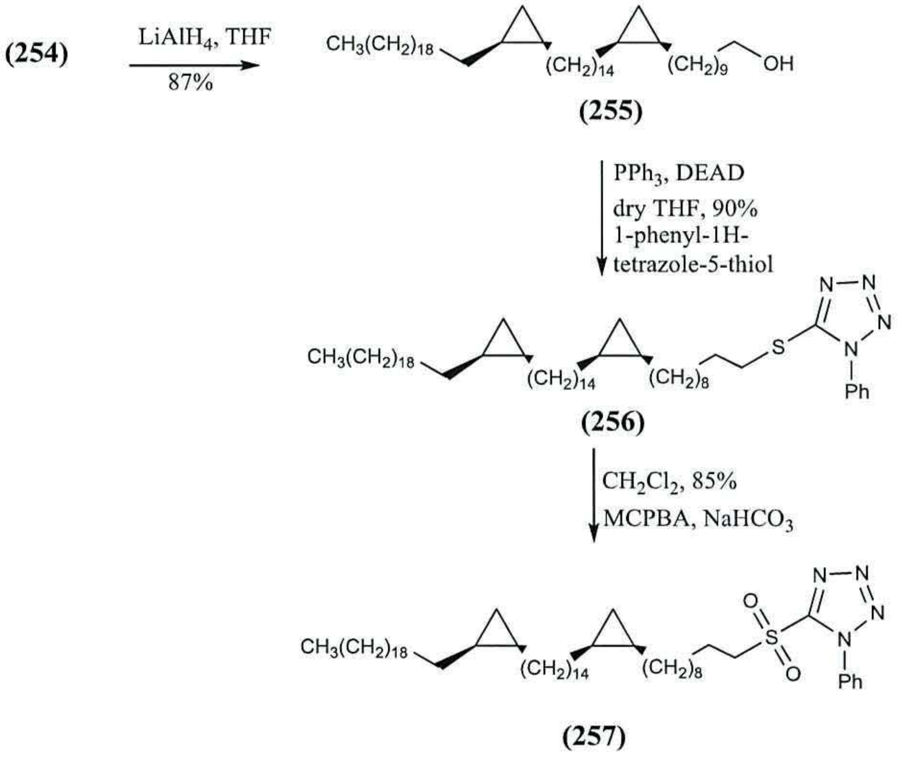
Figure 42: The signals of the eight cyclopropane protons of (254)

Table 19: The ^1H and ^{13}C NMR data for compound (254)

<table>
<tr><td colspan="6">
</td></tr>
<tr><td>Proton</td><td>δ</td><td>Multiplicity</td><td><i>J</i></td><td>Carbon</td><td>¹³C NMR</td></tr>
<tr><td>H_a</td><td>4.04</td><td>t</td><td>6.6</td><td>1</td><td>173.8</td></tr>
<tr><td>H_b</td><td>1.1</td><td>s</td><td></td><td>2</td><td>64.6</td></tr>
<tr><td>H_d</td><td>0.88</td><td>t</td><td>6.8</td><td>3</td><td>27.3</td></tr>
<tr><td>H_d</td><td>0.69 – 0.60</td><td>m</td><td></td><td>4</td><td>15.9</td></tr>
<tr><td>H_e</td><td>0.56</td><td>br.dt</td><td>4.1, 8.2</td><td>5</td><td>14.3</td></tr>
<tr><td>H_f</td><td>-0.33</td><td>br.q</td><td>5.4</td><td>6</td><td>11.1</td></tr>
</table>

2.8.10 Preparation of the intermediate sulfone

The ester group in **(254)** was deprotected with $LiAlH_4$ in THF to give the corresponding alcohol **(255)** in 97% yield (**Scheme 80**). The alcohol **(255)** was then reacted with 1–phenyl–1*H*–tetrazole–5–thiol in the presence of diethyl azodicarboxylate and triphenylphosphine in dry THF, to give a sulfane in a yield of 90% **(256)**. Finally this sulfane **(256)** was oxidised to the intermediate sulfone **(257)** using *meta*-chloroperbenzoic acid and sodium hydrogen carbonate in dichloromethane, in an excellent yield of 85% (**Scheme 80**).

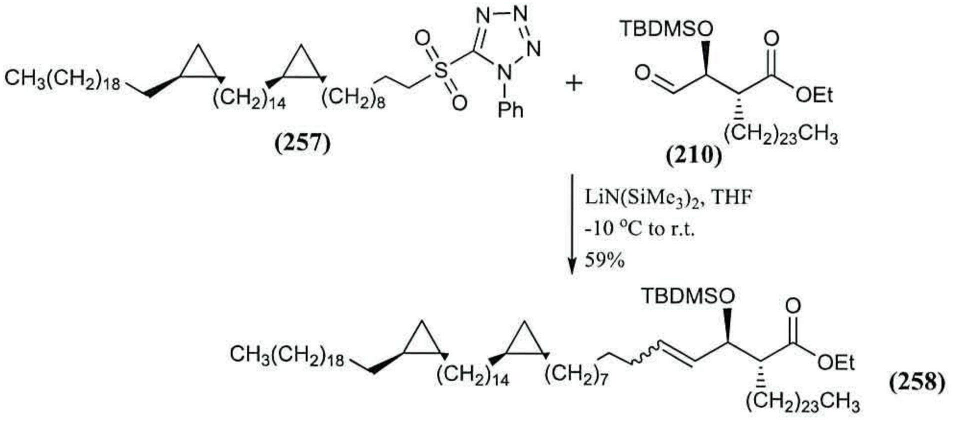


Scheme 80: Preparation of the intermediate sulfone (257)

The spectroscopic data for the sulfane **(256)** and the sulfone **(257)** were similar. The ^{1}H NMR spectrum of the sulfone **(257)** showed multiplets at δ 7.71–7.60 corresponding to the phenyl protons, and a triplet at δ 3.39 (*J* 7.4 Hz) for the two protons next to sulphur. The two sets of cyclopropane protons appeared as a multiplet at δ 0.71 – 0.59, a triplet of doublets at 0.55 (*J* 4.1, 8.2 Hz) and a quartet at δ -0.34 (*J* 5.1 Hz). The overall yield of this conversion was 66%.

2.8.11 The coupling of the sulfone (257) to the mycolic motif

A coupling reaction between the aldehyde (**210**) (prepared in **Section 2.7**) and the sulfone (**257**) using lithium *bis*(trimethylsilyl)amide as the base, gave the alkene (**258**) in 59% yield (**Scheme 81**).

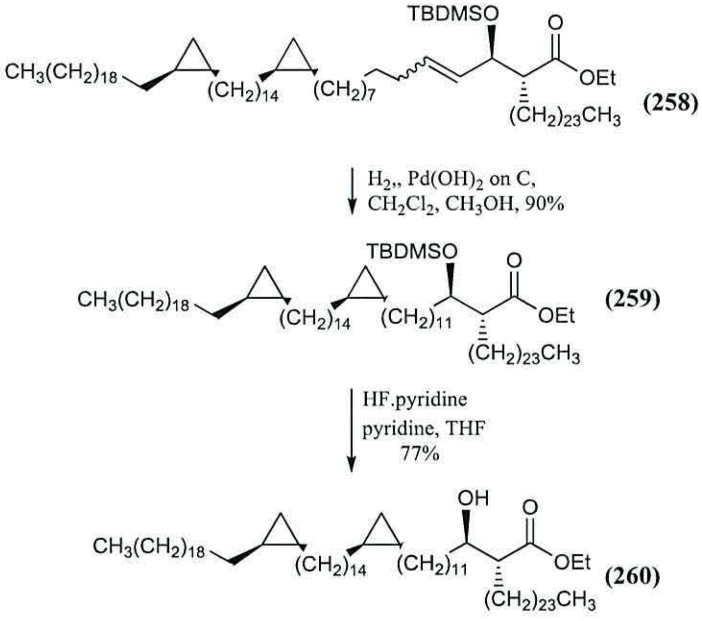


Scheme 81: Coupling of the sulfone (257) to the mycolic motif

The ^{1}H NMR spectrum of the product showed olefinic signals at δ 5.61 – 5.51 and 5.32 – 5.23 which confirmed that the olefination reaction had taken place.

2.8.12 Hydrogenation and deprotection of the unsaturated ester

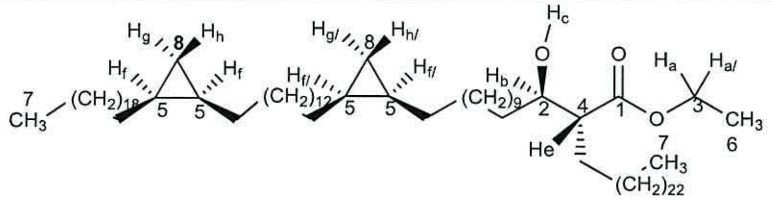
The alkene (**258**) was hydrogenated with hydrogen gas using palladium hydroxide on carbon as a catalyst in dichloromethane and methanol to give the saturated ester (**259**) in 90% yield (**Scheme 82**). On the other hand the hydrogenation in the literature was done by using dipotassium azo-dicarboxylate in dry THF then a solution of glacial acetic acid in dry THF was added dropwise over three days to complete the hydrogenation reaction in 60% yield.[195] In the new method in this work, the hydrogenation reaction was done in just 30 min.



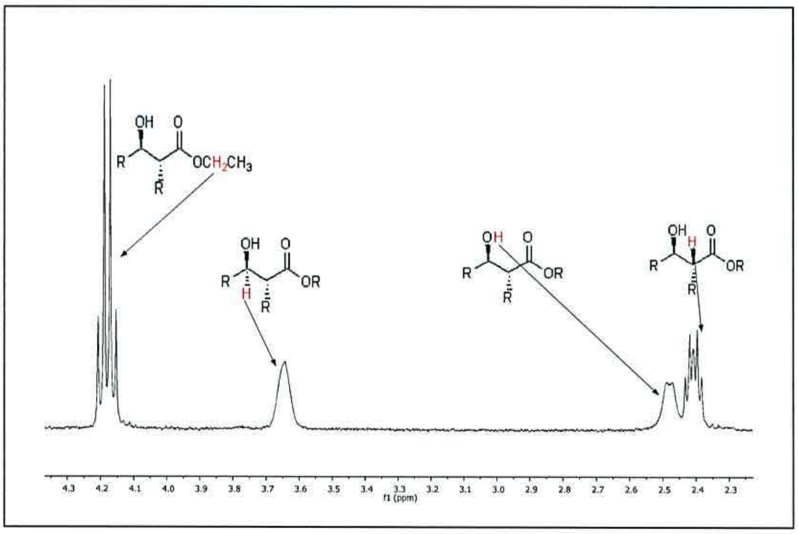
Scheme 82: Preparation of protected mycolic acid (260)

The NMR spectrum of the product did not show any signals for the double bonds and the other signals were as expected. The silyl protecting group in (**259**) was removed using HF.pyridine complex, giving the protected mycolic acid (**260**) in 77% yield (**Scheme 82**). The ^{1}H NMR and ^{13}C NMR spectra of (**260**) are given in **Table 20** and **Figure 43.** The data was identical to those in literature,[195] except the signal at δ 4.18 (2H, q, *J* 7.1 Hz OC$\underline{H}_2$CH$_3$) which belongs to the ethyl ester in this work while it appeared as a singlet at δ 3.68 (3H, s, OC$\underline{H}_3$) in the literature for the methyl ester group.[195]

Table 20: Selected ^{1}H and ^{13}C NMR data for the α-mycolic acid ethyl ester (260)



Proton	δ	Multiplicity	*J* (Hz)	Carbon	δ
H_a, H_a'	4.18	q	7.1	1	175.1
H_b	3.64	br s		2	72.5
H_c	2.48	d	7.4	3	60.5
H_e	2.41	dt	5.4, 9.4	4	51.0
2 x CH_3	0.88	t	6.8	5	15.9
H_f, H_f'	0.64	br s		6	14.5
H_g, H_g'	0.56	dt	4.1, 8.2	7	14.3
H_h, H_h'	– 0.34	q	5.1	8	11.1



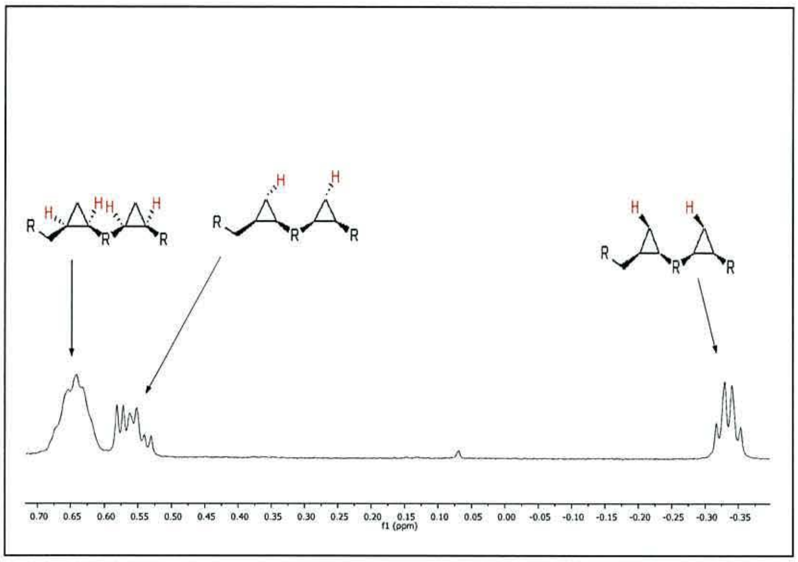


Figure 43: Selected signals in the ^1H NMR spectrum of (260)

3. Conclusion

The first aim of this study was a novel synthesis of gylcerol monomycolates (GroMM) from single synthetic mycolic acids. The preparation of several types of GroMM with different functional groups in both the proximal and distal positions of mycolic acid was detailed. Synthesising these compounds for the first time opens the way to understanding their properties and applications not only in immune mediated illnesses but also in other areas such as TB and viral inhibition. In order to achieve the esterification reaction between the glycerol and the corresponding mycolic acid, the first reactions to couple protected methoxy mycolic acid (**164**) to the glycerol (**163**), used DMAP and DCC to give the protected methoxy mycolic acid glycerol ester. A fully deprotected GroMM (**149**) was obtained using trifluoroacetic acid in dichloromethane (**Figure 44**).

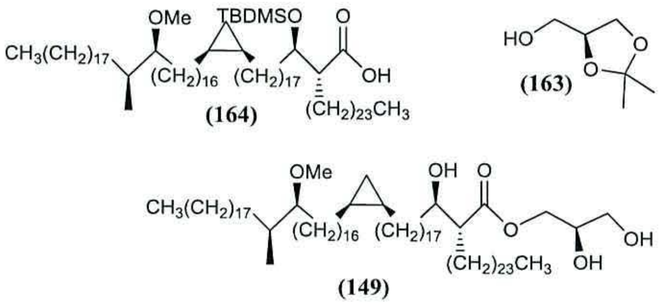
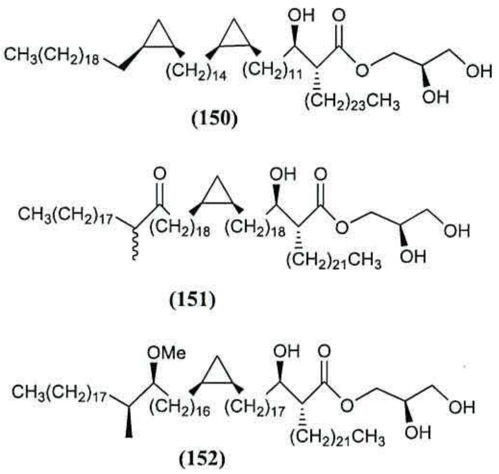


Figure 44: Synthesis methoxy mycolic acid glycerol ester (149)

In the second reaction for the synthesis of glycerol monomycolate from unprotected mycolic acid, it was necessary to convert the hydroxyl group in (**163**) into a good leaving group such as to *p*-toluenesulfonate (tosylate), and then couple with three different types of mycolic acid (**168**), (**170**), and (**172**) (supplied by Dr. Al-Dulayymi) using $CsHCO_3$. The protected GroMMs (**169**), (**171**), and (**173**) were obtained. The final products of GroMMs (**150**) - (**152**) were obtained after deprotection by using hydrochloric acid in THF and water (**Figure 45**).



(150)

(151)

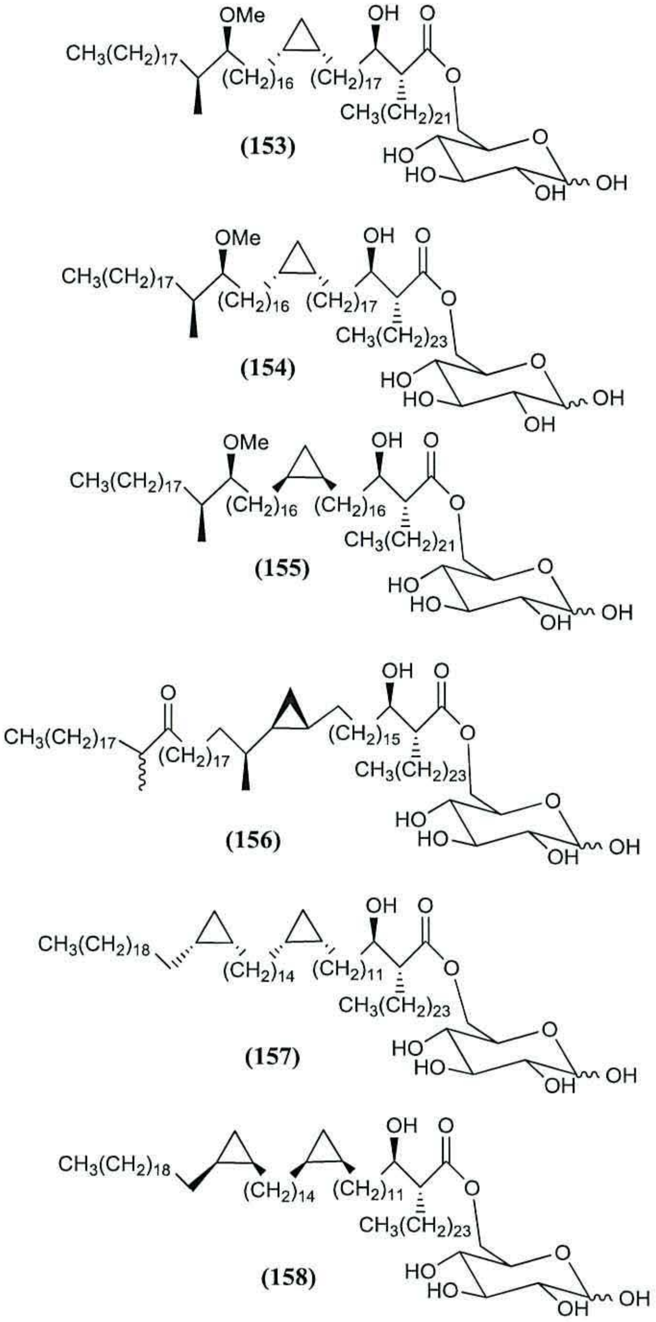
(152)

Figure 45: The glycerol monomycolates synthesised

The significant step in this part of synthesis mycolic acid glycerol ester is the deprotection reaction which was achieved successfully, because it might hydrolyse the ester group or open the cyclopropane ring in addition to the dioxolanyl group.

Preliminary biological activity tests were carried out in Brussels on using each of the four GroMM compounds **(149)** - **(152)** as antigens for TNF-α production. Unfortunately the results were very low and that might belong to the wrong selection of cytokines TNF-α production because the cytokines INF-γ production preferred as a signal for GroMMs than the cytokines TNF-α signals.[192] Another possible reason for the lacking of activity might belong to the stereochemistry of the chiral centre of the hydroxyl group in the glycerol unit. In addition to that the choice of the different mycolic acids might be another reason.

The second part of this work was the synthesis of six GMM compounds involving single synthetic mycolic acid, including *cis*-cyclopropane methoxy mycolic acid glucose esters **(153)** - **(155)**, *trans*-cyclopropane keto-mycolic acid glucose ester **(156)**, and *cis*-cyclopropane α-mycolic acid glucose esters **(157)** and **(158)** (**Figure 46**).



(153)

(154)

(155)

(156)

(157)

(158)

Figure 46: The glucose monomycolates synthesised

The modified esterification reaction was found to be the best for coupling in the synthesis of GMMs. This involved coupling between a tosylate (**178**) and different types of mycolic acid (supplied by Dr. Al-Dulayymi) using $CsHCO_3$ in dry DMF/THF in ratio 1:1. Hydrogen gas was used for hydrogenation at atmospheric pressure for protected GMM products to achieve the final GMMs (**Figure 46**). Palladium hydroxide on charcoal was used as a catalyst in CH_2Cl_2/CH_3OH as a solvent. The NMR data after the hydrogenation of the benzyl ethers showed that no reductive opening of the cyclopropane rings had occurred during this process.

Preliminary ELISA tests have been carried out by Dr. A. Jones on using each of the five GMM compounds **(153)** - **(157)** as antigens and a set of sera from TB-indigenous countries provided by the WHO and diagnosed as TB-positive or TB-negative based on a range of assays. The initial ELISA results using **(153)** - **(157)** showed a distinction between the TB+ and TB- serum for **(154)**, **(156)** and **(157)**, while **(153)**, **(155)** showed no distinction with the average responses for both the TB+ and TB- samples being almost the same (**Figure 25, Section 2.5.6**). The selectivity and specificity of **(157)** showed the best sensitivity (89%) which was the same as that observed for the best antigen (AD132) **see Table 12 page 95** and **(156)** gave the best specificity and **(153)** also gave a good sensitivity (83%), however the specificity was again low. Antigens **(154)** and **(157)** showed a high specificity of 84 and 93% respectively; however the sensitivity for these antigens was low (**Table 12, Section 2.5.6**).

The third part of this thesis was the development of a method for the synthesis of the α-alkyl-β-hydroxy fragment (mycolic motif) which is a common unit in all mycolic acids. The preparation of this part unit has so far proved to be the most difficult part of mycolic acid synthesis. The improvement of these prodecures was important in order to ease the hard work entailed in the overall synthesis of mycolic acids. The classic two approaches to the preparation of the *α*-alkyl-*β*-hydroxy unit have been reported in literature.[203, 204] Baird *et al.* [203] conducted the first approach of the chain extension using (2-benzeneoxyethyl) oxirane as the chiral intermediate. The (2-benzeneoxyethyl) oxirane **(64)** (**Figure 47**) was prepared from commercially available *L*-aspartic acid **(59)**, according to methods developed by Frick *et al.*[202]

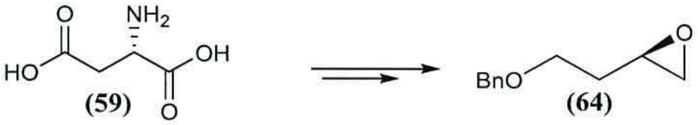
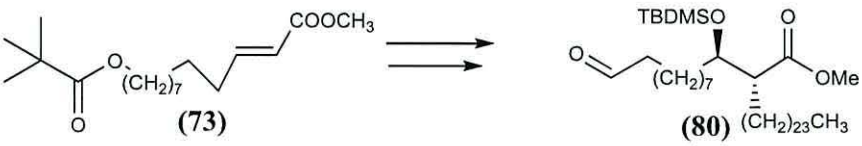


Figure 47: L-aspartic acid (59) and (2-benzeneoxyethyl) oxirane (64) strutures

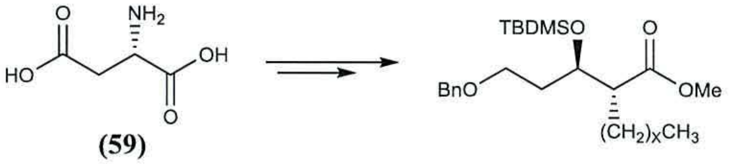
On the other hand Toschi *et al.*[185] was reported a short chain allylation was initially carried out followed by a further elongation by modified Julia-Kocienski reaction to achieve the intended chain length (**Figure 48**).



(73) **(80)**

Figure 48: The achieved (73) and (80) compounds by Toschi et al [185]

Koza *et al* [222] reported *L*-aspartic acid as the starting material for the synthesis of the (2-benzeneoxyethyl) oxirane intermediate and then a short chain Fräter allylation followed by a further Julia-Kocienski chain extension on the α-position (**Figure 49**).

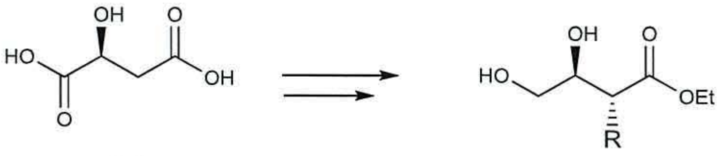


(59)

x = 21; (71)
x = 23; (72)

Figure 49: The achieved (59), (71), and (72) compounds by Koza et al [222].

Khan *et al.*[223] has prepared the β-hydroxy ester using *L*-malic acid which was esterified to give diethyl (*S*)-malate and then was alkylated with different allylic halides by Fräter allylation followed by selective reduction with $BH_3{\cdot}DMS$ to give diol (**85**) (**Figure 50**).



(81) **(85)**

Figure 50: The achieved (81) and (85) compounds by Khan et al [223].

In this thesis work *L*-malic acid was used as a cheap starting material (**Figure 51**).

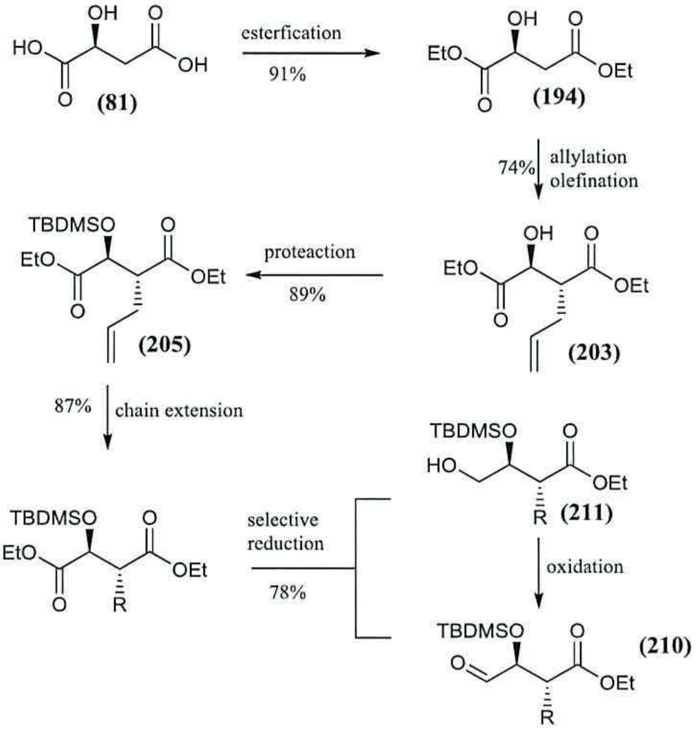


Figure 51: A New method for the preparation of the mycolic motif

This new method was successfully completed in 6 steps with an overall yield of 40%. In comparsion with the procedure by *Koza et al.* [222] which was achieved in 17 steps with an overall yield of 17%, the number of reaction steps has been reduced by 11 steps with a good percentage yield.

In addition, the final product (**210**) in this new method was successfully coupled with various functional groups of the meromycolate unit as explained earlier in **Section 2.7** and **Section 2.8**. On the other hand the final product (**85**) which prepared by Khan *et al* [223] was not successfully coupled with the various functional groups of the meromycolate unit because they couldnot protect only the secondary hydroxyl and leave the primary hydroxyl in (**85**) [212]

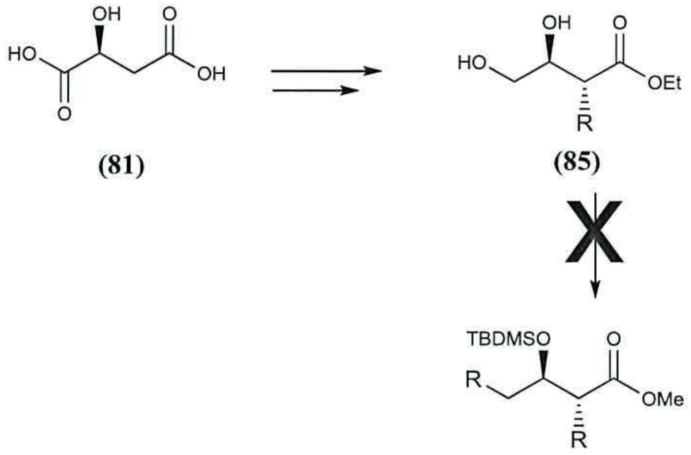


Figure 52: Unsucesfull coupling mycolic acid synthesis

As a result, the new method for the synthesis of the mycolic motif has been applied to prepare two mycolic acids (**159**) and (**160**) as in (**Figure 53**).

