DOCTOR OF PHILOSOPHY

Synthesis of mycolic acids and related sugar ester

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Award date:
2012

Awarding institution:
Bangor University

Synthesis of Mycolic Acids and related sugar ester

A thesis submitted to the Bangor University

for the degree of Doctor of Philosophy

by

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PRIFYSGOL
BANGOR
UNIVERSITY

2012

Acknowledgements

First of all, I would like to deeply thank my supervisor Professor Baird for all his guidance and help through these years. I would also like to sincerely thank Dr Juma'a Al-Dulayymi for his advice and help in the laboratory work. I would like to thank all the members of the Prof. Baird research group, past and present for their help and friendship. In addition, I would like to thank all the secretarial, technical and the entire staff at the School of Chemistry. I would also like to thank the government of Iraq, particularly the Ministry of Higher Education and Cultural Attaché for sponsoring me. Finally, I would also like to thank my family in Bangor and Iraq.

Contents

Abbreviations and acronyms

AIDS- Acquired Immune Deficiency Syndrome

aq. – aqueous

b – broad

BCG - Bacillus Calmette-Guérin

BT- benzothiazole

CID-MS - collision-induced dissociation mass spectrometry

d – doublet

DEAD- diethyl azodicarboxylate

dil. – dilute

DMAP – 4-dimethylaminopyridine

DME - Dimethoxyethane

DMF – dimethylformamide

EDCI- 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide

ELISA - enzyme-linked immunosorbent assay

FAS- Fatty Acid Synthase

GC – gas chromatography

HIV – human immunodeficiency virus

HMPA- hexamethylphosphorotriamide

HPLC – high performance liquid chromatography

hrs – hours

IMS – industrial methylated spirits

IR – infra-red

J - coupling constant

LDA- lithium *N, N*,-diisopropylamide

MDR-TB -multidrug-resistance tuberculosis

m - meta-

m - multiplet

M. marinum – Mycobacterium marinum

m.p. - melting point

mmaA1- methoxymycolic acid synthase 1

mmaA2- methoxymycolic acid synthase 2

M.Tb – Mycobacterium tuberculosis

MALDI - Matrix-Assisted Laser Desorption/Ionization

MALDI-TOF- Matrix Assisted Laser Desorption Ionization Time-Of-Flight

m-CPBA - *m*-chloroperbenzoic acid

MHz- megahertz

mmol - millimols

mol. equiv. – molecular equivalents

m.p – melting point

MS – mass spectrometry

NBS - *N*-bromosuccinimide

NMR – nuclear magnetic resonance

oxone - $2KHSO_5.KHSO_4.K_2SO_4$ ($KHSO_5$: potassium peroxomonosulfate)

ODP- *o*-phenylenediamine

PCC - pyridinium chlorochromate

Petrol - petroleum spirit (boiling point 40 to 60 ^{o}C)

ppm - parts per million

PPTS - pyridinium *p*-toluenesulfonate

PTSA - *p*-toluenesulfonic acid monohydrate

PT - phenyltetrazole

Pv - pivaloyl

q - quartet

s - singlet

sat. – saturated

SAM- S-adenosyl-L-methionine

t - triplet

TB – tuberculosis

TBDMS- *tert*-butyldimethylsilyl

TMM- – trehalose monomycolate

TDM – trehalose dimycolate

THP - tetrahydropyran

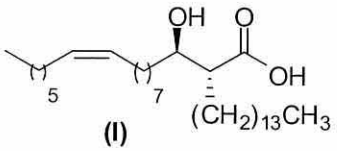
TLC – thin layer chromatography

v.br. - very broad

WHO – world health organisation

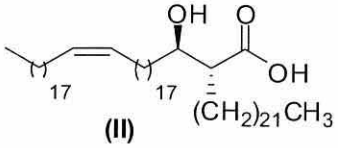
Abstract

This study involved the preparation of mycolic acid (**II**) containing a double bond in a *cis* configuration. This was attempted by hydrogenation of an alkyne, which was successful in model reactions, but gave no evidence of success when applied to the mycolic acid. The other strategy was the use of a Wittig reaction. In order to optimise the conditions for double bond synthesis, a small mycolic acid (**I**) was first synthesised since it is reported in *corynobacteria diphtheriae*.



(I)

Once the reaction conditions had been optimised, the same conditions were applied for the synthesis of mycolic acid (**II**) and mycolic acid (**III**).



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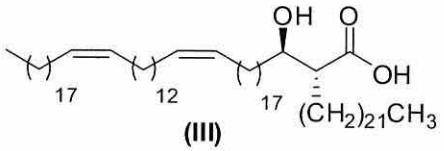
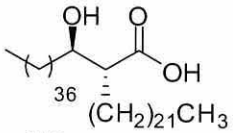


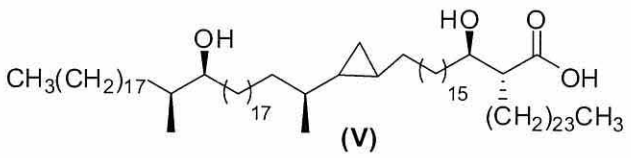
(III)

This was followed by unsuccessful attempt to synthesise saturated mycolic acid (**IV**).



(IV)

Three other mycolic acids were made; a hydroxy mycolic acid (**V**), which contains a hydroxy group in the meromycolate moiety, a keto mycolic acid (**VI**) containing a keto group in the meromycolate moiety and α-mycolic acid (**VII**), with two cyclopropane rings in the meromycolate moiety.



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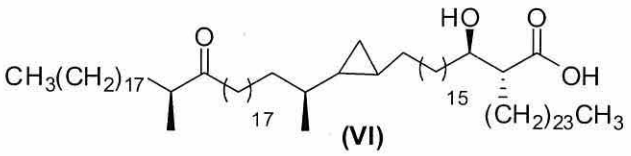

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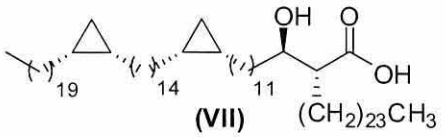


(VII)

In the last stages of this study, the synthesis of cord factors of mycolic acids became the focus. Two separate trehalose dimycolates (TDMs) of α-mycolic acids (**II** and **VII**) were synthesised, followed by the synthesis of the free trehalose monomycolate (TMM) of α-mycolic acid (**VII**), and the protected TMM of mycolic acid (**II**).

Introduction

1.1 Tuberculosis

Tuberculosis (TB) is a disease which has an ancient history.[1, 2] It has been known from the time of Hippocrates, who gave it a clinical description.[3] Moreover it became a public health problem three centuries ago, when cities became overcrowded and there was a lack of health care facilities. This led people to study the disease with the aim of finding a cure for it, with early studies starting in the 17th century.[4] 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 at that time, with one in four deaths in England being caused by TB and in France; one in six deaths was due to TB until the 20th century. The World Health Organisation (WHO) estimates that roughly one third of the world's population is currently infected with TB.[5]

Pioneering studies by Robert Koch determined that the bacillus causing this disease was *Mycobacterium tuberculosis,* leading him to receive a Nobel prize for this discovery. This led to the discovery of a vaccine against TB early in the 20th century. The discovery of the BCG vaccine, which was developed between 1905 and 1921,[6] and the improvement in living conditions occasioned a decline in the death rates from TB in developed countries towards the end of the 19th century. The discovery and use of anti TB drugs such as isoniazid (INH) and streptomycin, which began in the 1940's made the control of the disease appear possible, at least in developed countries.[3, 6] However, the belief that all of these improvements, the introduction of a vaccine and treatment with antibiotics would lead to the disappearance of the disease was only an illusion. TB infection rates in the western world began to rise again in the 1980's due to a number of factors, but mostly to the HIV/AIDS pandemic. The last decade saw the development of 80 million new cases and more than 30 million deaths due to TB.[7] Each year, there are approximately eight million new cases and three million deaths caused by TB. Of people infected with TB only 5-10 % develop active disease, although the percentage of HIV/AIDS sufferers co-infected with TB who develop active TB is much higher.[8] The cost of treatment of TB in high-burden countries increased dramatically between 2002 and 2010 (Figure (**1-1**)).[9]

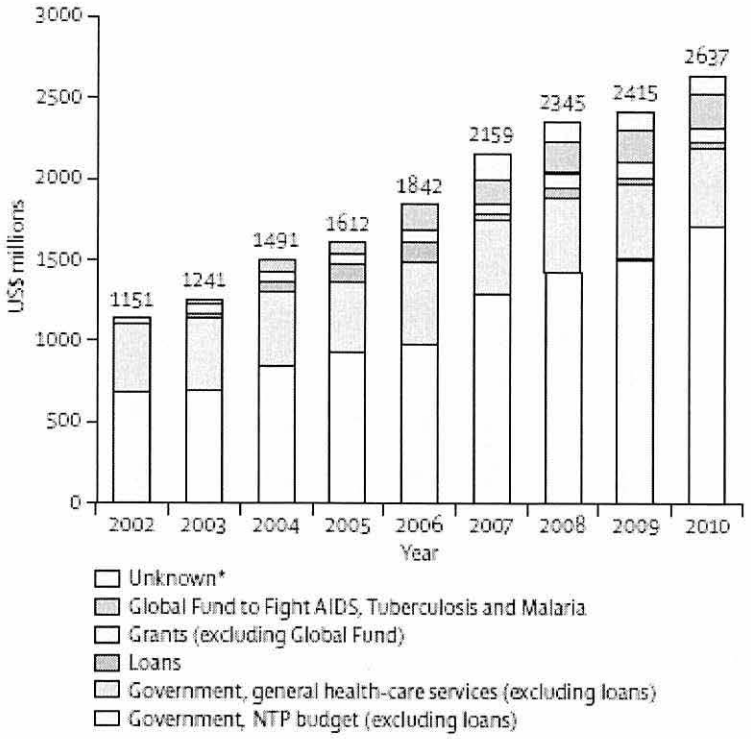


Figure (1-1): The cost of TB treatment between 2002 and 2010.[9]

An important reason for the failure to control TB is the fact that even in good treatment conditions, therapy must be continued for at least 6 months. This leads to patient non-compliance as the patients feel well after a few weeks of treatment and discontinue taking the anti TB drugs and may even be tempted to sell-on drugs in countries with a high prevalence of poverty. The use of the WHO implemented 'directly observed therapy short-course' (DOTS) does not solve the problem.[10] The reason for the prolonged (6-24 months) treatment of TB is that the chemotherapy kills the active bacteria in few days, but leaves subpopulations of "persisters"[11,12] which require a much more lengthy treatment. Therefore if the treatment is stopped at 3 months, relapse rates are high.[13]

1.2 Multi-Drug Resistance (MDR)

Early attempts to cure TB using streptomycin faced the emergence of unexpected problems, with many patients suffering relapses of TB with streptomycin resistant strains.[14] Since this time, strains of *M. tuberculosis* showing resistance to other anti-TB drugs have arisen. When a strain of TB is found to be resistant to two or more front line drugs (like INH, rifampin, PZA, ethambutol and streptomycin) it is termed

Multi-Drug Resistant (MDR). Over 0.5 million new cases of MDR TB were reported in 2008,[15] leading to the classification of the antibiotics used for TB treatment into five groups, with the recommendation that a design strategy for drug combinations should be used for each individual case of TB.[16] In the USA, primary drug resistance was 1-2 % in the 1950's, rose to 3 % in next decade and by 1970 had increased to 8.6 %.[3, 17] In the other parts of the world MDR-TB is a significant problem, with the range of MDR-TB in India, Pakistan and Central Haiti being between 20-30 %.[18,19]

1.3 The effect of HIV on TB

TB is one of the first secondary infections for HIV/AIDS patients.[20] WHO studies demonstrated that the countries with high incidence of AIDS/HIV also have a high rate of TB (Figure (**1-2**)), with 15 % of the 9.27 million new TB cases reported in 2007 being HIV positive.[21]

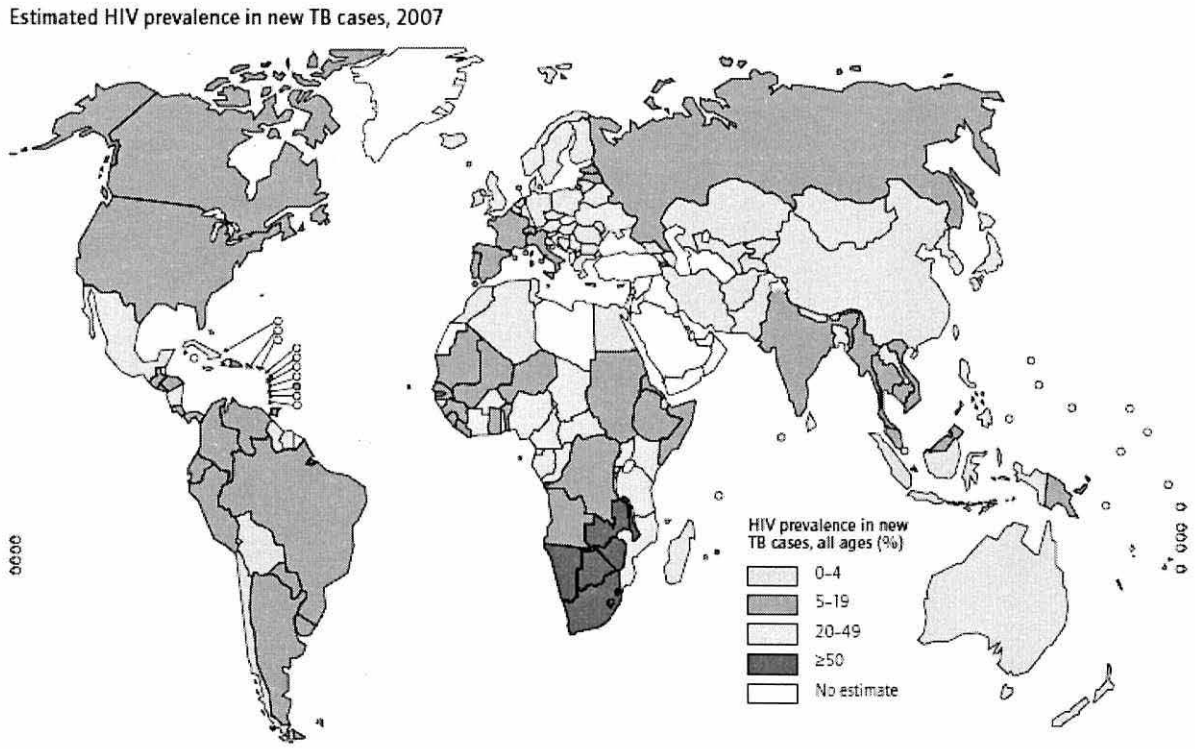


Figure (1-2): Fifteen countries with the highest estimated TB incidence rates and corresponding incidence rates of HIV-positive TB cases. [21]

1.4 Mycobacteria

There are many bacteria which belong to the same genus as *M. tuberculosis*, for example *Mycobacterium bovis*, *Mycobacterium smegmatis* and *Mycobacterium*

leprae. *M. bovis* is the causative agent of TB in cattle; *M. leprae* causes leprosy, while *M. smegmatis* is a non-pathogenic bacterium that is used for many *in vitro* experiments to model *M. tuberculosis* causing TB (Figure (**1-3**)). Part of the problem of treating mycobacterial infections is the high resistance to the majority of antibiotics that this type of bacterium displays. This resistance is thought to be due to the unique cell wall structure of mycobacteria.[22]

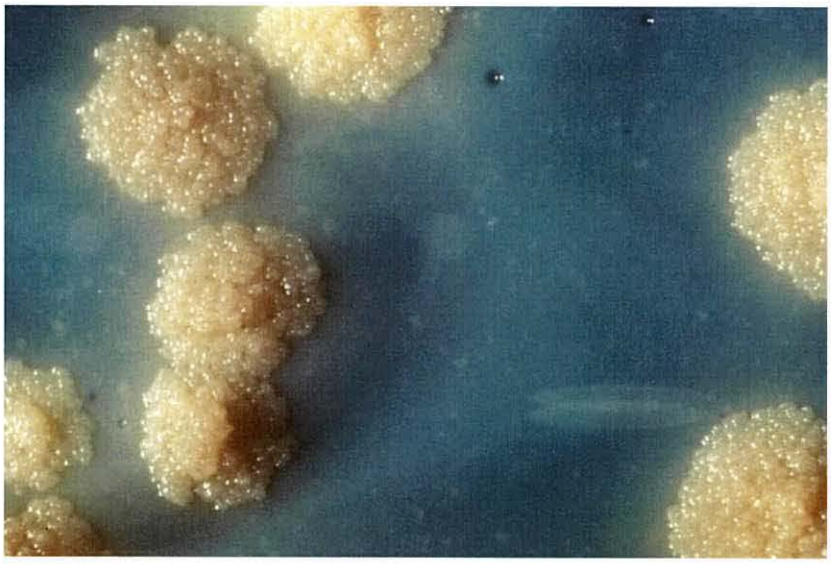


Figure (1-3): *M. tuberculosis* bacterial colonies.[23]

1.5 Cell wall components

Attempts to analyse the cell wall of mycobacteria in the early 1900's reported a very large quantity of lipid in the *tubercle bacilli* which 'appeared more than ordinarily important'. Three different groups of lipid were separated from the cell wall: wax, glycerides and phosphatides with the quantities depending on whether an acid or base extraction technique was used.[24] It has been reported that the lipid extract from the cell wall of *M. tuberculosis* contains more than one fatty acid which shows optical activity and is of high molecular weight.[25] In 1938 these fatty acids were named mycolic acids by Anderson,[26] who in the next year reported them to be hydroxy acids (see section (**1-6**)).[27] The cell wall of *M. tuberculosis* consists mainly of lipids or mycomembrane and other components such as peptidoglycan, arabinogalactan and inner membrane.[28] The layer of lipid in the cell wall provides *M. tuberculosis* with the ability to resists many drugs. For example, many organisms are killed if treated with 1 M sodium hydroxide solution, but *M. tuberculosis* cells still have the ability to grow

following treatment, if transferred to a suitable environment.[29] Numerous studies to classify the components of the cell wall showed that it contains a large number of lipids which make up around 60 %, making it difficult to understand how these lipids are arranged in the cell wall.[30] Many of these lipids and glycoconjugates, are arranged as a lipid bilayer in the cell wall (Figure (**1-4**)). Some of the lipid classes are considered as potential modulators of the membrane trafficking in the host cell.[31, 30]

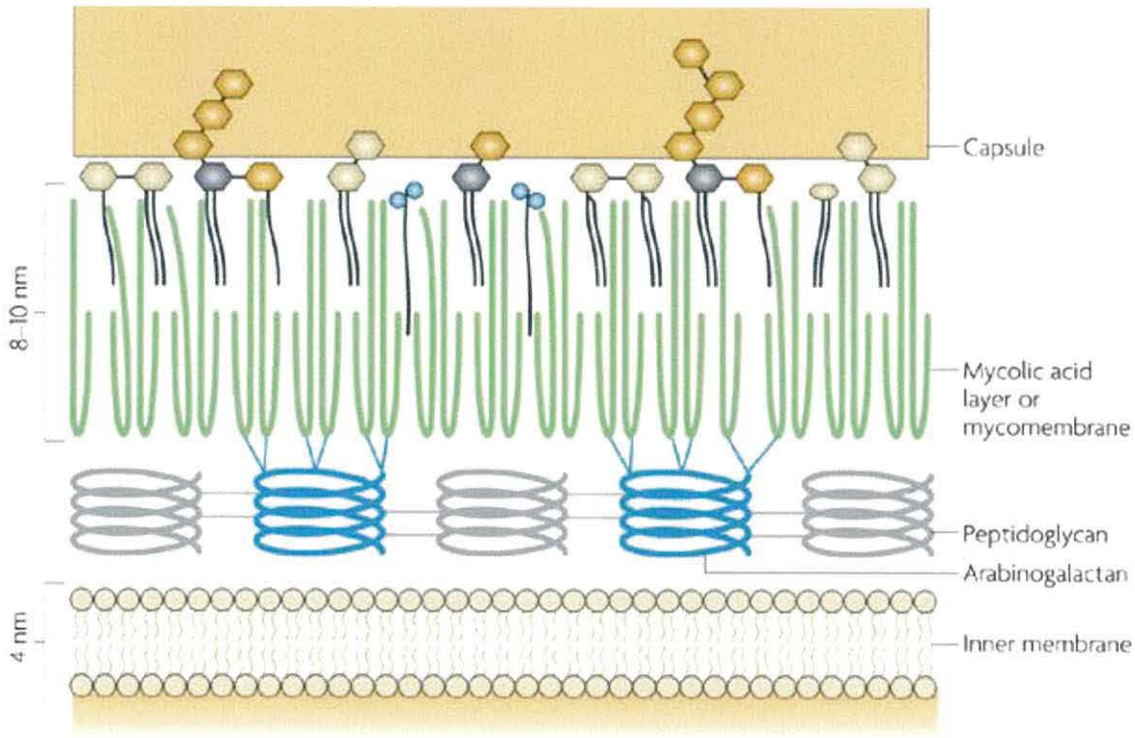


Figure (1-4): The mycobacterial cell wall[28]

The cell wall as shown in Figure (**1-4**) contains a capsule as the outer layer. The peptidoglycan is linked to the polysaccharide which is esterified to the distal end of the mycolic acid. Most of the hydrocarbon chains of the lipids are assembled as an asymmetric bilayer with an exceptional thickness.[22]

1.6 Mycolic acids

Mycolic acids have been reported since early in the last century,[26, 27] and the determination of their structure (**1**) was linked to the development of analytical tools for the determination of the structure of organic compounds. 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 structure for the main frame of mycolic acids, (Figure (**1-5**)).[32,33] In further studies Minnikin proposed the structure of the cell wall.[32, 34, 22] The mycolic acids have two key moieties, the main moiety called the meromycolate moiety and the other one called the mycolic motif. The mycolic motif is common for every mycolic acid and has the hydroxyl group and α-alkyl branch in the (*R,R*) configuration (Figure (**1-5**)).

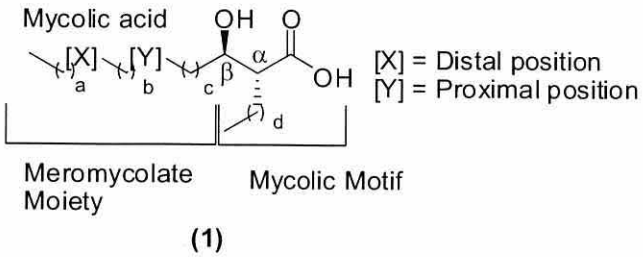


Figure (1-5): The general structure of a mycolic acid

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 (Figure (**1-6**)). The classification of mycolic acids depends on to the groups in this part of the mycolic acid, if both X and Y are cyclopropane rings or double bonds then they are known as α-mycolic acids (**2**), (**3**) and (**4**), if X is an α-methyl-β-keto (**5**) and (**6**) or a methyl-β-methoxy (**7**) and (**8**) then they are known as keto- and methoxy-mycolic acids and if X is an oxirane ring they are known as epoxy mycolic acids (**9**) and (**10**) (Figure (**1-6**)).[35-37] Barry *et al.* reported that the most common classes of mycolic acids are α-mycolic acids with double bonds or two cyclopropane rings.[38]

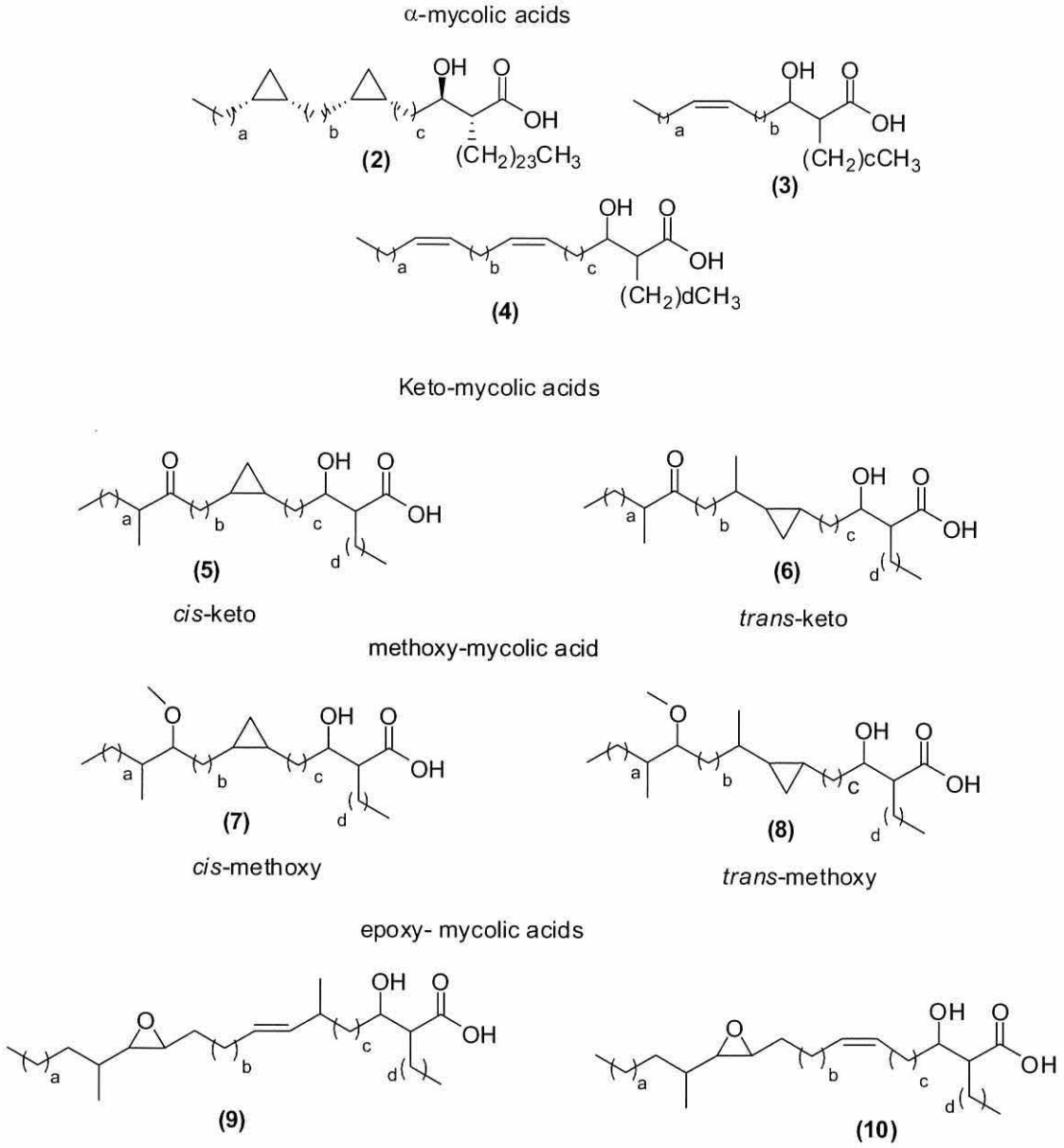


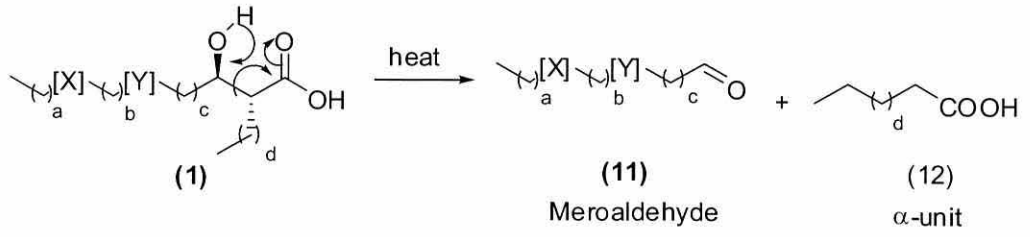
Figure (1-6): The major types of mycolic acids[34, 37, 39]

1.7 The chain length in the mycolic acid

Mycobacteria were reported not only to contain the three major types of mycolic acid, but also each one of those types is present as a number of different homologues. In the cell wall of *M. tuberculosis* over 500 mycolic acids with closely related chemical structures are present,[33] and this makes the separation process of mycolic acids extremely difficult.[38, 40] The three types of mycolic acid can be separated; however, separation of individual mycolic acids of a similar type is extremely time

consuming,[41, 42] and the most significant progress as been associated with the development of analytical tools.[32, 43, 44]

Since the discovery of mycolic acids there have been many studies in order to determine the chain lengths between the functional groups. The early attempts were done using GC with a high injector temperature, while Guerrant *et al.* attempted to analyse mycolic acids isolated from *nontuberculosis mycobacteria* using GC with two different injector temperatures. When the injector temperature was between 300 and 350 °C, thermal cleavage of the mycolic acids was observed and methyl esters with C_{22}, C_{24} or C_{26} chains were detected, but when it was set at 235 °C or lower the mycolic acids were heat stable and no methyl ester cleavage was observed.[44] Further studies adopted new tools for analysis like several techniques of mass spectrometry, in association with chromatography techniques for separation. Watanabe *et al.*[36] determined the location of the functional groups in the meromycolic acids (prepared by pyrolysis followed by oxidation using silver oxide Scheme **(1-1)** for 19 strains of the *M. tuberculosis* complex. The last step used MALDI-TOF mass spectrometry for the analysis of these fragments of mycolic acids.



Scheme (1-1): Pyrolytic cleavage of mycolic acid[38]

The location of the meromycolic acid functional groups, *i.e.* cyclopropane rings, double bonds, epoxide rings, keto groups, methoxy groups and the methyl branch, and the chain length were studied using CID mass spectrometry as well as other spectroscopic methods.[32, 36, 39, 44-46]

1.8 The stereochemistry of the functional groups

The stereochemistry of the functional groups in mycolic acids is still not completely clear. The two chiral centres of the mycolic motif (**13**) (α, β position) relative to the carboxylic group in all mycolic acids, are both *anti* to each other, and are in an *R,R*-configuration (Figure **(1-7)).**[35, 45, 47-49] The presence of the hydroxyl group and the

alkyl chain in this conformation is responsible for the packing in the molecules,[50] with the formation of hydrogen bonding between the carboxyl and the hydroxyl groups stabilising the ligand conformation between the long chains.[51-54] This configuration is necessary for the recognition the mycolic acids by T cells and the generation of an immune response which gives the mycolic acid derivatives antitumor properties.[55]

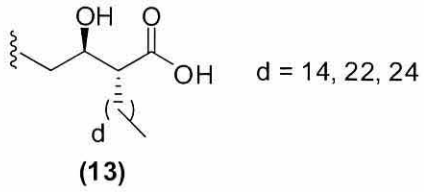


Figure (1-7): The stereochemistry of mycolic motif[43]

Little is known about the other chiral centres in the meromycolic part due to it being so difficult to determine the absolute configuration in such large molecules. A recent study on the stereochemistry of the methyl-keto (**14**), methoxy (**15**) and hydroxy (**16**) mycolic acids (in *M. smegmatis* modified genetically to resemble *M. tuberculosis*) showed that the methyl branch is in the (*S*) configuration (Figure (**1-8**)).[35, 56, 57] Furthermore it was found that the α-methyl-*trans*-epoxy mycolic acid (**17**) is in the *R* configuration and the methyl branch adjacent to the *trans* double bond is in the *R* configuration in *M. ulcerans* and *M. marinum*.[43, 47, 57, 58]

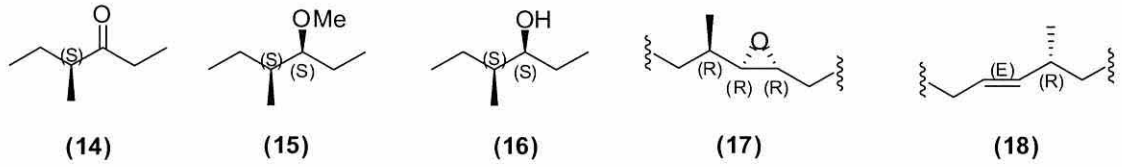


Figure (1-8): The stereochemistry of different groups in meromycolic acids[37, 43]

The configurations of the functional groups were deduced by measuring the molecular rotations ($[M]_D$), since spectroscopic methods like IR or NMR cannot be used to distinguish the absolute configuration, but using specific rotation showed values that could distinguish possible stereoisomers. Determination of the configuration of the stereocentres in natural mycolic acids was performed by measuring the M_D of the products of pyrolysis of the whole mycolic acid. These values were compared with those of similar fragments of synthetic mycolic acids which had a known stereochemistry.

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

The molecular rotations of the pyrolysis fragments of natural mycolic acids were also compared with the molecular rotation of corresponding synthetic fragments of mycolic acids with known stereochemistry. Also, a recent study proved that the α-methyl-*trans* cyclopropanes (**19**), (**20**) and (**21**) have essentially identical NMR spectra and the only way to differentiate between them is from the specific rotations, since the different stereoisomers give completely different values of molecular rotation (Figure (**1-9**)).

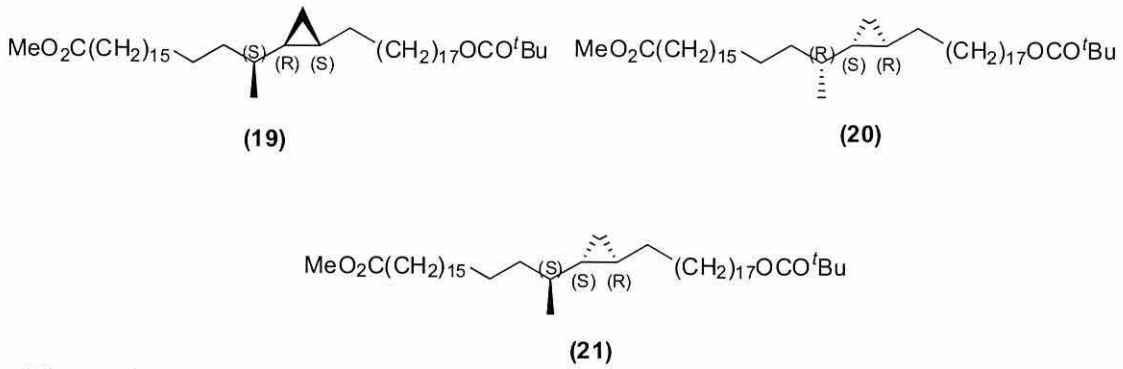


Figure (1-9): Different stereochemistry of α-methyl-*trans*-cyclopropane ester[58]

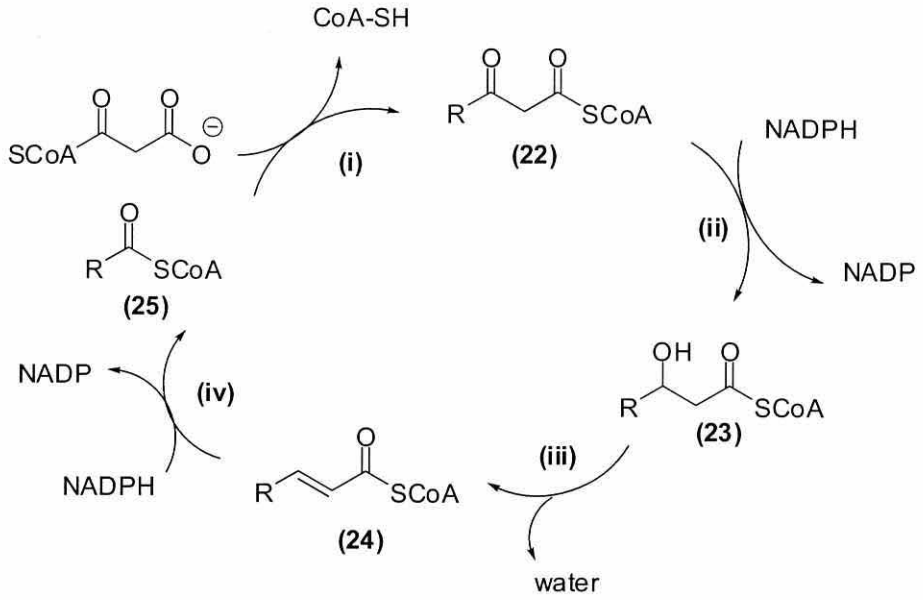
1.9 Biosynthesis of mycolic acids

The biosynthesis of mycolic acids has been the subject of many studies in order to find a drug for tuberculosis. Since the mycolic acids play an essential role in the *M. tuberculosis* host body,[38] the inhibition of the synthesis of mycolic acid could provide one of the most efficient antituberculous agents.[59] Fatty acid metabolism containing ~250 genes (in H37Rv strain) is involved, in contrast with *E. coli* metabolism which only involves 50 genes,[60,61] proving how these acids are essential for the survival of this bacterium.[29] Mycobacteria use three major steps for the biosynthesis of mycolic acids.

1.9.1 The synthesis of long fatty acid (FAS I)

Fatty acid synthesis involves a repeated cycle of enoyl reduction, dehydration, condensation and keto reduction to produce a fatty acid with a chain length of 16-18 carbons.[61] The acetyl-CoA forms the acyl chain (**22**) in each cycle, which is then used as a substrate. In fact in every cycle acetyl-CoA is used to add a C-2 unit, in order to obtain fatty acids with a C_{16}-C_{18} chain length (Scheme (**1-2**)). These processes are

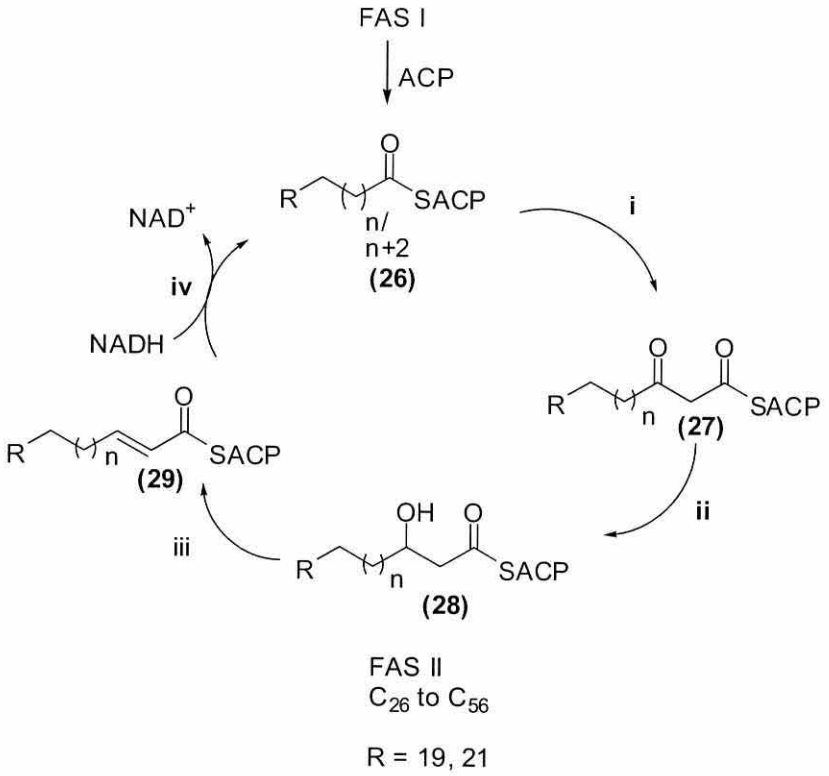
catalysed by two types of enzyme system.[62,38] There is also evidence of the involvement of malonyl-CoA sometimes instead of aceyl-CoA, which produces fatty acids with a chain length of C_{24}-C_{26} or longer.[63]



Scheme (1-2): FAS I process, i): condensation, ii): β-ketoreduction, iii): β-hydroxy dehydration, iv)**: enoyl reduction.**[57]

1.9.2 FAS II

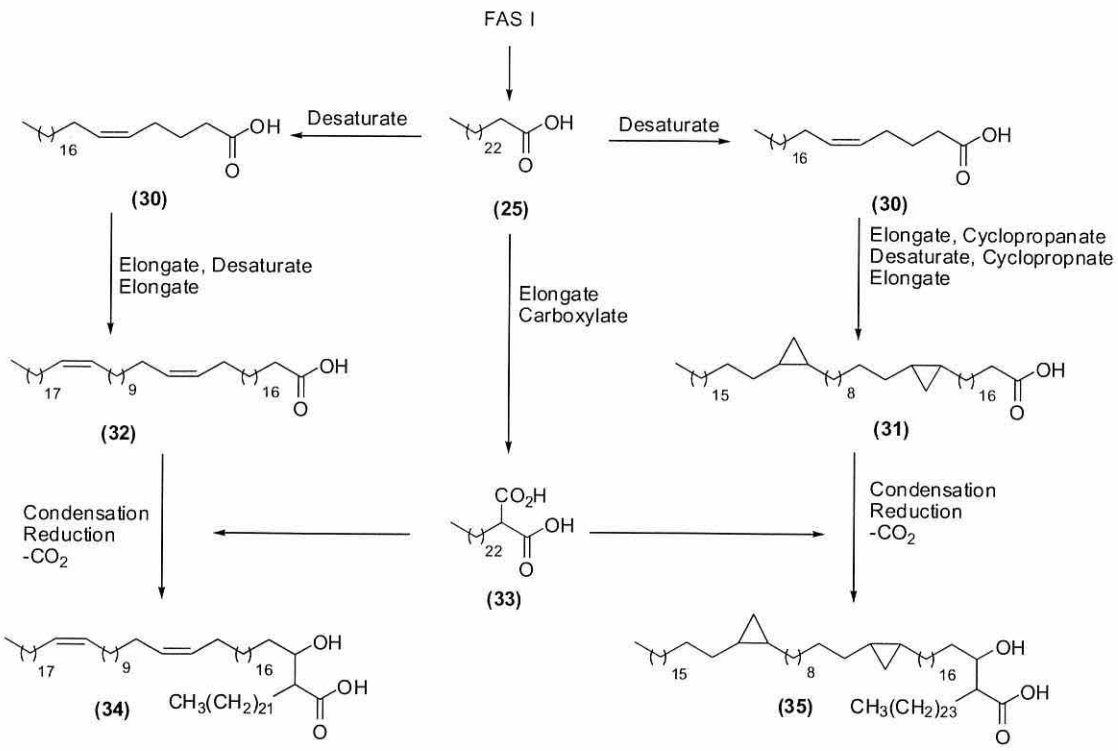
The product from FAS I compound **(25)** undergoes further elongation in another biosynthesis called FAS II. The enzymes involved in this process were a target for many drugs used for the treatment of tuberculosis, *e.g.* isoniazid, INH, thiolactomycin, and thiourea isoxyl, as these drugs work as inhibitors for them.[64-66] FAS II is used for the synthesis of long chain, up to 50 carbon units, in acyl carrier proteins (ACP). The enzymes in this system are able to recognise the mycolic acids as a substrate if they link to ACP.[67] Scheme (**1-3**) shows the steps of FAS II.[68]



Scheme (1-3): FAS II; i) β-ketoacyl-ACP synthase (KasA/B), ii) β-ketoacyl- ACP reductase, iii) β-ketoacyl-ACP dehydratase, iv) enoyl-ACP reductase.[68]

1.9.3 The condensation

The final step of the biosynthesis of mycolic acids is the condensation, which most researchers believe to be a Claisen-type condensation between the meromycolate (**31**) and (**32**) and alkyl dicarboxylic acid (**33**) units (Scheme (**1-4**)).[69] This type of condensation has been proved for short chain fatty acids.[70,47] Tracking of putative meromycolate in *M. tuberculosis* cultures was investigated in order to prove this route of biosynthesis.[71] A meromycolate chain was isolated from mutant *M. smegmatis* and gave evidence to support this theory.[72] Moreover a condensation of two fatty acids in order to form a mycolic acid was discovered, which strongly supported this theory and makes it a target for the development of new drugs for the treatment of any infection caused by mycobacteria.[73]

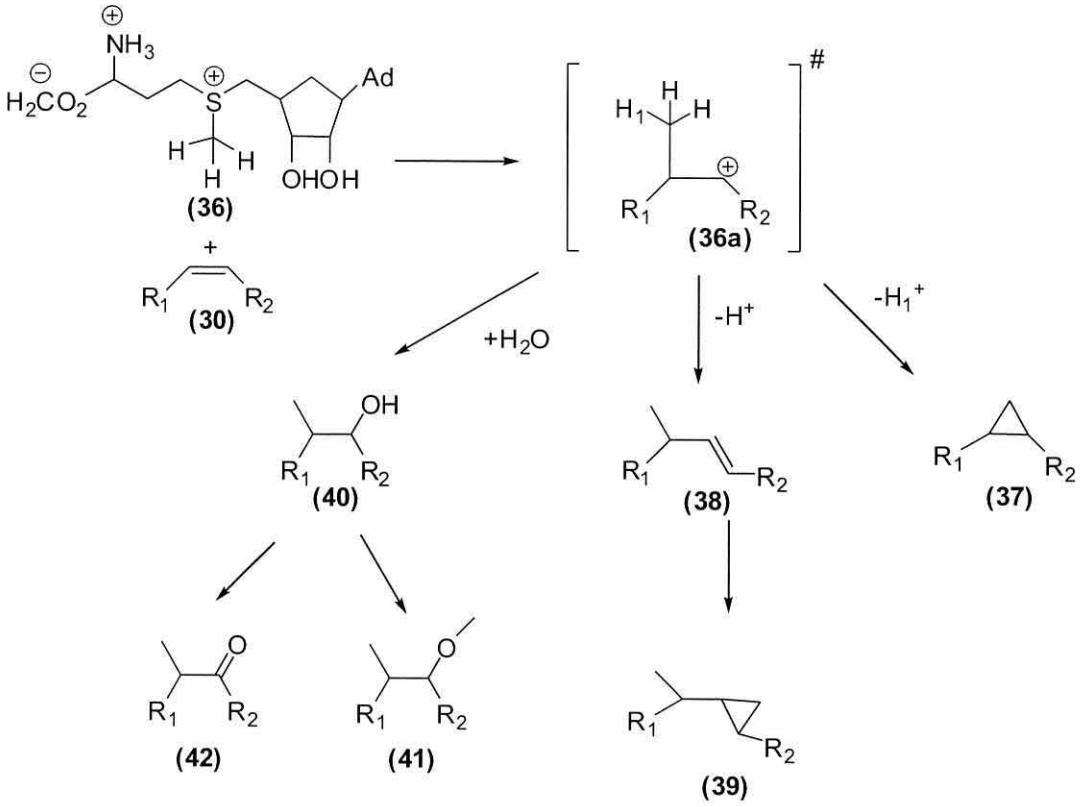


Scheme (1-4): The proposed pathway for biosynthesis of mycolic acid[69]

1.10 Functional group biosynthesis

The first step in the synthesis of functional groups in the meromycolic is the formation of a double bond, compound (**30**) (Scheme (**1-4**)). The desaturation process of compound (**25**) is carried out by Δ^5-desaturase in order to form *cis*-tetracos-5-enoic acid (**30**).[74] It was reported that the inhibition of this step inhibits the whole mycolic acid biosynthesis.[75] This gave strong evidence that the meromycolate unit is obtained through the desaturation step. Much is known about the hypotheses of functional groups in meromycolic acid and the enzymes involved in this process.[32, 76] In order to detect the methyl branch in mycolic acids, bacteria were grown in labelled media (^{14}C-methionine or ^{3}H- methionine), which indicated that the methyl group of methionine becomes the bridging methylene in the cyclopropane ring, the methyl branch next to the *trans* olefins, the carbon of the methoxy and keto moieties, all derivived from methionine via *S*-adenosyl-L-methionine (**SAM, 36**) (Scheme (**1-5**)). Barry *et al.*[38] reported that the intermediate cation (**36**) formed after adding the methyl group from (**SAM, 36**). This was followed by rearrangement of the intermediate (**36a**) by losing proton and led to *cis*-cyclopropane (**37**) or *trans*-olefin (**38**) which in a further step forms *trans* cyclopropane (**39**). The intermediate (**36**) adds water leading to hydroxyl

compound (**40**) and this then leads to methoxy (**41**) or keto (**42**) compounds.[32, 38, 77-79] The methyl branch in the oxygenated mycolic acid is believed to be in the (*S*) configuration, also the methyl branches in keto or methoxy mycolic acids are in the (*S*) configuration.[80]



Scheme (1-5): Formation of functional groups in the meromycolate.[38, 80]

1.11 The genes responsible for the biosynthesis of mycolic acids

The general structure of natural α-mycolic acids, which contain double bonds or cyclopropane rings in both the proximal and distal positions, as well as keto and methoxy mycolic acids has been the subject of numerous studies. Glickman *et al.*[81] demonstrated that the gene responsible for the *cis*-cyclopropanation is pcaA (**43**) and (**44**) and for the *trans*-cyclopropanation is cmaA2 (**46**) and (**48**), while the gene involved if the methyl branch is adjacent to the cyclopropane ring is mmaA1 (**46**) and (**48**) and if it is adjacent to the oxgygenated carbon the gene is mmaA4 (**45**), (**46**), (**47**) and (**48**) (Figure (**1-10**)).[77, 81-83]

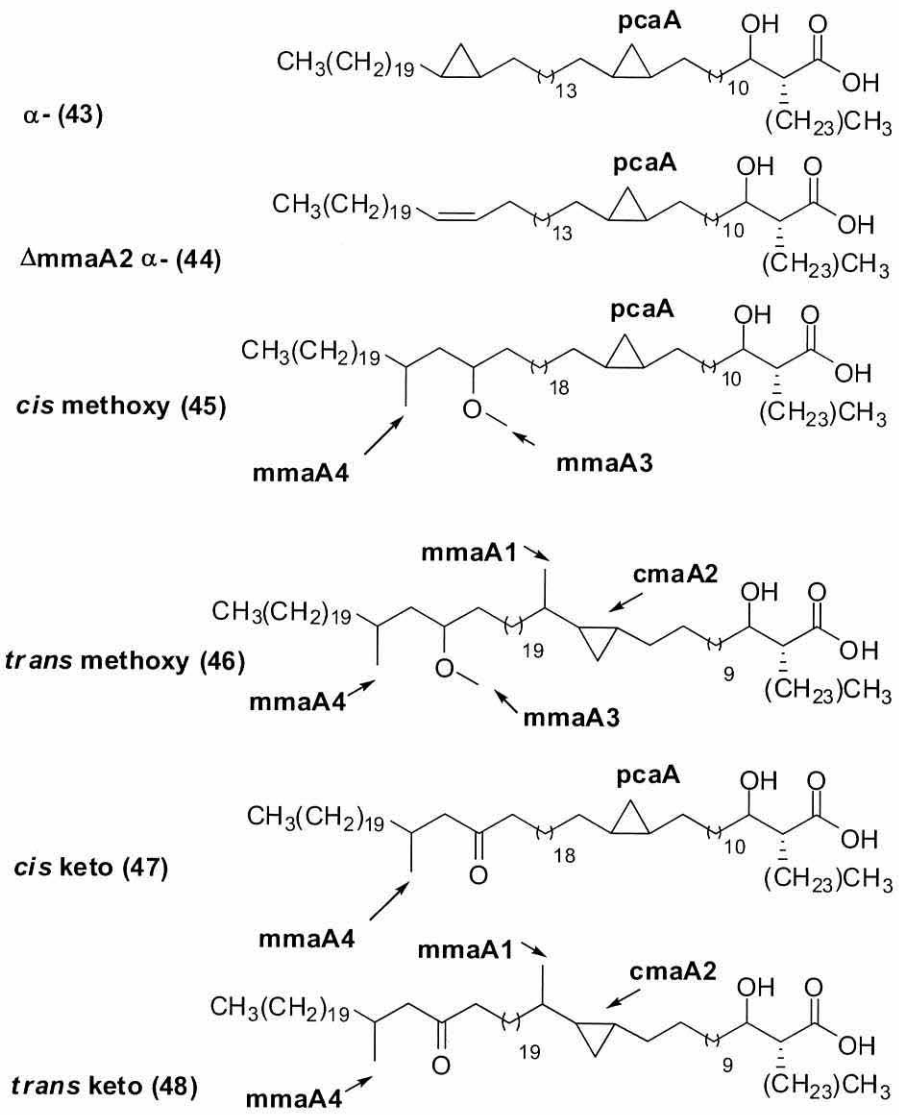


Figure (1-10): Genes involved in mycolic acid biosynthesis.[56, 80-82]

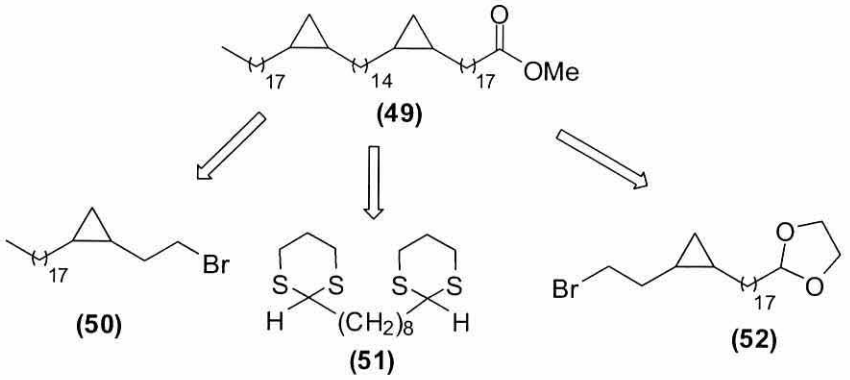
1.12 Synthesis of mycolic acids

In order to further understand the role mycolic acids play in the cell envelope and to study the stereochemistry of mycolic acids in the meromycolic moiety, synthetic mycolic acids need to be prepared, since the natural mycolic acids are only obtained are extremely hard to separate into pure single isomers Section (**1-6**).

Identifying the specific stereochemistry of the meromycolic moiety has attracted a lot of attention (Scheme (**1-5**)) shows that the functional groups are formed through the same intermediate **(36)** in the biosynthesis. The synthesis of mycolic acids allows new scope to compare the natural and synthetic mycolic acids.

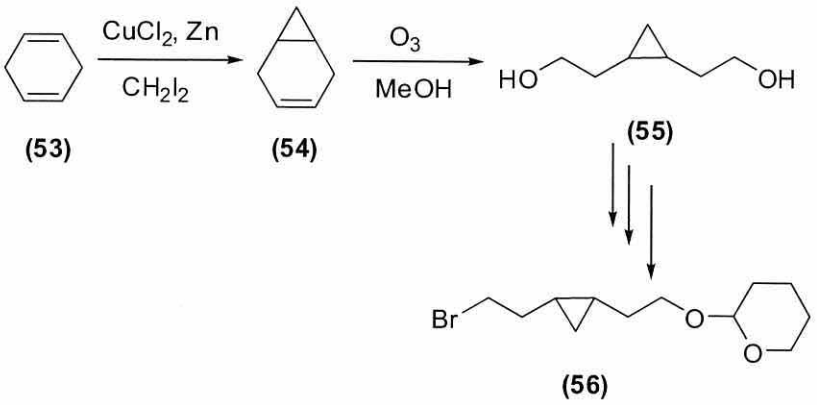
1.12.1 Previous synthesis

The major problem with the synthesis of mycolic acids is the insertion of the chiral centres in the correct stereochemistry and preventing them from changing during subsequent reactions. Gensler *et al.*[84] synthesised an α-meromycolic acid (**49**) which contained two *cis*-cyclopropane rings. Their strategy was to form the cyclopropanes in *cis*-configuration and then link the parts together using 1,3-dithianes (Scheme **(1-6)**).



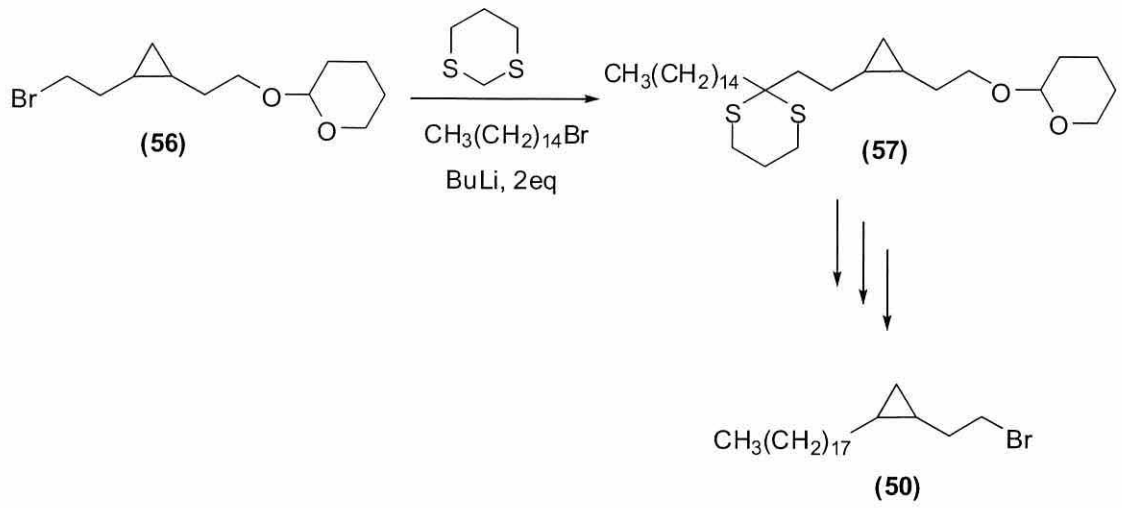
Scheme (1-6): The first synthesis of meromycolic acid (49).[84]

In order to obtain the *cis*-cyclopropane, a cyclization reaction between with 1,4-cyclohexadiene and diiodomethane in presence of cuprous chloride and zinc in dry ether was carried out. The product, compound **(54),** was oxidatively cleaved to give diol **(55)** and after a few steps to differentiate between the two hydroxyl groups, 2-((2-(bromo-methyl)cyclo-propyl)methoxy)tetrahydro-2H-pyran **(56)** was obtained (Scheme (**1-7**)).



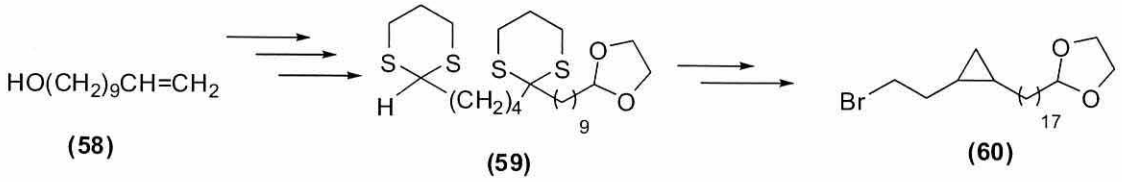
Scheme (1-7): Preparation of cyclopropanes with different functional groups[84]

The next step was the extension of the cyclopropane ring side chain using dithianes as the coupling reagent, followed by a few steps resulting in the replacement of the tetrahydro-pyranyloxy group with bromine to give compound (**58**) (Scheme (**1-8**)).



Scheme (1-8): Chain extension chain to give (50).[84]

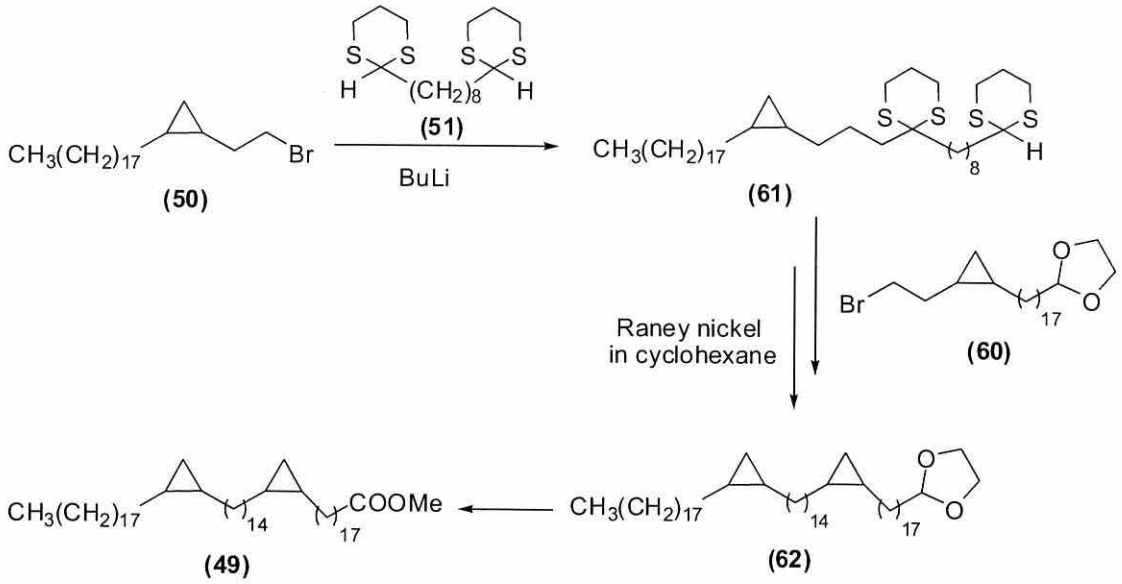
Once compound (**50**) had been prepared, synthesis of the second part was begun, starting with undec-10-en-1-ol (**58**) which was protected and coupled in order to extend the chain length. This was followed by a coupling reaction with compound (**56**) to obtain a cyclopropane ring with bifunctional groups and the right number of carbon atoms for the next stage of the synthesis (Scheme (**1-9**)).



Scheme (1-9): Preparation of cyclopropane (60)[85]

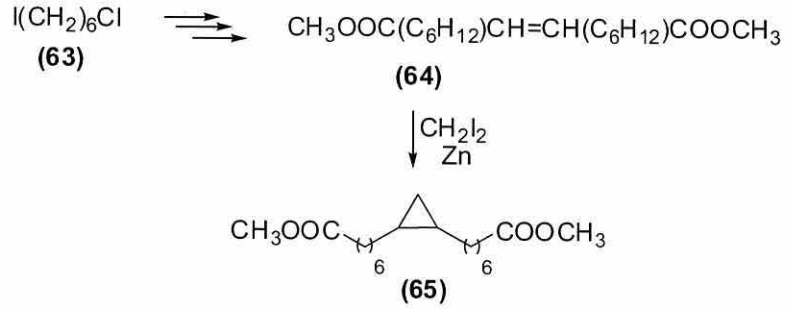
Before compound **(50)** could be coupled with compound **(60),** it was necessary to extend the side chain by coupling it with compound **(51),** forming compound (**61**), which when coupled with (**60**) resulted in a chain length matching that of the natural meromycolic acid. Desulfurization with Raney nickel resulted in the protected meromycolic acid (**62**) (Scheme (**1-10**)). The final step was deprotection of the acetal

and oxidation using ozone to give the meromycolic acid (**49**) as four different stereoisomers.



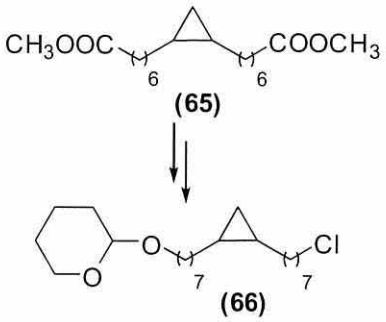
Scheme (1-10): Preparation of the meromycolate (49)[84]

Another approach to the synthesis of meromycolic ester **(49)** was demonstrated by the same authors.[85] This method has fewer steps and is also more reliable to scale up (Scheme (**1-11**)). The starting material was 1-chloro-6-iodohexane (**63**) which, after several steps was transformed into alkene **(64)** with two functional groups on either side of the double bond. This alkene was then used as the starting material for cyclization with diiodo-methane and zinc, resulting in compound (**65**) which has a cyclopropane ring in a *cis*-configuration.



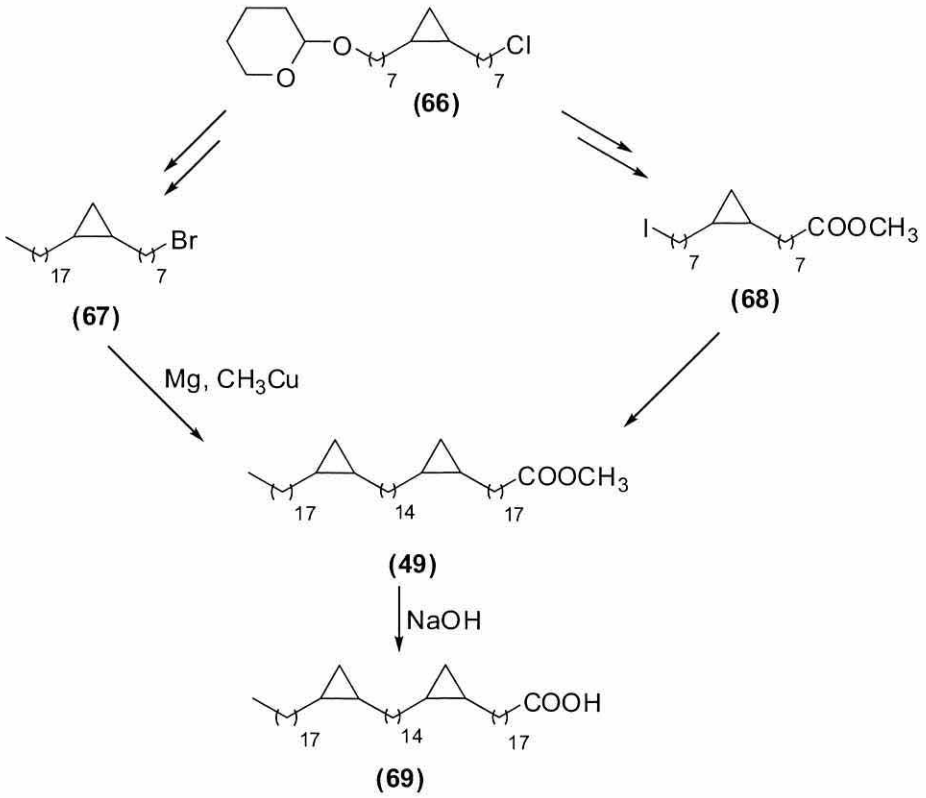
Scheme (1-11): The initial steps in the second Gensler *et al.* approach[85]

The next step was reduction of the functional groups, followed by a few steps to differentiate between the resulting alcohols to give the compound (**66**) (Scheme (**1-12**)).



Scheme (1-12): Replacing the functional groups[85]

After the unit (**66**) had been obtained, a Grignard reaction was used as the key step for extending the alkyl chain to obtain (**67**) and (**68**). These two fragments were then joined to obtain meromycolate (**49**), which was subjected to hydrolysis to give meromycolic acid (**69**) (Scheme (**1-13**)).

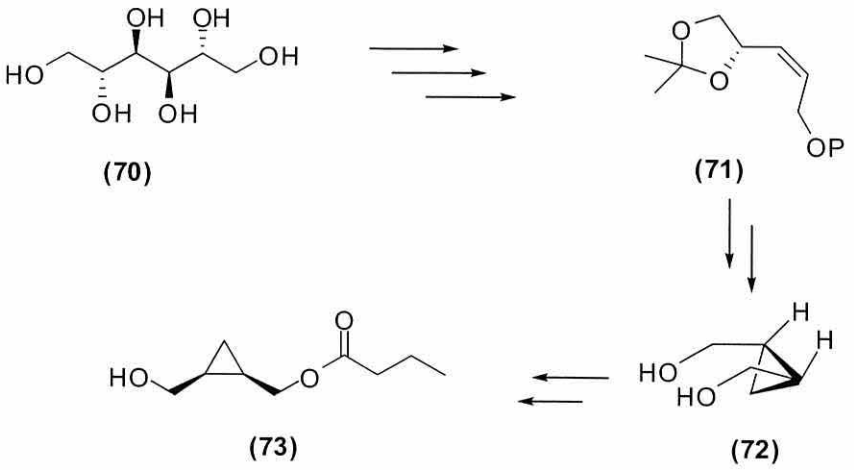


Scheme (1-13): Preparation of a meromycolic acid[85]

These two methods provided the first synthesis of meromycolic acid but there are still some problems with both of them. The last Grignard coupling in the second method gave a very poor yield and a lot of by product was obtained. The stereochemistry of the product is not defined either, since both of these methods produced four isomers of (**69**).