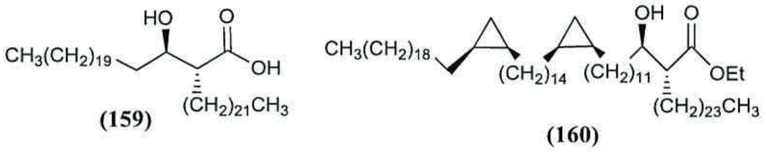


Figure 53: The mycolic acids (159) and (160) synthesised using the new method

In assessment, the mycolic acid is the most important part of synthesis of these glycerol and glucose monomycolates and the synthesis of mycolic acids take a very long time and steps to acheive Therefore; the work on the development of the new method for the synthesis of meromycolate fragments of mycolic acids in fewer steps will be the next target.

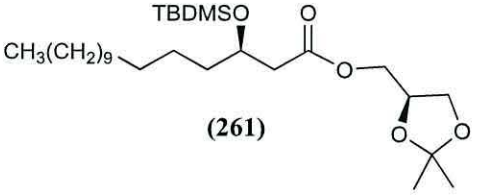
4. Experimental

4.1 General considerations

All chemicals were purchased from commercial suppliers. THF was distilled over sodium and benzophenone under nitrogen, while dichloromethane was distilled over calcium hydride. Petrol refers to the fraction bp 40–60 °C. All organic solutions were dried over anhydrous magnesium sulfate. 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. A cooling bath of methylated spirit and liquid nitrogen was used for reactions carried out at low temperatures. Silica gel and silica gel plates used for column chromatography and thin layer chromatography were obtained from Aldrich. 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). Optical rotations were measured as solutions in chloroform of known concentration using a Polar 2001 automatic polarimeter. NMR spectra were recorded either on an Advance 400 or 500 spectrometers; for ^{1}H spectra the machine was ran at 400MHz, and ^{13}C spectra were run at 125 MHz. Accurate mass MALDI data were obtained by Dr P. Gates, University of Bristol.

4.2 Experiments

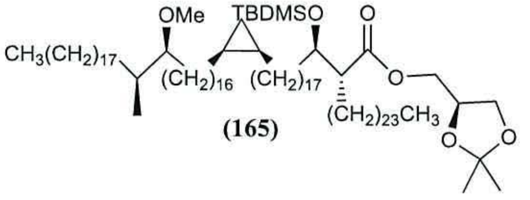
Experiment 1: ((*R*)-2,2-Dimethyl-1,3-dioxolan-4-yl)methyl(*R*)-3-((*tert*-butyldimethylsilyl)oxy)hexadecanoate (261)



(261)

(*R*)-3-((*tert*-Butyldimethylsilyl)oxy)hexadecanoic acid (25 mg, 0.06 mmol, supplied by Dr. Al Dulayymi) and DMAP (9 mg, 0.07 mmol) were added to a stirred solution of (*S*)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanol (**163**) (8 mg, 0.06 mmol) in CH_2Cl_2 (1 mL) then a solution of DCC (20.0 mg; 0.09 mmol) in CH_2Cl_2 (1 mL) was added dropwise with stirring under nitrogen atmosphere over 30 min then stirred for 1 h at room temperature. TLC showed no starting material was left. Precipitated dicyclohexylurea was filtered off and washed with CH_2Cl_2 (10 mL). The resulting solution was concentrated and the crude residue was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a semi-solid, *((R)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl(R)-3-((tert-butyldimethylsilyl)oxy)hexadecan-oate* (**261**) (28 mg, 87%), $[\alpha]_D^{23} - 5.5$ (*c* 1.2, $CHCl_3$), [Found $(M+Na)^+$: 523.4, $C_{28}H_{56}NaO_5Si$ requires: 523.4], which showed δ_H (500 MHz, $CDCl_3$): 4.35 – 4.27 (1H, m), 4.17 – 4.05 (4H, m), 3.74 (1 H, dd, *J* 8.5, 6.1 Hz), 2.51 – 2.42 (2H, m), 1.43 (3H, s), 1.36 (3H, s), 1.25 (23H, s), 0.95 – 0.80 (15H, m), 0.05 (3 H, s), 0.02 (3H, s); δ_C (126 MHz, $CDCl_3$): 171.8, 109.9, 73.7, 69.6, 66.7, 64.8, 42.6, 37.7, 32.1, 29.8, 29.7, 29.6, 29.5, 26.7, 25.9, 25.5, 25.1, 22.8, 18.1, 14.2, -4.4, -4.6; ν_{max}/ cm^{-1}: 2924, 2850, 1744, 1374, 1385.

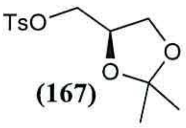
Experiment 2: ((*R*)-2,2-Dimethyl-1,3-dioxolan-4-yl)methyl (*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-18-((1*R*,2*S*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl)hexacosanoate (165)



(165)

(*R*)-2-((*R*)-1-((*tert*-Butyldimethylsilyl)oxy)-18-((1*R*,2*S*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl)hexacosanoic acid (**164**) (90 mg, 0.06 mmol supplied by Dr. Al Dulayymi) and DMAP (10 mg, 0.08 mmol) were added to a stirred solution of (*S*)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanol (10 mg, 0.07 mmol) in CH_2Cl_2 (2 mL). A solution of DCC (20 mg; 0.09 mmol) in CH_2Cl_2 (2 mL) was added dropwise with stirring under nitrogen atmosphere over 120 min then stirred for 1 h at room temperature. TLC showed no starting material was left. The reaction mixture was worked up and purified as before to give a semi-solid, *((R)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl(R)-2-((R)-1-((tert-butyldimethyl-silyl)oxy)-18-((1R,2S)-2-((17S,18S)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octa-decyl)hexacosanoate* (**165**) (70 mg, 72%), $[\alpha]_D^{24}$ - 6.7 (*c* 1.4, $CHCl_3$), [Found $(M+Na)^+$: 1505.5, $C_{97}H_{192}NaO_6Si$ requires: 1504.4], which showed δ_H (500 MHz, $CDCl_3$): 4.33 – 4.26 (1H, m), 4.11 (1H, dd, *J* 2.4, 5.4 Hz), 4.06 (1H, dd, *J* 6.4, 8.4 Hz), 3.94 – 3.87 (1H, m), 3.75 (1H, dd, *J* 6.0, 8.4 Hz), 3.34 (3H, s), 3.24 – 3.15 (1H, m), 2.98 – 2.92 (1H, m), 2.55 (1H, ddd, *J* 3.8, 6.9, 10.8 Hz), 2.00 – 1.01 ((162H, m including 1.43 (3H, s), 1.36 (3H, s)), 0.91 – 0.83 (18H, m), 0.69 – 0.61 (2H, m), 0.56 (1H, dt, *J* 3.7, 7.6 Hz), 0.04 (3H, s), 0.01 (3H, s), -0.34 (1H, q, *J* 5.2 Hz); δ_C (126 MHz, $CDCl_3$): 174.7, 109.8, 85.6, 73.7, 66.8, 64.4, 57.9, 55.9, 51.6, 35.5, 35.1, 33.95, 32.6, 32.1, 30.7, 30.4, 30.2, 30.1, 29.9, 29.8, 29.6, 29.5, 28.9, 28.0, 27.73, 26.9, 26.3, 26.2, 25.9, 25.6, 25.5, 24.9, 24.1, 22.9, 18.1, 15.9, 15.0, 14.3, 11.1, -4.2, -4.7; ν_{max}/cm^{-1}: 2918, 2849, 1726, 1465, 1256, 1178

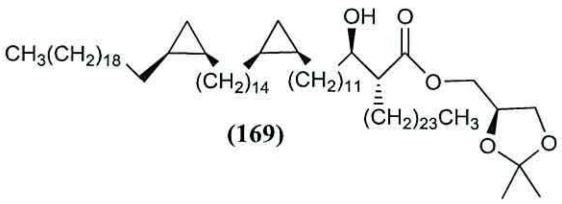
Experiment 3: (*R*)-(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl4-methylbenzenesulfonate (167)[244]



(167)

Pyridine (7 mL), DMAP (0.1g, 0.81 mmol) and tosyl chloride (3.4 g, 17.8 mmol) were added sequentially at 0 °C to a stirred solution of (*S*)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanol (**163**) (2.0 g, 15.1 mmol) in dichloromethane (30 mL). The mixture was allowed to reach room temperature then stirred for 18 h. The mixture was diluted with dichloromethane (30 mL) and quenched with hydrochloric acid (3 M, 10 mL). The organic phase was decanted and washed twice with sat. aq. $NaHCO_3$ and brine. The resulting solution was concentrated and the crude residue was purified by column chromatography eluting with petrol/ethyl acetate (5:1) to give a colourless oil, *(R)-(2,2-dimethyl-1,3-dioxolan-4-yl)methyl4-methylbenzenesulfonate* (**167**) (4.3 g, 88%), $[\alpha]_D^{23} - 12$ (*c* 0.9, $CHCl_3$) (*lit.*[224] $[\alpha]_D^{24}$ - 8.8), [Found $(M+Na)^+$: 309.1, $C_{13}H_{18}NaO_5S$ requires: 309.1]. Which showed δ_H (400 MHz, $CDCl_3$): 7.80 (2H, d, *J* 8.3 Hz), 7.35 (2H, d, *J* 8.0 Hz), 4.32 – 4.23 (1H, m), 4.08 – 3.94 (3H, m), 3.77 (1H, dd, *J* 5.1, 8.8 Hz), 2.45 (3H, s), 1.34 (3H, s), 1.31 (3H, s); δ_C (101 MHz, CDCl3): 145.2, 132.8, 130.0, 128.1, 110.2, 73.0, 69.6, 66.3, 26.7, 25.2, 21.8; ν_{max}/cm^{-1}: 2919, 2851, 1467, 1076, 856.

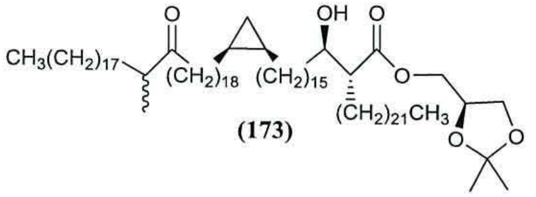
Experiment 4: ((*R*)-2,2-Dimethyl-1,3-dioxolan-4-yl)methyl(*R*)-2-((*R*)-1-hydroxy-12-((1*R*,2*S*)-2-(14-((1*R*,2*S*)-2-eicosylcyclopropyl)tetradecyl)cyclopropyl)dodecyl)hexacosanoate (169)



(169)

Dry cesium hydrogen carbonate (120 mg, 0.33 mmol) was added to a stirred solution of (*R*)-2-((*R*)-1-hydroxy-12-((1*R*,2*S*)-2-(14-((1*R*,2*S*)-2-icosylcyclopropyl)tetradecyl)cyclopropyl)dodecyl)hexacosanoic acid (**168**) (70 mg, 0.06 mmol, supplied by Dr. Al Dulayymi) in a mixture of THF:DMF (1:1, 4 mL) at room temperature. The mixture was left at room temperature for one hour then (*R*)-(2,2-dimethyl-1,3-dioxolan-4-yl)methyl 4-methylbenzenesulfonate (40 mg, 0.13 mmol) were added. The mixture was brought to 70 °C and left at this temperature for 18 h. TLC showed no starting material was left. The reaction mixture was cooled to room temperature then diluted with ethyl acetate (15 mL). The organic layer was separated and washed twice with water. The organic layer was dried over $MgSO_4$ and concentrated. The crude residue was purified by column chromatography eluting with petrol/ethyl acetate (5:1) to give a semi-solid, *((R)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl(R)-2-((R)-1-hydroxy-12-((1R,2S)-2-(14-((1R,2S)-2-icosylcyclopropyl)tetradecyl)cyclopropyl)dodecyl)-hexacosanoate* (**168**) (66 mg, 85%), $[\alpha]_D^{20}$- 4.7 (*c* 1.2, $CHCl_3$), [Found $(M+Na)^+$: 1274.2, $C_{84}H_{162}O_5Na$ requires: 1274.2]. The compound showed δ_H (400 MHz, $CDCl_3$): 4.27 – 4.20 (1H, m), 4.10 (1H, dd, *J* 3.3, 9.9 Hz), 4.06 (1H, dd, *J* 4.3, 9.9 Hz), 3.97 (1H, dd, *J* 6.5, 8.5 Hz), 3.65 (1H, dd, *J* 5.9, 8.5 Hz), 3.56 (1H, br s), 2.41 – 2.28 (2H, m), 1.38 – 0.98 ((140H, m including 1.34 (3H, s), 1.26 (3H, s)), 0.78 (6H, t, *J* 6.8), 0.61 – 0.50 (4H, m), 0.50 – 0.41 (2H, m), -0.43 (2H, q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$): 175.3, 109.8, 73.4, 72.3, 66.3, 64.4, 51.3, 35.6, 31.9, 30.2, 29.7, 29.7, 29.6, 29.5, 29.4, 28.7, 27.4, 26.7, 25.7, 25.3, 22.7, 15.7, 14.1, 10.9; ν_{max}/ cm^{-1}: 3430, 3011, 2925, 2886,1744, 1232, 759, 669.

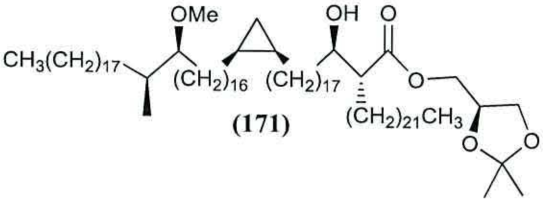
Experiment 5: ((*R*)-2,2-Dimethyl-1,3-dioxolan-4-yl)methyl(2*R*)-2-((1*R*)-1-hydroxy-16-((1*R*,2*S*)-2-(20-methyl-19-oxooctatriacontyl)-cyclopropyl)hexadecyl)tetracosanoate (173)



(**173**)

Dry cesium hydrogen carbonate (110 mg, 0.50 mmol) was added to a stirred solution of (2*R*)-2-((1*R*)-1-hydroxy-16-((1*R*,2*S*)-2-(20-methyl-19-oxooctatriacontyl)cyclopropyl)hexadecyl)tetracosanoic acid (**172**) (80 mg, 0.06 mmol, supplied by Dr. Al Dulayymi) in a mixture of THF:DMF (1:1, 3 mL) at room temperature. The mixture was left stirred at room temperature for one hour then (*R*)-(2,2-dimethyl-1,3-dioxolan-4-yl)methyl4-methylbenzenesulfonate (**167**) (40 mg, 0.13 mmol) was added .The mixture was brought to 70 °C and left at this temperature for 18 h. TLC showed no starting material was left. The reaction mixture was worked up and purified as before to give a semi-solid, *((R)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl (2R)-2-((1R)-1-hydroxy-16-((1R,2S)-2-(20-methyl-19-oxooctatriacontyl)cyclopropyl)hexadecyl)-tetracosanoate* (**172**) (64 mg, 85%), $[\alpha]_D^{24}$ - 8.1 (*c* 1.4, CHCl$_3$), [Found (M+Na)$^+$: 1346.9, C$_{88}$H$_{170}$NaO$_6$ requires: 1346.3], which showed δ$_H$ (400 MHz, CDCl$_3$): 4.40 – 4.32 (1H, m), 4.26 (1H, dd, *J* 5.9, 11.4 Hz), 4.20 (1H, dd, *J* 2.2, 5.4 Hz), 4.14 (1H, dd, *J* 4.6, 11.4 Hz), 4.12 – 4.07 (1H, m), 3.78 (1H, ddd, *J* 2.3, 5.7, 8.3 Hz), 3.73 – 3.65 (1H, m), 2.57 – 2.40 (4H, m), 1.66 – 1.11 ((118H, m including 1.46 (3H, s), 1.38 (3H, s)), 1.07 (3H, d, *J* 6.9 Hz), 0.90 (6H, t, *J* 6.8 Hz), 0.71 – 0.63 (2H, m), 0.62 – 0.54 (1H, m), -0.31 (1H, q, *J* 5.2 Hz); δ$_C$ (101 MHz, CDCl$_3$): 215.2, 175.3, 109.8, 73.5, 72.3, 66.2, 64.6, 51.5, 46.3, 41.1, 35.6, 35.5, 33.0, 31.9, 30.2, 29.7, 29.59, 29.5, 29.3, 28.7, 27.3, 26.7, 25.3, 23.7, 22.7, 16.3, 15.7, 14.1, 10.9; ν$_{max}$/ cm^{-1}: 3455, 2944, 2861, 1722, 1146, 1382, 1214, 1022.

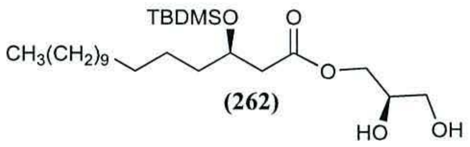
Experiment 6: ((*R*)-2,2-Dimethyl-1,3-dioxolan-4-yl)methyl(*R*)-2-((*R*)-1-hydroxy-18-((1*R*,2*S*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl)tetracosanoate (171)



(171)

Dry cesium hydrogen carbonate (100 mg, 0.40 mmol) was added to a stirred solution of (*R*)-2-((*R*)-1-hydroxy-18-((1*R*,2*S*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl)tetracosanoic acid (**170**) (70 mg, 0.05 mmol, supplied by Dr. Al Dulayymi) in a mixture of THF:DMF (1:1, 2 mL) at room temperature. The mixture was left at room temperature for one hour then (*R*)-(2,2-dimethyl-1,3-dioxolan-4-yl)methyl 4-methylbenzenesulfonate (**167**) (80 mg, 0.2 mmol) was added. The mixture was brought to 70 °C and left at this temperature for 18 h. TLC showed no starting material was left. The reaction mixture was worked up and purified as before to give a semi-solid, *((R)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl(R)-2-((R)-1-hydroxy-18-((1R,2S)-2-((17S,18S)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl)tetracosanoate* (**171**) (70 mg, 50%), $[\alpha]_D^{21}$ - 6.2 (*c* 0.9, $CHCl_3$), [Found $(M+Na)^+$: 1362.5, $C_{89}H_{174}NaO_6$ requires: 1362.3], which showed δ_H (400 MHz, $CDCl_3$): 4.37 – 4.29 (1H, m), 4.20 (1H, dd, *J* 3.3, 9.9 Hz), 4.16 (1H, dd, *J* 2.4, 8.0 Hz), 4.07 (1H, dd, *J* 6.5, 8.5 Hz), 3.75 (1H, dd, *J* 5.9, 8.4 Hz), 3.66 (1H, br s), 3.34 (3H, s), 2.99 – 2.92 (1H, m), 2.52 – 2.37 (2H, m), 1.48 – 1.06 ((155H, m including 1.44 (3H, s), 1.36 (3H, s)), 0.88 (6H, t, *J* 6.8 Hz), 0.85 (3H, d, *J* 6.9 Hz), 0.64 (2H, br s), 0.60 – 0.51 (1H, m), -0.34 (1H, q, *J* 5.3 Hz); δ_C (101 MHz, $CDCl_3$): 175.3, 109.8, 85.5, 73.4, 72.3, 66.3, 64.4, 57.7, 51.3, 35.5, 35.3, 32.4, 31.9, 30.8, 30.2, 30.0, 29.9, 29.7, 29.6, 29.5, 29.4, 29.3, 28.7, 27.6, 27.4, 26.7, 26.1, 25.7, 25.3, 22.7, 15.8, 14.9, 14.1, 10.9; ν_{max}/ cm^{-1}: 3511, 2918, 2849, 1726, 1465, 1256, 1178.

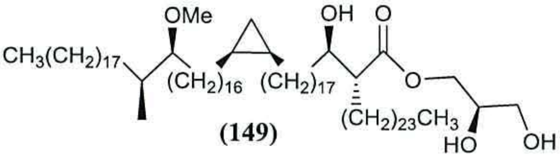
Experiment 7: (*R*)-2,3-Dihydroxypropyl(*R*)-3-((*tert*-butyldimethylsilyl)oxy)hexadecanoate (262)



(262)

Trifluoroacetic acid in dichloromethane (1:1) (0.4 mL) was added to a stirred solution of ((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl(*R*)-3-((*tert*-butyldimethyl-silyl)oxy)hexadecanoate (**261**) (20 mg, 0.05 mmol) in CH_2Cl_2 (2 mL) at 0 °C. The solution was stirred at 0 °C for 12 h. TLC showed no starting material was left. The reaction mixture was worked up and purified as before to give a semi-solid *(R)-2,3-dihydroxypropyl(R)-3-((tert-butyldimethylsilyl)oxy)hexadecanoate* (**262**) (17 mg, 88%) $[\alpha]_D^{24}$ - 8.1 (*c* 1.4, $CHCl_3$), [Found $(M+Na)^+$: 369.3, $C_{19}H_{38}NaO_5$ requires: 369.3], which showed δ_H (500 MHz, $CDCl_3$): 4.38 – 4.30 (2H, m), 4.28 – 4.22 (1H, m), 4.16 – 4.10 (2H, m), 4.01 – 3.92 (1H, m), 2.50 (1H, dd, *J* 3.1, 15.9 Hz), 2.37 (1H, dd, *J* 4.3, 15.9 Hz), 1.50 – 1.07 (27H, m), 0.80 (3H, t, *J* 6.9 Hz); δ_C (126 MHz, $CDCl_3$): 172.7, 68.6, 68.0, 67.50, 64.8, 41.6, 36.9, 32.0, 31.0, 29.9, 29.8, 29.7, 29.6, 29.5, 25.6, 22.8, 14.2; ν_{max}/cm^{-1}: 3525, 2926, 2844, 1757, 1362, 1385.

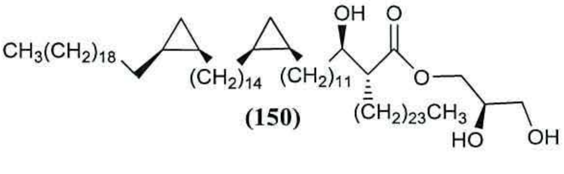
Experiment 8: (*R*)-2,3-Dihydroxypropyl(*R*)-2-((*R*)-1-hydroxy-18-((1*R*,2*S*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl)hexacosanoate (149)



(149)

Trifluoroacetic acid in dichloromethane (1:1) (0.4 mL) was added to a stirred solution of ((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl(*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)-oxy)-18-((1*R*,2*S*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)-octadecyl)hexacosanoate (**165**) (50 mg, 0.03 mmol) in CH_2Cl_2 (2 mL) at 0 °C. The solution was stirred at 0 °C for 12 h. TLC showed no starting material was left. The reaction mixture was worked up and purified as before to give a semi-solid, *(R)-2,3-dihydroxypropyl(R)-2-((R)-1-hydroxy-18-((1R,2S)-2-((17S,18S)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl)hexacosanoate* (**149**) (40 mg, 86%), $[\alpha]_D^{23}$ - 8.4 (*c* 1.1, $CHCl_3$), [Found $(M+Na)^+$: 1350.3199, $C_{88}H_{174}O_6Na$ requires: 1350.3203], which showed δ_H (500 MHz, C_6D_6): 4.25 (1H, dd, *J* 6.6, 11.4, Hz), 4.12 (1H, dd, *J* 3.9, 11.4, Hz), 3.81 – 3.71 (2H, m), 3.47 (1H. dd, *J* 4.2, 11.3, Hz), 3.40 (1H, dd, *J* 5.5, 11.4, Hz), 3.27 (3H, s), 3.03 – 2.97 (1H, m), 2.55 (1H, dd, *J* 9.0, 12.5, Hz), 1.37 (156H, s), 1.00 (3H, d, *J* 6.8 Hz), 0.93 (7H, t, *J* 6.6 Hz), 0.78 – 0.71 (2H, m), 0.68 (1H, dt, *J* 3.7, 7.6 Hz), -0.01 (1H, q, *J* 5.0 Hz); δ_C (126 MHz, C_6D_6): 175.2, 85.4, 73.2, 70.4, 65.5, 63.6, 57.6, 53.0, 35.9, 35.8, 33.0, 32.4, 30.9, 30.8, 30.6, 30.5, 30.3, 30.2, 30.1, 30.0, 29.9, 29.8, 29.3, 28.2, 28.0, 26.7, 26.0, 23.1, 16.3, 15.3, 14.4, 11.5; ν_{max}/cm^{-1}: 3499 2928, 2854, 1744, 1435, 1267, 1178.

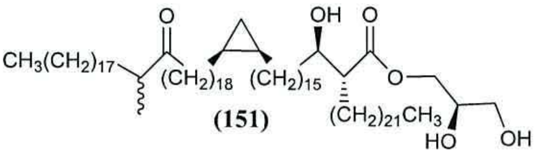
Experiment 9: (*R*)-2,3-Dihydroxypropyl (*R*)-2-((*R*)-1-hydroxy-12-((1*R*,2*S*)-2-(14-((1*R*,2*S*)-2-eicosylcyclopropyl)-tetradecyl)cycloprop-yl)dodecyl) hexacosanoate (150)



(150)

An aqueous solution of hydrochloric acid (2 M, 0.04 mL) was added to a stirred solution of ((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl(*R*)-2-((*R*)-1-hydroxy-12-((1*R*,2*S*)-2-(14-((1*R*,2*S*)-2-icosylcyclopropyl)tetradecyl)cyclopropyl)dodecyl)hexacosanoate (**169**) (55 mg, 0.04 mmol) in THF (25 mL) at room temperature. The reaction mixture was stirred at 40 °C for 16 h. TLC showed no starting material was left. The reaction mixture was quenched at room temperature with sat. aq. $NaHCO_3$ (10 mL), theproduct was extracted with CH_2Cl_2 (25 mL) and the aqueous phase was re-extracted with CH_2Cl_2 (3 × 25). The organic extracts were dried with $MgSO_4$ and concentrated. The crude residue was purified by column chromatography eluting with petrol/ethyl acetate (4:1) to give a semi-solid, *R)-2,3-dihydroxypropyl (R)-2-((R)-1-hydroxy-12-((1R,2S)-2-(14-((1R,2S)-2-icosylcyclopropyl)tetradecyl)cyclopropyl)-dodecyl)hexacosanoate* (**150**) (46 mg, 86%), $[\alpha]_D^{22}$ - 6.5 (*c* 0.9, $CHCl_3$), [Found $(M+Na)^+$: 1234.2005, $C_{81}H_{158}NaO_5$ requires: 1234.2001], which showed δ_H (400 MHz, $CDCl_3$): 4.30 – 4.26 (2H, m), 4.01 – 3.95 (1H, m), 3.76 – 3.61 (3H, m), 2.54 – 2.44 (1H, m), 1.49 – 1.08 (136H, m), 0.90 (6H, t, *J* 6.8 Hz), 0.71 – 0.63 (4H, m), 0.58 (2H, dt, *J* 3.7, 7.6 Hz), -0.31 (2H, q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$): 175.6, 73.1, 70.1, 65.4, 63.4, 52.3, 35.5, 32.1, 30.4, 29.9, 29.8, 29.7, 29.6, 29.5, 29.5, 28.9, 27.6, 25.6, 22.8, 15.9, 14.3, 11.1; ν_{max}/cm^{-1}: 3430, 3011, 2925, 2886,1744, 1232, 759, 669.

Experiment 10: (*R*)-2,3-Dihydroxypropyl (2*R*)-2-((1*R*)-1-hydroxy-16-((1*R*,2*S*)-2-(20-methyl-19-oxooctatriacontyl)cyclopropyl)hexadecyl)-tetracosanoate (151)

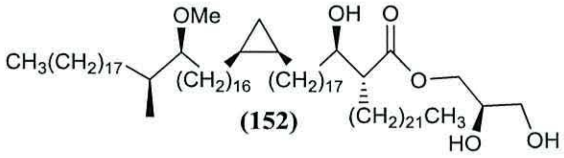


(151)

Hydrochloric acid (2 M, 0.04 mL) was added to a stirred solution of ((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl(2*R*)-2-((1*R*)-1-hydroxy-16-((1*R*,2*S*)-2-(20-methyl-19-oxo-octatriacontyl)cyclopropyl)hexadecyl)tetracosanoate (**173**) (60 mg, 0.04 mmol) in THF (30 mL) at room temperature. The reaction mixture was stirred was at 40 °C for 16 h. TLC showed no starting material was left. The reaction mixture was worked up and purified as before to give a semi-solid, *(R)-2,3-dihydroxypropyl (2R)-2-((1R)-1-hydroxy-16-((1R,2S)-2-(20-methyl-19-oxooctatriacontyl)cyclopropyl)hexa-*

decyl)tetracosanoate (**151**) (51 mg, 87%), $[\alpha]_D^{22}$ - 12 (*c* 1.1, $CHCl_3$), [Found $(M+Na)^+$: 1306.2569, $C_{85}H_{166}O_6Na$ requires: 1306.2577], which showed δ_H (400 MHz, $CDCl_3$): 4.30 (1H, dd, *J* 4.1, 11.8 Hz), 4.25 (1H, dd, *J* 6.7, 11.8 Hz), 4.03 – 3.94 (1H, m), 3.77 – 3.70 (2H, m), 3.65 (1H, dd, *J* 4.6, 10.5 Hz), 2.57 – 2.39 (4H, m), 1.76 – 1.10 (143H, m), 1.07 (3H, d, *J* 6.9 Hz), 0.90 (6H, t, *J* 6.8 Hz), 0.66 (2H, br s), 0.58 (1H, dt, *J* 3.7, 7.8 Hz), -0.31 (1H, q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$): 215.3, 175.4, 72.9, 69.7, 65.2, 63.3, 52.2, 46.3, 41.1, 37.9, 35.3, 33.0, 31.9, 30.2, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 28.7, 27.4, 27.3, 25.5, 23.7, 22.7, 16.3, 15.8, 14.1, 10.9; ν_{max}/cm^{-1}: 3455, 2944, 2861, 1722, 1146, 1382, 1214, 1022.

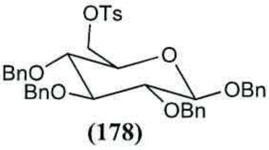
Experiment 11: (*R*)-2,3-Dihydroxypropyl (*R*)-2-((*R*)-1-hydroxy-18-((1*R*,2*S*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl) tetracosanoate (152)



(152)

Hydrochloric acid (2 M, 0.04 mL) was added to a stirred solution of ((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl(*R*)-2-((*R*)-1-hydroxy-18-((1*R*,2*S*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl)tetracosanoate (**171**) (65 mg, 0.05mmol) in THF (34 ml) at room temperature. The reaction mixture was stirred at 40 °C for 16 hrs. TLC showed no starting material was left. The reaction mixture was worked up and purified as before to give a semi-solid, *(R)-2,3-dihydroxypropyl(R)-2-((R)-1-hydroxy-18-((1R,2S)-2-((17S,18S)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl)tetracosanoate* (**152**) (56 mg, 88%), $[\alpha]_D^{24}$ - 9.3 (*c* 1.2, $CHCl_3$), [Found $(M+Na)^+$: 1322.2880, $C_{86}H_{170}NaO_6$ requires: 1322.2890], which showed δ_H (500 MHz, $CDCl_3$): 4.30 – 4.22 (2H, m), 3.99 – 3.92 (1H, m), 3.74 – 3.67 (2H, m), 3.62 (1H, dd, *J* 5.5, 11.4), 3.34 (3H, s), 2.98 – 2.92 (1H, m), 2.50 – 2.42 (1H, m), 1.74 – 1.01 (152 H, m), 0.88 (6H, t, *J* 6.9), 0.84 (3H, d, *J* 6.9), 0.69 – 0.60 (2H, m), 0.55 (1H, dt, *J* 3.9, 8.4 Hz), -0.34 (1H, q, *J* 5.1 Hz); δ_C (101 MHz, $CDCl_3$): 175.6, 85.6, 73.1, 70.1, 65.4, 63.4, 57.9, 52.3, 35.5, 35.4, 34.1, 32.5, 32.1, 30.6, 30.4, 30.1, 30.0, 29.9, 29.8, 29.7, 29.6, 29.5, 28.9, 27.7, 27.6, 26.3, 25.6, 25.1, 22.8, 15.9, 15.0, 14.3, 11.1; ν_{max}/cm^{-1}: 3511, 2918, 2849, 1726, 1465, 1256, 1178.

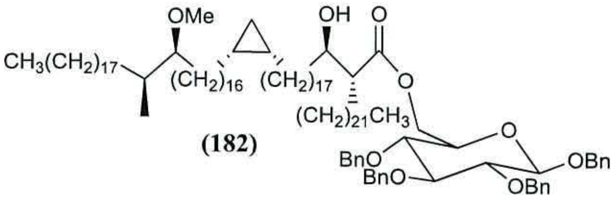
Experiment 12: ((2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5,6-Tetrakis(benzyloxy)tetrahydro-2*H*-pyran-2-yl)methyl 4-methylbenzenesulfonate (178)



(178)

Pyridine (7 mL), 4-(dimethylamino)pyridine (0.1g, 0.81 mmol) and tosyl chloride (4 g, 21 mmol) were sequentially added to a stirred solution of ((2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5,6-tetrakis(benzyloxy)tetrahydro-2*H*-pyran-2-yl)methanol (**177**) (2.0 g, 3.7 mmol) in dichloromethane (30 mL) at 0 °C. The mixture was allowed to reach room temperature and was stirred overnight. TLC showed no starting material was left and diluted with dichloromethane (30 mL). aq. hydrochloric acid (3 M, 10 mL) was added and the mixture was extracted with dichloromethane (3 × 30 ml). The combined organic layers were dried over $MgSO_4$ and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give a semi-solid, *((2R,3R,4S,5R,6R)-3,4,5,6-tetrakis(benzyloxy)-tetrahydro-2H-pyran-2-yl)methyl 4-methylbenzenesulfonate* (**178**) (2.1g, 82%): $[\alpha]_D^{22}$ - 3.3 (*c* 1.0, $CHCl_3$) (*lit.*[225] $[\alpha]_D^{22}$ - 4.4), [Found (M+Na)$^+$: 717.5, $C_{41}H_{42}NaO_8S$ requires: 717.2]. Which showed δ_H (400 MHz, $CDCl_3$): 7.71 (2H, d, *J* 8.2), 7.39 – 7.05 (22H, m), 4.85 (1H, d, *J* 3.5 Hz), 4.88 (1H, dd, *J* 3.5, 10.1 Hz), 4.75 (2H, t, *J* 10.9 Hz), 4.67 (1H, d, *J* 10.9 Hz), 4.61 (1H, d, *J* 10.9 Hz), 4.49 (1H, d, *J* 11.9 Hz), 4.41 (1H, d, *J* 10.9 Hz), 4.36 (1H, d, *J* 7.7 Hz), 4.18 (1H, d, *J* 10.3 Hz), 4.06 (1H, dd, *J* 4.5, 10.4 Hz), 3.53 (1H, t, *J* 8.5 Hz), 3.42 – 3.33 (3H, m), 2.31 (3H, s); δ_C (101 MHz, $CDCl_3$): 144.9, 138.4, 138.3, 137.7, 137.2, 133.0, 129.9, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 102.2, 84.6, 82.1, 77.0, 75.8, 75.1, 75.0, 72.8, 71.1, 68.8, 21.7. The ^1H, ^{13}C, and I.R spectra were identical to the literature.[225]

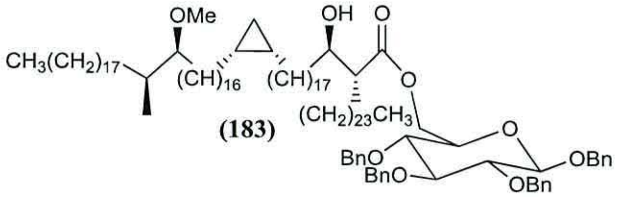
Experiment 13: (3,4,5,6-Tetrakis(benzyloxy)tetrahydro-2*H*-pyran-2-yl)methyl(2*R*)-2-((*R*)-1-hydroxy-18-((1*S*,2*R*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl)tetracosanoate (182)



(182)

Dry cesium hydrogen carbonate (120 mg, 0.62 mmol) was added to a stirred solution of (*R*)-2-((*R*)-1-hydroxy-18-((1*S*,2*R*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl)tetracosanoic acid (**179**) (70 mg, 0.05 mmol which was supplied by Dr.Al Dulayymi) in THF and DMF (1:1, 2.4 mL) at room temperature. The mixture was stirred at room temperature for one hour and ((2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5,6-tetrakis(benzyloxy)tetrahydro-2*H*-pyran-2-yl)methyl 4-methylbenzene sulfonate (**178**) (44 mg, 0.06 mmol) was added. The mixture was stirred at 70 °C for 18 h. TLC showed no starting material was left. Sat. aq. $NaHCO_3$ (10 mL) was added and extracted with dichloromethane (3 × 25 ml). The combined organic layers were dried over $MgSO_4$ and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (4:1) to give a semi-solid, *(3,4,5,6-tetrakis(benzyloxy)tetrahydro-2H-pyran-2-yl)methyl(2R)-2-((R)-1-hydroxy-18-((1S,2R)-2-((17S,18S)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)-octadecyl)tetracosanoate* (**182**) (61 mg, 62%): $[\alpha]_D^{22}$ + 14 (*c* 1.2, $CHCl_3$), [Found $(M+Na)^+$: 1770.8, $C_{117}H_{198}NaO_9$ requires: 1770.8], which showed δ_H (400 MHz, $CDCl_3$): 7.32 – 7.15 (20H, m), δ 4.88 (*J* 11.0 Hz), 4.87 (*J* 10.8 Hz), 4.84 (*J* 12.4 Hz), 4.81 (*J* 11.5 Hz), 4.71 (*J* 11.0 Hz), 4.63 (*J* 10.8 Hz), 4.56 (*J* 12.4 Hz) and 4.82 (*J* 11.5 Hz) ppm, 4.49 – 4.43 (2H, m), 4.14 (1H, dd, *J* 11.7, 4.6 Hz), 3.65 – 3.53 (2H, m), 3.48 – 3.38 (3H, m), 3.26 (3H, s), 2.92 – 2.85 (1H, m), 2.44 – 2.33 (2H, m), 1.71 – 0.96 (134H, m), 0.81 (6H, t, *J* 6.8 Hz), 0.77 (3H, d, *J* 6.9 Hz), 0.65 – 0.52 (2H, m), 0.53 – 0.44 (1H, m), -0.41 (1H, q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$): 175.4, 138.5, 138.4, 137.9, 137.3, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.04, 128.01, 127.9, 102.5, 85.6, 84.7, 82.4, 78.0, 75.9, 75.3, 75.1, 73.0, 72.5, 71.3, 63.0, 57.9, 51.5, 35.8, 35.5, 32.5, 32.1, 30.6, 30.4, 30.1, 30.1, 29.8, 29.8, 29.7, 29.6, 29.5, 28.9, 27.7, 27.6, 26.3, 26.0, 22.9, 15.9, 15.0, 14.3, 11.1; ν_{max}/cm^{-1}: 3535, 2922, 2844, 1726, 1465, 1256, 1178.

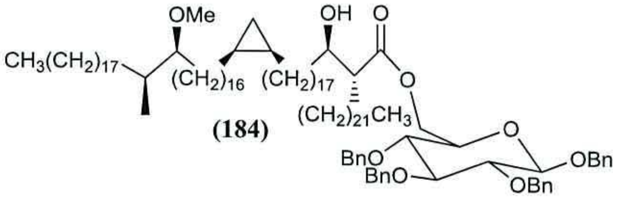
Experiment 14: (3,4,5,6-Tetrakis(benzyloxy)tetrahydro-2*H*-pyran-2-yl)methyl(2*R*)-2-((*R*)-1-hydroxy-18-((1*R*,2*S*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl)tetracosanoate (183)



(183)

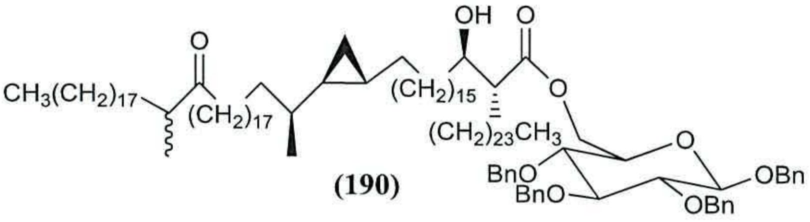
Dry cesium hydrogen carbonate (100 mg, 0.51 mmol) was added to a stirred solution of (*R*)-2-((*R*)-1-hydroxy-18-((1*S*,2*R*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatri acontyl)cyclopropyl)octadecyl)hexacosanoic acid (**180**) (61 mg, 0.04 mmol which was supplied by Dr.Al Dulayymi) in THF and DMF (1:1, 2.4 mL). The mixture was stirred at room temperature for one hour and ((2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5,6-tetrakis(benzyloxy)tetrahydro-2*H*-pyran-2-yl)methyl4-methylbenzenesulfonate (**178**) (37 mg, 0.05 mmol) was added. The mixture was brought to 70 °C and stirred at this temperature for 18 h. TLC showed no starting material was left. The mixture was worked up and purified as before to give a semi-solid, *(3,4,5,6-tetrakis-(benzyloxy)-tetrahydro-2H-pyran-2-yl)methyl(2R)-2-((R)-1-hydroxy-18-((1S,2R)-2-((17S,18S)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl)hexacosanoate* (**183**) (52 mg, 63%) $[\alpha]_D^{22}$ + 13 (*c* 1.1, $CHCl_3$), [Found (M+Na)$^+$: 1798.9, $C_{119}H_{202}NaO_9$ requires: 1798.5], which showed δ_H (400 MHz, $CDCl_3$): 7.32 – 7.15 (20H, m), δ 4.88 (*J* 11.0 Hz), 4.87 (*J* 10.8 Hz), 4.84 (*J* 12.4 Hz), 4.81 (*J* 11.5 Hz), 4.71 (*J* 11.0 Hz), 4.63 (*J* 10.8 Hz), 4.56 (*J* 12.4 Hz) and 4.82 (*J* 11.5 Hz) ppm, 4.49 – 4.43 (2H, m), 4.14 (1H, dd, *J* 11.7, 4.6 Hz), 3.65 – 3.53 (2H, m), 3.48 – 3.38 (3H, m), 3.26 (3H, s), 2.92 – 2.85 (1H, m), 2.44 – 2.33 (2H, m), 1.71 – 0.96 (138H, m), 0.81 (6H, t, *J* 6.8 Hz), 0.77 (3H, d, *J* 6.9 Hz), 0.65 – 0.52 (2H, m), 0.53 – 0.44 (1H, m), -0.41 (1 H, q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$): 175.4, 138.5, 138.4, 137.9, 137.3, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 102.5, 85.6, 84.7, 82.4, 78.0, 75.9, 75.3, 75.1, 73.0, 72.5, 71.3, 63.0, 57.9, 51.5, 35.8, 35.5, 32.5, 32.1, 30.6, 30.4, 30.1, 30.1, 29.8, 29.8, 29.7, 29.6, 29.5, 28.9, 27.7, 27.6, 26.3, 26.0, 22.9, 15.9, 15.0, 14.3, 11.1; ν_{max}/cm^{-1}: 3545, 2922, 2844, 1726, 1465, 1256, 1178.

Experiment 15: (3,4,5,6-Tetrakis(benzyloxy)tetrahydro-2*H*-pyran-2-yl)methyl(2*R*)-2-((*R*)-1-hydroxy-18-((1*R*,2*S*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl)tetracosanoate (184)


(184)

Dry cesium hydrogen carbonate (80.0 mg, 0.41 mmol) was added to a stirred solution of (*R*)-2-((*R*)-1-hydroxy-18-((1*R*,2*S*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl)tetracosanoic acid (**181**) (51 mg, 0.04 mmol which was supplied by Dr.Al Dulayymi) in THF and DMF (1:1, 2.4 mL) The reaction mixture was stirred at room temperature for one hour and ((2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5,6-tetrakis(benzyloxy)tetrahydro-2*H*-pyran-2-yl)methyl 4-m-ethylbenzenesulfonate (**178**) (37 mg, 0.05 mmol) was added. The mixture was brought to 70 °C and stirred at this temperature for 18 h. TLC showed no starting material was left. The reaction mixture was worked up and purified as before to give a semi-solid, *(3,4,5,6-tetrakis(benzyloxy)tetrahydro-2H-pyran-2-yl)methyl(2R)-2-((R)-1-hydroxy-18-((1R,2S)-2-((17S,18S)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl)-tetracosanoate* (**184**) (50 mg, 69%) $[\alpha]_D^{22}$ + 15 (*c* 1.1, $CHCl_3$), [Found $(M+Na)^+$: 1771.1, $C_{117}H_{198}NaO_9$ requires: 1770.5], which showed δ_H (400 MHz, $CDCl_3$): 7.32 – 7.15 (20H, m), δ 4.88 (*J* 11.0 Hz), 4.87 (*J* 10.8 Hz), 4.84 (*J* 12.4 Hz), 4.81 (*J* 11.5 Hz), 4.71 (*J* 11.0 Hz), 4.63 (*J* 10.8 Hz), 4.56 (*J* 12.4 Hz) and 4.82 (*J* 11.5 Hz) ppm, 4.49 – 4.43 (2H, m), 4.14 (1H, dd, *J* 11.7, 4.6 Hz), 3.65 – 3.53 (2H, m), 3.48 – 3.38 (3H, m), 3.26 (3H, s), 2.92 – 2.85 (1H, m), 2.44 – 2.33 (2H, m), 1.71 – 0.96 (134H, m), 0.81 (6H, t, *J* 6.8 Hz), 0.77 (3H, d, *J* 6.9 Hz), 0.65 – 0.52 (2H, m), 0.53 – 0.44 (1H, m), -0.41 (1H, q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$): 175.4, 138.5, 138.4, 137.9, 137.3, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.04, 128.01, 127.9, 102.5, 85.6, 84.7, 82.4, 78.0, 75.9, 75.3, 75.1, 73.0, 72.5, 71.3, 63.0, 57.9, 51.5, 35.8, 35.5, 32.5, 32.1, 30.6, 30.4, 30.1, 30.1, 29.8, 29.8, 29.7, 29.6, 29.5, 28.9, 27.7, 27.6, 26.3, 26.0, 22.9, 15.9, 15.0, 14.3, 11.1; ν_{max}/cm^{-1}: 3535, 2922, 2844, 1726, 1465, 1256, 1178.

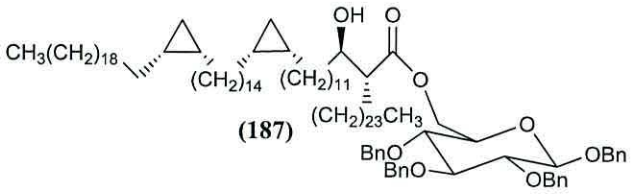
Experiment 16: ((2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5,6-Tetrakis(benzyloxy)-tetrahydro-2*H*-pyran-2-yl)methyl(2*R*)-2-((1*R*)-1-hydroxy-17-((1*R*,2*R*)-2-((2*S*)-22-methyl-21-oxotetracontan-2-yl)cyclopropyl)hept-adecyl)hexacosanoate (190)



(190)

Dry cesium hydrogen carbonate (70.0 mg, 0.36 mmol) was added to a stirred solution (2*R*)-2-((1*R*)-1-hydroxy-17-((1*R*,2*R*)-2-((2*S*)-22-methyl-21-oxotetracontan-2-yl)-cyclopropyl)heptadecyl)hexacosanoic acid (**188**) (43 mg, 0.03 mmol which was supplied by Dr.Al Dulayymi) in THF and DMF (1:1, 2.4 mL). The reaction mixture was stirred at room temperature for one hour and ((2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5,6-tetrakis(benzyloxy)tetrahydro-2*H*-pyran-2-yl)methyl4-methylbenzenesulfonate (**178**) (30 mg, 0.044 mmol) was added. The mixture was stirred at 70 °C for 18 h. TLC showed no starting material was left. The reaction mixture was worked up and purified as before to give a semi-solid, *((2R,3R,4S,5R,6R)-3,4,5,6-tetrakis(benzyloxy)-tetrahydro-2H-pyran-2-yl)methyl(2R)-2-((1R)-1-hydroxy-17-((1R,2R)-2-((2S)-22-methyl-21-oxotetracontan-2-yl)cyclopropyl)heptadecyl)hexacosanoate* (**190**) (35 mg, 58%), $[\alpha]_D^{22}$ + 11 (*c* 1.3, $CHCl_3$), [Found $(M+Na)^+$: 1824.7, $C_{121}H_{204}O_9Na$ requires: 1824.5], which showed δ_H (400 MHz, $CDCl_3$): 7.32 – 7.16 (20H, m), 4.86 (1H, d, *J* 3.6 Hz), 4.84 (1H, br s), 4.71 (1H, d, *J* 10.9 Hz), 4.63 (1H, d, *J* 10.8 Hz), 4.56 (1H, d, *J* 11.9 Hz), 4.52 (1H, d, *J* 10.9 Hz), 4.49 – 4.43 (2H, m), 4.15 (1H, dd, *J* 4.6, 11.7 Hz), 3.64 – 3.54 (2H, m), 3.50 – 3.38 (3H, m), 2.48 – 2.30 (5H, m), 1.71 – 1.03 (145H, m), 0.97 (3H, d, *J* 6.9 Hz), 0.85 – 0.78 (9H, m), 0.64 – 0.53 (1H, m), 0.43 – 0.32 (1H, m), 0.16 – -0.01 (2H, m); δ_C (101 MHz, $CDCl_3$): 215.2, 175.2, 138.4, 138.3, 137.7, 137.2, 128.5, 128.4, 128.3, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 102.3, 84.5, 82.3, 77.8, 75.7, 75.1, 74.9, 72.9, 72.3, 71.1, 62.9, 51.3, 46.3, 41.1, 38.1, 37.4, 35.6, 34.5, 33.0, 31.9, 30.1, 29.7, 29.6, 29.5, 29.4, 29.3, 27.5, 27.3, 27.2, 26.1, 25.9, 23.7, 22.7, 19.7, 18.6, 16.4, 14.1, 10.5; ν_{max}/cm^{-1}: 3525, 2944, 2861, 1722, 1146, 1382, 1214, 1022.

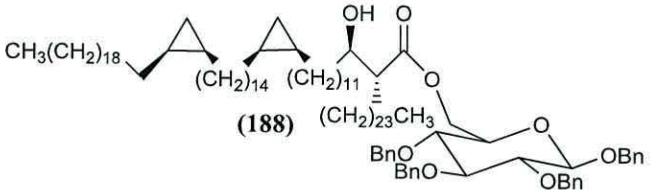
Experiment 17: ((2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5,6-Tetrakis(benzyloxy)-tetrahydro-2*H*-pyran-2-yl)methyl(*R*)-2-((*R*)-1-hydroxy-12-((1*S*,2*R*)-2-(14-((1*S*,2*R*)-2-icosylcyclopropyl)tetradecyl)cyclopropyl)dodecyl) hexacosanoate (187)



(187)

Dry cesium hydrogen carbonate (70.0 mg, 0.36 mmol) was added to a stirred solution of (*R*)-2-((*R*)-1-hydroxy-12-((1*S*,2*R*)-2-(14-((1*S*,2*R*)-2-icosylcyclopropyl)tetradecyl)cyclopropyl) dodecyl)hexacosanoic acid (**185**) (50 mg, 0.04 mmol which was supplied by Dr.Al Dulayymi) in THF and DMF (1:1, 2.4 mL) at room temperature. The mixture was stirred at room temperature for one hour and ((2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5,6-tetrakis(benzyloxy)tetrahydro-2*H*-pyran-2-yl)methyl 4-methylbenzene sulfonate (**178**) (36 mg, 0.04 mmol) was added. The mixture was brought to 70 °C and left at this temperature for 18 h. TLC showed no starting material was left. The reaction mixture was worked up and purified as before to give a semi-solid, *((2R,3R,4S,5R,6R)-3,4,5,6-tetrakis(benzyloxy)tetrahydro-2H-pyran-2-yl)methyl(R)-2-((R)-1-hydroxy-12-((1S,2R)-2-(14-((1S,2R)-2-icosylcyclopropyl)tetra-decyl)cyclopropyl)dodecyl)hexacosanoate* (**187**) (44 mg, 68%). $[\alpha]_D^{22}$ + 5.3 (*c* 0.9, $CHCl_3$), [Found $(M+Na)^+$: 1682.4, $C_{112}H_{186}NaO_8$ requires: 1682.4], which showed δ_H (400 MHz, $CDCl_3$): 7.31 – 7.16 (20H, m), 4.86 (1H, d, *J* 3.6 Hz), 4.84 (1H, br s), 4.83 – 4.77 (2H, m), 4.71 (1H, d, *J* 10.9 Hz), 4.63 (1H, d, *J* 10.9 Hz), 4.54 (2H, t, *J* 11.9 Hz), 4.49 – 4.43 (2H, m), 4.14 (1H, dd, *J* 4.5, 11.6 Hz), 3.63 – 3.55 (2H, m), 3.49 – 3.38 (3H, m), 2.44 – 2.34 (2H, m), 1.71 – 0.98 (134H, m), 0.81 (6H, t, *J* 6.8 Hz), 0.62 – 0.53 (4H, m), 0.48 (2H, dt 3.8, 7.6 Hz), -0.41 (2H, q, *J* 4.7); δ_C (101 MHz, $CDCl_3$) 175.2, 138.4, 138.3, 137.7, 137.2, 128.5, 128.4, 128.3, 128.1, 128.0, 127.9, 127.88, 127.85, 127.7, 102.3, 84.5, 82.3, 77.8, 77.3, 76.7, 75.7, 75.1, 74.9, 72.9, 72.3, 71.1, 62.9, 51.3, 35.6, 31.9, 30.2, 29.7, 29.6, 29.4, 29.3, 28.7, 27.5, 25.6, 22.7, 15.8, 14.1, 10.9; ν_{max}/cm^{-1}: 3447, 2925, 2886,1744, 1232, 759, 669.

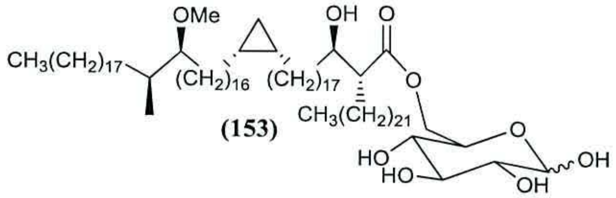
Experiment 18: (3,4,5,6-Tetrakis(benzyloxy)tetrahydro-2*H*-pyran-2-yl)methyl(2*R*)-2-((*R*)-1-hydroxy-12-((1*R*,2*S*)-2-(14-((1*R*,2*S*)-2-icosyl-cyclopropyl)tetradecyl)cyclopropyl)dodecyl)hexacosanoate (188)



(188)

Dry cesium hydrogen carbonate (122 mg, 0.87 mmol) was added to a stirred solution of (*R*)-2-((*R*)-1-hydroxy-12-((1*R*,2*S*)-2-(14-((1*R*,2*S*)-2-icosylcyclopropyl)tetradecyl)-cyclopropyl)dodecyl)hexacosanoic acid (**186**) (80 mg, 0.07 mmol which was supplied by Dr.Al Dulayymi) in THF and DMF (1:1, 3 mL) at room temperature. The reaction mixture was stirred at room temperature for one hour and ((2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5,6-tetrakis(benzyloxy)tetrahydro-2*H*-pyran-2-yl)methyl 4-methylbenzenesulfonate (**178**) (36 mg, 0.04 mmol) was added. The mixture was brought to 70 °C and left at this temperature for 18 h. TLC showed no starting material was left. The reaction mixture was worked up and purified as before to give a semi-solid, *(3,4,5,6-tetrakis-(benzyloxy)tetrahydro-2H-pyran-2-yl)methyl(2R)-2-((R)-1-hydroxy-12-((1R,2S)-2-(14-((1R,2S)-2-icosylcyclopropyl)tetradecyl)cyclopropyl)dodecyl)hexa-cosanoate* (**188**) (69 mg, 84%), $[\alpha]_D^{24}$ + 5.5 (*c* 1.1, $CHCl_3$), [Found $(M+Na)^+$: 1682.4, $C_{112}H_{186}NaO_8$ requires: 1682.4], which showed δ_H (400 MHz, $CDCl_3$) 7.31 – 7.16 (20H, m), 4.86 (1H, d, *J* 3.6 Hz), 4.84 (1H, br s), 4.83 – 4.77 (2H, m), 4.71 (1H, d, *J* 10.9 Hz), 4.63 (1H, d, *J* 10.9 Hz), 4.54 (2H, t, *J* 11.9 Hz), 4.49 – 4.43 (2H, m), 4.14 (1H, dd, *J* 4.5, 11.6Hz), 3.63 – 3.55 (2H, m), 3.49 – 3.38 (3H, m), 2.44 – 2.34 (2H, m), 1.71 – 0.98 (134H, m), 0.81 (6H, t, *J* 6.8 Hz), 0.62 – 0.53 (4H, m), 0.48 (2H, dt 3.8, 7.6 Hz), -0.41 (2H, q, *J* 4.7); δ_C (101 MHz, $CDCl_3$): 175.2, 138.4, 138.3, 137.7, 137.2, 128.5, 128.4, 128.3, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 102.3, 84.5, 82.3, 77.8, 77.3, 76.7, 75.7, 75.1, 74.9, 72.9, 72.3, 71.1, 62.9, 51.3, 35.6, 31.9, 30.2, 29.7, 29.6, 29.4, 29.3, 28.7, 27.5, 25.6, 22.7, 15.8, 14.1, 10.9; ν_{max}/cm^{-1}: 3447, 2925, 2886,1744, 1232, 759, 669.