1.12.2 Synthesis a single enantiomer

Al Dulayymi *et al.*[86] reported the first synthesis of a single enantiomer of meromycolic acid (**69**). This synthesis had two key steps: the first was the preparation of single enantiomers of cyclopropanes and the second step was the coupling of these units. D-mannitol (**70**) was used to prepare single enantiomers of cyclopropane. After several steps this gave the *cis*-alkene (**71**). Cyclopropanation of this alkene led to the *cis*-cyclopropane (**72**). In order to obtain a pure enantiomer of cyclopropane with two different functional groups (**73**), a pig liver esterase was employed for enzyme hydrolysis as described by Grandjean *et al.* (Scheme (**1-14**)).[86-88]

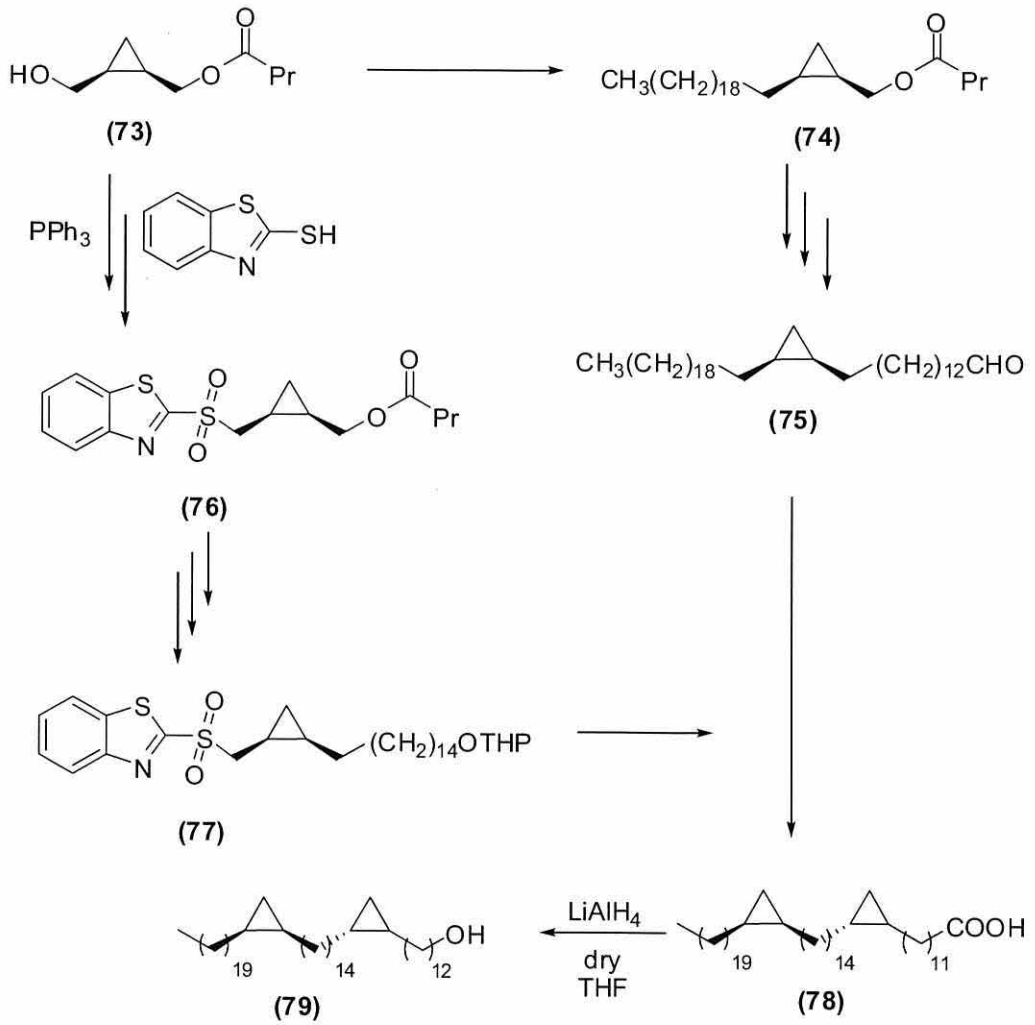


P= protecting group

Scheme (1-14): Preparation of a *cis*-cyclopropane intermediate[87-89]

The next steps were the modification of the cyclopropane rings for coupling to each other, by extending the side chains so that the correct carbon chain length would be achieved on joining the two parts. The cyclopropane ring with one protected hydroxyl (**73**) was oxidised to an aldehyde and coupled with a C-20 chain using a modified Julia reaction in order to make the terminal chain. The other ring was coupled from both sides and then was transformed into sulfone (**77**). The final step was the

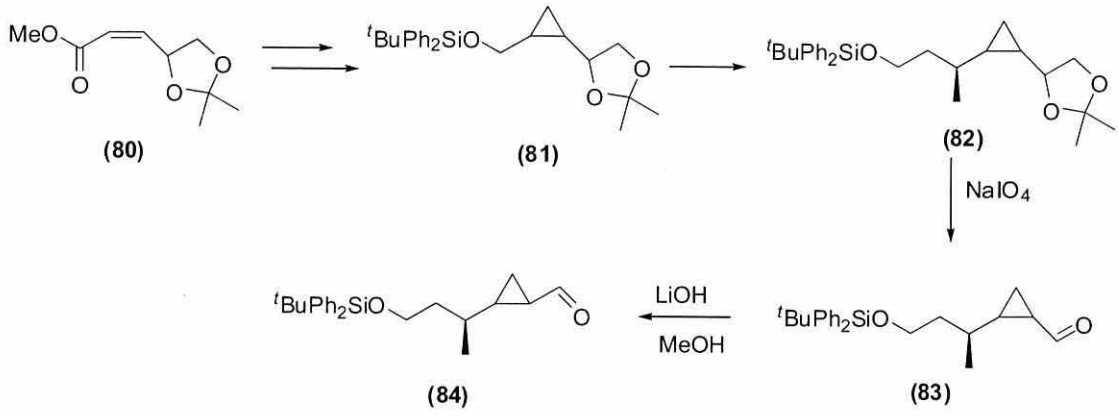
coupling of the two parts with each other, followed by hydrogenation to give the protected meromycolate, which was deprotected to give alcohol (**78**), and then oxidised to obtain meromycolic acid (**79**) (Scheme (**1-15**)).[90] There are many advantages to this method: the biggest advantage is the retention of the stereochemistry of the chiral centres through the synthesis; also, the overall yield using a Julia coupling reaction for joining the prepared parts (**73**), (**74**), (**75**), (**76**), and (**77**) gives is better. This led to this method being adopted in this research for the synthesis of complete mycolic acids.[90, 91]



Scheme (1-15): Synthesis of a pure enantiomer of meromycolic acid (Al Dulayymi *et al.*)[90]

In another improvement to this method, the cyclopropane ring was prepared with *trans* stereochemistry with an adjacent methyl branch to give compound (**82**) (Scheme (**1-16**)). A Wittig reaction was used to prepare alkene (**80**) which was then cyclopro-

panated using a Simmons-Smith reaction to give compound (**81**). After several more steps the *cis*-cyclopropane with a methyl branch (**83**) was epimerized into the *trans*-cyclopropane compound (**84**) by refluxing with base.[92]



Scheme (1-16): Preparation of *trans*-cyclopropanes.[93]

The preparation of meromycolic acid (**79**) and the ability to prepare *cis* and *trans* cyclopropane rings allowed the synthesis of meromycolic acid parts with different stereochemistries in recent studies (Figure (**1-11**)).[58]

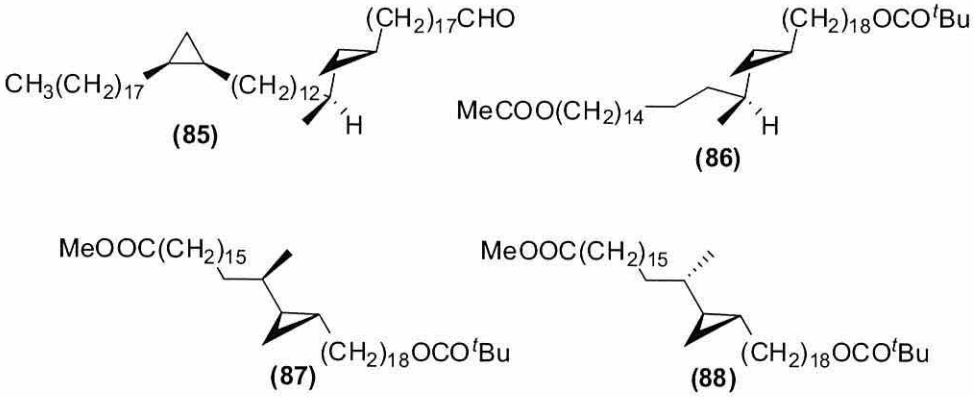


Figure (1-11): Meromycolates with different stereochemistry.[58, 94]

1.13 The progress in synthesis of a complete mycolic acid

The successful synthesis of meroycolate (**85**), (**86**), (**87**) and (**88**) led the same group to the synthesis of complete mycolic acids with different stereochemistries in order to compare them with natural mycolic acids. After solving the problem of synthesizing cyclopropanes with different stereochemistries and extending the chains between

groups using several methods, the problem of synthesizing the β-hydroxy-α-alkyl ester in an *R,R* configuration could be solved by several different methods.

1.14 Synthesis of the α-alkyl-β-hydroxy ester

1.14.1 Overview

The preparation of (2*R*,3*R*)-α-alkyl-β-hydroxy esters, in which the two chiral centres are opposite to each other, is critical for the synthesis of mycolic acids. Since this structure is common to every mycolic acid, it was the initial step in each synthesis. The variation in this 'mycolic motif' was the number of carbon atoms in the α-alkyl chain (Figure (**1-12**)).

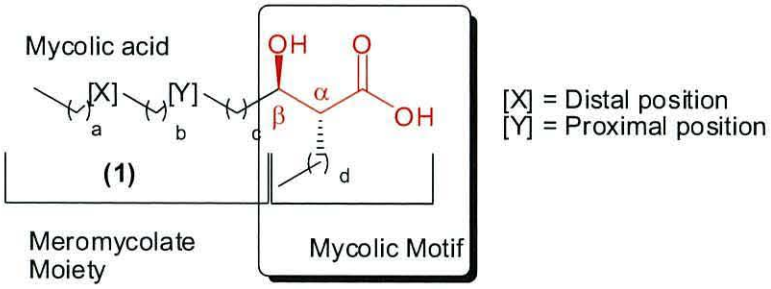


Figure (1-12): Motif side of mycolic acid

There are many methods reported for the preparation of (2*R*,3*R*)-α-alkyl-β-hydroxy esters (Figure (**1-13**)), with different starting materials and different catalysts. The first step is normally to prepare β-hydroxy esters (**89**) with the right stereochemistry. This is followed by insertion of the alkyl chain.

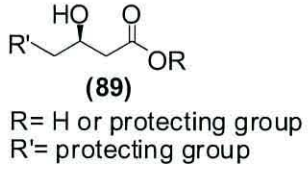
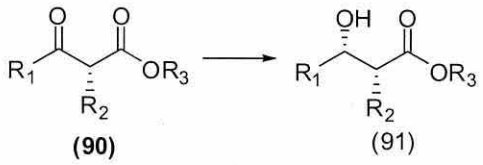


Figure (1-13): Structure of β-hydroxy esters

1.14.2 Enzyme catalysed reduction of β-ketoesters

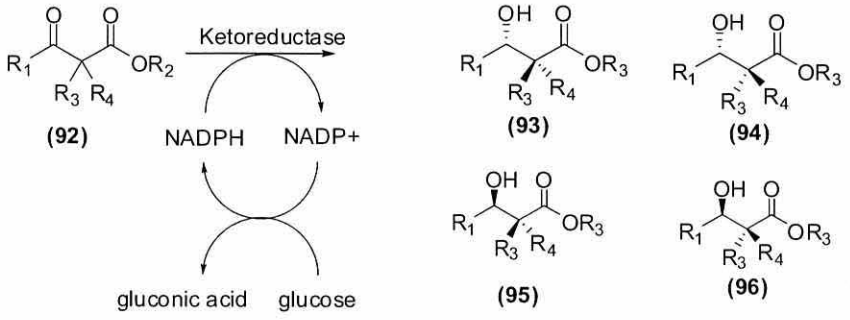
The advantage of this method is that it is cheap, since the starting materials were the β-ketoester (**90**) and several different enzymes, all of which are relatively inexpensive. Rodrı´guez *et al.* used *Escherichia coli* expressing enzymes from Baker's yeast in an

unsuccessful attempt at obtaining exclusively (*2R*,3*R*) configuration of the α-alkyl-β-hydroxy ester since they obtained *syn* configuration, (**91**) (Scheme (**1-17**)).[95]



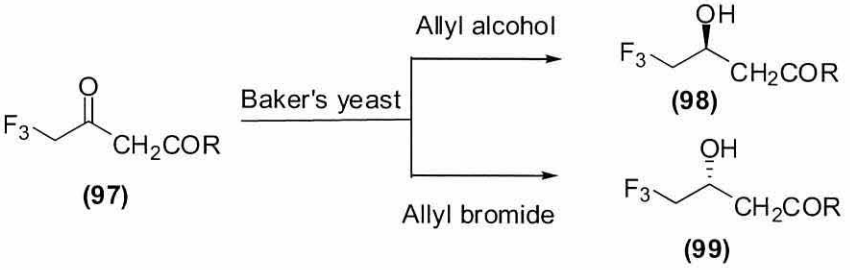
Scheme (1-17): A *syn* configuration of α-alkyl-β-hydroxy ester[95]

Another strategy which has been tested is the use of isolated NADPH as a catalyst for converting β-ketoester (**92**) into β-hydroxy ester. This method, however, produced a mixture of stereoisomers (**93**), (**94**), (**95**) and (**96**) (Scheme (**1-18**)).[96, 97]



Scheme (1-18): Reduction reaction using NADPH[96]

Other attempts at reducing the keto ester (**97**) involve using additives with the reaction mixture in order to control the stereochemistry of the product (**99**) and (**99**) (Scheme (**1-19**)).[98]

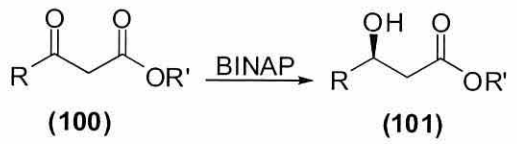


Scheme (1-19): The use of different additives[98]

1.14.3 Non-enzymatic methods for preparing β-hydroxy ester

1.14.3.1 The use of BINAP in preparing the β- hydroxy ester

A promising method is the use of a ruthenium-biarylbisphosphine catalyst (BINAP) to reduce β-keto esters (**100**) to β-hydroxy esters (**101**) or to reduce the α-alkyl-β-keto ester to an α-alkyl-β-hydroxyl ester with the right stereochemistry (Scheme (**1-20**)).[99] This showed the opportunity to obtain this type of compound with different stereochemistries, in a few steps.



Scheme (1-20): Preparation of β-hydroxy ester (102)[99]

1.14.3.2 BINAP complex overview

Transition metals have been used extensively as catalysts in organic synthesis. Knowles and Horner separately reported using this asymmetric catalyst for reduction of di-keto compounds.[100, 101]

The complex Ru-BINAP (**102**) (Figure (**1-14**)) when used as a catalyst for hydrogenation reactions gives a product with asymmetric induction. The stereochemistry depends on the complex used in the reaction and on the substrate used, with the (*R*)-BINAP (**103**) giving (*R*)-β-hydroxy ester (**105**) and (*S*)-BINAP (**103**) giving (*S*)-β-hydroxy ester (**109**) (Scheme (**1-21**)).[102-106]

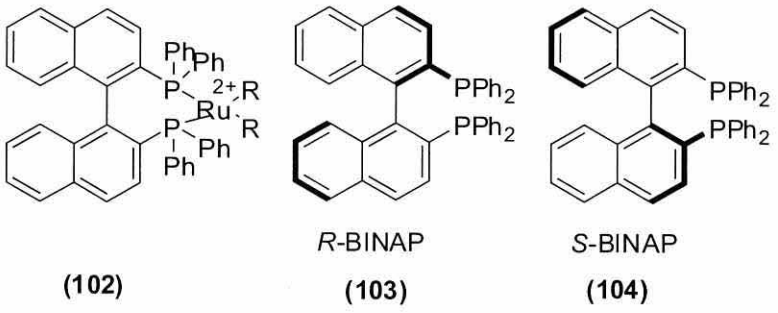
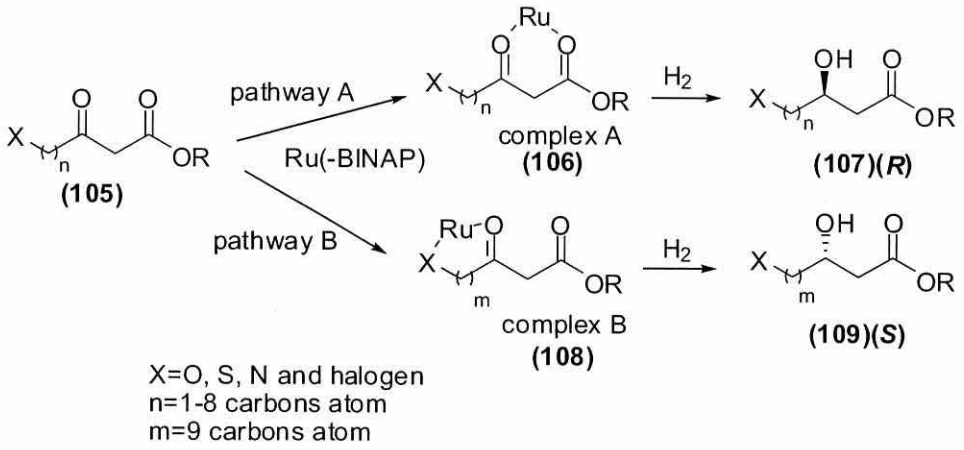


Figure (1-14): The stereochemistry of BINAP complexes

1.14.3.3 Reduction of a β-diketo ester

In reducing a ketoester, it has been reported that the structure of the compound affects the stereochemistry of the product (**107**) and (**109**).[99] If the alkyl chain in the C-4

positions is greater than nine carbons long, the enantioselectivity will decrease.[99] 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 (**1-21**)) clearly shows the same catalyst can gave different products, depending on the groups on the side functional substrate.[107] The formation of complex A, which is a coordination of the ruthenium of Ru-BINAP to the two carbonyls in the ester, hampers the formation of complex B, which is the pathway which leads to *S* isomers.[107, 108]



Scheme (1-21): Effect of different groups on the sterochemsistry of the product[99]

If the ruthenium in the BINAP complex forms a five-(**110**), six (**111**) or seven-(**112**) membered chelated ring, (Figure (**1-15**)), this leads to pathway B, which gives *S* isomers. The X in the complexes (**110**), (**111**) and (**112**) could be either oxygen, sulfur, nitrogen or a halogen.

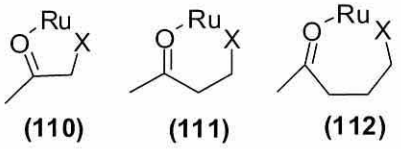
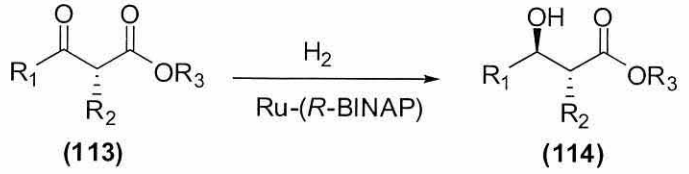


Figure (1-15): Ruthenium complex (pathway B).[107]

There are excellent literature accounts of the reduction of β-diketo esters (**113**) and how to control the stereochemistry of the product using BINAP complexes.[109] Furthermore there is other promising work described by Noyori for obtaining α-alkyl-β-hydroxy ester (**114**) by hydrogenation (Scheme (**1-22**)).[110]

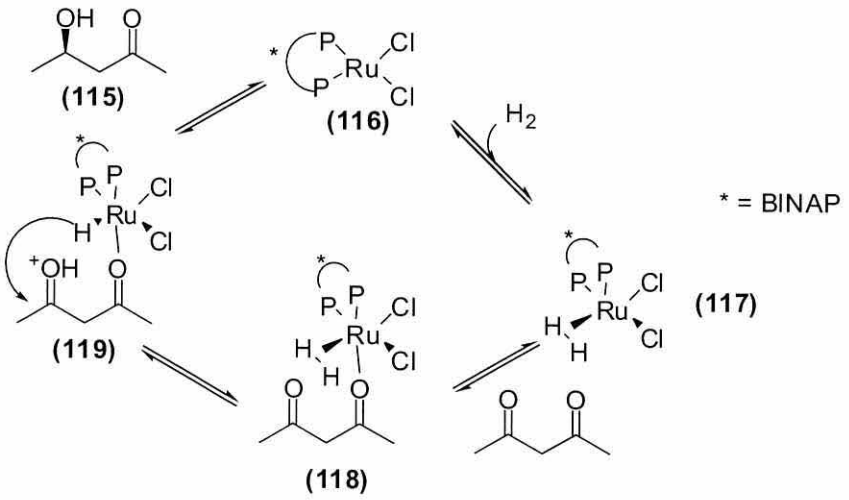


	R_1	R_2	R_3
1	Me	Me	Et
2	Me	NHCOMe	Me
3	Me	NHCOMe	*t*-Bu
4	Me	NHCO*i*-Pr	Me

Scheme (1-22): Preparation α-alkyl-β-hydroxy ester by BINAP.[106]

1.14.3.4 Mechanism of hydrogenation using BINAP as catalyst

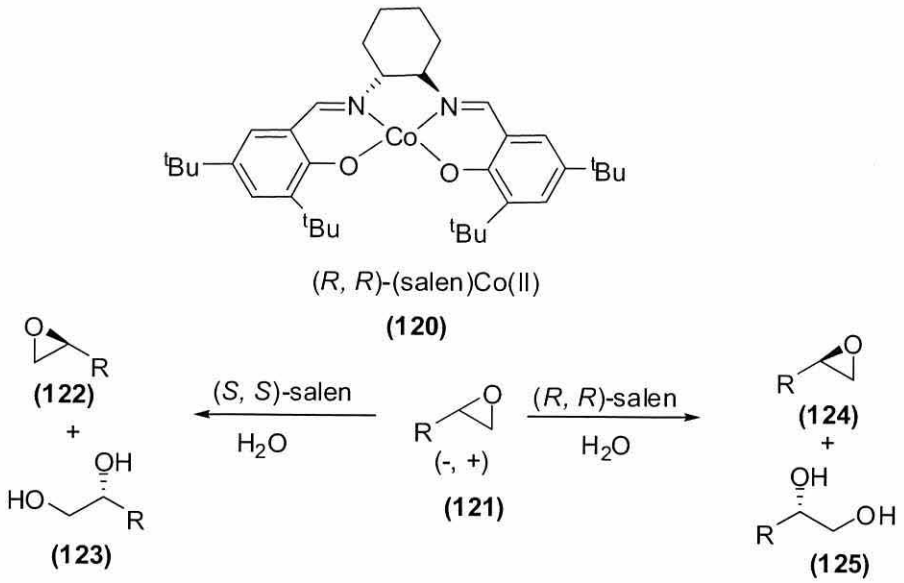
No specific mechanism has been proposed for the reduction of a 1,3-dicarbonyl substance using BINAP (**116**). The hydrogen activates BINAP as (**117**) and causes it to form a complex with ketoester (**118**), which rearranges to give complex (**119**). The metal allows the introduction of hydrogen to the carbonyl group forming 3-hydroxy-1-keto compound (**115**). Scheme (**1-23**) shows a suggested mechanism for this reaction.[111]



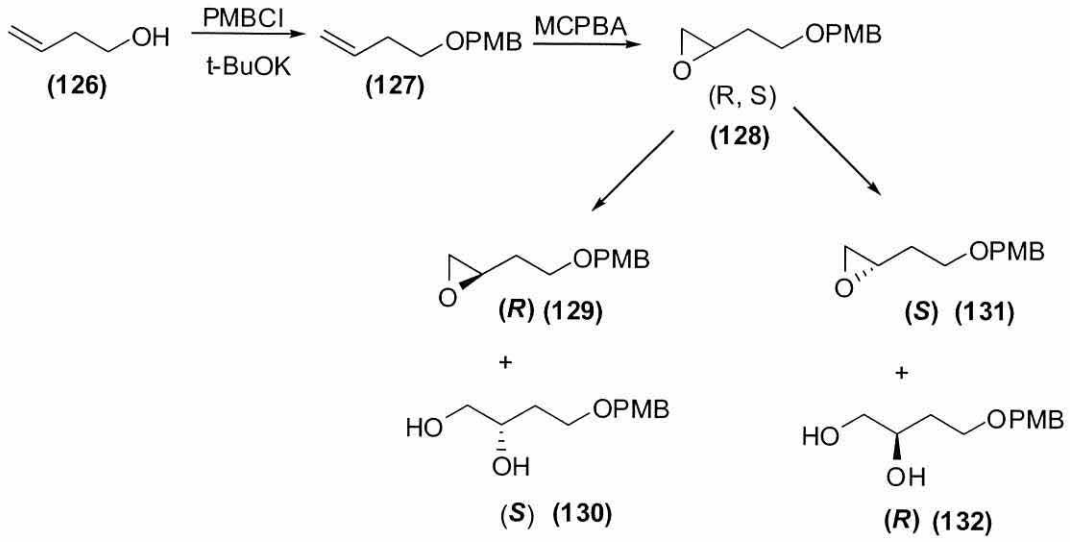
Scheme (1-23): A suggested mechanism for reducing a dicarbonyl compound.[111]

1.14.3.5 Another method for the preparation of β-hydroxy esters

The use of salen.Co(III) complex (**120**) for the production of a terminal epoxide rings with specific stereochemistry was reported recently (Scheme (**1-24**)). [112-114]



Scheme (1-24): The use of salen cobalt complexes[113]

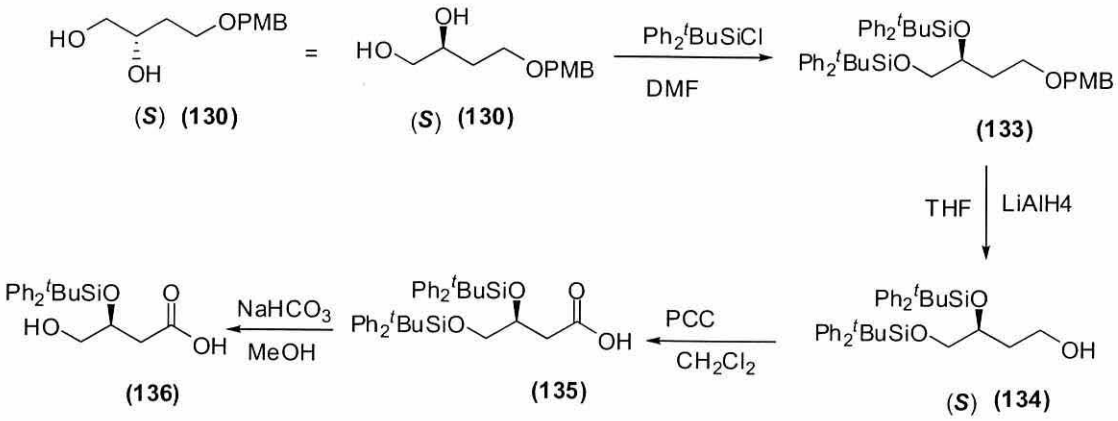


Scheme (1-25): Treatment of the epoxide ring with salen complex[115]

This method was used by Mori and his group for the synthesis of pheromones.[115] They employed the salen complex to obtain the desired compound with the right stereochemistry. Starting from 3-buten-1-ol (**126**) in three steps, they firstly protected the hydroxyl group with *p*-methoxylbenzyl (**127**) and then oxidised the double bond

with MCPBA to afford compound (**128**) as a mixture of *R* and *S* isomers. Finally, this mixture was treated with salen complex to give two different isomers of the same compound (**130**) and (**132**), (Scheme (**1-25**)).

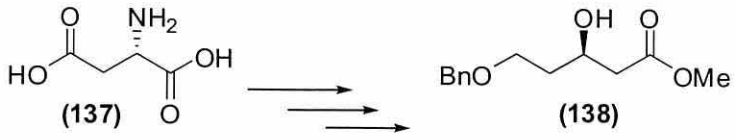
This method can provide the basis of a route to β-hydroxy acids by protecting the hydroxyl groups of (**130**) to with *tert*-butyldiphenylsilyl chloride to give compound (**133**), followed by selective deprotection of the the primary hydroxyl to give (**134**) and oxidation to give the β-hydroxy carboxylic acid (**135**) with the right stereochemistry, this follow by deprotection to the primary hydroxyl group and leave the secondary hydroxyl protected (**136**) (Scheme (**1-26**)).



Scheme (1-26): Preparation of β-hydroxy ester (136)

1.14.3.6 The aspartic acid route

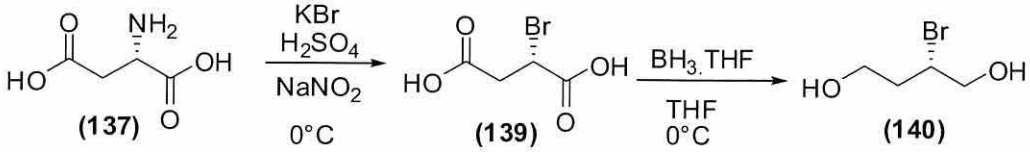
Preparation of the β-hydroxy ester (**137**) with the right stereochemistry starting from *L*.aspartic acid (**137**) is a method that can be easily scaled up. Aspartic acid has a chiral centre which was used to obtain the β-hydroxy ester (**138**) in the *R*-configuration in a few steps; this was the method which was used in this study (see Section (**2-3**)) (Scheme (**1-27**)).



Scheme (1-27): The preparation of β-hydroxy ester (138)

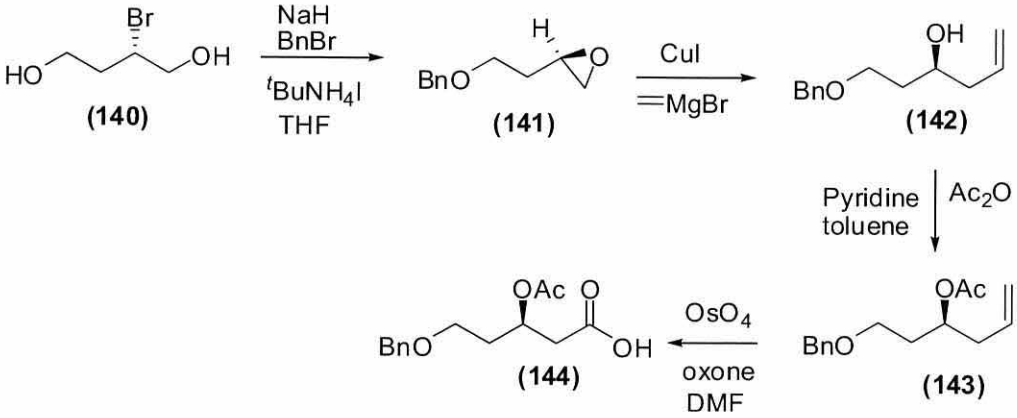
The first step in this route was to replace the amino group in the aspartic acid with a bromine group (**139**) by the use of KBr and $NaNO_2$ in H_2SO_4. This was followed by

reduction of the acid to the diol (**140**) using borane tetrahydrofuran, which is a mild reducing agent (Scheme (**1-28**)). [116]



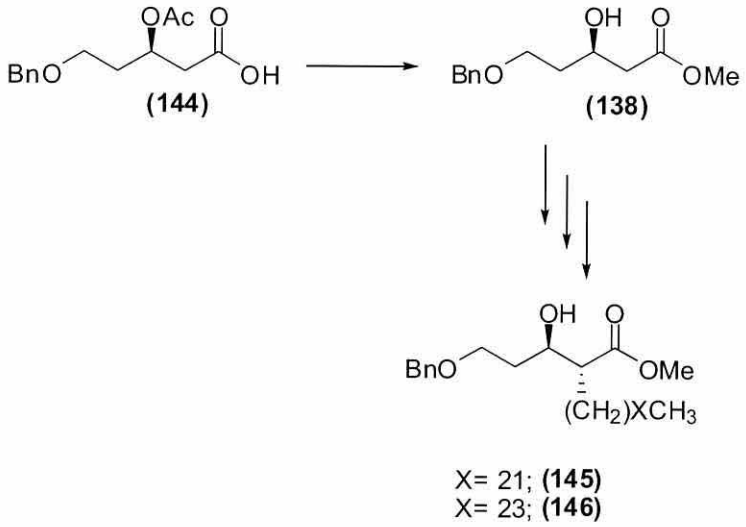
Scheme (1-28): Preparation of diol (140)[116]

The next step was cyclization to form the oxirane (**141**) on one side with simultaneous protection of the other hydroxyl with a benzyl protecting group. This was followed by a Grignard reaction to extend compound (**142**) with two carbon atoms. The next step was protection of the secondary alcohol with an acetyl group using acetic anhydride and pyridine as catalyst in dry toluene to form compound (**143**). This was followed by oxidative cleavage of the alkene (**143**) to form carboxylic acid (**144**) (Scheme (**1-29**)).



Scheme (1-29): Preparation of carboxylic acid (145)[93]

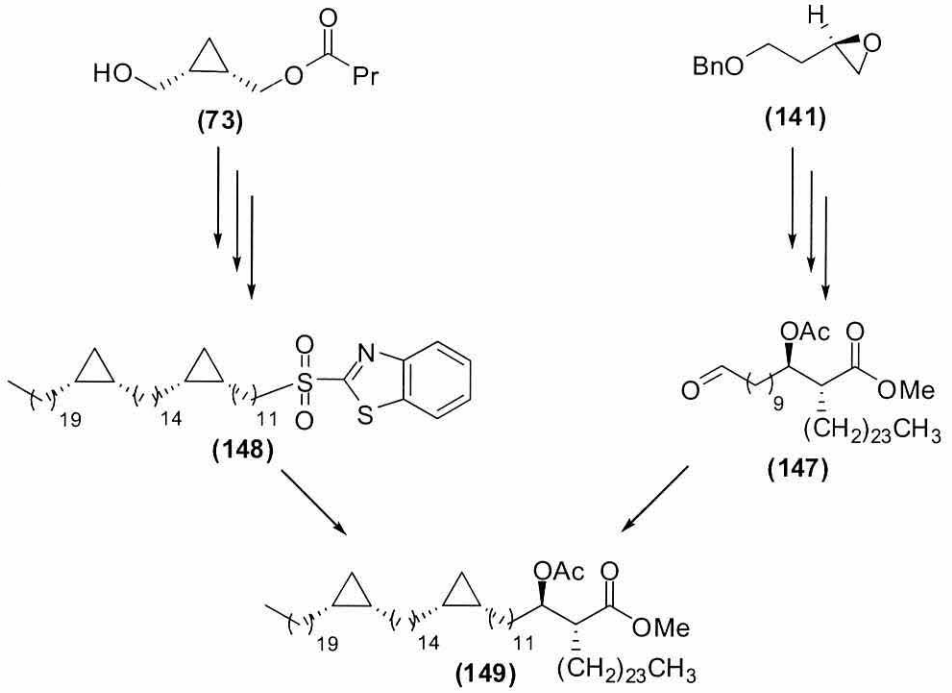
The next step was deprotection of the secondary hydroxyl and protection of the carboxylic acid group in one step by refluxing the acid (**144**) in methanol, which act as both reactant and solvent to form the ester (**138**). The next few steps were the insertion of the alkyl chain in the α-position with respect to the carboxylic acid group. This was done using a Fräter reaction for the insertion of an allyl chain in the α-position (see Ssction **3.6**) to give an alkene which was oxidatively cleaved to give an aldehyde. Coupling of the aldehyde with either a C-20 or C-22 sulfone followed by hydrogenation of the alkene led to the formation of the mycolic motif (**145**) and (**146**) respectively (Scheme (**1-30**)).



Scheme (1-30): Preparation of the mycolic motif with two different chain lengths[93, 117]

1.15 The first synthesis of a complete mycolic acid

The first fragment (**147**) was formed starting with the preparation of an epoxide (**141**) according to method described by Frick *et al.*[116] This was followed by opening of the ring and several other steps to obtain the target protected hydroxy aldehyde (**147**) (see Section **(1-14)**).[91, 116] The meromycolic sulfone (**148**) was prepared in many steps based on the synthesis of the cyclopropane ring in *cis*-stereochemistry (**73**), followed by extension of the chain length and coupling of the parts using Grignard, Julia and Wittig reactions as described in detail (see section **1.12.2**). The target sulfone (**148**) was coupled with aldehyde (**147**) using a modified Julia reaction followed by hydrogenation of the resulting alkenes in order to get the final full mycolic acid (**149**) with the β-hydroxyl and the carboxylic acid group still protected (Scheme **1-31**).[91, 86] Mycolic acid (**149**) with two *cis*-cyclopropane rings was chosen as it is the major mycolic acid of *M. tuberculosis*. A single enantiomer of (**149**) was obtained which had ^{1}H and ^{13}C NMR spectra which were essentially identical to those of a mixture of homologues of (**149**) protected at the hydroxyl and the carboxylic groups in which this mycolic acid was and the major component.[91] Because the synthesis of (**149**) by a similar route is a key part of this thesis, the various steps involved in preparing (**148**) and in coupling it to (**148**) are described in detail in Chapter 5 (Section **5-2**).



Scheme (1-31): Preparation of the protected mycolic acid (149).

1.16 Cord factors

In 1884, during Robert Koch's work on tubercle bacilli, he found it to form what he described as cords or filaments. These cords where found to be composed of mycolic acids esterified to a trehalose sugar forming a glycolipid. The toxic behaviour of these cords in mycobacteria was reported by Bloch[118, 119] when he extracted four different strains with petrol and tested this extract on mice. Cord factor obtained its name since it is responsible for the characteristic 'serpentine cord' appearance in *M. tuberculosis* colonies (Figure **(1-16)**).[120]

This characteristic cording appears to be limited to virulent tubercle bacilli colonies which are arranged in parallel bundles, while avirulent bacilli have a random orientation.[118, 119] It has also been noted that filament forming strains of mycobacteria absorb and fix "neutral red" dyes. This observation led to the hypothesis that some substance in the periphery of the virulent tubercle bacilli cell may be implicated in these two phenomena and also in the virulence of mycobacteria. This hypothesis stimulated the search for the causative substances of these extraordinary occurrences, leading to the discovery of cord factor (6,6'-dimycoloyl trehalose) and mycobacterial sulfolipids (multi acyltrehalose-2-sulfates).[121]

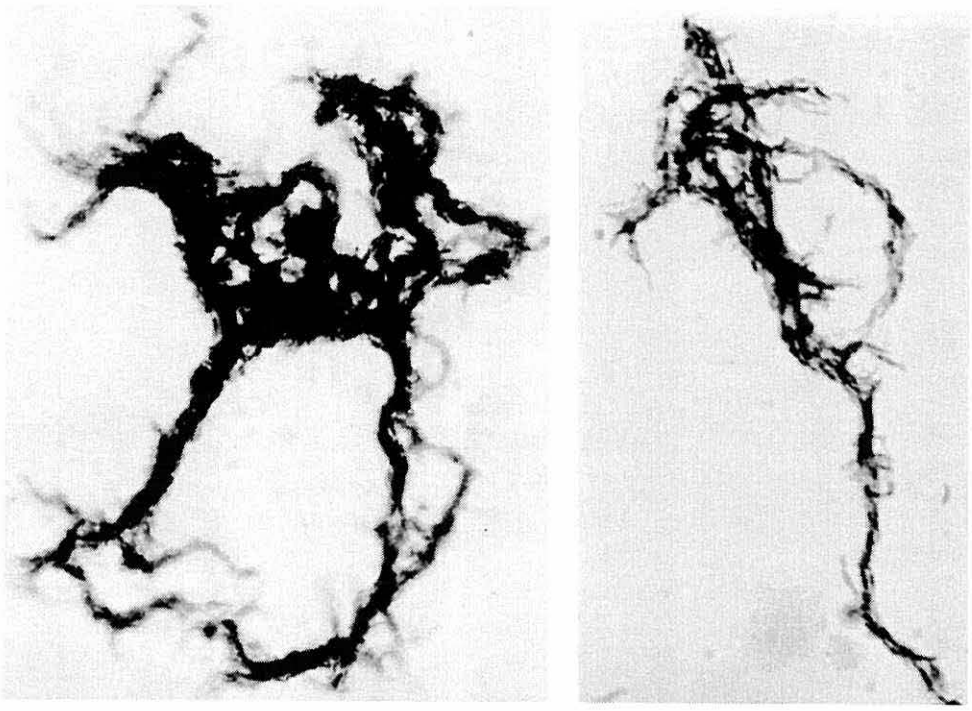


Figure (1-16): Microscopic morphology of *M. tuberculosis* showing different serpentine cording.[120]

At one point it was thought that the sulfolipids were responsible for the dye absorption, but later research has disproved this theory.[122] The structure of cord factor was confirmed by the work of Noll *et al.*[123, 124] Cord factor (**150**) isolated from *M. tuberculosis* was hydrolysed with alkali to give two parts mycolic acid and a non-reducing carbohydrate moiety. Following acid hydrolysis, the carbohydrate moiety yielded D-glucose. For further confirmation of the structure of the sugar it was converted into a crystalline acetate which was identified as α,α-trehalose octa-acetate, which confirmed that the sugar moiety was trehalose. The position of the mycolic acid attached to the trehalose sugar was clarified by methylation of the cord factor followed by saponification, resulting in hexa-methyltrehalose. This was followed by acid hydrolysis to give trimethylglucose (**151**), showing that the mycolic acid is attached to the trehalose sugar at 6,6' positions respectively (Figure (**1-17**)).[123] Other work has confirmed that the general structure of cord factor is an ester of two mycolic acid and a trehalose, since the ratio of mycolic acid to trehalose is 2:1, corresponding to 6,6′-dimycolate.[42, 119, 121, 123-127]

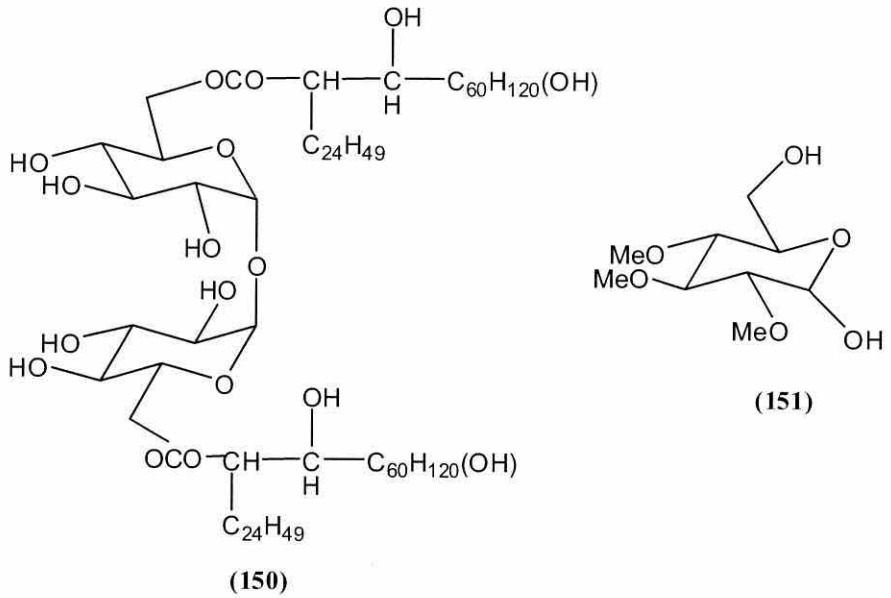


Figure (1-17): Structure of cord factor and protected trehalose proposed by Noll *et al.*[123]

Later elucidation of the structure of cord factor was carried out using different analytical tools to analyse samples extracted from different mycobacteria. All cord factors consist of a mycolic acids bound to trehalose, although the mycolic acid moiety of the cord factor varies greatly, even in the same cell wall.[123] Mass spectrometry is used widely for the determination of the structure of the mycolic acid found in each cord factor and analysis of the fragment pattern of the mass spectrum gives an idea of the degradation of the mycolic acid and what functional groups are contained in the molecule, especially in the mermycolic moiety. All these studies showed that TMM and TDM are among the most characteristic components of the cell wall in mycobacteria.[32, 118, 123] TMM and TDM, along with arabinogalactan and mycoloyl glycolipids or mycolate, having their own long alkyl chain, provide the cell wall of mycobacteria with an extremely hydrophobic surface. Fujita *et al.* used MALDI-TOF mass spectrometry for analysing the cell wall components of a few available types of mycobacteria and the TMM and TDM was confirmed in this way (Figure (**1-18**)).[128, 129]

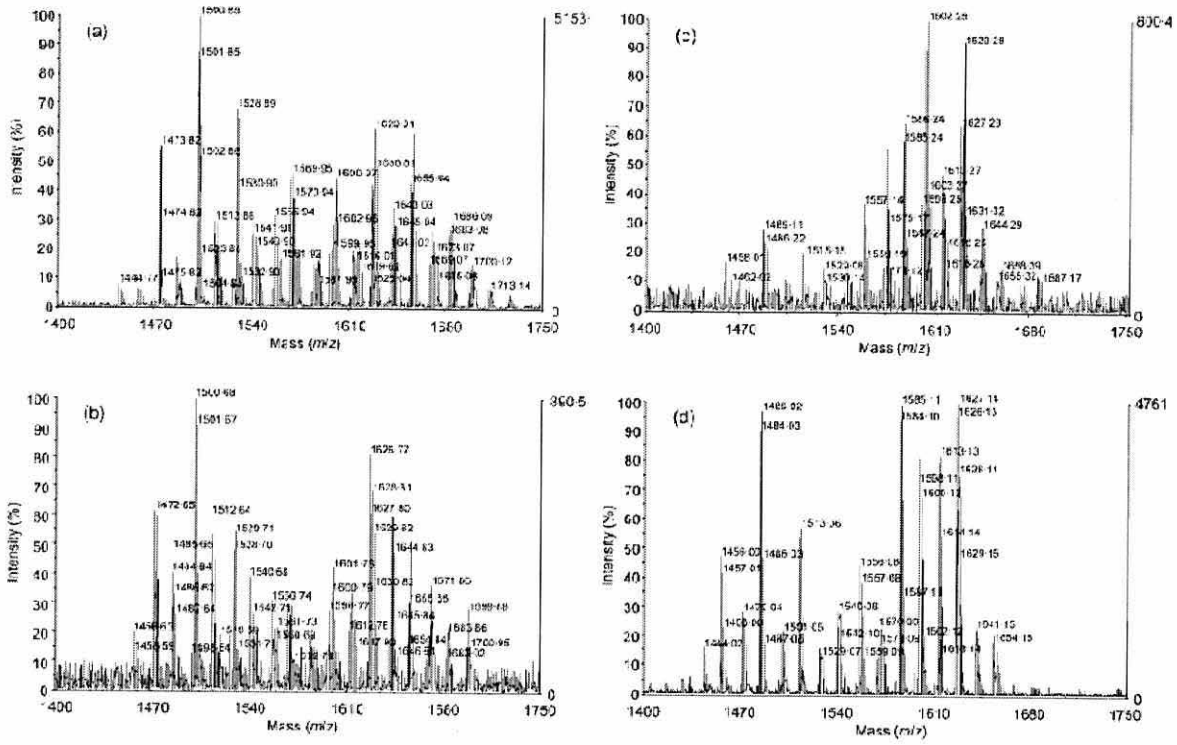


Figure (1-18): MALDI-TOF spectrum of TMM from *M. tuberculosis* (a), *M. tuberculosis* Aoyama B (b), *M. bovis* BCG Tokyo (c) and *M. bovis* BCG Connaught (d).[128]

Cord factor isolated from *Corynebacterium diphtheriae*[37b] was purified and prepared as a trimethylsilyl derivative. This was used for determining the structure of the cell wall compounds using electron-impact mass spectrometry (EIMS). Puzo *et al.* reported three different compounds from this study; the first was called true coryno-cord factor and the other two were 3-oxoacyl containing trehaloses, the difference between which was degree of saturation.[130] Kai *et al.* isolated TDM and TMM from *M. leprae* and *M. bovis* BCG and determined their structure using MALDI-TOF mass spectrometry (Figure (**1-19**)).[131]

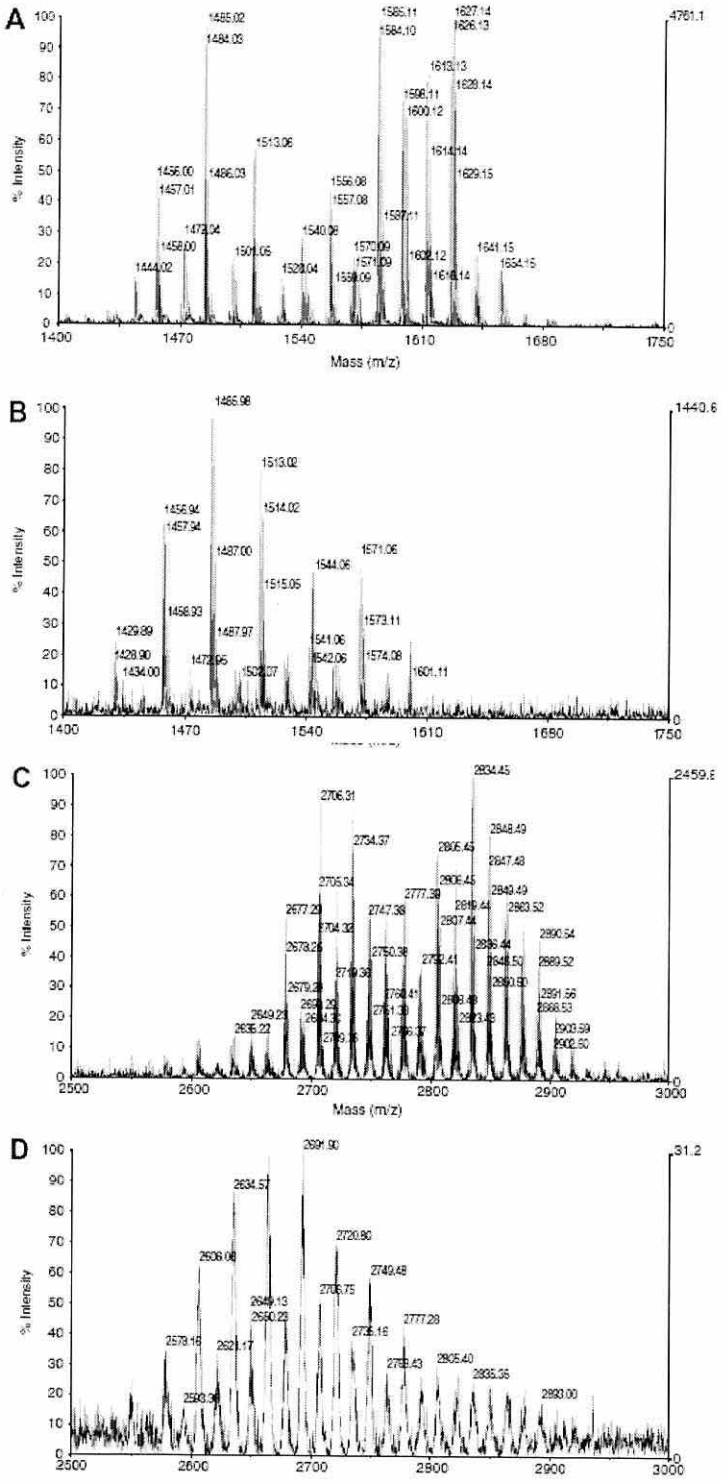


Figure (1-19): MALDI-TOF mass spectrum for TMM and TDM: A) TMM isolate from *M. bovis* BCG Connaught, B) TMM isolate from *M. leprae* Thai 53, C) TDM of isolate from *M. bovis* BCG Connaught, (D) TDM isolate from *M. leprae* Thai 53.[131]

Another effective tool that has been used for identification of cord factors is NMR. An attempt was made by Datta *et al.* [132] following the same procedure used by Puzo *et al.*[130] for the isolation of cord factor from *C*orynebacterium *matruchotii* and protecting it with trimethyl silyl groups. The synthetic cord factor (see Section (**1.23**) below)[132] gave an NMR spectrum very similar to that of the natural cord factor (Figure (**1-20**)).[130, 133]

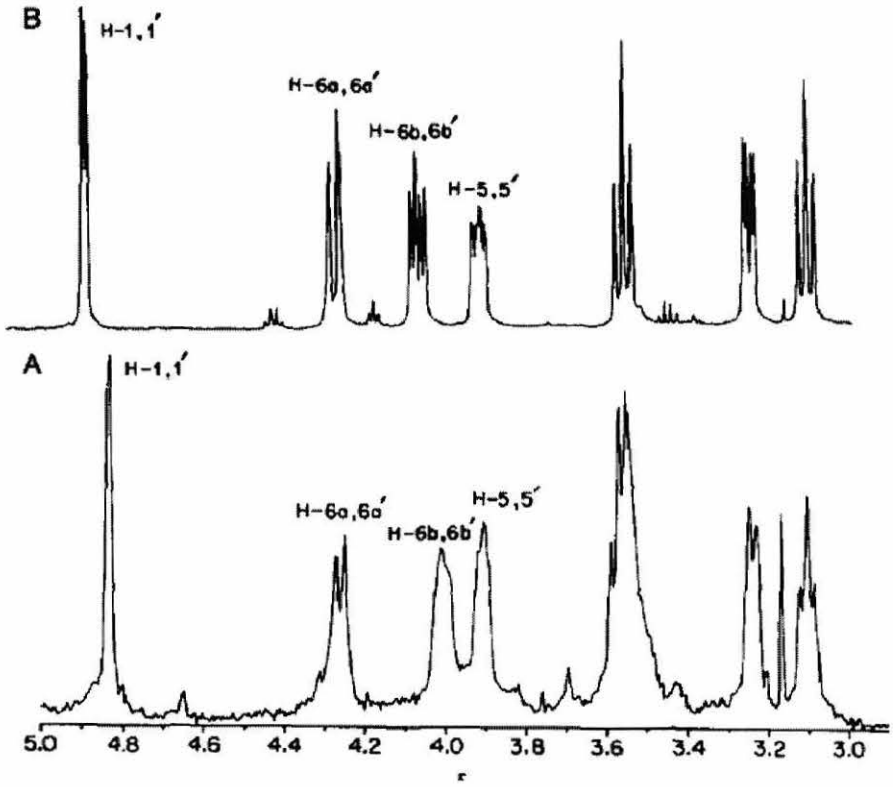


Figure (1-20): A comparison ^{1}H NMR between: A) purified cord factor from C. *matruchotii*, B) synthetic cord factor.[133]

1.17 Biosynthesis of cord factor

Takayama *et al.* proposed a hypothesis for the biosynthesis of the cord factor of the H37Ra strain of *M. tuberculosis* (Figure (**1-21**)).[134, 135] This proposal was based on the discovery of a mycolic acid attached to glycolipid 6-mycolyl-6'-acetyltrehalose (MAT).

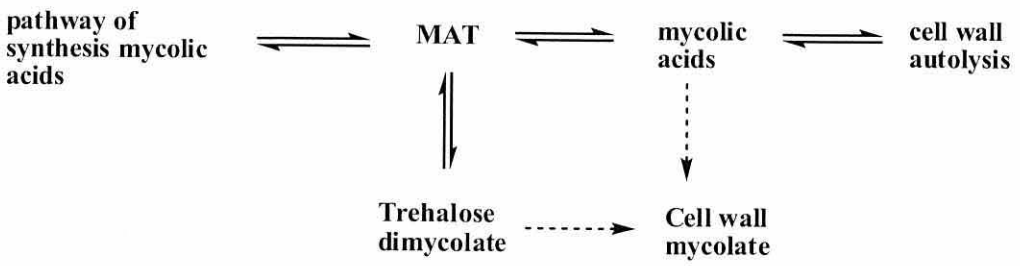


Figure (1-21): Proposed biosynthesis of cord factor [134]

MAT is the major mycolate containing free acid which is responsible for the transfer of the newly synthesised mycolic acids to the cell wall. The use of GC for detecting the substrate of growing H37Ra strain *M. tuberculosis,* led to the detection of MAT and the use of NMR and mass spectrometry led to the identification of the chemical structure of MAT (**152**) (Figure (**1-22**)), confirming that TMM is the initial step in the preparation of TDM.[135]

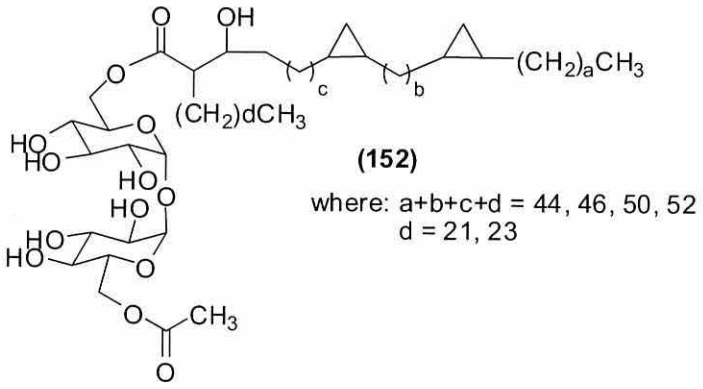


Figure (1-22): The structure of MAT[135]

1.18 Biological effects of cord factor

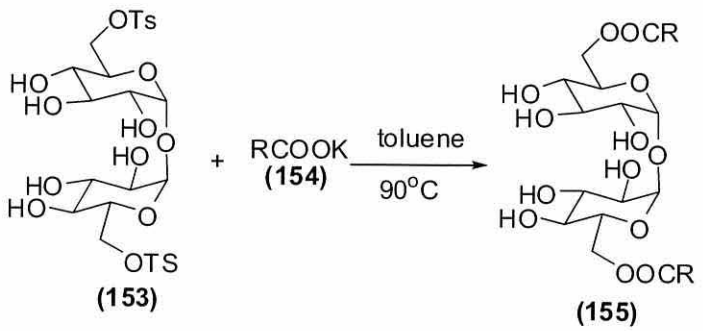
Cord factor causes an increase in antibody responses,[137] and a remarkable increase in DPNase activity in the liver, lungs and spleen when injected into mice.[138] Another study reported that cord factor caused an inhibition of the phosphorylation of NADPH and a loss of respiratory control in mouse liver by affecting mitochondrial membranes.[139] Rastogi *et al* [140] reported that cord factor coated *B. subtilis* caused inhibition of the immigration of blood leuckocytes, while a control group were unaffected. If cord factor is purified and then injected into mice, the animals die after a few injections.[140] In another study mice injected with cord factor from different mycobactria showed a lower humoral response with cord factor from BCG. Also, this

injection did not confer protection against[141] TB to the injected animals,[141] but it did demonstrate anti-tumor properties.[142] The toxic effect of cord factor has been the subject of numerous studies. Numata *et al.* [143] reported the toxic effects of cord factor in mice, with the majority of animals treated with TDM under three different protocols dying after only a few days of treatment. They dissolved the TDM in mineral oil and injected this into the mice. In the first protocol 10 μg of TDM was injected into the mice on days 0, 2, 4 and 6, causing 50 % of the mice to die by day 8. By day 9, the death toll had reached 75 % and by the tenth day the percentage mortality rose to 90 %, reaching 100 % by day 14. In the second protocol the mice were injected with 10 μg of TDM on days 0, 6, 8 and 12. By day 4, 16 % percent of the mice had expired and by the sixth day, 25 % had died and on day 8 only 50 % were left alive. By the eleventh day of the study, 90 % of the mice had succumbed to the toxic effects of TDM and finally on day 14 all of the mice had died. In the third protocol the mice were injected with 10 μg TDM on days 6 and 10 of the study. The percentage of dead mice by day 5 of the study was 16 % and by day 7 the percentage rose to 50 %. From day 9 until the end of the test, 75 % of the test subjects had expired.[143]

The toxicity of TMM was studied by Kato and Maeda[129] tested using the same methods as those used by Numata *et al.* They injected different concentrations of TMM in mineral oil into the mice using concentrations of 100, 200, 300, 400 and 500 μg, respectively. For the concentration of 500 and 400 μg, the percentage of dead mice by day 14 of the study was 20 % and by day 16 it had risen to 60 %. By the twentieth and last day of the study, 50 % of the mice had died, showing the same effect for both concentrations of TMM. For the 300 μg concentration, the percentage of dead mice by day 15 was 20 % and by day 18 it had risen to 30 % where it remained until the end of the experiment at day 20. For the 200 μg experiment, the percentage of the mice which had expired by day 16 of the study was 20 % and by day 18, 80 % of the mice had died, with no further deaths occurring by the end of the experiment at day 20. For the 100 μg concentration, 10 % of mice had died by day 6 and by day 9 the percentage had risen to 40 % where it remained static until the conclusion of the experiment at day 20.[129] 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.

1.19 Synthetic cord factors

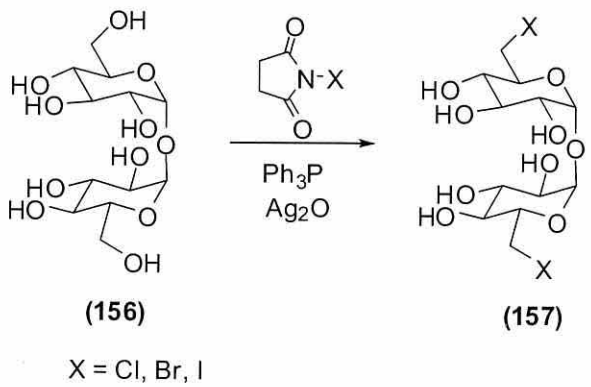
Attempts to synthesise cord factors started even before their structures had been confirmed. The first approach started during the 1950s, protecting the trehalose 6,6'-dihydroxyls with toluenesulfonyl (tosyl) groups (**153**) followed by nucleophilic displacement with a potassium salt of natural mycolic acid (**154**) in DMF.[144] This method gave a low yield and so was modified by changing the solvent to toluene and adding crown ether as catalyst.[145] TDM was obtained for two potassium salts of model mycolates, C-44 and C-32 (Scheme (**1-32**)). According to Polonsky *et al.*,[145] when the displacement was carried out in toluene and refluxed at a lower temperature in the presence of crown ether it alleviated the formation of 6-mycoloyl-3',6'-anhydrotrehalose (**155**).[145]



Scheme (1-32): The first attempt to prepare TDM[144] (RCOOK = natural mycolic acid potassium salt)

After this early attempt, research focused on finding a suitable method for protecting the hydroxy groups of the trehalose in order to optimise the yield. Trehalose is difficult to protect since it has two symmetrical D-glucopyranose units which are indistinguishable from one another.[145]

Hanessian *et al.* in their work on the synthesis of nucleosides introduced a method for the synthesis of halosaccharide using triphenylphosphine and *N*-halogenosuccinimide in DMF.[146] This method was also applied to the synthesis of 6-deoxy-6-halo-α, α-trehalose heptaacetates **(157)** starting from trehalose **(156)** in one step (Scheme (**1-33**)).[147]



Scheme (1-33): Synthesis of halo-trehalose

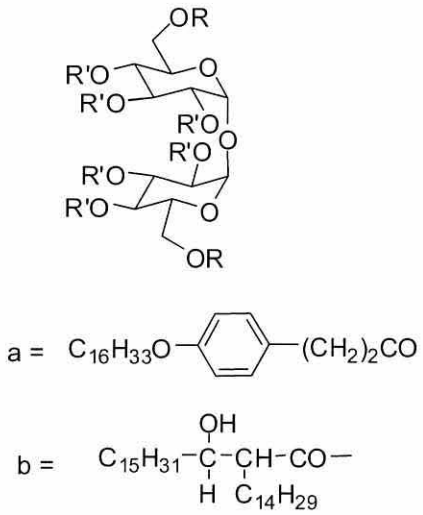
The use of this method for preparing halo-trehalose (**157**) led two groups independently to synthesize cord factor after protecting the secondary hydroxyl with trimethylsilyl followed by refluxing with natural potassium-mycolate in HMPT (see section (**1.22.1 D**) below).[148, 127]

1.19.1 Recent syntheses

All the recent syntheses of TDM are based on protecting the trehalose and then treating it either with mycolic acids or potassium salts of mycolic acids.

A) Hexabenzyl trehalose

The use of a benzyl protecting group for the hydroxy groups in trehalose is to prevent the side product, a 3,6-anhydrotrehalose sugar from forming. In a method used by Liav and Goren the trehalose was first derivatized with a trityl group in order to protect the 6,6'-position (**158**), and then the secondary hydroxyl groups were protected with benzyl groups (**159**). They next converted the ditrityl groups into dimesyl groups (**160**), since this is a better leaving group, and then coupled the compound (**160**) with a potassium salt of natural mycolic acid at 90 °C in HMPT (**161**). Finally the trehalose was deprotected by hydrogenation to obtain the TDM compound (**162**) (Scheme **(1-34)**).[149,150]

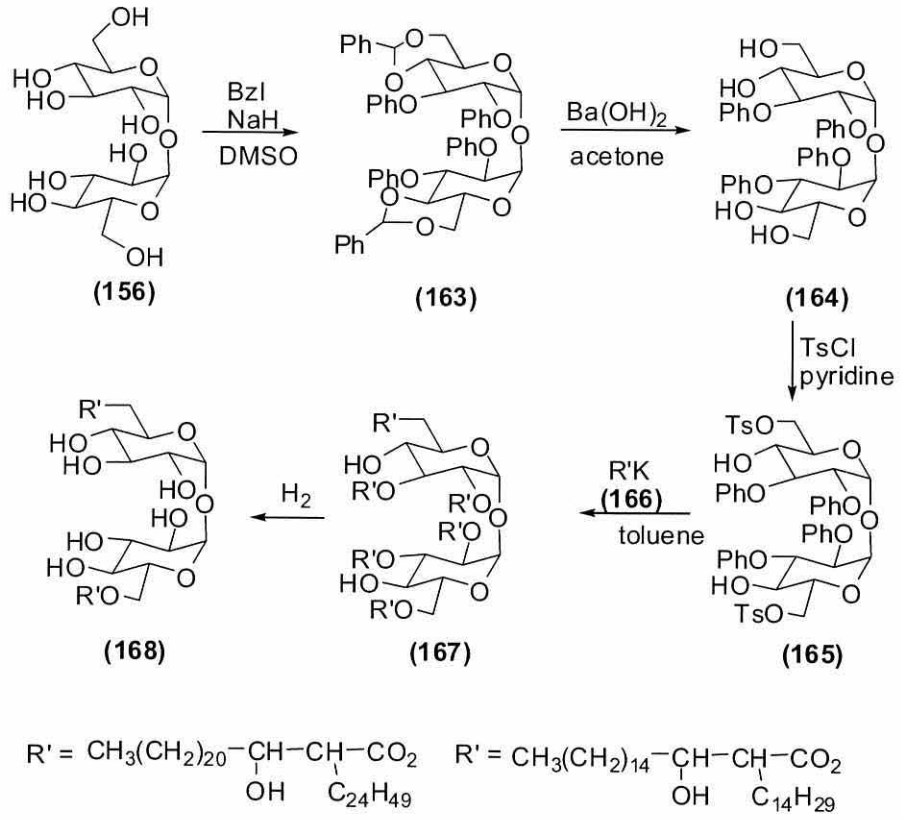


	R	R'
(158)	C-Ph_3	H
(159)	C-Ph_3	CH_2Ph
(160)	Ms	CH_2Ph
(161)	Natural mycolic acid	CH_2Ph
(162)	Natural mycolic acid	H

Scheme (1-34): Preparation of two TDMs[150, 151]

B) Tetrabenzyl trehalose

Another useful route for the preparation of TDM used 2,3,2',3'-tetra-O-benzyl trehalose. The initial step was the preparation of compound (**156**) by the benzylation of trehalose (**156**) using sodium hydride and benzyl halide in DMSO.[152] The next step was the hydrolysis of (**163**) to obtain compound (**164**) with a free hydroxy in the 6,6'-postions, followed by activation of this hydroxyl with a tosyl group, since this is a better leaving group. This was followed by coupling of the potassium of natural mycolic salt (**166**) with 4,6,4',6'-tetrabenzyltrehalose (**165**) and finally de-protecting the trehalose (**167**) by hydrogenolysis to obtain the TDM compound (**168**) (Scheme **(1-35)**).[121, 143, 149, 153]



Scheme (1-35): Another method for preparation of TDM.[154]

C) Synthesis via a Mitsunobu reaction

Perhaps one of the most useful methods for preparing cord factor is the Mitsunobu reaction. The simplest attempt was carried out by Bottle and Jenkins who synthesized a diester of trehalose and sucrose directly with no protection to the free sugar.[155] This was done by mixing palmitic acid, triphenylphosphine (TPP) and diisopropyl-azodicarboxylate (DIAD) in DMF and stirring overnight to obtain **(169)** and **(170)** in good yield (Figure (**1-23**)).[155]

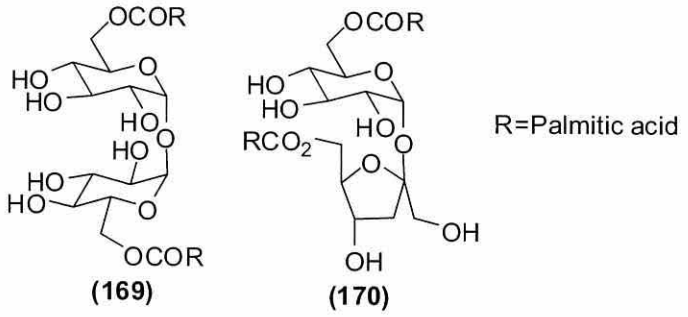
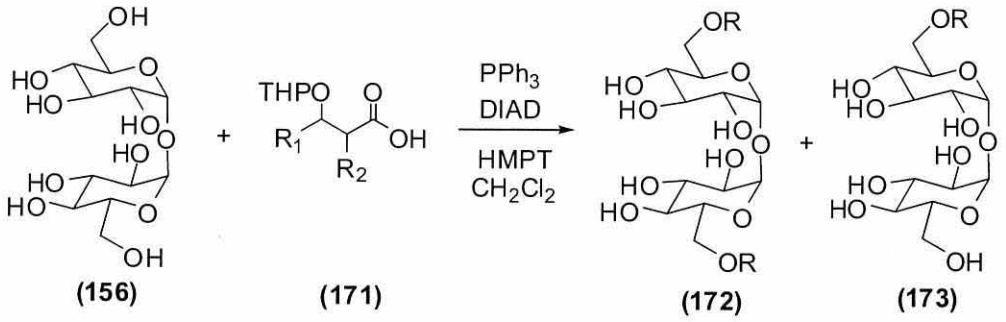


Figure (1-23): Dipalmitates of trehalose and sucrose

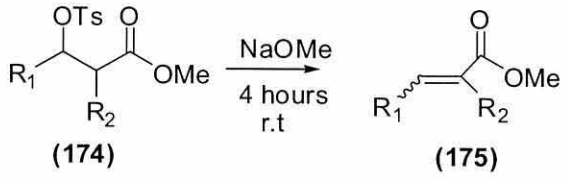
The application of this method to natural mixtures of mycolic acids did not result in the formation of the desired TDM or TMM due to the fact that the mycolic acid suffers from β-elimination in presence of the Mitsunobu reagents. In order to avoid the elimination reaction in the mycolic acid, the β-hyroxyl group was protected with THP and a Mitsunobu esterification reaction was carried out with this protected mycolic acid[1] (**171**) and free trehalose to give TDM (**172**) and TMM (**173**) in good yield (Scheme (**1-37**)).[156]



R = mycolic acid; $R_1 = C_{59}H_{117}$; $R_2 = C_{22}H_{45}$

Scheme (1-36): Synthesis cord factor via a Mitsunobu reaction

In order to test for the elimination reaction in the natural mycolic acid mixtures, Jenkins and Goren compared the results of mycolic acid after treatment with Mitsunobu reagents and the same mycolic acid after protecting the hydroxyl group with tosylate (**174**) (which is a good leaving group) and treating with sodium methoxide. Both of these reactions resulted in the formation of the same compound (**175**) (Scheme (**1-37**)).[156]



$R_1 = C_{59}H_{117}$; $R_2 = C_{22}H_{45}$

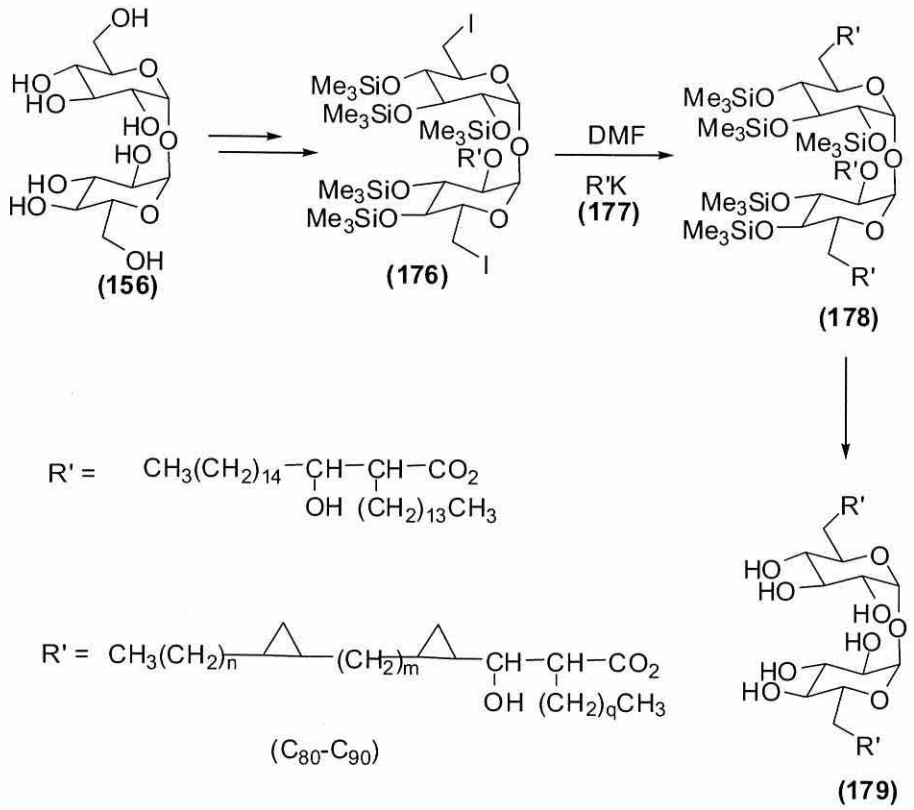
Scheme (1-37): An elimination reaction of mycolic acids[156]

---

[1] **Mixture of natural mycolic acid isolate from (BCG), with major isomer of $R_1=C_{59}H_{117}$; $R=C_{22}H_{45}$**

D) 2,3,4,2',3',4'-Hexakis-O-(trimethylsilyl)-α,α-trehalose

This is an early route for the synthesis of cord factor, the first attempts being made by Tocanne and Toubiana,[127, 148] in which the secondary hydroxyl groups of the trehalose sugar (**156**) were protected by trimethylsilyl,[157] and the primary hydroxyl in the 6,6'-position was replaced with a good leaving group (iodine) (**176**). The final coupling was between a potassium salt of natural mycolic acid (**177**) and the sugar moiety (**176**) forming protected TDM (**178**). After de-protecting the trehalose sugar from the trimethyl silyl protection groups, free TDM was formed (**179**) (Scheme (**1-38**)).[148]



Scheme (1-38): Preparation of TDM.[127, 148]

The difference between the reports of Toubiana *et al.* and Tocanne was the acids which were used for the synthesis of TDM, Table (**1-1**).[127, 148]

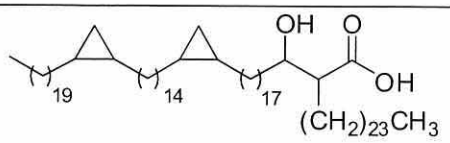
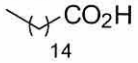
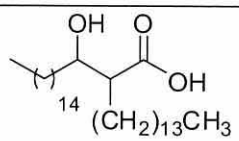
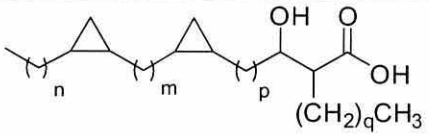
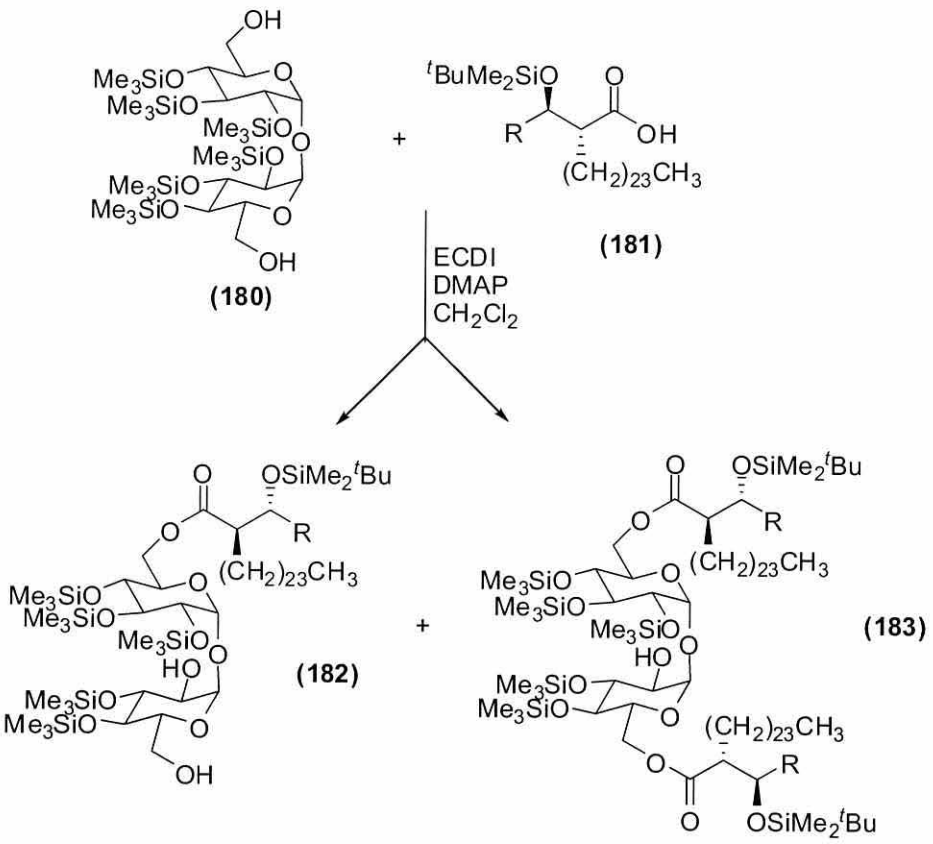
Toubiana *et al*[127]

Tocanne[148]



C_{80}-C_{90}

Table 1-1: The different mixtures of natural mycolic acids and models used by Toubiana *at al.*[127] and Tocanne[148] for the synthesis of 'TDM'

E) The total synthesis of cord factor

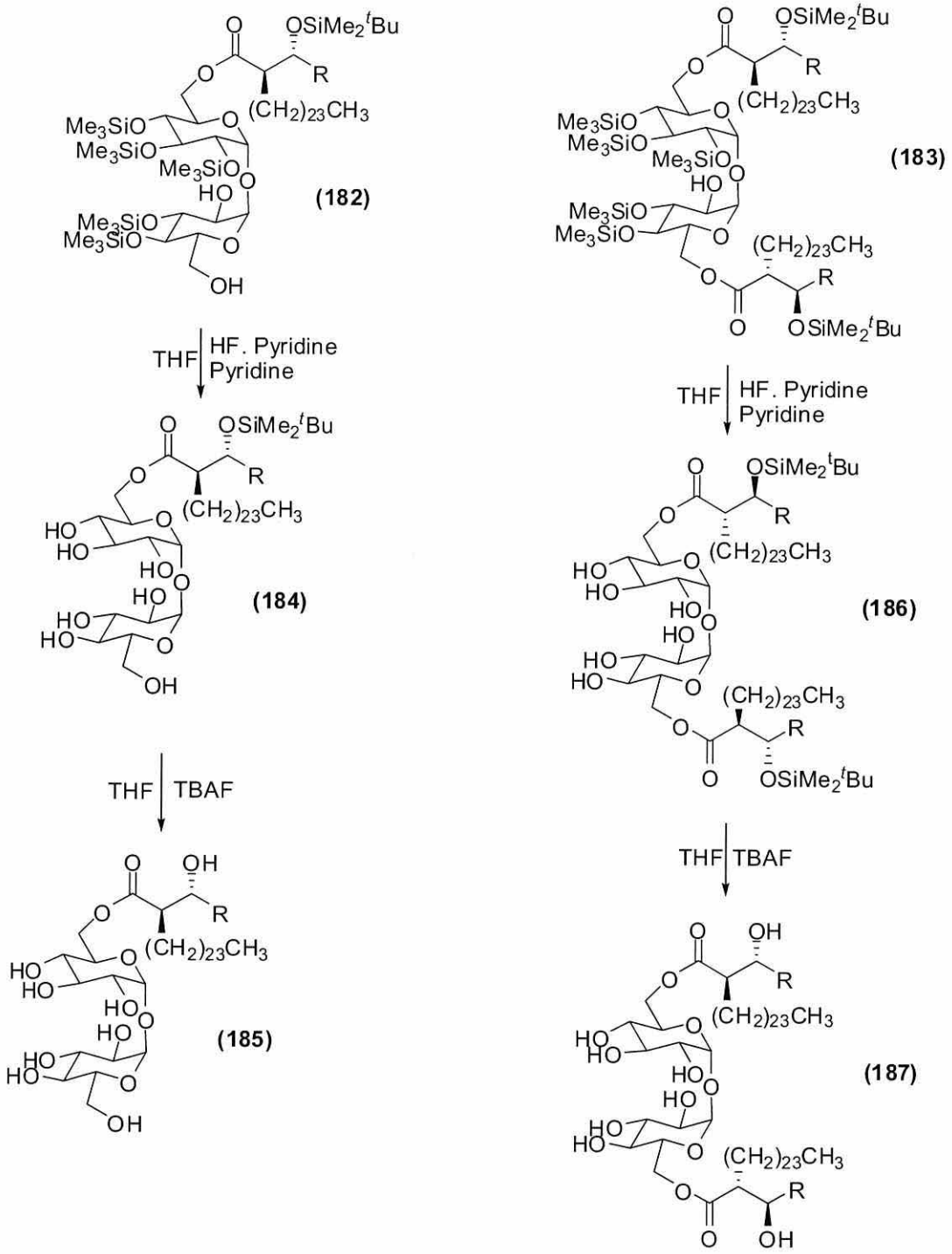
The first pure synthetic cord factor with a single synthetic mycolic acid of one absolute stereochemistry was completed by this group.[2] This was made using the synthetic mycolic acids in Table (**1-2**) and protecting the β-hydroxyl with TBDMS. The trehalose was protected with trimethyl silyl groups and then esterified for one week at room temperature (Scheme (**1-39**)).[158]

[2] Prof. M. S. Baird research group, (Uinversity of Bangor).