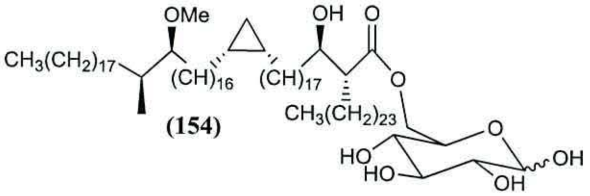
Experiment 19: (3,4,5,6-Tetrahydroxytetrahydro-2*H*-pyran-2-yl)methyl(2*R*)-2-((*R*)-1-hydroxy-18-((1*S*,2*R*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl)tetra-cosanoate (153)



(153)

Palladium hydroxide 10% on charcoal (6 mg) was added to a stirred solution of (3,4,5,6-tetrakis(benzyloxy)tetrahydro-2*H*-pyran-2-yl)methyl (2*R*)-2-((*R*)-1-hydroxy-18-((1*S*,2*R*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)-octadecyl)tetracosanoate (**182**) (45 mg, 0.02 mmol) in dry CH_2Cl_2/CH_3OH (2 mL, 1:1) at room temperature then the suspension was stirred for 18 h under a hydrogen atmosphere. The reaction was diluted with dichloromethane (20 ml), filtered through a bed of celite. The crude product was purified by column chromatography eluting with chloroform/methanol (10:1) to give a semi-solid, *(3,4,5,6-tetrakis(benzyloxy)-tetrahydro-2H-pyran-2-yl)methyl(2R)-2-((R)-1-hydroxy-12-((1R,2S)-2-(14-((1R,2S)-2-icosylcyclopropyl)tetradecyl)cyclopropyl)dodecyl)hexa-cosanoate* (**153**) (32 mg, 94%) as a mixture of two isomers (α, β) in ratio 1:1, $[\alpha]_D^{22}$ + 35 (*c* 1.2, $CHCl_3$), [Found $(M+Na)^+$: 1410.3035, $C_{89}H_{174}NaO_9$ requires: 1410.3050], which showed δ_H (400 MHz, $CDCl_3$): 5.09 (1H, d, *J* 3.8 Hz) for the α-isomer, 4.44 (1H, d, *J* 7.8 Hz) for the β-isomer, and for the mixture 4.41 – 4.34 (2H, m), 4.28 – 4.20 (2H, m), 3.97 – 3.90 (1H, m), 3.67 – 3.55 (4H, m), 3.49 – 3.41 (1H, m), 3.39 – 3.34 (2H, m), 3.34 – 3.31 (3H, m), 3.27 (6H, s), 3.19 – 3.13 (1H, m), 2.94 – 2.86 (2H, m), 2.43 – 2.31 (4H, m), 1.65 – 0.93 (241H, m), 0.81 (12H, t, *J* 6.9 Hz), 0.78 (4H, d, *J* 6.9 Hz), 0.62 – 0.53 (4H, m), 0.53 – 0.45 (2H, m), -0.41 (2H, q, *J* 5.2 Hz); δ_C (α, β isomer) (101 MHz, $CDCl_3$): 175.3, 96.7, 92.4, 85.7, 73.7, 72.63, 72.3, 70.6, 69.4, 63.7, 57.7, 52.9, 52.7, 35.4, 35.1, 32.4, 32.0, 30.5, 30.3, 30.0, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 28.8, 27.6, 27.4, 26.1, 25.5, 22.7, 15.8, 14.8, 14.1, 10.9; ν_{max}/cm^{-1}: 3456, 2919, 2850, 1728, 1148, 1106, 992, 721, 427.

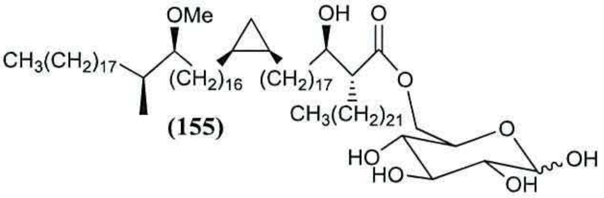
Experiment 20: (3,4,5,6-Tetrahydroxytetrahydro-2*H*-pyran-2-yl)methyl(2*R*)-2-((*R*)-1-hydroxy-18-((1*S*,2*R*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl)hexacosanoate (154)



(154)

Palladium hydroxide 10% on charcoal (6 mg) was added to a stirred solution of (3,4,5,6-tetrakis(benzyloxy)tetrahydro-2*H*-pyran-2-yl)methyl (2*R*)-2-((*R*)-1-hydroxy-18-((1*S*,2*R*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl)hexacosanoate (**183**) (46 mg, 0.02 mmol) in dry CH_2Cl_2/CH_3OH (2 mL, 1:1) at room temperature then the suspension was stirred for 18 h under a hydrogen atmosphere. The reaction mixture was worked up and purified as before to give a semi-solid, *(3,4,5,6-tetrahydroxytetrahydro-2H-pyran-2-yl)methyl(2R)-2-((R)-1-hydroxy-18-((1S,2R)-2-((17S,18S)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl)hexacosanoate* (**154**) (34 mg, 94%) as a mixture of two isomers (α, β) in ratio 1:1, $[\alpha]_D^{22}$ + 32 (*c* 1.3, $CHCl_3$), [Found $(M+Na)^+$: 1438.3348, $C_{91}H_{178}NaO_9$ requires: 1438.3363], which showed δ_H (400 MHz, $CDCl_3$): 5.09 (1H, d, *J* 3.8 Hz) for the α-isomer, 4.44 (1H, d, *J* 7.8 Hz) for the β-isomer, and for the mixture 4.41 – 4.34 (2H, m), 4.28 – 4.20 (2H, m), 3.97 – 3.90 (1H, m), 3.67 – 3.55 (4H, m), 3.49 – 3.41 (1H, m), 3.39 – 3.34 (2H, m), 3.34 – 3.31 (3H, m), 3.27 (6H, s), 3.19 – 3.13 (1H, m), 2.94 – 2.86 (2H, m), 2.43 – 2.31 (4H, m), 1.65 – 0.93 (241H, m), 0.81 (12H, t, *J* 6.9 Hz), 0.78 (4H, d, *J* 6.9 Hz), 0.62 – 0.53 (4H, m), 0.53 – 0.45 (2H, m), -0.41 (2H, q, *J* 5.2 Hz); δ_C (α, β isomer) (101 MHz, $CDCl_3$): 175.3, 96.7, 92.4, 85.7, 73.7, 72.63, 72.3, 70.6, 69.4, 63.7, 57.7, 52.9, 52.7, 35.4, 35.1, 32.4, 32.0, 30.5, 30.3, 30.0, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 28.8, 27.6, 27.4, 26.1, 25.5, 22.7, 15.8, 14.8, 14.1, 10.9, ν_{max}/cm^{-1}: 3456, 2919, 2850, 1728, 1468, 1148, 1106, 992, 721, 427.

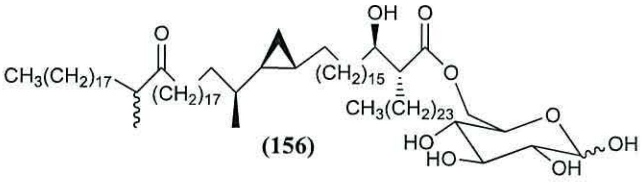
Experiment 21: (3,4,5,6-Tetrahydroxytetrahydro-2*H*-pyran-2-yl)methyl(2*R*)-2-((*R*)-1-hydroxy-18-((1*R*,2*S*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl)tetracosanoate (155)



(155)

Palladium hydroxide 10% on charcoal (5 mg) was added to a stirred solution of (3,4,5,6-tetrakis(benzyloxy)tetrahydro-2*H*-pyran-2-yl)methyl (2*R*)-2-((*R*)-1-hydroxy-18-((1*R*,2*S*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)-octadecyl)tetracosanoate (**184**) (41 mg, 0.02 mmol) in dry CH_2Cl_2/CH_3OH (2 mL, 1:1) at room temperature then the suspension was stirred for 18 h under a hydrogen atmosphere. The reaction mixture was worked up and purified as before to give a semi-solid, *(3,4,5,6-tetrahydroxytetrahydro-2H-pyran-2-yl)methyl(2R)-2-((R)-1-hydroxy-18-((1R,2S)-2-((17S,18S)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl)tetracosanoate* (**155**) (30 mg, 91%) as a mixture of two isomers (α, β) in ratio 1:1, $[\alpha]_D^{22}$ + 38 (*c* 1.2, $CHCl_3$), [Found $(M+Na)^+$: 1410.3062, $C_{89}H_{174}NaO_9$ requires: 1410.3050], which showed δ_H (400 MHz, $CDCl_3$): 5.09 (1H, d, *J* 3.8 Hz) for the α-isomer, 4.44 (1H, d, *J* 7.8 Hz) for the β-isomer, and for the mixture 4.41 – 4.34 (2H, m), 4.28 – 4.20 (2H, m), 3.97 – 3.90 (1H, m), 3.67 – 3.55 (4H, m), 3.49 – 3.41 (1H, m), 3.39 – 3.34 (2H, m), 3.34 – 3.31 (3H, m), 3.27 (6H, s), 3.19 – 3.13 (1H, m), 2.94 – 2.86 (2H, m), 2.43 – 2.31 (4H, m), 1.65 – 0.93 (241H, m), 0.81 (12H, t, *J* 6.9 Hz), 0.78 (4H, d, *J* 6.9 Hz), 0.62 – 0.53 (4H, m), 0.53 – 0.45 (2H, m), -0.41 (2H, q, *J* 5.2 Hz); δ_C (α, β isomer) (101 MHz, $CDCl_3$): 175.3, 96.7, 92.4, 85.7, 73.7, 72.63, 72.3, 70.6, 69.4, 63.7, 57.7, 52.9, 52.7, 35.4, 35.1, 32.4, 32.0, 30.5, 30.3, 30.0, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 28.8, 27.6, 27.4, 26.1, 25.5, 22.7, 15.8, 14.8, 14.1, 10.9, ν_{max}/cm^{-1}: 3456, 2919, 2850, 1728, 1468, 1148, 992, 721, 427.

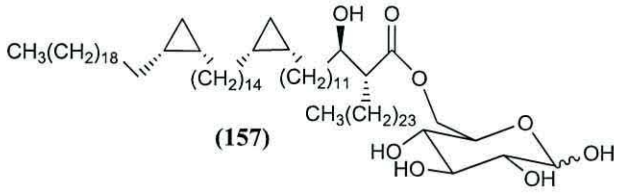
Experiment 22: (3,4,5,6-Tetrahydroxytetrahydro-2*H*-pyran-2-yl)methyl (2*R*)-2-((1*R*)-1-hydroxy-17-((1*R*,2*R*)-2-((2*S*)-22-methyl-21-oxotetracontan-2-yl)cyclopropyl)heptadecyl)hexacosanoate (156)



(156)

Palladium hydroxide 10% on charcoal (5 mg) was added to a stirred solution of (3,4,5,6-tetrakis(benzyloxy)tetrahydro-2*H*-pyran-2-yl)methyl(2*R*)-2-((1*R*)-1-hydroxy-17-((1*R*,2*R*)-2-((2*S*)-22-methyl-21-oxotetracontan-2-yl)cyclopropyl)heptadecyl)-hexacosanoate (**190**) (34 mg, 0.01 mmol) in dry CH_2Cl_2/CH_3OH (2 mL, 1:1) at room temperature then the suspension was stirred for 18 h under a hydrogen atmosphere. The reaction mixture was worked up and purified as before to give a semi-solid, *(3,4,5,6-tetrahydroxytetrahydro-2H-pyran-2-yl)methyl(2R)-2-((1R)-1-hydroxy-17-((1R,2R)-2-((2S)-22-methyl-21-oxotetracontan-2-yl)cyclopropyl)heptadecyl)hexacosanoate* (**156**) (25 mg, 92%) as a mixture of two isomers (α, β) in ratio 1:1, $[\alpha]_D^{23}$ + 15.72 (*c* 1.1, $CHCl_3$), [Found $(M+Na)^+$: 1465.3525, $C_{93}H_{180}NaO_9$ requires: 1464.3525], which showed δ_H (400 MHz, $CDCl_3$): 5.11 (1H, d, *J* 3.6 Hz) for the α-isomer, 4.46 (1H, d, *J* 7.8 Hz) for the β-isomer, and for the mixture 4.40 (2H, br d, *J* 11.8), 4.30 – 4.20 (2H, m), 3.99 – 3.91 (1H, m), 3.68 – 3.57 (3H, m), 3.50 – 3.43 (1H, m), (1H, t, *J* 9.4 Hz), 3.17 (1H, t, *J* 8.3 Hz) 2.46 (2H, q, *J* 6.9), 2.37 (4H, t, *J* 7.4), 1.65 – 1.03 (250 H, m), 0.99 (6H, d, *J* 6.9 Hz), 0.83 (18H, dt, *J* 4.4, 8.7, Hz), 0.67 – 0.55 (3H, m), 0.44 – 0.35 (2H, m), 0.18 – 0.01 (4H, m); δ_C (α, β isomer) (101 MHz, $CDCl_3$): 216.5, 175.2, 96.7, 92.4, 76.3, 74.6, 73.8, 72.6, 72.3, 70.6, 70.4, 69.32, 63.7, 63.6, 52.7, 46.4, 41.2, 38.2, 37.5, 35.1, 34.5, 33.1, 31.9, 30.1, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 27.4, 27.3, 27.3, 26.16, 25.5, 23.7, 22.7, 19.7, 18.6, 16.3, 14.1, 10.5; ν_{max}/cm^{-1}: 3501, 2917, 2855, 1730, 1471, 1151, 1112, 988, 725, 431.

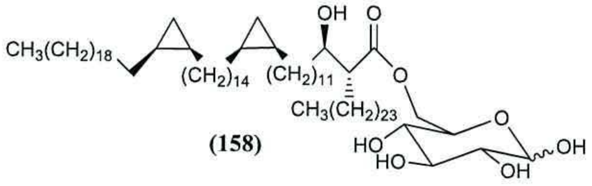
Experiment 23: (3,4,5,6-tetrahydroxytetrahydro-2*H*-pyran-2-yl)methyl(2*R*)-2-((*R*)-1-hydroxy-12-((1*S*,2*R*)-2-(14-((1*S*,2*R*)-2-icosyl-cyclopropyl)tetradecyl)cyclopropyl)dodecyl)hexacosanoate (157)



(157)

Palladium hydroxide 10% on charcoal (7 mg) was added to a stirred solution of (3,4,5,6-tetrakis(benzyloxy)tetrahydro-2*H*-pyran-2-yl)methyl (2*R*)-2-((*R*)-1-hydroxy-12-((1*S*,2*R*)-2-(14-((1*S*,2*R*)-2-icosylcyclopropyl)tetradecyl)cyclopropyl)dodecyl)-hexacosanoate (**187**) (44 mg, 0.02 mmol) in dry CH_2Cl_2/CH_3OH (2 mL, 1:1) at room temperature then the suspension was stirred for 18 h under a hydrogen atmosphere. The reaction mixture was worked up and purified as before to give a semi-solid, *(3,4,5,6-tetrahydroxytetrahydro-2H-pyran-2-yl)methyl(2R)-2-((R)-1-hydroxy-12-((1S,2R)-2-(14-((1S,2R)-2-icosylcyclopropyl)tetradecyl)cyclopropyl)dodecyl)hexacosanoate* (**157**) (35 mg, 96%) as a mixture of two isomers (α, β) in ratio 1:1, $[\alpha]_D^{24}$ + 16 (c 1.2, $CHCl_3$), [Found $(M+Na)^+$: 1322.2175, $C_{84}H_{162}NaO_8$ requires: 1322.2162], which showed δ_H (400 MHz, $CDCl_3$ δ_H (400 MHz, $CDCl_3$): 5.06 (1H, d, J 3.6 Hz) for the α-isomer, 4.41 (1 H, d, J 7.8) for the β-isomer, and for the mixture 4.39 – 4.32 (2H, m), 4.19 (2H, dd, J 5.6, 11.8 Hz), 3.94 – 3.86 (1H, m), 3.63 – 3.54 (3H, m), 3.46 – 3.38 (1 H, m), 3.36 – 3.26 (3 H, m), 3.26 – 3.19 (1H, m), 3.12 (1 H, t, J 8.3), 2.39 – 2.29 (2H, m), 1.61 – 0.95 (250H, m), 0.79 (16H, t, J 6.8), 0.55 (8H, br s), 0.50 – 0.43 (4H, m), -0.43 (4H, q, J 9.4, 5.1); δ_C (α, β isomer) (101 MHz, $CDCl_3$): 175.2, 96.6, 92.4, 76.3, 74.6, 73.8, 73.6, 72.5, 72.3, 70.6, 69.26, 63.6, 52.8, 35.0, 31.9, 30.2, 29.6, 29.4, 29.3, 28.7, 22.6, 15.7, 14.0, 10.8; ν_{max}/cm^{-1}: 3447, 2925, 2886,1744, 1232, 759, 669, 431.

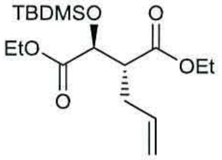
Experiment 24: (3,4,5,6-Tetrahydroxytetrahydro-2*H*-pyran-2-yl)methyl(2*R*)-2-((*R*)-1-hydroxy-12-((1*R*,2*S*)-2-(14-((1*R*,2*S*)-2-icosyl-cyclopropyl)tetradecyl)cyclopropyl)dodecyl)hexacosanoate (158)



(158)

Palladium hydroxide 10% on charcoal (6 mg) was added to a stirred solution of (3,4,5,6-tetrakis(benzyloxy)tetrahydro-2*H*-pyran-2-yl)methyl (2*R*)-2-((*R*)-1-hydroxy-12-((1*R*,2*S*)-2-(14-((1*R*,2*S*)-2-icosylcyclopropyl)tetradecyl)cyclopropyl)dodecyl)-hexacosanoate (50 mg, 0.02 mmol) in dry CH_2Cl_2/CH_3OH (2 mL, 1:1) at room temperature then the suspension was stirred for 18 h under a hydrogen atmosphere. The reaction mixture was worked up and purified as before to give a semi-solid, *(3,4,5,6-tetrahydroxytetrahydro-2H-pyran-2-yl)methyl(2R)-2-((R)-1-hydroxy-12-((1R,2S)-2-(14-((1R,2S)-2-icosylcyclopropyl)tetradecyl)cyclopropyl)dodecyl)hexacosanoate* (**188**) (34 mg, 92%) as a mixture of two isomers (α, β) in ratio 1:1, $[\alpha]_D^{22}$ + 14 (*c* 1.1, $CHCl_3$), [Found $(M+Na)^+$: 1322.2155, $C_{84}H_{162}NaO_8$ requires: 1322.2122], which showed δ_H (400 MHz, $CDCl_3$ δ_H (400 MHz, $CDCl_3$): 5.06 (1H, d, *J* 3.6 Hz) for the α-isomer, 4.41 (1 H, d, *J* 7.8) for the β-isomer, and for the mixture 4.39 – 4.32 (2H, m), 4.19 (2H, dd, *J* 5.6, 11.8 Hz), 3.94 – 3.86 (1H, m), 3.63 – 3.54 (3H, m), 3.46 – 3.38 (1 H, m), 3.36 – 3.28 (3 H, m), 3.23 – 3.19 (1H, m), 3.12 (1 H, t, *J* 8.3), 2.39 – 2.29 (2H, m), 1.61 – 0.95 (250H, m), 0.79 (16H, t, *J* 6.8), 0.55 (8H, br s), 0.50 – 0.43 (4H, m), 0.43 (4H, q, *J* 5.1); (the remaining signals were obscured by the α isomer); δ_C (α, β isomer) (101 MHz, $CDCl_3$): 175.2, 96.6, 92.4, 76.3, 74.6, 73.8, 73.6, 72.5, 72.3, 70.6, 69.26, 63.6, 52.8, 35.0, 31.9, 30.2, 29.6, 29.4, 29.3, 28.7, 22.6, 15.7, 14.0, 10.8; ν_{max}/cm^{-1}: 3447, 2925, 2886,1744, 1232, 759, 669, 431.

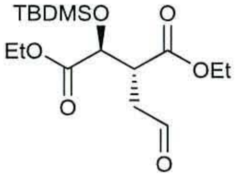
Experiment 25: Diethyl (2*R*,3*S*)-2-allyl-3-((*tert*-butyldimethylsilyl)-oxy)succinate (205)



(205)

tert-Butylchlorodimethylsilane (23.4 g, 0.15 mol.) was added to a stirred solution of diethyl (2*R*,3*S*)-2-allyl-3-hydroxysuccinate (**203**) (30.2 g, 0.13 mol.) in dry DMF (150 mL) and imidazole (20.3 g, 0.29 mol.) at 0 °C. The cooling bath was removed and the reaction was stirred at 70 °C for 24 h. TLC showed the reaction was complete. DMF was removed by flash distillation and water (100 mL) was added and extracted with dichloromethane (3 × 200 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) gave a thick colourless oil, *diethyl (2R,3S)-2-allyl-3-((tert-butyldimethylsilyl)oxy)succinate* (**205**) (40.1 g, 89%), $[\alpha]_D^{22} - 9.4$ (*c* 1.2, $CHCl_3$), [Found $(M+Na)^+$: 367.2. $C_{17}H_{32}NaO_5Si$ requires: 367.2], which showed δ_H (400 MHz, $CDCl_3$): 5.83 – 5.70 (1H, m), 5.13 – 5.01 (2H, m), 4.34 (1H, d, *J* 5.6 Hz), 4.27 – 4.03 (4H, m), 2.99 – 2.92 (1H, m), 2.53 – 2.41 (1H, m), 2.29 – 2.17 (1H, m), 1.29 (3H, t, *J* 7.1 Hz), 1.23 (3H, t, *J* 7.1 Hz), 0.88 (9H, s), 0.07 (3H, s), 0.04 (3H, s); δ_C (101 MHz, $CDCl_3$): 172.1, 171.8, 135.2, 117.2, 73.0, 61.0, 60.6, 50.0, 32.0, 25.7, 14.2, -4.8, -5.3; ν_{max}/cm^{-1}: 3082, 2930, 2861, 1730, 1651, 1468, 1370, 1261, 1182.

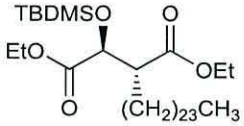
Experiment 26: Diethyl (2*S*,3*R*)-2-((*tert*-butyldimethylsilyl)oxy)-3-(2-oxoethyl)succinate (206)



(206)

Ozone was bubbled to a stirred solution of diethyl (2*R*,3*S*)-2-allyl-3-((*tert*-butyldimethylsilyl)oxy)succinate (**205**) (40 g, 11.6 mmol) and acetic acid (30 mL) in THF:MeOH (1:1) (200 mL) at -78 °C for 30 min, then the reaction mixture was allowed to reach room temperature and zinc powder (1 g) was added. TLC showed no starting material was left. Sat. aq. NH_4Cl (80 mL) (10 ml) and water (80 mL) were added and extracted with ethyl acetate (3 × 200 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) gave a thick colourless oil, *diethyl (2S,3R)-2-((tert-butyldimethylsilyl)oxy)-3-(2-oxoethyl)- succinate* (**206**) (38 g, 95%), (*c* 1.1, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 9.81 (1H, s), 4.47 (1H, d, *J* 4.0 Hz), 4.26 – 4.07 (4H, m), 3.52 – 3.45 (1H, m), 3.02 (1H, dd, *J* 9.1, 15.3 Hz), 2.64 (1H, dd, *J* 4.6, 15.3 Hz), 1.28 (3H, t, *J* 7.2 Hz), 1.23 (3H, t, *J* 7.0 Hz) 0.89 (9H, s), 0.10 (3H, s), 0.05 (3H, s); δ_C (101 MHz, $CDCl_3$): 199.9, 171.4, 170.7, 72.3, 61.4, 61.3, 44.2, 41.3, 25.7, 14.3, 14.2, -4.8, -5.3. ν_{max}/cm^{-1}: 3088, 2980, 2845, 2732, 1738, 1459, 1381, 1318, 1249, 1182, 1095, 1045, 1006.

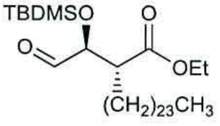
Experiment 27: Diethyl (2*S*,3*R*)-2-((*tert*-butyldimethylsilyl)oxy)-3-(2-oxoethyl)succinate (209)



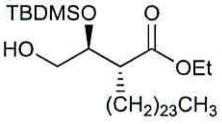
(209)

Lithium bis-(trimethylsilyl) amide (129.2 mL, 203.7 mmol.) was added to a stirred solution of diethyl (2*S*,3*R*)-2-((*tert*-butyldimethylsilyl)oxy)-3-(2-oxoethyl)succinate (**206**) (25.1 g, 72.3 mmol.) and 5-(henicosylsulfonyl)-1-phenyl-1*H*-tetrazole (**207**) (50.7 g, 97.8 mmol.) in dry THF (600 mL) at –10 °C under nitrogen and stirred at –10 °C for 1 h. TLC showed no starting material was left; the reaction was quenched with sat. aq. NH_4Cl (250 mL). The aqueous layer was separated and extracted with petrol/ethyl acetate (20:1, 3 × 100 mL) and the combined organic layers were dried over $MgSO_4$ and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a thick yellow oil, *diethyl(2S,3R)-2-((tert-butyldimethylsilyl)oxy)-3-((E/Z)-tetracos-2-en-1-yl)-succinate* (**208**) (34.2 g; 73%) as a mixture of isomers. Palladium hydroxide on carbon (10%, 1.0 g) was added to the above succinate in CH_2Cl_2/CH_3OH (500 mL, 1:1) and the suspension was stirred overnight under hydrogen atmosphere. The suspension 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 (20:1) to give a thick colourless oil, *diethyl (2S,3R)-2-((tert-butyldimethyl-silyl)oxy)-3-tetracosylsuccinate* (**209**) (33.2 g; 95%), $[\alpha]_D^{22}$ – 8.4 (*c* 1.2, $CHCl_3$), [Found (M-^{t}Bu)$^+$: 583.4758. $C_{34}H_{68}O_5Si$ requires: 584.4836], which showed δ_H (400 MHz, $CDCl_3$): 4.31 (1H, d, *J* 6.8 Hz), 4.25 – 4.07 (4H, m), 2.81 (4H, ddd, *J* 4.6, 6.8, 10.0 Hz), 1.42 – 1.17 (51H, m), 0.87 (12H, s), 0.05 (3H, s), 0.03 (3H, s); δ_C (101 MHz, $CDCl_3$) 172.9, 172.2, 74.0, 61.0, 60.6, 50.6, 32.1, 29.9, 29.8, 29.7, 29.6, 3.7, 29.5, 29.4, 27.8, 27.4, 25.7, 22.8, 14.3, 14.2, 14.1, -4.9, -5.4; ν_{max}/cm^{-1}: 3088, 2928, 2860, 1740, 1460, 1380, 1252, 1174, 1000, 1011, 969, 836, 810, 775.