Scheme (1-39): Preparation of TDM and TMM[158]

The TDM and TMM obtained were protected both on the hydroxyl group of the mycolic acid and on the trehalose moiety. In order to deprotected them, a two step procedure was carried out. The first step was deprotection of the trehalose trimethylsilyl ethers, and the second was the deprotection of the mycolic acid hydroxyl group, obtaining pure enantiomer of TDM and TMM (Scheme (**1-40**)).[158]



Scheme (1-40): The deprotection steps for TDM and TMM[158]

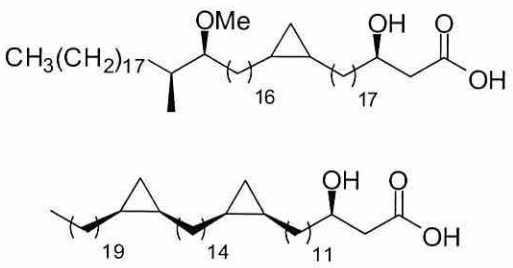


Table (1-2): Synthetic mycolic acids used to prepared TDM and TMM[158]

F) Synthesis of mirror pseudo cord factor

The difference between mirror pseudo cord factor (**188**) and cord factor (**189**) is the arrangement around the ester linkage (Figure (**1-24**)).

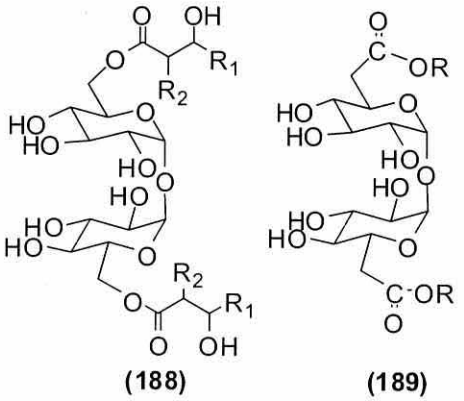
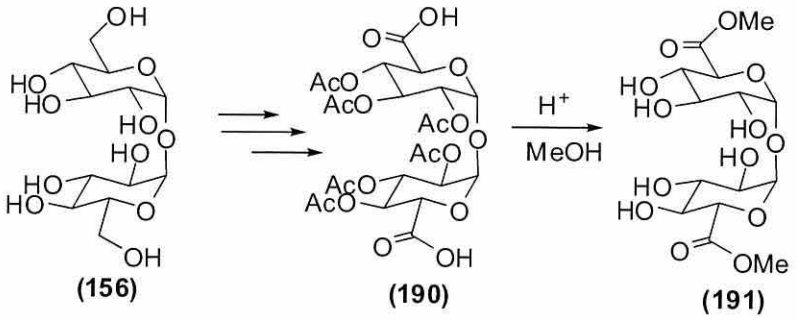


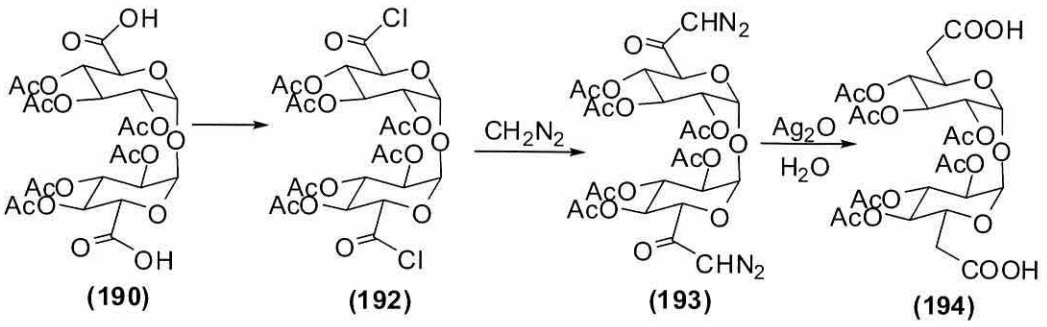
Figure (1-24): Cord factor and mirror pseudo cord factor

An early attempt for the preparation of pseudo cord factor was performed by Goren and Jaing.[151] Their method was based on the protection of the secondary hydroxyls in trehalose (**156**) followed by oxidation of the 6,6'-primary hydroxyl, forming a dicarboxylic acid. The first step was the protection of the hydroxyl groups with acetyl, followed by a platinum-catalysed oxidation to give **(190)**. The final step was the simultaneous deprotection and esterification of **(190),** which resulted in the pseudo cord factor **(191)** (Scheme (**1-41**)).[151]



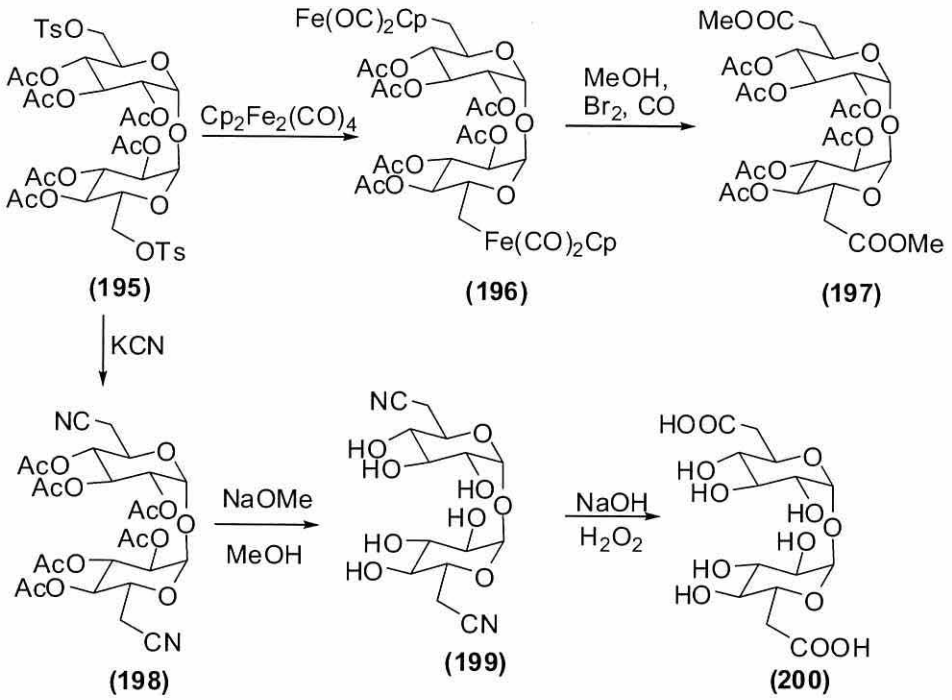
Scheme (1-41): Preparation of pseudo cord factors[151]

Another development for the preparation of pseudo cord factors was made by Baer and his group, who added a carbon atom to the trehalose sugar in the 6 and 6' positions, since in natural cord factors, the trehalose sugar is linked to the mycolic acid at these two positions. They synthesised (**194**), (**197**) and (**200**) in a few steps for use as building blocks instead of (**190**). The first approach was the preparation of 2,3,4,2',3',4'-hexaacetyl dicarboxylic trehalose (**190**) which was then treated with thionyl chloride to give **(192)**, followed by reaction with diazomethane to give **(193)**. The oxidation of compound (**193**) gave a dicarboxylic acid with an extra carbon in the dicarboxylate protected trehalose (**194**) (Scheme (**1-42**)).[159-161]



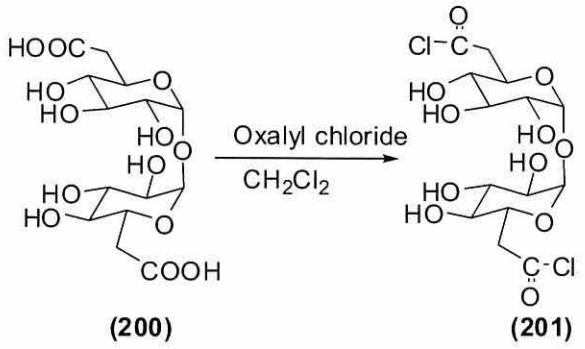
Scheme (1-42): Preparation of intermediate (194)[160, 161]

This same group also developed another method for elongation at the 6 and 6' positions of trehalose. The initial step was the protection of trehalose with acetyl groups at the 2,3,4,2,'3, and '4- positions, followed by replacement of the 6,6'-position hydroxyl groups with tosylate groups to give compound (**195**). This was then treated with an iron complex to replace the tosylate group and give compound (**196**). The final step was the addition of a carbon atom by treating the reaction mixture with carbon monoxide to produce (**197**) as the desired compound (Scheme **(1-43)**).[160,142]



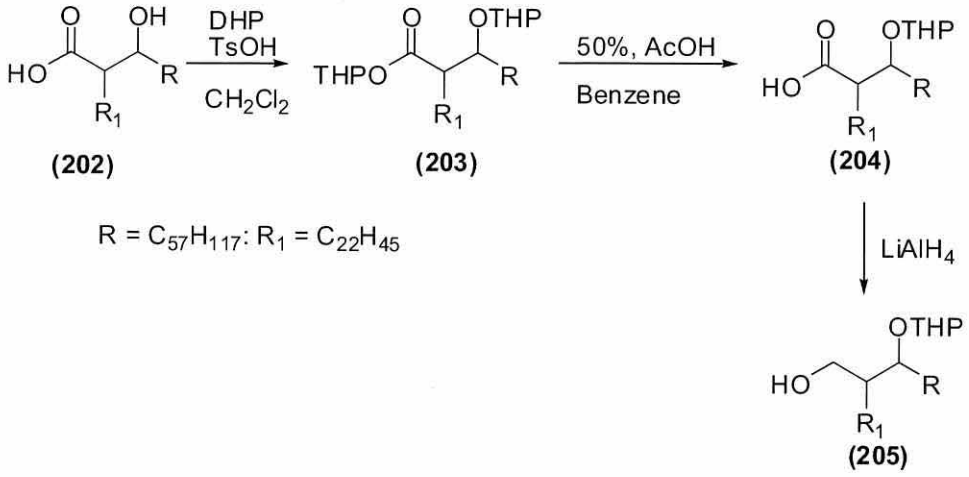
Scheme (1-43): An alternative approach for the preparation of trehalose dicarboxylate.[160, 142]

In the third method, the same intermediate (**195**) was used and the tosylate group was replaced with a nitrile group, followed by hydrolysis in order to deprotect the trehalose sugar to form compound **(199)**. The last steps were an oxidation using hydrogen peroxide to obtain (**200**), followed by transformation of the carboxylic acid **(200)** into the acid chloride **(201)** in order to facilitate coupling between the potassium salt of dicarboxylate trehalose (**200**) and the alcohol (**205**) (Scheme (**1-44**)).[142]



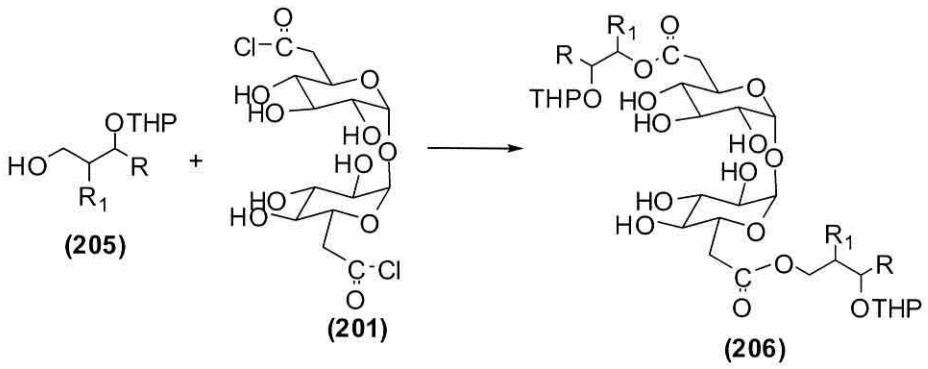
Scheme (1-44): Preparation of the trehalose derivative

The second moiety of "mirror" pseudo cord factor is the mycolic acid which was converted into an alcohol (**205**). The β-hydroxy group was protected using THP (**203**), followed by reduction using lithium aluminum hydride to obtain the alcohol (**205**) (Scheme (**1-45**)).[142]



Scheme (1-45): Reduction of the mycolic acid

The last step was the coupling of the trehalose chloride derivative (**201**) with the alcohol (**205**) to obtain the mirror pseudo cord factor (**206**) (Scheme (**1-46**)).



Scheme (1-46): The preparation of pseudo cord factor

2. Project Aims

The project consists of five parts. The first target was the synthesis of unsaturated mycolic acids (**207**), (**208**) and (**209**), since the mycolic acids (**208**) and (**209**) are reported in *M. smegmatis* and mycolic acid (**207**) was reported in *Corynebacterium diphtheriae*. This was accompanied by an attempted synthesis of the saturated mycolic acid (**210**). The second part was the synthesis of oxygenated mycolic acids, hydroxy and keto mycolic acids (**211**) and (**212**) since these mycolic acids were reported as component of the *M. tuberculosis* cell wall. This was followed by synthesis of α-mycolic acid (**213**) which is present in *M. tuberculosis*. The fourth part of this project was the synthesis of cord factors of α-mycolic acid (**213**) to give TDM (**215**) and TMM (**214**). The final target was the synthesis of cord factors of the unsaturated mycolic acid (**208**) to obtain TDM (**216**) and TMM (**217**) (Figure **(2-2)**).

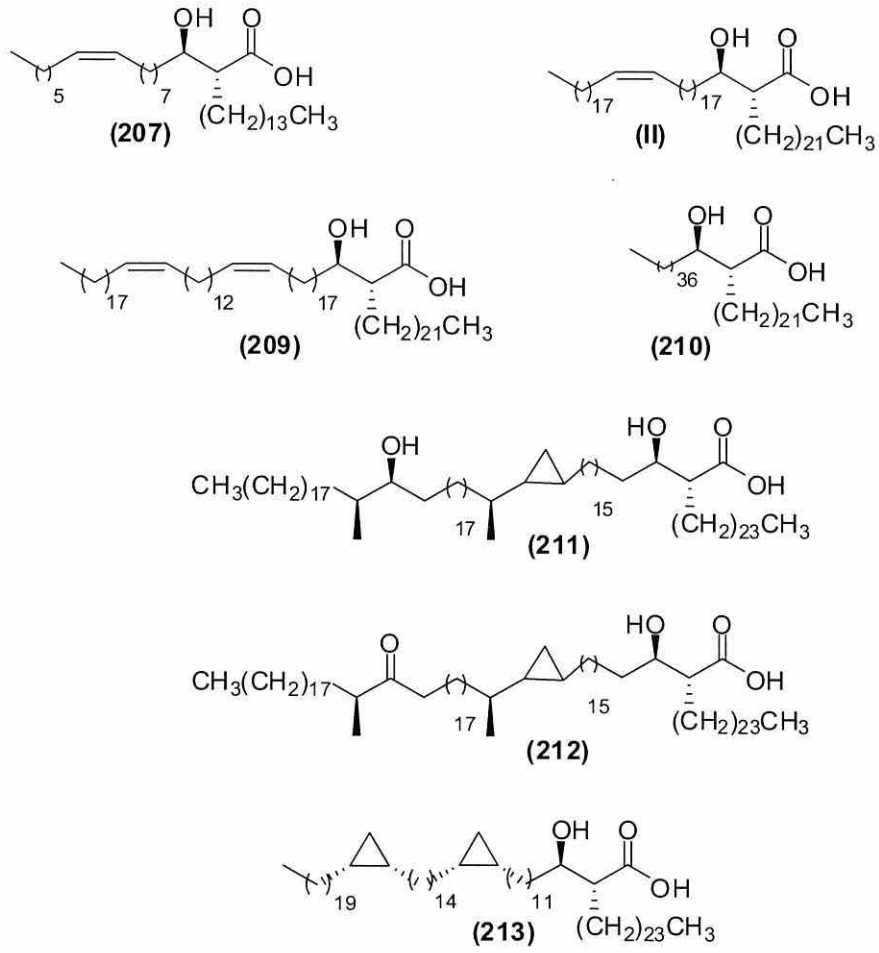


Figure (2-1): Mycolic acids to be synthesised in this study

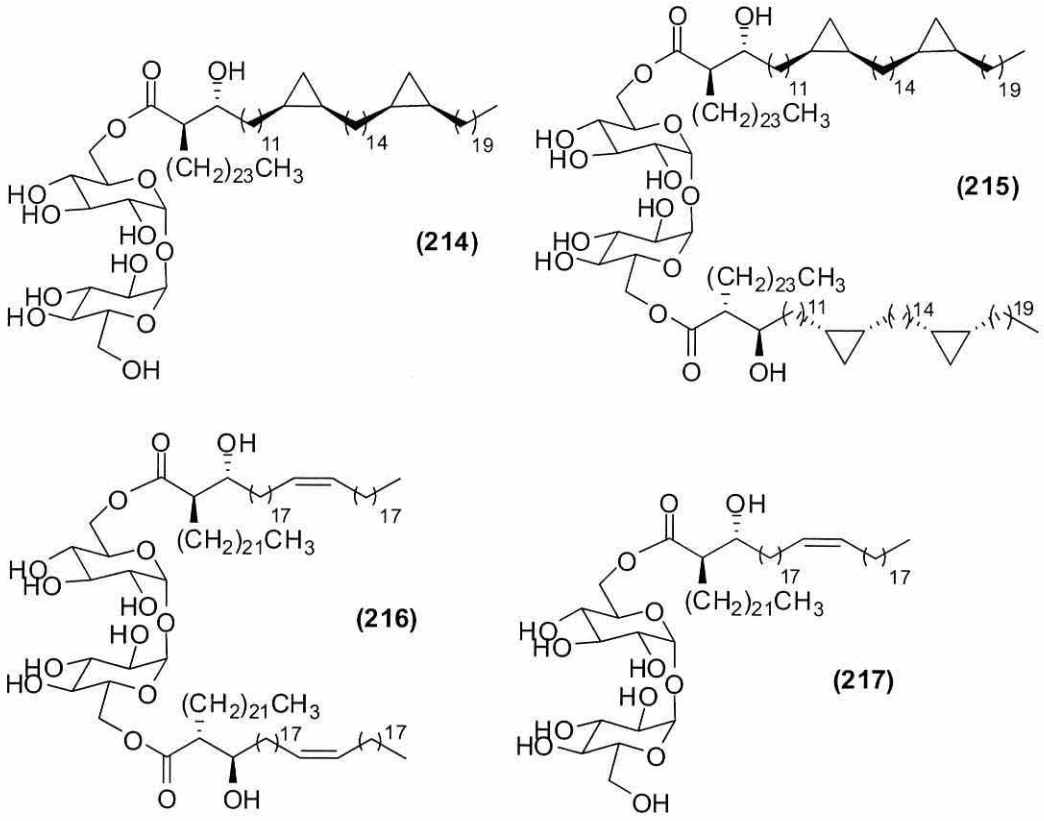


Figure (2-2): Target cord factors of α- mycolic acid

The work carried out towards each of these targets is presented in the following four chapters, Chapter 3 covering alkene mycolic acids, Chapter 4 covering oxygenated mycolic acids, Cahpter 5 the synthesis of an α-mycolic acid, and Chapter 6 the synthesis of cord factors.

Results and Discussion

3. Synthesis of alkene mycolic acids

3.1 Natural alkene mycolic acids

The desaturation of fatty acids in the cell wall of mycobacteria is the first step for the introduction of functional groups into the meromycolate chain (see **Section (1.9.3)**) for further detail). The desaturation step occurs during the elongation of the meromycolate chain.[38] Different types of mycolic acids containing a double bond in the proximal position have been reported in the cell wall of mycobacteria. These may contain either one double bond as in (**218**), two double bonds as in (**219**) and (**220**), a double bond in conjunction with an epoxy ring as in (**221**) and (**222**), or a double bond in conjunction with a cyclopropane ring as in (**223**) Figure (**3-1**).[37]

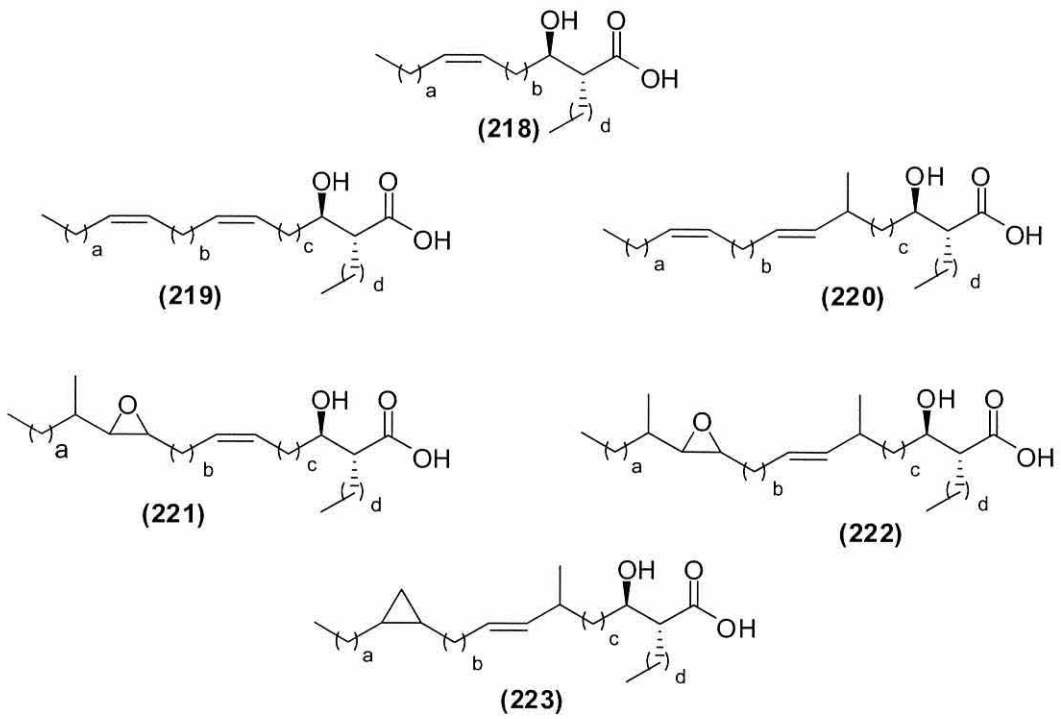
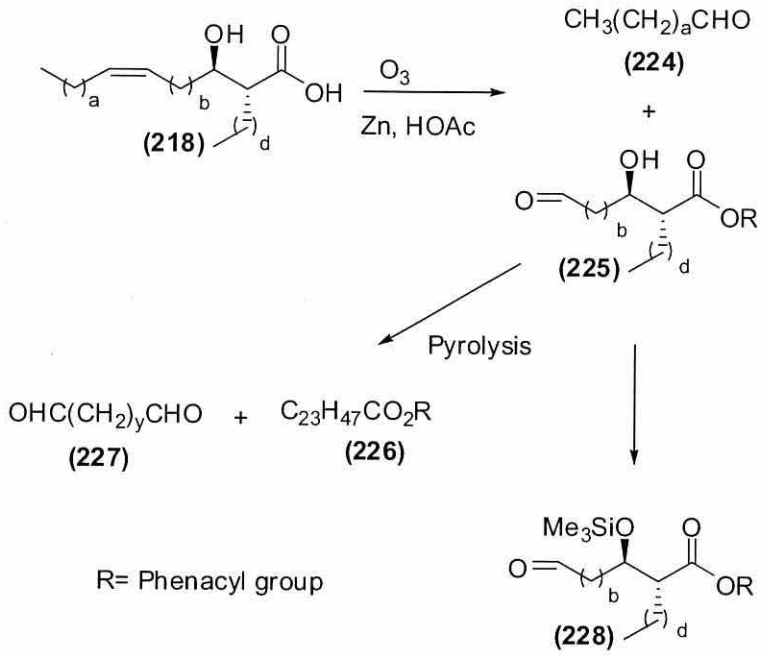


Figure (3-1): The structure of unsaturated mycolic acid[37]

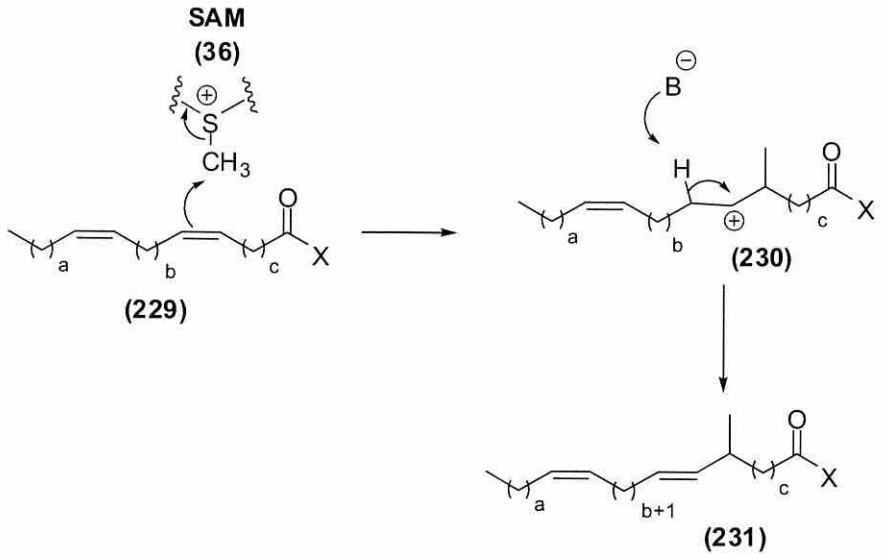
Kaneda *et al.* [162] found the use of GC-MS was insufficient for the differentiation between double bonds and cyclopropane rings in the meromycolate chain, since the mass numbers of the derivatives of both the cyclopropanated and olefinic compounds were identical in his study.[162] Thus, Kaneda *et al.*[162] used hydrogenation in a neutral solvent in order to distinguish between a double bond and a cyclopropane ring, since

cyclopropane rings will only hydrogenate under acidic conditions. The total molecular weight will increase by two or four mass units if the mycolic acid contains either one or two double bonds, while the molecular mass will remain the same following the hydrogenation of a sample of mycolic acid containing cyclopropane and no double bond.[162] The first two unsaturated mycolic acids (**218**) and (**219**) are the major components of the *M. smegmatis* mycolic acids and were studied by Gray *et al.* [163] in early 1980s who showed that, the α-carbon chain lengths is 22 in both acids and a + b = 32, 34, 36 and 38, leading to an odd carbon number in the meromycolate, in mycolic acid (**218**). In mycolic acid (**219**), a+b+c = 44, 46, 48, 50 and 52, leading to an even number in the meromycolate chain, while position of the methyl branch of mycolic acid (**220**) is said to be 'arbitrary'.[163] Baba *et al.* [164] showed that in *M. smegmatis*, the α- mycolic acid (**218**) contained between 60 and 66 carbons atom and the mycolic acid (**219**) between 72 and 81 carbons, while the epoxy mycolates contained between 73 and 81 carbons.[164] Determination of the double bond location was made by oxidation of natural mycolic acid (**218**) using ozonolysis, followed by decomposition using powdered zinc in 50 % acetic acid and resulting in aldehydes (**224**) and (**225**). The aldehyde (**224**) gave an indication of the terminal chain length. Analysis of fragment (**225**) was carried out directly, or after modification to its silyl ether (**228**), since trimethyl silylation prevents the pyrolysis of compound (**225**) if it is injected into a GC. The analysis of the fragments (**226**) and (**227**) showed the mean isomers of unsaturated mycolic acid in *M. smegmatis* cell wall were: a = 17; b = 17 or a = 19; b = 15, while d = 22 (Scheme (**3-1**)).[165]



Scheme (3-1): Pyrolysis of natural mycolic acid (218)

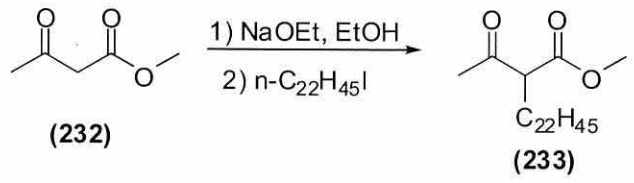
These studies led to the suggestion that the *cis*-diene (**229**) is converted into the *trans*-diene (**231**). This must be achieved by a route which shifts the double bond one position further from the end of meromycolic acid. The suggested mechanism involved the double bond electron attacking SAM, taking a methyl group from SAM (**36**) and then intermediate (**230**) is neutralized to give the diene (**231**) (Scheme (**3-2**)).



Scheme (3-2): Suggested mechanism for formation of the α-methyl-*trans*-alkene

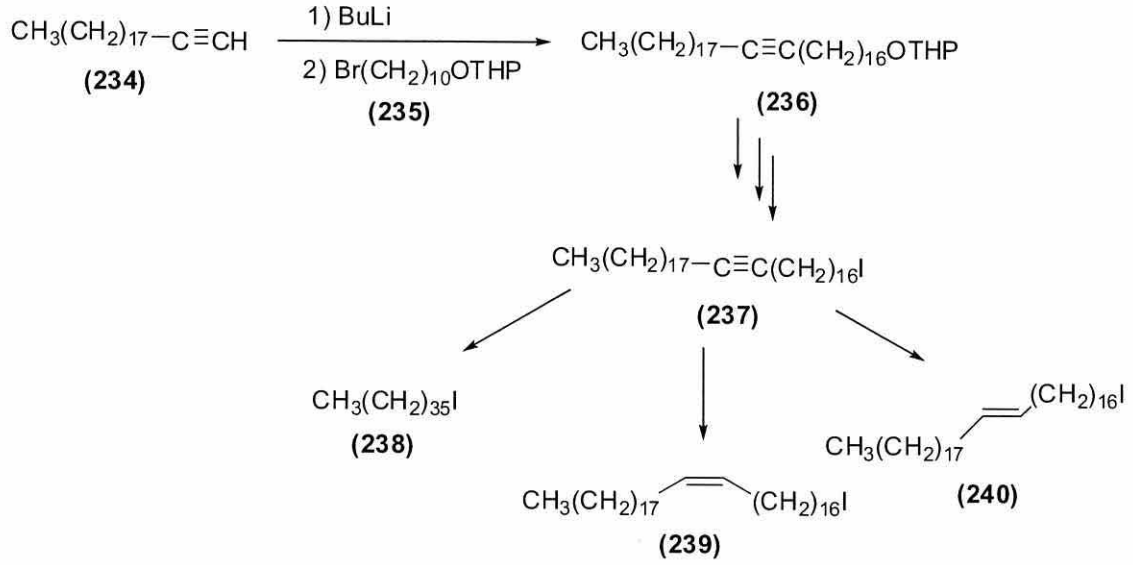
3.2 Previous synthetic approaches

An early attempt to synthesise alkene-containing mycolic acids was begun in the 1980s by Huang *et al.*[166] Their strategy was to synthesise parts of the desired mycolic acid (**218**) and link those parts using different methods. The first part to be prepared was the 'mycolic motif' starting from β-diketo ester (**232**) and inserting the α-alkyl chain using sodium ethoxide as catalyst since the two adjacent carbonyl groups make the α-protons more acidic and thus increase the ease with which the α-alkyl chain can be inserted (**233**) (Scheme (**3-3**)).[166] This gave (**232**) as a racemate.



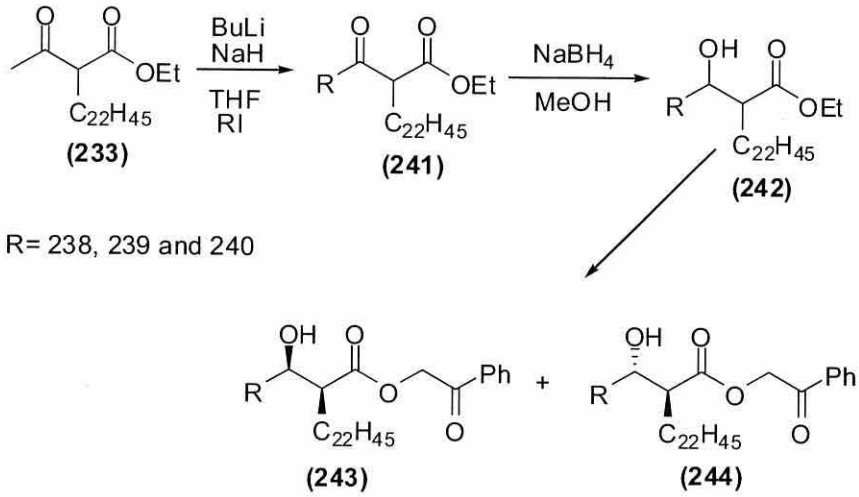
Scheme (3-3): Preparation of compound (243)[166]

The second part of Huang's synthesis was the coupling of the alkyne **(234)** with a C_{16} chain **(235)** protected with THP from one side and bromide on the other side. The product (**236**) was converted in several standard steps into iodide (**237**). This compound was hydrogenated using three different catalysts to give three compounds, a saturated compound (**238**) and two alkenes, *cis*-(**239**) and *trans*-(**240**) (Scheme (**3-4**)).[166]



Scheme (3-4): The preparation of meromycolate (239) and related compounds[166]

The next step was the coupling of compound (**233**) with compounds (**238**), (**239**) and (**240**) respectively, using butyllithium and sodium hydride in dry THF to give compounds (**241**). These were reduced using sodium borohydride to give a mixture of four stereoisomers of mycolic acids (**242**). An attempt to separate this mixture using HPLC resulted in two isomers for each acid, an erythro (**243**) and a threo (**244**) mycolate each as a racemate (Scheme (**2-6**)). Using (**239**) as starting material this led after HPLC to a racemic mixture of the natural alkene mycolic acid (**207**) (Scheme (**3-5**)).



Scheme (3-5): Separation of synthetic diastereomeric mycolic acid and analogues with HPLC[166]

3.3 The synthesis of single stereoisomers of unsaturated mycolic acids

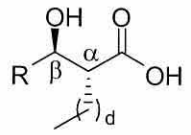
As seen above, the previous synthesis of unsaturated mycolic acid (**207**) gave a racemic mixture, and even then only after HPLC separation. The first target of this work was therefore to synthesise a singler enantiomer without the need for HPLC separation. This would be achieved by synthesis of the myolic motif with a single (correct) absolute stereochemistry (see Section **3.4**) followed by linking this to the meromycolate by different methods.

3.4 Preparation of the Mycolic Motif

The mycolic motif is the common unit in all mycolic acids, and contains an alkyl group and a hydroxyl group which are in an *anti* configuration with respect to each other (Figure (**3-2**)). As described in the Introduction, there are a number of ways of

doing this (Section **1.14**). The most successful involve preparing a single enantiomer of a β-hydroxy acid with no chain at the α-position and then introducing that chain. This approach was also followed in this work, but a number of potential changes to the exisiting method were first studied.

The first problem examined in this project was the preparation of the unsubstituted β-hydroxy acid (Sections **3.5.1**); the second was the preparation of several different (2*R*,3*R*)-α-alkyl-β-hydroxy esters (Figure (**3-2**)) as in Section (**3.5.2**).



R = corynomycolate moiety

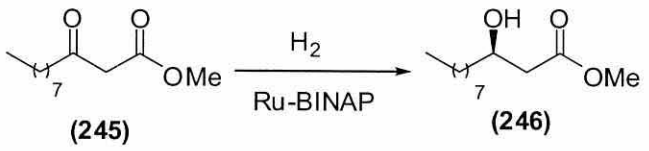
Figure (3-2): Motif side of mycolic acid

3.5 The preparation of unsubstituted β-hydroxyacids

Two methods for preparing these acids were studied, asymmetric hydrogenation using BINAP (see Section **3.5.1**), and the use of an enantiomerically pure starting material.

3.5.1 BINAP hydrogenation

In order to refine the conditions of hydrogenation to use BINAP to obtain the desired β-hydroxyesters in the correct stereochemistry, a model reaction was carried out. The first step was the preparation of dry solvent, since BINAP is sensitive to oxygen and moisture.[167] The methanol was refluxed over iodine-activated magnesium and then distilled.[168] This was followed by de-gassing the solvent using a freezing-melting method. Hydrogenation of keto-ester (**245**) was carried out using Ru-BINAP with an excess of hydrogen for 72 hours and 3 atomsphere pressure (Scheme (**3-6**)).



Scheme (3-6): Hydrogenation of the keto ester (245) using BINAP as catalyst

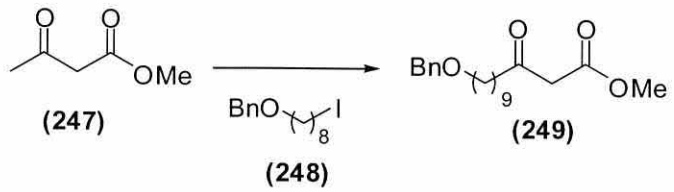
The desired ester (**246**) was obtained in very low yield (7 %). This might be due to the need to dilute the reaction mixture for this hydrogenation compared with the literature conditions because of the size of the pressure vessel.[169] The specific rotation

for the ester (**246**) was $[\alpha]_D^{24}$ -4.89 (c = 1.3 $CHCl_3$) which compares favourably with ester (**138**) which had a specific rotation of $[\alpha]_D^{28}$ -9.15 (c = 1.58, $CHCl_3$).

3.5.2 The preparation of the keto ester intermediate for the mycolic motif

In order to test the BINAP catalysed hydrogenation on other keto esters, compound (**249**) was prepared. The benzyloxy protected chain would eventually be used to couple to the meromycolate fragment to from the full mycolic acid; the long chain was designed to hamper any coordination between BINAP and the oxygen at the end of the chain (see section **1.14.3**).

The coupling reaction between methyl acetoacetate (**247**) with compound (**248**), was carried out using very strong base, sodium hydride and *tert*-butyllithium to de-protonate the methyl acetoacetate (**247**) and generate an anion to attack the ((8-iodooctyloxy)methyl)benzene (**248**). The formation of the resulting compound (**249**) was confirmed by the proton NMR spectrum which gave a multiplet between 7.33-7.26 ppm and singlet at 4.49 ppm for the two protons to the benzyl group. The CH_2 adjacent to the oxygen showed a triplet at 3.4 ppm (J = 6.3 Hz). The two protons between the two carbonyls showed a singlet at 2.51 ppm (Scheme (**3-7**)).



Scheme (3-7): Coupling aceto-acetate with protected C-8 chain

Although the model reaction only proceeded in 7 % yield, it is clear that intermediates such as (**249**) are very easy to prepare in bulk. It therefore does seem that, if technical difficulties with the availability and use of high pressure hydrogenation apparatus of the correct scale can be overcome, this approach may save a great deal of time. The established aspartate route described below was used in the rest of this work.

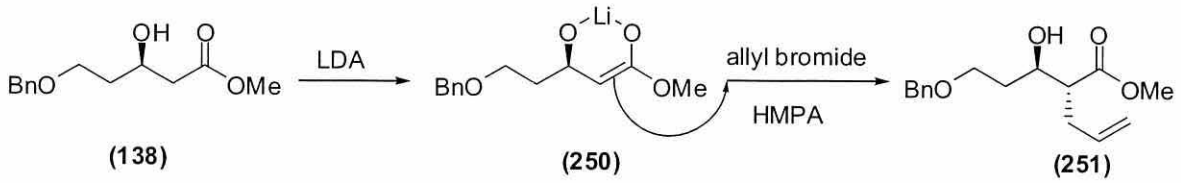
3.5.3 The aspartic acid route

The ester (**138**) was prepared in this study following the standared literature methods (see Section **1.14.3.6**). The experimental procedures are described in the Appendix (see page250).

3.6 Introduction of the α-alkyl chain: the Fräter reaction

The stereocontrolled insertion of the alkyl chain into a β-hydroxyester demonstrated by Fräter *et al.*[170-172] was used to prepare the required α-alkyl-β-hydroxyesters. Previous attempts to introduce the full length alkyl chain in one step had always failed completely of given very low yields. A further attempt was made to insert a chain of 22 carbons, again without success. The previous strategy of introducing an allyl group and then chain extending was therefore adopted.

The first step of this reaction is the generation of LDA (2 mol eq) *in situ* by reacting diisopropylamine with MeLi at -78 °C, followed by addition of the ester (**138**) (prepared as described in the Introduction) at –62 °C and stirring for 2 hours at this temperature to ensure the generation of the chelated (*Z*)-enolate complex (**250**) (Scheme (**2-9**)). The mixture was allowed to stir for 2 hours between -60 °C to -10 °C and then allyl iodide in HMPA was added, which allowed the enolate (**250**) to attack the allyl from the *anti* side, forming the α-alkyl-β-hydroxy in the (*R, R*) configuration.[173] Dugger *et al.* reported that the yield and configuration of this reaction depend on the ester used for the alkylation.[174] The formation of the resulting compound (**251**) was confirmed by the proton NMR spectrum which showed a doublet of triplets at 2.6 ppm (J = 8.8, 5.65 Hz) for the α-proton. For the alkene protons it showed a triplet of doublets of doublets at 5.8 ppm (J = 13.85, 10.1, 6.95 Hz) for the CH in the alkene and a triplet of doublet of doublet at 5.12 ppm (J = 17.35, 12.6, 1.9 Hz). The terminal protons gave a triplet of doublets of doublets at 5.12 ppm (J = 17.35, 12.6, 1.9 Hz). The proton adjacent to the hydroxyl group showed a doublet of doublets of triplets at 4.2 ppm (J = 12.6, 6.3, 4.75 Hz). The benzyl group showed signals in the aromatic region with a multiplet of five protons between 7.34-7.27 ppm and a singlet at 4.49 ppm for two protons. The ^{13}C NMR showed a carbonyl carbon at 173.56 ppm and five aromatic carbons between 138.38 and 127.45 ppm. The alkene carbons were seen at 127.38 and 116.24 ppm respectively, and one signal was seen at 72.79 ppm for the carbon adjacent to the hydroxyl group. There were also signals at 51.60 and 51.20 ppm for the methoxy carbon and α-carbon respectively (Scheme (**3-8**)).[175]

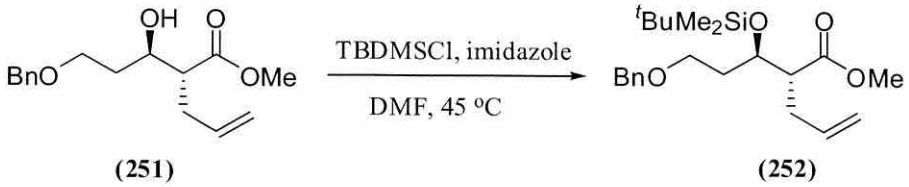


Scheme (3-8): The insertion of the α-allyl chain

The allyl chain was then extended using methods described in Section (**3.6.5**).

3.6.1 Protection of the hydroxyl group of compound (251)

In order to prepare compound (**251**) for the next step, the protection of the hydroxyl group with *tert*-butyldimethylsilyl (TBDMS) group was necessary. This protection group was chosen since it has been reported to be very stable through many reaction protocols,[176] and is not susceptible to solvolysis in protic solvent even in the presence of acid or base.[177] The secondary alcohol (**251**) was mixed with imidazole and *tert*-butyldimethylsilyl chloride in DMF and stirred for 18 hours at 45 °C. The formation of protected compound (**252**) was confirmed by the proton NMR spectrum which gave a singlet at 0.89 for the *tert*-butyl group, and broad singlet at 0.0 ppm for the two methyls groups adjacent to the silyl group. The ^{13}C NMR gave a signal at -4.68 ppm and -4.93 ppm for the two carbons attaches to the silyl (Scheme (**3-9**)). The specific rotation of the product was found to be $[\alpha]_D^{24}$ -15.07 (c = 1.87, $CHCl_3$).

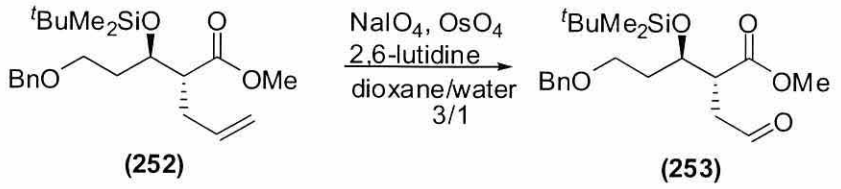


Scheme (3-9): The protection of the hydroxyl group

3.6.2 Extension of the α-alkyl chain

The majority of mycolic acids have an α-alkyl chain with a C_{22} or a C_{24} length chain. In this study the mycolic acids which were prepared had C_{14}, C_{22} and C_{24} length alkyl chain. In order to extend the α-alkyl, the allyl group of (**252**) was first converted into the aldehyde (**253**) and this was then chain extended a modified Julia-Kocienski olefination reaction. This was chosen, since it gives a better yield and is easy to handle

compared with other reactions used for extending the alkyl chain such as Wittig and Grignard reactions. The olefin (**252**) was oxidised to the aldehyde (**253**) (Scheme (**3-10**)).

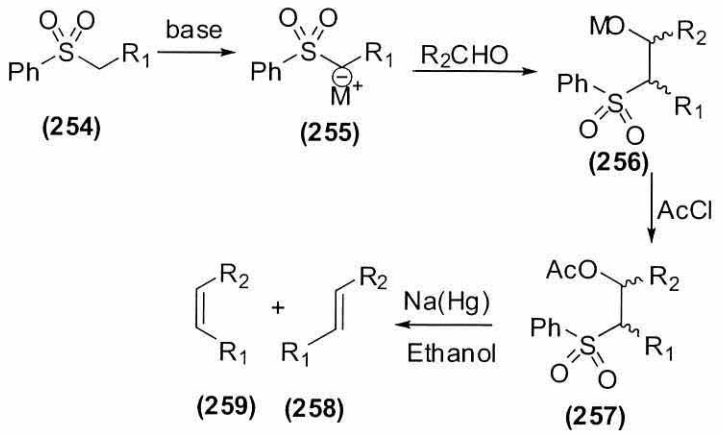


Scheme (3-10): Oxidation of the olefin to give aldehyde (253)

It has been reported that the oxidation of an olefin using osmium tetroxide and sodium periodate suffers from a low yield; however, the addition of 2,6-lutidine has been shown to improve the yield since it suppresses any side reactions.[178] A mixture of OsO_4 (0.23 mmol), $NaIO_4$ (50.84 mmol), olefin (**252**) (12.71 mmol) and lutidine (25.42 mmol) was stirred in dioxane-water (3:1) for 2.5 hours, forming the aldehyde (**253**) in 78 % yield. The formation of the resulting aldehyde (**253**) was confirmed from the proton NMR spectrum which gave a singlet at 9.74 ppm for the aldehyde proton and lacked signals in the olefinic region. The carbon NMR spectrum gave a signal at 200.45 ppm for the aldehyde carbon and a signal at 172.40 ppm for the ester carbonyl. The infra red spectrum gave an absorbance at 1737 cm^{-1} for the carbonyl groups, and the specific rotation was $[\alpha]_D^{26}$ -18.42 (c = 0.97, $CHCl_3$).

3.6.3 The modified Julia-Kocienski reaction

Formation of a double bond by reaction between an aldehyde and phenylsulfone on reaction with base was published by Julia and Paris and became known as the classical Julia Olefination.[179] This method was developed by Lythgoe and Kocienski[180-183] to give the 'modified Julia reaction'. The method can be described in four different steps: firstly metallation of a phenylsulfone (**254**) with non-nucleophile base, adding the aldehyde to the metalt complex (**255**), and forming intermediate (**256**) followed by acylation of the β-alkoxysulfone (**257**) and finally elimination reaction for β-alkoxysulfone (**257**) with an electron donor producing the alkene in the *trans* configuration (**258**) and in the *cis* configuration (**259**) (Scheme (**3-11**)).[184-186]



Scheme (3-11): The classical Julia Olefination mechanism.[186, 184]

A development of the Julia olefination was done by Julia and his team.[187] They replaced the phenylsulfone by other heteroarylsulfones (Figure (**3-3**)) in a modified Julia olefination.[186]

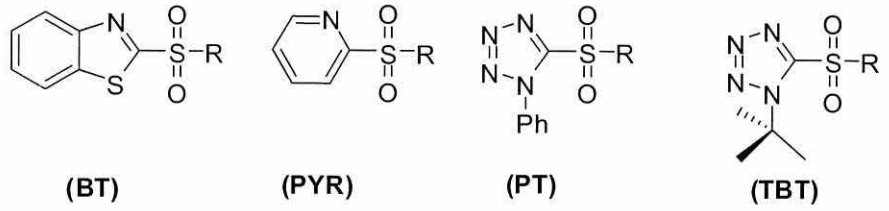
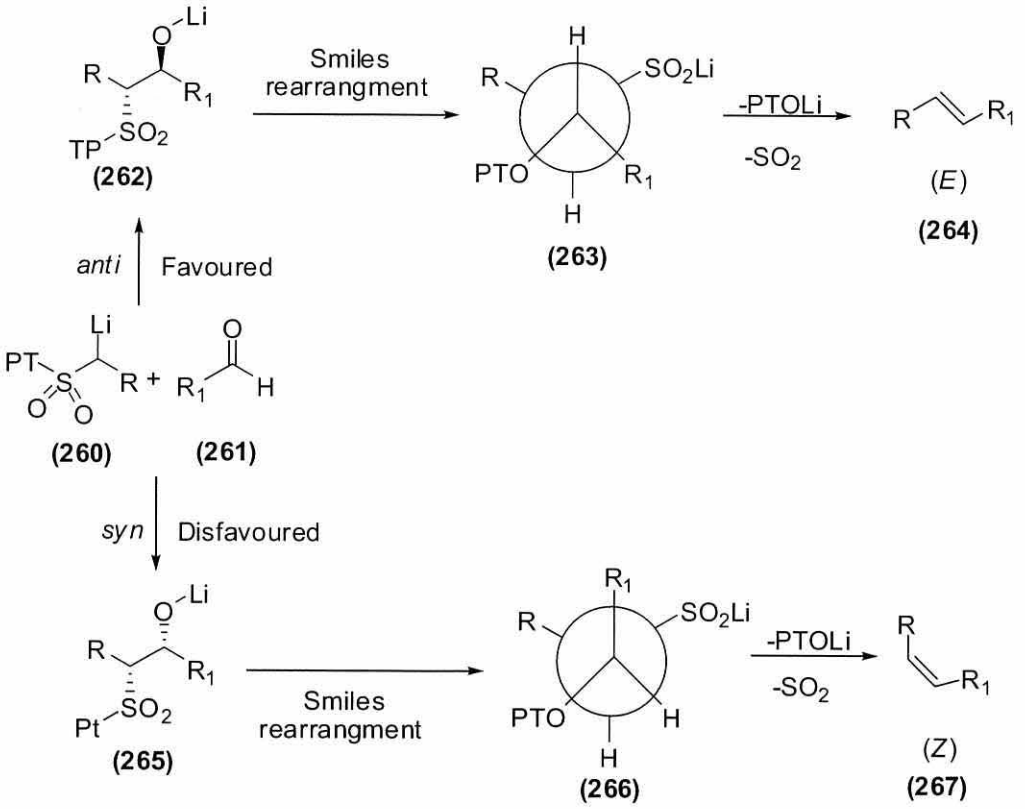


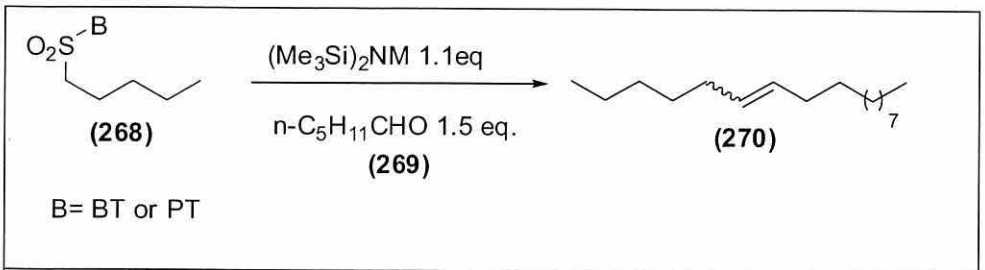
Figure (3-3): The heterocyclic sulfones used in the modified Julia olefination.[185, 186]

In this project, 1-phenyl-1*H*-tetrazole-5-thiol was used in order to prepare a sulfide which was then oxidised in good yield to the corresponding sulfone.[185] This heterocyclic compound was used in preference to the other heterocyclic compounds since BT reportedly gives a dimer through self-condensation and the sulfide of PYR is unstable at room temperature.[188, 189] In Julia reactions both (*Z*) and (*E*) isomers are obtained with the (*E*) isomer beings favoured. The reason for giving the (*E*) alkene is that there is a kinetically controlled addition between the aldehyde (**261**) and sulfone (**260**), leading to the formation of anti-β-alkoxysulfones (**262**) more than *syn*-β-alkoxysulfones (**265**) (Scheme (**2-12**)).[186] The sequence of the two stages for the reaction mechanism between the Smiles rearrangement and the elimination are sterocontrolled essentially to give the (*E*) isomer (**264**) (Scheme (**3-12**)).[186]



Scheme (3-12): Steroselectivity of the Julia reaction.[186]

The ratio of *E/Z* isomers in the Julia reaction depends on many different conditions. The solvent and the base used in the reaction mixture have a remarkable effect on this ratio since it can change from 1/1 to 1/10 in different solvents.[190, 191] The base which is used has been reported to have a similar effect on the isomeric ratio of the product since the proportion of (*E*) isomers depends on the base used, with the proportion increasing K>Na>Li.[192] Table (**2-1**) shows the effect of base and solvent used in the reaction on the ratio of *E/Z* isomers. Blakemore *et al.* reported the effect of solvent and the catalyst base on the *E/Z* ratio in a Julia reaction used to couple aldehyde (**269**) and two different heterocyclic sulfones (**268**) (Table (**3-1**)).[185]#



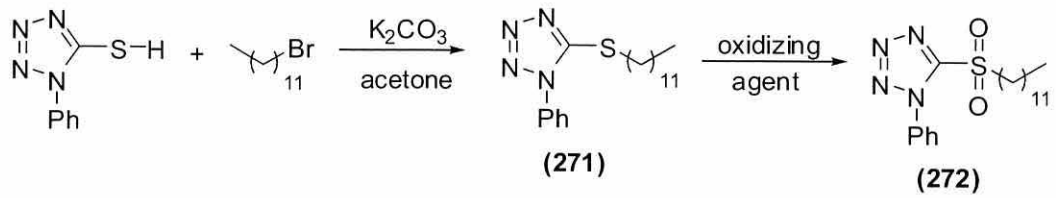
Entry	solvent	M	(BT)		(PT)	
			% Yield	*E/Z*	% Yield	*E/Z*
1	PhMe	Li	5	40/60	55	57/43
2		Na	29	51/49	80	59/41
3		K	15	47/53	13	64/36
4	Et_2O	Li	7	43/57	76	73/27
5		Na	17	53/47	90	57/43
6		K	68	51/49	30	72/28
7	THF	Li	42	60/40	97	75/25
8		Na	0	0	89	76/24
9		K	24	55/45	71	86/14
10	DME	Li	3	55/45	95	77/23
11		Na	27	77/23	92	86/14
12		K	6	75/25	71	94/6

Table (3-1): The effect of solvent and base on the *E/Z* ratio on coupling with BT- and PT-sulfone[185]

3.6.4 Preparation of the sulfone (272)

The mycolic motif was required with three different α-alkyl chain lengths in this study, in turn requiring three different sulfones; the literature procedure was used to prepare C_{20} and C_{22} sulfones.[93, 175] The C_{12} sulfone was prepared as in Scheme (**2-13**).

1-Bromododecane was stirred with 1-phenyl-1*H*-tetrazole-5-thiol in the presence of potassium carbonate in acetone at room temperature overnight. The resulting sulfide (**271**) was purified by re-crystallization from methanol/acetone (1:1). The formation of the product (**271**) was confirmed by the proton NMR spectrum, which gave a multiplet for the five aromatic between 7.75-7.62 ppm and a triplet at 3.37 (J = 7.25 Hz) for the CH_2 group adjacent to sulfur. The carbon NMR spectrum gave signals for the phenyl group between 133-123 ppm and for the carbon on the tetrazole at δ 155ppm (Scheme (**3-13**)).

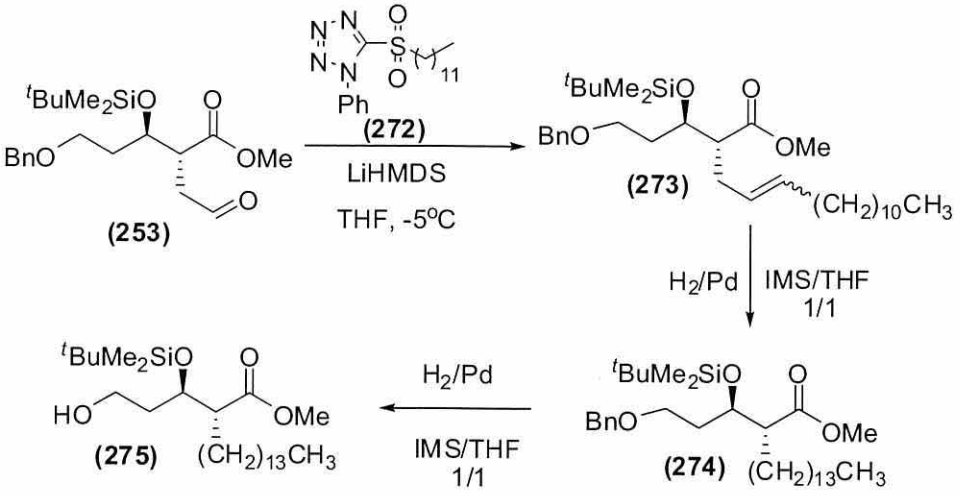


Scheme (3-13): Preparation the sulfone (272)

The next step was the oxidation of the sulfide using a suitable oxidising agent. The sulfone (**272**) was prepared by oxidation of the sulfide (**271**) using ammonium molybdate (VI) tetrahydrate and hydrogen peroxide as oxidant, in a mixture of THF and acetone (1:1) since this reagent requires a polar solvent. The formation of the products (**272**) was confirmed by the proton NMR, with the CH_2 adjacent to sulfur shifting from 3.4 ppm to 3.7 ppm.

3.6.5 The chain extension

The next step was reaction of the aldehyde (**253**) with the sulfone (**272**) and lithium bis(trimethyl silyl)amide (LiHMDS) at -5 °C and then at room temperature for 1 hour. The formation of product, alkene (**273**), was verified by the proton NMR which gave two multiplet signals between 5.46-5.32 ppm and between 5.33-5.18 ppm with an integration of 1 proton each, relating to the alkene protons. The carbon NMR spectrum showed the alkenes (**273**) carbon signals between 132-127 ppm since the alkene is a mixture of (*E/Z*) isomers (Scheme (**3-14**)).



Scheme (3-14): Preparation of the Mycolic Motif (275)

The alkene (**273**) was hydrogenated using hydrogen gas and palladium on carbon as catalyst in THF and IMS (1:1). The product (**274**) showed a disappearance of the alkene proton in the proton NMR spectrum, but still showed signals for the benzyl group with a multiplet between 7.33-7.31 ppm and a singlet at 4.44 ppm, but the reaction mixture stopped absorbing hydrogen at this point. The products (**274**) was purified by column chromatography eluting with petrol/ether (1:2) and the hydrogenation was continued, until the proton NMR showed the lack of benzyl signals in the product (**275**); the infra red spectrum showed a very wide absorbance at 3435cm^{-1} for the hydroxyl group (Scheme (**3-14**)).

In the same way, two other mycolic motifs, (**145**) and (**146**) with different required chain lengths were prepared by the known method. (See Appendix p 250 for experimental details).[93]

3.7 Synthesis of the complete mycolic acid (208)

3.7.1 General approaches

The initial goal of this project was the synthesis of mycolic acid (**208**). The mycolic motif (**145**) was prepared as presented in Section (**1.14.3**) and was the first problem solved in this project. The meromycolate moiety contains one functional group, a double bond. Among many possible methods for synthesising the the (*Z*)-double bond, three were tested in this work.

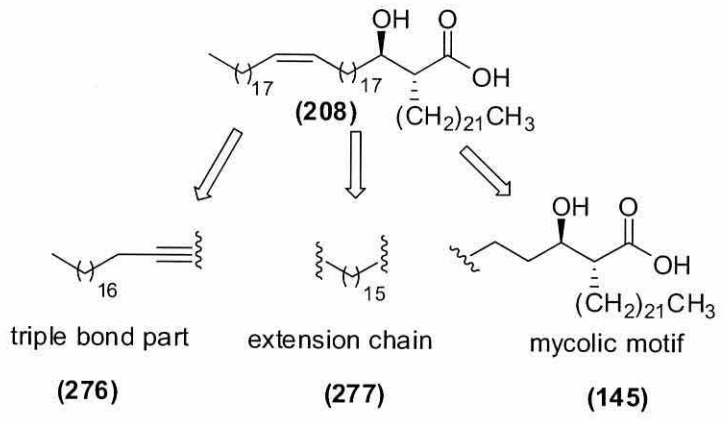
- Hydrogenation of a triple bond in the presence of a catalyst.

-The Wittig reaction, since it has been reported to favour the *cis*-isomer.

- The Julia reaction for comparison with the Wittig reaction.

3.7.2 The alkyne route

In the first approach, the alkene was to be introduced by selective hydrogenation of an alkyne. The strategy for the preparation of mycolic acid (**208**) was to link three parts as in Scheme (**3-15**). The first part was the mycolic motif (**145**) which contains two chiral centres in (*R, R*) configuration as described in Section (**1.14.3**). It is reported that in the major natural compnent the chain between the β-hydroxyl group and the double bond contains seventeen carbons as in (**208**).[37] Thus, it was necessary to prepare a bifunctional chain 15 carbons (**277**) in length for coupling with alkyne (**276**) to give the correct (natural) chain length. Finally, a triple bond with an eighteen carbon chain was required in order to match the structure of the natural product (Scheme (**3-15**)).