Experiment 28: Ethyl (*R*)-2-((*S*)-1-((*tert*-butyldimethylsilyl)oxy)-2-oxoethyl)hexacosanoate (210), (211)



+



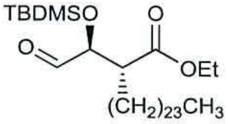
(210) **(211)**

A solution of diethyl (2*S*,3*R*)-2-((*tert*-butyldimethylsilyl)oxy)-3-tetracosylsuccinate (**209**) (10.0 g, 15.6 mmol) and magnesium bromide etherate (6.0 g, 23.2 mmol) in methylene chloride (500 mL) was stirred at room temperature for 1 h and then cooled to -55 °C. Diisobutylaluminum hydride (34.3 mL of a 1 M solution in toluene) was added dropwise via syringe (one drop every 8-10 s) at -55 °C. The reaction mixture was allowed to warm to 0 °C. Methanol (30 mL) and freshly prepared sat. aq. sodium sulfate decahydrate (30 mL) were added at 0 °C and stirred at r.t. for 2 h. The organic phase was separated and water layer was extracted with methylene chloride (3 × 250 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 (20:1) gave the first fraction a semi-solid, *ethyl (R)-2-((S)-1-((tert-butyldimethylsilyl)oxy)-2-oxoethyl)hexacosanoate* (**209**) (3.1 g, 33%), $[\alpha]_D^{22}$ – 6.4 (c 0.8, $CHCl_3$), [Found (M-^{t}Bu)$^+$: 539.4467, $C_{32}H_{63}O_4Si$ requires: 539.4459], which showed δ_H (400 MHz, $CDCl_3$): 9.62 (1H, d, *J* 1.8 Hz), 4.22 – 4.06 (2H, m), 4.04 (1H, dd, *J* 1.7, 5.2), 2.77 (1H, ddd, *J* 2.9, 5.7, 11.2), 1.83 – 1.70 (H, m), 1.52 – 1.38 (1H, m), 1.25 (50H, s), 0.89 (12H, s), 0.06 (3H, s), 0.05 (3H, s); δ_C (101 MHz, $CDCl_3$):

203.0, 172.1, 78.0, 60.8, 49.2, 32.1, 29.9, 29.8, 29.7, 29.6, 29.5, 27.5, 27.4, 25.7, 22.8, 14.4, 14.3, -4.4, -5.0; ν_{max}/cm^{-1}: 2924, 2855, 1740, 1460.

The second fraction was ethyl *(R)-2-((S)-1-((tert-butyldimethylsilyl)oxy)-2-hydroxyethyl)hexacosanoate* (**211**) (4.2 g, 45%), $[\alpha]_D^{22} - 8.1$ (*c* 1.2, CHCl₃), [Found (M-^{t}Bu)$^+$: 541.4676, $C_{32}H_{64}O_4Si$ requires: 541.4652], which showed δ_H (400 MHz, CDCl₃): 4.20 – 4.05 (2H, m), 3.95 – 3.88 (2H, m), 3.61 (2H, br d, *J* 3.6 Hz), 2.74 – 2.64 (1H, m), 1.97 (1H, d, *J* 5.3 Hz), 1.64 – 1.51 (2H, m), 1.51 – 1.37 (1H, m), 1.24 (51H, s), 0.87 (12H, s), 0.08 (3H, s), 0.05 (3H, s); δ_C (101 MHz, CDCl₃): 174.6, 74.0, 63.9, 60.4, 49.3, 32.1, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 28.4, 27.6, 25.8, 22.8, 14.4, 14.3, -4.3, -4.9; ν_{max}/cm^{-1}: 3363, 2925, 2854, 1738, 1465, 1254, 1056.

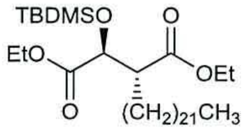
Experiment 29: Ethyl (*R*)-2-((*S*)-1-((*tert*-butyldimethylsilyl)oxy)-2-oxoethyl)hexacosanoate (210)



(210)

Ethyl (*R*)-2-((*S*)-1-((*tert*-butyldimethylsilyl)oxy)-2-hydroxyethyl)hexacosanoate (**211**) (0.5 g, 0.9 mmol.) in dichloromethane (4 mL) was added to a stirred suspension of pyridinium chlorochromate (0.6 g, 2.6 mmol.) in dichloromethane (20 mL) in portions at room temperature. The mixture was stirred for 2 h. TLC showed no starting material was left. The mixture was diluted with petrol/ether (5:1, 200 mL) and filtered through a bed of Celite on silica washed with warm ether (400 mL). The filtrate was evaporated and concentrated to obtain the crude product. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) gave a semi-solid, *ethyl (R)-2-((S)-1-((tert-butyldimethylsilyl)oxy)-2-oxoethyl)hexacosanoate* (**210**) (0.4 g, 82%), which showed ^{1}H, ^{13}C and IR spectra identical to the experiment **28**.

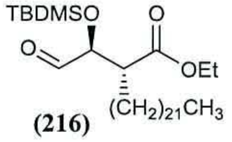
Experiment 30: Diethyl (2*S*,3*R*)-2-((*tert*-butyldimethylsilyl)oxy)-3-docosylsuccinate (215)



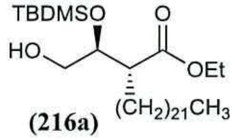
(215)

Lithium bis-(trimethylsilyl) amide (63.8 mL, 203.7 mmol.) was added to a stirred solution of diethyl (2*S*,3*R*)-2-((*tert*-butyldimethylsilyl)oxy)-3-(2-oxoethyl)succinate (**206**) (13.1 g, 56.0 mmol.) and 5-(nonadecylsulfonyl)-1-phenyl-1*H*-tetrazole (**213**) (21.4 g, 45.0 mmol.) in dry THF (400 mL), at –10 °C under nitrogen atmosphere and stirred at r.t. 2 h. TLC showed the reaction was complete and sat. aq NH_4Cl (250 mL) was added. The aqueous layer was separated and extracted with petrol/ethyl acetate (10:1, 3 × 100 mL). The combined organic layers were dried over $MgSO_4$ and solvent was evaporated to give a crude product. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a thick yellow oil, *diethyl (2S,3R)-2-((tert-butyldimethylsilyl)oxy)-3-((E/Z)-docos-2-en-1-yl)succinate* (**214**) (16.5 g; 72%) as a mixture of *E/Z* isomer. Palladium hydroxide on carbon (10 % 1.0 g) was added to the above succinate in CH_2Cl_2/CH_3OH (500 mL, 1:1) and the suspension was stirred overnight under hydrogen. The suspension was filtered over a bed of Celite and washed with ethyl acetate (100 mL). The filtrate was concentrated and purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a thick oil, *diethyl (2S,3R)-2-((tert-butyldimethylsilyl)oxy)-3-docosylsuccinate* (**215**) (15.9 g, 96%) $[\alpha]_D^{22}$ – 7.4 (*c* 1.1, $CHCl_3$), [Found (M-^{t}Bu)$^+$: 555.4434, $C_{32}H_{63}O_5Si$ requires: 555.4443], which showed δ_H (400 MHz, $CDCl_3$): 4.31 (1H, d, *J* 6.8 Hz), 4.25 – 4.07 (4H, m), 2.81 (4H, ddd, *J* 4.6, 6.8, 10.0 Hz), 1.42 – 1.17 (47H, m), 0.87 (12H, s), 0.05 (3H, s), 0.03 (3H, s); δ_C (101 MHz, $CDCl_3$): 172.9, 172.2, 74.0, 61.0, 60.6, 50.6, 32.1, 29.9, 29.8, 29.7, 29.6, 3.7, 29.5, 29.4, 27.8, 27.4, 25.7, 22.8, 14.4, 14.3, 14.2, -4.9, -5.4; ν_{max}/cm^{-1}: 2931, 2850, 1767, 1746, 1472, 1434, 1350.

Experiment 31: Ethyl (*R*)-2-((*S*)-1-((*tert*-butyldimethylsilyl)oxy)-2-oxoethyl)tetracosanoate (216), (216a)



(216)

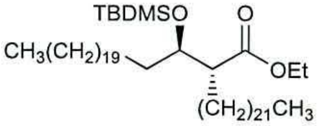


(216a)

A solution of diethyl (2*S*,3*R*)-2-((tert-butyldimethylsilyl)oxy)-3-docosylsuccinate (**215**) (5.0 g, 8.1 mmol) and magnesium bromide etherate (3.1 g, 12.2 mmol) in methylene chloride (250 mL) was stirred at room temperature for 1 h. Diisobutylaluminum hydride (17.9 mL of a 1 M solution in toluene) was added dropwise via syringe (one drop every 8-10 s) at -55 °C. The reaction mixture was allowed to warm to 0 °C. Methanol (20 mL) and sat. aq. sodium sulfate decahydrate (20 mL) were added and stirred at r.t. for 2 h. The organic phase was separated and water layer was extracted with methylene chloride (3 × 150 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give the first fraction as semi-solid, *ethyl (R)-2-((S)-1-((tert-butyldimethylsilyl)oxy)-2-oxoethyl)-tetracosanoate* (**216**) (1.6 g, 34%), $[\alpha]_D^{22}$ – 4.4 (c 1.2, $CHCl_3$), [Found (M-^{t}Bu)$^+$: 511. 4178, $C_{32}H_{59}O_4Si$ requires: 511.4182], which showed, δ_H (400 MHz, $CDCl_3$): 9.62 (1H, d, *J* 1.8 Hz), 4.22 – 4.06 (2H, m), 4.04 (1H, dd, *J* 1.7, 5.2 Hz), 2.77 (1H, ddd, *J* 2.9, 5.7, 11.2 Hz), 1.83 – 1.70 (1H, m), 1.52 – 1.38 (1H, m), 1.25 (44 H, s), 0.89 (12H, s), 0.06 (3H, s), 0.05 (3H, s); δ_C (101 MHz, $CDCl_3$): 203.0, 172.1, 78.0, 60.8, 49.2, 32.1, 29.9, 29.8, 29.7, 29.6, 29.4, 29.5, 27.3, 27.2, 25.7, 22.8, 14.4, 14.3, -4.4, -5.0;ν_{max}/cm^{-1}: 2924, 2855, 1740, 1460.

The second fraction was a semi-solid, *ethyl (R)-2-((S)-1-((tert-butyldimethylsilyl)-oxy)-2-hydroxyethyl)tetracosanoate* (**216a**) (1.9 g, 41%), $[\alpha]_D^{22}$ – 6.6 (c 1.1, $CHCl_3$), [Found (M-^{t}Bu)$^+$: 513. 4348, $C_{32}H_{61}O_4Si$ requires: 513.4334], which showed δ_H (400 MHz, $CDCl_3$): 4.20 – 4.05 (2H, m), 3.95 – 3.88 (2H, m), 3.61 (2H, d, *J* 3.6 Hz), 2.74 – 2.64 (1H, m), 1.97 (1H, d, *J* 5.3 Hz), 1.64 – 1.51 (2H, m), 1.51 – 1.37 (1H, m), 1.24 (51H, s), 0.87 (12H, s), 0.08 (3H, s), 0.05 (3H, s); δ_C NMR (101 MHz, $CDCl_3$): 174.6, 74.0, 63.9, 60.4, 49.3, 32.1, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 28.4, 27.6, 25.8, 22.8, 14.4, 14.3, -4.3, -4.9; ν_{max} /cm^{-1}: 3363, 2925, 2854, 1738, 1465, 1254, 1056.

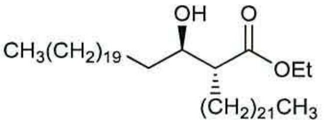
Experiment 32: Ethyl (2*R*,3*R*)-3-((*tert*-butyldimethylsilyl)oxy)-2-docosyltetracosanoate (218)



(218)

Lithium bis-(trimethylsilyl) amide (0.6 mL) was added to a stirred solution of ethyl (*R*)-2-((*S*)-1-((tert-butyldimethylsilyl)oxy)-2-oxoethyl)tetracosanoate (**216**) (0.2 g, 0.3 mmol.) and 5-(nonadecylsulfonyl)-1-phenyl-1*H*-tetrazole (**213**) (0.9 g, 0.4 mmol) in dry THF (25 mL) at –10 °C under nitrogen and stirred at r.t. for 2 h. TLC showed the reaction was complete. Sat. aq. ammonium chloride (25 mL) was added and extracted with petrol/ethyl acetate 10:1 (3 × 100) mL) and dried over $MgSO_4$ and the solvent was evaporated to obtain a crude product. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (40:1) to give a thick yellow oil, *ethyl (2R,3R,E/Z)-3-((tert-butyldimethylsilyl)oxy)-2-docosyltetracos-5-enoate* (**217**) (0.24 g, 82%), as a mixture. This was dissolved in THF/IMS (50 mL, 1:1) and palladium (10% on carbon) (0.1 g) was added, and the suspension was stirred overnight under a hydrogen atmosphere. The suspension was filtered over a bed of Celite and washed with ethyl acetate (20 mL). The filtrate was concentrated and purified by column chromatography eluting with petrol/ethyl acetate (40:1) to give a thick colourless oil, *ethyl (2R,3R)-3-((tert-butyldimethylsilyl)oxy)-2-docosyl-tetracosanoate* (**218**) (0.22 g, 91%), $[\alpha]_D^{22}$ – 3.4 (*c* 1.1, $CHCl_3$), [Found (M-^{t}Bu)$^+$: 777.7520, $C_{50}H_{102}O_3Si$ requires: 777.7519], which showed δ_H (400 MHz, $CDCl_3$): 4.18 – 4.05 (2H, m), 3.94 – 3.87 (1H, m), 2.49 (1H, ddd, *J* 3.8, 7.0, 10.8 Hz), 1.61 – 1.14 (85H, m), 0.93 – 0.82 (15H, m), 0.04 (3H, s), 0.02 (3H, s); δ_C (101 MHz, $CDCl_3$) 174.8, 73.4, 60.1, 51.7, 33.8, 32.0, 30.0, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 27.9, 27.6, 25.9, 23.9, 22.9, 14.3, -4.2, -4.8; ν_{max}/cm^{-1}: 2925, 2854, 1741, 1465, 1378, 1254, 1178, 1096, 1005.

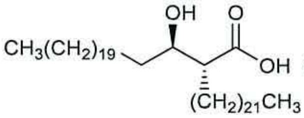
Experiment 33: Ethyl (2*R*,3*R*)-2-docosyl-3-hydroxytetracosanoate (219)



(219)

Ethyl (2*R*,3*R*)-3-((tert-butyldimethylsilyl)oxy)-2-docosyltetracosanoate (**218**) (0.22 g, 0.02 mmol.), was dissolved in dry THF (4.0 mL) in a dry polyethylene vial under nitrogen at room temperature and stirred. Pyridine (0.1 mL) and HF pyridine (0.7 mL) were added, and the reaction was stirred for 17 h at 40 ˚C. TLC showed that the starting material had almost gone. Sat. aq. NH_4Cl (20 ml) petrol/ ethyl acetate (10:1, 30 mL) were added and extracted. The aqueous layer was separated and re-extracted with ethyl acetate 10:1 (3 × 25 mL). The organic layer was washed with brine and dried over $MgSO_4$. 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, *ethyl (2R,3R)-2-docosyl-3-hydroxytetracosanoate* (**219**) (0.16g, 88%), mp. 45 ˚C, $[\alpha]_D^{22}$ + 8.2 (*c* 0.8, $CHCl_3$), [Found Found $(M\text{-}H_2O)^+$: 702.7251, $C_{50}H_{102}O_3Si$ requires: 702.7253], which showed δ_H (400 MHz, $CDCl_3$): 4.20 (2H, q, *J* 7.1 Hz), 3.72 – 3.63 (1H, m), 2.51 (1H, d, *J* 8.3 Hz), 2.48 – 2.39 (1H, m), 1.80 – 1.69 (1H, m), 1.66 – 1.57 (1H, m), 1.54 – 1.40 (3H, m), 1.39 – 1.20 (80H, m), 0.90 (6H, t, *J* 6.8 Hz); δ_C (101 MHz, $CDCl_3$) 176.0, 72.5, 60.5, 51.0, 35.9, 32.1, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 27.5, 25.9, 22.8, 14.3; ν_{max} /cm^{-1}: 3500, 2950, 2920, 2855, 1741, 1473, 1463, 1377, 1180.

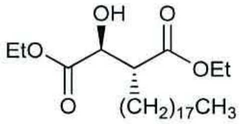
Experiment 34: (2*R*,3*R*)-2-Docosyl-3-hydroxytetracosanoic acid (220)



(220)

Lithium hydroxide monohydrate (0.02 g, 0.476 mmol) was added to a stirred solution of ethyl (2*R*,3*R*)-2-docosyl-3-hydroxytetracosanoate (**219**) (0.16 g, 0.22 mmol) in MeOH:THF:H_2O, (1:3:1, 4 mL) at room temperature. The mixture reaction was stirred at 45 ˚C for 18 h. TLC showed no starting material was left. The reaction was cooled to room temperature and diluted with petrol/ethyl acetate (7:2, 5 mL). The aqueous layer was separated and re-extracted with petrol/ethyl acetate (5:2, 3 × 20mL). The combined organic layers were dried over $MgSO_4$ and filterted. The filtrate was concentrated and purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a white solid, *(2R,3R)-2-docosyl-3-hydroxytetra-cosanoic acid* (**220**) (0.14g, 84%), mp. 52 – 54 °C, $[\alpha]_D^{22}$ + 5.1 (c 1.2, $CHCl_3$), [Found $(M+Na)^+$: 715.6, $C_{46}H_{92}NaO_3$ requires: 715.7], which showed δ_H (500 MHz, $CDCl_3$): 3.76 – 3.68 (1H, m), 2.54 – 2.43 (1H, m), 1.80 – 1.70 (1H, m), 1.67 – 1.59 (2H, m), 1.57 – 1.43 (3H, m), 1.28 – 1.20 (78H, m), 0.88 (6H, t, *J* 6.8 Hz), δ_C (101 MHz, $CDCl_3$): 174.6, 72.3, 50.7, 35.7, 32.1, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 27.5, 25.9, 22.8, 14.3; ν_{max} /cm^{-1}: 3580, 3390, 2916, 2849, 1744, 1465, 1381, 1047.

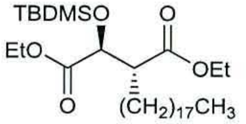
Experiment 35: Diethyl (2*S*,3*R*)-2-hydroxy-3-octadecylsuccinate (212)



(212)

Butyllithium solution (15.1 mL, 1.6 M in hexanes) was added to a stirred solution of diisopropylamine (3.7 ml, 262.3 mmol) in dry THF (25 mL) at 0 °C and stirred for 30 min. diethyl (*S*)-2-hydroxysuccinate (**187**) (2.0 g, 10.5 mmol) in dry THF (10 mL) was added –55 °C and stirred for 1 hr. 1-Iodooctadecane (5.9 g, 15.7 mmol) in dry THF (10 ml) was added via syringe at –55 °C and stirred for 1 h and warmed slowly to -20 °C, and stirred at that temperature for 1 h and at room temperature for 1 h. Sat. aq. NH_4Cl (50 mL) was added and extracted. The aqueous layer was re-extracted with ethyl acetate (3 × 50mL) and the combined organic layers dried over $MgSO_4$. The solvent was evaporated and the crude product was purified by column chromatography eluting with petrol/ethyl acetate (5:1) gave a semi-solid, *diethyl (2S,3R)-2-hydroxy-3-octadecylsuccinate* (**212**) (1.5 g, 32%), $[\alpha]_D^{22}$ + 6.4 (*c* 0.8, $CHCl_3$), [Found $(M+Na)^+$: 465.4, $C_{26}H_{50}O_5Na$ requires: 465.4], which showed δ_H (400 MHz, $CDCl_3$): 4.32 – 4.20 (3H, m), 4.16 (1H, dd, *J* 1.9, 7.1 Hz), 4.12 (1H, dd, *J* 1.8, 7.1 Hz), 3.19 (1H, d, *J* 7.6 Hz), 2.83 (1H, dt, *J* 3.6, 7.4 Hz), 1.90 – 1.77 (1H, m), 1.71 – 1.60 (1H, m), 1.43 – 1.16 (38H, m), 0.87 (3H, t, *J* 6.8 Hz); δ_C (101 MHz, $CDCl_3$) 173.6, 173.0, 71.2, 61.8, 60.8, 48.7, 32.0, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 28.2, 27.5, 22.8, 14.3, 14.2; ν_{max} /cm^{-1}: 3521, 2955, 2920, 2855, 1711, 1473, 1463, 1377, 1180.

Experiment 36: Diethyl (2*S*,3*R*)-2-((*tert*-butyldimethylsilyl)oxy)-3-octadecylsuccinate (212a)



(212a)

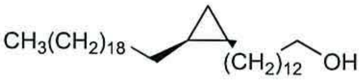
Imidazole (0.22 g, 2.9 mmol) was added to the stirred solution of diethyl (2*S*,3*R*)-2-hydroxy-3-octadecylsuccinate (**212**) (0.60 g, 1.0 mmol) in dry DMF (15 mL) at room temperature. The mixture was cooled to 0 °C followed by the addition of *tert*-butyldimethylchlorosilane (0.25 g, 1.60 mmol). The cooling bath was removed and the reaction was stirred at 70 °C for 18 h. TLC showed no starting material was left and DMF was removed by flash distillation at 50 °C and 1mm/Hg. The residue was dissolved in water (25 mL) and extracted with dichloromethane (3 × 50 mL). The combined organic extracts were washed with water and brine and then dried over $MgSO_4$, and concentrated to obtain a crude product. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a thick colourless oil, *diethyl (2S,3R)-2-((tert-butyldimethylsilyl)oxy)-3-octadecylsuccinate* (**212a**) (0.70 g, 93%), $[\alpha]_D^{22} - 11$ - 11 (*c* 1.1, $CHCl_3$), [Found $(M+Na)^+$: 579.4, $C_{32}H_{64}NaO_5Si$ requires: 579.4], which showed δ_H (400 MHz, $CDCl_3$): 4.28 (1H, d, *J* 6.8 Hz), 4.22 – 4.02 (4H, m), 2.77 (1H, ddd, *J* 4.7, 6.8, 10.0 Hz), 1.43 – 1.13 (38H, m), 0.90 – 0.77 (12H, m), 0.02 (3H, s), -0.00 (3H, s); δ_C (101 MHz, $CDCl_3$): 172.9, 172.2, 74.0, 61.0, 60.5, 50.6, 32.1, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 27.8, 27.4, 25.7, 22.8, 14.4, 14.3, 14.2, -4.9, -5.3; ν_{max} /cm^{-1}: 2950, 2925, 2855, 1719, 1468, 1459, 1377, 1180.

Experiment 37: (1*R*,2*S*)-2-Icosylcyclopropane-1-carbaldehyde (242)

(242)

((1*R*,2*S*)-2-icosylcyclopropyl)methanol (**234**) (8.5 g 24.1 mmol) in dichloromethane (50 mL) was added to a stirred suspension of pyridinium chlorochromate (12.9 g, 60.3 mmol.) in dichloromethane (300 mL) at r.t and stirred for 2 h. TLC showed no starting material was left. The mixture was diluted with petrol/ether (2:1, 300 mL) and filtered through a bed of Celite on Silica, then washed with warm ether (400 mL) and the filtrate was evaporated to give a residue which was purified by column chromatography on silica eluting with petrol/ethyl acetate (2:1) to give *(1R,2S)-2-icosylcyclopropane-1-carbaldehyde* (**242**) (8.7 g, 97%), mp. 45–47˚C, $[\alpha]_D^{22}$ - 8.5 (c 1.64, $CHCl_3$) (*lit*[221] $[\alpha]_D^{22}$ - 3.9 (*c* 1.22, $CHCl_3$)). The compound showed δ_H, δ_C and ν_{max} identical to the literature.[221]

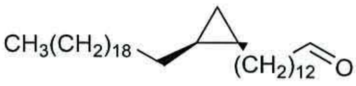
Experiment 38: 13-((1*R*,2*S*)-2-Icosylcyclopropyl)tridecan-1-ol (245)



(245)

Lithium bis-(trimethylsilyl) amide (34.5 mL, 36.6 mmol.) was added to a stirred solution of (1*R*,2*S*)-2-icosylcyclopropane-1-carbaldehyde (8.5 g, 24.9 mmol) and 12-((1-phenyl-1*H*-tetrazol-5-yl)thio)dodecyl pivalate (**240**) (13.9 g, 31.1 mmol) in THF (400 mL) at 0 ˚C. The reaction was worked up as before. Column chromatography eluting with petrol/ether (20:1) to give a white solid of *(E/Z)-13-((1R,2S)-2-icosylcyclopropyl)tridec-12-en-1-yl pivalat* (**243**) (7.7g; 55 %), as a mixture. **(243)** (7.7 g, 12.7 mmol) in THF (30 mL) was added to a stirred solution of lithium aluminium hydride (0.72 g, 19.1 mmol.) in THF (100 mL) at 0 °C and the mixture was refluxed for 3 h. TLC showed no starting material was left. Sat.aq. sodium sulphate decahydrate (100 mL) was added dropwise and stirred at r.t until white solid was formed. THF (40 mL) was added and the mixture was filtered through a bed of celite and washed with THF (50 mL) the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ ethyl acetate (5:2) to give, *(E/Z)-13-((1R,2S)-2-icosylcyclopropyl)tridec-12-en-1-ol* (**244**) (11.0 g: 97%). Hydrazine monohydrate (50 mL), acetic acid (5 mL) and sat. aq. copper sulfate (5 mL) were added to a stirred solution of (**244**) (11.0 g, 21.2 mmol) in I P E (500 mL) at 50 °C , then sodium meta periodate solution (40.0 g, 21.2 mmol) in hot water (150 mL) was carefully added over a period of 2.5 h at 80 °C. The reaction was worked as before, the aqueous layer was separated and extracted with petrol/ethyl acetate (1:1, 3 × 500 mL) and the combined organic layers were dried and concentrated. The crude product was purified by column chromatography eluting with petrol/ ethyl acetate (5:1) to give, *13-((1R,2S)-2-icosylcyclopropyl)tridecan-1-ol* (**245**) (9.7 g; 88%), m.p.73–75˚C , $[\alpha]_D^{22}$ - 2.1 (*c* 1.2, $CHCl_3$) (*lit.*[221] $[\alpha]_D^{22}$ - 2.0 (*c* 1.03, $CHCl_3$)) which showed δ_H, δ_C and ν_{max} identical to the literature.[221]

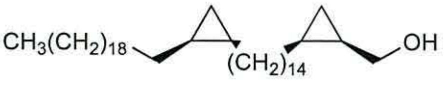
Experiment 39: 13-((1*R*,2*S*)-2-Icosylcyclopropyl)tridecanal (246)



(246)

13-((1*R*,2*S*)-2-Icosylcyclopropyl)tridecan-1-ol (**245**) (3.0 g, 5.7 mmol) in dichloromethane (50 mL) was added to a stirred suspension of pyridinium chlorochromate (3.1 g, 14.4 mmol) in dichloromethane (60 mL) in portions at r.t and stirred for 2 h. TLC showed no starting material was left. The mixture was diluted with petrol/ether 2:1 (300 mL) and filtered through a bed of Celite on Silica, then washed with warm ether (400 mL) and the filtrate was evaporated to give a residue which was purified by column chromatography eluting with petrol/ethyl acetate (2:1) to give, *13-((1R,2S)-2-icosylcyclopropyl)tridecanal* (**246**) (2.6 g, 89%), mp 61-64 ˚C, *lit.* 64-66 ˚C, $[\alpha]_D^{20}$ + 2.2 (*c* 1.2, $CHCl_3$) (*lit.*[221] $[\alpha]_D^{22}$ + 1.6 (*c* 1.03, $CHCl_3$)) which showed δ_H, δ_C and ν_{max} identical to the literature.[221]

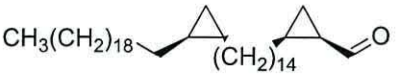
Experiment 40: ((1*R*,2*S*)-2-(14-((1*R*,2*S*)-2-Icosylcyclopropyl) tetradecyl) cyclopropyl)methanol (250)



(250)

Lithium bis(trimethylsilyl) amide (13.6 mL, 13 mmol) was added dropwise to a stirred solution of 13-((1*R*,2*S*)-2-icosylcyclopropyl)tridecanal (**246**) (2.6 g, 6.9 mmol) and ((1*R*,2*S*)-2-((1-phenyl-1H-tetrazol-5-ylsulfonyl)methyl)cyclopropyl) methyl butyrate (**247**) (3.5 g, 7.53 mmol) in dry THF (30 mL) under nitrogen at -10 ˚C. The reaction was allowed to reach r.t and stirred for 5 h. TLC showed no starting material was left. Sat.aq. ammonium chloride (50 mL) and petrol/ether (1:1, 100 mL) were added. The organic layer was separated and aqueous layer was re-extracted with petrol/ether (1:1, 2 × 75 mL). The combined organic layers were dried and evaporated to give a solid residue which was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give, *((1R,2S)-2-((E/Z)-14-((1R,2S)-2-icosylcyclopropyl)tetradec-13-en-1-yl)cyclopropyl)methyl butyrate* (**248**) (3.2 g, 68%). $LiAlH_4$ (0.2 g) was added to stirred THF (100 mL) at 0 °C under nitrogen to check THF dryness. Then further $LiAlH_4$ (1.1 g, 26 mmol) was added. A solution of (**248**) (3.2 g, 4.47 mmol) in dry THF (20 mL) was added dropwise at 0 °C and the mixture was refluxed for 3 h. TLC showed no starting material was left. Sat.aq. sodium sulphate decahydrate (20 mL) was added dropwise at 0 °C and stirred at r.t until white solid was formed. THF (40 mL) was added and the mixture was filtered through a bed of celite dried and the solvent evaporated. The crude product was purified by column chromatography eluting with petrol/ether (5:1) to give, *((1R,2S)-2-((E/Z)-14-((1R,2S)-2-icosylcyclopropyl)tetradec-13-en-1-yl)cyclopropyl)methanol* (**249**) (2.4 g, 85%). Sodium (meta) periodate (22.3 g, 97 mmol) in hot water (60 mL) was added over 90 min at 70-80 °C, to a stirred solution of (**249**) (2.4 g, 4.0 mmol) in isopropyl alcohol (250 mL), acetic acid (1.5 mL), sat.aq. Copper sulphate (1.5 mL) and hydrazine hydrate (22 mL). The mixture was stirred for 2 h at r.t. Water (100 mL) and petrol/ether (5:1, 250 mL) were added and extracted. The aqueous layer was re-extracted with warm petrol/ether (5:1, 3 × 100 mL) and dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (5:1) to give a waxy solid, *((1R,2S)-2-(14-((1R,2S)-2-icosylcyclo-propyl)tetradecyl)cyclopropyl)-methanol* (**250**) (2.2 g, 91%), $[\alpha]_D^{23}$ + 3.3 (*c* 1.2, $CHCl_3$) (*lit.*[221]$[\alpha]_D^{32}$ − 0.3 (*c* 2.03, $CHCl_3$), [Found $(M+Na)^+$: 611.6, $C_{41}H_{80}ONa$ requires: 611.6]. The compound showed δ_H (400 MHz, $CDCl_3$) 3.65 (1H, dd, *J* 7.1, 11.3 Hz), 3.58 (1H, dd, *J* 8.1, 11.3 Hz), 1.51 – 1.04 (78H, m), 0.92–0.83 (5H, m, including a triplet resonating at δ 0.88 with *J* 6.8 Hz), 0.74 – 0.67 (1H, m), 0.67 – 0.61 (2H, m), 0.60 – 0.52 (1H, m), -0.04 (1H, q, *J* 5.35 Hz), -0.34 (1H, q, *J* 5.35 Hz); δ_C (101 MHz, $CDCl_3$) 63.5, 32.1, 30.4, 30.3, 29.9, 29.8, 29.7, 29.5, 28.9, 28.7, 22.8, 18.3, 16.3, 16.0, 14.27, 11.0, 9.6; ν_{max} (cm^{-1}): 3370, 2850, 1464, 1370, 1170, 1064, 1037, 964.