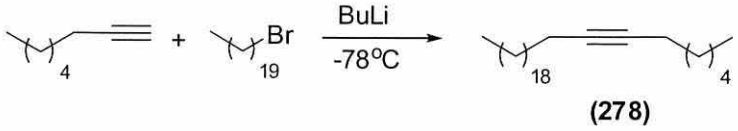
Scheme (3-15): Retrosynthesis of mycolic acid (208)

3.7.2.1 Hydrogenation of a triple bond

Hydrogenation of a triple bond to obtain an alkene in a *cis*-stereochemistry was developed by Campbell and Eby[193] who reported the use of Raney nickel or colloidal palladium, since a mild catalyst is required to prevent the hydrogenation from continuing past the alkene stage.[193] There is also a report of the synthesis of unsaturated fatty acids following the same method, with Raney nickel as the catalyst for the hydrogenation of the corresponding alkyne.[194] The preparation of a *cis*-alkene has also been reported by Li *et al.*[195] using nickel acetate tetrahydrate and sodium borohydride .[195-197]

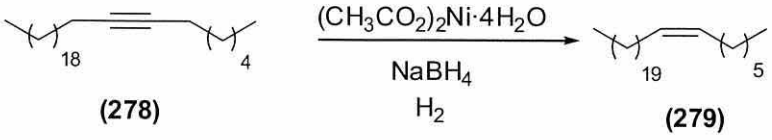
3.7.2.2 Model hydrogenation reaction

An alkyne was prepared as a model. In order to form this alkene, *n*-butyllithum (0.066 mmol) was added to 1-octyne (11.06 mmol) at –78 °C in dry THF and stirred for 2 hours, followed by adding 1-bromoeicosane (5.53 mmol), after which the reaction was stirred overnight at room temperature. The alkyne product (**278**) was verified by proton NMR, which it gave a triplet at 2.14 ppm for the CH_2 adjacent to the triple bond. The carbon NMR gave two signals at 80.23 and 80.22 ppm for the alkyne carbons, (Scheme (**3-16**)).[198,199,200]



Scheme (3-16): Preparation of model alkyne

The preparation of the *cis*-alkene used the method of Li *et al.*[195] This was done by mixing nickel acetate tetrahydrate (3.83 mmol) and sodium borohydride (0.38 mmol) in absolute ethanol, followed by the addition of ethylene diamine, and octacos-7-yne (**278**) (3.83 mmol) in THF (4 ml). The reaction was monitored until it absorbed the right amount of hydrogen to reduce one double bond. The product (**279**) was confirmed from the proton NMR spectrum which showed a wide triplet for two protons at 5.36 ppm (J = 4.7 Hz). The ^{13}C NMR spectrum showed signals at δ 129.91 and 129.89 ppm for the alkene carbons. The formation of the *cis*-alkene was further confirmed by the infrared spectrum which gave an absorbance at 721 cm^{-1} for the *cis*-isomer (Scheme (**3-17**)).[201]

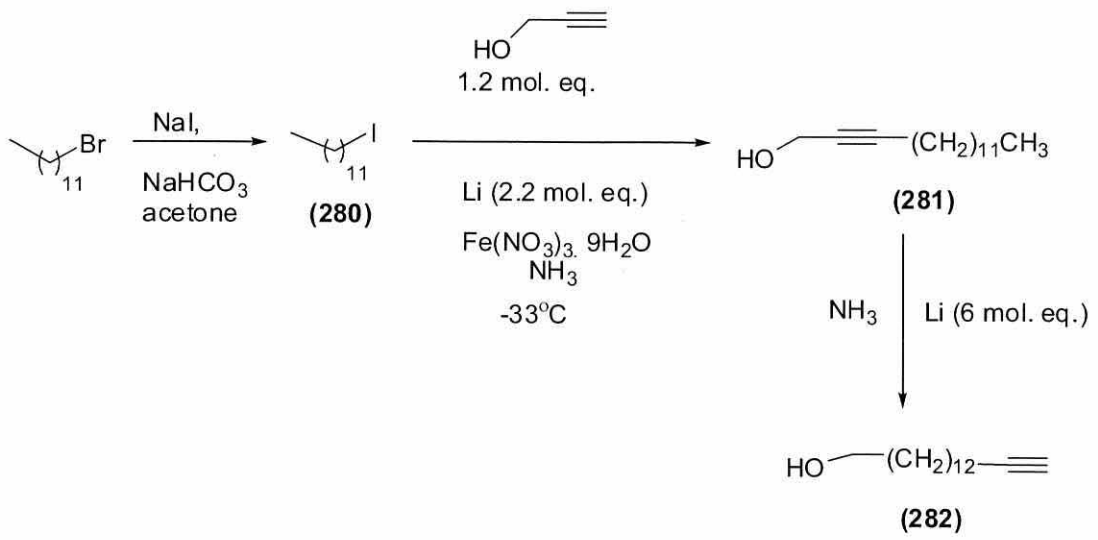


Scheme (3-17): Hydrogenation of alkyne (278) to alkene (279)

3.7.2.3 The bifunctional chain

In order to form the extended chain between the alkene and the hydroxyacid of (**208**), a C-15 chain with functional groups at 1 and 15-positions, (**277**), was required (Scheme (**3-18**)). To prepare it, 1-bromododecane was first stirred with sodium iodide

in acetone in the presence of sodium bicarbonate to produce the corresponding iodide. Proton NMR was used to confirm the formation of the (**280**) which gave a triplet at 3.2 ppm for the CH_2 group adjacent to iodine. The 1-iodododecane produced (80.2 mmol) was reacted with the dianion of prop-2-yn-1-ol (89.19 mmol), following a modification of a method reported by Vaughn *et al.*[202] The reaction initially started with the preparation of the very strong base; lithium wire was added in portions to liquid ammonia. A deep blue colour was observed, and ferric nitrate (0.2 g) was added and left to stir for 30 minutes, to prepare lithium amide. Prop-2-yn-1-ol (5 g, 89.19 mmol) in dry ether (Scheme (**2-19**)); it was necessary to add 2 mol.eq. of the base or more since the terminal proton of the alkyne is less acidic (pKa = 25)[203] than the alcohol proton (pKa = 13.6).[204] Addition of the iodide led to reaction at the softer acetylide carbon rather than the alkoxide oxygen.



Scheme (3-18): Preparation of the bifunctional C-15 chain

The formation of the product (**281**) was verified by the proton NMR spectrum which gave a triplet for $(CH_2)_a$ adjacent to the hydroxyl group at 4.25 ppm (J = 2.25 Hz), and a triplet of triplets for $(CH_2)_b$ adjacent to the triple bond at 2.21 ppm (J = 7.25, 1.9 Hz) due to the adjacent to $(CH_2)_c$, and the other $(CH_2)_a$ across the triple bond (Figure (**3-4**)). The carbon NMR gave signals at 86.69 and 51.44 ppm for the alkyne carbon and the carbon next to the oxygen, respectively. The terminal methyl group showed a triplet at 0.88 ppm (J = 6.95 Hz).

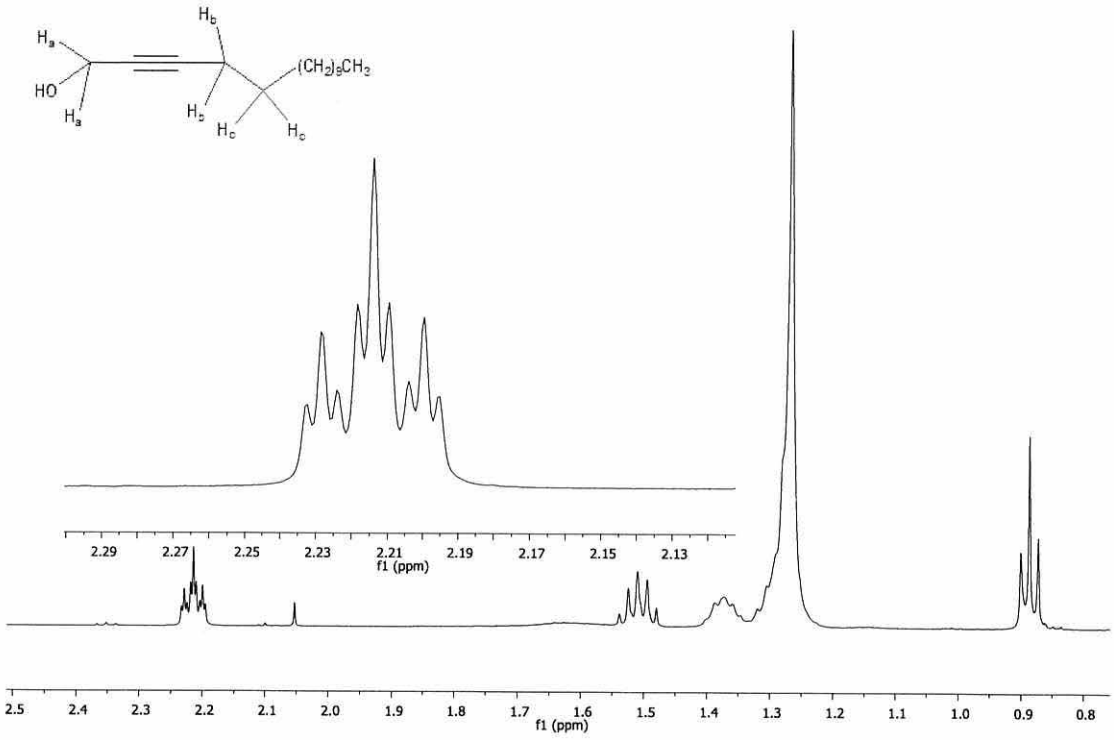


Figure (3-4): Proton NMR spectrum for compound (281)

The final step was the transfer of the triple bond to the end of the chain using a 'zipper reaction', which required the use of a very strong base.[205, 206] This reaction was first reported as an effective method for the transfer of a triple bond along a hydrocarbon chain.[207] The initial step of this reaction was the preparation of the strong base, since it is required to transfer the triple bond from one end of the chain to the other. The preparation of this base using sodium hydride and 1,3-diaminopropane has been reported; one disadvantage of this procedure is the use of sodium hydride which is hazardous and has a short shelf life.[208] An alternative method developed for the preparation of the base was the treatment of potassium amide in liquid ammonia with 1,3-propanediamine at 80 °C.[209] In this study, the base was prepared by treating 1,3-diaminopropane with lithium wire and heating the solution to 70 °C until a blue colour was discharged. This was followed by adding potassium-*tert*-butoxide (6.25 g, 55 mmol) and pentadec-2-yn-1-ol (3.13 g, 139.6 mmol). The reaction was quenched by pouring into ice water.[208]

The successful formation of the product (**282**) was confirmed by proton NMR which showed a triplet for the $(CH_2)_a$ group adjacent to oxygen at 3.54 ppm (J = 6.65 Hz). The alkyne proton CH_c showed a triplet at 1.88 ppm (J = 2.5 Hz) and the $(CH_2)_b$ group

adjacent to the alkyne group gave a triplet of doublets at 2.11 ppm (J = 7.25, 2.5 Hz) demonstrating the lack of a terminal methyl group (Figure (**3-5**)). The carbon NMR gave signals at 84.54 ppm and 67.94 ppm for the alkyne carbons and 62.57 ppm for the carbon next to the oxygen.

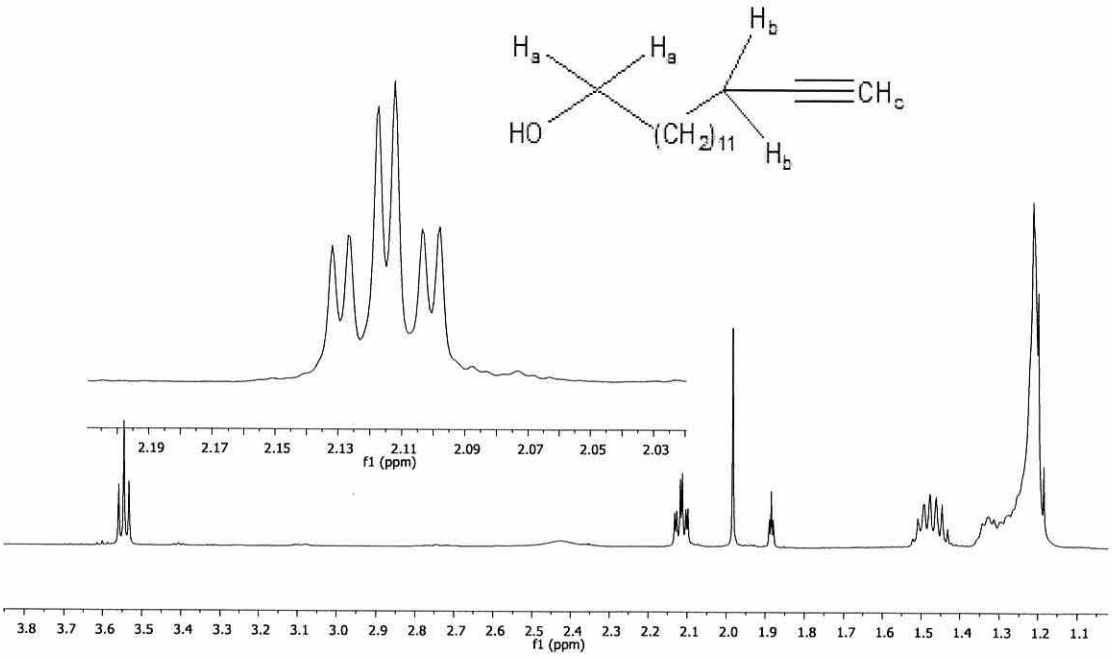
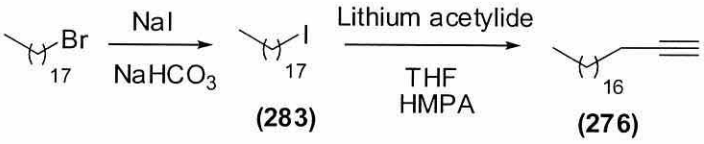


Figure (3-5): Proton NMR for compound (282)

3.7.2.4 Preparation of the terminal part (276)

The third part was the preparation of the terminal alkyne chain (**276**) with a C-20 chain length. This was synthesized by treating 1-bromo-octadecane with sodium iodide in acetone in the presence of sodium hydrogen carbonate, in order to replace the bromine with iodine for the next reaction since it is a better leaving group. The formation of 1-iodo-octadecane (**283**) was confirmed by proton NMR which gave a triplet for the CH_2 adjacent to the iodide at 3.2 ppm (J = 6.95 Hz). This reaction was followed by a reaction between the lithium acetylide complex and 1-iodo-octadecane (**283**) to give (**276**) which was confirmed by proton NMR which gave a triplet for the acetylene proton at 1.94 ppm (J = 2.5 Hz) and a triplet of doublets for the CH_2 adjacent to the triple bond due at 2.18 ppm (J = 7.25, 2.85 Hz) due to the adjacent CH_2 group and the allyl effect through the triple bond for the acetylene proton. The ^{13}C

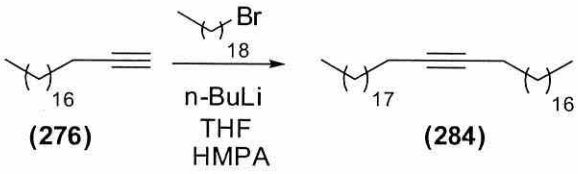
NMR showed a signal at 84.80 and 68.00 ppm for the triple bond carbons (Scheme (**3-20**)).[210]



Scheme (3-20): Preparation of the model alkyne (276)

3.7.2.5 Studies of the chain extension

The next key step of the proposed synthesis required the coupling of the alkyne (**276**) to a short chain mycolic motif (**145**). Initially a model reaction was carried out to ensure that the coupling of the alkyne to a model alkyl bromide, nonadecyl bromide, was not affected by the very long alkyl chains involved.



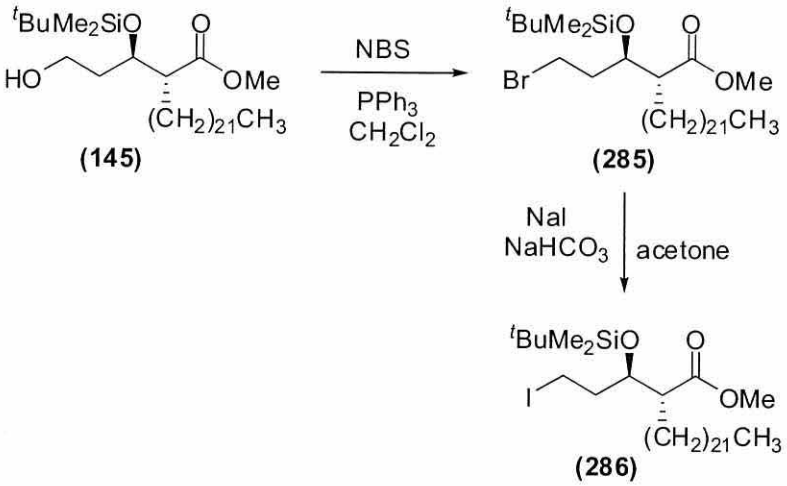
Scheme (3-21): a model reaction with alkyne (276)

The product was obtained in 74 % yield as confirmed by proton NMR with both CH_2 adjacent to the triple bond on both sides giving a doublet of triplets at 2.20 ppm (J = 6.95, 2.55 Hz) and 2.16 ppm (J = 6.95, 1.6 Hz) (Scheme (**3-21**)).

Despite the relatively good yield of this reaction, it was decided to couple the mycolic motif (**145**) with eicos-1-yne (**276**). In order to do this, it was necessary to change the hydroxyl group to iodide since the iodine is a better leaving group. This was done in two steps (Scheme (**3-22**)).

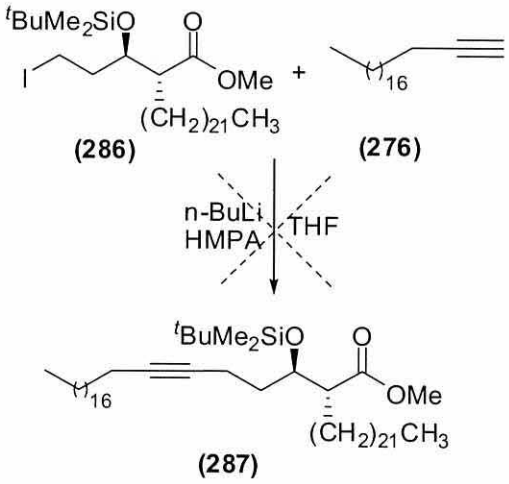
First, the hydroxyl group was exchanged for bromide using *N*-bromosuccinimide (1.14 mmol) and triphenylphosphine (1 mmol). The formation of the product (**285**) was verified by the proton NMR spectrum, which gave a triplet for the CH_2 adjacent to the bromide at 3.43 ppm (J = 6.6 Hz). The ^{13}C NMR showed the disappearance of the signal adjacent to oxygen. The specific rotation was $[\alpha]_D^{24}$ +5.80 (c = 0.98, $CHCl_3$). Finally, the bromide was replaced with iodide using sodium iodide and sodium hydrogen carbonate in acetone. The formation of the product (**286**) was

confirmed by the proton NMR spectrum which gave a triplet for CH_2 the adjacent to iodide at 3.34 ppm (J = 6.6 Hz). The specific rotation was found to be $[\alpha]_D^{20}$ + 6.22 (c = 1.02, $CHCl_3$) (Scheme (**3-22**)).



Scheme (3-22): Replacing hydroxyl group with iodine

A coupling reaction was attempted between eicos-1-yne and (*R*)-2-[(*R*)-1-(*tert*-butyl-dimethyl-silanyloxy)-3-iodo-propyl]-tetracosanoic acid methyl ester (**286**) using the same conditions used in (Scheme (**2-21**)) without obtaining the desired compound (**287**) (Scheme (**3-23**)).

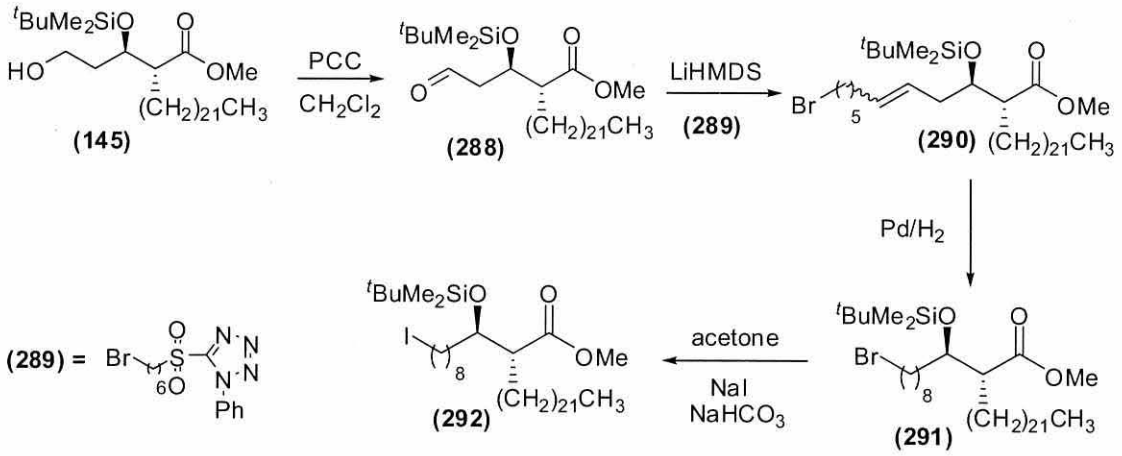


Scheme (3-23): Attempted coupling between compounds (276) and (286)

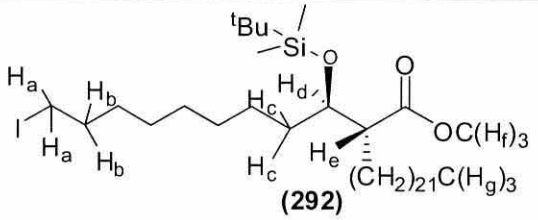
Since this model was not successful, another approach was tried. In order to test the hypothesis that steric- or stereoelectronic effects of the *tert*-butyldimethylsilyl group hinder the reaction with compound (**286**), a chain extension was performed on the

mycolic motif to make the side chain longer and thus increase the distance between the iodide and the motif.

An extension to the side chain was done using a six carbon unit (Scheme (**3-23**)). Initially, the mycolic motif (**145**) was oxidised using PCC in dichloromethane. The formation of the resulting aldehyde (**288**) was confirmed by proton NMR which gave a triplet for the aldehyde proton at 9.72 ppm (J = 2.2 Hz), while the protons of the CH_2 group adjacent to the aldehyde group gave a multiplet between 2.59-2.49. The carbon NMR showed a signal at 201.06 and 173.95 ppm for the carbonyl carbon of the aldehyde and the carboxylic acid, respectively. The specific rotation of (**288**) was $[\alpha]_D^{26}$ -4.98 (c = 1.23, $CHCl_3$). A Julia coupling was applied to the aldehyde by adding lithium bis(trimethylsilyl) amide at –5 °C to a solution of tetrazole (**289**) and ester (**288**) in dry THF. The formation of the product (**290**) was confirmed by proton NMR which gave a multiplet between 5.44-5.35 ppm for the alkene proton, while the CH_2 adjacent to the bromide showed a triplet at 3.33 ppm (J = 6.95). The ^{13}C NMR gave alkene carbons signals at 133.05 and 124.92 ppm, further confirming the formation of alkene (**290**). The next step was the hydrogenation of compound (**290**) using palladium on carbon (10 %) in a hydrogen atmosphere. The formation of compound (**291**) was verified by proton NMR which gave a doublet of doublets of triplets for the proton for the β-chiral centre at 3.91 ppm (J = 9.15, 6.95, 4.75 Hz), the CH_2 adjacent to the bromide showed a triplet at 3.41 ppm (J = 6.95 Hz) and the proton at the α-chiral centre gave a doublet of doublets of doublets at 2.49 ppm (J = 10.7, 6.9, 3.45 Hz) with a lack of any peak in the alkene region. The specific rotation was $[\alpha]_D^{24}$ -7.17 (c = 0.85, $CHCl_3$). The final step was replacing the bromide with iodide by stirring compound (**291**) with sodium iodide and sodium hydrogen carbonate in acetone (Scheme (**3-24**)). The formation of the product (**292**) was confirmed by the use of the proton NMR; data shown in Table (**3-2**). The specific rotation was $[\alpha]_D^{24}$ -4.77 (c = 0.55, $CHCl_3$).



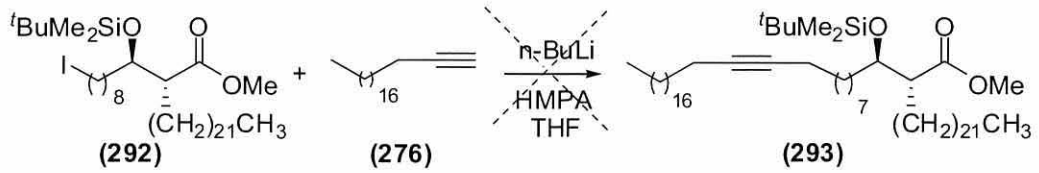
Scheme (3-24): Preparation of compound (292)



H_x	δ	Multiplicity	Integration	J (H_z)
H_a	3.18	t	2	9
H_b	1.82	q	2	6.95
H_c	1.57-1.47	m	2	-
H_d	3.93	ddt	1	10.1, 6, 4.1
H_e	2.52	ddd	1	9.45, 8.5, 3.45
H_f	3.62	s	3	-
H_g	0.88	t	3	6.95
SiMe	0.04	s	3	-
SiMe	0.02	s	3	-

Table (3-2): NMR analysis of compound (292)

n-Butyllithium (0.73 mmol) was added to eicos-1-yne (**276**) (0.98 mmol) in dry THF at 0 °C. The mixture was stirred for 2 hours to deprotonate the alkyne (**276**) proton and the iodide (**293**) in HMPA was added. Work up of the reaction did not show any evidence for the formation of the desired compound (**293**) (Scheme (**3-25**)).



Scheme (3-25): Model coupling reaction

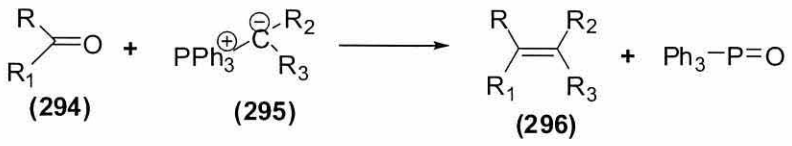
Because these reactions did not appear to work with a substrate containing the mycolate motif, an alternative approach was then followed.

3.7.3 The Wittig approach

The second approach to obtain the target *cis*-mycolic acid (**208**) involved a Wittig reaction. In order to optimise the conditions, it was decided to synthesize a smaller mycolic acid (**207**) first. Mycolic acid (**207**) has been reported in *Corynebacterium diphtheriae* and is a small target in comparison with mycolic acid (**207**) which has the same functional group in the meromycolic portion.[37b]

3.7.3.1 The Wittig reaction

The Wittig reaction is the key step in this strategy. This, in general, is a reaction between an aldehyde or a ketone (**294**) with a triphenyl phosphonium ylide (**295**), forming an alkene in the *cis* or *trans* configuration (Scheme (**3-26**)). A high selectivity for an (*E*) or (*Z*) alkene can be achieved, depending on the conditions employed in the reaction or the substance of the starting material.

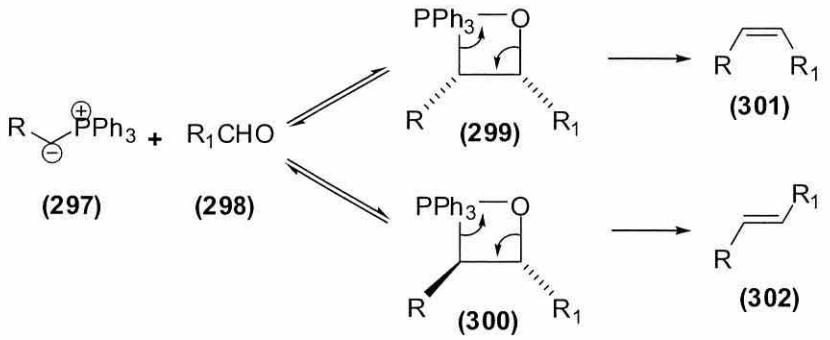


R, R_1, R_2 and R_3 = alkyl or H

Scheme (3-26): General scheme of the Wittig reaction

The Wittig reaction in general gives product (**296**) in the *Z*-configuration unless the ylid (**295**) is stabilised by an electron withdrawing substituent. It has been reported

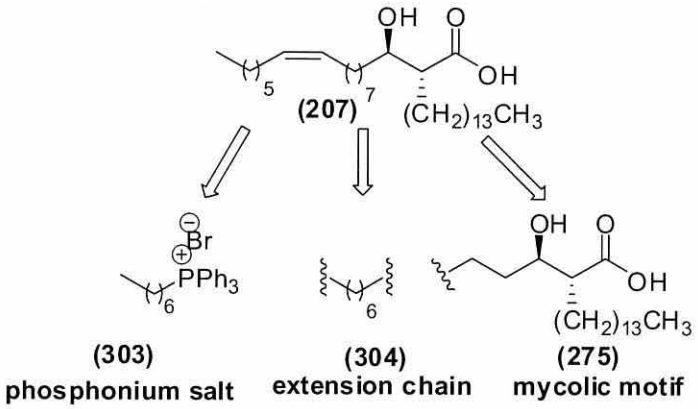
that the stereochemistry of the product is dependent on the oxaphosphetane elimination step. The initial step in the Wittig reaction treats the phosphonium salt with a base to form an ylid (**297**). The stability of that ylide leads to either the (*Z*) or (*E*) configuration of the alkenes (**301**) and (**302**); a non-stabilised ylide (**297**) leads to the (*Z*) isomer, while a more stable gives the (*E*) isomer.[211] The stability of the ylide (**297**) is due to the R-group in the phosphonium salt; this contains an electron withdrawing group and makes the ylide (**297**) more stable.[203,212] Other conditions can affect the stereochemistry of the product of a Wittig reaction.[213] The solvent used in the reaction can affect the stereochemistry of the product. Reitz *et al.* demonstrated the effect of the solvent and in general concluded that THF, DME, ether and *tert*-butyl methyl ether are the solvents of choice for higher yields of (*Z*) isomers, while alcohols and DMSO should be avoided.[214] The base used to generate the anion from the phosphonium salt can affect the (*Z/E*) ratio of the product. Potassium bases give a higher ratio of *cis* isomers to *trans* isomers, while sodium bases are less selective and lithium bases give a higher ratio of *trans* to *cis* isomers (Scheme (**3-27**)).[212, 213, 215]



Scheme (3-27): Forming *cis* and *trans* isomers in the Wittig reaction[203]

3.7.3.2 Synthesis of (207)

The strategy planned for the preparation of this mycolic acid (**207**) used the Wittig reaction as the key step (Scheme (**2-25**)). The first part, *i.e.* the mycolic motif (**275**), had already been prepared in section (**2-11**). The second part was the preparation of an extension to the chain with a C-6 bifunctional chain starting with C-6 diol (**304**). The third part was the preparation of a phosphonium salt (**303**) with the right number of carbons atoms in its chain (Scheme (**3-28**)).

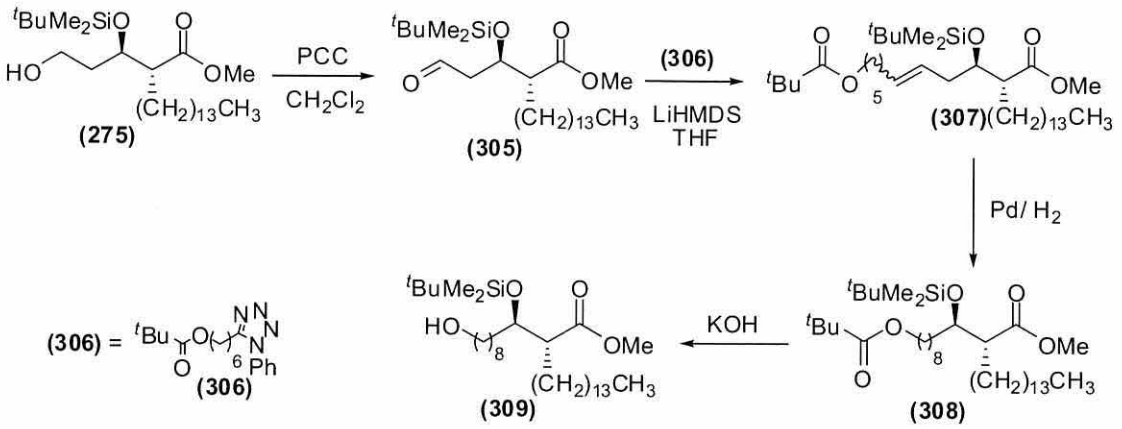


Scheme (3-28): Strategy for the synthesis mycolic acid (207)

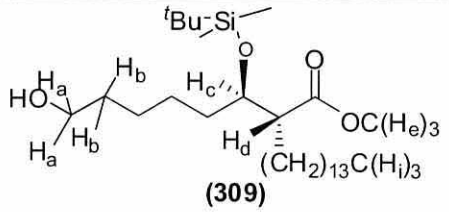
In order to prepare the mycolic motif with the right number of carbons in the side chain (Scheme (**2-26**)), oxidation of the mycolic motif (**275**) was done using PCC. The formation of the aldehyde (**305**) was confirmed using proton NMR which gave a triplet for the aldehyde proton at 9.80 ppm (J = 2.55 Hz). The carbon NMR spectrum provided other evidence, showing carbonyl carbons at 201.16 and 173.98 ppm for the aldehyde and carboxylic acid carbonyl, respectively. The aldehyde (**305**) was coupled to the 6-(1-phenyl-1H-tetrazol-5-yl)hexyl pivalate (**306**) and LiHMDS as a base was added in dry THF. The formation of the alkene (**307**) was verified by the proton NMR spectrum, which gave a multiplet for the two alkene protons between 5.47-5.43 ppm, while the CH_2 adjacent to the oxygen gave a triplet at 4.05 ppm (J = 6.3 Hz) and a singlet for the *t*-butyl of the silyl ether was seen at 0.86 ppm. The ^{13}C NMR gave signals for the two carbonyl carbons at 175.04 and 174.85 ppm. The alkene carbons gave a signal for the *trans* alkene carbons at 133.22 and 131.59 ppm and for the *cis* alkene carbons at 125.27 and 124.81 ppm.

The next step was hydrogenation to reduce the double bond using palladium on carbon (10%) as the catalyst in a hydrogen atmosphere. After one hour of hydrogenation, the formation of the product (**308**) was confirmed by the proton NMR spectrum which showed no signals in the alkene region. The CH_2 adjacent to the oxygen showed as a triplet at 4.04 ppm (J = 6.65 Hz), while the protons at the chiral centres showed a doublet of triplets for the β-chiral proton at 3.90 ppm (J = 9.75, 5.05 Hz), and a doublet of doublets of doublets at 2.52 ppm (J = 10.75, 6.95, 3.8 Hz). The carbon NMR showed the disappearance of any alkene carbons with an absence of

signals in the alkene region. The specific rotation was $[\alpha]_D^{23}$ –4.01 (c = 1.52, $CHCl_3$) (Scheme (**3-29**)).



Scheme (3-29): Extension of the side chain of the mycolic motif to give (309)

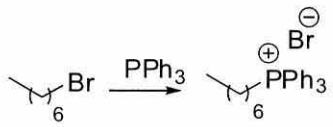


H_x	Δ	Multiplicity	Integration	J (H_z)
H_a	3.63	t	2	6.65
H_b	1.56	q	2	6.6
H_c	3.89	dt	1	6.95, 5.05
H_d	2.52	ddd	1	10.75, 6.95, 3.8
H_e	3.65	s	3	-
SiMe	0.04	s	3	-
SiMe	0.01	s	3	-
^tBu	0.86	s	9	-
H_i	3.65	t	3	6.95

Table (3-3): NMR analysis for compound (309)

The hydrogenation was followed by hydrolysis of compound (**308**) using potassium hydroxide in a solution of THF: MeOH: H_2O in the ratio 10: 10: 1. The product (**309**) was confirmed by the proton NMR spectrum which is shown in Table (**3-3**). The infrared spectrum showed broad absorbance at 3435 cm^{-1} due to the hydroxyl group. The specific rotation for compound (**309**) was $[\alpha]_D^{24}$ -3.07 (c = 1.43, $CHCl_3$) (Scheme (**3-26**)).

The third part of the strategy in Scheme (**2-26**) was the preparation of the phosphonium salt (**303**). This was done by refluxing 1-bromoheptane and triphenylphosphine in toluene for three days. The product (**303**) gave a multiplet in the proton NMR spectrum for the 15 aromatic protons between 7.82-7.36 ppm, while the CH_2 adjacent to phosphorus gave a multiplet between 3.72-3.67 ppm (Scheme (**3-30**)).

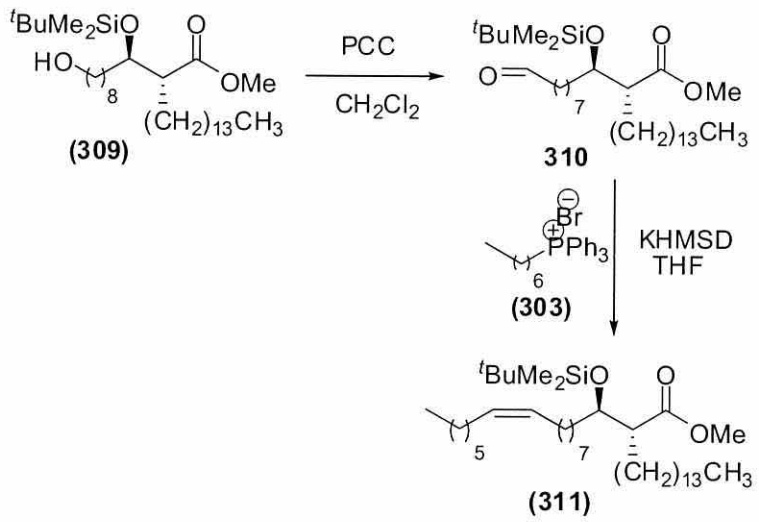


(303)

Scheme (3-30): Preparation of phosphonium salt (303)

The first step of forming aldehyde (**310**) in order to couple it with the ylid from phosphonium salt (**303**) was the oxidation of alcohol (**309**) with PCC in CH_2Cl_2 at room temperature; the reaction was monitored by TLC until the starting material had disappeared. The proton NMR spectrum resulting aldehyde (**310**) gave a triplet for the aldehyde proton at 9.76 ppm (J = 1.55 Hz) and the protons of the CH_2 group adjacent to the aldehyde group gave a triplet of doublets at 2.42 ppm (J = 7.25, 1.55 Hz). The ^{13}C NMR spectrum gave a signal at 202.83 ppm and 175.08 ppm for the two carbonyl groups of the aldehyde and ester, respectively. The specific rotation for the aldehyde was $[\alpha]_D^{24}$ -7.09 (c = 1.72, $CHCl_3$).

The first step of the Wittig reaction was the preparation of the ylid by treating the phosphonium salt (**303**) with a potassium bis(trimethylsilyl)amide solution (1.0 M in THF) since this gives more of the *cis*-isomer compared to a lithium bis(trimethylsilyl) amide solution.[214, 215] The addition of the base was done at -10 °C and the mixture was left to stir for 30 minutes before the aldehyde (**310**) was added to the reaction mixture (Scheme (**3-31**)).



Scheme (3-31): Preparation of protected *cis*-alkenemycolic acid (311)

The formation of the product (**311**) was confirmed by the proton NMR spectrum which showed a doublet of triplets for the *cis* alkene protons at 5.35 ppm while the *trans*-alkene isomer protons gave a multiplet between 5.4-5.38 ppm (Figure (**3-6**)). The ratio of *trans* alkene protons to *cis* alkene protons was 2.1:0.31. The proton at the β-chiral centre gave a doublet of doublets of doublets at 3.91 ppm (J = 8.8, 6.95, 4.7 Hz) while the proton at the α-chiral centre showed a doublet of doublets of doublets at 2.53 ppm (J = 11, 7.25, 3.8 Hz). The carbon NMR spectrum gave peaks at 130.38 and 130.27 ppm for the *trans* alkene carbons and 129.92 and 129.81 ppm for the *cis* alkene carbons, as well as a peak at 175.14 ppm for the carbonyl carbon. The infrared spectrum gave absorbances at 836 and 775 cm^{-1} and no peak was displayed in the *trans* alkene[201] in region between 900–1000 cm^{-1}. The specific rotation was $[\alpha]_D^{24}$ -5.76 (c = 1.18, $CHCl_3$).

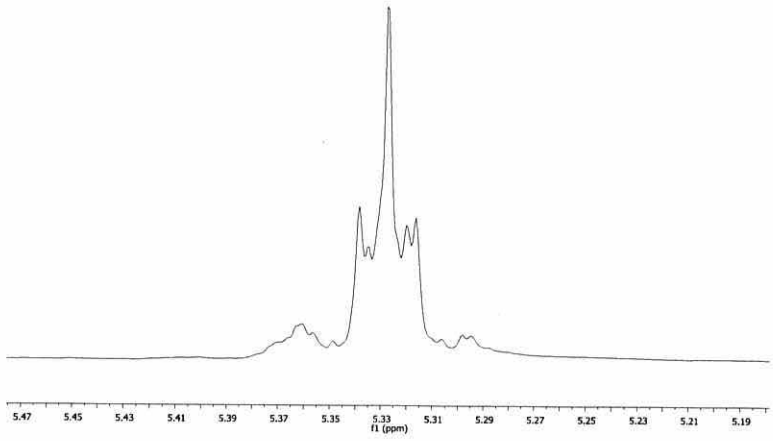
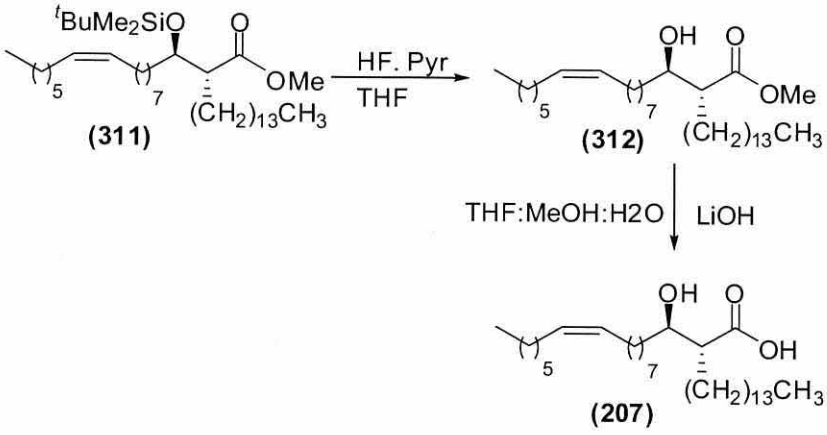


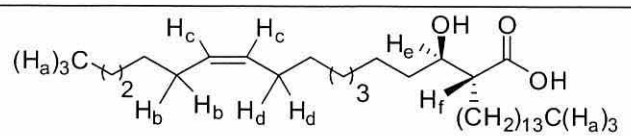
Figure (3-6): Proton NMR for the alkene protons of compound (311)

The mycolic acid (**311**) was protected at the β-hydroxy and carboxylic acid groups. An HF.pyridine complex was used to deprotect the β-hydroxy group since the TBDMS group required acid media for its removal; this complex provides mild conditions compared to the alternative reagent, acetic acid. The use of formic acid gives a slower reaction than HF. pyridine if employed under the same conditions.[216] Compound (**311**) was therefore stirred at 45 °C overnight with pyridine (0.2 ml) and HF.pyridine (1.5 ml). The formation of the product (**312**) was confirmed by the proton NMR spectrum which demonstrated the disappearance of the nine proton singlet at 0.89 ppm and the signals belonging to the methyl groups adjacent to the silyl group. Moreover, the product gave a molecular ion in the MALDI MS 531.475 as expected for (**312**). The specific rotation was $[\alpha]_D^{24}$ +7.10 (c = 1.71, $CHCl_3$).



Scheme (3-32): Final steps for the preparation of the mycolic acid (207)

The final step was the hydrolysis of the ester (**312**), in order to obtain the carboxylic acid (**207**). For the hydrolysis of the ester (**312**), lithium hydroxide was chosen as the reagent since it is mild base compared to sodium hydroxide and potassium hydroxide. Also, Yuen *et al.* reported a better conversion for lithium hydroxide.[217] The hydrolysis of ester (**312**) was performed by stirring it with lithium hydroxide monohydrate (15 mol. eq.) in a mixture of THF, water and MeOH at 45 °C overnight (Scheme (**3-32**)). The formation of the product was confirmed by the proton NMR spectrum; the data are presented in Table (**3-4**). The specific rotation was $[\alpha]_D^{22}$ +11.27 (c = 0.51, $CHCl_3$).



(207)

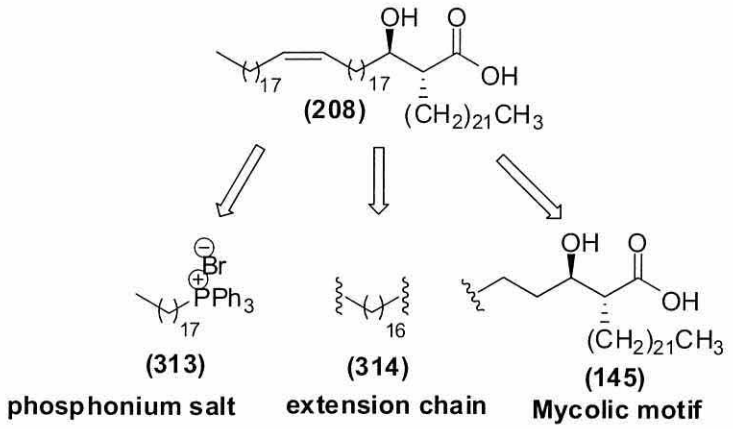
H_x	Δ	Multiplicity	Integration	J (H_z)
H_a	0.88	t	6	4.7
H_b	1.74-1.67	m	1	-
H_c	5.35-5.33	m	2	-
H_d	1.53-1.44	m	2	-
H_e	3.71	dt	1	9.5, 5.05
H_f	2.44	dt	1	9.15, 5.35

Table (3-4): NMR analyses for mycolic acid (207)

3.7.4 Synthesis of mycolic acid (208)

3.7.4.1 Overview

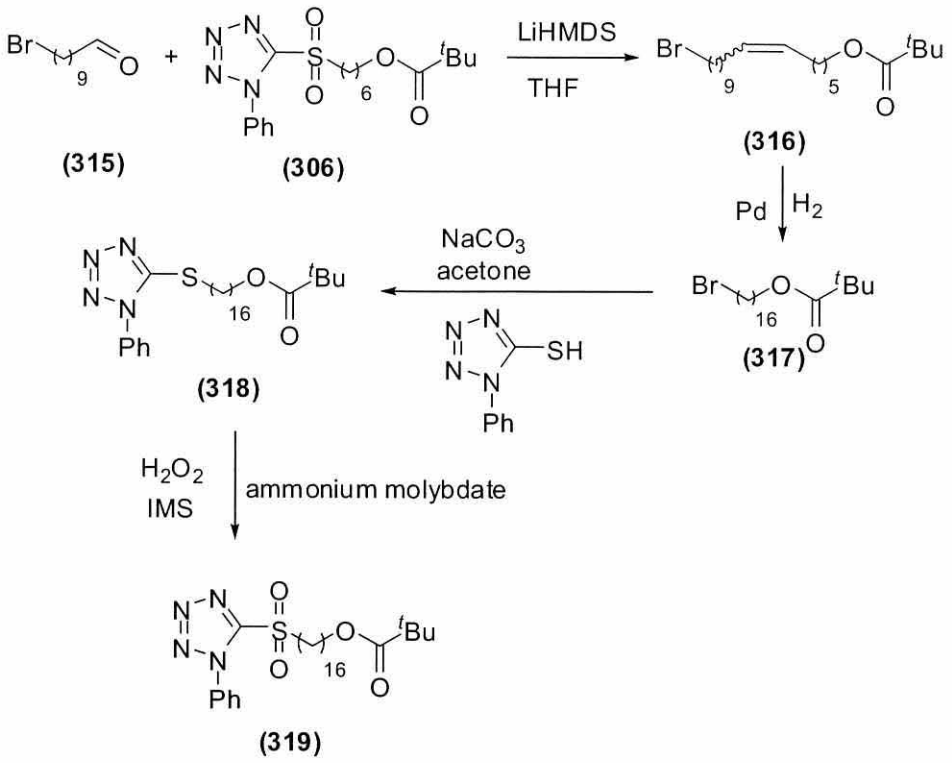
Since the strategy used for the synthesis of the mycolic acid (**207**) was successful, the same method was applied for the mycolic acid (**208**). The mycolic motif (**145**) was prepared with a C-22 side chain using exactly the same steps as for the preparation of the previous mycolic motif (**145**) described in Section (**1.14.3**). Similarly, the phosphonium salt (**313**) was prepared with the correct chain length and the two portions were linked using Scheme (**3-33**).



Scheme (3-33): Suggested strategy for the preparation of mycolic acid (208)

3.7.4.2 Preparation of the extended chain (314)

A Julia reaction was used to prepare a bifunctional chain for extending the mycolic motif (**145**). The first reaction was a coupling between bromodecanal (**315**) and 6-((1-phenyl-1H-tetrazol-5-yl)sulfonyl)hexyl pivalate (**306**) in dry THF in the presence of LiHMDS. The formation of alkene (**316**) was confirmed by proton NMR which gave a multiplet for the alkene protons between 5.40-5.29 ppm while the CH_2 adjacent to the oxygen gave a triplet at 4.02 ppm (J = 6.6 Hz) and the CH_2 adjacent to bromine showed a triplet at 3.37 ppm (J = 6.6 Hz). The carbon NMR showed a peak at 178.41 ppm for the carbonyl group and peaks at 130.71, 130.20, 129.64 and 129.15 ppm for the *trans* and *cis* isomers of the alkene (Scheme (**3-34**)).

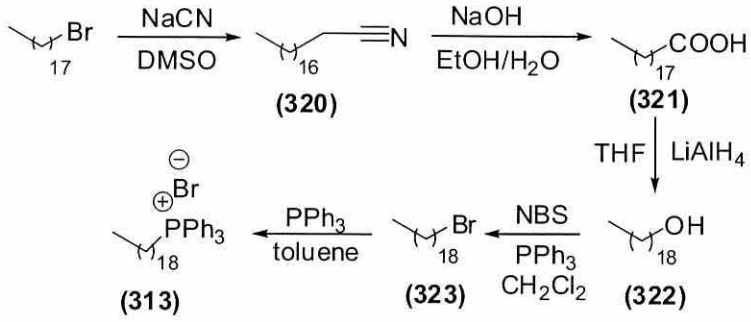


Scheme (3-34): Preparation of the C-16 bifunctional chain

The next step was the hydrogenation of compound (**316**) using Pd on carbon (10 %) in a hydrogen atmosphere. The proton NMR spectrum of the product (**317**) showed the disappearance of any alkene signal. The CH_2 adjacent to the oxygen gave a triplet at 4.02 ppm (J = 6.65 Hz) and the CH_2 adjacent to the bromine gave a triplet at 3.38 ppm (J = 6.6 Hz). The carbon NMR spectrum confirmed the absence of any signal in the alkene region. In the next reaction, compound (**317**) was treated with 1-phenyl-1*H*-tetrazole-5-thiol in acetone in the presence of anhydrous potassium carbonate. The sulfide product (**318**) was confirmed by the proton NMR spectrum which showed a multiplet for the phenyl group between 7.53-7.45 ppm, while the CH_2 adjacent to the oxygen gave a triplet at 3.98 ppm (J = 6.6 Hz) and the CH_2 adjacent to the sulfur gave a triplet at 3.33 ppm (J = 7.55 Hz). The ^{13}C NMR gave a signal at 178.33 ppm for the tetrazole carbon and peaks for the phenyl group at 154.27, 133.63, 129.84 and 129.57 ppm.

Finally, the sulfide (**318**) was oxidised to obtain sulfone (**309**) by dissolving it in IMS and ammonium molybdate (VI) tetrahydrate in 35 % H_2O_2, added at 0 °C. The formation of the sulfone was confirmed by proton NMR in which the CH_2 adjacent to the sulfur appeared as triplet at 3.75 ppm (J = 7.9 Hz) (Scheme (**3-34**)).

The phosphonium salt (**313**) has a nineteen carbon chain. In order to obtain this chain length, an extension to 1-bromoeicosane by one carbon was necessary. This was done by stirring 1-bromoeicosane (0.13 mmol) with sodium cyanide (0.39 mmol) at 60 °C. The formation of the product (**320**) was confirmed by the proton NMR spectrum which showed the CH_2 group adjacent to the nitrile group as a triplet at 2.33 ppm (J = 7.25 Hz). The ^{13}C NMR gave a peak at 119.78 ppm for the nitrile carbon (Scheme (**2-36**)).[210, 218] This was followed by hydrolysis of the nitrile group using sodium hydroxide in a mixture of EtOH and water (10:1.5). The formation of the resulting nonadecanoic acid (**321**) was confirmed by the proton NMR spectrum which showed the CH_2 group adjacent to the carboxylic acid as a triplet at 3.35 ppm (J = 7.6 Hz). The carbon NMR gave a peak at 178.87 ppm for the carboxylic acid carbon. The next step was reducing the acid (**321**) to alcohol (**322**) using lithium aluminium hydride in THF (Scheme (**3-35**)).

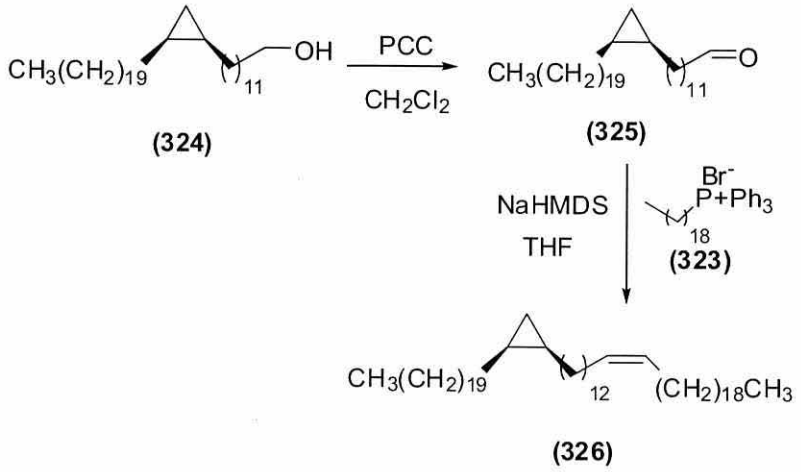


Scheme (3-35): Preparation of the phosphonium salt (313)

The resulting alcohol (**322**) was characterised by the proton NMR spectrum which gave a triplet for the CH_2 group adjacent to the hydroxyl group at 3.63 ppm (J = 6.6 Hz). The ^{13}C NMR gave a signal at 63.03 ppm for the carbon adjacent to the hydroxyl group (Scheme (**2-37**)). This was followed by replacement of the hydroxyl group with a bromine, using *N*-bromosuccinimide (76.7 mmol) with triphenylphosphine (58.8 mmol) in dichloromethane. The resulting product (**323**) was identified by the proton NMR spectrum which gave a triplet for the CH_2 adjacent to the bromine at 3.43 ppm (J = 6.9 Hz). The ^{13}C NMR gave a peak at 34.01 ppm for the carbon adjacent to the bromine. The final step in the phosphonium salt preparation involved refluxing 1-bromononadecane (**323**) with triphenylphosphine for four days to give the product (**313**); this was identified by the proton NMR spectrum which gave a multiplet

between 7.79-7.68 ppm for the phenyl groups and a triplet 3.80 (13.55 Hz) for the CH_2 adjacent to the phosphorus.

Since the mycolic acid (**208**) has a long chain, a model reaction was carried out in order to optimise the conditions of the Wittig reaction (Scheme (**3-36**)). The alcohol (**324**) was oxidised using PCC in dichloromethane for 2.5 hours, to give aldehyde (**325**); this was identified by the proton NMR spectrum which gave a triplet for the aldehyde proton at 9.77 ppm (J = 1.9 Hz) while the CH_2 adjacent to the carbonyl gave a doublet of triplets at 2.42 ppm (J = 7.25, 1.85 Hz) and the cyclopropane ring gave a multiplet between 0.52-0.49 ppm. A doublet of triplets was seen for the proton at 0.42 ppm (J = 8.2, 4.1 Hz) and a broad quartet at 0.47 ppm (J = 5.35 Hz). The ^{13}C NMR gave a signal at 202.95 ppm for the carbonyl carbon. The infrared spectrum showed an absorbance 1712 cm^{-1} for the carbonyl group.



Scheme (3-36): The model Wittig reaction

In order to couple the aldehyde (**325**) with the phosphonium salt (**313**), the salt was reacted with NaHMDS for 30 minutes. It has been noted that the phosphonium salt precipitates at low temperatures; therefore, the reaction was performed at room temperature. The base was added to the phosphonium salt (**313**), then the aldehyde (**325**) was added, resulting in the formation of (*1S,2S*)-1-((*Z*)-docos-2-enyl)-2-eicosylcyclopropane (**326**). This was confirmed by proton the NMR spectrum which gave a broad triplet at 5.36 ppm (J = 4.6 Hz) for the alkene proton, and the cyclopropane ring protons were seen as a multiplet between 0.67-0.64 ppm for the two protons H_a, and the other protons in the cyclopropane ring were seen as a doublet of triplets for the H_b at 0.57 ppm (J = 8, 4 Hz) and as a quartet for H_c at -0.31 ppm (J =

5.2 Hz) (Figure (**3-7**)). The infrared spectrum showed an absorbance at 720 cm^{-1} associated with *cis*-alkene bending.

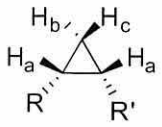
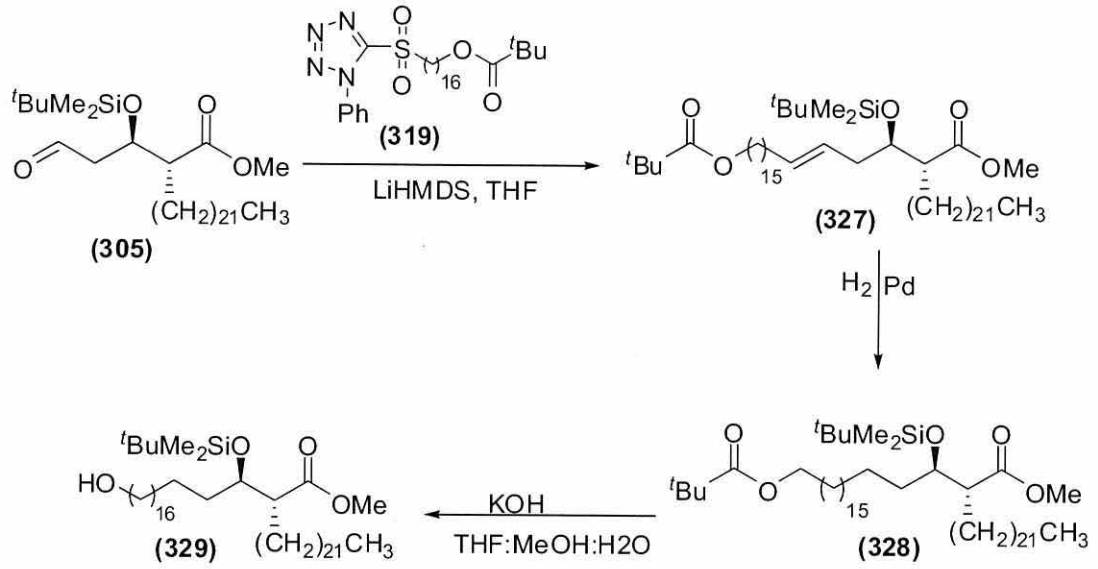


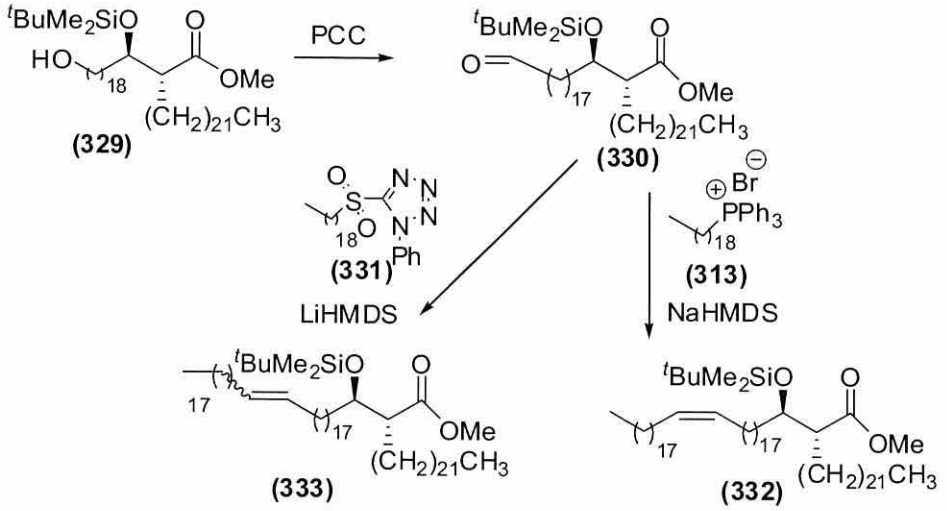
Figure (3-7): Differentiation of *cis*-cyclopropane protons

In order to apply the Wittig reaction to couple the two parts of the mycolic acid to obtain (**207**), a Julia reaction was first employed to link aldehyde (**305**) with sulfone (**319**) using LiHMDS as a base. The formation of the resulting alkene was confirmed by the proton NMR spectrum which gave a multiplet for the alkene protons between 5.44-5.41 ppm, while the CH_2 adjacent to the oxygen gave a triplet at 4.02 ppm (J = 6.65 Hz). The carbon NMR spectrum showed two carbonyl carbon signals at 179.49 and 175.04 ppm for the protecting group and the carboxylic acid, respectively, and the alkene carbons showed at 133.68, 132.06, 124.74 and 124.24 ppm for the *cis* and *trans* isomers. This was followed by hydrogenation of the alkene (**327**) using Pd on carbon (10 %) in a hydrogen atmosphere. The formation of the product (**328**) was verified by proton NMR which showed the disappearance of any signal in the alkene area, while the CH_2 adjacent to the oxygen gave a triplet at 4.04 ppm (J = 6.6Hz). The final step was the hydrolysis of the *t*-butyl protecting group, which was done by stirring compound (**328**) and potassium hydroxide (30.8 mmol) in THF:MeOH:H_2O in a ratio of 10:10:1 and refluxing for three hours. The formation of the alcohol (**329**) was confirmed by the proton NMR spectrum which showed the disappearance of a singlet at 1.2 ppm for the *t*-butyl group. The CH_2 group adjacent to the oxygen gave a triplet at 3.61 ppm (J = 6.6 Hz). The infrared spectrum further confirmed the presence of the hydroxyl group, showing a very broad peak at 3384 cm^{-1} (Scheme (**3-37**)).



Scheme (3-37): The preparation of compound (329)

The alcohol (**329**) was oxidised with PCC in dichloromethane for 2 hours, resulting in aldehyde (**330**); this was confirmed by the proton NMR spectrum which displayed the aldehyde proton as a triplet at 9.77 (1.9 Hz) and the CH_2 adjacent to the aldehyde carbonyl was seen as a doublet of triplets at 2.44 ppm (J = 7.55, 1.9 Hz). The specific rotation was $[\alpha]_D^{24}$ -1.09 (c 1.35, $CHCl_3$).



Scheme (3-38): Mycolic acid preparation by Wittig and Julia reactions

The aldehyde (**330**) was coupled to the phosphonium salt (**313**) using the Wittig reaction. The phosphonium salt (**313**) was treated with NaHMSD since this gives more of the *cis* isomer and stirred for 30 min, before adding the aldehyde (**330**) in dry THF to the reaction mixture at room temperature. The formation of the resulting

alkene (**332**) was confirmed by the proton NMR spectrum which gave a doublet of triplets at 5.35 ppm (J = 11.65, 5.65 Hz) for the double bond protons (Figure **(3-7)**), while the protons at the β-chiral centre were seen as a triplet of doublets at 3.91 ppm (J = 7, 4.4 Hz). The α-chiral proton was seen as a doublet of doublets of doublets at 2.53 ppm (J = 11.05, 7.25, 3.8 Hz). The carbon NMR showed the carbonyl carbon at 175.14 ppm and the alkene carbon showed at 129.89 ppm (Figure **(3-8)).**

In order to compare the results, the coupling was also carried out using the Julia reaction (Scheme (**3-38**)). A Julia reaction was carried out by mixing aldehyde (**330**) with sulfone (**331**) in THF and adding LiHMSD at –10 °C. The formation of the alkene (**333**) was confirmed by the proton NMR spectrum which gave a multiplet for the *trans*-alkene protons between 5.4–5.38 ppm, and for the *cis*-alkene protons between 5.36–5.34 ppm; shown in (Figure (**3-8**)). The proton at the β-chiral centre gave a doublet of triplets at 3.86 ppm (J = 6.65, 4.7 Hz), while the proton at the α-chiral gave a doublet of doublets of doublets at 2.48 ppm (J = 11, 7.25, 3.75 Hz). The ^{13}C NMR spectrum showed the carbonyl carbon at 175.14 ppm and the alkene carbons at 130.36 ppm for the *trans* isomer and 129.89 ppm for *cis* the isomer (Figure (**3-9**).

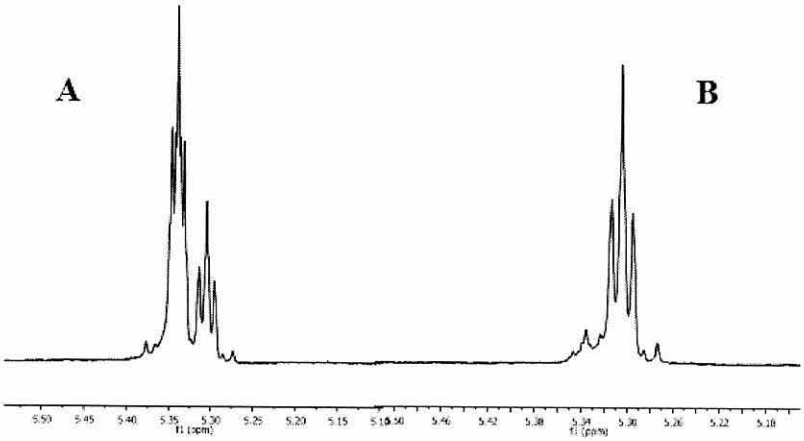


Figure (3-8): Proton NMR spectra (A) of the (333) alkene via Julia reaction and alkene prepared via Wittig reaction (B) compounds (332)

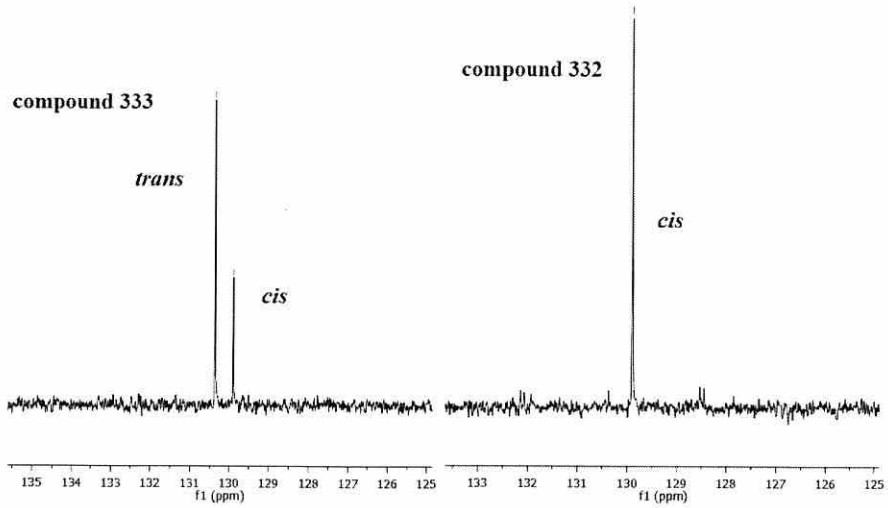
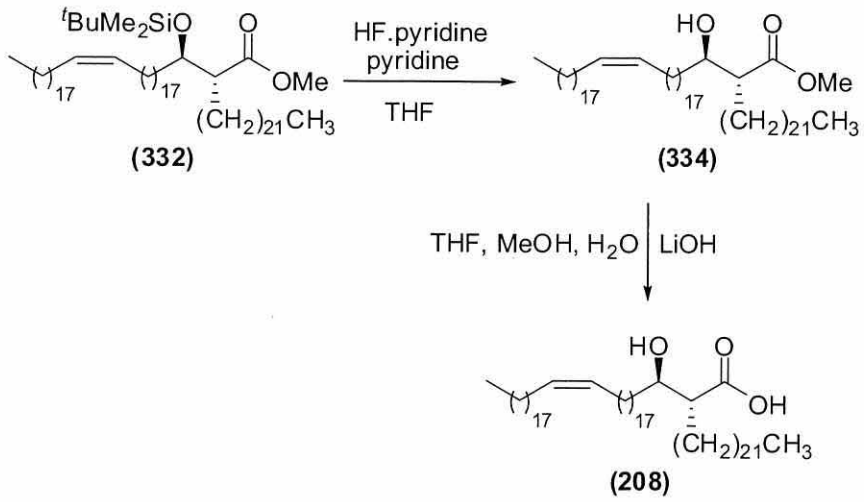


Figure (3-9): ^{13}C NMR spectra for the alkene prepared via Julia reaction and alkene prepared via Wittig reactionisomers, compounds (332) and (333)

The protected mycolic acid (**332**), formed in the Wittig reaction, was deprotected in two steps to give the free mycolic acid (**208**). In order to remove the silyl group from the β-hydroxyl, compound (**332**) was stirred with a mixture of pyridine (0.3 ml) and an HF.pyridine complex (1 ml) in dry THF at 40 °C for 18 hours. The resulting compound (**332**) was confirmed by proton NMR which gave a multiplet for the alkene protons between 5.31-5.24 ppm and showed the disappearance of the *t*-butyl protons at 0.87 ppm as well as the disappearance of dimethyl signals at 0.05 ppm and 0.02 ppm, respectively.

The final step was the hydrolysis of the methyl ester using lithium hydroxide monohydrate as described in Section (**2.15.2**). The formation of the free mycolic acid (**208**), identical to the major component reported in the natural mixture from *M. smegmatis*,[37] was verified by proton NMR which gave a multiplet for the alkene protons between 5.34-5.25 ppm and the protons at the β-chiral showed as a doublet of triplets at 3.70 ppm (J = 8.2, 4.95 Hz). The proton at the α-chiral gave a doublet of triplets at 2.43 ppm (J = 8.8, 5.35 Hz).

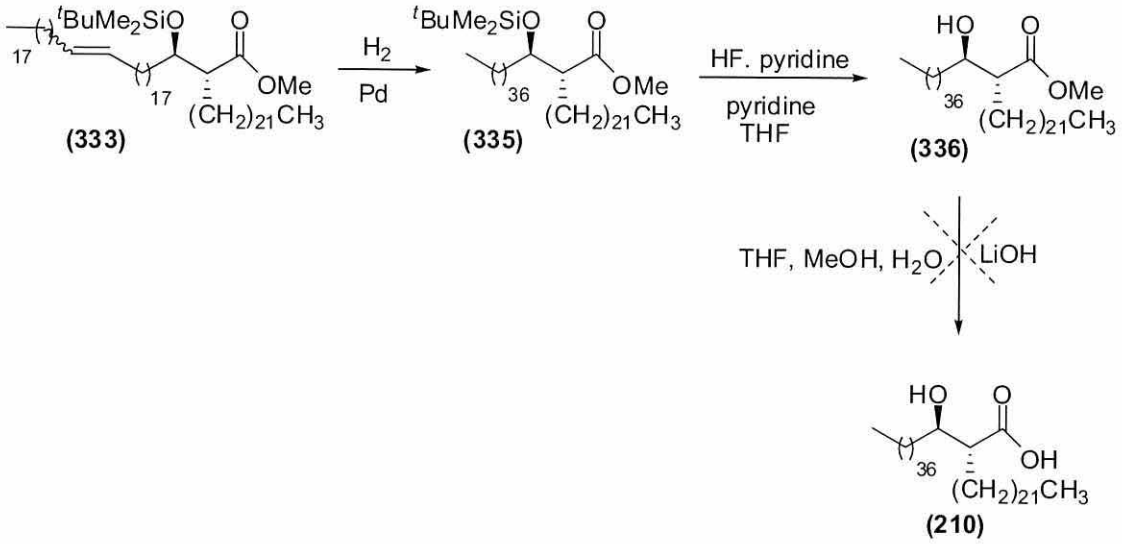


Scheme (3-39): Preparation of mycolic acid (208)

The ^{13}C NMR spectrum showed the carbonyl carbon 175.14 ppm and the alkene carbons were seen at 129.90 ppm. The MALDI of **(208)** also confirmed that the hydrolysis was successful as the value obtained for the mass ion was *m/z*1066.0, which is in concurrence with the expected mass for **(208)** with the formula $C_{69}H_{138}O_3SiNa$ (*m/z* 1066.0) (Scheme (**4-14**)). The specific rotation was $[\alpha]_D^{21}$ +3.43 (c = 0.97, $CHCl_3$) (Scheme (**3-39**)).

3.8 Attempted synthesis of a saturated mycolic acid

In order to prepare a saturated mycolic acid (**210**) to compare its properties with the unsaturated examples present in nature, hydrogenation of the double bond and two steps of deprotection were carried out. The first step was the hydrogenation of compound un-natural *trans*-isomer (**333**) used Pd on carbon (10 %) in a hydrogen atmosphere. The formation of the product (**335**) was confirmed by the proton NMR spectrum which showed a triplet of doublets at 3.91 ppm (J = 7.1, 4.55 Hz) for the β-chiral centre and for the α-chiral showed a doublet of doublets of doublets at 2.55 ppm (J = 10.9, 7.15, 3.75 Hz) with a lack of signals in the alkene region. The carbon NMR spectrum confirmed the disappearance of any alkene carbons in the product (**335**).



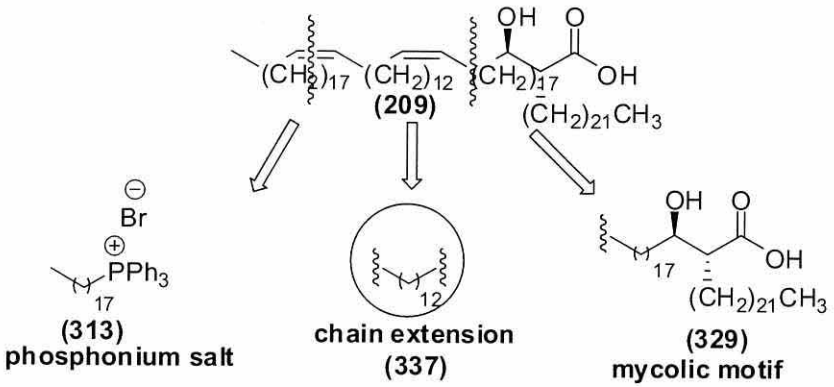
Scheme (3-40): Preparation of mycolic acid (210)

The next reaction was desilylation as described above (see Section (**2.12.1**)) by the use of an HF.pyridine complex with pyridine. The structure of the product was verified by proton NMR spectrum which demonstrated the lack of signals belonging to *t*-butyl and methyl groups adjacent to the silyl and by MALDI MS gave *m/z* 953.95 as expected. However, the final step, hydrolysis of compound (**336**) (Scheme (**3-40**)) did not give the desired compound (**210**) possibly because it was very difficult to dissolve under the reaction conditions; therefore, modified reaction conditions were studied, focusing on changing the solvent to either propanol or DMF, without any success.

3.9 Preparation diene containing mycolic acid (209)

3.9.1 Overview

The preparation of mycolic acid (**207**) and mycolic acid (**208**) encouraged the preparation of the diene mycolic acid (**209**), which has been reported in *Mycobacterium fortuitum.*[37] The difficulty encountered in the preparation of this particular mycolic acid (**209**) is the insertion of the two *cis*-double bonds in the meromycolic moiety. In planning for the strategy to be followed, it was noted that the mycolic acid (**209**) has the same general carbon framework as the mycolic acid (**208**). The same fragments were therefore used and a linking carbon chain was added between them (Scheme (**3-41**)).

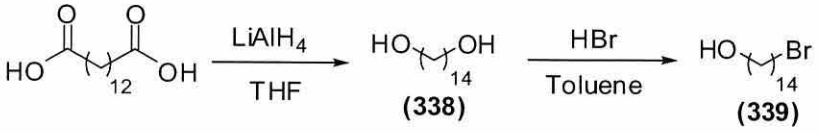


Scheme (3-41): Strategy for the synthesis mycolic acid (209)

3.9.2 Preparation of the chain extension fragment

In order to prepare the chain extension fragment (**337**), a twelve carbon bifunctional chain was necessary, so 1,12-dodecanedicarboxylic acid was chosen as the starting material. The first step was the reduction of the two carbonyl groups by treating 1,12-dodecane-dicarboxylic acid (3.09 mmol) with $LiAlH_4$ (3 eq.) in THF. The structure of the resulting compound (**338**) was verified by the proton NMR spectrum which gave a triplet for the two CH_2 groups adjacent to the hydroxyl group at 3.64 ppm (J = 6.6 Hz). The carbon NMR spectrum showed the disappearance of all carbonyl groups.

The next reaction was the bromination of one of the hydroxyl groups using hydrobromic acid in toluene. The success of the reaction was confirmed by the proton NMR spectrum which gave a triplet for the CH_2 adjacent to oxygen at 3.63 ppm (J = 6.6 Hz) and the CH_2 adjacent to bromide gave a triplet at 3.40 ppm (J = 6.9 Hz) (Scheme (**3-42**)). (See Appendix p 250)

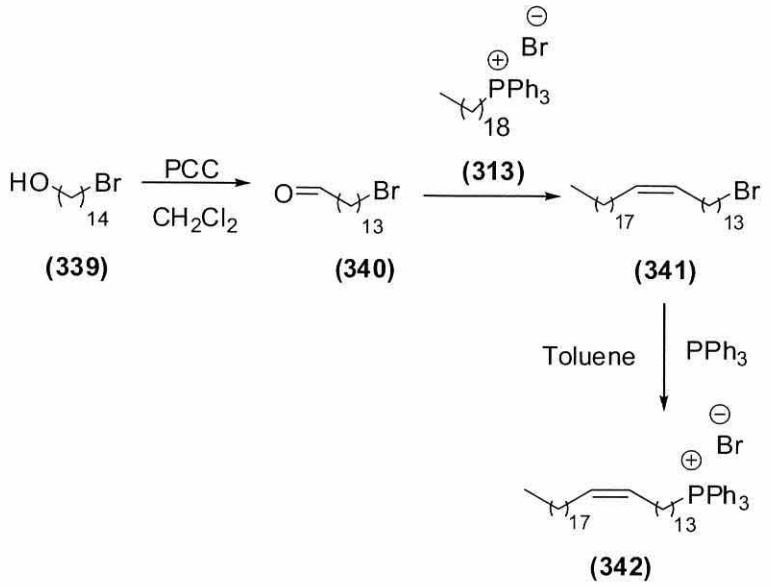


Scheme (3-42): Preparation of (339)

3.9.3 Extension of the meromycolate chain by the Wittig reaction

In order to simultaneously extend the chain of (**339**) and insert one of the *cis* double bonds, a Wittig reaction was carried out. 14-Bromotetradecan-1-ol (**339**) was oxidised with PCC in dichloromethane. The formation of the aldehyde (**340**) was confirmed by the proton NMR spectrum which gave a triplet at 9.72 ppm (J = 1.9 Hz)

for the aldehyde proton, and the CH_2 adjacent to the bromine showed as a triplet at 3.36 ppm (J = 4.1Hz). The ^{13}C NMR gave a peak at 202.75 ppm for the carbonyl carbon. The infrared spectrum showed a peak at 1727 cm^{-1} due to the carbonyl group. For the Wittig reaction, the ylide was generated by treating phosphonium salt (**313**) with NaHMDS for 30 minutes. This was followed by adding the aldehyde (**340**) in dry THF. The formation of the product (**341**) was verified by the proton NMR spectrum which showed a broad triplet at 5.35 ppm (J = 4.75 Hz) for the double bond protons and a triplet at 3.41 ppm (J = 6.95 Hz) for the CH_2 group adjacent to the bromide. The ^{13}C NMR spectrum showed the alkene (**341**) peaks at 129.89 and 129.86 ppm which confirmed that the product (**345**) was in the *cis* configuration rather than the *trans* configuration by comparison with *cis* and *trans* compounds prepared earlier (Scheme (**3-43**)).



Scheme (3-43): Preparation of phosphonium salt (342)

The final step was the preparation of the phosphonium salt (**342**) by refluxing (*Z*)-1-bromo-dotriacont-14-ene (**341**) with triphenylphosphine in toluene. The resulting compound (**342**) was verified by proton NMR which gave a multiplet for the protons of the phenyl groups between 7.70-7.34 ppm, while the alkene protons showed a multiplet between 5.40-5.35 ppm. The CH_2 group adjacent to the phosphorous gave a triplet at 3.41 ppm (J = 6.9 Hz) (Scheme (**3-43**)).

3.9.4 Preparation of complete mycolic acid (209)

In order to prepare mycolic acid (**209**), another Wittig reaction was carried out. The ylide was prepared by treating the phosphonium salt (**342**) with NaHMDS in dry THF for 30 minutes. The aldehyde (**330**) was added and the mixture was stirred for 18 hours. The formation of the product was confirmed by the proton NMR spectrum which gave a multiplet for the minor *trans* isomer between 5.39-5.37 ppm, while the major *cis* isomer showed as a broad triplet at 5.35 ppm (J = 4.7 Hz) in a ratio 1/10.3 (Figure (**3-10**)); the proton at the β-chiral centre gave a doublet of triplets at 3.91 ppm (J = 6.95, 4.75 Hz), while the α-chiral centre proton gave a doublet of doublets of doublets at 2.53 ppm (J = 11.05, 7.25, 3.8 Hz) and the *t*-butyl of silyl group gave a singlet at 0.86 ppm. The ^{13}C NMR spectrum gave peaks for the minor *trans* isomer at 133.80 and 133.64 ppm and for the major *cis* isomer at 129.88, 128.68, 128.49 and 128.44 ppm. The α-carbon and the methoxy group gave peaks at 51.56 and 51.21 ppm, respectively. The infrared spectrum confirmed the existence of both the *trans* and *cis* isomers, giving peaks at 836 and 774 cm^{-1} (Scheme (**2-44**)).

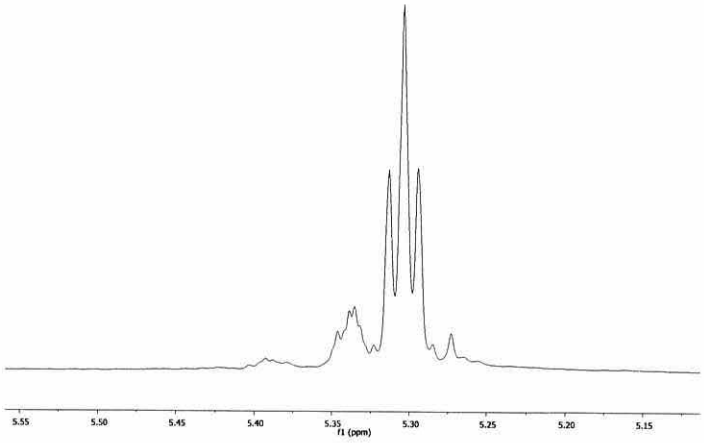
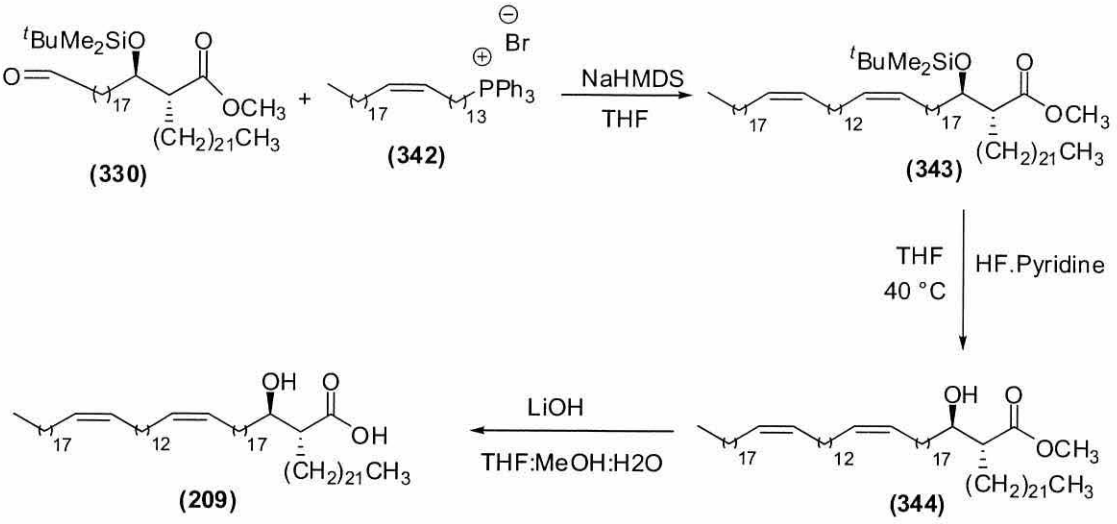


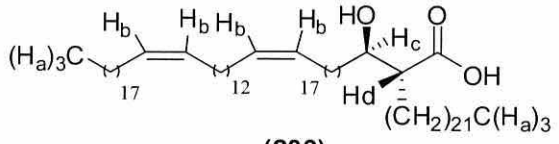
Figure (3-10): Partial proton NMR for compound (343)

In order to remove the silyl group from the hydroxyl, the HF.pyridine complex was employed, using the same reaction conditions as detailed earlier. The resulting compound (**344**) was characterised was by the proton NMR spectrum which showed the disappearance of signals at 0.86 ppm for the *t*-butyl group and 0.00 ppm for the methyl groups adjacent to the silyl. The final reaction was the hydrolysis of the methyl ester (**344**) using LiOH (15 mol. eq.) in a mixture of THF, MeOH and H_2O in

the ratio 10:1:1. The success of the reaction was confirmed by the proton NMR spectrum, shown in Table (**3-5**). The ^{13}C NMR further confirmed the disappearance of the methoxyl group, giving only one peak at 50.84 ppm for the α-chiral centre (Scheme (**3-44**)). [175]



Scheme (3-44): Preparation of mycolic acid (209)



(209)

H_x	Δ	Multiplicity	Integration	J (H_z)
H_a	0.88	t	6	6.65
H_b	5.37-5.33	m	4	-
H_c	3.72	dt	1	7.25, 4.1
H_d	2.46	dt	1	10.1, 5.35

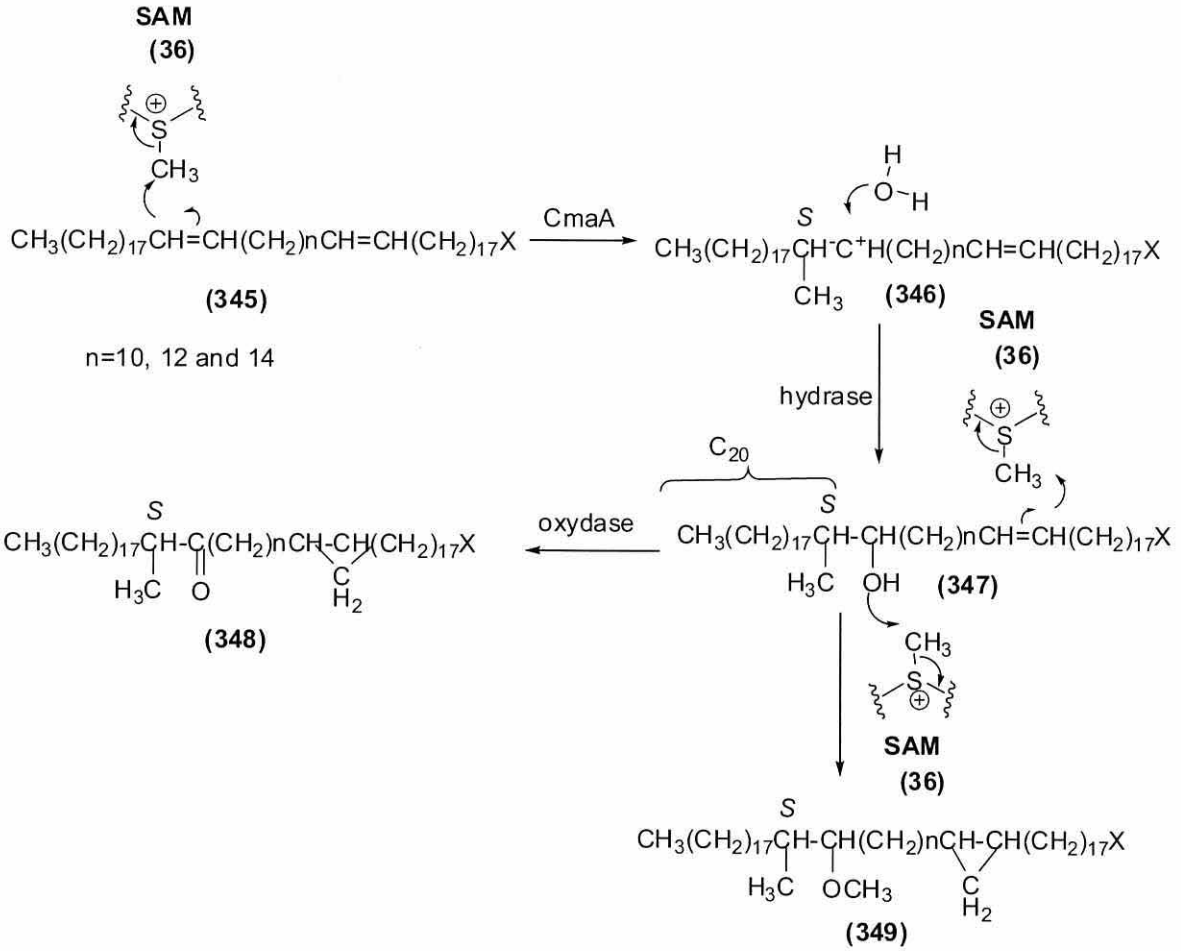
Table (3-5): Proton NMR analysis for mycolic acid (209)

This represents the first synthesis of a diene containing mycolic acid. The biological activity of this compound is now being assessed by others.

4-Preparation of hydroxy and keto mycolic acids (211) and (212) containing α-methyl-*trans*-cyclopropanes

4.1 Natural Keto Mycolic acid

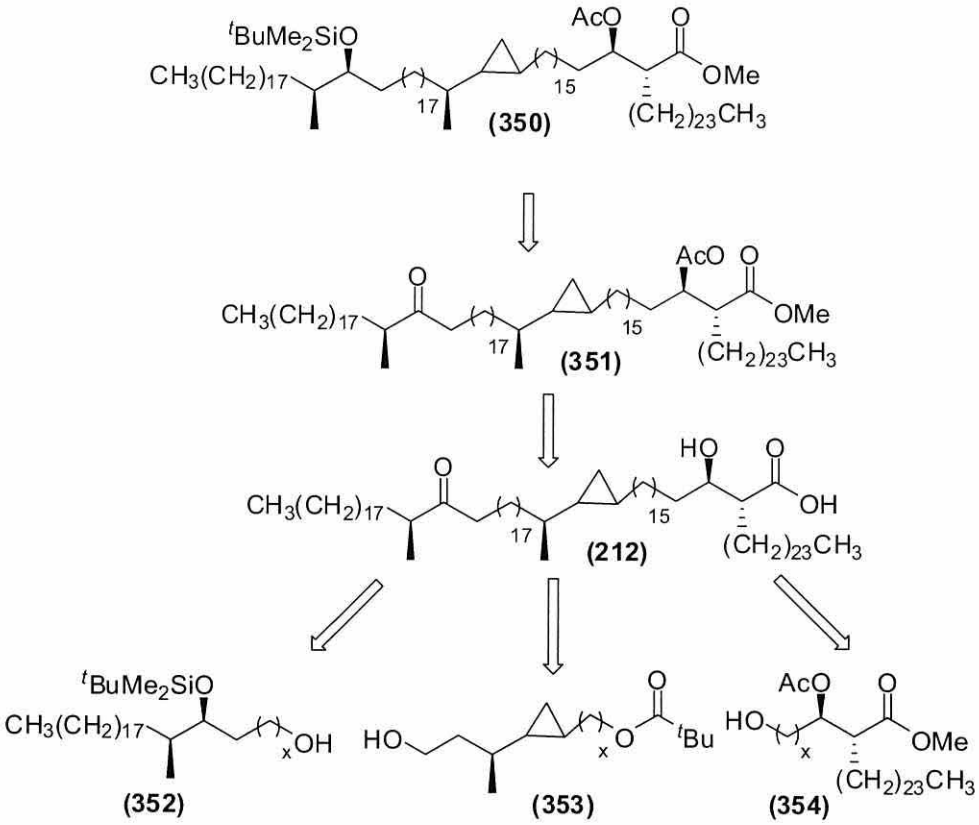
Ketomycolic acids are the major oxygenated mycolic acids in the cell wall of mycobacteria such as *M. bovis*. The biosynthetic pathway of keto and methoxy groups has been described previously in Section (**1.9.3**).[219] Quémard *et al.* showed that when a gene cluster (cmaA) isolated from *M. bovis* which is responsible for the synthesis of the keto groups in the cell wall[80, 82, 220, 221] is inserted into *M. smegmatis* it confers upon *M. smegmatis* the ability to synthesis keto and hydroxy mycolic acids. Hydroxy mycolic acids have only been found in small quantities in *M. bovis* BCG and *M. tuberculosis* following meticulous examination of all the mycolic-like fatty acids of these mycobacteria and there is evidence that they are on the biosynthetic pathway to keto mycolic acids.[43] Dubnau *et al.* isolated oxygenated mycolic acids from genetically modified *M. smegmatis*. The majority of these oxygenated mycolic acids were found to be methoxy and keto mycolic acids, along with what was called an 'unknown' compound. After analysing it by EI mass spectrometry it was found to be a hydroxy mycolic acid. This led to a proposed mechanism for the biosynthesis of oxygenated mycolic acids (Scheme (**4-1**).[56]



Scheme (4-1): Proposed mechanism for biosynthesis of keto, methoxy and hydroxymycolic acids[56]

4.2 Previous syntheses of ketomycolic acids containing α-methyl-*trans*-cyclopropanes

In an earlier syntheses of ketomycolic acids carried out in this laboratory, such as that of (**212**) shown in Scheme (**4-2**), the final step was the deprotection of the MA methyl ester to give the free mycolic acid using lithium hydroxide monohydrate.[117, 222, 223]

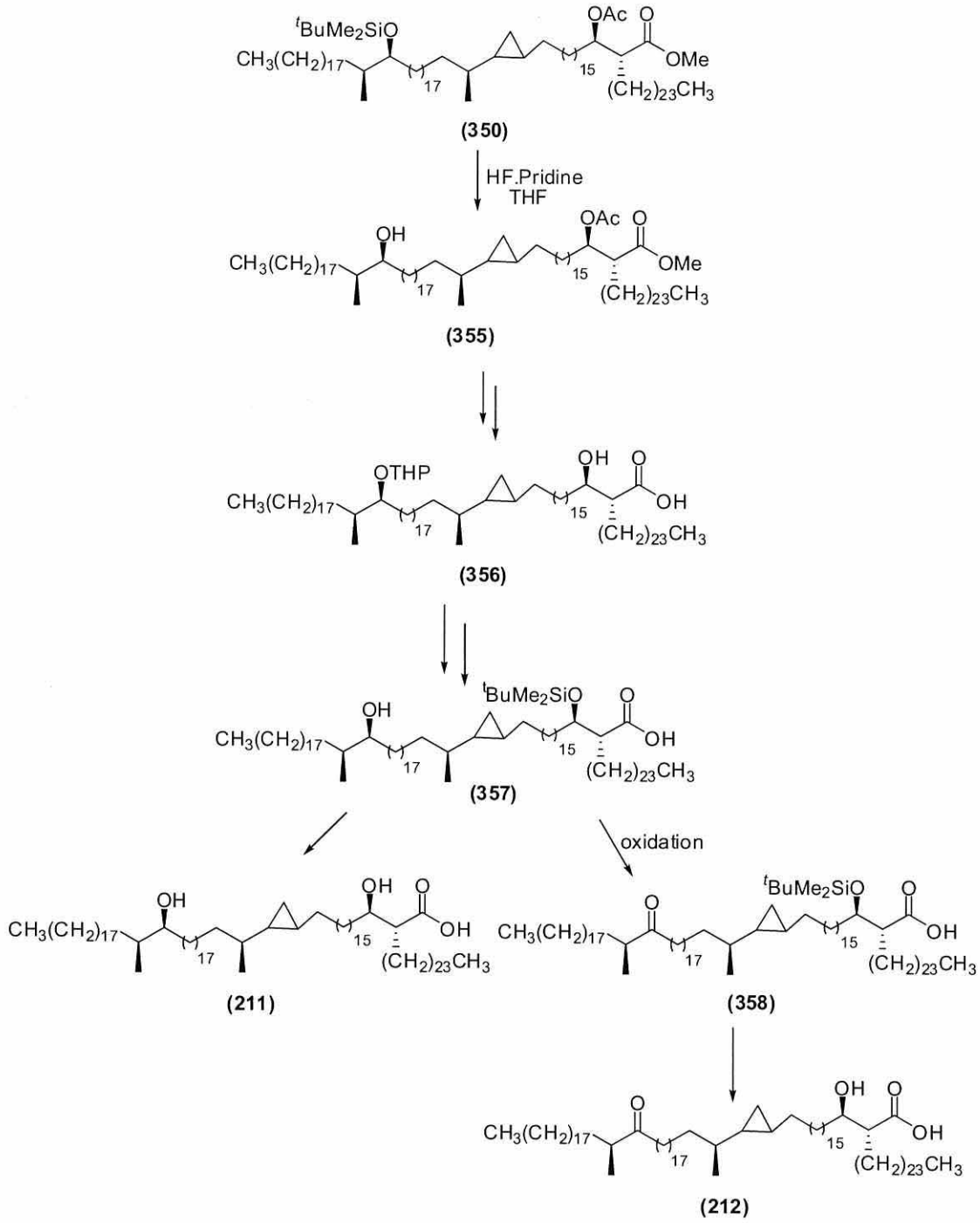


Scheme (4-2): Strategy followed for the earlier syntheses of mycolic acids (212)[117, 223]

In this deprotection step, the base caused epimerisation of the methyl group adjacent to the carbonyl group. The target of the present work was to prepare (**212**) as a single, nature identical, stereoisomer. This suggested the need for a different choice of protecting groups for the secondary hydroxyl groups in both meromycolate and mycolic motif in order to differentiate between those group and ensure that the last step of deprotection could be conducted in acid instead of base media.

4.3 The synthetic strategy for preparing single epimers of unprotected ketomycolic acids

In the earlier approach, deprotection of the keto-MA (**351**) led to epimerisation adjacent to the carbonyl group. In the approach to be adopted in the present work (Scheme (**4-3**)), the mycolic acid (**350**) having different protecting groups was first prepared from two available fragments. By switching the protecting groups on the two alcohol positions it would be possible to oxidise the alcohol of the mero-chain to a carbonyl group, a reaction known not to cause epimerisation, and then to complete the deprotection under acidic conditions.



Scheme (4-3): Strategy for the synthesis of mycolic acids (211) and (212)

There are many protecting groups that can be removed using acid. The benzyl ether group requires HBr or HOAc (or hydrogenation) for deprotection, which might affect the cyclopropane ring.[224] Another protecting group is a tetrahydropyran (THP) group which resists basic media, and is cheap and easy to handle.[176] The other protecting group used in this study was TBDMS, since it is a very stable protecting group but is

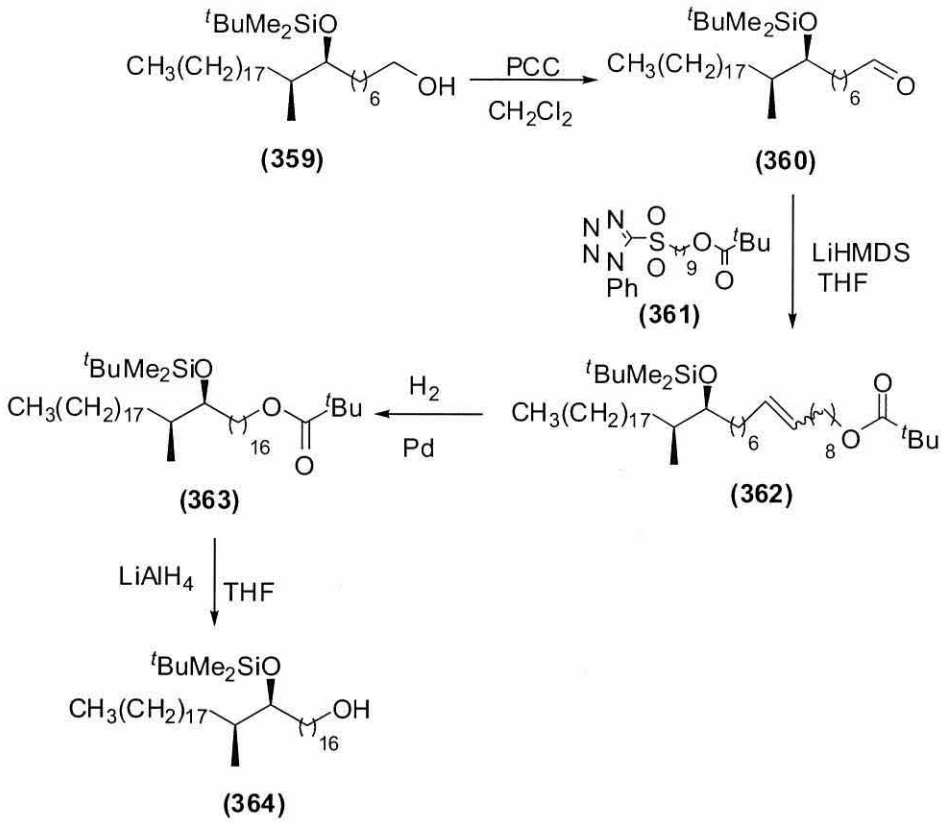
readily removed using HF.pyridine complex.[176] The planned route to the mycolic acids (**211**) and (**212**) therefore replaced the silyl ether with an acetyl group in the mycolic motif (**354**) since the deprotection method in basic media is the same for the hydroxy protecting group as for carboxylic acid. The next step linked the two moieties of mycolic acid, forming protected mycolic acid (**350**) via the Julia reaction. This was followed by replacing the TBDMS protecting group with a THP group and deprotecting the motif part in basic media which deprotected both the hydroxyl group and the carboxylic group (**356**). The following steps reprotected the hydroxyl group in the mycolic motif with a TBDMS group and deprotected the hydroxyl group in the meromycolic moiety by the use of a mild acid, *p*-toluenesulfonic acid (PTSA) (**357**).

4.4 Preparation of protected hydroxymycolic acid (350)

Compound (**350**) was prepared by the same procedure as reported earlier, by linking the three fragments set out in Scheme (**4-2**). The synthesis is described briefly below. The experimental data are presented for completeness in the Appendix (p 250).[222]

4.4.1 Preparation of the α-methyl hydroxy fragment (352)

In order to repeat the first part of the synthesis described above, compound (**359**)[225] was oxidised with PCC in dichloromethane. The product (**360**) was confirmed by the proton NMR spectrum which gave a triplet for the aldehyde proton at 9.76 ppm (J = 1.65 Hz). The ^{13}C NMR spectrum showed a peak at 202.9 ppm for the aldehyde carbon. The optical rotation was $[\alpha]_D^{25}$ –8.44 (c = 1.01, $CHCl_3$). A Julia reaction was employed to couple the aldehyde (**360**) with sulfone (**361**) (1.3 mol. eq.) in THF with LiHMDS (8.7 mmol, 1.06 M) as base. The product alkene (**362**) was hydrogenated without characterisation with Pd on carbon (10 %) in a hydrogen atmosphere. The product (**363**) was characterised by using proton NMR which showed the CH_2 adjacent to the oxygen as a triplet at 4.05 (6.6 Hz), while the CH adjacent to the silyl ether displayed a doublet of triplets at 3.50 ppm (J = 6.3, 3.5 Hz) and the α-methyl showed a doublet at 0.81 ppm (J = 6.65 Hz). The ^{13}C NMR spectrum showed the carbonyl carbon at 178.63 ppm and the carbons adjacent to the oxygen gave peaks at 75.87 and 64.45 ppm, respectively. The optical rotation was $[\alpha]_D^{25}$ –5.42 (c = 1.23, $CHCl_3$) (Scheme (**4-4**)).

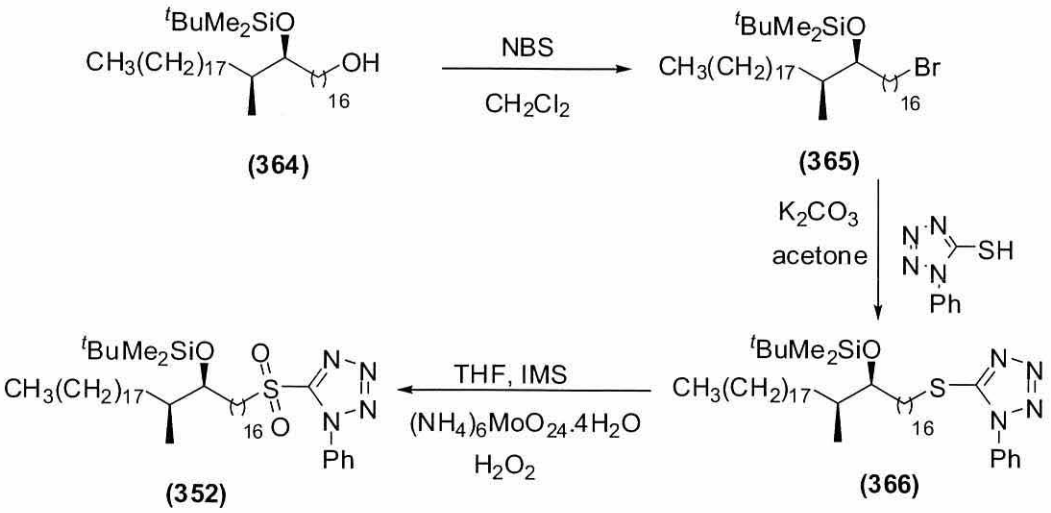


Scheme (4-4): Preparation of the α-methyl hydroxy unit

The final step was deprotection of the primary hydroxyl group (**363**). In order to deprotect the hydroxyl group in compound (**363**), $LiAlH_4$ was used in THF. The alcohol (**364**) was confirmed by the proton NMR spectrum which showed a triplet for the CH_2 group adjacent to the hydroxyl group at 3.64 ppm (J = 6.65 Hz) and the reduction of the lack of singlet at 1.02 ppm to the *t*-butyl. The ^{13}C NMR spectrum showed the loss of the peak corresponding to the carbonyl carbon. The infrared spectrum showed a very broad absorbance at 3323 cm^{-1} corresponding to OH stretching. The optical rotation was $[\alpha]_D^{24}$ –5.4 (c = 1.01, $CHCl_3$) (Scheme (**4-4**)).

In order to link the α-methyl-β-hydroxy unit (**364**) with the cyclopropane containing fragment, it had to be converted into a sulfone. The hydroxyl group in compound (**364**) was changed to bromine using NBS in dichloromethane. The resulting compound (**365**) was confirmed by the proton NMR spectrum which showed the CH_2 group adjacent to the bromide as a triplet at 3.41 ppm (J = 6.95 Hz). The ^{13}C NMR spectrum gave one peak for the carbon adjacent to the oxygen at 75.88 ppm which belongs to the carbon adjacent to the protected oxygen with the silyl ether. The

infrared spectrum showed the disappearance of the signal belonging to the hydroxyl group. The specific rotation was $[\alpha]_D^{23}$ –5.2 (c = 1.08, $CHCl_3$). This was followed by treating compound (**365**) with 1-phenyl-1H-tetrazol-5-thiol in acetone. The formation of the product (**366**) was verified by the proton NMR spectrum which gave a multiplet peak for the phenyl group between 7.60-7.53 ppm, and the CH_2 group adjacent to sulfur gave a triplet at 3.39 ppm (J = 7.55 Hz). The carbon NMR spectrum showed the tetrazole carbon at 154.47 ppm. The aromatic carbons appeared at 133.78, 130.00, 129.72 and 123.382 ppm. Finally, the oxidation of the sulfide with ammonium heptamolybdate (VI) tetrahydrate in THF:IMS (1:1) was undertaken. The formation of sulfone (**352**) was confirmed by the proton NMR spectrum which gave a triplet at 3.73 ppm (J = 7.85 Hz) for the CH_2 group adjacent to the sulfur atom. The specific rotation was $[\alpha]_D^{22}$ –3.74 (c = 1.15, $CHCl_3$) (Scheme ((**4-5**)).



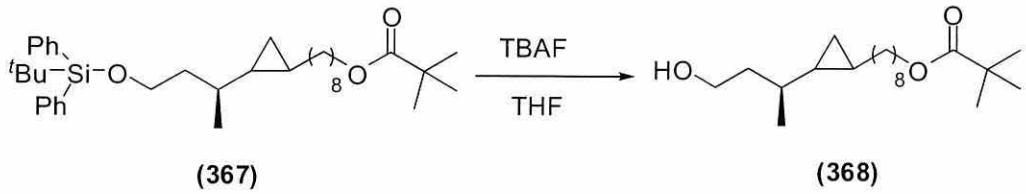
Scheme (4-5): Preparation of sulfone (352)

4.3.2 Preparation of the cyclopropane part

TBAF was used to deprotect compound (**367**)[3] in dry THF. The success of the reaction was confirmed by the proton NMR spectrum which gave a triplet for the CH_2 adjacent to the hydroxyl at 4.04 ppm (J = 6.65 Hz), while the other CH_2 adjacent to the oxygen gave a multiplet between 3.75-3.67 ppm, and the α-methyl gave a doublet at 0.94 ppm (J = 6.65 Hz). The cyclopropane ring showed multiplet signals between 0.7-0.64 ppm and between 0.34-0.27 ppm, representing one proton each and two

[3] Thankfully donated by C. Theunissen, prof. M. S. Baird group (Bangor University)

protons between 0.08-0.03 ppm. The infrared spectrum showed an absorbance at 3386 cm^{-1} for the hydroxyl group (Scheme (**4-6**)).



Scheme (4-6): Desilylation of compound (368)

4.3.3 The Julia reaction

In order to link the two parts (**352**) and (**368**) of the mycolic acid (**212**), a Julia reaction was carried out. The first step was to oxidise alcohol (**368**) to the corresponding aldehyde (**369**). The resulting aldehyde (**369**) was characterised by the proton NMR which gave a doublet of doublets for the aldehyde proton at 9.78 ppm (J = 2.2, 2 Hz) (Figure (**4-1**)). The CH_2 group adjacent to the carbonyl had non-equivalent protons; the first one gave a doublet of doublets of triplets at 2.52 ppm (J = 6.3, 2.9, 1.95 Hz), while the second one gave a doublet of doublets of triplets at 2.38 ppm (J = 9.44, 7.55, 1.9 Hz). The α-methyl gave a doublet at 1.03 ppm (J = 6.6 Hz).

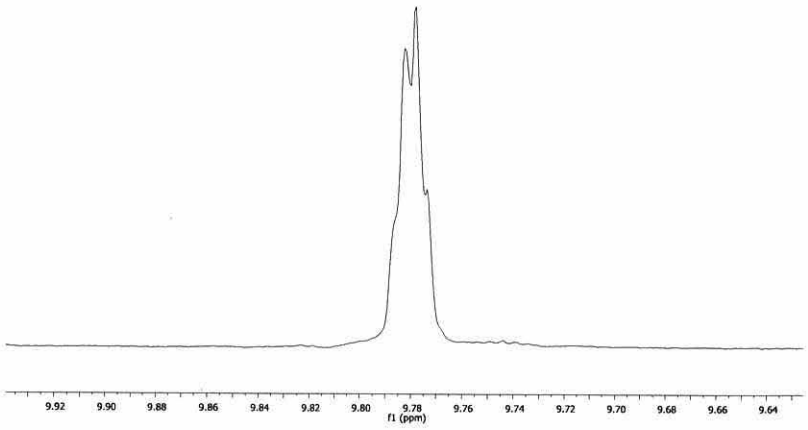
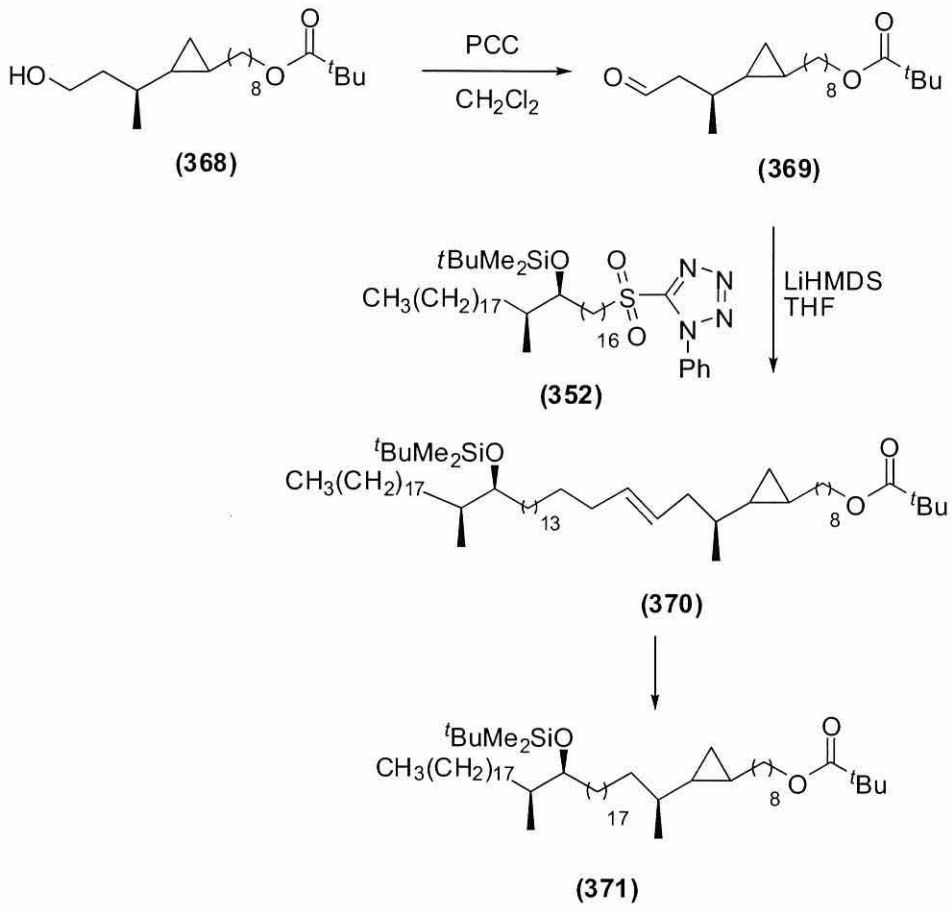


Figure (4-1): Aldehyde proton for compound (369)

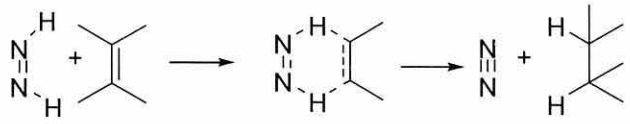
The next step was to treat the aldehyde (**369**) with sulfone (**352**) and LiHMDS as base. The success of the coupling reaction was verified by the proton NMR spectrum which

gave a multiplet between 5.39-5.36 ppm for the alkene protons. The chiral centre adjacent to the silyl ether gave a doublet of triplets at 3.48 ppm (J = 6.3, 3.9 Hz). The ^{13}C NMR spectrum showed the carbonyl carbon at 178.61 ppm and alkene carbon signals at 131.42, 130.42, 128.85 and 128.40 ppm belonging to both *cis* and *trans* isomers were observed (Scheme (**4-7**)).



Scheme (4-7): Preparation of meromycolic acid (371)

The presence of cyclopropane in compound (**371**) led to the use of mild conditions for hydrogenation using di-imide generated *in situ* (Scheme (**4-8**)).[226, 227]



Scheme (4-8): Mechanism of hydrogenation using di-imide

There are several methods for the preparation of this reducing agent, including:

- Oxidation of hydrazine using hydrogen peroxide
- Oxidation of hydrazine using oxygen and Cu(II)
- Fragmentation of azodicarboxylic acid

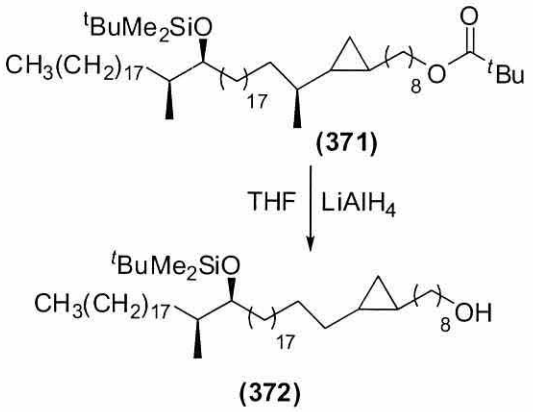
In this study, the third method was followed to prepare the reagent.[228]The azodicarboxylic acid is generarated by reaction of dipotassium azodicarboxylate with acetic acid and decarboxylates *in situ*. The preparation of the potassium salt involved the addition of azodicarbonamide in portions to a stirred solution of potassium hydroxide in distilled water at 0° C. The resulting bright yellow solution was left to stir for 45 minutes at that temperature, followed by a filtration and washing process. The product was maintained at 0 °C until use. The mechanism of hydrogenation using dipotassium azodicarboxylate has been reported by Nakatsuka *et al.*[229]

The hydrogenation was carried out twice in order to completely saturate the alkene and obtain the saturated product (**371**) which was verified by the proton NMR spectrum which demonstrated the absence of any alkene proton signals. For the CH_2 adjacent to the oxygen, a triplet at 4.05 ppm (J = 6.6 Hz) was seen, for the CH adjacent to the silyl ether, a doublet of triplets at 3.5 ppm (J = 3.45, 5.95Hz) was observed, and for the two *t*-butyl groups, singlets at 1.20 and 0.89 ppm were obtained. The α-methyl gave a doublet at 0.79 ppm (J = 6.6 Hz), and the cyclopropane ring gave a multiplet between 0.68-0.61 ppm for one of the CH_2 groups of the cyclopropane ring and 0.45-039 ppm for the other proton in the CH_2 group of the cyclopropane ring and 0.18-0.06 ppm for the two CH groups of the cyclopropane ring and the proton adjacent to the ring (3H, m). The two methyl groups adjacent to the silyl gave singlets at 0.03 ppm and 0.02 ppm (Scheme (**4-6**)).

4.3.4 Deprotection of the meromycolic moiety

Compound (**371**) was protected with *t*-butyl ester in order to deprotect the ester. A reduction reaction was employed rather than hydrolysis to prevent any epimerisation at the chiral centres. Lithium aluminium hydride (2 mol.eq.) was employed in THF at 0 °C (Scheme (**4-9**)). The resulting compound (**372**) was characterised by the proton NMR spectrum, showing the CH_2 group adjacent to the hydroxyl as a triplet at 3.64 ppm (J = 6.65 Hz) and the CH group adjacent to the silyl ether as a doublet of

doublets of doublets at 3.50 (3.45, 6, 9.45 Hz). The α-methyl groups gave a doublet at 0.91 ppm (J = 6.25 Hz) and 0.81 ppm (J = 6.6 Hz) (Figure **(4-2)**).



Scheme (4-9): Reduction of compound (372)

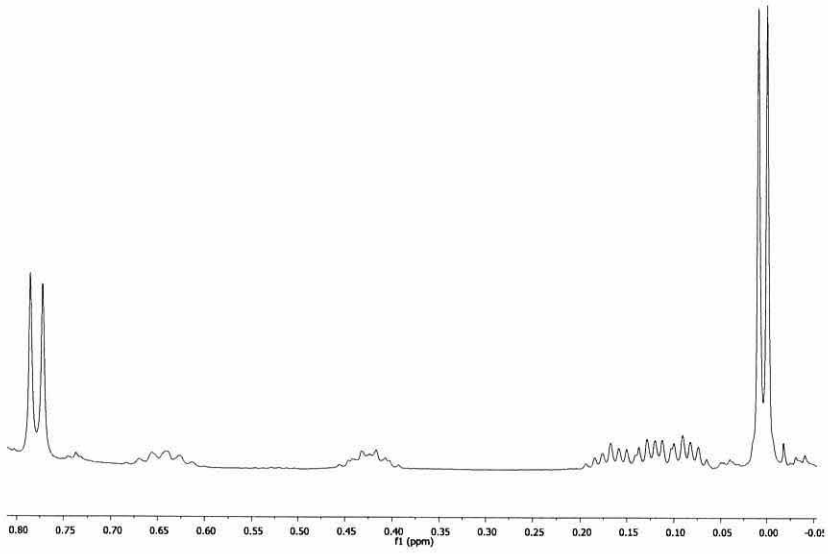
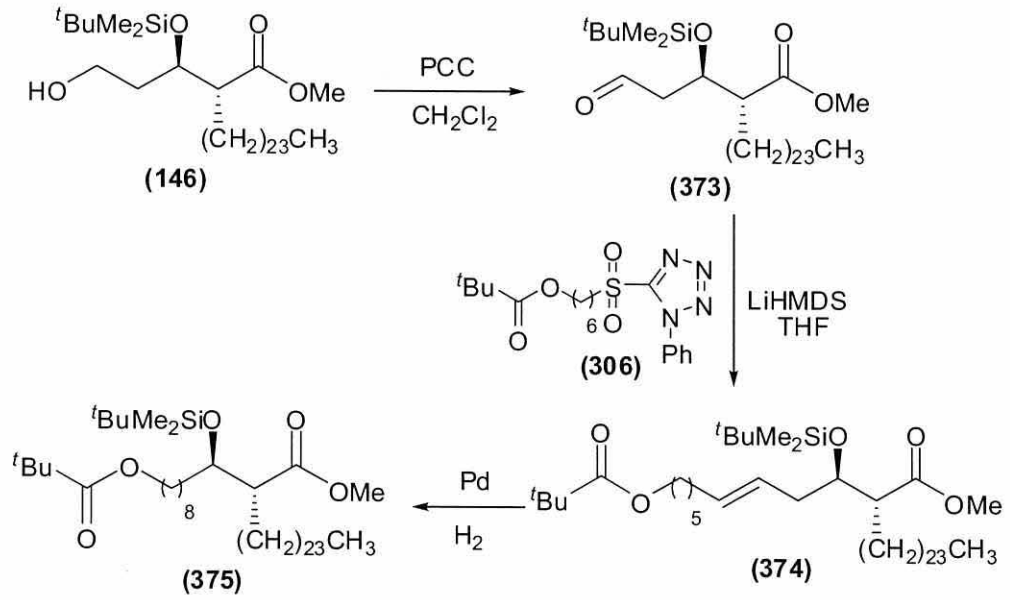


Figure (4-2): NMR for the meromycolic moiety (372)

4.3.5 Preparation of the mycolic motif

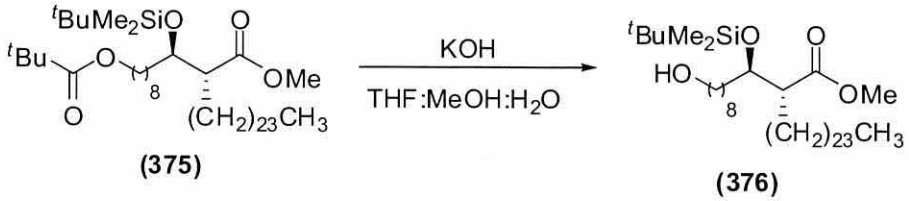
The third part of mycolic acids (**211**) and (**212**), according to the strategy described above, requires that the mycolic motif (**146**) be extended by six carbon atoms. The mycolic motif (**146**) was first oxidised using PCC. The formation of aldehyde (**373**) was confirmed by the proton NMR spectrum which showed the aldehyde proton as a doublet of doublets at 9.83 ppm (J = 1.8, 2.9 Hz) since the adjacent CH_2 group had non-equivalent protons. The proton adjacent to the silyl ether gave a doublet of

triplets at 4.43 ppm (J = 4.8, 6.2 Hz) and proton in the α-chiral centre showed a doublet of doublets of doublets at 2.68 ppm (J = 1.6, 4.5, 6.3 Hz). A Julia reaction was then carried out in order to couple the aldehyde (**373**) and 2,2-dimethyl-propionic acid 6-(1-phenyl-1H-tetrazole-5-sulfonyl)-hexyl ester (**306**) using LiHMDS in dry THF. The resulting compound (**374**) was characterised by the proton NMR spectrum which gave a multiplet for the alkene protons between 5.47-5.43 ppm, while the CH_2 group adjacent to the oxygen gave a triplet at 4.04 ppm (J = 6.6 Hz). The protons on the chiral centres gave a multiplet for the β-centre between 3.94-3.87 ppm and a doublet of doublets of doublets at 2.52 ppm (J = 11.2, 7.45, 3.65 Hz). The ^{13}C NMR provided confirmation, showing the two carbonyl carbons at 178.56 and 175.06 ppm, with two signals belonging to *t*-butyl at 1.19 and 0.86 ppm with the integration of nine protons each. The alkene carbons showed at 133.25, 131.61, 125.20 and 124.75 ppm for both *cis* and *trans* isomers. This was followed by hydrogenation by the use of palladium on carbon (10 %) in a hydrogen atmosphere. The formation of product (**375**) was confirmed by the proton NMR spectrum which showed the disappearance of any signal belonging to alkene hydrogen. The ^{13}C NMR also showed the lack of any signal in the alkene region. The specific rotation was $[\alpha]_D^{25}$ - 3.52 (c = 1.87, $CHCl_3$) (Scheme (**4-10**)).



Scheme (4-10): Preparation of the chain extended mycolic motif moiety (375)

The last step in the preparation of the mycolic motif was the hydrolysis of compound (**375**) in order to deprotect the primary hydroxyl. Potassium hydroxide (15 mol. eq.) was used to hydrolyse compound (**375**) in a mixture of THF:MeOH:H_2O at a ratio of 10:10:1. The product (**376**) was characterised by proton NMR which gave a doublet of triplets at 3.91 ppm (J = 6.95, 4.75 Hz) for the β-chiral centre, while the α-chiral centre showed a doublet of doublets of doublets at 2.52 ppm (J = 3.75, 7.25, 11 Hz). The CH_2 group adjacent to the oxygen gave a triplet at 3.64 ppm (J = 6.6 Hz). The absence of signals for the *t*-butyl ester group supplied additional confirmation of hydrolysis since there was one singlet for nine protons at 0.86 ppm belonging to the *t*-butyl of silyl ether. The ^{13}C NMR confirmed the success of hydrolysis, showing one peak for the carbon carbonyl at 175.12 ppm. The infrared spectrum showed a very broad absorbance at 3357 cm^{-1} associated with the hydroxyl group (Scheme (**4-11**)). The specific rotation was $[\alpha]_D^{20}$ -5.34 (c = 1.97).



Scheme (4-11) Hydrolysis of compound (376)

In these steps, the preparation of the mycolic motif (**376**) was carried out for coupling with the meromycolic motif (**372**) via a Julia reaction. The protecting group of the secondary hydroxyl was changed from TBDMS to an acetyl group as explained above.

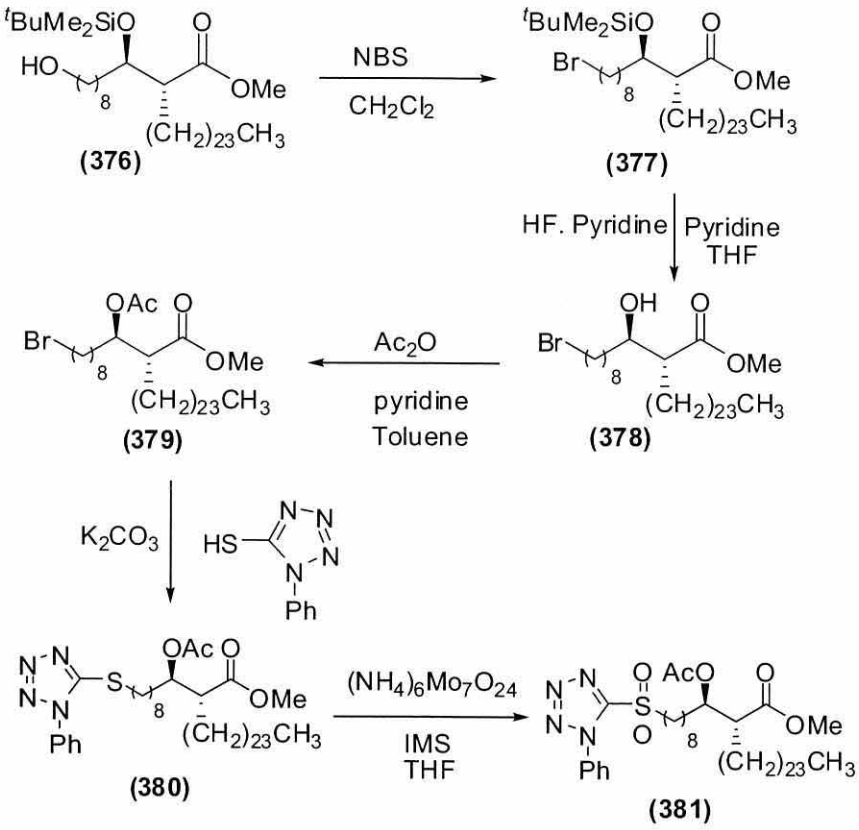
First, the primary hydroxyl group was changed to bromine using NBS in dichloromethane. The formation of compound (**377**) was confirmed by the proton NMR spectrum which showed a doublet of triplets for the CH adjacent to the secondary hydroxyl at 3.91 ppm (J = 6.9, 5.05 Hz) and the CH_2 adjacent to the bromide gave a triplet at 3.40 ppm (J = 6.95). The α-chiral centre proton showed a doublet of doublets of doublets at 2.52 ppm (J = 11, 7.25, 3.75 Hz), while the silyl group showed a singlet at 0.86 ppm to the *tert*- butyl with the integration of nine protons, and the two methyl groups gave a two singlets at 0.04 and 0.02 ppm with an integration of three protons each. The ^{13}C NMR showed dimethyl-silyl at -4.37 and -

4.93 ppm. The specific rotation was $[\alpha]_D^{20}$ −1.95 (c = 1.12, $CHCl_3$). The next step was deprotection of the silyl group using HF.pyridine complex in the presence of pyridine in dry THF. The product (**378**) was verified by the proton NMR spectrum which showed the lack of signals at 0.89 ppm corresponding to *tert*-butyl and 0.00 ppm corresponding to dimethyl groups. The ^{13}C NMR spectrum confirmed the disappearance of signals below zero belonging to dimethylsilyl. The infrared spectrum confirmed the deprotection, showing very broad absorbance at 3528 cm^{-1} corresponding to the hydroxyl group.

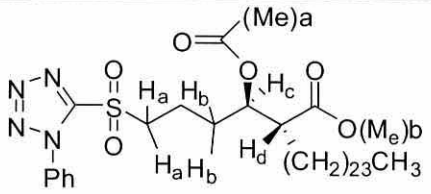
This was followed by the protection of the secondary hydroxyl by an acetate group using acetic anhydride and anhydrous pyridine in dry toluene. The resulting compound (**379**) was confirmed by proton NMR showing a doublet of doublets of doublets for the proton adjacent to the secondary hydroxyl at 5.08 ppm (J = 11, 6.95, 2.85 Hz) since proximity to the acetate group made it more acidic. The CH_2 adjacent to the bromide gave a triplet at 3.39 ppm (J = 6.9 Hz), and the α-chiral proton gave a doublet of doublets of doublets at 2.61 ppm (J = 10.7, 6.6, 4.1 Hz). The carbon spectrum gave two signals at 173.60 and 170.32 ppm belonging to the carbons of the carbonyl groups. The infrared spectrum showed the lack of any free hydroxyl group in the product (Scheme (**4-12**)).

The next step was the preparation of the sulfide (**380**) by the use of 1-phenyl-1H-tetrazol-5-thiol and potassium carbonate stirred overnight in acetone. The product (**380**) was verified by the proton NMR spectrum which gave a multiplet of five phenyl protons between 7.58-7.53 ppm and the CH_2 group adjacent to the bromine gave a triplet at 3.39 ppm (J = 7.25Hz). The ^{13}C NMR showed the tetrazole carbon at 154.46 ppm and the phenyl group at 133.77, 130.03, 129.73 and 123.84 ppm.

The final step was the oxidation of the sulfide (**380**) to sulfone (**381**) using ammonium molybdate (VI) tetrahydrate in 35% H_2O_2. The product (**381**) was confirmed by the proton NMR spectrum illustrated in Table (**4-1**).



Scheme (4-12): Preparation of the mycolic motif (381)



H_x	δ	Multiplicity	Integration	J (H_z)
H_a	3.72	t	2	7.9
H_b	1.97-1.91	m	2	-
H_c	5.08	ddd	1	10.7, 6.95, 3.8
H_d	2.61	ddd	1	10.75, 6.65, 4.1
$(Me)_a$	2.03	s	3	-
$(Me)_b$	3.67	s	3	-

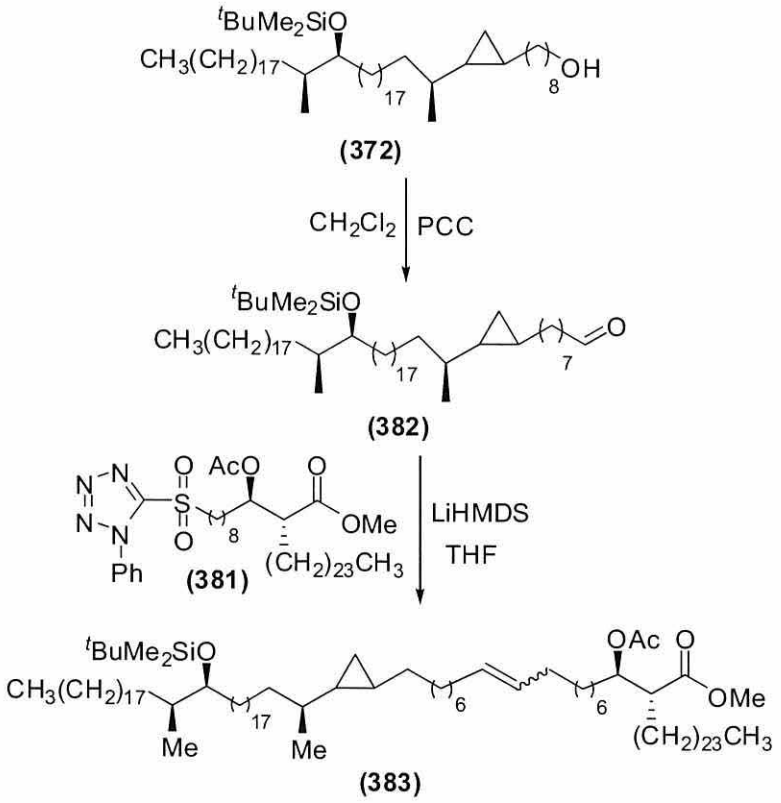
Table (4-1): Proton NMR analysis of compound (381)

4.3.6 The coupling reaction

The mycolic motif sulfone (**381**) prepared above had a different protecting group on the secondary hydroxyl group from that on the secondary hydroxyl on the meromycolic moiety (**372**). A Julia reaction was carried out in order to link these two fragments.

Oxidation of the alcohol (**372**) was first performed using PPC in dichloromethane. The resulting aldehyde (**382**) was confirmed by proton NMR which gave a triplet for the aldehyde proton at 9.77 ppm (J = 1.9 Hz). The proton adjacent to the silyl ether gave a multiplet between 3.52–3.49 ppm while the CH_2 group adjacent to the aldehyde group showed a doublet of triplets at 2.43 ppm (J = 1.9, 7.3 Hz). The α-methyl group gave a doublet at 0.80 ppm (J = 7.0 Hz). The carbon NMR spectrum showed a carbonyl carbon at 202.9 and the carbon adjacent to the silyl ether at 75.9 ppm. The infrared spectrum showed the absorbance of the carbonyl group at 1742 cm $^{-1}$.

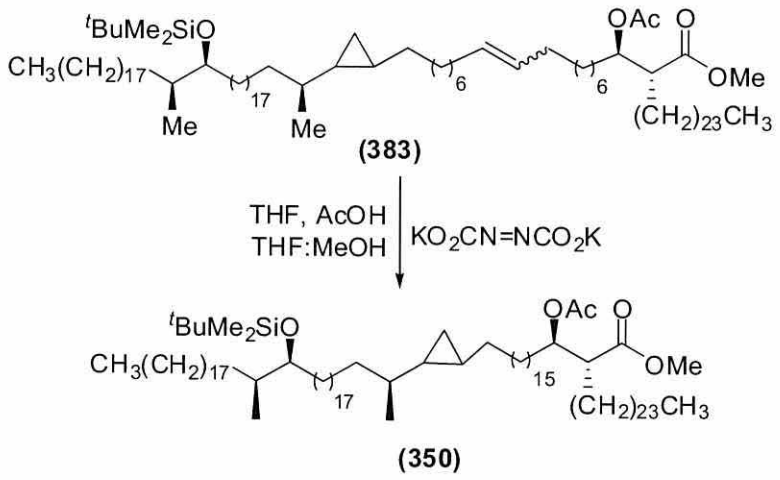
This was followed by coupling the aldehyde (**382**) with sulfone (**381**) via a Julia reaction using LiHMDA in dry THF (Scheme (**4-13**)).



Scheme (4-13): Preparation of protected hydroxymycolic acid (383)

The formation of the alkene (**383**) was confirmed by the proton NMR spectrum which gave a multiplet between 5.39-5.35 ppm for the alkene protons, and for the β-chiral centre proton adjacent to the acetyl group showed a doublet of doublets of doublets at 5.09 ppm (J = 11.05, 7.55, 3.8 Hz). The proton adjacent to the silyl ether gave a doublet of triplets at 3.50 ppm (J = 6.3, 3.45 Hz), while the α-chiral centre showed a doublet of doublets of doublets at 2.62 ppm (J = 10.75, 6.65, 4.1 Hz). The cyclopropane ring gave a multiplet between 0.70-0.65 ppm for one proton, one proton showed a multiplet between 0.48-0.44 ppm and three protons gave a multiplet between 0.21-0.1 ppm which included a proton adjacent to the α-methyl group. The ^{13}C NMR spectrum showed the two carbonyl carbons at 173.63 and 170.31 ppm and the alkene carbons at 130.43, 130.22 and 129.76 ppm for both *cis* and *trans* alkene isomers.

The next step was the hydrogenation of the double bond in compound (**383**), in order to obtain the saturated protected mycolic acid (**350**). Dipotassium azodicarboxylate was used for hydrogenation since it does not affect the cyclopropane ring.[226, 227] The formation of saturated compound (**350**) was confirmed by the proton NMR spectrum which is illustrated Table (**4-2**). The MALDI of **(350)** also confirmed that the hydrogenation was successful as the value obtained was 1474.6, which is in agreement with the expected exact mass for **(350)** with the formula $C_{96}H_{190}NaO_5Si$ (Scheme (**4-14**)).

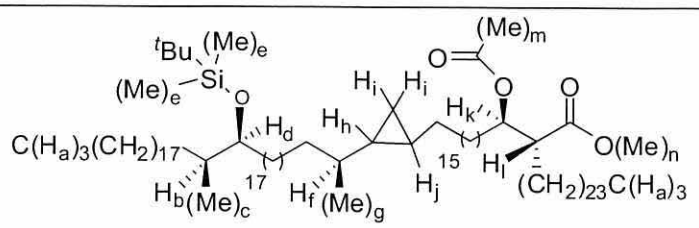


Scheme (4-14): Hydrogenation of alkene (383)

The protected mycolic acid (**350**) was prepared from three fragments and links them via Julia reaction. The molecular rotation of the first fragment (**352**) was calculated using the equation: $M_D = \alpha_D$ x (Mol. Wt./100)

The M_D of the first fragment (**352**) was found as -3.74(858.67/100) = -32.11. The second fragment (**369**) M_D was found as +49.96; while the third fragment (**381**) molecular rotation was found as +13.56.

The theoretical molecular rotation was calculated by adding the rotation of each chiral fragment to give M_D = +31.41, while the M_D for compound (**350**) was found as +44.27, proving the synthesis of compound (**350**) without epimerisation.



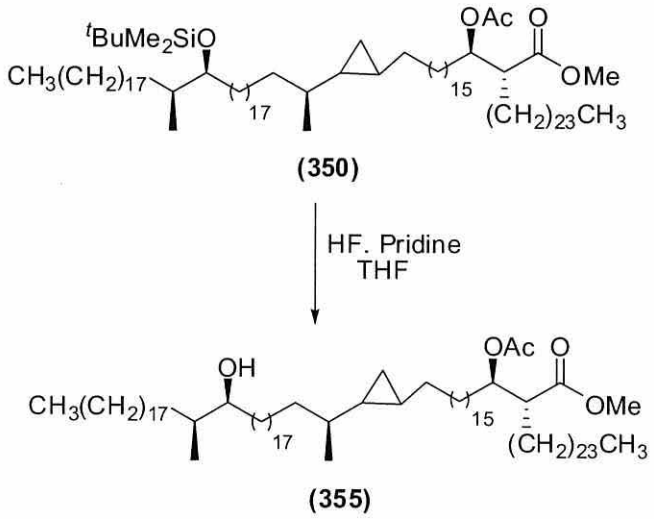
(350)

H_x	δ	Multiplicity	Integration	J (H_z)
H_a	0.87	t	6	8.2
H_b	2.32-2.26	m	1	-
$(Me)_c$	0.8	d	3	6.2
H_d	3.50	ddd	1	9.45, 6.3, 2.8
$(Me)_e$	0.03	s	3	-
$(Me)_f$	0.02	s	3	-
^tBu	0.88	s	9	-
H_f	0.70-0.62	m	1	-
$(Me)_g$	0.90	d	3	6.95
H_j	0.22-0.17	m	1	-
H_h	0.48-0.41	m	1	-
H_i	0.16-0.09	m	2	-
H_l	2.62	ddd	1	10.7, 6.95, 4.1
$(Me)_m$	3.68	s	3	-
H_k	3.50	ddd	1	9.45, 6.3, 2.8
$(Me)_n$	2.03	s	3	-

Table (4-2): Proton NMR analysis of compound (350)

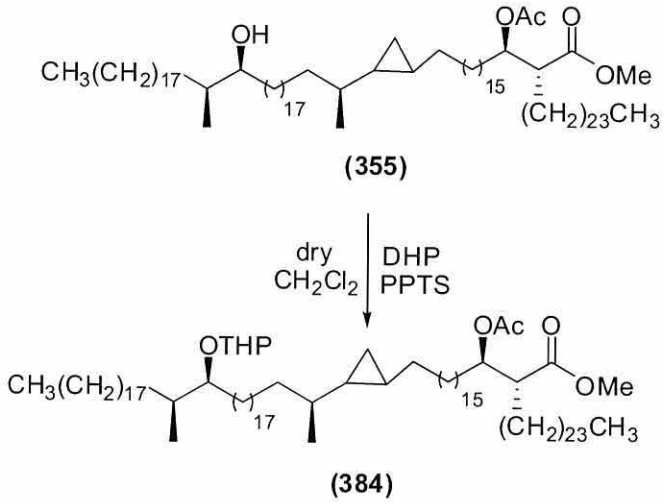
4.3.7 Deprotecting the silyl group

The final steps to obtain mycolic acids (**211**) and (**212**) involved deprotecting compound (**350**). In order to deprotect compound (**350**), the HF.pyridine complex and pyridine in dry THF was employed at 45° C. The resulting compound (**355**) was confirmed by proton NMR which showed a doublet of doublets of doublets at 5.09 ppm (J = 11.05, 8.2, 4.1 Hz) for the chiral proton adjacent to the acetyl group. The proton adjacent to the free hydroxyl showed a doublet of triplets at 3.50 ppm (J = 7.25, 4.1 Hz). There was a disappearance of the signals belonging to *t*-butyl and dimethyl silyl groups. The carbon spectrum demonstrated the disappearance of the signal of the carbon adjacent to the silyl group. The infrared spectrum showed a very broad of absorbance corresponding to a hydroxyl group at 3474 cm^{-1} (Scheme (**4-15**)).