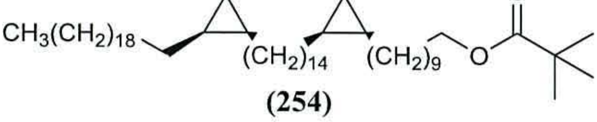
Experiment 41: (1*R*,2*S*)-2-(14-((1*R*,2*S*)-2-Icosylcyclopropyl)-tetradecyl)cyclopropane-1-carbaldehyde (251)



(251)

Pyridinium chlorochromate (1.8 g, 8.5 mmol) was stirred in dichloromethane (40 mL), and ((1*R*,2*S*)-2-(14-((1*R*,2*S*)-2-icosylcyclopropyl)tetradecyl)cyclopropyl)-methanol (**250**) (2.0 g, 3.4 mmol) in dichloromethane (35 mL) was added in portions at r.t. The mixture was stirred for 2 h then TLC showed no starting material was left, the mixture was diluted with petrol/ether 5:1 (250 mL) and filtered through a bed of celite on silica, then washed with warm ether (400 ml) and the filtrate was evaporated to give a residue which was purified by column chromatography on silica eluting with petrol/ethyl acetate (10:1) to give semi-soled, *(1R,2S)-2-(14-((1R,2S)-2-icosylcyclopropyl)-tetradecyl)cyclopropane-1-carbaldehyde* (**250**) (1.6 g, 84%); $[\alpha]_D^{22}$ + 5.3 (*c* 1.3, $CHCl_3$), [Found $(M+Na)^+$: 609.6, $C_{41}H_{80}NaO$ requires: 609.5950]. The compound showed δ_H (400 MHz, $CDCl_3$): 9.35 (1H, d, *J* 5.6 Hz), 1.91 – 1.81 (1H, m), 1.66 – 1.54 (2H, m), 1.55 – 1.44 (2H, m), 1.43 – 1.07 (67H, m), 0.88 (3H, t, *J* 6.8 Hz), 0.69 – 0.60 (2H, m), 0.59 – 0.51 (1H, m), -0.34 (1H, q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$): 201.9, 32.1, 30.4, 30.1, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 28.9, 28.4, 28.0, 27.4, 25.0, 22.9, 16.0, 15.0, 14.3, 11.1; ν_{max} /cm^{-1} : 2933, 2860, 1778, 1485, 1380, 1341, 1151.

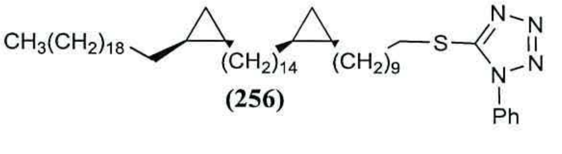
Experiment 42: 10-((1*R*,2*S*)-2-(14-((1*R*,2*S*)-2-Icosylcycloprop-yl)tetradecyl)cyclopropyl)decyl pivalate (254)



(254)

Lithium bis(trimethylsilyl) amide (2.0 ml, 2.4 mmol) was added to a stirred solution of (1*R*,2*S*)-2-(14-((1*R*,2*S*)-2-icosylcyclopropyl)tetradecyl)cyclopropane-1-carbaldehyde (**251**) (1.5 g, 2.5 mmol) and 9-((1-phenyl-1H-tetrazol-5-yl)sulfonyl)nonyl pivalate (**252**) (0.9 g, 2.2 mmol) in dry THF (20 mL) at -10 °C. The reaction mixture was allowed to reach r.t and then stirred for 2 h. TLC showed no starting material was left. Petrol/ethyl acetate (10:1) (75 mL) and sat. aq. NH_4Cl (40 ml) were added. The organic layer was separated and the aqueous layer was re-extracted with petrol/ethyl acetate (10:1) (2 × 100 mL). The combined organic layers were dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give, *(E/Z)-10-((1R,2S)-2-(14-((1R,2S)-2-icosylcyclopropyl)tetradecyl)cyclopropyl)dec-9-en-1-yl pivalate* (**253**) (1.7 g, 85%) as mixture. Potasium azodicarboxilate (3.0 g, 15.0 mmol) was added to a stirred solution of the pivalate (1.7 g, 2.1mmol) in THF (25 mL) and methanol (30 ml) at 0 °C. The reaction mixture was stirred then acetic acid (5 mL) in THF (5 mL) was added dropwise at a rate of 1 ml/15 min. The reaction turned bright yellow and was left stirring for 16 h at r.t. The addition procedure was repeated as described above with further potassium azodicarboxilate, acetic acid and the solution was stirred for another 16 h. The mixture was then poured slowly into saturated aqueous solution of $NaHCO_3$ (50 mL). The organic layer was separated and the aqueous layer was re-extracted with petrol/ ethyl acetate (10:1) (2 × 100 mL). The compound organic layers were purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give waxy sold, *10-((1R,2S)-2-(14-((1R,2S)-2-icosyl-cyclopropyl)tetradecyl)-cyclopropyl)decyl-pivalate* (**254**) (1.5 g, 88%), $[\alpha]_D^{20}$ + 4.3 (*c* 0.9, $CHCl_3$), [Found $(M)^+$: 798.8197, $C_{55}H_{106}O_2$ requires: 798.8192], which showed δ_H (400 MHz, $CDCl_3$): 4.04 (2H, t, *J* 6.6 Hz), 1.67 – 1.57 (2H, m), 1.42 – 1.12 (89H, m, including a singlet 9 Hat δ 1.19), 0.88 (3H, t, *J* 6.8 Hz), 0.69 – 0.60 (4H, m), 0.56 (2H, td, *J* 2.0, 6.2 Hz), -0.33 (2H, q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$): 173.8, 64.6, 32.1, 30.4, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 28.9, 28.8, 27.3, 26.1, 22.8, 15.9, 14.3, 11.1; ν_{max} /cm^{-1} : 2923, 2854, 1744, 1462, 1377, 1338, 1156.

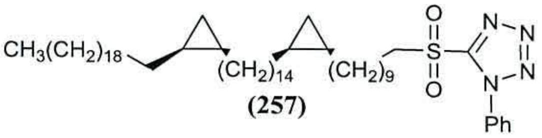
Experiment 43: 5-((10-((1*R*,2*S*)-2-(14-((1*R*,2*S*)-2-Icosylcyclopropyl) tetradecyl)cyclopropyl)decyl)thio)-1-phenyl-1H-tetrazole (256)



(256)

A solution of 10-((1*R*,2*S*)-2-(14-((1*R*,2*S*)-2-icosylcyclopropyl)tetradecyl)cyclopropyl)decyl pivalate (**254**) (0.8 g, 1.1 mmol.) in dry THF (20 mL) was added dropwise at 0 °C to a suspension solution of lithium aluminum hydride (0.09 g, 2.3 mmol)) in THF (10 mL) was added and the mixture was refluxed for 3 h. TLC showed no starting material was left. The mixture was cooled to 0 °C and quenched with a sat.aq, sodium sulphate decahydrate (10 ml) which was added dropwise and stirred at r.t for 30 min then THF (20 ml) was added and the mixture was filtered through a bed of Celite and dried. The solvent was evaporated to give a semi-solid, *10-((1R,2S)-2-(14-((1R,2S)-2-icosylcyclopropyl)tetradecyl)cyclopropyl)decan-1-ol* (**255**) (0.9 g, 90%). Diethyl azodicarboxylate (0.25 g, 1.4 mmol) in dry THF (6 mL) was added to a stirred the alcohol (0.8 g, 1.1 mmol.), triphenylphosphine (0.38 g, 1.4 mmol.) and 1–phenyl–1*H*–tetrazole–5–thiol (0.25 g, 1.4 mmol) in dry THF (7 mL) at 0 °C. The reaction mixture was stirred at room temperature for 18 h. The solvent was evaporated and the residue was diluted with petrol/ethyl acetate (5:2) and reflexed for 30 min then filtered. The filtrate was evaporated to obtain the crude product which was purified by column chromatography eluting with petrol/ethyl acetate (15:1) to give a waxy solid, *5-((10-((1R,2S)-2-(14-((1R,2S)-2-icosylcyclopropyl)-tetradecyl)cyclopropyl)decyl)thio)-1-phenyl-1H-tetrazole* (**256**) (0.9 g, 90%), $[\alpha]_D^{25}$ + 4.5 (c 1.1, CHCl$_3$), [Found (M+Na)$^+$: 897.8, C$_{57}$H$_{102}$NaN$_4$S requires: 897.8], which showed δ_H (400 MHz, CDCl$_3$): 7.61 – 7.50 (5H, m), 3.39 (2H, t, *J* 7.4 Hz), 1.87 – 1.76 (2H, m), 1.31 – 1.19 (80H, m), 0.88 (3H, t, *J* 6.8Hz), 0.71 – 0.59 (4H, m), 0.60 – 0.51 (2H, m), -0.34 (2H, q, *J* 5.1 Hz); δ_C (101 MHz, CDCl$_3$): 130.2, 129.9, 124.0, 33.5, 32.1, 30.4, 29.9, 29.7, 29.5, 29.2, 28.9, 22.8, 15.9, 14.3, 11.1; ν_{max}/ cm^{-1}: 3060, 2990, 2920, 2851, 1600, 1508, 1470, 1389, 1238, 1019, 821, 752, 698, 546, 451, 451, 435.

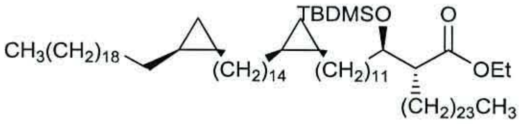
Experiment 44: 5-((10-((1*R*,2*S*)-2-(14-((1*R*,2*S*)-2-Icosylcyclopropyl)-tetradecyl)cyclopropyl)decyl)sulfonyl)-1-phenyl-1H-tetrazole (257)



(257)

A solution of *meta*-chloroperbenzoic acid 70 % (0.7 g, 3.9 mmol) in CH$_2$Cl$_2$ (20 ml) previously dried with MgSO$_4$ was added to a stirred solution of 5-((10-((1*R*,2*S*)-2-(14-((1*R*,2*S*)-2-icosylcyclopropyl)tetradecyl)cyclopropyl)decyl)thio)-1-phenyl-1*H*-tetrazole (**256**) (0.8 g, 0.9 mmol) and NaHCO$_3$ (0.3 g, 3.6 mmol) in CH$_2$Cl$_2$ (50 mL) at r.t. The reaction mixture was stirred for 18 h at r.t. TLC showed no starting material was left. Aq. NaOH (5 %, 40 mL) was added at r.t and stirred for 2 h. The organic layer was separate and the aqueous layer was re-extracted with warm CH$_2$Cl$_2$ (3 × 100), dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give, *5-((10-((1R,2S)-2-(14-((1R,2S)-2-icosylcyclopropyl)tetradecyl)cyclopropyl)decyl)sulfonyl)-1-phenyl-1H-tetrazole* (**257**) (0.7 g, 85%), $[\alpha]^{22}_{D}$ - 14 (*c* 0.9, $CHCl_3$), [Found $(M+Na)^+$: 929.8. $C_{57}H_{102}NaN_4SO$ requires: 929.8], which showed δ_H (400 MHz, $CDCl_3$): 7.61 – 7.56 (3H, m), 7.56 – 7.50 (2H, m), 3.39 (2H, t, *J* 7.4 Hz), 1.87 – 1.76 (2H, m), 1.31 – 1.19 (80H, s), 0.88 (3H, t, *J* = 6.8 Hz), 0.71 – 0.59 (4H, m), 0.60 – 0.51 (2H, m), -0.34 (2H, q, *J* 5.1 Hz); δ_C (101 MHz, $CDCl_3$): 130.2, 129.9, 124.0, 32.1, 30.4, 29.9, 29.7, 29.5, 29.2, 28.9, 22.8, 15.9, 14.3, 11.1; ν $_{max}$/ cm^{-1}: 2995, 2920, 2841, 1500, 1475, 1348, 1149, 768, 718, 687 cm^{-1}.

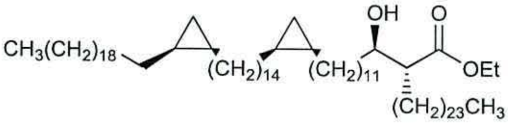
Experiment 45: Ethyl (*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-12-((1*R*,2*S*)-2-(14-((1*R*,2*S*)-2-icosylcyclopropyl)tetradecyl)cyclopropyl)-dodecyl)hexacosanoate (259)



(259)

Lithium bis–(trimethylsilyl) amide (1.2 mL) was added a stirred solution of 5-((10-((1*R*,2S)-2-(14-((1*R*,2*S*)-2-icosylcyclopropyltetradecylcyclopropyldecylsulfonyl)-1-phenyl-1*H*-tetrazole (**257**) (0.6 g, 0.6 mmol) and ethyl (*R*)-2-((*S*)-1-((*tert*-butyl-dimethylsilyl)oxy)-2-oxoethyl)hexacosanoate (**210**) (0.4 g, 0.7 mmol) at –10 ˚C under nitrogen. The mixture reaction was allowed to reach room temperature and stirred for 2 h. TLC showed no starting material was left. Water (20 mL) was added and extracted with petrol/ether (5:1, 3 × 15 mL). The combined organic layers were washed with sat aq. NH_4Cl (2 x 5 mL), dried over $MgSO_4$ and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give, *ethyl (R)-2-((R, E/Z)-1-((tert-butyldimethylsilyl)oxy)-12-((1R,2S)-2-(14-((1R,2S)-2-icosylcyclopropyl)tetradecyl)-cyclo-propyl)dodec-2-en-1-yl)hexacosanoate* (**258**) (0.5 g; 59%) as a mixture. The mixture was dissolved in dry CH_2Cl_2/CH_3OH (50 mL, 1:1), and palladium hydroxide on carbon (10%, 15 mg) was added and the suspension was stirred for 30 min under hydrogen atmosphere. The suspension was filtered on a bed of Celite, washed with ethyl acetate (50 mL). The filtrate was concentrated and purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a semi-solid, *ethyl (R)-2-((R)-1-((tert-butyldimethylsilyl)oxy)-12-((1R,2S)-2-(14-((1R,2S)-2-icosylcyclopropyltetradecyl-cyclopropyl)dodecyl)hexacosanoate* (**259**) (0.45 g; 90%), $[\alpha]_D^{24}$ + 4.1 (*c* 1.2, $CHCl_3$), [Found $(M+Na)^+$: 1302.3, $C_{86}H_{170}NaO_3Si$ requires: 1302.3], which showed δ_H (400 MHz, $CDCl_3$): 4.20 – 4.06 (2H, m), 3.95 – 3.86 (1H, m), 2.56 – 2.46 (1H, m), 1.68 – 1.03 (144 H, m), 0.91 – 0.84 (15H, m), 0.71 – 0.60 (4H, m), 0.60 – 0.51 (2H, m), 0.04 (3H, s), 0.02 (3H, s), -0.34 (2H, q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$): 174.8, 73.4, 60.1, 51.7, 33.8, 32.1, 30.4, 30.1, 29.9, 29.8, 29.7, 29.6, 29.5, 28.9, 27.9, 27.6, 25.9, 24.0, 22.8, 15.9, 14.4, 14.3, 11.1, -4.2, -4.8; ν_{max}/ cm^{-1}: 2923, 2853, 1742, 1464, 1254.

Experiment 46: Ethyl (*R*)-2-((*R*)-1-hydroxy-12-((1*R*,2*S*)-2-(14-((1*R*,2*S*)-2-icosylcyclopropyl)tetradecyl)cyclopropyl)dodecyl)hexacosanoate (260)



(260)

Fluoride-pyridine complex as ~70% hydrogen fluoride (2 mL) was added a stirred solution of ethyl (*R*)-2-((*R*)-1-((tert-butyldimethylsilyl)oxy)-12-((1*R*,2*S*)-2-(14-((1*R*,2*S*)-2-icosylcyclopropyl)tetradecyl)cyclopropyl)dodecyl)hexacosanoate (**259**) (0.4 g, 0.3 mmol) and anhydrous pyridine (0.3 mL) in dry THF (15 mL) at 5 °C in a dry polyethylene vial equipped with an acid proof rubber septum. The mixture was stirred at 45 °C for 18 h. TLC showed no starting material was left, the mixture was diluted with petrol/ethyl acetate (10:1, 100 ml) and neutralized by pouring it slowly into sat. aq. sodium bicarbonate until no more CO_2 was liberated. The product was extracted with warm petrol/ethyl acetate (10:1) (2 × 100 mL), and then the combined organic layers were washed with brine (25 mL). The organic layer was dried and the solven.t The crude product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give a waxy solid, *ethyl (R)-2-((R)-1-hydroxy-12-((1R,2S)-2-(14-((1R,2S)-2-icosylcyclopropyl)tetradecyl)cyclopropyl)dodecyl)hexa-cosanoate* (**260**) (0.28 g, 77%), $[\alpha]_D^{22}$ + 5.2 (*c* 0.9, $CHCl_3$), [Found $(M+Na)^+$: 1180.0, $C_{80}H_{162}NaO_2$ requires: 1178.2], which showed δ_H (400 MHz, $CDCl_3$): 4.18 (2H, q, *J*

7.1 Hz), 3.64 (1H, br s), 2.48 (1H, br d, *J* 7.4), 2.41 (2H, dt, *J* 5.4, 9.4 Hz), 1.78 – 1.01 (127H, m), 0.88 (6H, t, *J* 6.8 Hz), 0.65 – 0.63 (4H, m), 0.56 (2H, dt, *J* 4.1, 8.2 Hz), -0.34 (2H, q, *J* 5.1 Hz), δ_C (101 MHz, $CDCl_3$): 175.1, 72.5, 60.5, 51.0, 35.9, 32.1, 30.4, 29.9, 29.8, 29.7, 29.6, 29.5, 28.9, 27.5, 25.9, 22.9, 15.9, 14.5, 14.3, 11.1; ν_{max}/ cm^{-1}: 3425, 3011, 2915, 2860, 1744,1460, 1200, 673.

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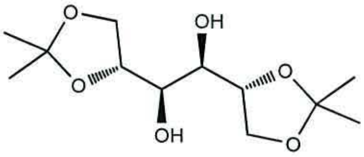
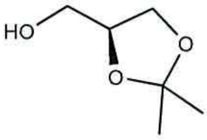
6. Appendices

Appendix 1: (1*R*,2*R*)-1,2-bis((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)ethane-1,2-diol



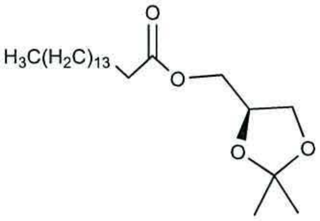
Anhydrous zinc chloride (41.6 g, 305.8 mmol) was added to a stirred solution of *D*-mannitol (20.1 g, 109.8 mmol) in acetone (250 mL) at room temperature. The milky solution was formed and stirred with a mechanical stirrer at room temperature until it became clear (3 h). The mixture was poured in sat. aq. potassium carbonate (200 mL) and stirred for 1 h and filtered. The residue was re-extracted with CH_2Cl_2 (3 × 300 mL), dried over anhydrous $MgSO_4$ and the solvent was evaporated. The crude product was purified by re-crystallization from hexane/dichloromethane (9:1) to give white soled *(1R,2R)-1,2-bis((R)-2,2-dimethyl-1,3-dioxolan-4-yl)ethane-1,2-diol* (25.2 g, 87%). which showed δ_H, δ_C and ν_{max} identical to the literature.[224, 226]

Appendix 2: (*S*)-(2,2-Dimethyl-1,3-dioxolan-4-yl)methanol



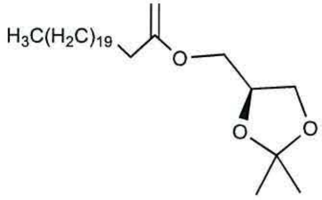
Dry lead tetracetate (8.0 g, 6.7 mmol) was added portion wise to a stirred solution of (1*R*,2*R*)-1,2-bis((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)ethane-1,2-diol (5.0 g, 19.0 mmol) in dry THF (100 mL) at 10 °C. The mixture was stirred for 30 min in an ice bath and an additional 30 min at room temperature. After filtering through a bed of celite and cooling in an ice bath, a solution of $NaBH_4$ (1.5 g, 37.2 mmol) in 4% NaOH (100 mL) was added dropwise with vigorous stirring while maintaining the temperature below 5 °C and stirred for 30 min at these temperature and 5 h at room temperature. Solid ammonium chloride was added to the solution until pH 8. The THF was removed under reduced pressure then the resulting aqueous solution was saturated with NaCI. After extracting with ethyl acetate (3 × 300), the organic layer was washed with 5% NaOH (50 mL) and sat. aq. NaCl (50 mL), dried over $MgS0_4$ and the solvent was evaporated, The crude product was purified by flash distillation at 0.5 mmHg and 40 °C to give a colorless oil, *(S)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanol* (3.5 g, 96 %), $[\alpha]_D^{24}$ – 5.2(c 1.4, $CHCl_3$)(*lit.*[224, 226] $[\alpha]_D^{24}$ - 8.4); which showed δ_H (500 MHz, $CDCl_3$): 4.28 – 4.22 (1H, m), 4.05 (1H, dd, *J* 6.6, 8.2 Hz), 3.81 (1H, dd, *J* 6.5, 8.2 Hz), 3.75 (1H, dd, *J* 3.5, 11.6 Hz), 3.60 (1H, dd, *J* 5.0, 11.6 Hz), 1.85 (1H, s), 1.45 (3H, s), 1.39 (3 H, s); δ_C NMR (101 MHz, $CDCl_3$): 109.3, 76.3, 65.7, 62.9, 26.5, 25.1. The ^1H, ^{13}C, and I.R spectra were identical to the literature.[224, 226]

Appendix 3: (*R*)-(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl palmitate



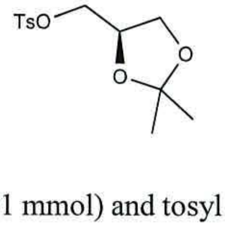
Palmitic acid (0.42 g, 1.60 mmol) and DMAP (0.18 g, 1.40 mmol) was added a stirred solution of (*S*)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanol (0.2 g, 1.50 mmol) in CH_2Cl_2 (3 mL). A solution of DCC (0.34g, 1.60 mmol) in CH_2Cl_2 (3 mL) was added dropwise with stirring under nitrogen atmosphere over 30 min. TLC showed no starting material was left. Precipitated dicyclohexylurea was filtered off and washed with CH_2Cl_2 (10 mL). The resulting solution was concentrated and the crude residue was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give semisolid, *(R)-(2,2-dimethyl-1,3-dioxolan-4-yl)methyl palmitate* (0.5 g, 89 %), $[\alpha]_D^{22}$– 9.5(c 1.1, $CHCl_3$) (*lit.*[224] $[\alpha]_D^{22}$ - 10; which showed δ_H (400 MHz, $CDCl_3$): δ 4.35 – 4.28 (1H, m), 4.16 (1H, dd, *J* 4.7, 11.5 Hz), 4.13 – 4.03 (2H, m), 3.74 (1H, dd, *J* 6.2, 8.4 Hz), 2.34 (2H, t, *J* 7.6 Hz), 1.68 – 1.57 (2H, m), 1.43 (3H, s), 1.37 (3H, s), 1.25 (23H, s), 0.88 (3H, t, *J* 6.9 Hz); δ_C (101 MHz, $CDCl_3$): 173.3, 109.6, 73.5, 66.2, 64.3, 33.9, 31.8, 29.6, 29.5, 29.5, 29.4, 29.3, 29.2, 29.1, 29.0, 26.5, 25.2, 24.8, 22.7, 14.0; ν_{max}/ cm^{-1}: 2930, 2854, 1740,1380, 1390 cm^{-1}.