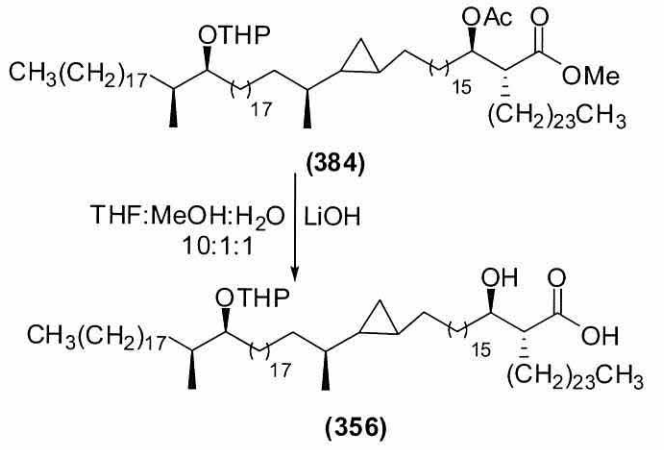
Scheme (4-15): Desilylation of compound (350)

Reprotecting the hydroxyl group with dihydro-2H-pyran was now carried out as described above (Section (**4.3**)). Stability in basic media was necessary, to allow the deprotection of the methyl ester and acetyl groups without losing the advantage of differentiating between the two hydroxyl groups. Freshly distilled dihydro-2H-pyran was added to a solution of compound (**355**) in dry dichloromethane in the presence of pyridium *p*-toluene sulfonate. The formation of the protected compound (**384**) with a THP group was confirmed by proton NMR which gave a triplet of doublets at 4.65 ppm (J = 16.4, 3.45 Hz) and a multiplet of two protons between 3.49-3.43 ppm for the THP group. The infrared spectrum showed the disappearance of any signal belonging to the hydroxyl group (Scheme ((**4-16**)).



Scheme (4-16): Protection with a THP group

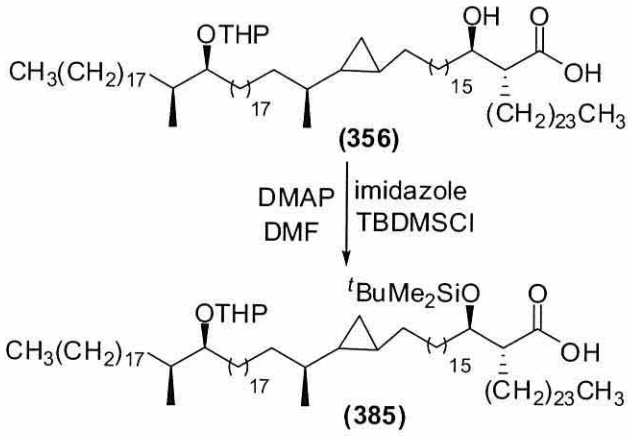
The next step was the hydrolysis of the protected mycolic compound (**384**) using lithium hydroxide in a mixture of THF:MeOH:H_2O at a ratio of 10:1:1. The reaction was stirred at 45 °C overnight (Scheme (**4-17**)). The formation of the deprotected compound (**356**) was confirmed by the proton NMR spectrum which gave a multiplet between 3.96-3.90 ppm for proton adjacent to the free hydroxyl group, since it became less acidic after removing the acetyl group, thus shifting to the upper field. The disappearance of any signal belonging to the acetyl or methoxy groups was observed. The infrared spectrum showed broad absorbance at 3519 cm^{-1} corresponding to the hydroxyl group.



Scheme (4-17): Hydrolysis of protected compound (384)

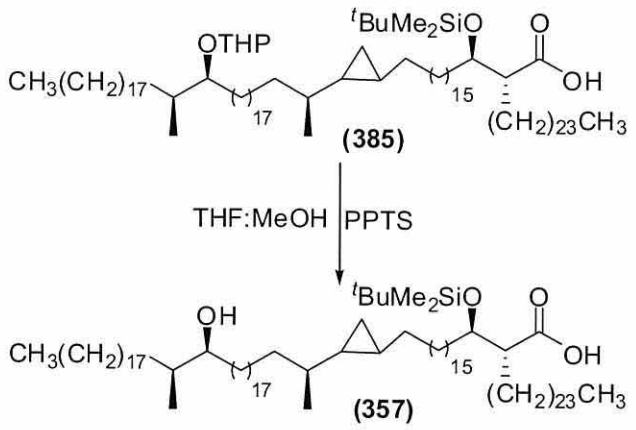
The next step was reprotecting compound (**356**) with a silyl group on the β-hydroxyl group of the mycolic motif (Scheme (**4-18**)). This was carried out by the use of *t*-

butylsilyl chloride and imidazole in dry DMF at 70 °C, leading to proection of both acid and alcohol groups. The crude product was stirred with potassium carbonate in a mixture of THF:MeOH: H_2O in a ratio of 10:1:1 in order to deprotect the acid and leave the hydroxyl protected. The formation of compound (**385**) was confirmed by the proton NMR spectrum which showed a singlet for the nine *t*-butyl protons at 0.93 and singlets at 0.15 and 0.14 ppm for the dimethyl groups. The ^{13}C NMR confirmed the presence of the silyl group and gave two signals for dimethyl carbons at -4.25 and -4.87 ppm.



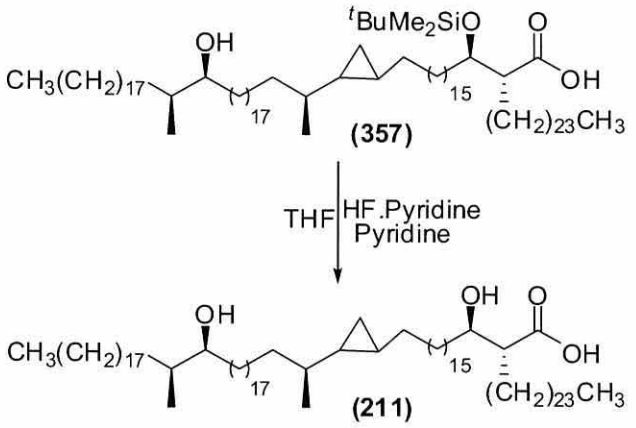
Scheme (4-18): Preparation of the protected compound (385)

The two secondary hydroxyl groups were now protected with two different protection groups. In order to remove the THP group, PPTS was used (Scheme (**4-19**)).[230] PPTS was added to a stirred solution of compound (**385**) in a mixture of THF:MeOH in a ratio of 10:1. The reaction was monitored using TLC until completion. The product (**357**) was verified by the proton NMR spectrum which demonstrated the lack of any signal belong to the THP group. The infrared spectrum confirmed the deprotection, showing a very broad absorbance at 3485 cm^{-1} corresponding to the hydroxyl group.



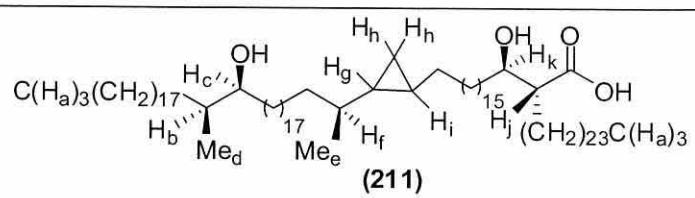
Scheme (4-19): Deprotection of the secondary alcohol (385)

In order to obtain the free hydroxyl mycolic acid (**211**), deprotection of the silyl group was carried out. The protected compound (**357**) was stirred with the HF.pyridine complex in the presence of pyridine in dry THF (Scheme (**4-20**)).



Scheme (4-20): Preparation of the free acid (211)

This showed identical sperta to those obtained earlier.[222] The free hydroxyl mycolic acid (**211**) was confirmed by the NMR proton spectrum as shown in Table (**4-3**).

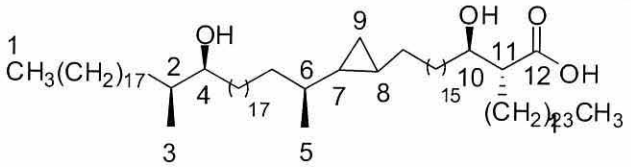


(211)

H_x	δ	Multiplicity	Integration	J (H_z)
H_a	0.88	t	6	5.7
H_b	1.63-1.60	m	1	-
H_c	3.73–3.69	m	1	-
Me_d	0.87	d	3	6.95
Me_e	0.90	d	3	4.15
H_f	0.48-0.43	m	1	-
H_g				
H_h	0.16-0.08	m	2	-
H_i	0.69-0.63	m	1	-
H_k	3.54–3.51	m	1	-
H_j	2.45	dt	1	8.8, 5.4

Table (4-3): Analysis of NMR data for mycolic acid (211)

The ^{13}C NMR spectrum provided additional confirmation of the success of the deprotection, shown in Table (**4-4**). The specific rotation for the free acid was $[\alpha]_D^{16}$ -1.05 (c = 0.55, $CHCl_3$).



(211)

Carbon No.	ppm	Carbon No.	ppm
1	14.20	7	19.72
2	38.8	8	10.53
3	18.62	9	13.62
4	72.2	10	75.5
5	18.62	11	50.8
6	38.18	12	178.8

Table (4-4): The ^{13}C NMR analysis of compound (211)

In order to obtain the non-epimerised keto mycolic acid (**212**), an oxidation to alcohol (**357**) was carried out using PCC in dichloromethane at room temperature (Scheme (**4-21**)). The formation of compound (**358**) was determined by the proton NMR spectrum which gave a doublet of doublets of doublets at 3.82 ppm (J = 7.9, 5.35, 2.55 Hz). The β-chiral centre proton gave a doublet of triplets at 2.41 ppm (J = 7.25, 2.2 Hz), the proton adjacent to the carbonyl in the meromycolic part gave a multiplet at 2.55-2.49 ppm and the CH_2 group adjacent to the distal position gave a triplet of doublets at 2.41 ppm (J = 7.25, 2.2 Hz) (Figure (**4-3**)). The two α-methyl groups gave a doublet at 1.06 (J = 6.9 Hz) and 0.90 ppm (J = 6.2 Hz). The *t*-butyl of the silyl group gave a singlet at 0.94 ppm. The specific rotation was $[\alpha]_D^{21}$ + 6.33 (c = 0.71, $CHCl_3$).

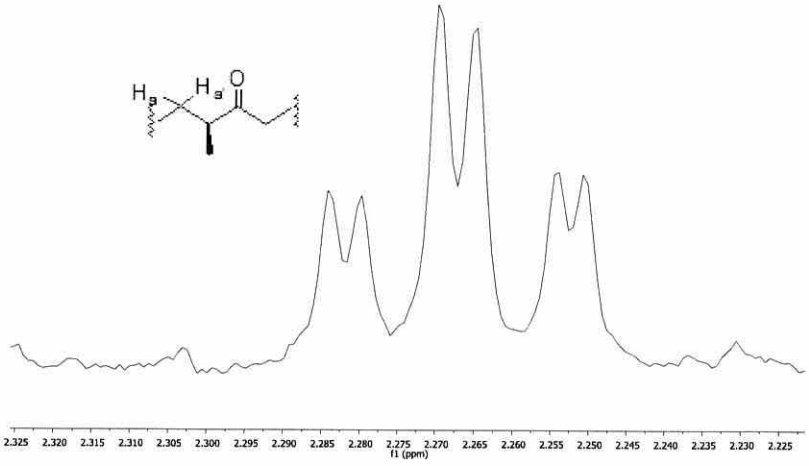
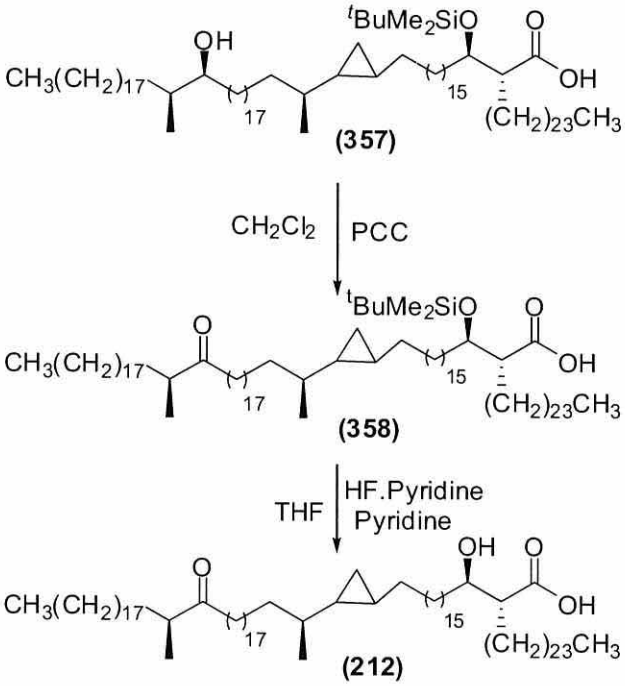
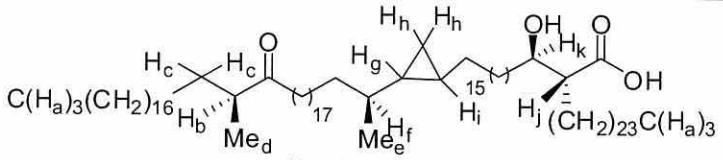


Figure (4-3): Proton NMR of the CH_2 group adjacent to methyl group in (358)



Scheme (4-21): Preparation of mycolic acid (212)

The deprotection of mycolic acid (**358**) was carried out as in Scheme (**4-21**), and the resulting compound (**212**) was determined by the proton NMR spectrum which gave the data illustrated in Table (**4-5**) and Figure **(4-4)**.



(211)

H_x	δ	Multiplicity	Integration	J (H_z)
H_a	0.88	t	6	6.65
H_b	1.77-1.70	m	1	-
H_c	2.29	td	2	9.45, 1.9
Me_d	0.90	d	3	6.95
Me_e	1.06	d	3	6.95
H_g	0.47-0.42	m	1	-
H_i	0.21-0.17	m	1	-
H_h	0.16-0.08	m	2	-
H_k	3.71	dt	1	7.9, 4.75
H_j	2.38	m	1	-

Table (4-5): NMR analysis of the mycolic acid (212)

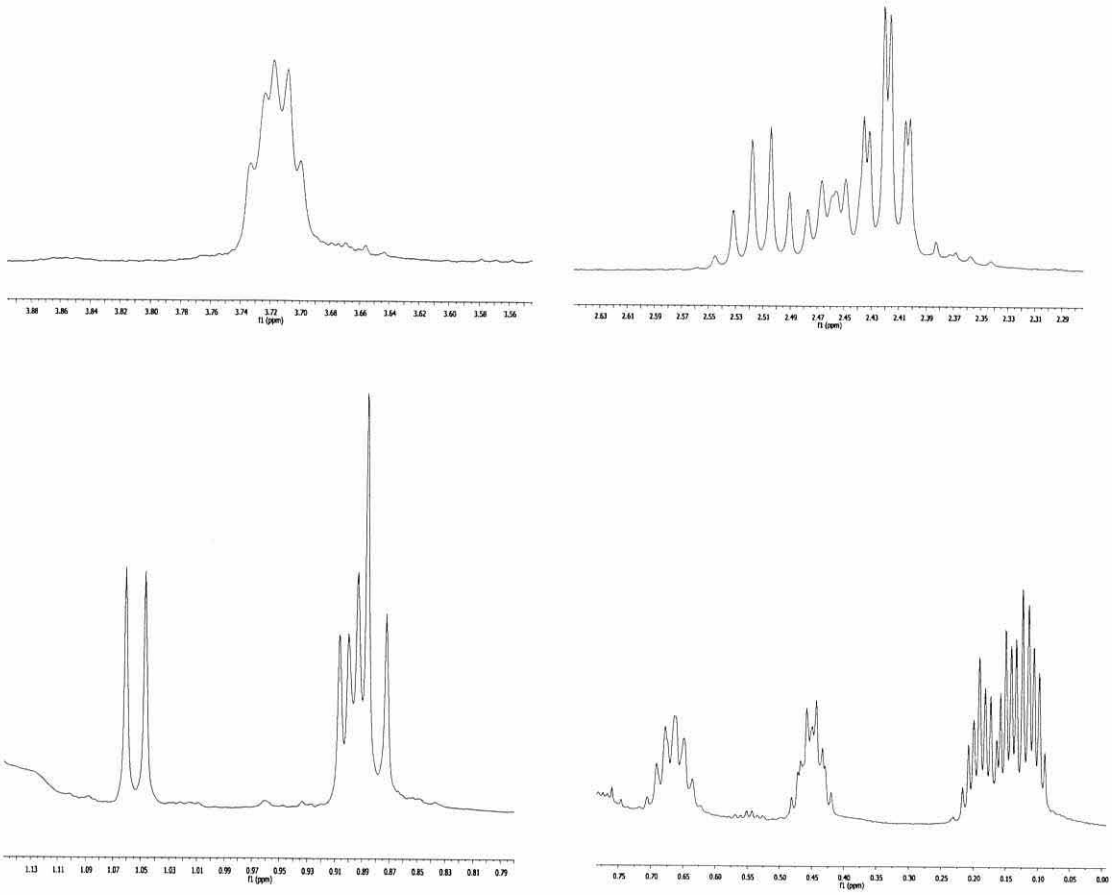
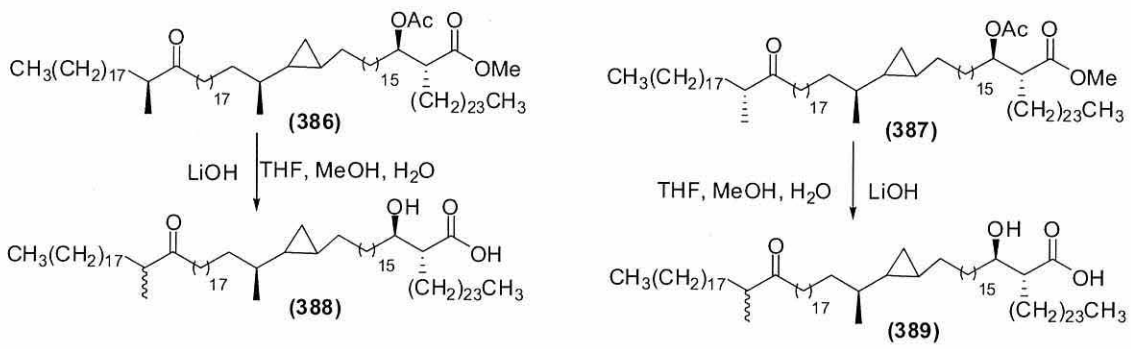


Figure (4-4): Proton NMR for mycolic acid (212)

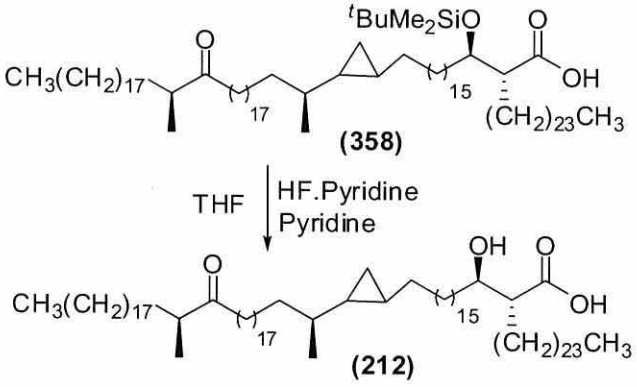
In previous studies, two different diastereoisomers of protected α-methyl-*trans*-keto mycolic acids have been synthesised. The only difference between these two is that in the one, (**386**) the stereochemistry of the methyl branch adjacent to the ketone was in *S*-configuration, while in (**287**) the methyl branch was in *R*-configuration. In both studies, an excess of lithium hydroxide monohydrate was used in the final deprotection step. Before the final deprotection was carried out, the molecular rotations for compounds (**386**) and (**387**) were +121.78 and +41.39 respectively.[93, 222] Following base hydrolysis of the methyl ester and acetyl protecting group, the free mycolic acids (**388**) and (**389**) both displayed a similar molecular rotation, giving +67.79 and +60.63, respectively. Thus when deprotected in this way, the stereochemistry adjacent to the ketone has become a mixture of epimers.



Scheme (4-22): The base deprotection step

Quémard *et al.* demonstrated the molecular rotation for the *R, R*-β-hydroxy-α-alkyl carboxylic acid to be M_D +40, and showed the *S*-methyl keto fragment to be M_D +44. The third fragment of mycolic acid (**212**) is the *trans*-cyclopropane ring fragment which was found in this study to have a molecular rotation of M_D +49.96, therefore; the theoretical M_D for the mycolic acid (**212**) can be found by adding the rotations of the fragments to each other: (+40) + (+44) + (+49.96) = +133.96, which rounds up to give M_D +134.

In this study the final deprotection step was conducted in acidic media, since the protecting group used was a silyl group which is easily removed with the use of HF. pyridine complex. The molecular rotation of the free acid (**212**) was M_D +134.96, compares favourably with the calculated molecular rotation of M_D +134, inferring that the free mycolic acid had been obtained without epimerisation of the α-methyl of the distal position.



Scheme (4-23): Acidic deprotection step

The mycolic acid (**212**) was prepared in all of these studies in order to discover if the stereochemistry has any effect on its biological activities. Having now successfully obtained a single stereoisomer of the α-methyl-*trans*-keto mycolic acid, it would be interesting to compare the biological effects of this single stereoisomer with the effects of the epimerised α-methyl-*trans*-keto mycolic acids prepared in earlier studies.

5-Preparation of an α-mycolic acid

One aim of this work was to prepare trehalose esters of mycolic acids, and in particular of an α-mycolic acid (**213**) identical to the major component in *M. tuberculosis*. This chapter briefly describes the synthesis of that α-mycolic acid, using a minor variation of an existing method Figure (**5-1**).

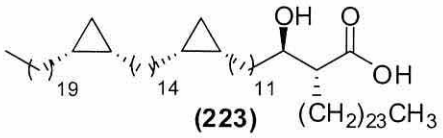


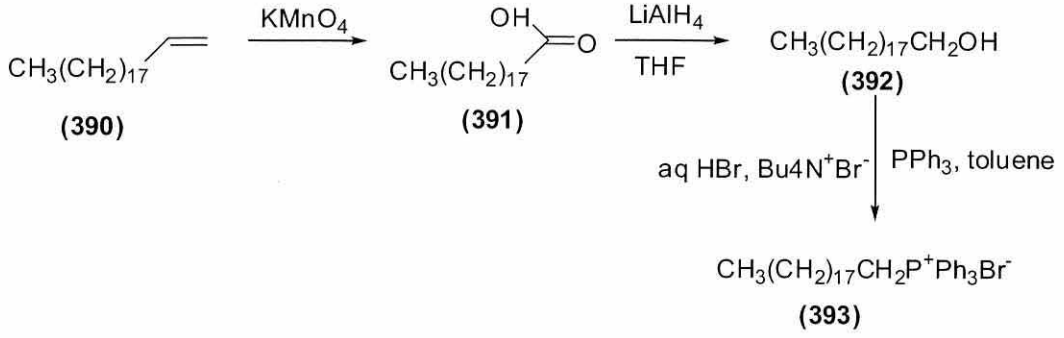
Figure (5-1): α-mycolic acid (213)

5.1 Natural α-mycolic acids

α-Mycolic acids are the major mycolic acids in the cell wall of mycobacteria such as *M. tuberculosis, M. microti, M. kansasii* and *M. avium*. The ratio of α-mycolic acid to oxygenated mycolic acid (both methoxy and keto) was shown to be 1:0.06 in some studies.[39] The biosynthetic pathway of α-mycolic acid groups has been described previously in Section (**1.11**).[219] George *et al.* showed that the gene *cma1* is responsible for the biosynthesis of the cyclopropane groups in the cell wall.[231] Yuan *et al.* illustrated that the conversion of the distal *cis*-double bond of *M. smegmatis* to a *cis*-cyclopropane ring occurs upon the action of single gene, *cma1*.[221] Researchers have confirmed that the biosynthesis of cyclopropane rings in the cell wall involves the transfer of a methyl group from SAM (**36**) to the double bond.[79, 80, 221, 231, 232]

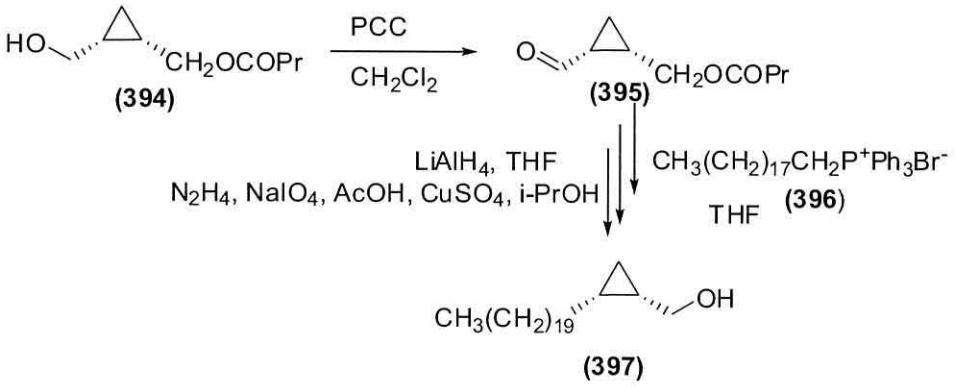
5.2 Previous synthesis of (213)

The first synthesis of (**213**) involved the synthesis of portions of the mycolic acid, followed by linking of the fragments through either Julia or Wittig reactions. The first fragment was prepared by oxidation of alkene (**390**) to form carboxylic acid (**391**). This was reduced to alcohol (**392**) and then converted in two steps to give the phosphonium salt (**393**) (Scheme (**5-1**)).



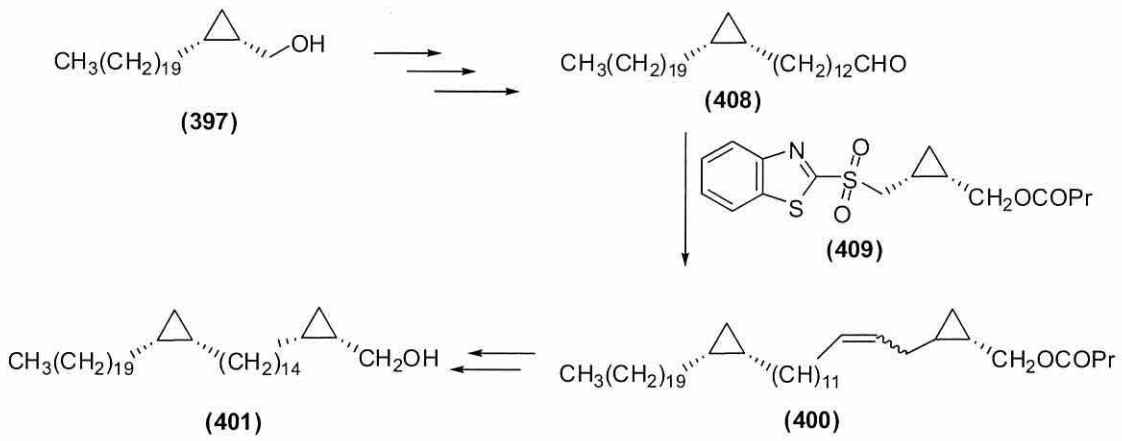
Scheme (5-1): Preparation of compound (393)

The next fragment prepared was the cyclopropane ring in a *cis*-configuration (**394**) which was oxidised to give aldehyde (**395**) and then coupled with phosphonium salt (**396**) through a Wittig reaction. This was followed by hydrogenation and deprotection to form alcohol (**397**) (Scheme (**5-2**)).



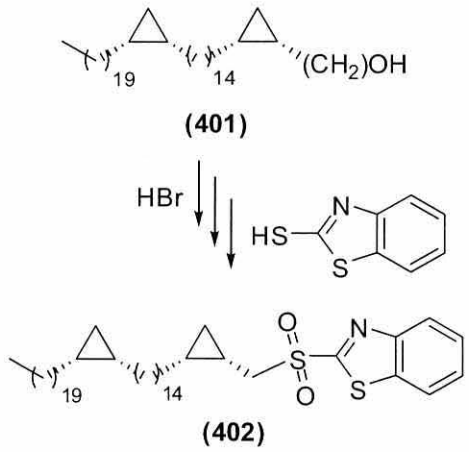
Scheme (5-2): The preparation of alcohol (397)

This alcohol (**397**) was homologated to give (**398**) by coupling it with a C_{12} chain which was followed with a few steps involving hydrogenation, reduction and oxidation to eventually yield aldehyde (**398**). The second fragment was prepared from the same cyclopropane compound (**394**) which was converted into sulfone (**399**) and then coupled with the first fragment (**398**), forming meromycolate alcohol (**401**) after hydrogenation and reduction (Scheme (**5-3**)).



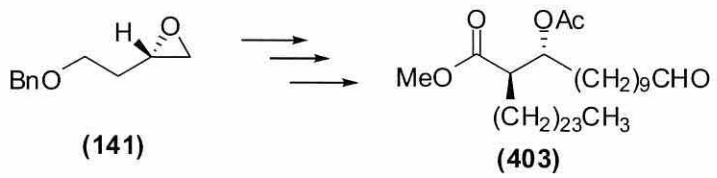
Scheme (5-3): Preparation of alcohol (401)

The alcohol (**401**) was converted into sulfone (**402**) by replacing the hydroxyl group with a bromide group, which was then exchanged for a sulfide group and then oxidised to give sulfone (**402**) (Scheme (**5-4**)).



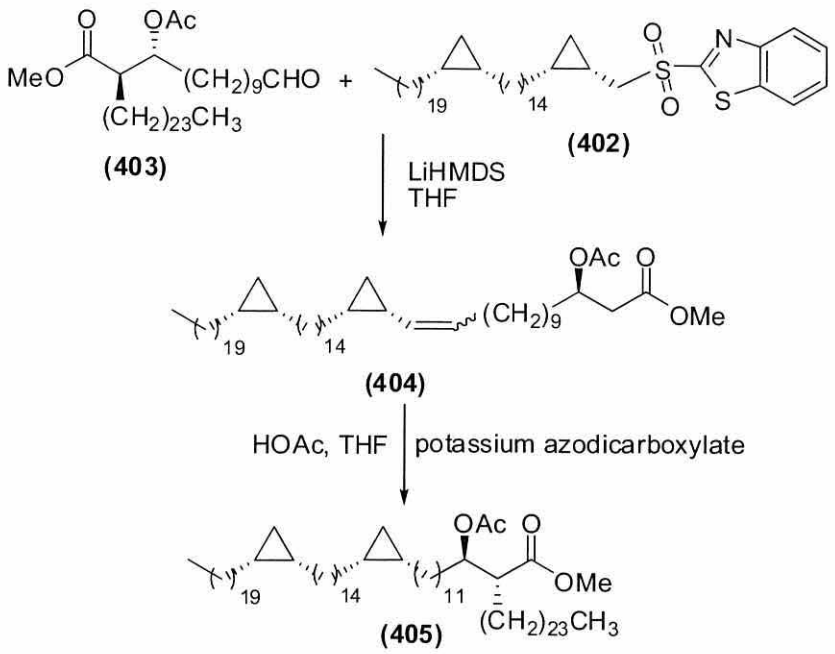
Scheme (5-4): The preparation of sulfone (402)

The mycolic motif (**403**) fragment was prepared from *L*-aspartic (see Section **1.14.3** for details) (Scheme (**5-5**)).



Scheme (5-5): Preparation of mycolic motif aldehyde fragment

The final steps involved coupling of the sulfone (**402**) with aldehyde (**403**), resulting in alkene (**404**). This was hydrogenated to yield the enantiomerically pure protected mycolic acid (**405**) (Scheme (**5-6**)).



Scheme (5-6): The preparation of the α-mycolic acid derivative (405)

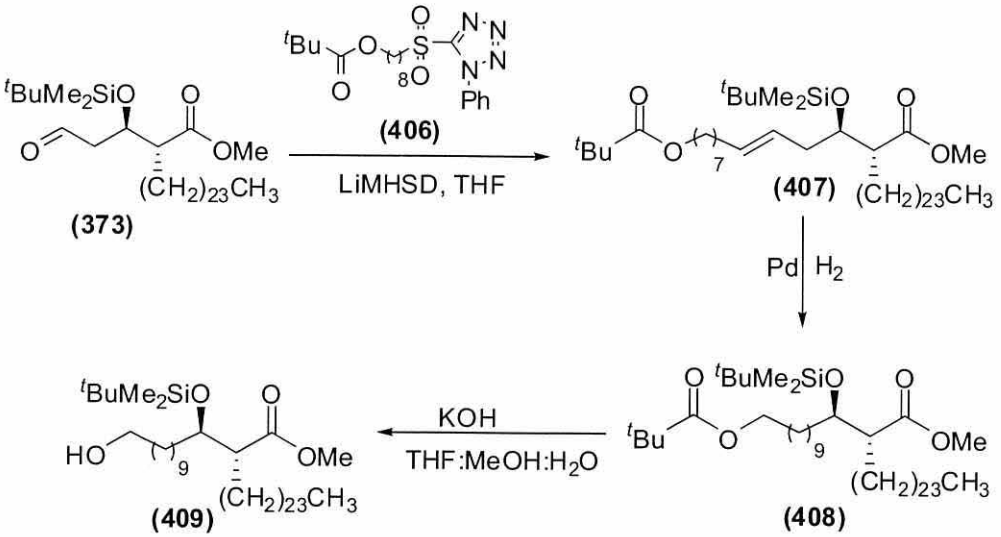
There is no report in the literature of the deprotection of (**405**) to give the free mycolic acid.

5.3 The synthesis of α-mycolic acid (213)

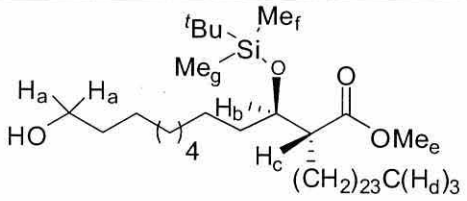
5.3.1 Preparation of the mycolic motif

The aldehyde (**373**) was coupled with sulfone (**406**) via a standard Julia reaction (Scheme (**5-7**)). The formation of the resulting alkene (**407**) was confirmed using proton NMR which gave a multiplet for the alkene protons between 5.43-5.36 ppm. The CH_2 group adjacent to the oxygen showed a triplet at 4.02 ppm (J = 6.3 Hz) and the proton adjacent to the silyl ether gave a doublet of triplets at 3.87 ppm (J = 7.9, 3.45 Hz). The ^{13}C NMR spectrum showed the carbon of the carbonyl groups at 174.95 and at 174.76 ppm. The next step was hydrogenation of the double bond using palladium on carbon (10 %) in a hydrogen atmosphere. The product (**408**) was characterised by proton NMR which gave a doublet of triplets at 3.90 ppm (J = 6.3, 4.4 Hz) for the β-chiral centre proton and the α-chiral centre proton showed a doublet of doublets of doublets at 2.51 ppm (J = 10.7, 6.9, 3.45 Hz). The CH_2 group adjacent

to the oxygen gave a triplet at 4.03 ppm (J = 6.95 Hz), with the lack of any signal in the alkene region.



Scheme (5-7): Preparation of the silyl-protected mycolic motif (409)



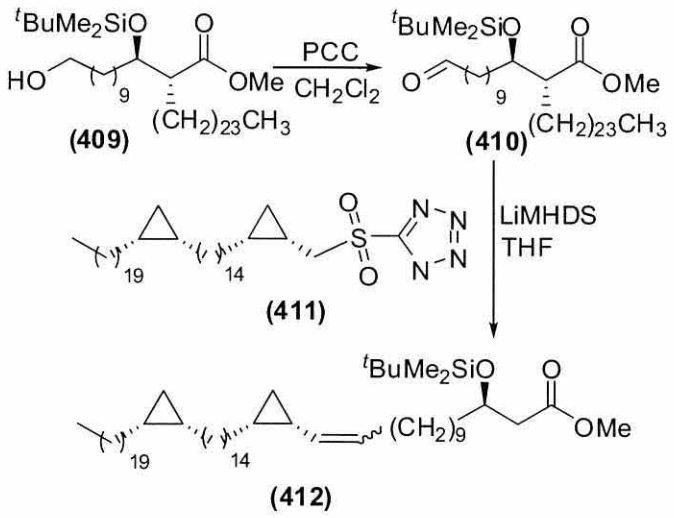
H_x	δ	Multiplicity	Integration	J (H_z)
H_a	3.63	t	2	7.9
H_b	3.91	dt	1	9.8, 4.4
H_c	2.52	ddd	1	10.75, 7.25, 3.8
Me_f	0.04	s	3	-
Me_g	0.02	s	3	-
^tBu	0.89	t	9	10.75
Me_e	3.65	s	3	-

Table (5-1): Proton NMR analysis of compound (409)

The final step was deprotection of the primary alcohol using hydrolysis. The formation of the free alcohol (**409**) was verified by the proton NMR spectrum which is presented in Table (**5-1**). The ^{13}C NMR showed one peak for the carbon of the carbonyl group at 175.11 ppm. The infrared spectrum provided additional confirmation with broad absorbance at 3368 cm^{-1}. The specific rotation of compound (**409**) was $[\alpha]_D^{24}$ -4.46 (c = 1.06, $CHCl_3$) (Scheme (**5-7**)).

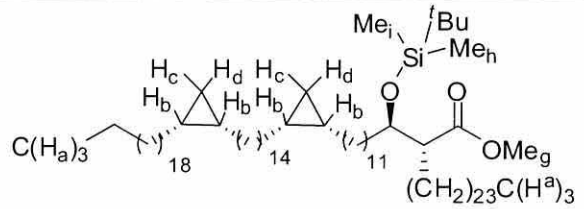
5.3.2 The coupling reaction

An oxidation reaction was carried out on the alcohol (**409**) by the use of PCC in dichloromethane. The formation of the aldehyde (**410**) was verified by proton NMR which gave a triplet at 9.74 (1.9 Hz) for the aldehyde proton, while the CH_2 group adjacent to the aldehyde carbonyl showed a doublet of triplets at 2.42-2.40 ppm (J = 1.59, 7.35 Hz). This was followed by a coupling reaction of this with sulfone (**411**) (donated thankfully by Dr. J. R. Al Dulayymi, Chemistry, Bangor University) (Scheme (**5-8**)). The formation of the resulting alkene (**412**) was confirmed by the proton NMR spectrum which showed multiplets at 5.54-5.38 and at 5.20-5.01 ppm for the two alkene protons. The proton adjacent to the silyl ether gave a doublet of triplets at 3.86 ppm (J = 6.95, 4.75 Hz), and the α-chiral centre gave a doublet of doublets of doublets at 2.53 ppm (J = 10.7, 6.95, 3.8 Hz). The two cyclopropane rings gave a multiplet between 0.66-0.64 ppm for four protons, a doublet of triplets for two protons at 0.56 ppm (J = 7.9, 3.8 Hz) and a quartet at -0.32 ppm (J = 5.35) for two protons.



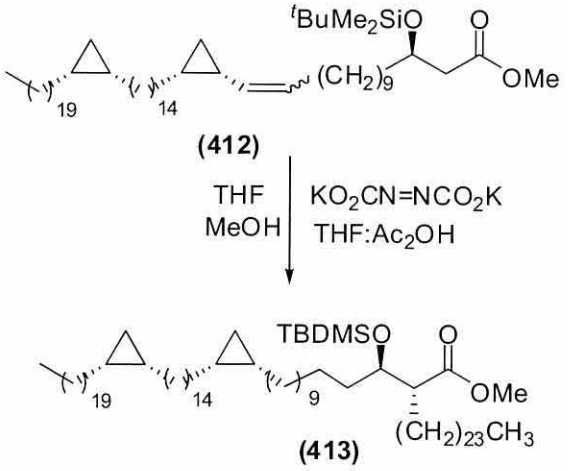
Scheme (5-8): Julia reaction to prepare the protected mycolic acid (412)

In order to saturate the alkene (**412**), dipotassium azodicarboxylate with acetic acid and THF (1:1) was used as before (Scheme (**5-9**)). The resulting compound (**413**) was verified by the proton NMR spectrum shown in Table (**5-2**). The ^{13}C NMR spectrum showed the lack of any carbon in the alkene region.



H_x	δ	Multiplicity	Integration	J (H_z)
H_a	0.89	t	6	6.9
H_b	0.67-0.64	m	4	-
H_c	-0.32	q	2	5.35
H_d	0.58	dt	2	3.75, 7.85
H_e	3.91	dt	1	7.25, 4.7
H_f	2.53	ddd	1	11.05, 7.25, 3.8
Me_g	0.05	s	3	-
Me_h	0.02	s	3	-
Me_i	0.87	s	9	-
H_j	3.66	s	3	-

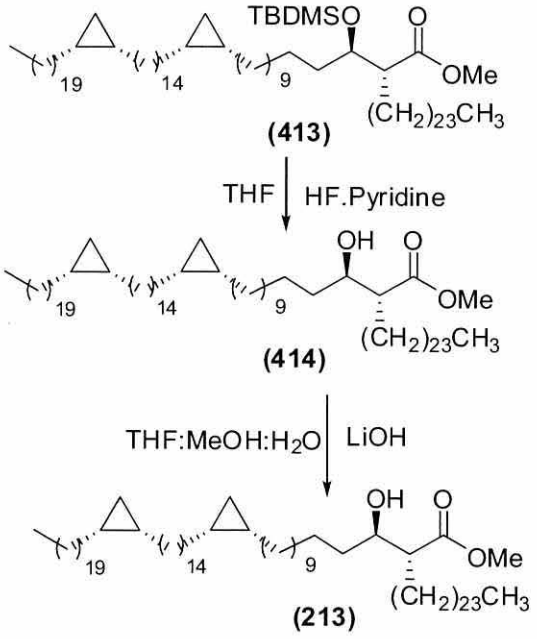
Table (5-2): NMR analysis of compound (413)



Scheme (5-9): Hydrogenation of alkene (412)

5.3.3 Deprotection mycolic acid (413)

To obtain the free mycolic acid (**213**), two deprotection steps were carried out (Scheme (**5-10**)). The first step was the desilylation using the same method as described above. The formation of the resulting compound (**414**) was confirmed by the proton NMR spectrum which showed the lack of a signal belonging to *t*-butyl dimethyl silyl ether at 0.87 ppm and around 0 ppm for the dimethyl groups (Scheme (**5-10**)).



Scheme (5-10): The preparation of unprotected mycolic acid (213)

The final step was hydrolysis using lithium hydroxide as previously described. The free mycolic acid (**213**) was characterised using proton NMR, showing a multiplet at 3.75-3.71 ppm for the proton adjacent to the hydroxyl group, while the α-proton gave a doublet of triplet at 2.48 ppm (J = 10.1, 5.4 Hz). The region of interest was between 0.6 and -0.3 ppm, belonging to the *cis* cyclopropane rings, gave a multiplet for four protons at 0.67-0.64 ppm, a doublet of triplets for two protons at 0.57 ppm (J = 8.2, 4.1 Hz) and a quartet at -0.32 ppm (J = 5.05 Hz). The terminal CH_3 group showed a triplet at 0.88 ppm (J = 6.65 Hz) (Figure **(5-2)**).

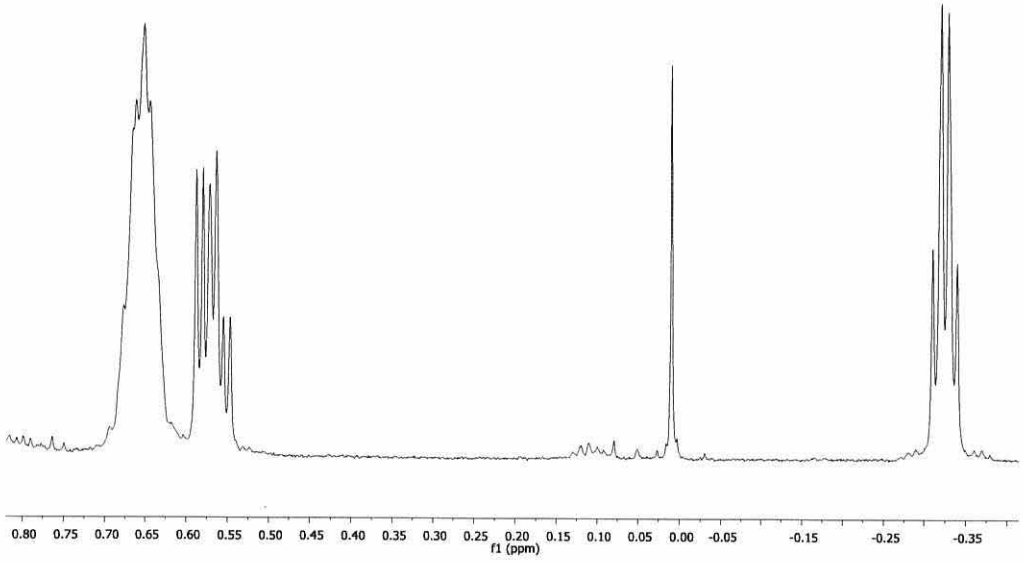


Figure (5-2): The cyclopropane proton NMR signals in the mycolic acid (213)

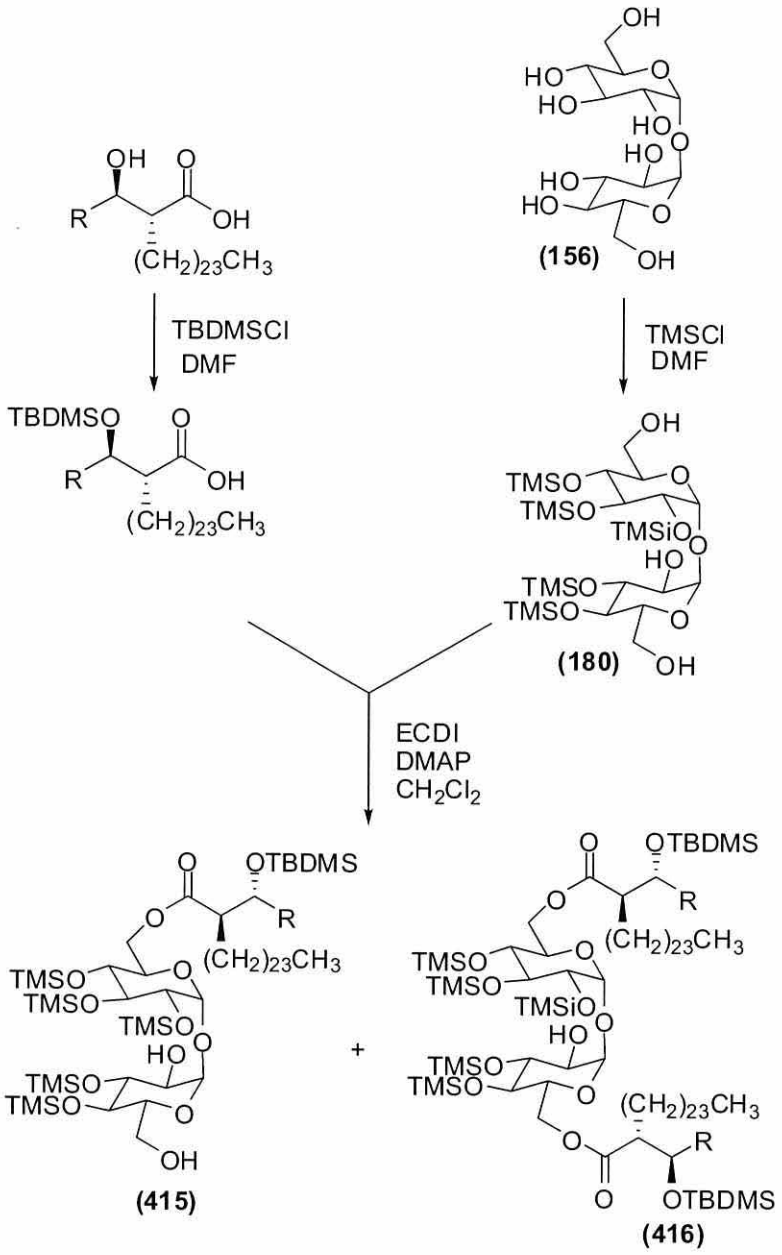
The mycolic acid (**213**) was used for synthesis the corresponding cord factor as described in Chapter 6.

6. Synthesis of trehalose esters ('cord factors')

In mycobacterial cell walls, some of the mycolic acid is esterified to trehalose, making cord factors. If two mycolic acids are esterified to trehalose, this forms trehalose-6,6'-dimycolates (TDMs), and if one mycolic moiety is esterified to trehalose, this forms trehalose monomycolates (TMMs).

The synthesis of trehalose esters of mycolic acids carried out in this study followed the same procedure as described in Section (**1.22.1**). The initial step of this procedure contains two major steps of protection, the first to the hydroxyl group of mycolic acid with TBDMS and the second to the trehalose sugar with TMS as shown in Scheme (**6-3**). The protected trehalose sugar donated by Dr. Maximiliano Maza-Iglesias [4] and the mycolic acids was carried out following the stranded method as in Scheme (**6-1**).[158, 233]

[4]Maza Iglesias , Maximiliano, University of Bangor



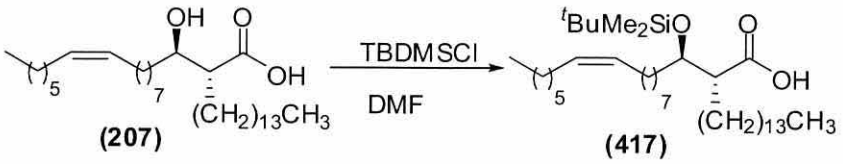
Scheme (6-1): Planned route for synthesis of cord factor

The aim of this work was to prepare sugar esters of the mycolic acids (**207**), (**208**), (**212**) and (**213**) prepared above. This required protection of the secondary β-hydroxy group in each of them as in Scheme (**3-9**). The mycolic acid (**212**) did not need this step since it was already protected with *t*-butyldimethsilyl ether.

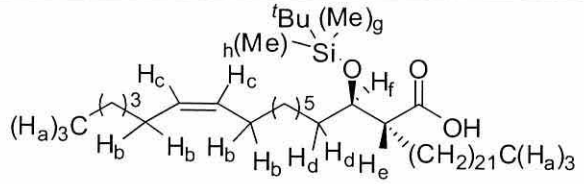
6.1 Selective protection of the hydroxy group of mycolic acids

In order to prepare a cord factor from the mycolic acid (**207**), it was necessary to protect the β-hydroxyl group using TBDMS. In the same procedure as for compound

(**251**) (Section **3.6.1**), the mycolic acid (**207**) was mixed with imidazole and *tert*-butyldimethylsilyl chloride in DMF and stirred for 18 hours at 45 °C. The crude product was protected at both the β-hydroxyl and the carboxylic acid; therefore it was dissolved in a mixture of THF, water and methanol and potassium carbonate was added and stirred overnight at 45 °C. This deprotects the acid group. The formation of compound (**417**) was confirmed by the proton NMR spectrum which gave a singlet for the *t*-butyl at 0.90 ppm and for the two methyl groups adjacent to the silyl group a singlet for three protons at 0.11 ppm and another singlet for three protons at 0.09 ppm, Table (**6-1**) and (Scheme (**6-2**)).



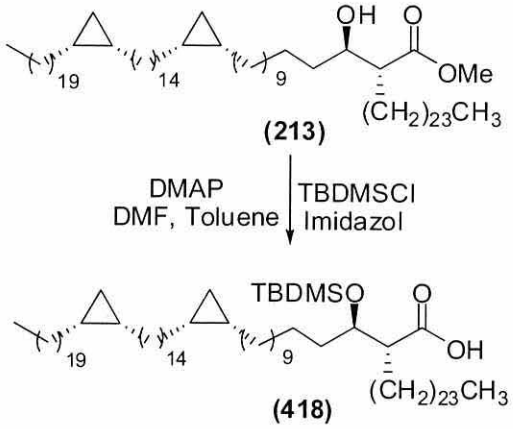
Scheme (6-2): Protected mycolic acid (417)



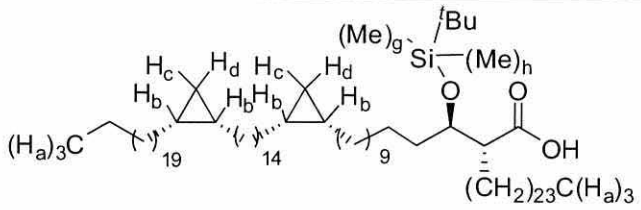
Hc_x	δ	Multiplicity	Integration	J (H_z)
H_a	0.89	t	6	7.5
H_b	2.01	q	4	7.14
H_c	5.35	t	2	5.56
H_d	1.54	m	2	-
H_e	2.53	dt	1	9.7, 4.7
H_f	3.86	dt	1	9.8, 5.95
Me_g	0.11	s	3	-
Me_h	0.09	s	3	-
^tBu	0.90	s	9	-

Table (6-1): Proton NMR analysis for protected mycolic acid (417)

Mycolic acid (**213**) was protected in the same way by stirring at 70 °C with *t*-butyldimethylsilylchloride in the presence of imidazole and 4-dimethylaminopyridine in a mixture of dry DMF and dry toluene 1:1.3. The reaction was monitored using TLC; after 24 hours, no starting material remained. The solvent was removed under vacuum and the residue was dissolved in a mixture of THF:MeOH:H_2O in a ratio of 10:1:1 and stirred overnight with potassium carbonate at 45°C in order to desilylate the protected carboxylic acid and leave the secondary hydroxyl protected. The formation of the required compound (**418**) was confirmed by the proton NMR spectrum (given in Table (**6-2**)). The ^{13}C NMR gave signals at -4.23 and -4.85 ppm for the methyl carbon adjacent to the silyl group. The specific rotation was $[\alpha]_D^{24}$ +1.64 (c = 1.76, $CHCl_3$) (Scheme (**6-3**)).



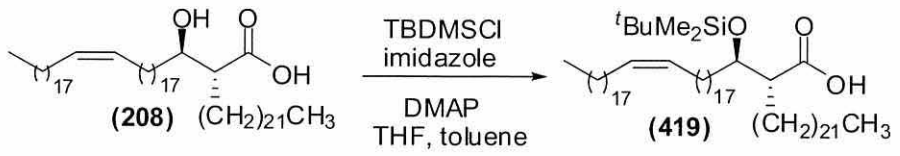
Scheme (6-3): Protected mycolic acid (418)



H_x	δ	Multiplicity	Integration	J (H_z)
H_a	0.89	t	6	6.65
H_b	0.67-0.64	m	4	-
H_c	-0.31	q	2	5.4
H_d	0.57	dt	2	8.2, 4.1
H_e	3.83	ddd	1	7.9, 5.05, 2.55
H_f	2.53	ddd	1	9.15, 6.35, 2.85
$(Me)_g$	0.15	s	3	-
$(Me)_h$	0.14	s	3	-
^tBu	0.93	s	9	-

Table (6-2): Proton NMR analysis for compound (418)

The same procedure was repeated for the mycolic acid (**208**) (Scheme (**6-4**)). The protected mycolic acid (**419**) was confirmed by proton NMR shown in Table (**6-3**) and (Figure (**6-2**)).



Scheme (6-4): Protection of the mycolic acid (419)

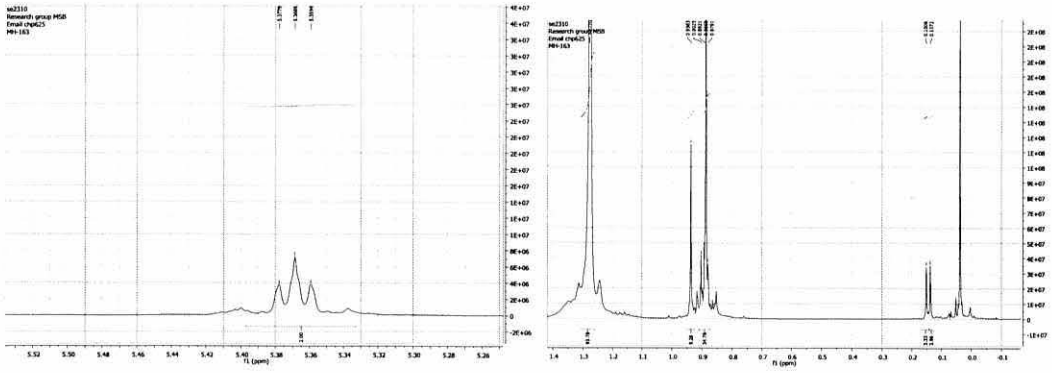
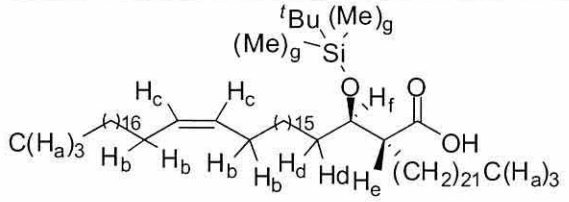


Figure (6-2): Proton NMR for compound (419)

The carbon spectrum provided additional evidence for the protection showing signals at -4.26 ppm and -4.90 ppm respectively for the dimethyl silyl groups. The infrared spectrum showed very broad absorbance at 3433 cm^{-1} for the hydroxyl group and a medium band for carbonyl group at 1706 cm^{-1}.

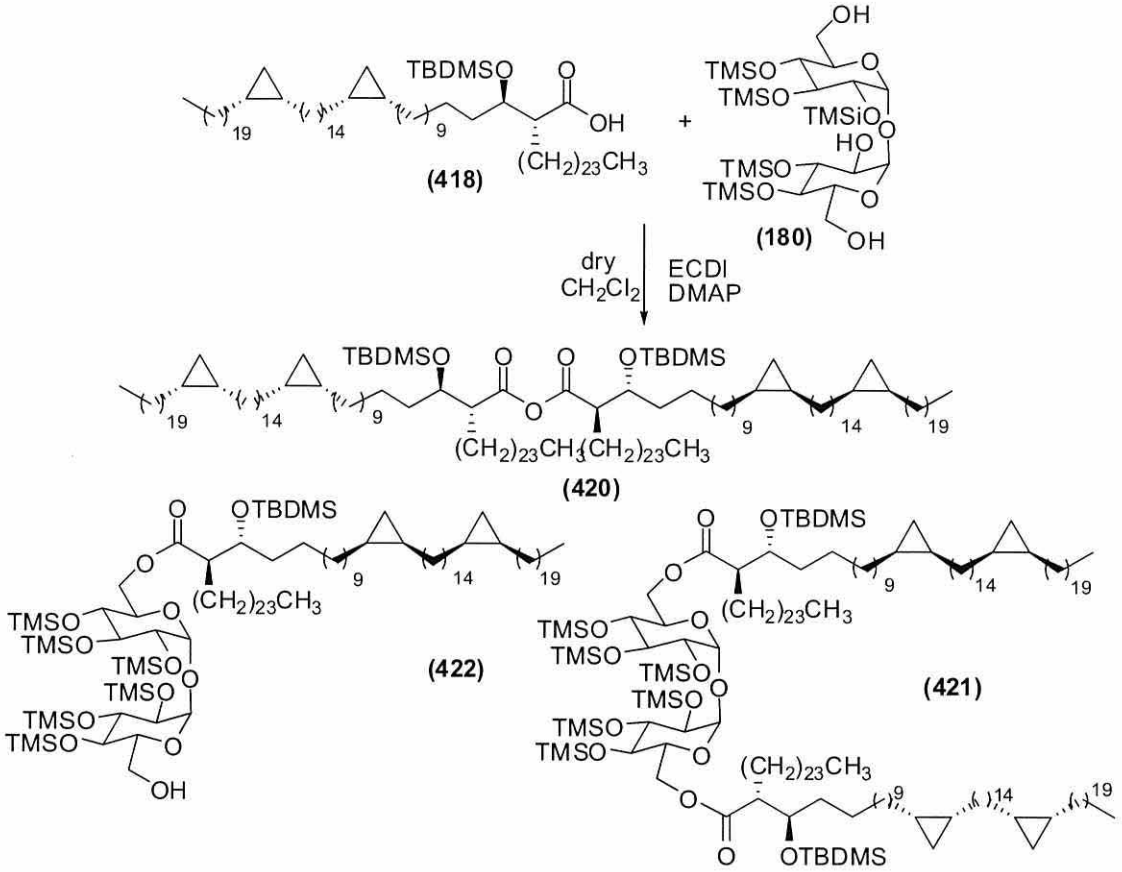


H_x	δ	Multiplicity	Integration	J (H_z)
H_a	0.87	t	6	3.85
H_b	2.02	v br q	4	6.6
H_c	5.35	br t	2	4.7
H_d	1.72-1.64	m	2	-
H_e	2.53	ddd	1	9.15, 5.35, 3.75
H_f	3.85	dt	1	7.25, 5.05
Me_g	0.06	br s	6	-
^{t}Bu	0.92	s	9	-

Table (6-3): Proton NMR analysis for protected mycolic acid (419)

6.2 Coupling of protected α-mycolic acid to protected trehalose

This involves an esterification reaction between protected mycolic acid (**414**) and protected trehalose (**180**) in the presence of EDCI and DMAP as catalysts which have been reported to improve the yield.[234] The reaction mixture was stirred at room temperature for 7 days, at which point TLC showed no starting material remaining. The solvent was evaporated and the resulting first fraction of carboxylic anhydride (**420**), the second fraction of TDM (**421**) and the third fraction of TMM (**422**) were formed (Scheme (**6-5**)).



Scheme (6-5): Synthesis of cord factor derivatives (421, 422) from an α-mycolic acid

The proton NMR for the mycolic anhydride (**420**) showed a doublet of triplets for the two protons adjacent to the silyl ether at 3.92 ppm (J = 11.35, 5.7 Hz), and for the α-carbon proton gave a doublet of triplets at 2.60 ppm (J = 11.05, 5.05 Hz). The four cyclopropane rings protons showed a multiplet of eight protons between 0.66-0.64 ppm, a doublet of triplets at 0.58 ppm (J = 7.85, 4.1 Hz) for another four protons and a broad quartet at -0.31 ppm (J = 5.4 Hz). The terminal methyl groups gave a triplet at 0.89 ppm (3.75Hz), while the *t*-butyl groups of the protecting groups showed a singlet at 0.87 ppm with the integration of 18 protons. MALDI confirmed the formation of the mycolic acid anhydride, giving a mass of *m/z* 2509.6 as expected.

TDM (**421**) showed for the sugar moiety a doublet for the two acetal protons at 4.86 ppm (J = 2.85 Hz); the rest of the sugar protons and the 10 β-hydroxyl protons resonated at 4.37, 4.04 - 3.89 and 3.39 ppm. The α-protons of the mycolic acid showed a doublet of triplets at 2.55 ppm (J = 9.8, 4.75 Hz). The *t*-butyl groups gave a singlet at 0.88 ppm for 18 protons. The terminal CH_3 groups gave a triplet at 0.89 ppm (12H, t, J = 4.75 Hz) and to the methyl groups of the protecting groups showed

as singlets at 0.166, 0.15 and at 0.14 ppm with 18 protons each. The cyclopropane ring protons showed a multiplet for eight protons between 0.56 (8H, m), a doublet of triplets for four protons at 0.57 ppm (J = 8.5, 3.8 Hz) and a quartet at -0.31 ppm (J = 5.35 Hz). The ^{13}C NMR showed the carbonyl carbon at 173.84 ppm, the anomeric carbon signal at 94.83 ppm and the rest of the sugar carbons between 73.55 and 70.73 ppm. MALDI MS showed an $[M + Na]^+$ of *m/z* 3266.3, while the calculated value was *m/z* 3266.9.

The third fraction was TMM (**422**), and the proton NMR was more complicated than that of TDM due to the lack of the symmetry in the TMM. The hemiacetal protons gave a doublet at 4.9 and at 4.85 ppm. The rest of sugar carbons resonated at 4.35, 4.07, 3.99, 3.96-3.94 and 3.91 ppm. The proton adjacent to the silyl ether showed a doublet of triplets at 3.84 ppm (J = 6.6, 3.45 Hz). The α-proton of mycolic acid gave a doublet of doublets of doublets at 2.55 ppm (J = 9.45, 5.56, 3.45 Hz). The cyclopropane rings gave a multiplet for four protons between 0.67-0.64 ppm, a doublet of triplets for two protons at 0.57 ppm (J = 4.1, 8.2 Hz) and a quartet for two protons at -0.32 ppm (J = 5 Hz). The *t*-butyl gave a singlet at 0.88 ppm for nine protons. The methyl groups of the protection groups resonated at 0.17, 0.16, 0.156, 0.151, 0.15 and 0.12 ppm with the integration nine protons for each. The MALDI MS showed a molecular ion at 2032.13 as expected.

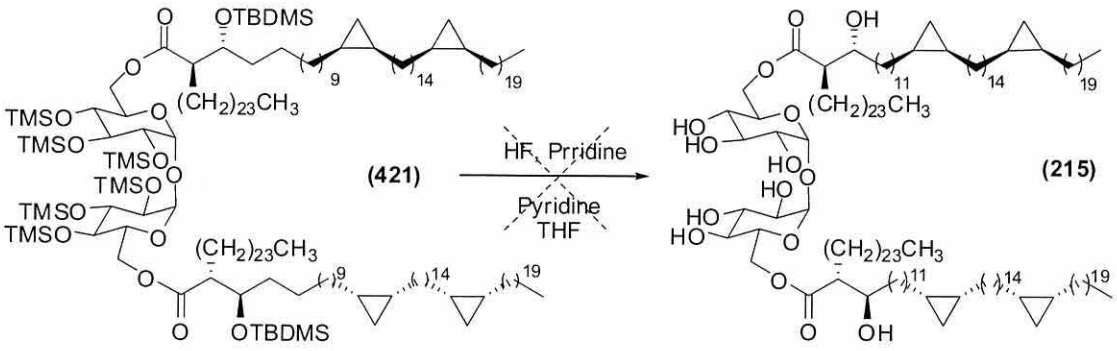
6.3 Final deprotection to give free cord factors containing α-mycolic acid

In order to obtain free cord factor, deprotection both trimethylsilyl and *t*-butyldimethylsilyl protecting groups was necessary.

6.3.1 Attempted one-step deprotection

This was attempted using the method applied above for deprotecting *t*-butyl dimethyl silyl to remove the TMS-group as well. Treatment with the HF.pyridine complex overnight led to complete loss of starting material and no observable product (Scheme (**6-6**)).

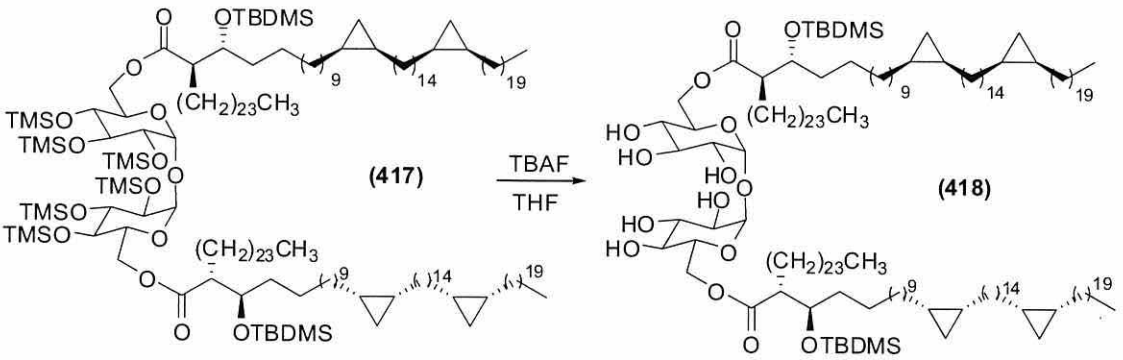
Therefore, a two-step deprotection process was carried out on both TDM and TMM using the method described earlier (see Section (**1.22.1**)).[158]



Scheme (6-6): Attempted one-step deprotection of TDM (421)

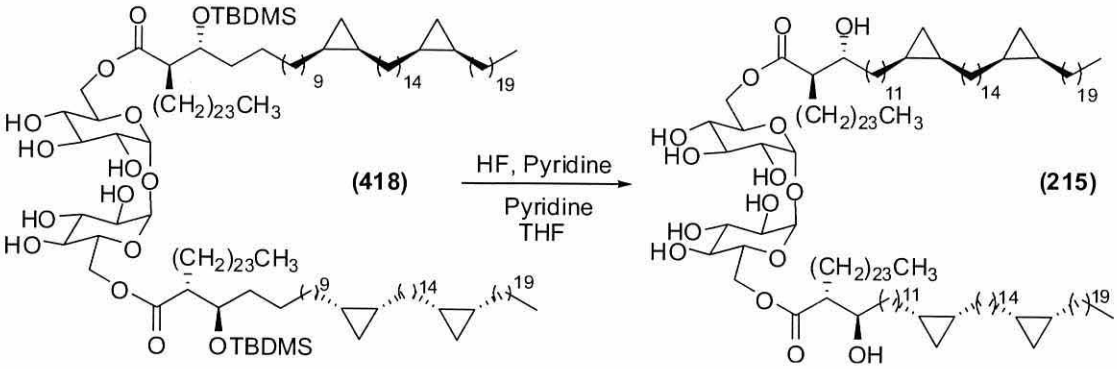
6.3.2 Two-step deprotection of TDM

The first step of the deprotection process involved deprotecting the trimethyl silyl ether groups from the sugar moiety using TBAF. TDM was stirred with TBAF at room temperature for 1 hour (Scheme (**6-7**)). The success of the reaction was confirmed by the proton NMR spectrum which gave for the hemiacetal protons a doublet at 4.99 ppm (J = 3.45 Hz); the rest of the sugar protons resonated at 4.22, 3.88, 3.82, 3.71, 3.45 and 3.25 ppm with the integration two protons per signal. The mycolic acid moiety gave to the protons adjacent to the silyl ether a doublet of triplets at 3.39 ppm (J = 9.45, 3.45 Hz). The α-protons showed a doublet of doublets of doublets at 2.46 ppm (J = 10.1, 6.35, 3.5 Hz). The cyclopropane rings gave a multiplet for eight protons between 0.56-0.55 ppm, a doublet of triplets for two protons at 0.47 ppm (J = 8.2, 4.1 Hz) and a quartet for two protons at -0.41 ppm (J = 5.05 Hz). The *t*-butyl of the protecting group showed a singlet at 0.77 ppm and the methyl groups of the protecting group showed singlets at -0.04 and at -0.06 ppm for six protons each. No signal belonging to trimethyl silyl ether was observed.



Scheme (6-7): Deprotection of the trehalose moiety (417)

The final step was deprotection of the *t*-butyl silyl ether from the β-hydroxy group in mycolic acid (**423**), as described above (see Section (**2.12.1**)) using the HF.pyridine complex. The formation of the resulting free TDM (**215**) was confirmed by the proton NMR spectrum which gave for the hemiacetal protons a doublet at 4.88 ppm (J = 3.15 Hz). The rest of the sugar protons resonated at 4.24, 6.16, 3.99, 3.61, 3.51-3.48 and at 3.33 ppm with two protons integrated in each signal. The protons adjacent to the β-hydroxyl of the mycolic acid showed a doublet of doublets at 3.91 ppm (J = 11.35, 6.65 Hz). The α-protons of the mycolic moiety showed doublet of doublets of doublets at 2.25 ppm (J = 12, 7.9, 4.75 Hz). The terminal CH_3 groups gave a triplet at 0.71 ppm (J = 1.6 Hz) with the integration of 12 protons. The cyclopropane rings gave a multiplet between 0.49-0.47 ppm with an integration of eight protons, at 0.39 ppm (J = 7.9, 4.1 Hz) for four protons and at -0.49 ppm (J = 5.05 Hz) for the other four protons. The ^{13}C NMR showed the carbonyl carbon at 175.08 ppm and the anomeric carbon gave a signal at 93.37 ppm. The rest of the sugar carbons resonated at 72.63 and at 70.18 ppm (Scheme (**6-8**)). MALDI MS showed an $[M + Na]^+$ of 2833.9, while the calculated value was 2833.9.

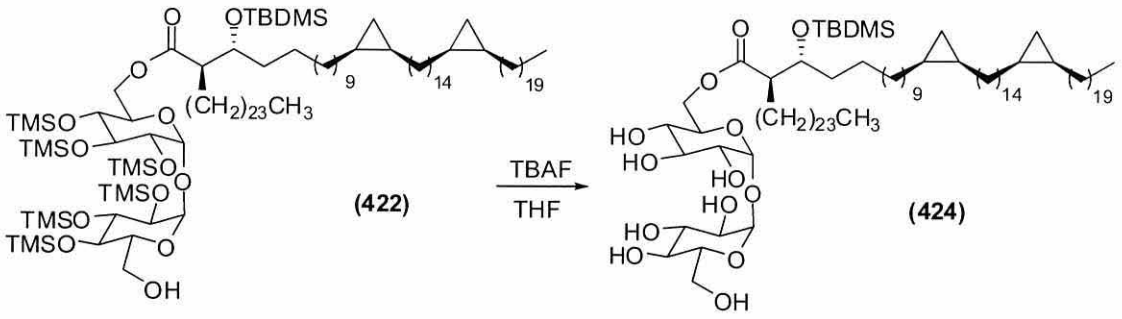


Scheme (6-8): Preparation of complete cord factor

6.3.3 Deprotection of TMM (214)

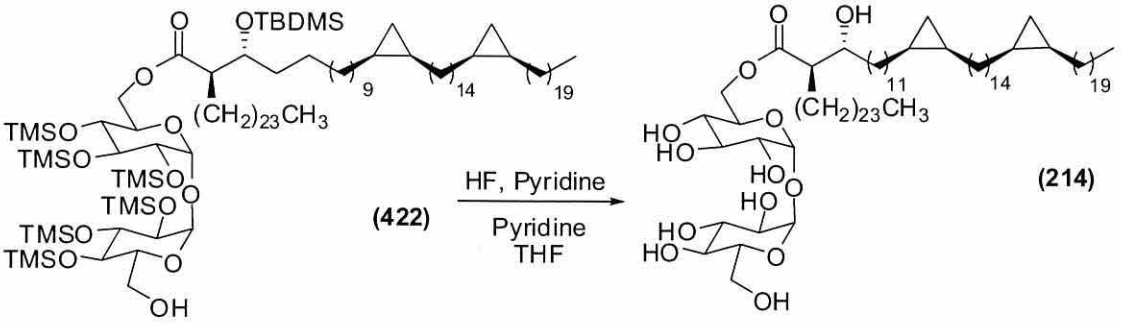
Following the same procedures described above for the TDM, a two-step deprotection was carried out (Scheme (**1-42**)). The first step used TBAF to deprotect the sugar moiety, and the success of this step in forming compound (**424**) was confirmed by proton NMR which gave a doublet for the hemiacetal protons at 4.93 ppm (J = 3.5Hz) and signals for the rest of the sugar protons between 4.15 and at 2.86 ppm. The mycolic acid moiety showed for the α-proton a doublet of doublets at 2.40 ppm (J =

10.4, 6.65, 3.8 Hz), and the α-proton gave a doublet of doublets of doublets at 2.25 ppm (J = 12, 7.9, 4.75Hz). The cyclopropane rings gave a multiplet for four protons between 0.51-0.49 ppm, a doublet of triplets at 0.39 ppm (J = 8.2, 4.1 Hz) and a quartet at -0.48 ppm (J = 5.05 Hz). The *t*-butyl group gave a singlet at 0.70 with an integration of nine protons, and the dimethyl of the protecting group gave two singlets at -0.10 and -0.12 ppm with an integration of three protons for each peak (Scheme (**6-9**)).



Scheme (6-9): Deprotection of the trehalose moiety

The final step removed the *t*-butyldimethyl silyl ether by the method described above (see Section (**1.2.1**)). The product (**214**) was characterised by the use of proton NMR which showed the same spectrum as for protected compound (**424**) except for the absence of peaks belonging to the protecting group (Scheme (**6-10**)).



Scheme (6-10): The preparation of deprotected TMM (224)

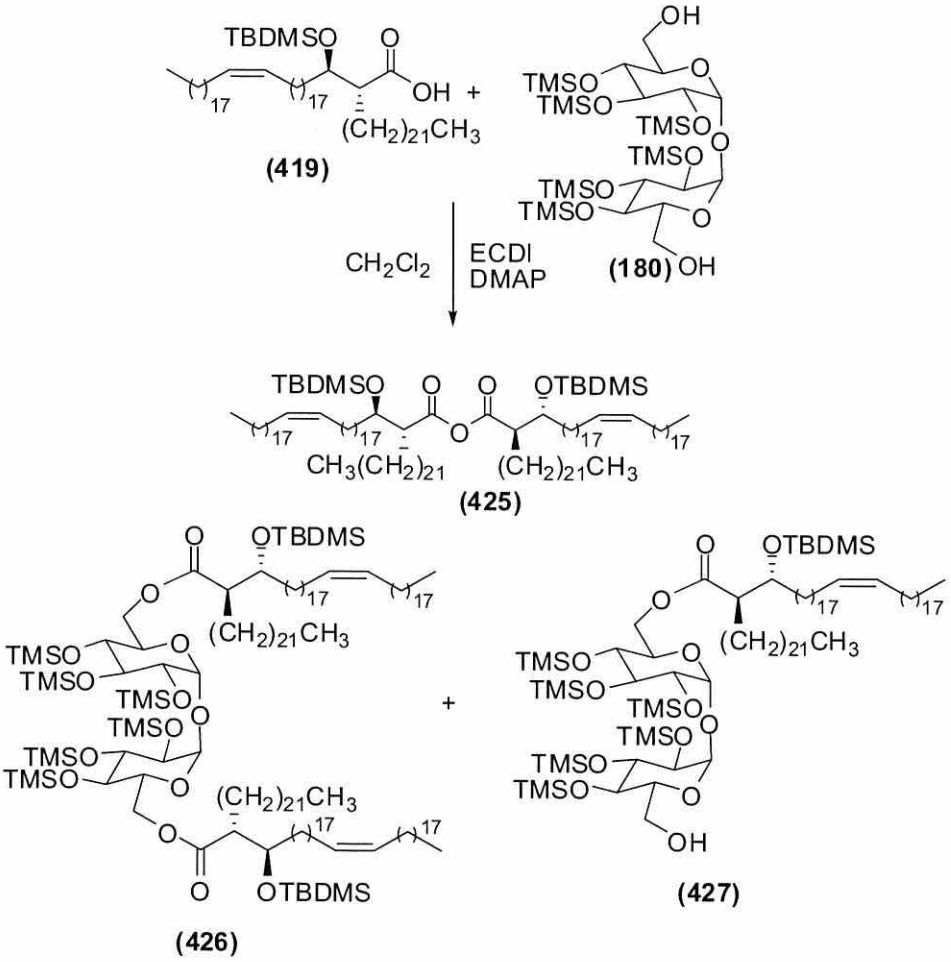
The synthesis of TDM (**215**) and TMM (**214**) complements earlier syntheses of TDM (**187**) and TMM (**185**), the difference being in the stereochemistry of cyclopropane ring in each case. A study of their effects as antigens against antibodies present in the serum of TB patients is being carried out to determine whether the stereochemistry matters.

6.4 Synthesis of a cord factor derived from an alkene mycolic acid

6.4.1 The coupling reaction

The protected mycolic acid (**419**) was used to prepare the first cord factors with unsaturated mycolic acids, TDM (**216**) and TMM (**217**). The same method employed to prepare TDM (**214**) and TMM (**215**) (Scheme (**2-83**)) was adopted to prepare TDM (**216**) and TMM (**217**).

The productwas separated into three fractions by column chromatography. The first fraction was identified by proton NMR spectrum as the anhydride (**425**), showing a broad triplet at 5.35 ppm (J = 4.7 Hz) for the alkene protons integrating to four protons, while the protons adjacent to the silyl ether gave a doublet of doublets at 3.96 ppm (J = 11.05, 6.6 Hz) with the integration of two protons. The α-protons gave a doublet of doublets of doublets at 2.55 ppm (J = 13.6, 9.8, 3.8 Hz) with the integration of two protons (Scheme (**6-11**)). MALDI MS showed an $[M + Na]^+$ of 2063.0, while the calculated value was 2063.0.



Scheme (6-11): Preparation of unsaturated trehalose ester derivatives (426, 427)

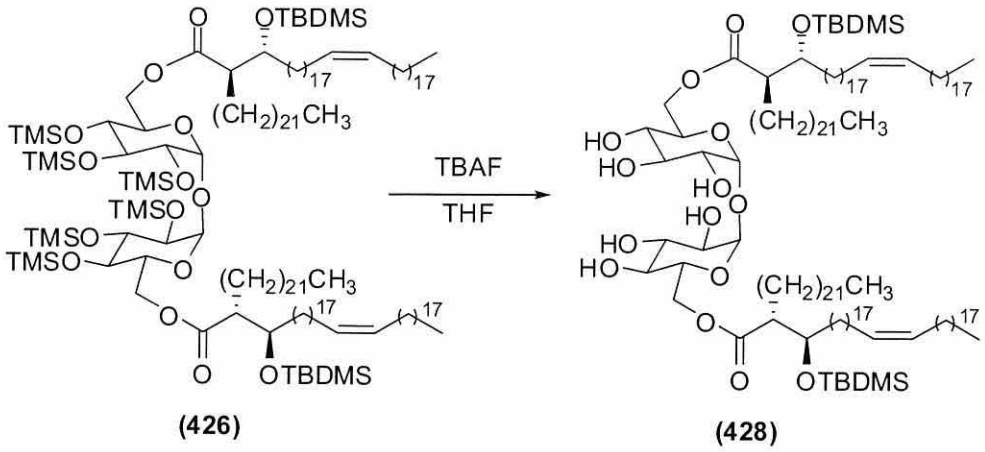
The second fraction was protected TDM (**426**) as was confirmed by the proton NMR spectrum which gave a triplet at 5.20 ppm (J = 4.75 Hz) for the alkene protons with the integration of four protons, while the two hemiacetal protons gave a doublet at 4.69 ppm (J = 2.85 Hz). The rest of the sugar protons resonated between 4.20 ppm to 3.21 ppm, including two protons of β-mycolic acid which were adjacent to the silyl ether. The α-protons of the carboxylic acid of the mycolic acid showed a doublet of doublets of doublets at 2.39 ppm (J = 14.15, 10.05, 4.7 Hz) with an integration of two protons. The trimethyl silyl ether protecting groups gave signals at 0.00, -0.01 and -0.02 ppm with an integration of 18 protons for each peak. The *t*-butyl group gave a singlet at 0.72 ppm with an integration of 18 protons and the methyl groups of the TBDMS gave a broad singlet at -0.10 ppm.

The third fraction was protected TMM (**427**), which showed a triplet at 5.33 ppm with an integration of two, for the alkene protons. The hemiacetal protons gave doublets at 4.91 and at 4.84 ppm each integrating to one proton. The rest of the sugar protons resonated between 4.06 and 3.40 ppm. The proton in the β-carbon in the mycolic acid give a doublet of triplets at 3.99 ppm (J = 6.3, 2.5 Hz). The α-chiral centre gave a doublet of doublets of doublets at 2.55 ppm (J = 9.1, 5.35, 3.15 Hz). The terminal methyl groups gave a triplet at 0.88 ppm (J = 6 Hz) with the integration of six protons. The trimethyl silyl ether protecting groups appeared at 0.17, 0.159, 0.155, 0.14 and 0.12 ppm with an integration of nine protons for every peak, and the TBDMS protecting group of the mycolic acid gave a singlet at 0.87 ppm. The dimethyl silyl resonated at 0.052 and 0.05 ppm with the integration three protons per peak.

6.4.4 Attempted deprotection of TDM (436)

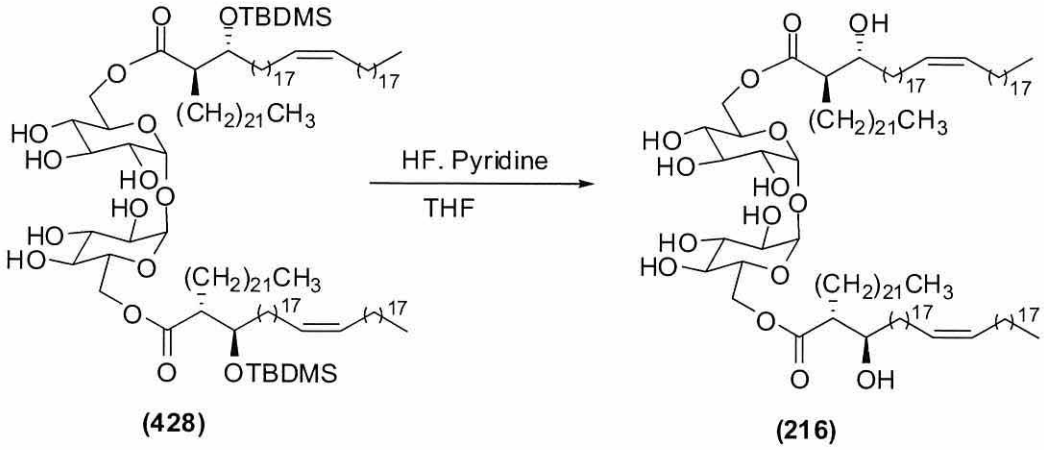
The first deprotection step involved the use of TBAF in order to remove the trimethyl silyl protecting group from the sugar. The formation of the product (**428**) was confirmed by the proton NMR spectrum which showed the lack of any peak belonging to the trimethyl silyl group (Scheme (**6-12**)).

The following step was the removal of the *t*-butyldimethylsilyl ether from the mycolic acid of compound (**428**) using HF.pyridine and pyridine.



Scheme (6-12): Deprotection of compound (426)

The formation of the TDM (**216**) was confirmed by proton NMR which gave a triplet for four protons at 5.24 ppm (J = 4.4 Hz) for the alkene protons, while the hemiacetal gave a doublet for two protons at 4.93 ppm (J = 3.45 Hz). The rest of the sugar protons resonated between 4.06 and at 3.17 ppm. The protons adjacent to the β-hydroxyl of mycolic acid gave a doublet of triplets for two protons at 3.56 ppm (J = 7.9, 3.15 Hz). The ^{13}C NMR gave a peak for the carbonyl carbon at 175.50 ppm and for the alkene carbons at 129.85 ppm. The hemiacetal carbon resonated at 95.05 ppm and the rest of sugar carbons resonated between 72.47 and 69.87 ppm. MALDI MS showed $[M + Na]^+ = 2158.4$, while the calculated value was 2158.6 (Scheme (**6-13**). The cord factor TDM (**216**) and other α-mycolic acid cord factor order to discover if the stereochemistry has any effect on their biological activities.

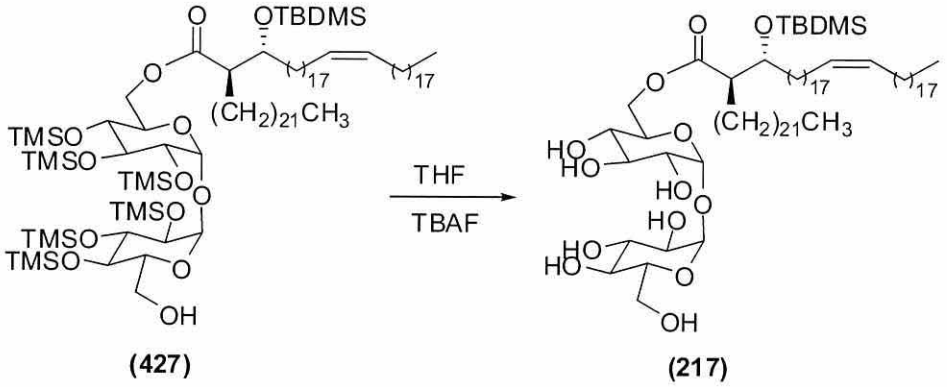


Scheme (6-13): Preparation of unsaturated cord factor (216)

Having an early detection method for TB means that less time is spent in quarantine and anti-TB therapy is able to start sooner.[235] A mixture of natural mycolic acids can be used for the serodiagnosis for TB run in an ELISA plate assay, although the selectivity is not accurate enough for commercial application. Patients with HIV and TB co-infection, retain high levels of antibodies to cord factors, making this method attractive in co-infected patients, but the natural mixtures of mycolic acids used was still not sufficient for accurate serodiagnosis. [236] It has been reported that cholesterol antibodies an TB antibodies may both interact with mycolic acids, interfering with the accuracy when testing patients for TBA more sensitive diagnosis method can be achieved by using synthetic enantiomers of single MA instead of natural extracts which contain a complex mixture of mycolic acids. A specific synthetic MA antigen could give more accurate data and give greater distinction between TB positive and TB negative patient sera.[235] One reason for making the compounds above is to test the antigenic activity of natural and synthetic MA using TB positive and TB negative serum samples with ELISA. Furthermore these studies will demonstrate the role of the functional groups of the mycolic acids within the different structures.[236]

6.3.2 Deprotection of TMM (427)

The deprotection of (**427**) firstly involved the use of TBAF in order to remove the trimethyl silyl protecting groups on the trehalose. The formation of the product (**217**) was confirmed by the proton NMR spectrum which showed the lack of any peak belonging to the trimethyl silyl group. The alkene proton showed a triplet (J = 4.7 Hz) while the trehalose sugar protons resonated at 5.007, 4.46, 4.25, 4.16, 3.70, 3.39 and 3.32 ppm. The proton on the α-chiral centre to the acid showed a multiplet between 2.35-2.31 ppm and the proton on the β-chiral centre gave a multiplet between 3.90-3.86 ppm. The MALDI mass as expected showed *m/z* 1484.1478 with molecular formula $C_{80}H_{156}O_{13}SiNa$ (Scheme (**6-14**)).



Scheme (6-14): The first step in the deprotection of TMM (427)

At this stage only a very small quantity of protected TMM remained and therefore the final deprotection step was not carried out.

7. Conclusions

The aim of this study was the preparation of several types of mycolic acid from pathogenic and non-pathogenic mycobacteria with different functional groups in both the proximal and distal positions (Figure (**7-1**).

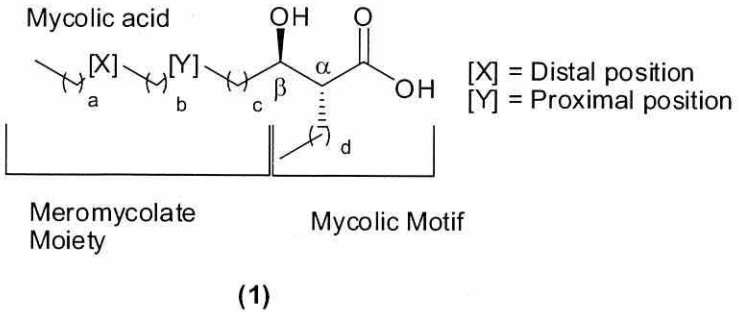


Figure (7-1): General structure of mycolic acids

The purpose of synthesising this array of mycolic acids was to provide single enantiomers of different types of mycolic acids for biological testing to determine what effect (if any) the different mycolic acid types has on the biological activity and also how this is affected by a change in stereochemistry.

This work involved the successful synthesis of six mycolic acids, and the attempted synthesis of a seventh saturated mycolic acid, without success. This was followed by the synthesis of four cord factors and many intermediates for use in future synthesis of mycolic acids and cord factors.

The first aim of this study was to synthesise the α'-mycolic acid (**208**) which is present in *M. smegmatis*, which is used quite often to study mycobacteria due to this being a non pathogenic mycobacterial species, (Figure (**7-2**)).

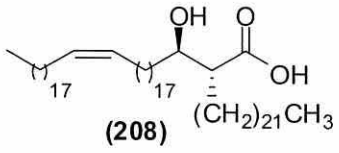
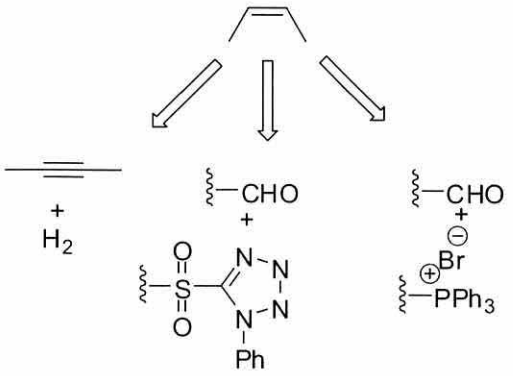


Figure (7-2): Mycolic acid (208)

In order to obtain the target molecule (**208**), the synthesis led to fragments which were then linked using different methods. The first part to be synthesised was the β-hydroxy-α-alkyl ester, which is the common unit in all mycolic acids. This was

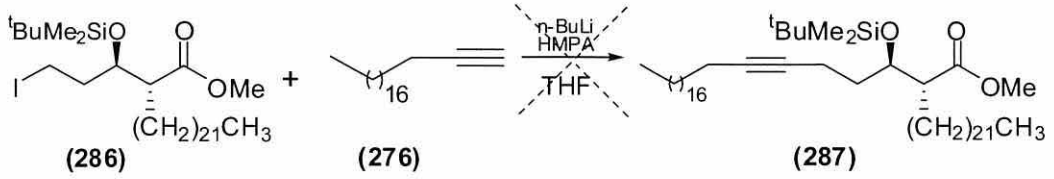
synthesised through literature methods and also an alternative synthesis of this portion using an asymmetric hydrogenation method was tested. This method involved the hydrogenation of a β-diketo compound using BINAP as catalyst and gave promising results. It which would be recommended to test the application of this method to compound (**249**) for the preparation of the hydroxy ester (**138**) with the right stereochemistry and it would also be interesting to apply this method for the preparation of the hydroxy ester using the alternative catalyst, Co(salen), see Section (**1.14.3**). Unfortunately, due to time constrains, it was not possible to optimise this method for the synthesis of β-hydroxy ester (**138**) in this study.

Three methods were tested for the synthesis of a double bond with *cis* stereochemistry: hydrogenation of a triple bond, a Julia reaction and a Wittig reaction, Scheme (**7-1**).



Scheme (7-1): General approach for synthesis of a *cis* double bond

The hydrogenation of the triple bond gave a double bond in *cis* stereochemistry in model compounds. However, attempts at coupling the triple bond (**276**) with the iodo-motif unit (**286**) for the synthesis of mycolic acid were unsuccessful Scheme (**7-2**)).



Scheme (7-2): A coupling attempt between (276) and (286)

The Julia reaction gave a mixture of both *cis* and *trans* isomers, with the *trans* isomer being the major. The third method to be tested in this study for the synthesis of a *cis* double bond was a Wittig reaction. In order to optimise the conditions for this reaction, mycolic acid (**207**) (Figure (**7-3**)) was chosen for synthesis, since it is a small alkene and present in *Corynebacterium diphtheriae.*

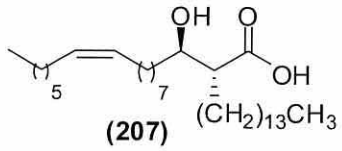


Figure (7-3): Mycolic acid (207)

Following the successful synthesis of the small alkene (**207**), the same reaction conditions were successfully applied for the synthesis of mycolic acid (**208**), and for the synthesis of the diene mycolic acid (**209**) (Figure (**7-4**)).

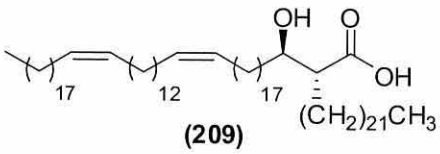


Figure (7-4): Mycolic acid (209)

Since the completion of this work, Coxon and Berretta have reported the synthesis of a di-alkene carboxylic acid in which a Wittig reaction was successfully utilised for the synthesis of the *cis*-double bonds, (Figure (**7-5**)).[237]

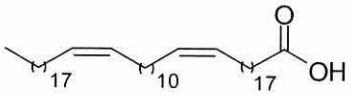


Figure (7-5): Synthetic meromycolic acid

It would also be interesting to prepare the *trans*-isomer of (**208**) and compare the biological effects of these two isomers.

The second part of this work was the synthesis of two oxygenated mycolic acids, the hydroxy mycolic acid (**211**) and the keto mycolic acid (**212**) (Figure (**7-6**)).

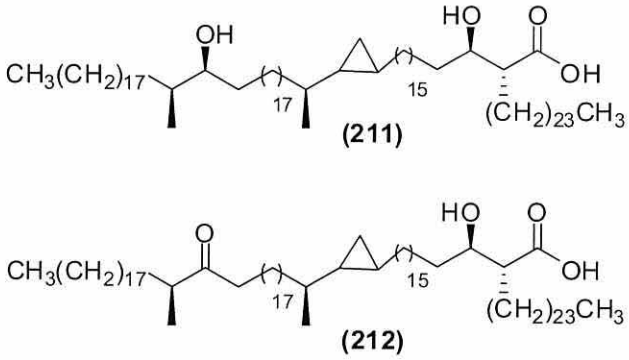
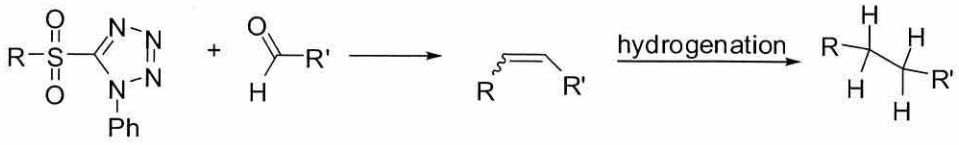


Figure (7-6): Oxygenated mycolic acids (211) and (212)

The best method for linking the different mycolic acid portions to each other was a Julia reaction followed by hydrogenation, since this gave the best yields. The hydrogenations were carried out using palladium on carbon under a hydrogen atmosphere when there was no cyclopropane in the compound, otherwise di-imide was used for hydrogenation which is a mild hydrogenation method which does not cause hydrogenolysis of the cyclopropane ring (Scheme (**7-2**)).[88]



Scheme (7-2): General Julia reaction

Another challenge was found in the final deprotection step of the mycolic acid methyl ester (**386**) (Figure (**7-7**)), which was found to cause problems with the retention of the stereochemistry when carried out in base media.

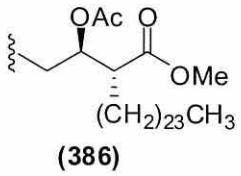
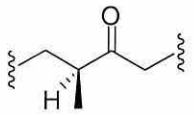


Figure (7-7): partil structure of actylated mycolic acid methyl ester (386)

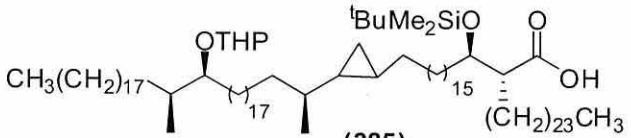
The hydrogen atom adjacent to the carbonyl in the distal position is acidic (**386**) (Figure (**7-8**)), and therefore is easily lost in base media, leading to epimerisation of the methyl group adjacent to the carbonyl group when deprotection of the methyl ester was carried out in base media in the last step of synthesis.



(386)

Figure (7-8): partial methyl branched keto structure (386)

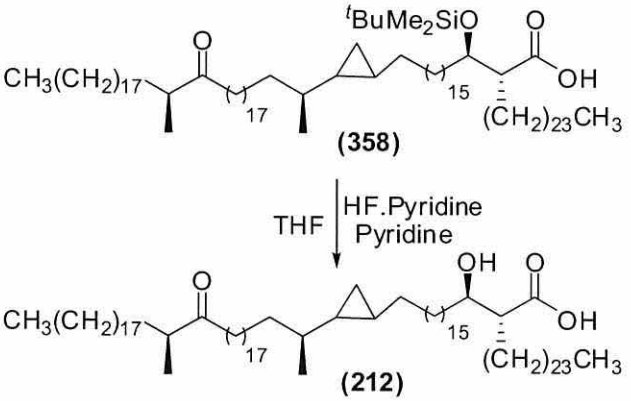
In order to keep the stereochemistry of these groups, the secondary alcohol group was protected with a different protecting group from the hydroxyl group of the mycolic motif (**385**) (Figure (**7-9**)).



(385)

Figure (7-9): Two different groups were used to protect the secondary alcohol in compound (385)

This was followed by a series of protection and de-protection procedures, with the use of an acid in the last deprotection step of the mycolic motif to avoid epimerisation of the methyl group adjacent to the ketone group of mycolic acid (**212**). Comparison of the molecular rotation of keto mycolic acid (**212**) with an epimerised keto mycolic acid (**212**) and a non-epimerised protected keto mycolic acid (**358**) indicated that this method was successful in preventing the epimerisation of the methyl group adjacent to the ketone, Scheme (**7-3**)).



Scheme (7-3): Acidic deprotection of compound (358)

The final mycolic acid prepared in this study was the α-mycolic acid (**213**) (Figure (**7-10**)); this was done using the same methods used for the preparation of the mycolic acids above. The last deprotection step could be performed in basic media since this meromycolic acid contained a cyclopropane ring in both the distal and proximal positions and therefore epimerisation was not a problem.

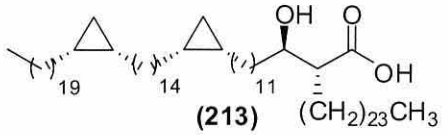


Figure (7-10): Dicyclopropyl mycolic acid (213)

The successful synthesis of these mycolic acids led to a further step which was the synthesis of cord factors, (Figure (**7-11**)).

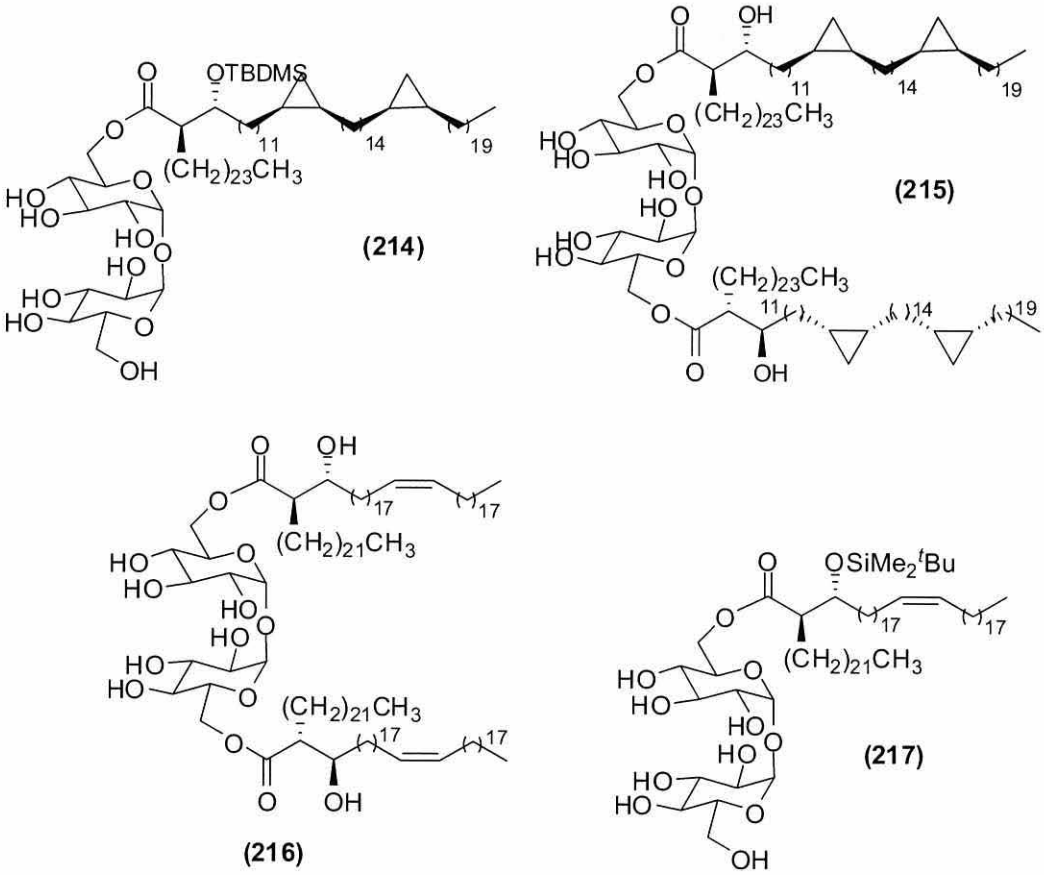


Figure (7-11): Cord factors synthesised in this study

The first step in the synthesis of the cord factors was the protection of the secondary hydroxyl group in both the mycolic acid and the trehalose sugar with two different

protecting groups, followed by an esterification reaction to obtain protected cord factor. This was followed by two deprotection steps, since deprotection in one step using HF. pyridine complex failed. The first step of deprotection was using TBAF in order to deprotect the trehalose sugar followed by deprotection of the mycolic acid using HF. pyridine complex. The esterification step using the method employed in this study was very slow, therefore it would be useful to try the Mitsunobu reaction for the synthesis of cord factors, since this method seems to provide a very fast reaction compared to the method which was applied in this study. The Mitsunobu method may also be a better method to use, since the method followed in this study sometimes results in the formation of mycolic anhydride and TDM without forming TMM. Another way in which this synthesis could be improved would be to find a protecting group for the secondary hydroxyl of both the mycolic acid and trehalose sugar so that the de-protection can be carried out in one step to obtain the desired cord factor.

All of the mycolic acids and cord factors synthesised in this study are undergoing biological assessment in order in order to distinguish the value of these compounds for the detection TB. The protocol used for testing of these compounds is as follows: firstly a solution of the sample (mycolic acid or cord factor) in hexane is transferred into ELISA plates and left until the hexane has evaporated. This is followed by coating of the ELISA plate with Casein, after which a serum is added to the plate and the excess serum is washed off. This was followed by the addition of a secondary antibody, normally IgG, followed by washing to remove excess antibody. Inorder to visualise the results of the ELISA test, OPD is added which creates a colour if a TB positive antibody is present in the serum. This can be measured using a colorimeter to confirm the assistance of TB antibodies in the serum.[235]

The initial ELISA result using mycolic acid (**209**) showed poor sensitivity, since the TB positive and negative absorbance appeared similar in the detection, (Figure (**7-12**)).

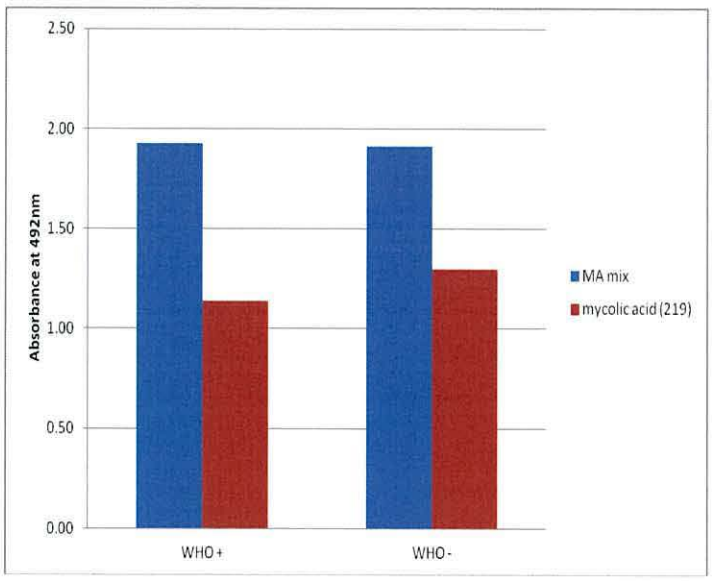


Figure (7-12): Average responses of ELISA assays using mycolic acid (209) to TB-positive sera (WHO+) and TB-negative sera (WHO-)

Similar testing of the other products of this study is on-going; initial results results are promising. Thus it appears that, with cord factors (**214**) and (**215**), there is greater differentiation between TB positive and TB negative samples than there is for the natural mixture of TDM isolated from cells.[158]

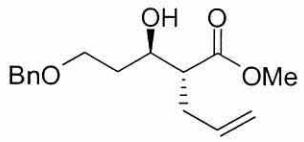
Future work will also involve studies of other biological effects of the compounds prepared in this work, for potential therapeutic application. The results of these tests will guide future synthetic targets. In any event, the protected mycolic acids (**207**) and (**211**) prepared in this work should be converted into their corresponding cord factors, while the mycolic acid (**209**) needs to be prepared on a larger scale, before allowing it to be processed to form cord factor.

Experimental

All chemicals were purchased from Aldrich Chemical Co. Ltd, Lancaster Synthesis Ltd, or Avocado Chemical Co. Ltd. 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. Organic solutions were dried over anhydrous magnesium sulfate and solvents were removed at 14 mmHg; residual traces of solvent were finally removed at 0.1 mmHg. All glassware used in anhydrous reactions was dried for not less than 5 h in a 250 °C oven.

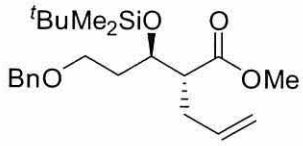
Column chromatography was conducted under medium pressure using silica gel (BDH, particle size 33–70 mm); TLC was carried out on pre-coated Kieselgel 60 F254 (Art. 5554; Merck) plates. Optical rotations were measured as solutions in chloroform of known concentration using a Polar 2001 automatic polarimeter. Melting points were measured using a Gallenkamp melting point apparatus. Infra-red spectra were recorded as KBr discs (solids) or thin films on NaCl windows or using a Perkin Elmer 1600 series FT-IR spectrometer. NMR spectra were recorded either on a Bruker AC 250 spectrometer with 5 mm Dual probe or on a Bruker Advance 500 spectrometer with 5 mm BBO probe as solutions in deuterated chloroform ($CDCl_3$) if not differently indicated. Chemical shifts are quoted in δ relative to chloroform (δ 7.27 ppm), and $CDCl_3$ (δ 77.0 ppm). Mass spectra were obtained using a Bruker MicroTOF time of flight mass spectrometer with ESI source. Matrix assisted laser desorption ionisation (MALDI) were obtained using a Bruker Daltonics *Reflex IV*.

Experiment 1: (*R*)-2-((*R*)-3-Benzyloxy-1-hydroxy-propyl)-pent-4-enoic acid methyl ester (251)



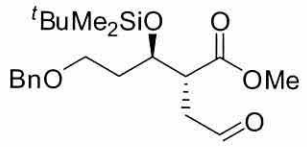
Diisopropylamine (9.8 g, 97.04 mmol) was dissolved in dry THF (100 ml) and cooled to –78 °C. MeLi (87.3 ml, 56.69 mmol, 1.5M) was added and the mixture was stirred and allowed to reach +16 °C over 30 min. before being re-cooled to –61 °C and (*R*)-5-benzyloxy-3-hydroxy-pentanoic acid methyl ester **(138)** (10.09 g, 42.19 mmol) in dry THF (50 ml) was added. The mixture was stirred at –45 °C for 1 hour, –20 °C for 40 min. and then at –20 °C to –10 °C for 20 min. before re-cooling to –62 °C and allyl iodide (5.8 ml, 63.28 mmol) in dry THF (20 ml) and HMPA (14.68 ml, 84.38 mmol) were added and the mixture was stirred at –45 °C for 1 hour, –45 °C to –20 °C for 30 min. and then –20 °C for 30 min. and the reaction mixture was cooled to –25 °C, quenched with sat. aq. ammonium chloride (70 ml) and extracted with petrol/ethyl acetate (1:1, 3x100 ml), dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (2:1) to give a colourless oil, (*R*)-2-((*R*)-3-benzyloxy-1-hydroxy-propyl)-pent-4-enoic acid methyl ester (9.55 g, 33.85 mmol, 81 %), $[\alpha]_D^{24}$ -4.8 (c = 1.12, $CHCl_3$) {Found *m/z* $[M + H]^+$: 279.1582, $C_{16}H_{23}O_4$ requires: 279.1591}. This showed δ_H (500 MHz, $CDCl_3$): 7.34-7.27 (5H, m), 5.8 (1H, tdd, J = 13.85, 10.1, 6.95 Hz), 5.12 (2H, tdd, J = 17.35, 12.6, 1.9 Hz), 4.49 (2H, s), 4.2 (1H, ddt, J = 12.6, 6.3, 4.75 Hz), 3.92-3.75 (2H, m), 3.7 (3H, s), 2.6 (1H, dt, J = 8.8, 5.65 Hz), 2.47-2.35 (2H, m), 1.86-1.83 (2H, m); δ_C: 173.56, 138.38, 135.83, 128.21, 127.45, 127.38, 116.24, 72.79, 70.14, 66.12, 51.60, 51.20, 33.61; ν_{max}/cm^{-1}:3489, 3065, 3030, 2950, 2863, 1736, 1642, 1496, 1454, 1438, 1366, 1241, 1197,1169, 1099, 1028, 996, 917, 738, 698.[117]

Experiment 2: (*R*)-2-[(*R*)-3-Benzyloxy-1-(*tert*-butyl-dimethyl-silanyloxy)-propyl]-pent-4-enoic acid methyl ester (252)



Imidazole (5.22 g, 76.75 mmol) was added to a stirred solution of (*R*)-2-((*R*)-3-benzyloxy-1-hydroxy-propyl)-pent-4-enoic acid methyl ester **(251)** (8.57 g, 30.7 mmol) in dry DMF (100 ml) at room temperature, followed by addition of *tert*-butyldimethylchlorosilane (6.05 g, 39.91 mmol). The mixture was stirred at 45 °C for 18 hours. When TLC showed that the reaction was complete, it was quenched with water (350 ml) and extracted with dichloromethane (3x200 ml). The combined organic layers were washed with water (200 ml), dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ether (4:1) to give a colourless oil, (*R*)-2-[(*R*)-3-benzyloxy-1-(*tert*-butyl-dimethyl-silanyloxy)-propyl]-pent-4-enoic acid methyl ester (9.82 g, 24.96 mmol, 84 %), $[\alpha]_D^{24}$ -15.07 (c = 1.87, $CHCl_3$) {Found *m/z* $[M + Na]^+$: 415.2256, $C_{22}H_{36}NaO_4Si$ requires: 415.2275}. This showed δ_H (500 MHz, $CDCl_3$): 7.33-7.25 (5H, m), 5.78 (1H, ddt, J = 13.9, 10.1, 6.95 Hz,), 5.06 (1H, dd, J = 17, 1.25) *trans*, 4.99 (1H, dd, J = 10.1, , 1.25 Hz) *cis*, 4.5 (2H, br t, J = 9.45 Hz), 4.15 (1H, q, J = 6 Hz), 3.6 (3H, s), 3.6 (1H, td, 9.45, 6.3 Hz), 3.5 (1H, td, J = 9.1, 3.15 Hz), 2.7-2.66 (1H, m), 2.4-2.3 (2H, m), 1.8 (2H, dd,J = 9.75, 6.3 Hz), 0.89 (9H, s), 0.00 (6H, s); δ_C: 173.56, 138.38, 135.83, 128.21,127.45, 127.38, 116.24, 72.79, 70.14, 66.12, 51.6, 51.2, 33.61, 31.25, 25.65, 17.86, -4.68, -4.93; ν_{max}/cm^{-1} :3030, 2929, 2856, 1736, 1642, 1496, 1436, 1254, 1101, 836, 776, 698, 664.[117]

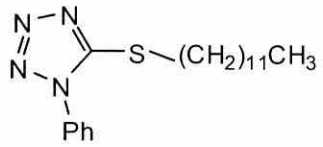
Experiment 3: (5-Benzyloxy-3-(tert-butyl-dimethyl-silanyloxy)-2-(2-oxo-ethyl)-penta-noic acid methyl ester (253)



2,6-Lutidine (3 ml, 25.42 mmol), OsO_4 2.5 % in 2-methyl-2-propanol (2.9 ml, 0.23 mmol), and then $NaIO_4$ (10.87 g, 50.84 mmol) were added to a stirred solution of (*R*)-2-[(*R*)-3-benzyloxy-1-(*tert*-butyl-dimethyl-silanyloxy)-propyl]-pent-4-enoicacid ethyl ester (**252**) (5.0 g, 12.71 mmol) in 1,4-dioxane–water (160 ml, 3:1) at room temperature. The reaction was stirred at 25 °C for 2 hours. When TLC showed that the reaction was complete, water (300 ml) and dichloromethane (300 ml) were added and the product was extracted. The water layer was re-extracted with dichloromethane (2x100 ml) and the combined organic layers were washed with brine

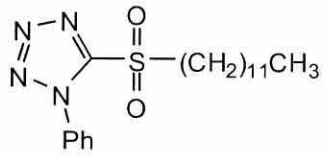
(200 ml) and dried. The solvent was evaporated and the crude product was purified by column chromatography eluting with petrol/ether (2:1) to give a colourless oil, (2*R*,3*R*)–5–benzyloxy–3-(*tert*–butyl-dimethyl-silanyloxy)-2-(2-oxo-ethyl)-pentanoic acid methyl ester (**253**) (3.82 g, 78 %), $[\alpha]_D^{26}$ -18.42 (c 0.97, $CHCl_3$) {Found *m/z* $[M+Na]^+$: 395.2244, $C_{21}H_{34}O_5Si$ requires: 395.2248}. This showed ^{1}HNMR (500MHz, TMS, $CDCl_3$): 9.74 (1H, s), 7.29-7.20 (5H, m), 4.43 (2H, q, J = 11.7 , 6.3 Hz), 4.20 (1H, quintet, J = 4.4 Hz), 3.61 (3H, s), 3.46-3.40 (2H, m), 3.18-3.15 (1H, m), 2.94 (1H, dd, J = 10.4 Hz), 2.65 (1H , dd, J = 14.8 Hz), 1.62-1.58 (2H, m) , 0.80 (9H, s), 0.01 (6H, broad s); δ_C 200.45, 172.40, 138.36, 128.35, 127.58, 127.56, 72.85, 68.82, 66.12, 51.6, 51.99, 45.28, 40.04, 33.73, 25.65, 17.93, -4.74, -4.87; ν_{max}/cm^{-1} : 3442, 3030, 2930, 2857, 1737, 1452, 1362, 1316, 1098, 837, 736, 698.

Experiment 4: 5-(Dodecylthio)-1-phenyl-1H-tetrazole (271)



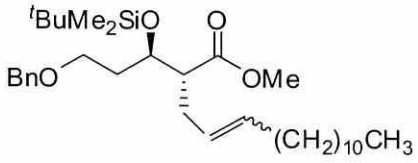
1-Phenyl-1*H*-tetrazole-5-thiol (4.5 g, 25.2 mmol), 1-bromododecane (6 g, 24.07 mmol), anhydrous potassium carbonate (5 g, 36.1 mmol) and acetone (70 ml) were mixed together and stirred vigorously at 60 °C for 15 hours. When TLC indicated complete removal of the thiol, the inorganic salts were filtered off and washed well with acetone. The acetone solution was evaporated to a small bulk and dissolved in dichloromethane (50 ml). The solution was washed with water (30 ml) and the aqueous layer was re-extracted with dichloromethane (2x25 ml). The combined organic phases were washed with water (30 ml), dried and the solvent was evaporated. The crude product was recrystallised from acetone (15 ml) and methanol (15 ml) to give a white solid of 5-(dodecylthio)-1-phenyl-1H-tetrazole (**271**) (8.46 g, 24.45 mmol, 76 %), m.p. 52-54°C {Found *m/z* $[M + H]^+$: 347.392 $C_{19}H_{29}N_4S$ requires: 347.2269 (MALDI)}. This showed δ_H (500 MHz, $CDCl_3$): 7.58–7.51 (5H, m), 3.38 (2H, t, J = 7.55 Hz), 1.82 (2H, pent., J = 7.6 Hz), 1.43 (2H, pent., J = 6.95 Hz), 1.25 (16H, br s), 0.87 (3H, t, J = 6.6 Hz; δ_C: 154.44, 133.71, 129.68, 123.77, 33.31, 31.83, 29.53, 29.46, 29.36, 29.26, 29.01, 28.96, 28.57, 22.60, 14.04; ν_{max}/cm^{-1}: 3019, 2924, 2854, 1596, 1523.

Experiment 5: 5-(Dodecylsulfonyl)-1-phenyl-1H-tetrazole (282)



A solution of ammonium molybdate (VI) tetrahydrate (13.57 g, 10.98 mmol) in 35 % H_2O_2 (25 ml), prepared and cooled in an ice bath, was added to a stirred solution of the 5-(dodecylthio)-1-phenyl-1H-tetrazole (**271**) (8.46 g, 24.4 mmol) in THF (40 ml) and IMS (40 ml) at 10 °C and stirred at room temperature for 2 hours. A further solution of ammonium molybdate (VI) tetrahydrate (6.5 g, 5.25 mmol) in 35 % H_2O_2 (10 ml) was added and the mixture was stirred at room temperature for 18 hours. The mixture was poured into water (100 ml) and extracted with CH_2Cl_2 (1x70 ml, 3x50 ml). The combined organic phases were washed with water (60 ml), dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (5:1) to obtain the title compound as a white semi-solid, (8.46 g, 91 %) {Found *m/z* $[M + Na]^+$: 401.1992 $C_{19}H_{30}O_2N_4S$ requires: 401.1995}. This showed δ_H (500MHz, $CDCl_3$): 7.70-7.68 (2H, m), 7.64-7.58 (3H, m), 3.73 (2H, t, J = 7.85 Hz), 1.95 (2H, pent., J = 7.85 Hz), 1.49 (2H, pent., J = 6.95 Hz), 1.26 (16 H, br s), 0.88 (3H, t, J = 6.6 Hz); δ_C: 153.49, 133.06, 131.45, 129.71, 125.07, 55.98, 31.92, 31.71, 29.70, 29.66, 29.64, 29.57, 29.46, 29.41, 29.36, 29.21, 29.02, 28.9, 28.15, 22.69, 21.95, 14.12; ν_{max}/cm^{-1}: 2915, 2847, 1490, 1463, 1338, 1145, 760, 689.

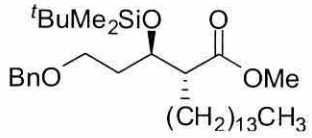
Experiment 6: (*E/Z*)-(*R*)-2-[(*R*)-3-Benzyloxy-1-(*tert*-butyldimethylsilanyloxy)-propyl]- hexadec-4-enoic acid methyl ester (273)



Lithium bis(trimethylsilyl)amide (9.33 ml, 9.89 mmol) was added to a stirred solution of (5-benzyloxy-3-(*tert*-butyldimethylsilanyloxy)-2-(2-oxo-ethyl)-pentanoic acid methyl ester (**253**) (2 g, 5.07 mmol) and 5-(dodecylsulfonyl)-1-phenyl-1H-tetrazole (**272**) (2.49 g, 6.59 mmol) in dry THF at -10 °C. The reaction turned bright yellow and was left to reach room temperature and stirred for one hour under a nitrogen

atmosphere, when TLC showed no starting material was left, the reaction was quenched by addition of sat. aq. NH_4Cl. The product was extracted with petrol/ethyl acetate (2:1, 3x50 ml), dried over $MgSO_4$, filtered and evaporated. The crude product was purified by column chromotography over silica gel, eluting with petrol/ethyl acetate (10:1). This gave the title compound (2.27 g, 4.15 mmol, 82 %) as a colourless oil {Found *m/z* $[M + Na]^+$: 569.8892, $C_{33}H_{58}O_4Si$ requires: 569.8967}. This showed: δ_H (500MHz, $CDCl_3$): 7.34-7.27 (5H, m), 5.46-5.38 (1H, m), 5.33-5.23 (1H, m), 4.49 (2H, s), 4.12 (1H, pent, J = 7.25 Hz), 3.64 (3H, s), 3.60-3.53 (2H, m), 2.60 (1H, dt, J = 13.55, 6.3 Hz), 3.31-2.23 (2H, m), 1.95 (2H, J = 6.35 Hz), 1.86-1.81 (2H, m), 1.28 (17H, br s), 0.89 (13H, s), 0.05 (6H, br s); δ_C: 173.97, 138.47, 138.46, 132.77, 131.90, 128.28, 127.53, 127.45, 126.83, 72.88, 70.41, 66.22, 52.23, 52.13, 51.13, 51.21, 33.74, 33.68, 32.51, 30.39, 29.66, 29.62, 29.57, 29.50, 29.46, 29.33, 29.07, 27.25, 25.93, 25.09, 22.66, 17.93, 14.16, -4.60, -4.62, -4.86, -4.88; ν_{max}/cm^{-1}: 3070, 3036, 2925, 2855, 1745, 1497, 1471, 1436, 1362, 1255, 1198, 1169, 1100, 1049, 970, 939, 837, 777, 734, 697.

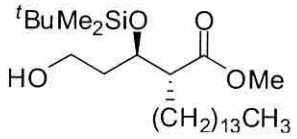
Experiment 7: (*R*)-2-[(*R*)-1-(*tert*-Butyl-dimethylsilanyloxy)-3-ethoxypropyl]-hexade-canoate acid methyl ester (274)



Palladium 10 % on carbon (0.5g) was added to a stirred solution of (*E/Z*)-(*R*)-2-[(*R*)-3-benzyloxy-1-(*tert*-butyl-dimethyl-silanyloxy)-propyl]-hexadec-4-enoic acid methyl ester (**273**) (2.27g, 4.15 mmol) in IMS (50 ml) and (10) THF. Hydrogenation was carried out for 3 hours. The solution was filtered over a bed of celite and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) and then (5:1) to give a colourless oil (*R*)-2-[(*R*)-1-(*tert*-butyl-dimethyl-silanyloxy)-3-ethoxy-propyl]hexadecanoate acid methyl ester (**274**) (2.15 g, 94 %), $[\alpha]_D^{21}$ +3.29 (c = 1.68, $CHCl_3$) {Found *m/z* $[M + Na]^+$: 571.4119, $C_{33}H_{60}O_4SiNa$ requires: 571.4158}. This showed δ_H (500MHz, $CDCl_3$): 7.34-7.27 (5H, m), 4.49 (2H, s), 4.07 (1H, dd, J = 6.3, 5.05 Hz), 3.65 (3H, s), 3.57 (2H, dt, J = 6.65, 2.25 Hz), 2.55 (1H, ddd, J = 10.75, 6.95, 4.1Hz), 1.25 (37H, br s), 0.89 (3H, t, J = 6.95Hz), 0.049 (3H, s), 0.042 (3H, s); δ_C: 174.70, 138.48, 128.28, 127.53, 127.45,

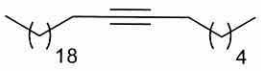
66.17, 52.02, 51.25, 33.65, 31.90, 29.67, 29.65, 29.63, 29.62, 29.58, 29.43, 29.34, 27.86, 27.23, 25.71, 22.67, 17.92, 14.09, -4.60, -4.91; ν_{max}/cm^{-1}: 3028, 2926, 2855, 1739, 1461, 1361, 1253, 1195, 1167, 1100, 938, 836, 775, 732, 696.

Experiment 8: (*R*)-2-[(*R*)-1-(*tert*-Butyl-dimethyl-silanyloxy)-3-hydroxy-propyl]-hexa-decanoate acid methyl ester (275)



Palladium 10 % on carbon (0.5 g) was added to a stirred solution of (*R*)-2-[(*R*)-1-(*tert*-Butyl-dimethyl-silanyloxy)-3-ethoxy-propyl]-hexadecanoate acid methyl ester (**274**) (2.15 g, 4.69 mmol) in IMS (100 ml) and (10 ml) THF. Hydrogenation was carried out for one day. The solution was filtered over a bed of celite and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) and then (5:1) to give a colourless oil (1g, 55 %), $[\alpha]_D^{21}$ – 1.79 (c = 1.45 in $CHCl_3$) {Found *m/z* $[M+H]^+$: 449.3376, $C_{21}H_{34}O_5Si$ requires: 459.386963}. This showed ^{1}HNMR (500MHz, TMS, $CDCl_3$): 4.27 (1H, dt, J = 5.35, 1.9Hz), 3.66 (3H, s), 2.62 (1H, ddd, J = 10.75, 6.95, 3.8Hz), 1.61-1.52(2H, m), 1.51-1.45(2H, m), 1.25 (27H, br s), 0.87 (3H, t, J = 3.45 Hz), 0.86 (9H, s), 0.09(3H, s), 0.05(3H, s). ^{13}C NMR (500 MHz, TMS, $CDCl_3$) δ=174.62, 64.65, 59.41, 51.61, 51.34, 35.30, 31.88, 31.70, 29.63, 29.61, 29.58, 29.53, 29.51, 29.39, 29.31, 27.83, 26.38, 25.66, 22.64, 17.93, 17.82, 14.13, -4.37, -4.56; ν_{max}/cm^{-1}: 3435, 2926, 2854, 1739, 1470, 1443, 1258, 1174, 1093, 836.

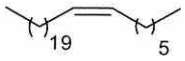
Experiment 9: Octacos-7-yne (278)



n-Butyllithum (4.49 ml, 0.066 mmol) was added to 1-octyne (1.2 g, 11.06 mmol) at -78 °C in dry THF (50 ml) and stirred for 2 hours at this temperature before addition of 1-bromoeicosane (2 g, 5.53 mmol) mixed with HMPA (1.92 ml, 11.06 mmol) in dry THF (5 ml). The reaction mixture was stirred for 24 hours at room temperature and then quenched with sat. aq. NH_4Cl and then extracted with petrol/ether (5:2, 3x50 ml). The organic layers were dried over $MgSO_4$, evaporated. The product was purified by

column chromatography eluting with petrol to obtain tetracont-19-yne (2.3 g, 4.11 mmol, 74 %) as white solid, m.p. 55-57 °C {Found *m/z* $[M + Na]^+$: 427.4282, $C_{29}H_{56}Na$ requires: 427.4279}. This showed δ_H (500MHz, $CDCl_3$): 2.14 (4H, t, J = 6.6 Hz,), 1.48 (4H, q, J = 6.65 Hz), 1.41-1.34 (4H, m), 1.26 (38H, s, chain), 0.90 (3H, t, J = 6.9 Hz), 0.89 (3H, t, J = 6.95 Hz); δ_C: 80.23, 80.22, 31.94, 31.39, 29.71, 29.66, 29.64, 29.58, 29.37 br, 29.18, 29.15, 28.88, 28.55, 28.70, 22.58, 18.77, 14.11, 14.04; ν_{max}/cm^{-1}: 2931, 2860, 2212, 1713, 1676, 1465, 1166, 726.

Experiment 10: Tetradec-7-ene (279)



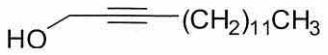
To a vigorously stirring solution of nickel acetate tetrahydrate (0.1 g, 3.83 mmol) in absolute ethanol (7 ml) under a hydrogen atmosphere, a solution of sodium borohydride (0.014 g, 0.38 mmol) in ethanol (0.8 ml) was added. Ethylene diamine (0.06 ml) was added, followed by a solution of octacos-7-yne (**278**) (1.5 g, 3.83 mmol) in THF (4 ml). After the required amount of hydrogen had been absorbed, the black solution was diluted with ether (45 ml) and filtered over a bed of celite and silica gel before being washed many times with a mixture of petrol/ether (10:1). The solvent was evaporated and the crude product was purified by column chromatography eluting with petrol to yield tetradec-7-ene (1.4 g, 3.54 mmol, 96 %) as white solid, m.p. 56-58 °C {Found *m/z* $[M + Na]^+$: 429.4446, $C_{29}H_{58}Na$ requires: 429.4436}. This showed δ_H (500MHz, $CDCl_3$): 5.36 (2H, t, J = 4.7 Hz), 2.03 (4H, q, J = 6.3 Hz), 1.27 (46H, br s, chain), 0.89 (3H, t, J = 6.65 Hz), 0.89 (3H, t, J = 6.9 Hz); δ_C: 129.91, 129.89, 31.94, 31.80, 29.79, 29.76, 29.71, 29.66, 29.57, 29.36, 29.33, 29.00, 27.22, 22.69, 22.66, 14.09, 14.08; ν_{max}/cm^{-1} : 2920, 2852, 2360, 1465, 1377, 721.

Experiment 11: 1-Iodododecane (290)

1-Bromododecane (15 g, 60.18 mmol), sodium iodide (27.05 g, 180.54 mmol) and sodium hydrogen carbonate (20.22 g, 240.73 mmol, 99%) were dissolved in acetone (500 ml) and refluxed for 3 hours and left overnight. The solvent was evaporated and the product was extracted with dichloromethane. The organic layer was dried over

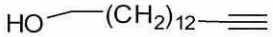
$MgSO_4$ and the product was purified by column chromatography, eluting with petrol to yield 1-iodododecane (17.74 g, 59.88 mmol, 99 %). This showed δ_H (500MHz, $CDCl_3$): 3.19 (2H, t, J = 5 Hz), 1.84 (2H, q, J = 5 Hz), 1.40 (2H, pent., J = 10 Hz), 1.38 (16H, br s), 0.90 (3H, t, J = 5 Hz); δ_C: 33.65, 31.97, 30.59, br 29.69, 29.62, 29.50, 29.41, 28.62, 22.74, 14.17, 6.96; ν_{max}/cm^{-1}: 2924, 2853, 1609, 1493, 1465, 1426, 1182, 1203, 1166, 824, 720 cm^{-1}.[238] Which was identical to the literature values.

Experiment 12: Pentadec-2-yn-1-ol (281)



Liquid ammonia (200 ml) was decanted into a 3 neck (500 ml) round-bottomed flask surrounded with cotton wool and fitted with a liquid nitrogen/IMS condenser, protected with a soda lime tube. Lithium wire (1.36 g, 0.196 mol) was washed with petrol and added in 1 cm portions over 30 min, with a deep blue colour being observed. Ferric nitrate (0.2 g) was then added and the solution stirred with a mechanical stirrer for 30 mins, then prop-2-yn-1-ol (5 g, 89.19 mmol) in dry ether (10 ml) was added over 30 min. The resultant mixture was then stirred for 1 hour, and then 1-iodo-octadecane (**280**) (23.77 g, 80.2 mmol) in dry ether (10 ml) was added over 30 mins. The reaction was stirred for 3 hours, keeping the condensers temperature maintained. The reaction was then left without stirring for 18 hours to allow the ammonia to evaporate. The reaction mixture was then diluted with ethyl acetate (250 ml) and the mixture quenched with 10 % sulfuric acid (50 ml). The aqueous layer was re-extracted with ethyl acetate (3x100 ml), the combined organic extracts were dried over $MgSO_4$ and the solvent evaporated to give a crude dark brown oil, which was purified via column chromatography eluting with petrol/ethyl acetate (10:1) to give a yellow oil, pentadec-2-yn-1-ol (**281**) (3.13 g, 13.9 mmol, 38 %). This showed δ_H (500 MHz, $CDCl_3$): 4.25 (2H, t, J = 2.25 Hz), 2.21 (2H, tt, J = 7.25, 1.9 Hz), 1.50 (2H, q, J = 6.95 Hz), 1.37 (2H, q, J = 6.95 Hz), 1.26 (17H, s), 0.88 (3H, t, J = 6.95 Hz); δ_C: 86.69, 51.44, 31.89, 29.64, 29.61, 29.50, 29.33, 29.12, 28.86, 28.59, 22.67, 18.71, 14.09; ν_{max}/cm^{-1}: 3017, 2954, 2916, 2851, 1470, 1216, 1136, 1019, 758, 717. The data were identical to those in the literature.[239]

Experiment 13: Pentadec-14-yn-1-ol (282)

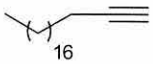


Lithium wire (0.58 g, 83.76 mmol) was added in (1 cm) portion to dry 1, 3-diamino-propane (70 ml, 0.838 mol) under a nitrogen atmosphere and stirred for 30 min. The mixture was heated to 70 °C until the blue colour discharged. The mixture was cooled to 25 °C and potassium-*tert*-butoxide (6.25 g, 55 mmol) was added and the mixture was left to stir for 20 min at room temperature, then pentadec-2-yn-1-ol (**281**) (3.13 g, 139.6 mmol) was added dropwise over 30 min. The mixture was stirred for 45 min. then poured into ice water (300 ml). The product was extracted with ethyl acetate (3x70ml), the combined organic layers were dried over $MgSO_4$ and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (5:1) to yield the title compound (2.11 g, 68 %). This showed δ_H (500MHz, $CDCl_3$): 3.54 (2H, t, J = 6.65 Hz), 2.11 (2H, td, J = 7.25, 2.5 Hz), 1.88 (1H, t, J = 2.5 Hz), 1.47 (4H, q, J = 8.2 Hz), 1.32 (2H, q, J = 7.25 Hz), 1.21 (17H, s); δ_C: 84.54, 67.94, 62.57, 32.63, 31.77, 29.48, 29.45, 29.39, 29.36, 29.33, 29.21, 28.96, 28.61, 28.34, 25.66, 22.53, 18.22, 13.94; ν_{max}/cm^{-1}: 3282, 2918, 2850, 1470, 1215, 1058, 757, 628. The data were identical to those in the literature.[239]

Experiment 14: 1-Iodo-octadecane (283)

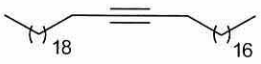
1-Bromo-octadecane (10 g, 29.99 mmol) in acetone (300 ml) was added to sodium iodide (13.48 g, 89.98 mmol) and sodium hydrogen carbonate (10.07 g, 11.99 mmol) and refluxed for 3 hours before being left to stir overnight at room temperature. The reaction mixture was then evaporated. The crude product was purified by column chromatography eluting with petrol. This gave the title compound (10.29 g, 27.05 mmol, 96 %). m.p. = 32-33 °C, while the literature showed 33-35 °C. This showed: δ_H (500MHz, $CDCl_3$): 3.2 (2H, t, J = 6.95 Hz), 1.83 (2H, q, J = 6.95 Hz), 1.38 (2H, q, J = 6.95 Hz), 1.27 (28H, s, chain), 0.89 (3H, t, J = 6.95 Hz); δ_C: 33.6, 31.94, 30.53, 29.71, 29.67, 29.63, 29.57, 29.44, 29.38, 28.56, 22.71, 14.13; ν_{max}/cm^{-1}: 2920, 2851, 1465, 1170, 720.[240]

Experiment 15: Eicos-1-yne (276)



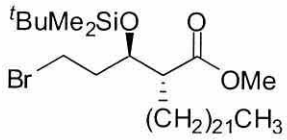
Lithium acetylide (EDA) complex (0.968 g, 10.5 mmol) was dissolved in dry THF (40 ml). 1-iodo-octadecane (**283**) (2 g, 5.258 mmol) was dissolved in (5 ml) dry THF and HMPA (1.82 ml, 10.5 mmol) were added to the reaction mixture. The reaction mixture was stirred for 3 hours before being quenched with water (50 ml) and extracted with dichloromethane (3x75 ml). The combined organic layers were dried over $MgSO_4$ and evaporated. The crude product was purified with column chromatography eluting with petrol to give the title compound as a white solid (1.02 g, 73 %), m.p. = 32-34°C {Found *m/z* $[M + Na]^+$: 428.1854, $C_{20}H_{38}Na$ requires: 428.1916}. This showed: δ_H (500MHz, $CDCl_3$): 2.18 (2H, td, J = 7.25, 2.85 Hz), 1.94 (1H, t, J = 2.5 Hz), 1.43 (2H, q, J = 7.85 Hz), 1.31 (2H, q, J = 6.65 Hz), 1.18 (28H, s), 0.81 (3H, t, J = 6.95 Hz); δ_C: 84.80, 68.00, 31.93, 29.70, 29.66, 29.61, 29.51, 29.36, 29.12, 28.77, 28.50, 22.69, 18.40, 14.11; ν_{max}/cm^{-1}:2924, 2854, 2361, 1741, 1464, 1361, 1255, 1169, 1069, 938, 835, 776.

Expriment 16: Tetracont-19-yne (284)



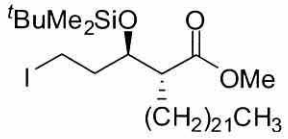
n-Butyllithum (4.49 ml, 0.066 mmol) was added to eicos-1-yne (**276**) (1.2 g, 11.06 mmol) at -78 °C in dry THF and stirred for 2 hours at this temperature before addition of 1-bromoeicosane (2 g, 5.53 mmol) mixed with HMPA (1.92 ml, 11.06 mmol) in dry THF (5 ml). The reaction mixture was stirred for 24 hours at room temperature, and then quenched with sat.aq. NH_4Cl, and then extracted with petrol/ether (5:2, 3x25 ml). The combine organic layers were dried over $MgSO_4$, evaporated. The product was purified by column chromatography eluting with petrol to yield tetracont-19-yne (**284**) (2.3 g, 4.11 mmol, 74 %). This showed δ_H (500MHz, $CDCl_3$): 2.20 (2H, dt, J = 6.95, 2.55 Hz), 2.16 (2H, dt, J = 6.95, 1.6 Hz), 1.47 (4H, q, J = 7.6 Hz), 1.38-1.34 (4H, m), 1.26 (60H, s, chain), 0.9 (6H, t, J = 6.95 Hz); δ_C: 80.25, 31.92, 29.70, 29.66, 29.64, 29.61, 29.58, 29.36, 29.18, 28.87, 22.69, 18.76, 4.1; ν_{max}/cm^{-1}:2931, 2860, 2212, 1713, 1676, 1465, 1166, 726.