Appendix 4: (*R*)-(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl docosanoate



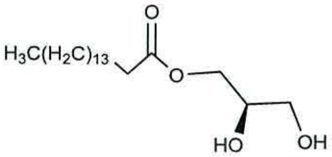
To a stirred solution of (*S*)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanol (0.2g, 1.50 mmol) in CH_2Cl_2 (3 mL), was added behenic acid (0.6 g, 1.70 mmol) and DMAP (0.2g, 1.60 mmol), then a solution of DCC (0.4 g, 1.90 mmol) in CH_2Cl_2 (3 mL) was added dropwise under nitrogen atmosphere over 30 min. TLC showed no starting material was left. The reaction mixture was worked up and purified as before to give a semisolid, *(R)-(2,2-dimethyl-1,3-dioxolan-4-yl)methyl docosanoate* (0.6g, 88%), $[\alpha]_D^{22}$ - 9.1(*c* 1.3, $CHCl_3$) (*lit.*[224] $[\alpha]_D^{21}$ - 12; which showed δ_H (400 MHz, $CDCl_3$): 4.32 – 4.24 (1H, m), 4.14 (1H, dd, *J* 4.8, 11.5 Hz), 4.10 – 4.01 (2H, m), 3.71 (1H, dd, *J* 6.2, 8.4Hz), 2.31 (2H, t, *J* 7.5 Hz), 1.66 – 1.54 (2H, m), 1.41 (3H, s), 1.34 (3H, s), 1.23 (35 H, s), 0.85 (3 H, t, *J* 6.8 Hz); δ_C (101 MHz, $CDCl_3$): 173.4, 109.7, 73.6, 66.3, 64.4, 34.0, 31.9, 29.8, 29.7, 29.5, 29.4, 29.3, 29.2, 29.1, 29.0, 26.6, 25.3, 24.8, 22.6, 14.0; ν_{max}/ cm^{-1}: 2930, 2854, 1740,1380, 1390.

Appendix 5: (*R*)-(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl 4-methylbenzenesulfonate



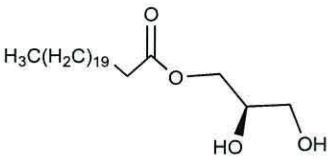
Pyridine (7 mL), DMAP (0.1g, 0.81 mmol) and tosyl chloride (3.4 g, 17.8 mmol) were added sequentially at 0 °C to a stirred solution of (*S*)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanol (2.0 g, 15.1 mmol) in dichloromethane (30 mL). The mixture was allowed to reach room temperature then stirred for 18 h. The mixture was diluted with dichloromethane (30 mL) and quenched with hydrochloric acid (3 M, 10 mL). The organic phase was decanted and washed twice with sat. aq. $NaHCO_3$ and brine. The resulting solution was concentrated and the crude residue was purified by column chromatography eluting with petrol/ethyl acetate (5:1) to give a colourless oil, *(R)-(2,2-dimethyl-1,3-dioxolan-4-yl)methyl 4-methylbenzenesulfonate* (4.3g, 88%), $[\alpha]_D^{23}$ - 12(c 0.9, $CHCl_3$) (*lit.*^{224} $[\alpha]_D^{24}$ -8.8); which showed δ_H (400 MHz, $CDCl_3$): 7.80 (2H, d, *J* 8.3 Hz), 7.35 (2H, d, *J* 8.0 Hz), 4.32 – 4.23 (1H, m), 4.08 – 3.94 (3H, m), 3.77 (1H, dd, *J* 5.1, 8.8 Hz), 2.45 (3H, s), 1.34 (3H, s), 1.31 (3H, s); δ_C (101 MHz, CDC13): 145.2, 132.8, 130.0, 128.1, 110.2, 73.0, 69.6, 66.3, 26.7, 25.2, 21.8; ν_{max}/cm^{-1}: 2919, 2851, 1467, 1076, 856.

Appendix 6: (*R*)-2,3-Dihydroxypropyl palmitate



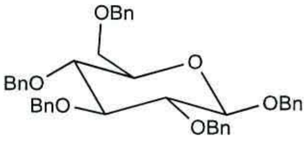
A solution of (*R*)-(2,2-dimethyl-1,3-dioxolan-4-yl)methyl palmitate (0.4 g, 1.08 mmol) in CH_2Cl_2 (25 mL) was stirred in the presence of trifluoroacetic acid (1 mL) at 0 °C for 12 h TLC showed no starting material was left.The resulting solution was evaporated, and the traces of trifluoroacetic acid was removed under high vacuum. The crude residue was purified by column chromatography eluting with petrol/ethyl acetate (2:1) to give a semisolid *(R)-2,3-dihydroxypropyl palmitate* (0.3 g, 85%), $[\alpha]_D^{24}$ – 12 (c 0.9, $CHCl_3$) (*lit.*^{224} $[\alpha]_D^{22}$) -15); which showed δ_H (400 MHz, $CDCl_3$): 4.20 (1H, dd, *J* 4.7, 11.6 Hz), 4.14 (1H, dd, *J* 6.1, 11.6 Hz), 3.96 – 3.88 (1H, m), 3.69 (1H, dd, *J* 3.9, 11.5 Hz), 3.59 (1H, dd, *J* 5.8, 11.5 Hz), 2.34 (2H, t, *J* 7.6 Hz), 1.68 – 1.57 (2H, m), 1.25 (26H, s), 0.87 (3H, t, *J* 6.8 Hz); δ_C (101 MHz, CDC13): 174.5, 70.4, 65.3, 63.5, 34.3, 32.1, 30.0, 29.9, 29.8, 29.9, 29.6, 29.5, 29.4, 29.3, 29.2, 25.0, 22.8, 14.2; ν_{max}/cm^{-1}: 3455, 2930, 2854, 1740,1380, 1390.

Appendix 7: (*R*)-2,3-dihydroxypropyl docosanoate



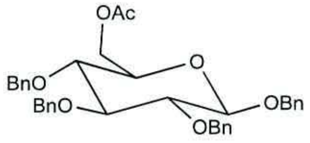
Trifluoroacetic acid in dichloromethane (1:1) (2 mL) was added a stirred solution of (*R*)-(2,2-dimethyl-1,3-dioxolan-4-yl)methyl docosanoate (0.5 g, 1.1 mmol) in CH_2Cl_2 (25 mL) at 0 °C. The solution was stirred at 0 °C for 12 h. TLC showed no starting material was left. The reaction mixture was worked up and purified as before to give a semisolid, *(R)-2,3-dihydroxypropyl docosanoate* (0.4 g, 85%)$[\alpha]_D^{24}$ - 14(c 1.2, $CHCl_3$) (*lit.*[224] $[\alpha]_D^{24}$ - 15); which showed δ_H (400 MHz, $CDCl_3$): 4.20 (1H, dd, *J* 4.7, 11.6 Hz), 4.14 (1H, dd, *J* 6.1, 11.6 Hz), 3.96 – 3.88 (1H, m), 3.69 (1H, dd, *J* 3.9, 11.5 Hz), 3.59 (1H, dd, *J* 5.8, 11.5 Hz), 2.34 (2H, t, *J* 7.6 Hz), 1.68 – 1.57 (2H, m), 1.25 (38H, s), 0.87 (3H, t, *J* 6.8 Hz); δ_C (101 MHz, CDC13): 174.5, 70.4, 65.3, 63.5, 34.3, 32.0, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 25.0, 22.8, 14.2; ν_{max}/cm^{-1}: 3455, 2930, 2854, 1740,1380, 1390.

Appendix 8: (2*R*,3*R*,4*S*,5*R*,6*R*)-2,3,4,5-Tetrakis(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran



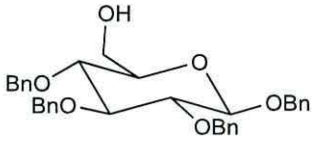
Sodium hydride (2.0 g of 60% dispersion in mineral oil, 83 mmol which was washed twice with hexane) was added to a stirred solution of *D*-glucose (3.0 g, 16.6 mmol) in anhydrous DMF (90 mL) at room temperature. The suspension was stirred for 30 min at room temperature then benzyl bromide (7 mL, 57 mmol) was added dropwise over a 5 min period at 0 °C, and after 10 min the ice bath was removed. The reaction mixture was stirred at at room temperature 2.5 h. Sodium hydride (3.0 g, 16.6 mmol) and benzyl bromide (7 mL, 57 mmol) were added consecutively at 0 °C. The mixture was stirred at room temperature 18 h, then quenched with methanol (10 mL). DMF was removed under reduced pressure at 55 °C. The residue was dissolved in CH_2Cl_2 (80 mL) and water (40 mL) and brine (40 mL) were added and extracted with CH_2Cl_2 (3 × 50 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 9:1) to give white solid, *(2R,3R,4S,5R,6R)-2,3,4,5-tetrakis(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran* (5.0 g, 66%) mp 83–83.5°C, (*lit*[225] 83–84 °C), $[\alpha]_D^{21} - 7.2$ (c 1.3, $CHCl_3$), (*lit*[225] $[\alpha]_D^{21} - 8.8$); which showed δ_H (400 MHz, $CDCl_3$): 7.45 – 7.27 (27H, m), 7.21 – 7.12 (3H, m), 5.05 – 4.92 (3H, m), 4.88 – 4.52 (9H, m), 3.79 (1H, br d, *J* 10.9 Hz), 3.73 (1H, dd, *J* 4.6,10.8 Hz), 3.66 (2H, t, *J* 7.1 Hz), 3.59 – 3.53 (1H, m), 3.53 – 3.47 (1H, br m); δ_C (101 MHz, $CDCl_3$): 138.7, 138.5, 138.3, 138.2, 137.6, 128.6, 128.5, 128.4, 128.3, 128.1, 128.0, 127.9, 127.8, 127.7, 102.7, 84.9, 82.4, 78.0, 75.8, 75.1, 75.0, 73.6, 71.3, 69.0. The ^{1}H, ^{13}C, and I.R spectra were identical to the literature.[225]

Appendix 9: ((2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5,6-Tetrakis(benzyloxy) tetrahydro-2H-pyran-2-yl)methyl acetate



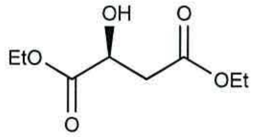
(2*R*,3*R*,4*S*,5*R*,6*R*)-2,3,4,5-Tetrakis(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran (3.0 g, 5.1 mmol) in acetic acid/acetic anhydride (1:1, 15 mL) was added dropwise to a stirred solution of zinc chloride (3.0 g, 22 mmol) and acetic acid/acetic anhydride (1:1, 15 mL) at 0 °C. The reaction mixture was stirred at room temperature for 1.5 h. TLC showed that the reaction was almost complete. Cold water (100 mL) was added and extracted with CH_2Cl_2 (3 × 50). The combined organic layers were dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (5:1) to give white solid, *((2R,3R,4S,5R,6R)-3,4,5,6-tetrakis(benzyloxy)tetrahydro-2H-pyran-2-yl)methyl acetate* (2, 2 g, 80%), mp 116–117 °C (*lit.*[225] 114–115 °C) $[\alpha]_D^{22}$ 3.0 (*c* 1.2 $CHCl_3$) (*lit.*[225] $[\alpha]_D^{22} - 2.8$; which showed δ_H (400 MHz, $CDCl_3$) 7.33 – 7.15 (20H, m), 4.93 – 4.84 (3H, m), 4.79 (1H, d, *J* 10.8 Hz), 4.71 (1H, d, *J* 10.9 Hz), 4.65 (1H, d, *J* 10.9 Hz), 4.59 (1H, d, *J* 11.9 Hz), 4.49 (1H, d, *J* 10.8 Hz), 4.44 (H, d, *J* 7.8 Hz), 4.29 (1H, dd, *J* 1.8, 11.9 Hz), 4.17 (1H, dd, *J* 4.7, 11.9 Hz), 3.60 (1H, t, *J* 8.8 Hz), 3.53 – 3.39 (3H, m), 1.99 (3H, s); δ_C (101 MHz, $CDCl_3$): 166.4, 138.4, 138.2, 137.7, 137.1, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 102.4, 84.6, 82.2, 75.7, 75.0, 74.9, 72.8, 71.2, 22.2. The ^1H, ^{13}C, and I.R spectra were identical to the literature.[225]

Appendix 10: ((2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5,6-Tetrakis(benzyloxy) tetrahydro-2H-pyran-2-yl)methanol



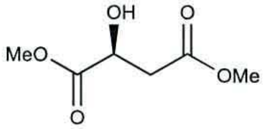
Sodium methoxide (0.025 M) in methanol (30 mL) was added to a stirred solution of ((2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5,6-tetrakis(benzyloxy)tetrahydro-2H-pyran-2-yl)methylacetate (2.0 g, 3.43 mmol) in (10 mL) at room temperature and stirred for 5 h. The reaction mixture was poured into ice water (200 mL) and stirred for 30 min. The resulting precipitate was filtered and washed with sat. aq. $NaHCO_3$ (30 mL) and water (30 mL). The precipitate dried under high vacuum and extracted with CH_2Cl_2 (3 × 30). The combined organic layers were dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (3:1) to give white needles, *((2R,3R,4S,5R,6R)-3,4,5,6-tetrakis(benzyloxy)tetrahydro-2H-pyran-2-yl)methanol* (1.9 g, 97%): mp 104–106°C (*lit.*[225] 102–104°C), $[\alpha]_D^{22}$ – 8.0 (*c* 1.1, $CHCl_3$) (*lit* [225] $[\alpha]_D^{22}$ – 8.8); which showed δ_H (400 MHz, $CDCl_3$): 7.32 – 7.16 (20H, m), 4.90 – 4.86 (2H, m), 4.84 (1H, d, *J* 4.3 Hz), 4.79 (1H, d, *J* 10.9 Hz), 4.73 (1H, d, *J* 10.9 Hz), 4.66 (1H, d, *J* 10.9 Hz), 4.62 (1H, d, *J* 11.9 Hz), 4.57 (1H, d, *J* 10.9 Hz), 4.50 (1H, d, *J* 7.8 Hz), 3.80 (1H, ddd, *J* 2.7, 5.8, 11.8 Hz), 3.68 – 3.57 (2H, m), 3.50 (1H, t, *J* 9.3 Hz), 3.41 (1H, t, *J* 9.3 Hz), 3.29 (1H, ddd, *J* 2.8, 4.5, 9.5 Hz), δ_C (101 MHz, $CDCl_3$): 138.5, 138.3, 137.9, 137.2, 128.6, 128.5, 128.4, 128.3, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 102.8, 84.5, 82.3, 77.5, 75.7, 75.1, 75.0, 74.9, 71.6, 62.0. The ^1H, ^{13}C, and I.R spectra were identical to the literature.[225]

Appendix 11: Diethyl- (*S*)-2-hydroxysuccinate



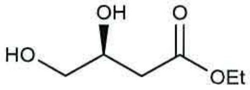
Con. H_2SO_4 (3 mL) was added to the a stirred solution of *L*-malic acid (50 g, 0.37 mol.) in ethanol (300 mL) and the mixture was refluxed for 3 h at 92 ˚C. The reaction was monitored by TLC until there was no more staring material was left. Sat. aq. $NaHCO_3$ (100 mL) was added and extracted with CH_2CL_2 (3 × 200 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was evaporated. The crude product was purified by flash distillation at 90 – 120 ˚C and 1mm/Hg to give a colourless oil, *diethyl (S)-2-hydroxysuccinate* (65 g, 91%), $[\alpha]_D^{22}$ + 12 (c 1.1, $CHCl_3$) (*lit.*[227]$[\alpha]_D^{22}$+ 9.1(*c* 2.2, EtOH)); which showed δ_H (400 MHz, $CDCl_3$) 4.45 (1 H, t, *J* 5.3 Hz), 4.23 (2 H, dd, *J* 2.3, 7.1 Hz), 4.13 (2 H, q, *J* 7.1), 2.81 (1 H, dd, *J* 4.5, 16.3 Hz), 2.74 (1 H, dd, *J* 6.1, 16.3 Hz), 1.31 – 1.18 (6 H, m); δ_C (101 MHz, $CDCl_3$): 173.7, 170.4, 67.2, 61.7, 60.8, 38.7, 13.1, and I.R spectra were identical to the literature.[227]

Appendix 12: Dimethyl (*S*)-2-hydroxysuccinate



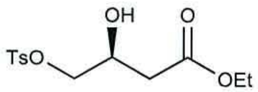
Con. H_2SO_4 (3 mL) was added to a stirred solution of *L*-malic acid (50 g, 0.37 mol.) was in methanol (300 mL); and the mixture was refluxed for 3 h at 92 ˚C. The reaction was monitored by TLC until there was no staring material was left. The excess methanol was evaporated and then sat. aq. $NaHCO_3$ (100 mL) was added to neutralize the acid. The product was separated and aq layer was extracted with CH_2CL_2 (3 × 200 mL) then the combined organic layers were dried over $MgSO_4$ and concentrated. The crude product was purified by flash distillation at 90 – 120 ˚C and 1mm/Hg to give a colourless oil, *dimethyl (S)-2-hydroxysuccinate* (55 g, 91%), $[\alpha]_D^{23}$ + 11 (c 1.1, $CHCl_3$) (*lit.*[228] $[\alpha]_D^{22}$ + 9.1(c 2.2, EtOH)); which showed δ_H (400 MHz, $CDCl_3$): 4.49 (1H, dd, *J* 4.4, 6.1 Hz), 3.80 (3H, s), 3.70 (3 , s), 2.86 (1H, dd, *J* 4.4, 16.4 Hz), 2.78 (1H, dd, *J* 6.2, 16.4 Hz); δ_C (101 MHz, $CDCl_3$): 173.7, 171.0, 67.3, 52.6, 51.9, 38.5, 38.5, and I.R spectra were identical to the literature.[228]

Appendix 13: Ethyl (*S*)-3,4-dihydroxybutanoate



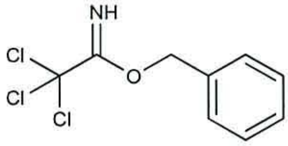
Borane-dimethylsulphide complex (40.5 mL, 0.07 mmol, 2M) was added dropwise over 10 minutes to a stirred solution of diethyl (*S*)-2-hydroxysuccinate (15.0 g, 79 mmol) in dry THF (100 mL) at 20 °C. The reaction was stirred at this temperature for 30 minutes until the evolution of hydrogen ceased and then cooled to 10 °C in an ice bath then the mixture was stirring vigorously for 10 minutes then NaBH$_4$ (0.1 g, 2.65 mmol) was added in one portion; this caused an exothermic reaction which was allowed to subside before the cooling water bath was removed. The reaction was stirred at room temperature until the starting material disappeared as shown by TLC. Ethanol (50 mL) and p-TsOH (0.5 g, 2.65 mmol) were added at 5 °C and stirred for 30 min. The solvent was evaporated and the residue was re-dissolved in toluene/IMS (1:1, 50 mL) and evaporated again (this process was repeated three times). The crude product was purified by column chromatography eluting with petrol/ethyl acetate (19:1) to give a colourless oil, *ethyl (S)-3,4-dihydroxybutanoate* (10.2 g; 87%), $[\alpha]_D^{22}$ + 7.2(c 1.1, CHCl$_3$) (*lit.*[229] $[\alpha]_D^{22}$ + 6.2(c 0.5, CHCl$_3$)); which showed δ_H (400 MHz, CDCl$_3$): 4.21 – 4.10 (3H, m), 3.67 (1H, dd, *J* 2.9, 11.3 Hz), 3.52 (1H, dd, *J* 6.3, 11.3 Hz), 2.54 (1 , dd, *J* 8.3, 16.3 Hz), 2.48 (1H, dd, *J* 6.4, 16.3 Hz), 1.27 (3 , t, *J* 7.1); δ_C (101 MHz, CDCl$_3$): 172.7, 68.7, 65.8, 61.0, 37.8, 14.2, and I.R spectra were identical to the literature.[229]

Appendix 14: Ethyl (*S*)-3-hydroxy-4-(tosyloxy)butanoate



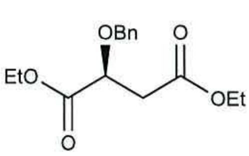
Tosylchloride (4.6 g, 24.2 mmol) was added in one portion to a stirred solution of ethyl (*S*)-3,4-dihydroxybutanoate (3.0 g, 20.2 mmol) and triethylamine (4.1 g, 40.6 mmol) in dry CH$_2$Cl$_2$ at –20 °C, and the solution was kept in the fridge overnight. The solvent was evaporated, and the residue was washed with water and extracted with ethyl acetate (3 × 20 mL). The combined organic layers were dried and concentrated to obtain the crude product. The crude was purified by column chromatography eluting with petrol/ethyl acetate (3:1) to give a thick yellow oil, *ethyl (S)-3-hydroxy-4-(tosyloxy)butanoate* (4.5 g; 73%), $[\alpha]_D^{22}$ – 5.4(c 1.7, $CHCl_3$); which showed δ_H (400 MHz, $CDCl_3$): 7.80 (2H, d, *J* 8.1 Hz), 7.36 (2H, d, *J* 8.0 Hz), 4.28 – 4.21 (1H, m), 4.15 (2H, q, *J* 7.1 Hz), 4.04 (2H, d, *J* 5.2 Hz), 2.61 – 2.47 (2H, m), 2.45 (3H, s), 1.26 (3H, t, *J* 7.1 Hz); δ_C (101 MHz, $CDCl_3$): 171.6, 145.3, 132.7, 130.1, 128.1, 72.2, 66.1, 61.2, 4.59, 2.78, 14.2; ν_{max}/ cm^{-1}: 3445, 2974, 1755, 1600, 1355, 1177, 1097, 985.

Appendix 15: Benzyl 2,2,2-trichloroacetimidate



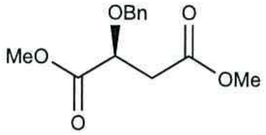
Aqueous potassium hydroxide solution (100 mL of 50%) was added to a stirred solution of benzyl alcohol (10.0 g, 0.09 mol) in dichloromethane (100 mL) in the presences of tetrabutylammonium hydrogen sulphate (15 mg) at room temperature. The resulting mixture was vigorously stirred at (-15 to -10 °C), trichloroacetonitrile (18.0 g, 0.11 mol) was added dropwise, and the reaction mixture was further stirred for 30 min then allowed to reach room and extracted with dichloromethane (2 × 100 mL). The combined organic layers were dried over anhydrous $MgSO_4$. The crude product was purified by flash distillation at 125 °C to give a colourless oil (22.0 g 95%), *benzyl 2,2,2-trichloroacetimidate* which showed ^1H, ^{13}C and IR spectrum identical to the literature.[228]

Appendix 16: Diethyl (*S*)-2-(benzyloxy)succinate



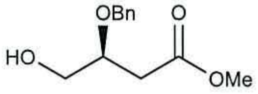
Benzyl 2,2,2-trichloroacetimidate(16.3 g, 65.0 mmol) was added to a stirred solution of diethyl (*S*)-2-hydroxysuccinate (5.0 g, 26.3 mmol) in CH_2Cl_2 (35 mL) then the mixture was cooled to –20 °C under nitrogen followed by the addition of trifluoroacetic acid (2.7 g, 24.1mmol) over half an hour and the reaction mixture was kept in the freezer for overnight. The mixture was lift to reach room temperature and cyclohexane (70 mL) was added. The mixture was filtered and the filtrate was washed with sat. aq. $NaHCO_3$ (50 mL) and the product was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried and concentrated to obtain the crude product which was purified by column chromatography, eluting with petrol/ethyl acetate (10:2) to give a thick yellow oil, *diethyl (S)-2-(benzyloxy) succinate* (6.3 g; 84%), $[\alpha]_D^{20} - 55$ (c 0.8, $CHCl_3$) (*lit.*[206, 229] $[\alpha]_D^{22} - 68$ (c 1.1, $CHCl_3$)); which showed δ_H (400 MHz, $CDCl_3$): 7.40 – 7.28 (5H, m), 4.77 (1H, d, *J* 11.4 Hz), 4.54 (1H, d, *J* 11.4 Hz), 4.39 (1H, dd, *J* 5.2, 7.8 Hz), 4.27 – 4.18 (2H, m), 4.16 – 4.10 (2H, m), 2.85 – 2.72 (2H, m), 1.29 (3H, t, *J* 7.1 Hz), 1.24 (3H, t, *J* 7.2 HZ); δ_C (101 MHz, $CDCl_3$): 171.5, 170.2, 137.4, 129.1, 128.5, 128.2, 128.1, 127.9, 74.8, 73.2, 61.0, 38.2, 29.3, 14.3; ν_{max}/cm^{-1} 3022, 2955, 17380, 1489, 1440, 1362, 1277, 1170, 1110, 1018, 740, 695.

Appendix 17: Dimethyl (*S*)-2-(benzyloxy)succinate



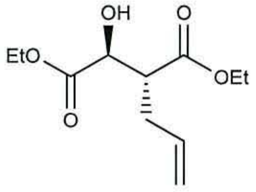
Benzyl 2,2,2-trichloroacetimidate (32.6 g, 65.0 mmol) was added to a stirred solution of dimethyl (S)-2-hydroxysuccinate (10.0 g, 26.3 mmol) in CH_2Cl_2 (100 mL) and the mixture was cooled to – 20 °C under nitrogen then trifluoroacetic acid (5.4g, 47.3mmol) was added over 30 min and the reaction mixture was kept in the freezer for overnight. The mixture was left to reach room temperature and cyclohexane (200 mL) was added. The mixture was filtered and the filtrate was washed with sat. aq. $NaHCO_3$ (75 ml) and the product was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layers were dried and concentrated to obtain the crude product which was purified by column chromatography, eluting with petrol/ethyl acetate (10:2) to give a thick yellow oil, *dimethyl (S)-2-(benzyloxy)succinate* (13.2 g; 85%), $[\alpha]_D^{20} - 45$ (c 1.2, $CHCl_3$) (*lit.*[206, 229] $[\alpha]_D^{22}$ - 68 (c 1.1, $CHCl_3$)); which showed δ_H (400 MHz, $CDCl_3$): 7.39 – 7.28 (5H, m), 4.77 (1H, d, *J* 11.4 Hz), 4.54 (1H, d, *J* 11.4 Hz), 4.40 (1H, dd, *J* 5.2, 7.6 Hz), 3.77 (3H, s), 3.69 (3H, s), 2.87 – 2.77 (2H, m); ^{13}C and IR spectrum identical to the literature.[206, 229]

Appendix 18: Methyl (*S*)-3-(benzyloxy)-4-hydroxybutanoate



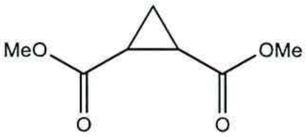
To a stirred solution of dimethyl (*S*)-2-(benzyloxy)succinate (1.1 g, 3.96 mmol) in CH_2Cl_2 (50 mL) was added $MgBr_2.OEt_2$ (1.1 g, 4.48 mmol) and the resulting mixture was stirred at room temperature for 1 h. The solution was cooled to -78 °C, and DIBAL-H (9.92 mL 1.0 M solution in toluene, 9.92 mmol) was added dropwise over 90 min via syringe. The mixture was stirred 30 min at -78 °C and 2 hrs at 0 °C. Methanol (5 mL) and saturated (aq) Rochelle's salt solution (30 ml) were added at 0 °C. The reaction mixture was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (3:2) gave a thick yellow oil, *methyl (S)-3-(benzyloxy)-4-hydroxybutanoate* (0.4 g; 47%), $[\alpha]_D^{20}$ 4.4 (c 1.1, $CHCl_3$) (*lit*[229] $[\alpha]_D^{20} - 2.1$ (c 1.1, $CHCl_3$)); which showed δ_H (400 MHz, $CDCl_3$): 7.39 – 7.28 (5H, m), 4.65 (1H, d, *J* 11.5 Hz), 4.58 (1H, d, *J* 11.5 Hz), 4.03 – 3.94 (1H, m), 3.76 (1H, dd, *J* 2.4, 11.6 Hz), 3.69 (3H, s), 3.60 (1H, dd, *J* 5.1, 11.7 Hz), 2.69 (1H, dd, *J* 6.7, 15.7 Hz), 2.58 (1H, dd, *J* 6.0, 15.7 Hz); δ_C (101 MHz, $CDCl_3$): 171.9, 138.0, 128.5, 127.9, 127.8, 76.4, 72.1, 63.8, 51.8, 36.5; and IR spectra identical to the literature.[229]

Appendix 19: Diethyl (2*R*,3*S*)-2-allyl-3-hydroxysuccinate



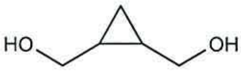
BuLi (151.3 mL, 241.3 mmol 1.6 m) was added to a stirred solution of diisopropylamine (38.1 mL, 262.3 mmol) in dry THF (150 mL) at 0 °C. After 30 min, the solution was cooled to –78 °C and diethyl (*S*)-2-hydroxysuccinate (20.1 g, 105.2 mmol) in dry THF (50 mL) was added and stirred at -78 for 1 h. Allyl bromide (25.2 g, 210.0 mmol) was added and stirred at -78 for 1 h. The mixture was slowly warmed to -20 °C and stirred at that temperature for 1 h. TLC analysis showed a trace of starting material was left, so another additional portion of allyl bromide (19.2 g, 157.5 mmol) was added and stirred at -20 °C for 1 hr and then at 0 °C for 1 h. TLC showed that the reaction was almost complete. Sat. aq. $NaHCO_3$ (50 ml) and water (100 ml) were added and extracted ethyl acetate (3 × 150 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (2:1) which was repeated three times to give a pure single enantiomer as a thick yellow oil, *diethyl (2R,3S)-2-allyl-3-hydroxysuccinate* (18.1 g, 74%), $[\alpha]_D^{20}$ + 10 (c 0.8, $CHCl_3$) (*lit.*[223] $[\alpha]_D^{20}$ +15 (c 1.4, $CHCl_3$)); Which showed δ_H (400 MHz, $CDCl_3$): 5.88 – 5.76 (1H, m), 5.22 – 5.08 (2H, m), 4.31 – 4.21 (3H, m), 4.18 – 4.11 (2H, m), 3.16 (1H, d, *J* 7.0Hz), 2.96 (1H, ddd, *J* 3.1, 6.7, 8.4 Hz), 2.68 – 2.58 (1H, m), 2.49 – 2.39 (1H, m), 1.30 (3H, t, *J* 7.1 Hz), 1.23 (3H, t, *J* 7.1 Hz); δ_C (101 MHz, $CDCl_3$):173.5, 171.9, 134.9, 118.0, 70.2, 61.8, 60.8, 48.1, 32.1, 14.1, 14.0, and IR spectra identical to the literature.[223]

Appendix 20: Dimethyl cyclopropane-1,2-dicarboxylate



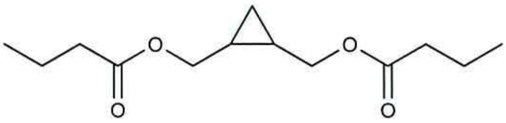
Sodium methoxide and (144 g, 2.66 mol) was added portion wise over 1.5 hrs to stirred solution of methyl acrylate (552 mL, 6 mol) and of methyl chloroacetate (234 mL, 2.66 mol) in a two neck flask fitted with mechanical stirrer between 18 and 30 °C through the addition and the mixture was stirred for 1 h. at room temperature. Water (600 mL) was added and organic layer was separated. The aqueous layer was re-extracted with dichloromethane (2 × 250 mL). The combined organic phases were then washed with saturated brine solution (2 × 250 mL) and dried over magnesium sulphate then filtrate and concentrate The crude product was purified by flash distillation at 65 – 80 °C to give a colourless oil, *dimethyl 2,2'-(cyclopropane-1,2-diyl)diacetate* (225 g, 58%); Which showed δ_H (500 MHz, $CDCl_3$) 3.68 (6H, s), 2.10 – 2.00 (2H, m), 1.70 – 1.62 (1H, m), 1.24 (1H, td, *J* 5.0, 8.4 Hz), δ_C (126 MHz, $CDCl_3$) δ 170.5, 52.3, 21.5, 11.9; and IR spectra identical to the literature.[230]

Appendix 21: Cyclopropane-1,2-diyldimethanol



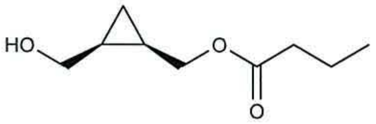
Dimethyl 2,2'-(cyclopropane-1,2-diyl)diacetate (50.0 g, 268.8 mmol) in THF (200 mL) was added dropwise to a stirred solution of lithium aluminium hydride (16.9 g, 0.671 mol) in THF (500 mL) at –5 °C. After that the reaction mixture was refluxed for 2.5 hrs. TLC showed no starting material was left. Freshly prepared sat. aq. sodium sulfate decahydrate (60 mL) wsa added slowly at 5 °C. The reaction mixture was filtered and on a bed of celite and the precipitate washed with THF (100 mL). The solvent was evaporated and the crude product was purified by column chromatography eluting with petrol/ethyl acetate (1:1) gave a thick oil, *cyclopropane-1,2-diyldimethanol* (24.2 g; 88%); which showed δ_H (500 MHz, $CDCl_3$): 4.03 (1H, d, *J* 5.0 Hz), 4.01 (1H, d, *J* 4.9 Hz), 3.25 – 3.14 (2H, m), 1.34 – 1.21 (2H, m), 0.75 (1H, td, *J* 5.1, 8.2 Hz), 0.16 (1H, q, *J* 5.2 Hz). δ_C (126 MHz, $CDCl_3$): 62.9, 17.5, 8.6; and IR spectra identical to the literature.[230]

Appendix 22: Cyclopropane-1,2-diylbis(methylene) dibutyrate



A mixture of cyclopropane-1,2-diyldimethanol (60.0 g, 0.58 mol) and butyric anhydride (170.6 g, 1.07 mol) was refluxed at 120 °C for 1 h. Aq. sodium hydroxide (40.0 g in 500 mL water) was added at room temperature and extracted with dichloromethane (3 × 250 mL). The combined organic layers were washed with sat. aq. sodium bicarbonate and dried over $MgSO_4$ and filtered. The filtrate was concentrated to give crude product. The crude product was purified by high vacuum flash distillation at 110–145°C to give a colourless oil of *cyclopropane-1,2-diylbis(methylene) dibutyrate* (130.0 g; 91%); which showed δ_H (500 MHz, $CDCl_3$): 4.24 (1H, d, *J* 5.6 Hz), 4.21 (H, d, *J* 5.6 Hz), 3.96 (1 H, d, *J* 7.2 Hz), 3.93 (1H, d, *J* 7.6 Hz), 2.28 (4H, t, *J* 7.4 Hz), 1.70 – 1.59 (4H, m), 1.35 – 1.27 (2H, m), 0.94 (6H, t, *J* 7.4 Hz), 0.90 – 0.86 (1H, m), 0.33 (1H, q, *J* 5.5 Hz); δ_C (126 MHz, $CDCl_3$): 173.7, 64.3, 36.3, 18.6, 14.8, 13.8, 8.8; and IR spectra identical to the literature.[230]

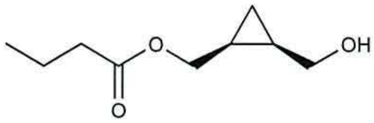
Appendix 23: ((1*R*,2*S*)-2-(hydroxymethyl)cyclopropyl)methyl butyrate



Ethylene glycol (200 mL) in distilled water (350 mL) was cooled to 3°C under nitrogen, lipase (4.5 g) was added and the pH was about 6.8. Cyclopropane-1, 2-diylbis(methylene) dibutyrate (50.0 g, 0.20 mol.) was added, hydrolysis began and the pH became low due to the formation of butyric acid. The pH was restored to 6.5 by cautiously adding sodium hydroxide solution (1.0 M) but still maintaining the temperature below 3°C. Further lipase (1 g) was added to the reaction mixture after 1 hour, whilst the pH and temperature were still maintained at 6.5 and 3 °C respectively. A constant pH of 6.5 without addition of more sodium hydroxide was an indication that hydrolysis had stopped and that the reaction was complete. The total volume of sodium hydroxide solution used was (140 mL) and the total time of the reaction was 5-6 hrs. The mixture was filtered through a bed of celite, washed with water (100 mL) and then with ethyl acetate (200 mL). Sat. aq. sodium bicarbonate (100 mL) and sat. aq.NH_4Cl (100 mL) was added and the mixture was extracted with ether (3 × 300 mL). The combined organic layers were dried over $MgSO_4$ and concentrated to obtain the crude product. The crude product was purified by column chromatography eluting with petrol/ether (1:1) to give a colourless oil, *((1R,2S)-2-(hydroxymethyl)cyclopropyl)methylbutyrate* (33.6 g; 94%), $[\alpha]_D^{22}$ + 22(c 1.2, $CHCl_3$) (*litt* [230] $[\alpha]_D^{22}$+ 18 (c

1.6, $CHCl_3$)); which showed δ_H (500 MHz, $CDCl_3$): 4.48 (1H, dd, *J* 5.6, 12.0 Hz), 3.85 (1H, dd, *J* 5.6, 12.0 Hz), 3.80 (1H, dd, *J* 9.7, 12.0 Hz), 3.39 (1H, dd, *J* 9.3, 12.0 Hz), 2.30 (2H, t, *J* 7.4 Hz), 1.71 – 1.60 (2H, m), 1.37 – 1.22 (2H, m), 0.95 (3H, t, *J* 7.4 Hz), 0.84 (1H, dt, *J* 5.2, 8.4 Hz), 0.22 (1H, q, *J* 5.5 Hz); δ_C (126 MHz, $CDCl_3$): 173.8, 64.5, 62.7, 36.4, 18.8, 18.6, 14.5, 13.7, 7.8; and I.R spectra were identical to the literature.[230]

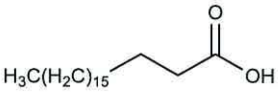
Appendix 24: ((1*S*,2*R*)-2-(hydroxymethyl)cyclopropyl)methyl butyrate



A solution of 2,2,2-triflouroethyl butyrate in isopropyl ether was prepared by dissolving 2,2,2-trifluoroethanol (50.0 g, 0.50 mol) in isopropyl ether (500 mL), and then butyric anhydride (92 mL, 0.58 mol) was added. trimethylsilyl trifluoromethane sulfonate (2 mL) was added at 4 °C, causing an exothermic reaction which raised the temperature to 25 °C. The mixture was stirred at room temperature for 1.5 h until GC showed the absence of butyric anhydride. Aqueous sodium hydroxide (350 mL, 1.25M) and sodium hydrogen carbonate (25 g) were added and the mixture was stirred for 15 min. The aqueous phase was separated and extracted with isopropanol (3 × 75 mL). The combined organic phases were washed with brine (300 mL), dried over magnesium sulfate and concentrated. Assuming a 90% yield, the I P E solution was estimated to contain (76.5 g) of 2,2,2-triflouroethyl butyrate. Cyclopropane-1,2-diyldimethanol (30.0 g, 0.29 mol) was dissolved in THF (120 mL) and added to the above I P E solution of 2,2,2-triflouroethyl butyrate (76.5 g, 0.45 mol) and then lipase (20.0 g) was added. The reaction was stirred at room temperature for over 72 h, after which GC showed a low diol content. Then it was filtered through a bed of celite, washed thoroughly with I P E and evaporated to give a yellow liquid residue, which was dissolved in dichloromethane (250 mL) and washed with sat. aq. sodium hydrogen carbonate (150 mL). The organic layer was separated and dried over MgSO4 and evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (3:1) to give a colourless oil, *((1S,2R)-2-(hydroxymethyl)cyclopropyl)methyl butyrate* (33.1 g; 63%), $[\alpha]_D^{20}$- 23(c 1.2, $CHCl_3$) (*litt.*[230] $[\alpha]_D^{24}$ – 18 (c

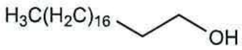
1.4, $CHCl_3$)); which showed δ_H (500 MHz, $CDCl_3$): 4.48 (1H, dd, J 5.6, 12.0 Hz), 3.85 (1H, dd, J 5.6, 12.0 Hz), 3.80 (1H, dd, J 9.7, 12.0 Hz), 3.39 (1H, dd, J 9.3, 12.0 Hz), 2.30 (2H, t, J 7.4 Hz), 1.71 – 1.60 (2H, m), 1.37 – 1.22 (2H, m), 0.95 (3H, t, J 7.4 Hz), 0.84 (1H, dt, J 5.2, 8.4 Hz), 0.22 (1H, q, J 5.5 Hz); δ_C (126 MHz, $CDCl_3$): 173.8, 64.5, 62.7, 36.4, 18.8, 18.6, 14.5, 13.7, 7.8; and I.R spectrums were identical to the literature.[230]

Appendix 25: Nonadecanoic acid



Potassium permanganate (80 g, 506 mmol) was added in portions over 2 h to a mechanically stirred solution of eicosene (50 g, 178.67 mmol), water (1000 mL), acetic acid (20 mL) hexadecyltrimethylammonium bromide (6 g, 16.4 mmol) and sulphuric acid 1 M (120 mL) in dichloromethane (1000 mL) at 3˚C.The mixture was stirred for 16 h at room temperature, then quenched carefully with saturated aq sodium metabisulphite until a clear solution was obtained. The organic layer was separated and the aqueous layer was re-extracted with dichloromethane (3 × 200 mL). The combined organic layers were dried; evaporated and recrystallized. The solid was filtered, washed with cold dichloromethane (60 ml) and petroleum (50 ml) and dried to give, *nonadecanoic acid* as a white solid (50 g, 94%) (mp 65–67 ˚C, *litt.*[221]: mp 67–68 ˚C), The compound showed δ_H , δ_C and ν_{max} identical to the literature.[221]

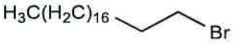
Appendix 26: Nonadecan-1-ol



Nonadecanoic acid (50 g, 175.7 mmol) in THF (900 mL) was added dropwise over 15 min to a suspension of lithium aluminium hydride (10 g, 261.6 mmol) in tetrahydrofuran (300 mL) at -10˚C. The mixture was refluxed for 1 h, then quenched carefully with freshly prepared sat aq. sodium sulfate decahydrate (40 mL) followed by the addition of anhydrous magnesium sulphate (10 g). The mixture was stirred vigorously for 10 min, filtered through a bad of celite and washed with tetrahydrofuran

(250 mL). The filtrate was evaporated and the residue was recrystallized from methanol (500 mL) and water (30 mL) at -10°C for 12 h then washed with cold methanol (50 mL) to give, *1-nonadecanol* as a white solid (47. g, 95%) (mp 60–62 °C, *lit.*[221]: mp 62 °C); which showed δ_H (500 MHz, $CDCl_3$): 3.64 (2H, t, *J* 6.6 Hz), 1.60 – 1.53 (2H, m), 1.49 (2H, s), 1.36 – 1.20 (31H, m), 0.88 (3H, t, *J* 6.9 Hz). δ_C (126 MHz, $CDCl_3$): 63.3, 33.0, 32.1, 29.9, 29.8, 29.7, 29.5, 25.9, 22.8, 14.26; and I.R spectrums were identical to the literature.[221]

Appendix 27: 1-Bromononadecane



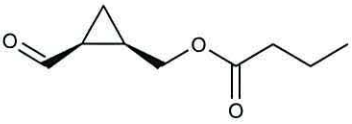
1-Nonadecanol (40.0 g, 144.34 mmol) was dissolved in toluene (300 mL) and then added to a stirred solution of hydrobromic acid (48%, 200 mL) and tetrabutylammonium bromide (1.6 g). The mixture was refluxed overnight. The reaction mixture was left to reach room temperature and sat aq. sodium bicarbonate (80 mL) was added carefully and extracted. The aqueous layer was re-extracted with petrol / ether (5:1, 3 × 200 mL) and dried. The solvent was evaporated and the crude product was purified by column chromatography eluting with petroleum / ethyl acetate (20:1) gave a white solid, *1-bromononadecane* (48.7 g, 93%), mp 35–37 °C (*lit.*[221] mp 37 °C); which showed δ_H (500 MHz, $CDCl_3$): 3.40 (2H, t, *J* 6.9 Hz), 1.89 – 1.81 (2H, m), 1.46 – 1.38 (2H, m), 1.26 (30H, s), 0.88 (3H, t, *J* 6.9 Hz); δ_C (126 MHz, $CDCl_3$): 34.1, 33.0, 32.1, 29.9, 29.8, 29.7, 29.6, 29.5, 28.9, 28.3, 22.8, 14.2; and I.R spectra were identical to the literature.[221]

Appendix 28: Nonadecyltriphenylphosphonium bromide

1-Bromononadecane (29.0 g, 83.5 mmol) was added to a stirred solution of triphenylphosphine (33.0 g, 125 mmol) in toluene (250 mL). The mixture was refluxed for 120 h. The solvent was evaporated and petroleum ether (100 ml) was added and again evaporated. The residue was treated with diethyl ether (150 ml) and stirred for 1 hr; by this time slurry of fine crystals had formed. These were filtered, washed well with ether and dried to give a white solid, *Nonadecyltriphenylphosphoniumbromide*

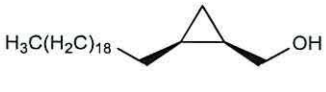
(43.5 g, 85%); which showed δ_H (500 MHz, $CDCl_3$): 7.90 – 7.82 (6H, m), 7.81 – 7.75 (3H, m), 7.73 – 7.65 (6H, m), 3.95 – 3.74 (2H, m), 1.62 (4H, s), 1.32 – 1.12 (31H, m), 0.87 (H, t, *J* 6.9 Hz); δ_C (126 MHz, $CDCl_3$): 135.1, 135.0, 133.8, 133.7, 130.6, 130.5, 118.9, 118.2, 32.0, 30.6, 30.4, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 23.1, 22.7, 15.3, 14.2; and I.R spectrums were identical to the literature.[221]

Appendix 29: ((1*R*,2*S*)-2-formylcyclopropyl)methyl butyrate



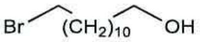
A solution of ((1*R*,2*S*)-2-(hydroxymethyl)cyclopropyl)methyl butyrate (12 g, 0.06 mol) in dichloromethane (170 mL) was added in portions to a stirred solution of pyridinium chlorochromate (40 g, 0.18 mol) and molecular service (6 g) in dichloromethane (714 mL). The mixture turned black during the reaction. The reaction mixture was stirred for 2 h at room temperature then TLC petrol/ethyl acetate (5:1) showed that the reaction was completed. The reaction was diluted with petrol/ethyl acetate (600 mL) and filtered through a bed of silica and celite and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (5:2) to give a colourless oil, *((1R,2S)-2-formylcyclopropyl)methyl butyrate* (10 g; 84%) was obtained, $[\alpha]_D^{20}$ + 72 (c 1.5, $CHCl_3$) (*lit.*[230] $[\alpha]_D^{24}$ + 88 (c 2.1, $CHCl_3$)); which showed δ_H (500 MHz, $CDCl_3$): 9.44 (1H, d, *J* 4.4 Hz), 4.42 (1H, dd, *J* 6.1, 12.0 Hz), 3.86 (1H, dd, *J* 9.3, 11.9 Hz), 2.18 (2H, t, *J* 7.4 Hz), 2.02 – 1.96 (1H, m), 1.84 – 1.75 (1H, m), 1.53 (2H, sext, *J* 7.4 Hz), 1.29 – 1.24 (1H, m), 1.23 – 1.19 (1H, m), 0.84 (3H, t, *J* 7.4 Hz); δ_C (126 MHz, $CDCl_3$): 199.9, 173.3, 62.2, 35.9, 26.6, 22.4, 18.3, 13.5, 12.6, and I.R spectrums were identical to the literature.[230]

Appendix 30: ((1*R*,2*S*)-2-Eicosylcyclopropyl)methanol



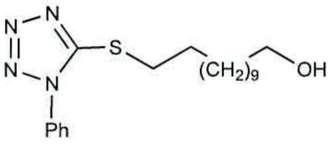
Lithium bis–(trimethylsilyl) amide (100 mL) was added to a stirred solution of octadecanyltriphenyl phosphonium bromide (42.8 g, 0.07 mol) in tetrahydrofuran (600 mL) at –30 ˚C. The mixture was allowed to reach room temperature and stirring for 2 h ((1R,2S)-2-formylcyclopropyl)methyl butyrate (10.0 g, 0.05 mol) in tetrahydrofuran (80 mL) was added at –10 ˚C and stirred at reach room temperature for 18 h. Sat. aq. ammonium chloride (100 mL) and then petrol/ethyl acetate (1:1, 200 mL) was added and mixture was extracted. The aqueous layer was re–extracted with petrol/ethyl acetate (1:1, 2 × 120 mL). The combined organic layers were washed with saturated aqueous sodium hydroxide (150 mL), dried with magnesium sulphate and filtered. The solvent was evaporated and the crude product was purified by column chromatography eluting with petrol/ethyl acetate (10:1 to give a colourless oil, *((1R,2R)-2-((E/Z)-eicos-1-en-1-yl)cyclopropyl)methyl butyrate* (19.0 g; 77%) as a mixture. Potassium hydroxide (7.2 g) in methanol (80 mL) was added to a stirred solution of the above ester (19.0 g) in tetrahydrofuran (250 mL) at room temperature. The reaction was stirred at 40 ˚C for 3 h and water (100 mL) and petrol/ethyl acetate (1:1, 250 mL) were added at room temperature. The aqueous layer was separated and re-extracted with petrol/ethyl acetate (2 × 100 mL). The combined organic layers were washed with brine (100 mL), dried and evaporated to give a white solid, *((1R,2R)-2-((E/Z)-icos-1-en-1-yl)cyclopropyl)methanol* (17.0 g, 95%). Sat. aq. sodium (meta)periodate (97.0 g, 0.45 mol) in hot water was added dropwise over a period of 1.5 h to a stirred solution of the above alcohol (16.0 g), hydrazine hydrate (64 mL), acetic acid (2.5 mL) and sat. aq. copper sulphate (2.5 mL) in 2–propanol (500 mL) at 70 – 80 ˚C. The mixture was stirred for 2 h at room temperature and sat. aq. ammonium chloride (100 mL) in small portions. The combined organic layer was extracted with petrol/ethyl acetate (1:1, 3 × 200 mL) dried over anhydrous magnesium sulphate. The solvent was evaporated and the crude product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give a white solid, *((1R,2S)-2-icosylcyclopropyl)methanol* (16.0 g; 95%), m.p. 55˚C, $[\alpha]_D^{24}$+ 13 (c 0.9, $CHCl_3$); which showed δ_H (500 MHz, $CDCl_3$): 3.62 (1H, dd, *J* 7.1, 11.3 Hz), 3.56 (1H, dd, *J* 8.0, 11.3 Hz), 1.50 – 1.35 (3H, m), 1.33 – 1.15 (36H, m), 1.14 – 1.03 (1H, m), 0.86 (3H, t, *J* 6.9 Hz), 0.68 (1 H, dt, *J* 4.6, 8.3 Hz), -0.06 (1H, q , *J* 5.4 Hz). δ_C (126 MHz, $CDCl_3$): 63.4, 32.0, 30.3, 29.8, 29.8, 29.7, 29.5, 28.7, 22.8, 18.3, 16.3, 14.2, 9.6; ν_{max} /cm^{-1}: 3315, 1468, 1380, 1045, 1008, 727. The ^{1}H, ^{13}C, and I.R spectrums were identical to the literature.[221]

Appendix 31: 12–bromo–1–dodecanol



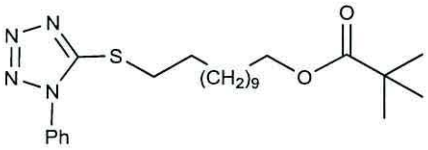
Hydrogen bromide (48%, 60 mL) was added to a stirred solution of 1,12–Dodecanediol (50 g, 0.24 mol) in toluene (500 mL) at room temperature. The reaction mixture was refluxed for 17 h at 120 °C. The toluene layer was separated and evaporated, and the crude product was purified by column chromatography eluting with petrol/ethyl acetate (5:2) to give a waxy solid of *12–bromo–1–dodecanol* (40 g 70%), which showed ^{1}H, ^{13}C, and I.R spectrums were identical to the literature.[231]

Appendix 32: 12-((1-phenyl-1H-tetrazol-5-yl)thio)dodecan-1-ol



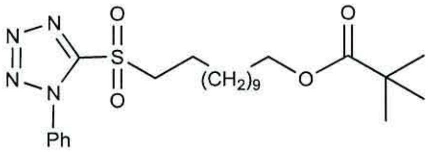
12–Bromo–1–dodecanol (45 g, 0.18mol) was added to a stirred solution of 1–phenyl–1H–tetrazole–5–thiol (33.2 g, 0.18 mol) and potassium carbonate (52 g, 0.37mol) in HPLC grade acetone (500 mL). The mixture was stirred at room temperature for one hour and then refluxed at 70 °C for another 2 h. The reaction mixture was allowed to reach room temperature and stirred overnight. TLC showed no staring material was left. The inorganic salts were filtered off and washed with acetone. The filtrate was evaporated and the residue was treated with dichloromethane (250 mL) and water (100 mL). The aqueous layer was separated and extracted with dichloromethane (2 × 150 mL) and the combined organic layers were washed with water (2 × 300 mL), dried and evaporated to give waxy solid, *12-((1-phenyl-1H-tetrazol-5-yl)thio)dodecan-1-ol* (62 g, 93%); Which showed δ_H (500 MHz, $CDCl_3$): 7.63 – 7.49 (5H, m), 3.63 (2H, t, *J* 6.6), 3.38 (2H, t, *J* 7.4), 1.81 (2H, quintet, *J* 7.4 Hz), 1.55 (2H, quintet, *J* 7.4 Hz), 1.48 – 1.39 (2H, m), 1.38 – 1.19 (16H, m); δ_C (126 MHz, CDCl3): 154.7, 133.9, 130.2, 129.9, 124.0, 63.9, 33.5, 32.9, 29.7, 29.6, 29.5, 29.2, 29.1, 28.7, 25.8; ν_{max} /cm^{-1}: 3552, 2940, 2850, 1486, 1291, 1152, 456, 450, 443, 437, 405; ^{1}H, ^{13}C, and I.R spectrums were identical to the literature.[231]

Appendix 33: 12-((1-phenyl-1H-tetrazol-5-yl)thio)dodecyl pivalate



Trimethyl acetyl chloride (24.2 g, 0.20 mol) and dimethylamino pyridine (2.0 g) were added to a stirred solution of 12-((1-phenyl-1H-tetrazol-5-yl)thio)dodecan-1-ol (61 g, 0.37 mol) dichloromethane (250 ml) at 5 °C, and triethylamine (25.5 g, 0.25 mol). The reaction mixture was allowed to reach room temperature and stirred overnight. TLC showed no starting material was left. Water (250 mL) and dichloromethane (300 mL) were added atroom temperature. The aqueous layer was separated and re–extracted with dichloromethane (3 × 300 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 (5:1) to give a colourless oil, *12-((1-phenyl-1H-tetrazol-5-yl)thio)dodecyl pivalate* (66.0 g, 94%), The compound showed δ_H (500 MHz, $CDCl_3$): 7.63 – 7.46 (5H, m), 4.02 (2H, t, *J* 6.6 Hz), 3.37 (2H, t, *J* 7.4 Hz), 1.80 (2H, quintet, *J* 7.0 Hz), 1.59 (2H, quintet, *J* 7.4 Hz), 1.45 – 1.38 (2H, m), 1.36 – 1.21 (14H, m), 1.17 (9H, s); δ_C (126 MHz, $CDCl_3$): 178.7, 154.6, 133.9, 130.1, 129.8, 123.9, 64.5, 38.8, 33.5, 29.6, 29.5, 29.3, 29.2, 29.1, 28.7, 28.6, 27.3, 26.6, 26.0; ν_{max} /cm^{-1}: 2948, 2850, 1741, 1490, 1286, 1152, 456, 450, 443, 437, 405; ^1H, ^{13}C, and I.R spectrums were identical to the literature.[231]

Appendix 34: 12-((1-Phenyl-1H-tetrazol-5-yl)sulfonyl)dodecyl pivalate



Ammonium molybdate VI tetrahydrate (96.3 g, 0.07 mol) was dissolved in cold hydrogen peroxide (35%, w/w, 120 mL) and was gradually added to a stirred solution of 2,2–dimethylpropanoic acid 12–(1–phenyl–1H–tetrazole–5–ylsulfanyl)dodecyl ester (69.1 g, 0.14 mol) in IMS (600 mL) at 10 °C. The reaction mixture was allowed to reach room temperature and stirred for 2 h, and then another portion of ammonium molybdate VI tetrahydrate (48 g, 0.03mol) in cold hydrogen peroxide was added (35%, w/w, 60 mL). The reaction mixture was stirred for 18 h. The mixture was poured into water (2.5 litres) and extracted with dichloromethane (3 × 400 mL). The compound organic layers were washed with more water (2 × 400 mL) and dried. The solvent was evaporated and the crude product was purified by column chromatography eluting with with petrol/ethyl acetate (5:2), to give a semi solid, *12-((1-phenyl-1H-tetrazol-5-yl)sulfonyl)dodecyl pivalate* (70.0 g, 93%), which showed ^{1}H, ^{13}C, and I.R spectrums were identical to the literature.[231]