Experiment 17: (*R*)-2-[(*R*)-3-Bromo-1-(*tert*-butyldimethylsilanyloxy)-propyl]-tetracosanoic acid methyl ester (285)



N-Bromosuccinimide (0.2 g, 1.14mmol) was added in portions over 5 min. to a stirred solution of (*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilanyloxy)-3-hydroxypropyl]-tetracosanoic acid methyl ester (**145**) (0.5 g, 0.87 mmol) and triphenylphosphine (0.26 g, 1 mmol) in dichloromethane (10 ml) at -10°C. The mixture was stirred at room temperature for 1 hour when TLC showed complete reaction, then quenched with sat. aq. sodium metabisulfate and extracted with dichloromethane. The organic layer was dried over $MgSO_4$ and purified by column chromatography eluting solvent with petrol/ethyl acetate (10:1), to give (*R*)-2-[(*R*)-3-bromo-1-(*tert*-butyldimethylsilanyloxy)-propyl]-tetracosanoic acid methyl ester (**285**) (0.5 g,0.78 mmol, 90 %), $[\alpha]_D^{24}$ 5.80 (c = 0.98, $CHCl_3$) {Found *m/z* $[M + Na]^+$: 633.9021, $C_{34}H_{69}BrO_3SiNa$ requires: 633.8994}. This showed δ_H (500MHz, $CDCl_3$): 4.09 (1H, dt, J = 6.95, 3.8 Hz), 3.67 (3H, s), 3.43 (2H, t, J = 6.6 Hz), 2.54 (1H, ddd, J = 9.45, 5.7, 3.8 Hz), 2.09-2.03 (1H, m), 2.00-1.94 (1H, m), 1.63-1.57 (1H, m), 1.53-1.49 (1H, m), 1.25 (40H, s), 0.88 (12H, t, J = 6.65 Hz), 0.11 (3H, s), 0.08 (3H, s); δ_C: 173.95, 51.59, 51.38, 36.83, 31.93, 29.70, 29.66, 29.62, 29.58, 29.55, 29.42, 29.36, 27.99, 26.49, 25.70, 22.67, 22.64, 17.92, 14.08, -4.58, -4.84; ν_{max}/cm^{-1}: 2927, 2854, 1741, 1464, 1361, 1255, 1195, 1168, 1073, 1005, 958, 837, 776, 720.

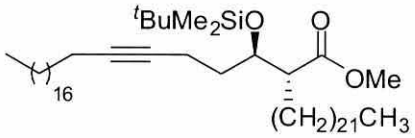
Experiment 18: (*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-3-iodopropyl]-tetracosanoic acid methyl ester (286)



(*R*)-2-[(*R*)-3-Bromo-1-(*tert*-butyldimethylsilanyloxy)-propyl]-tetracosanoic acid methyl ester (**285**) (1.3 g, 2 mmol), sodium iodide (0.92 g, 6 mmol) and sodium hydrogen carbonate (0.68 g, 8 mmol) were dissolved in acetone (20 ml) and refluxed for 3 hours and then left overnight; the solvent was evaporated and the residue was extracted with dichloromethane. The organic layer was dried over $MgSO_4$ and the

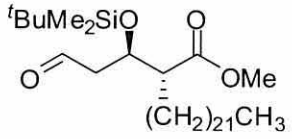
solvent was evaporated. The product was purified by column chromatography eluting with petrol/ethyl acetate (10:1), to give (*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilanyloxy)-3-iodopropyl]-tetra-cosanoic acid methyl ester (**286**) (1.15 g, 1.68 mmol, 82 %), $[\alpha]_D^{20}$ +6.22 (c 1.02, $CHCl_3$) {Found *m/z* $[M + Na]^+$:703.7314 $C_{34}H_{69}IO_3SiNa$ requires: 703.3958}. This showed ^{1}HNMR (500MHz, TMS, $CDCl_3$): 3.86 (1H, dt, J = 6.95, 5.05 Hz), 3.60 (3H, s), 3.24 (2H, t, J = 6.6 Hz), 2.48 (1H, ddd, J = 10.01, 6.95, 3.15 Hz), 1.80 (2H, q, J = 2.85 Hz), 1.53-1.41 (2H, m), 1.38-1.33 (2H, m), 1.21 (38H, s), 0.83 (3H, t, J = 6.65 Hz), 0.81 (9H, s), 0.00 (3H, s), -0.02 (3H, s); δ_C: 174.98, 73.19, 51.52, 51.11, 33.68, 33.61, 32.81, 31.92, 29.70, 29.67, 29.63, 29.57, 29.54, 29.51, 29.43, 29.36, 29.34, 29.23, 28.67, 28.12, 27.81, 27.46, 25.72, 23.64, 22.67, 17.92, 14.08, -04.41, -4.9; ν_{max}/cm^{-1}: 2921, 2851, 1738, 1463, 1361, 1250, 1168, 1066, 935, 836, 778.

Experiment 19: (*2R,3R*)-Methyl 3-(*tert*-butyldimethylsilyloxy)-2-docosylpentacos-6-ynoate (287)



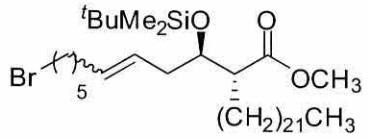
n-Butyllithum (0.89 ml, 1.43 mmol) was added to eicos-1-yne (**276**) (0.3 g, 1.1 mmol) at -78 °C in dry THF and stirred for 2 hours at this temperature before addition of (*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilanyloxy)-3-iodopropyl]-tetracosanoic acid methyl ester (**286**) (0.15 g, 22 mmol) mixed with HMPA (1.92 ml, 11.06 mmol) in dry THF (5 ml). The reaction mixture was stirred for 24 hours at room temperature, and then quenched by addition of sat. aq. NH_4Cl, and extracted with petrol/ether (5:2, 3x25 ml). The organic layer was dried over $MgSO_4$, evaporated and the product was purified by column chromatography eluting with petrol/ethyla cetate (5:1) with none of the desired product being obtained.

Experiment 20: (*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-3-oxopropyl]-tetra-cosanoic acid methyl ester (298)



(*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-3-hydroxypropyl]-tetracosanoic acid methyl ester (**145**) (2.01 g, 3.51 mmol) in dichloromethane (25 ml) was added at room temperature to a stirred solution of PCC (1.89 g, 12.29 mmol) in dichloromethane (100 ml). During the addition a black colour appeared. The mixture was stirred at room temperature for 2.5 hours until TLC showed the reaction was complete. Ether (300 ml) was added and the mixture was filtered through a bed of silica gel. The solvent was evaporated and the crude product was purified by column chromatography eluting with petrol/ether (4:1) to give a colourless oil of the title compound, (1.92 g, 3.37 mmol, 96 %) $[\alpha]_D^{26}$ -4.98 (c 1.23, $CHCl_3$) {Found *m/z* [M + Na]$^+$: 591.4774 $C_{34}H_{68}NaO_4Si$ requires: 591.4779}. This showed δ_H (500MHz, $CDCl_3$): 9.72 (1H, t, J = 2.2 Hz), 4.36 (1H, br., q, J = 6 Hz), 3.59 (3H, s), 2.59-2.49 (3H, m), 1.57-1.48 (1H, m), 1.46-1.4 (1H, m), 1.35-1.13 (41H, m), 0.80 (3H, t, J = 7 Hz), 0.78 (9H, s), 0.002 (3H, s), -0.008 (3H, s); δ_C: 201.06, 173.95, 68.79, 52.22, 51.43, 48.07, 31.90, 29.68, 29.64, 29.60, 29.52, 29.47, 29.36, 29.34, 27.72, 27.00, 25.58, 22.66, 17.83, 14.06, -4.69, -4.96; ν_{max}/cm^{-1}: 2924, 2856, 1734, 1466, 1362, 1255, 1198, 1167, 590, 477.

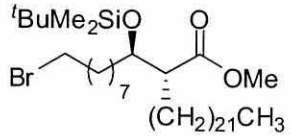
Experiment 21: (*R*)-2-[(*R*)-9-Bromo-1-(*tert*-butyl-dimethylsilanyloxy)-non-4-enyl] -tetracosanoic acid methyl ester (290)



5-(6-Bromohexylsulfonyl)-1-phenyl-1H-tetrazole (**289**) (1.82 g, 4.63 mmol) was dissolved in dry THF (30 ml) and a solution of (*R*)-2-[(*R*)-1-(*tert*-butyl-dimethyl-silanyloxy)-3-oxo-propyl]-tetracosanoic acid methyl ester (**288**) (1.76 g, 3.09 mmol) in dry THF (10 ml) was added at room temperature. This solution was cooled to –10 °C and lithium bis-(trimethylsilyl) amide (5.66 ml, 6.95 mmol, 1.06 M) was added at between –10 °C and –4 °C. The solution was allowed to reach room temperature and stirred for 1.5 hours until TLC analysis indicated that the reaction was complete. The reaction was quenched by adding sat. aq. ammonium chloride (25 ml). The organic phase was separated and the water layer was extracted with ethyl acetate (2x75 ml). The combined organic layers were dried and the solvent was evaporated. The crude

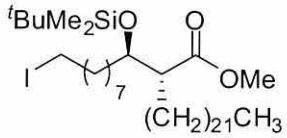
product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give a colourless oil of the title compound (1.74 g, 81 %). This showed δ_H (500MHz, $CDCl_3$): 5.44-5.35 (2H, m), 3.9-3.83 (1H, m), 3.59 (3H, s), 3.33 (2H, t, J = 6.95 Hz), 2.49 (1H, ddd, J = 11.35, 7.9, 4.1 Hz), 2.28-2.14 (2H, m), 2.0-1.95 (2H, m), 1.80 (2H, q, J = 6.95 Hz) , 1.39-1.31 (4H, m), 1.20 (42H, br s, chain), 0.3 (3H, t, J = 6.3 Hz), 0.81 (9H, s), 0.00 (3H, s), -0.02 (3H, s); δ_C: 174.78, 133.05, 124.92, 73.14, 51.51, 51.14, 41.36, 38.6, 37.32, 36.36, 35.81, 33.19, 33.5, 33.15, 32.74, 32.42, 32.07, 29.98, 29.94, 28.19, 27.94, 27.66, 25.84, 22.68, 17.65, 15.64, 14.54, -4.18, -5.11; ν_{max}/cm^{-1}: 2923, 2853, 1739, 1462, 1361, 1253, 1193, 1075, 835, 775.

Experiment 22: (*R*)-2-[(*R*)-9-Bromo-1-(*tert*-butyldimethylsilanyloxy)-nonyl]-tetra-cosanoic acid methyl ester (291)



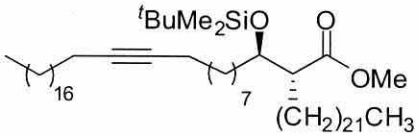
Palladium 10 % on carbon (0.5 g) was added to a stirred solution of 11-bromo-3-(*tert*-butyl-dimethyl-silanyloxy)-2-docosyl-undec-5-enoic acid methyl ester (**290**) (1.65 g, 2.3 mmol) in IMS (35 ml) and THF (35 ml). Hydrogenation was carried out for 2 hours. The solution was filtered over a bed of celite and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ether (20:1) to give a white semi-solid (*R*)-2-[(*R*)-9-bromo-1-(*tert*-butyl-dimethyl-silanyl-oxy)-nonyl]-tetra-cosanoic acid methyl ester (**291**) (1.04 g, 1.46 mmol, 63 %), $[\alpha]_D^{24}$ -7.17 (c = 0.85, $CHCl_3$) {Found *m/z* $[M + Na]^+$: 767.5372 $C_{40}H_{81}BrNaO_3Si$ requires: 767.5341}. This showed δ_H (500MHz, $CDCl_3$): 3.91 (1H, ddt, J = 9.15, 6.95, 4.75 Hz), 3.66 (3H, s), 3.41 (2H, t, J = 6.95 Hz), 2.49 (1H, ddd, J = 10.7, 6.9, 3.45 Hz), 1.85 (2H, quintet, J = 6.95), 1.48-1.40 (4H, m), 1.26 (50H, br s), 0.88 (3H, t, J = 6.95 Hz), 0.85 (9H, s), 0.05 (3H, s), 0.02 (3H,s); δ_C: 175.08, 73.21, 51.58, 51.22, 33.94, 33.67, 32.82, 31.92, 31.86, 29.82, 29.65, 29.52, 29.44, 29.23 ,28.69, 27.48, 27.46, 25.82, 23.70, 22.68, 28.19, 27.83, 27.48, 27.46, 22.68, 14.08, -4.37, -4.92; ν_{max}/cm^{-1}:2925, 2854, 1741, 1463, 1253, 1166, 863, 775, 721.

Experiment 23: (*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-9-iodo-nonyl]-tetracosanoic acid methyl ester (292)



(*R*)-2-[(*R*)-9-Bromo-1-(*tert*-butyldimethylsilanyloxy)-nonyl]-tetracosanoic acid methyl ester (**291**) (0.77 g, 1.07 mmol), sodium iodide (0.48 g,3.21 mmol) and sodium hydrogen carbonate (0.36 g, 4.28 mmol) were dissolved in acetone (20 ml) and refluxed for 3 hours and left to stir overnight. The solvent was evaporated and the residue was extracted with dichloromethane. The combined organic layers were dried over $MgSO_4$ and the solvent was evaporated. The crude product was purified by column chromatography, eluting with petrol/ethyl acetate (10:1), to give a colourless oil of (*R*)-2-[(*R*)-1-(*tert*-butyl-dimethyl-silanyloxy)-9-iodo-nonyl]-tetracosanoic acid methyl ester (**292**) (1.15 g, 1.68 mmol, 82 %), $[\alpha]_D^{24}$ -4.77 (c = 0.55, $CHCl_3$) {Found *m/z* $[M + Na]^+$: 787.4952 $C_{40}H_{81}IO_3SiNa$ requires: 787.4897}. This showed δ_H (500MHz, $CDCl_3$): 3.93 (1H, ddt, J = 10.1, 6, 4.1 Hz), 3.62 (3H, s), 3.18 (2H, t, J = 9 Hz), 2.52 (1H, ddd, J = 9.45, 8.5, 3.45 Hz), 1.82 (2H, q, J = 6.95 Hz), 1.57-1.47 (2H, m), 1.43-1.36 (2H, m), 1.26 (50H, s, chain), 0.88 (3H, t, J = 6.95 Hz), 0.86 (9H, s), 0.04 (3H, s), 0.02 (3H, s); δ_C: 175.05, 73.17, 51.56, 33.67, 33.64, 31.86, 30.02, 29.82, 29.70, 29.66, 29.52, 29.36, 28.46, 27.81), 27.48, 25.74, 23.69, 22.68, 17.96, 14.08, -4.37, -4.93; ν_{max}/cm^{-1}: 2921, 2851, 1738, 1463, 1361, 1250, 1168, 1066, 935, 836, 778.

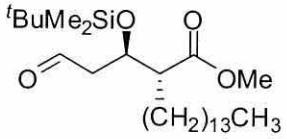
Experiment 24: Methyl (*2R,3R*)-3-(*tert*-butyldimethylsilyloxy)-2-docosylhentriacont-12-ynoate (293)



n-Butyllithum (0.37 ml, 1.17 mmol) was added to eicos-1-yne (**286**) (0.27 g, 0.98 mmol) at -78 °C in dry THF and stirred for 2 hours at this temperature before addition of (*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilanyloxy)-3-iodopropyl]-tetracosanoic acid methyl ester (**302**) (0.15 g, 22 mmol) mixed with HMPA (1.92 ml, 11.06 mmol) in dry THF (5 ml). The reaction mixture was stirred for 24 hours at room temperature, and then quenched with sat.aq. NH_4Cl, and then extracted with petrol/ether (5:2). The

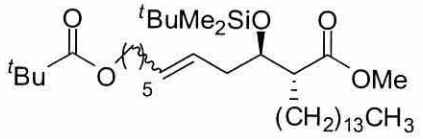
organic layer was dried over $MgSO_4$, evaporated and the product was purified by column chromatography eluting with petrol/ethyl acetate (5:1) with none of the desired product being obtained.

Experiment 25: (*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-3-oxo-propyl]-hexade-canoic acid methyl ester (305)



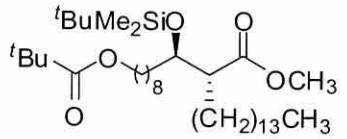
(*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-3-hydroxy-propyl]-hexadecanoate acid methyl ester (**275**) (0.29 g, 0.63 mmol) in dichloromethane (10 ml) was added to a stirred suspension of PCC (0.34 g, 1.58 mmol) in dichloromethane (20 ml). During the addition a black colour appeared. The reaction mixture was stirred for 2.5 hours at room temperature, when TLC showed complete reaction. The mixture was poured into a mixture of petrol/ethyl acetate (1:1). The mixture was filtered through a bed of silica gel and washed with ethyl acetate (2x20 ml). The solvent was evaporated and the product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give a colourless oil, (*R*)-2-[(*R*)-1-(*tert*-butyl-dimethyl-silanyloxy)-3-oxo-propyl]-hexadecanoic acid methyl ester (**305**), (0.22 g, 76 %), $[\alpha]_D^{23}$ -5.79 (c = 1.26) {Found *m/z* $[M + H]^+$: 479.3791, $C_{26}H_{51}O_4Si$ requires: 479.7742}. This showed δ_H (500MHz, $CDCl_3$): 9.80 (1H, t, J = 2.55 Hz), 4.43 (1H, dt, J,6, 1 Hz), 3.67 (3H, s), 2.66 (2H, dd, J = 4.7, 1.55 Hz), 2.61-2.56 (2H, m), 1.60-1.50 (2H, m), 1.24 (23H, br s), 0.87 (3H, t, J = 6.6 Hz), 0.85 (9H, s), 0.07 (3H, s), 0.06 (3H, s); δ_C: 201.16, 173.98, 68.82, 61.68, 60.26, 52.26, 51.58, 51.48, 48.10, 36.71, 36.06, 31.91, 31.57, 29.63, 29.59, 29.52, 29.49, 29.42, 29.37, 29.34, 29.04, 28.92, 27.74, 27.65, 27.38, 27.03, 26.90, 25.87, 25.83, 25.61, 22.66, 22.58, 20.42, 18.73, 17.86, 14.28, 11.39, -4.65, -4.92; ν_{max}/cm^{-1}: 2926, 2855, 1735, 1464, 1362, 1255, 1196, 1168, 1097, 1005, 837, 777.

Experiment 26: (*R*)-2-[(*E/Z*)-(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-9-(2,2-dimethyl-propionyloxy)-non-3-enyl]-hexadecanoic acid methyl ester (307)



Lithium bis(trimethyl silyl)amide (0.88 ml, 0.94 mmol) was added to a stirred solution of (*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilanyloxy)-3-oxo-propyl]-hexadecanoic acid methyl ester (**305**) (0.22 g, 0.48 mmol) and 6-(1-phenyl-1H-tetrazol-5-ylsulfonyl)hexyl pivalate (**306**) (0.24 g, 0.62 mmol) in dry THF at -10 °C. The reaction turned bright yellow and was left to reach room temperature and stirred for one hour under a nitrogen atmosphere. When TLC showed no starting material was left the reaction was quenched by addition of sat. of NH_4Cl. The product was extracted with petrol/ethyl acetate (2:1, 3x50 ml), dried over $MgSO_4$, filtered and evaporated. The crude product was purified by column chromotography, eluting with petrol/ethyl acetate (10:1) to obtain the title compound (**317**) (2.1 g, 73.36 mmol, 95 %) {Found *m/z* $[M + H]^+$: 647.4991 $C_{37}H_{71}O_5SiNa$ requires: 647.5046}. This showed δ_H (500MHz, $CDCl_3$): 5.47-5.43 (2H, m), 4.05 (2H, t, J = 6.3 Hz), 3.95-3.88 (1H, m), 3.65 (3H, s), 2.52 (1H, ddd, J = 11.05, 7.6, 3.8 Hz), 2.33-2.15 (2H, m), 1.38-1.37 (6H, m), 1.29-1.25 (25H, m), 1.20 (9H, s), 0.88 (3H, t, J = 7.25 Hz), 0.86 (12, s), 0.05 (3H, s), 0.02 (3H, s); δ_C: 175.04, 174.85, 133.22, 131.59, 125.27, 124.81, 73.60, 73.16, 64.36, 64.30, 51.55, 51.39, 51.16, 38.71, 32.58, 31.91, 29.68, 29.63, 29.57, 29.50, 29.42, 29.34, 29.21, 29.05, 28.56, 28.48, 27.73, 27.70, 27.62, 27.20, 25.73, 25.66, 25.49, 22.67, 17.95, 14.08, -4.27, -4.30; ν_{max}/cm^{-1}: 2926, 2855, 1732, 1462, 1362, 1284, 1192, 1157, 1076, 973, 836, 812, 776.6.

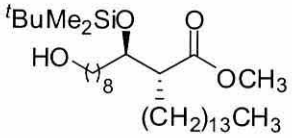
Experiment 27: (*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-9-(2,2-dimethyl-propionyloxy)-nonyl]-hexadecanoic acid methyl ester (308)



Palladium 10 % on carbon (0.1 g) was added to a stirred solution of (*R*)-2-[(E/Z)-(*R*)-1-(*tert*-butyldimethylsilanyloxy)-9-(2,2-dimethylpropionyloxy)-non-3-enyl]-hexadecanoic acid methyl ester (**307**) (0.16 g, 0.255 mmol) in IMS (20 ml). Hydrogenation was carried out for 1 hour. The solution was filtered over a bed of celite and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (5:1) to give a colourless oil (0.12 g, 0.19 mmol, 72 %), (*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilanyloxy)-9-(2,2-dimethylpropionyloxy)nonyl]hexadecanoic acid methyl ester (**308**), $[\alpha]_D^{23}$ –4.01 (c = 1.52, $CHCl_3$)

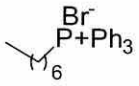
{Found $[M + H]^+$: 649.5100, $C_{37}H_{73}O_5SiNa$ requires: 649.5203}. This showed δ_H (500MHz, $CDCl_3$): 4.04 (2H, t, J = 6.65 Hz), 3.90 (1H, dt, J = 9.75, 5.05 Hz), 3.65 (3H, s), 2.52 (1H, ddd, J = 10.75, 6.95, 3.8 Hz), 1.63-1.54 (3H, m), 1.25 (31H, vbr s), 1.19 (12H, s), 0.88 (3H, t, J = 8.85 Hz), 0.86 (12H, s), 0.04 (3H, s), 0.02 (3H, s); δ_C: 178.60, 175.05, 64.42, 51.59, 51.18, 38.71, 33.66, 31.91, 29.74, 29.67, 29.66, 29.63, 29.62, 29.56, 29.44, 29.43, 29.34, 29.18, 29.04, 28.62, 27.82, 27.45, 27.19, 25.90, 25.82, 25.74, 23.78, 22.67, 22.59, 17.96, 14.08, 11.38, -4.38, -4.93; ν_{max}/cm^{-1}: 2927, 2855, 1732, 1463, 1362, 1284, 1254, 1156, 836, 775.

Experiment 28: (*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-9-hydroxy-nonyl]-hexade-canoic acid methyl ester (309)



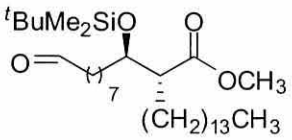
(*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-9-(2,2-dimethylpropionyloxy)-nonyl]-hexadecanoic acid methyl ester (**308**) (1.07, 1.7 mmol) was added to a stirred solution of potassium hydroxide (1.43 g, 25.5 mmol) dissolved in a mixture of THF:MeOH:H_2O (10:10:1, 21 ml). The mixture was refluxed at 70 °C and monitored by TLC. After 3 hours, the TLC showed no starting material left and the reaction was quenched with water and extracted with ethyl acetate (3x300 ml), dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (5:2) to give a semi-solid of the title compound (**309**) (0.78 g, 84 %), $[\alpha]_D^{24}$ -3.07 (c = 1.43, $CHCl_3$) {Found *m/z* $[M + Na]^+$: 565.4544,$C_{32}H_{66}O_4SiNa$ requires: 565.4628}. This showed δ_H (500MHz, $CDCl_3$): 3.89 (1H, dt, J = 6.95, 5.05 Hz), 3.65 (3H, s), 3.63 (2H, t, J = 6.65 Hz), 2.52 (1H, ddd, J = 10.75, 6.95, 3.8 Hz), 1.56 (2H, q, J = 6.6 Hz), 1.24 (39H, s), 0.87 (3H, t, J = 6.9 Hz), 0.86 (9H, s), 0.04 (3H, s), 0.01 (3H, s); δ_C: 175.33, 73.17, 63.02, 62.91, 51.56, 51.22, 33.62, 32.77, 31.50, 29.73, br 29.67, 29.65, 29.63, 62.56, 29.56, 29.50, 29.42, 29.33, 27.82, 27.45, 26.80, 25.73, 25.68, 23.68, 22.66, 17.96, 14.09, -4.40, -4.96; ν_{max}/cm^{-1}:3435, 2927, 2857, 1742, 1644, 1466, 1435, 1366, 1254, 1174, 1074, 836.

Experiment 29: (Heptyl)triphenylphosphonium bromide (303)



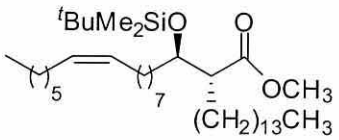
1-Bromononane (20 g, 10.303 mmol) was added to stirred solution of triphenylphosphine (43.93 g, 167 mmol) in toluene (150 ml). The mixture was refluxed for 3 days and the solvent was evaporated. The residue was washed with diethyl ether (50 ml) and left to dry to obtain the title compound (**303**) (30.5 g, 67.96 mmol, 62 %), m.p. 132-134 °C {Found *m/z* $[M]^+$: 361.6849. $C_{25}H_{30}P$ requires: 361.208 (MALDI)}. This showed δ_H (500MHz, $CDCl_3$): 7.82-7.36 (15H, m), 3.72-3.67 (2H, m), 1.59 (4H, q, J = 3.15 Hz), 1.23-1.12 (6H, m), 0.78 (3H, t, J = 6.6 Hz); δ_C: 134.94, 134.92, 133.72, 133.58, 133.50, 130.46, 130.35, 31.20, 30.31, 30.19, 28.71, 22.92, 22.53, 22.49, 22.36, 13.87; ν_{max}/cm^{-1}:2928, 2858, 1587, 1484, 1438, 1113, 996, 923, 748, 723, 691.

Experiment 30: (*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-9-oxononyl]-hexadecanoic acid methyl ester (310)



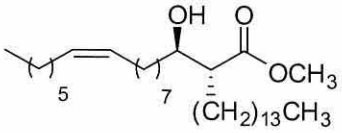
(*R*)-2-[(*R*)-1-(*tert*-Butyl-dimethyl-silanyloxy)-9-hydroxy-nonyl]-hexadecanoic acid methyl ester (**309**) (0.3 g, 0.55 mmol) in dichloromethane (5 ml) was added at room temperature to a stirred solution of PCC (0.3 g, 1.38 mmol) in dichloromethane (20 ml). During the addition a black colour appeared. The reaction was stirred at room temperature for 2 hours until TLC showed the reaction was complete. The reaction mixture was poured into (25 ml) petrol/ethyl acetate (10:1) and filtered through a bed of silica gel. 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 of the title compound (0.26 g, 86 %), $[\alpha]_D^{24}$ -7.09 (c 1.72, $CHCl_3$) {Found *m/z* $[M + Na]^+$: 563.4492, $C_{32}H_{64}O_4SiNa$ requires: 563.4574}. This showed δ_H (500MHz, $CDCl_3$): 9.76 (1H, t, J = 1.55 Hz), 3.90 (1H, ddd, J = 9.1, 6.4, 5 Hz), 3.66 (3H, s), 2.52 (1H, ddd, J = 11, 7.25, 3.75 Hz), 2.42 (2H, td, J = 7.25, 1.55 Hz), 1.63 (2H, q, J = 6.9 Hz), 1.25 (36H, br. s), 0.88 (3H, t, J = 6.6 Hz), 0.86 (9H, s), 0.04 (3H, s), 0.02 (3H, s); δ_C: 202.83, 175.08, 73.13, 51.57, 51.23, 43.91, 33.88, 31.59, 31.91, 29.67, 29.66, 29.63, 29.62, 29.60, 29.56, 29.43, 29.34, 29.28, 29.09, 27.83, 27.44, 25.74, 23.69, 22.67, 22.03, 17.96, 14.18, -4.39, -4.93; ν_{max}/cm^{-1}: 2924, 2853, 1741, 1464, 1372, 1249, 1167, 1048, 836, 775.

Experiment 31: Methyl (*2R,3R,Z*)-3-(*tert*-butyldimethylsilyloxy)-2-tetradecyl-octadec-11-enoate (311)



Sodium bis(trimethylsilyl)amide (0.81 ml, 0.81 mmol, 1.0M in THF) was added to a stirred solution of (heptyl)triphenylphosphonium bromide (**303**) (0.27 g, 0.76 mmol) at room temperature under a nitrogen atmosphere. The mixture was stirred for 30 min and then (*R*)-2-[(*R*)-1-(*tert*-butyl-dimethyl-silanyloxy)-9-oxo-nonyl]-hexadecanoic acid methyl ester (**310**) (0.26 g, 0.48 mmol) in dry THF (5 ml) was added. The mixture was stirred for 3 hours, when TLC showed no starting material was left. The reaction was quenched with sat. aq. NH_4Cl (10 ml) and the product was extracted with petrol/ethyl acetate (20:1, 3×20 ml). The combined organic layers were dried over $MgSO_4$, filtered and the solvent was evaporated. The product was purified by column chromatography, eluting with petrol/ethyl acetate (40:1) to give the title compound (**309**) (0.29 g, 50 %), $[\alpha]_D^{24}$ -5.76 (c = 1.18, $CHCl_3$) {Found *m/z* $[M + Na]^+$: 645.711, $C_{39}H_{78}O_3SiNa$ requires: 645.561 (MALDI)}. This showed δ_H (500MHz, $CDCl_3$): *trans* alkene 5.4-5.38 (2H, m), *cis* alkene 5.35 (2H, dt, J = 10, 5 Hz), 3.91 (1H, ddd, J=8.8, 6.95, 4.7Hz), 3.66 (3H, s), 2.53 (1H, ddd, J = 11, 7.25, 3.8 Hz) , 2.02 (4H, q, J = 5.9 Hz), 1.25 (46H, br.s), 0.89 (6H, t, J = 6.95 Hz), 0.86 (9H, s), 0.04 (3H, s), 0.02 (3H, s); δ_C: 175.13, 130.38, 130.27, 129.92, 129.81, 73.22, 51.57, 51.21, 33.67, 31.92 , 29.83, 29.77, 29.70, 29.61, 29.58, 29.56, 29.44, 29.36, 29.31, 27.83, 27.50, 27.21 , 25.75, 23.68, 22.69, 17.97, 14.11, -4.37, -4.93; ν_{max}/cm^{-1}: 2923, 2852, 1741, 1468, 1437, 1361, 1179, 1120, 836, 775, 720, 695.

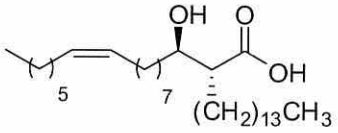
Experiment 32: Methyl (*2R,3R,Z*)-3-hydroxy-2-tetradecyloctadec-11-enoate (312)



(*Z*)-(*2R,3R*)-3-(*tert*-Butyldimethylsilanyloxy)-2-tetradecyl-nonadec-11-enoic acid methyl ester (**311**) (0.29 g, 0.44 mmol) was stirred in dry THF (20 ml) in dry polyethylene vial under a nitrogen atmosphere at room temperature pyridine (0.2 ml) and HF.pyridine (1.5 ml) were added and the mixture was stirred for 18 hours at 45

°C. The reaction was diluted with petrol/ ethyl acetate (1:1, 10 ml) and neutralized with sat. aq. $NaHCO_3$. The mixture was separated and the aqueous layer was re-extracted with petrol/ethyl acetate (1:1, 2x20 ml). The combined organic layers were washed with brine, dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) gave (*Z*)-(*2R,3R*)-3-(hydroxy)-2-tetradecyl-nonadec-11-enoic acid methyl ester (**312**) as a colourless oil (0.20 g, 86 %), $[\alpha]_D^{24}$ +7.10 (c = 1.71, $CHCl_3$) {Found *m/z* $[M + Na]^+$: 531.475, $C_{33}H_{64}O_3Na$ requires: 531.4753}. This showed: δ_H (500MHz, $CDCl_3$): *trans* protons 5.34-5.33 (2H, m), *cis* protons 5.30 (2H, dt, J = 5.7, 3.5 Hz), 3.71 (3H, s), 3.65 (1H, dt, 7.55, 3.15 Hz), 2.43 (1H, dt, J = 5.35, 9.15 Hz), 2.01 (2H, q, J = 6.3 Hz), 1.74-1.67 (1H, m), 1.60-1.56 (1H, m), 1.25 (47H, br s), 0.88 (6H, t, J = 4.75 Hz); δ_C: 176.21, 129.92, 129.79, 72.72, 60.36, 51.48, 50.94, 35.67, 31.90, 31.79, 29.71, 29.66, 29.63, 29.60, 29.54, 29.49, 29.48, 29.44, 29.40, 29.33, 29.20, 28.96, 27.40, 27.17, 27.19, 25.70, 22.66, 22.63, 14.16, 14.08; ν_{max}/cm^{-1}: 3444, 2925, 2854, 1720, 1645, 1464, 1377, 1195, 1167, 1052, 721.

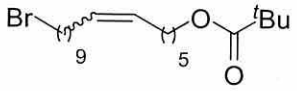
Experiment 33: (*2R,3R,Z*)-3-Hydroxy-2-tetradecyloctadec-11-enoic acid (207)



(*Z*)-(*2R,3R*)-3-(Hydroxy)-2-tetradecyl-nonadec-11-enoic acid methyl ester (**312**) (0.24 g, 0.45 mmol) in THF (8 ml), water (0.8 ml), MeOH (0.7 ml) was stirred and LiOH (0.28 g, 6.8 mmol) was added. The mixture was stirred for 18 hours at 45 °C. When TLC showed no starting material was left, the mixture was dissolved in warmed petrol/ethyl acetate 5:1 (50 ml) and an aqueous solution of 5 % HCl was added until the water layer was pH 1-2. The organic phase was then re-extracted (2x 50 ml) and the combined organic layers were dried, evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate eluting with petrol/ethyl acetate (7:3) to give the title compound (0.20 g, 0.38 mmol, 86 %), $[\alpha]_D^{22}$ +11.27 (c 0.51, $CHCl_3$) {Found *m/z* $[M + Na]^+$: 517.5306, $C_{32}H_{62}O_3Na$ requires: 517.4596 (MALDI)}. This showed δ_H (500MHz, $CDCl_3$): 5.35-5.33 (2H, m), 3.71 (1H, dt, J = 9.5, 5.05 Hz), 2.44 (1H, dt, J = 9.15, 5.35 Hz), 2.02 (4H, q, J = 6.6 Hz), 1.74-1.67 (1H, m), 1.63-1.56 (1H, m), 1.53-1.44 (2H, m), 1.25 (44H, br s), 0.88 (6H, t, J = 4.7 Hz);

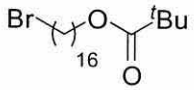
δ_C: 180.57, 129.92, 129.76, 60.48, 51.08, 35.33, 31.91, 31.77, 29.73, 29.71, 29.69, 29.67, 29.65, 29.60, 29.53, 29.49, 29.42, 29.35, 29.24, 28.96, 27.32, 27.20, 27.18, 25.67, 22.67, 22.64, 14.43; ν_{max}/cm^{-1}: 3539, 2918, 2842, 2364, 1687, 1462, 1375, 1206, 965, 719.

Experiment 34: 16-Bromohexadec-6-enyl pivalate (316)



Lithium bis(trimethyl silyl)amide (39.13 ml, 41.4 mmol) was added to a stirred solution of 10-bromodecanal (**315**) (5 g, 21.27 mmol) and 6-((1-phenyl-1H-tetrazol-5-yl)sulfonyl)hexyl pivalate (**306**) (10.85 g, 27.6 mmol) in dry THF at -10 °C. The reaction turned bright yellow and was left to reach room temperature and stirred for one hour under a nitrogen atmosphere. When TLC showed no starting material was left the reaction was quenched by addition of sat. aq. NH_4Cl. The product was extracted with petrol/ether (1:2, 3x150 ml), dried over $MgSO_4$, filtered and evaporated. The crude product was purified by column chromotography, eluting with petrol/ether (20:1) to obtain the title compound (7.8 g, 91 %). This showed δ_H (500MHz, $CDCl_3$): 5.40-5.29 (2H, m), 4.02 (2H, t, J = 6.6 Hz), 3.37 (2H, t, J = 6.6Hz), 1.96 (2H, 5.65 Hz), 1.83 (2H, q, J = 6.9Hz), 1.59 (2H, q, J = 7.25 Hz), 1.33-1.29 (18H, br m), 1.17 (9H, s); 178.41, 130.71, 130.20, 129.64, 129.15, 64.31, 38.60, 33.66, 33.61, 32.64, 32.47, 32.21, 29.61, 29.49, 29.38, 29.34, 29.31, 29.16, 29.12, 29.01, 28.76, 28.61, 28.52, 27.72, 27.54, 27.11, 26.87, 25.80; ν_{max}/cm^{-1}:2922, 2853, 2301, 1726, 1654, 1543, 1479, 1460, 1397, 1365, 1284, 1157, 1034, 968, 771, 722, 644.

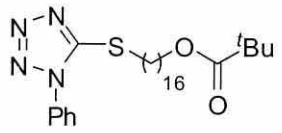
Experiment 35: 16-Bromohexadecyl pivalate (317)



Palladium 10 % on carbon (1 g) was added to a stirred solution of 16-bromohexadec-10-en-1-yl pivalate (**316**) (9.8 g, 24.29 mmol) in IMS (80 ml) and (20 ml) THF. Hydrogenation was carried out for 1 hour. The solution was filtered over a bed of celite and the solvent was evaporated to give pure colourless oil, 16-bromohexadecyl pivalate[93] (**327**) (8.34g, 85 %) {Found *m/z* $[M + Na]^+$: 427.2188 $C_{21}H_{41}BrO_2Na$ requires: 427.2187}. This showed δ_H (500MHz, $CDCl_3$): 4.02 (2H, t, J = 6.65 Hz),

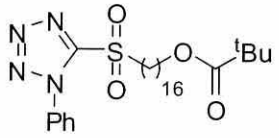
3.38 (2H, t, J = 6.6 Hz), 1.83(2H, q, J = 7.25 Hz), 1.60 (2H, q, J = 6.6 Hz), 1.40 (2H, q, J = 6.9 Hz), 1.24 (22H, br s), 1.18 (9H, s); δ_C: 178.50, 64.37, 41.24, 38.64, 36.01, 33.82, 32.79, 31.87, 31.53, 29.63, 29.60, 29.58, 29.57, 29.55, 29.50, 29.46, 29.39, 29.31, 29.17, 28.99, 28.72, 28.56, 28.13, 27.61, 27.24, 27.13, 25.85, 22.62, 22.59, 22.54, 20.38, 19.36, 18.68, 14.24, 11.34; ν_{max}/cm^{-1}: 2925, 2854, 1730, 1479, 1463, 1397, 1365, 1284, 1156, 1034.

Experiment 36: 2,2-Dimethyl-propionic acid 16-(1-phenyl-1H-tetrazol-5-ylsulfanyl)-hexadecyl ester (318)



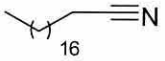
1-Phenyl-1*H*-tetrazole-5-thiol (3.29 g, 18.5 mmol), 2,2-dimethyl-propionic acid 16-bromo-hexadecyl ester (8.34 g, 20.5 mmol) (**317**), anhydrous potassium carbonate (4.26 g, 30.8 mmol) and acetone (100 ml) were vigorously stirred and refluxed at 60 °C for 15 hours. When TLC indicated complete removal of the thiol, the inorganic salts were filtered off and washed well with acetone. The acetone solution was evaporated to a small bulk and dissolved in dichloromethane (70 ml). The solution was washed with water (50 ml) and the aqueous layer was re-extracted with dichloromethane (2x50 ml). The combined organic phases were washed with water (30 ml), dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (5:1), to give 2,2-dimethyl-propionic acid 16-(1-phenyl-1H-tetrazol-5-ylsulfanyl)-hexadecyl ester (**318**) (8.01 g, 77 %) {Found *m/z* $[M + Na]^+$: 525.3356, $C_{28}H_{46}N_4O_2SNa$ requires: 525.3234}. This showed δ_H (500MHz, $CDCl_3$): δ = 7.53-7.45 (5H, m), 3.98 (2H, t, J = 6.6 Hz), 3.33 (2H, t, J= 7.55 Hz), 1.76 (2H, pent., J = 7.9 Hz), 1.55 (2H, pent., J 6.6 Hz),1.38 (2H, pent., J = 5.95 Hz), 1.20-1.17(22H, br. m), 1.34 (9H, s); δ_C: 178.33, 154.27, 133.63, 129.84, 129.57, 123.64, 64.23, 38.7, 33.17, 29.45, 29.43, 29.42, 29.36, 29.32, 29.25, 29.03, 28.92, 28.84, 28.45, 28.43, 27.00 25.72; ν_{max}/cm^{-1}: 2923, 2849, 1723, 1597, 1499, 1479, 1386, 1282, 1156, 760, 693.

Experiment 37: 2,2-Dimethyl-propionic acid 16-(1-phenyl-1H-tetrazole-5-sulfonyl)-hexadecyl ester (319)



A solution of ammonium molybdate (VI) tetrahydrate (8.52 g, 6.9 mmol) in 35 % H_2O_2 (23 ml), prepared and cooled in an ice bath, was added to a stirred solution of the 2,2-dimethyl-propionic acid 16-(1-phenyl-1H-tetrazol-5-ylsulfanyl)-hexadecyl ester (**328**) (7.71 g, 15.3 mmol) in THF (40 ml) and IMS (40 ml) at 10 °C and stirred at room temperature for 2 hours. A further solution of ammonium molybdate (VI) tetrahydrate (8.52g, 6.9 mmol) in 35 % H_2O_2 (23 ml) was added and the mixture was stirred at room temperature for 18 hours. The mixture was poured into water (100 ml) and extracted with CH_2Cl_2 (1x70 ml, 3x50 ml). The combined organic phases were washed with water (60 ml), dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (5:1) to obtain the title compound as semi-solid, (8.46 g, 91 %) {Found *m/z* $[M + Na]^+$: 525.3356, $C_{28}H_{46}N_4O_2SNa$ requires: 525.3234}. This showed δ_H (500 MHz, $CDCl_3$): 7.71-7.69 (2H, m), 7.64-7.61 (3H, m), 4.05 (2H, t, J = 6.6 Hz), 3.75 (2H, distorted t, J = 7.9 Hz), 1.95 (2H, pent., J = 7.8 Hz), 1.62 (2H, pent., J = 6.65 Hz), 1.5 (2H, pent., J = 6.65 Hz), 1.35-1.25 (22H, m), 1.2 (9H, s); δ_C: 178.67, 153.51, 133.07, 131.46, 129.72, 125.08, 64.23, 56.03, 38.73, 29.62, 29.56, 29.52, 29.46, 29.23, 29.2, 28.9, 28.82, 28.62, 28.15, 27.22, 25.91, 21.95; ν_{max}/cm^{-1}: 2928, 2864, 1728, 1498, 1480, 1462, 1344, 1284, 1155, 762, 688.

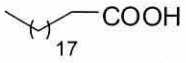
Experiment 38: Nonadecanenitrile (320)



Sodium cyanide (19.16 g, 0.39 mmol) was added to a stirred solution of 1-bromoeicosane (43.47g, 0.13mmol) in DMSO (300 ml) and the mixture was heated to 60 °C for 3 h. When TLC show no starting material left, water (500 ml) was added and the mixture was extracted with ethyl acetate (3x300ml). The organic layer was dried over $MgSO_4$, evaporated and the product was re-crystallized from petrol to obtain nonadecanenitrile (**330**) (36.1 g, 99 %), m.p.30-32 °C {Found $[M + Na]^+$: 277.2916, $C_{19}H_{37}NSi$ requires: 277.2770}. This showed δ_H (500 MHz, $CDCl_3$): 2.33

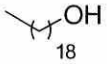
(2H, t, J = 7.25 Hz), 1.65 (2H, pent. J = 7.25 Hz), 1.44 (2H, pent., J = 6.95 Hz), 1.24 (28H, br m), 0.88 (3H, t, J 6.95 Hz); δ_C: 119.78, 31.89, 29.66, 29.60, 29.55, 29.47, 29.32, 29.26, 28.73, 28.63, 25.35, 22.65, 17.08, 14.06; ν_{max}/cm^{-1}:3315, 2922, 2853, 1465, 1240, 720, 628.

Experiment 39: Nonadecanoic acid (321)



Sodium hydroxide (30.9 g, 773 mmol) was dissolved in mixture of ethanol (330 ml) and water (47 ml). Nonadecanenitrile (**320**) (36.01 g, 128.8 mmol) was added and the mixture was refluxed for 3 hours under N_2. The reaction was quenched with dil HCl (5 %) and extracted with petrol/ethyl acetate (1:1). The combined organic layers were dried over $MgSO_4$ and evaporated; re-crystallization from 10:1 petrol/ethyl acetate gave nonade-canoic acid (**321**) (14.24 g, 41 %) as a colourless solid, m.p. 48-50 °C {Found *m/z* $[M + Na]^+$: 321.2754, $C_{19}H_{38}O_2$ requires: 321.2764}. This showed δ_H (500 MHz, $CDCl_3$): 3.35 (2H, t, J = 7.6 Hz), 1.64 (2H, pent. J = 7.25 Hz), 1.39-1.21 (31H, br m), 0.88 (3H, t, J = 6.65 Hz); δ_C: 178.87, 33.89, 31.92, 29.69, 29.66, 29.63, 29.58, 29.46, 29.43, 29.36, 29.31, 29.24, 29.20, 29.07, 25.50, 24.71, 22.68, 14.11; ν_{max}/cm^{-1}: 2921, 2846, 1706, 1463, 1412, 1307, 926, 719.

Experiment 40: Nonadecan-1-ol (322)



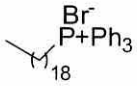
Nonadecanoic acid (**321**) (16.22 g, 54.3 mmol) in THF (50 ml) was added dropwise over 15 min to a suspension of lithium aluminium hydride (4.23 g, 108.6 mmol) in THF (250 ml) at 0 °C. The mixture was allowed to reach room temperature and refluxed for 1 hour, when TLC show no starting material, then cooled to 0 °C and sat. aq. sodium sulfate was added until a white precipitate formed. THF (50 ml) was added and the mixture was stirred at room temperature for 30 min and then the $MgSO_4$, was filtered off through a bed of silica gel and the solvent was evaporated. Recrystallisation from petrol/ethyl acetate (10:1) gave nonadecan-1-ol (**322**) (14.56 g, 94 %) as a colourless solid, m.p. 48-50 °C {Found *m/z* $[M + Na]^+$: 284.3403, $C_{19}H_{40}O$ requires: 284.3074}. This showed δ_H (500 MHz, $CDCl_3$): 3.63 (2H, t, J = 6.6 Hz), 1.56 (2H, pent., J = 6.6 Hz), 1.36-1.22 (33H, br m), 0.88 (3H, t, J = 6.6 Hz); δ_C: 63.03,

42.18, 33.74, 32.81, 31.91, 29.87, 29.68, 29.61, 29.59, 29.49, 29.43, 29.34, 26.88, 25.74, 22.67, 14.08; ν_{max}/cm^{-1}: 2917, 2849, 1467, 1060, 825.

Experiment 41: 1-Bromo-nonadecane (323)

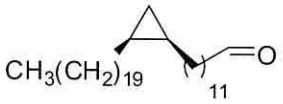
N-Bromosuccinimide (13.66 g, 76.7mmol) was added in portions over 5 min. to a stirred solution of nonadecan-1-ol (**322**) (14.56 g, 51.17 mmol) and triphenylphosphine (15.43 g, 58.8 mmol) in dichloromethane (300 ml) at -10 °C. The reaction mixture was stirred at room temperature for 1hour, when TLC show the reaction was complete. It was quenched with sat.aq. sodium metabisulfate and extracted with dichloromethane (200x3 ml). The organic layer was dried over $MgSO_4$. The crude product was purified by column chromatography eluting with petrol/ethyl acetate eluting with petrol/ethyl acetate (10:1), to obtain 1-bromo-nonadecane (**323**) (10.14 g, 57 %) as a colourless solid, m.p. 35-37 °C {Found *m/z* $[M + Na]^+$: 347.2132, $C_{19}H_{39}Br$ requires: 347.2136}. This showed δ_H (500 MHz, $CDCl_3$): 3.43 (2H, t, J = 6.9 Hz), 1.86 (2H, pent., 6.9 Hz), 1.40 (2H, pent., 6.95 Hz), 1.32-1.22(30H, br m), 0.89 (3H, t, J = 6.65 Hz); δ_C: 34.01, 32.86, 31.92, 29.69, 29.61, 29.54, 29.44, 29.36, 28.77, 28.18, 22.68, 14.10; ν_{max}/cm^{-1}: 2924, 2853, 1465, 1377, 1255, 720.

Experiment 42: Nonadecyltriphenylphosphonium bromide (313)



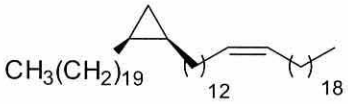
1-Bromononadecane (**323**) (9.03 g, 25.9 mmol) was added to a stirred solution of triphenylphosphine (10.22 g, 38.9 mmol) in toluene (80 ml). The reaction mixture was refluxed for 4 days. The solvent was evaporated and petrol (50 ml) was added, filtered, and again evaporated. The residue was treated with diethyl ether (50 ml) and stirred for 1 hour, and filtered to give the product, nonadecyltriphenylphosphonium bromide (**313**) (15.15 g, 95.64 %) as a colourless solid {Found *m/z* $[M + Na]^+$: 529.3966, $C_{37}H_{55}P$ requires: 529.3963}. This showed δ_H (500 MHz, $CDCl_3$): 7.79-7.68 (15H, m), 3.80 (2H, t, J = 13.55 Hz), 1.63 (4H, v br s), 1.32-1.14 (31H, br m), 0.87 (3H, t, J = 6.65 Hz); δ_C: 134.92, 134.9, 133.76, 133.69, 133.61, 130.49, 130.39, 128.73, 128.43, 118.82, 118.14, 31.87, 30.47, 30.34, 29.65, 29.59, 29.55, 29.5, 29.3, 29.23, 29.15, 23.1, 22.7, 22.67, 22.63, 14.06.

Experiment 43: 12-(2-Eicosyl-cyclopropyl)-dodecanal (325)



12-(2-Eicosyl-cyclopropyl)-dodecan-1-ol (**324**) (0.2 g, $0.38x10^{-3}$mmol) in dichloromethane (5 ml) was added at room temperature to a stirred solution of PCC (0.2 g, $0.95x10^{-3}$ mmol) in (20 ml) dichloromethane. During the addition a black colour appeared. The reaction was stirred at room temperature for 2 hours when TLC showed the reaction was complete. The reaction mixture was poured into (25 ml) petrol/ethyl acetate (10:1) and filtered through a bed of silica gel. The solvent was evaporated and the crude product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to obtain the title compound (**325**) as a colourless oil (0.11 g, $0.2x10^{-3}$ mmol, 55 %). This showed δ_H (500 MHz, $CDCl_3$): 9.77 (1H, t, J = 1.9 Hz), 2.42 (2H, dt, J = 7.25, 1.85 Hz), 1.66-1.60 (2H, m), 1.26 (56H, s, chain) 0.88 (3H, t, J = 6.95 Hz), 0.52-0.49 (2H, m, cyclopropane proton), 0.42 (1H, dt, J = 8.2, 4.1 Hz, cyclopropane proton), 0.47 (1H, br q, J = 5.35 Hz, cyclopropane proton); δ_C: 202.95, 43.92, 31.92, 30.21, 29.69, 29.65, 29.58, 29.42, 29.35, 29.17, 28.71, 22.68, 22.09, 15.77, 14.11, 10.91; ν_{max}/cm^{-1}: 2914, 2848, 1712, 1466, 718.

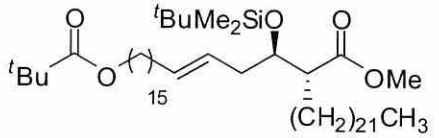
Experiment 44: (*1S,2S*)-1-((*Z*)-Docos-2-enyl)-2-eicosylcyclopropane (326)



Nonadecyltriphenylphosphonium bromide (**313**) (0.16 g, $0.72x10^{-3}$ mol) was dissolved in dry THF (4 ml) under a nitrogen atmosphere and sodium bis (trimetylsilyl) amide (0.35 ml, $0.35x10^{-3}$ mol, 1.0 M in THF) was added at room temperature. The mixture was stirred for 20 min and then (0.11 g, $0.211x10^{-3}$ mol) of 12-(2-eicosyl-cyclopropyl)-dodecanal (**325**) was dissolved in (2 ml) dry THF and added drop wise and the mixture was stirred for 30 min at room temperature. The reaction mixture was cooled to 0 °C and quenched by addition of sat.of NH_4Cl, extracted with petrol/ethyl acetate (10:1, 3x30). The combined organic layers were dried over $MgSO_4$ and the solvent was evaporated. The crude product was purified by column chromatography eluting with chloroform to obtain 1-eicosyl-2-tritriacont-13-enylcyclo-propane (**326**) (0.8 g, $1.02x10^{-4}$ mol, 48 %) as white solid, $[\alpha]_D^{24}$ +13.62 (c = 0.53, $CHCl_3$), m.p. = 45-46 °C. This showed δ_H (500 MHz, $CDCl_3$): 5.36 (2H, br t, J

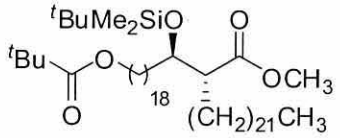
= 4.6 Hz), 2.02 (4H, pent., J = 5.7 Hz), 1.38-1.23 (93H, m, chain), 0.88 (6H, t, 6.8 Hz), 0.67-0.64 (2H, m, cyclopropane), 0.57 (1H, dt, J = 8, 4 Hz, cyclopropane), -0.31 (1H, q, J = 5.2 Hz, cyclopropane proton); δ_C: 129.89, 31.92, 30.22, 29.77, 29.70, 29.60, 29.57, 29.36, 29.32, 28.72, 28.15, 27.21, 22.69, 15.77, 14.11, 10.9; ν_{max}/cm^{-1}:3005, 2956, 2923, 2853, 1466, 1377, 1075, 720.

Experiment 45: (*E/Z*)-(*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-3-benzyloxy-propyl] -tetracos-4-enoic acid methyl ester (327)



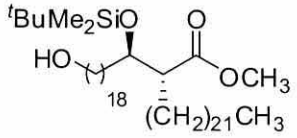
Lithium bis(trimethyl silyl)amide (6.1 ml, 6.56 mmol) was added to a stirred solution of (*R*)-2-[(*R*)-1-(*tert*-butyl-dimethyl-silanyloxy)-3-oxo-propyl]-tetracosanoic acid methyl ester (**305**) (3.82 g, 9.68 mmol) and 2,2-dimethyl-propionic acid 16-(1-phenyl-1H-tetrazole-5-sulfonyl)-hexadecyl ester (**319**) (2.34 g, 4.37 mmol) in dry THF at -10 °C. The reaction turned bright yellow and was left to reach room temperature and stirred for one hour under a nitrogen atmosphere. When TLC showed no starting material, the reaction was quenched by addition of sat. NH_4Cl. The product was extracted with petrol/ether (1:2, 3x 150 ml), dried over $MgSO_4$, filtered and the solvent was evaporated and the crude product was purified by column chromotography, eluting with petrol/ether (20:1) to obtain the title compound (4.92 g, 7.41 mmol, 79 %) as a colourless oil {Found *m/z* $[M + H]^+$: 659.5409, $C_{41}H_{73}O_4Si$ requires: 659.5426}. This showed δ_H (500 MHz, $CDCl_3$): 5.44-5.41 (2H, m), 4.02 (2H, t, J = 6.65 Hz), 3.84 (1H, dt, J = 7.55, 4.4 Hz), 3.63 (3H, s), 2.51 (1H, ddd, J = 10.7, 8.2, 4.1 Hz), 2.28-2.14 (2H, m), 1.59 (2H, q = 6.6 Hz), 1.23 (31H, br s), 1.17 (12H, s), 0.86 (3H, 6 Hz), 0.84 (9H, s), 0.03 (3H, s), 0.00 (3H, s); δ_C: 179.49, 175.04, 133.68, 132.06, 124.74, 124.24, 64.36, 60.27, 51.45, 51.28, 38.64, 37.34, 32.71, 31.88, 29.66, 29.66, 29.61, 29.53, 29.48, 29.43, 29.38, 29.31, 29.19, 28.58, 27.67, 27.59, 27.13, 25.87, 25.67, 22.63, 22.54, 20.91, 17.89, 14.12, -4.34, -5.10; ν_{max}/cm^{-1}: 2934, 2858, 1741, 1465, 1366, 1287, 1191, 1077, 976, 831, 809, 778.

Experiment 46: (*R*) – 2 - [(*R*) – 1 - (*tert*-Butyldimethylsilanyloxy)-19-(2,2-dimethylpropionyloxy)-nonadecyl]-tetracosanoic acid methyl ester (328)



Palladium 10 % on carbon (0.5 g) was added to a stirred solution of (*E*/*Z*)-(*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilanyloxy)-3-benzyloxy-propyl]-tetracos-4-enoic acid methyl ester **(327)** (2.1 g, 2.39x10^{-3} mmol) in IMS (20 ml) and (10 ml) THF. Hydrogenation was carried out 1 hour. The solution was filtered over a bed of celite and the solvent was evaporated to give a pure colourless oil of [(*R*)–1-(*tert*-butyl-dimethyl-silanyloxy)-19-(2,2-dimethyl-propionyloxy)-nonadecyl]-tetracosanoic acid methyl ester **(328)** (1.8 g, 85 %), $[\alpha]_D^{20}$ -11.19 (c 1.51, $CHCl_3$) {Found *m/z* $[M + Na]^+$: 901.8007, $C_{55}H_{110}O_5SiNa$ requires: 901.8015}. This showed δ_H (500 MHz, $CDCl_3$): 4.04 (2H, t, J = 6.6Hz), 3.9 (1H, br. td, J = 7, 4.75 Hz), 3.65 (3H, s), 2.53 (1H, ddd, J = 10.7, 6.9, 3.45 Hz), 1.64-1.59 (2H, m), 1.57-1.24 (74H, m), 1.2 (9H, s), 0.89 (3H, t, J = 7 Hz), 0.86 (9H, s), 0.04 (3H, s), 0.02 (3H, s); δ_C: 178.57, 175.06, 73.24, 64.44, 51.59, 51.15, 38.71, 33.71, 31.92, 29.82, 29.69, 29.65, 29.59, 29.57, 29.52, 29.43, 29.35, 29.23, 28.63, 27.83, 27.48, 27.19, 25.91, 25.76, 23.75, 22.67, 17.97, 14.08, -4.37, -4.92; ν_{max}/cm^{-1}: 2924, 2856, 1734, 1466, 1362, 1255, 1198, 1167, 590, 477.

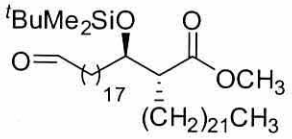
Experiment 47: (*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-19-hydroxy-non-adecyl]-tetracosanoic acid methyl ester (329)



(*R*)-2-[(*R*)-1-(*tert*-Butyl-dimethyl-silanyloxy)-19-(2,2-dimethyl-propionyloxy)-non-adecyl]-tetracosanoic acid methyl ester (**328**) (1.81 g, 2.07x10^{-3} mmol) was added to a stirred solution of potassium hydroxide (1.72 g, 30.8 mmol) dissolved in a mixture of THF:MeOH:H_2O (10:10:1, 315 ml). The mixture was refluxed at 70 °C and monitored by TLC. After 3 hours, the TLC showed no starting material was left and the reaction was quenched with water and extracted with ethyl acetate (3x300 ml), dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ether (2:1 and then 1:1) to give a semi-solid compound, (*R*)-2-[(*R*)-1-(*tert*-butyl-dimethyl-silanyloxy)-19-hydroxy-nonadecyl]-tetracosanoic acid methyl ester (**329**) (1.38 g, 84 %), $[\alpha]_D^{20}$ -11.19 (c = 1.51, $CHCl_3$)

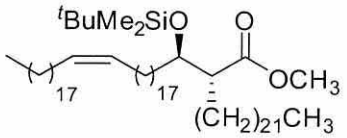
{Found *m/z* $[M + Na]^+$: 817.7401, $C_{50}H_{102}O_4SiNa$ requires: 817.7440}. This showed δ_H (500 MHz, $CDCl_3$): 3.89 (1H, td, J = 7.25, 4.7 Hz), 3.63 (3H, s), 3.61 (2H, t, J = 6.6 Hz), 2.51 (1H, ddd, J = 11.05, 7.25, 3.8 Hz), 1.7 (1H, br, s), 1.54 (2H, pent., J = 6.3 Hz), 1.23 (74H, br, s), 0.86 (3H, t, J = 6.9 Hz), 0.84 (9H, br, s), 0.02 (3H, s), 0.001 (3H, s); δ_C: 175.07, 73.18, 62.91, 60.30, 51.52, 33.64, 31.78, 31.89, 29.77, 29.66, 29.61, 29.58, 29.53, 29.51, 29.42, 29.39, 29.32, 27.77, 27.45, 26.86, 22.64, 14.03, -4.43, -5.00; ν_{max}/cm^{-1}: 3384, 2923, 2853, 1741, 1464, 1361, 1254, 1195, 1166, 1070, 836, 775, 720.

Experiment 48: (*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-19-oxononadecyl]-tetra-cosanoic acid methyl ester (330)



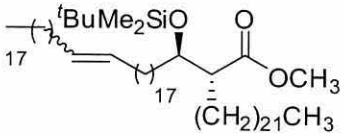
2-[1-(*tert*-Butyldimethylsilanyloxy)-19-hydroxynonadecyl]-tetracosanoic acid methyl ester (**329**) (0.25 g, 0.314 mmol) in dichloromethane (5 ml) was added at room temperature to a stirred solution of PCC (0.17 g, 0.785 mmol) in dichloromethane (30 ml). During the addition a black colour appeared. The reaction was stirred at room temperature for 2 hours, when TLC showed the reaction was complete. The reaction mixture was poured into (50 ml) petrol/ethyl acetate (10/1) and filtered through a bed of silica gel. 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 (0.23 g, 92 %), $[\alpha]_D^{24}$ -1.09 (c = 1.35, $CHCl_3$) {Found *m/z* $[M + Na]^+$: 816.48, $C_{50}H_{100}O_4SiNa$ requires: 816.41 (MALDI)}. This showed δ_H (500 MHz, $CDCl_3$): 9.77 (1H, t, J = 1.9 Hz), 3.92 (1H, br.td, J = 6.6, 4.75 Hz), 3.66 (3H, s), 2.55 (1H, ddd, J = 10.7, 6.95, 3.5 Hz), 2.44 (2H, dt, J = 7.55, 1.9 Hz), 1.63 (2H, pent., J =7 Hz), 1.55-1.20 (72H, br. m), 0.88 (3H, t, J = 6.6 Hz), 0.87 (9H, s), 0.04 (3H, s), 0.02 (3H, s); δ_C: 202.9, 175.13, 73.22, 51.58, 51.21, 43.91, 33.68, 31.92, 29.82, 29.69, 29.65, 29.58, 29.43, 29.35, 29.17, 27.83, 27.48, 25.76, 23.71, 22.67, 22.09, 17.97, 14.10, -4.37, -4.93; ν_{max}/cm^{-1}: 2924, 2853, 1741, 1464, 1372, 1249, 1167, 1048, 836, 775.

Experiment 49: (*Z*)-(*R*)-3-(*tert*-Butyldimethylsilanyloxy)-octatriacont-20-enoic -2-tetracosanoic acid methyl ester (332)



Sodium bis(trimethylsilyl) amide (1.75 ml, 1.57 mmol, 1.0M in THF) was added to a stirred solution of nonadecyltriphenylphosphonium bromide (**313**) (0.476 g, 0.76 mmol) at room temperature under a nitrogen atmosphere. The mixture was stirred for 30 min and then (*R*)-2-[(*R*)-1-*tert*-butyldimethylsilanyloxy)-19-oxononadecyl] tetracosanoic acid methyl ester (**320**) (0.31 g, 0.39 mmol) in dry THF (15 ml) was added. The mixture was stirred for 3 hours, when TLC showed no starting material was left, and then the reaction was quenched with sat. aq. NH_4Cl (10 ml) and the product was extracted with petrol/ethyl acetate (20:1, 3×20 ml). The combined organic layers were dried over $MgSO_4$, filtered and the solvent was evaporated. The product was purified by chromatography over silica gel, eluting with petrol/ethyl acetate (40:1) to give methyl (*2R,3R,Z*)-3-(*tert*-butyldimethylsilyloxy)-2-docosyltetracont-21-enoate **(332)** (0.255 g, 62 %), $[\alpha]_D^{24}$ -2.45 (c = 1.18, $CHCl_3$) {Found *m/z* $[M + Na]^+$: 1066.0319. $C_{69}H_{138}O_3SiNa$ requires: 1066.0307}. This showed δ_H (500 MHz, $CDCl_3$): 5.35 (2H, dt, J = 11.65, 5.65 Hz), 3.91 (1H, br.td, J = 7, 4.4 Hz), 3.66 (3H, s), 2.53 (1H, ddd , J = 11.05, 7.25, 3.8 Hz), 2.02 (4H, br.q, J = 7 Hz), 1.6-1.2 (106H, br.m), 0.89 (6H, t, J = 6.6 Hz), 0.87 (9H, s), 0.05 (3H, s), 0.02 (3H, s); δ_C: 175.14, 129.89, 73.22, 51.57, 51.21, 33.67, 31.92 , 29.83, 29.77, 29.70, 29.61, 29.58, 29.56, 29.44, 29.36, 29.31, 27.83, 27.50, 27.21 , 25.75, 23.68, 22.69, 17.97, 14.11, -4.37, -4.93; ν_{max}/cm^{-1}: 2923, 2852, 1741, 1468, 1437, 1361, 1179, 1120, 836, 775, 720, 695.

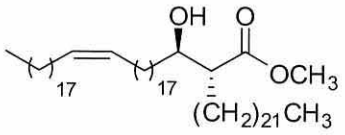
Experiment 50: (*E/Z*)-(*R*)-3-(*tert*-Butyldimethylsilanyloxy)-octatriacont-20-enoic -2- tetracosanoic acid methyl ester (333)



Lithium bis(trimethyl silyl)amide (0.5 ml, 5.38×10^{-4} mmol) was added to a stirred solution of (*R*)-2-[(*R*)-1-(*tert*-butyl-dimethyl-silanyloxy)-19-oxo-nonadecyl]-

tetracosanoic acid methyl ester (**330**) (0.19 g, 2.44x10^{-4} mmol) and 5-(octadecane-1-sulfonyl)-1-phenyl-1H-tetrazole (**331**) (0.17 g, 3.66x10^{-4} mmol) in dry THF (5 ml) at -10 °C. The reaction turned bright yellow and was left to reach room temperature and stirred for one hour under a nitrogen atmosphere, when TLC showed no starting material was left the reaction was quenched by addition of sat. NH_4Cl. The product was extracted with petrol/ether (1:2, 3x25 ml) dried over $MgSO_4$, filtered and evaporated. The crude product was purified by column chromotography over silica gel, eluting with petrol/ether (20:1), to obtain the title compound (0.18 g, 70 %) {Found *m/z* $[M + Na]^+$: 1066.0248. $C_{69}H_{138}O_3SiNa$ requires: 1066.0308}. This showed δ_H (500 MHz, $CDCl_3$): (major isomer) 5.4-5.38 (2H, m), 3.86 (1H, br.dt, J = 6.65, 4.7 Hz), 3.61 (3H, s), 2.48 (1H, ddd, J = 11, 7.25, 3.75 Hz), 1.93-1.90 (4H, br.q, J = 6 Hz), 1.5-1.15 (104H, m), 0.84 (6H, t, J = 7 Hz), 0.82 (9H, s), 0.00 (3H, s), -0.025 (3H, s); (minor isomer) 5.36-5.34 (2H, m), 1.97 (4H, br.q, J = 6.65 Hz) (the remaining signals obscured by the major isomer); δ_C: (both isomers): 175.14, 130.36(*trans* isomer), 129.89(cis isomer), 73.22, 51.57, 51.2, 33.68, 32.6, 31.92, 29.83, 29.78, 29.7, 29.66, 29.6, 29.58, 29.57, 29.54, 29.53, 29.44, 29.36, 29.32, 29.2, 27.82, 27.5, 27.2, 25.75, 23.69, 22.68, 22.6, 17.97, 14.1, -4.38, -4.94; ν_{max}/cm^{-1}: 2922, 2851, 2360, 1741, 1464, 1361, 1252, 1193, 1165, 1070, 1005, 965, 835, 774, 719.

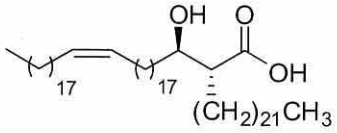
Experiment 51: Methyl (*2R, 3R, Z*)-2-docosyl-3-hydroxytetracont-21-enoate (334)



(*Z*)-(*R*)-3-(*tert*-Butyldimethylsilanyloxy)-octatriacont-20-enoic-2-tetracosanoic acid methyl ester (**332**) (0.4 g, 4.1 mmol) was stirred in dry THF (10 ml) in a dry polyethylene vial under a nitrogen atmosphere at room temperature. Pyridine (0.3 ml) and HF.pyridine complex (1 ml) were added and the mixture was stirred for 18 hours at 40 °C. The reaction mixture was diluted with petrol/ethyl acetate (1:1, 10 ml) and neutralized with sat.aq. $NaHCO_3$. The mixture was separated and the aqueous layer was re-extracted with petrol/ethyl acetate (1:1, 2x 20 ml). The combined organic layers were washed with brine, dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate eluting with petrol/ethyl acetate (10:1) gave methyl (2*R*,3*R*,*Z*)-2-docosyl-3-hydroxytetracont-21-enoate **(334)** as a white solid (0.32 g, 87 %), m.p: 44 - 46 °C,

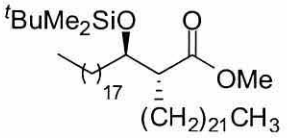
$[\alpha]_D^{20}$ +4.65 (c = 1.83, $CHCl_3$) {Found *m/z* $[M + Na]^+$: 951.9458, $C_{63}H_{124}NaO_3$ requires: 951.9443}. This showed δ_H (500 MHz, $CDCl_3$): 5.31-5.24 (2H, m), 3.71 (3H, s), 3.60-3.58 (1H, m), 2.38 (1H, br.td, J = 9.15, 5.35 Hz), 1.94 (4H, q, J = 6.6 Hz), 1.66-1.61 (1H, m), 1.56-1.48 (4H, m), 1.42-1.2 (102H, br. m), 0.89 (6H, t, J = 6.6 Hz); δ_C: 176.21, 129.89, 72.31, 51.48, 50.95, 35.70, 31.92, 29.77, 29.69, 29.60, 29.57, 29.49, 29.42, 29.35, 29.32, 27.42, 27.21, 25.72, 22.67, 14.09; ν_{max}/cm^{-1}: 3516, 2917, 2849, 1713, 1463, 1169, 719.

Experiment 52: (*2R, 3R, Z*)-2-Docosyl-3-hydroxytetracont-21-enoic acid (208)



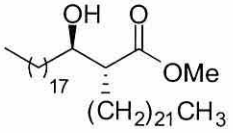
Lithium hydroxide monohydrate (0.3 g, 5.24 mmol) was added to a stirred solution of methyl ester (**334**) (0.32 g, 0.3 mmol) in THF (10 ml), methanol (1 ml) and water (1.5 ml) at room temperature. The mixture was stirred at 40 °C for 18 hours, when TLC showed no starting material was left. The reaction mixture was cooled down to room temperature and diluted with petrol/ethyl acetate (5:2, 10 ml) and acidified to pH = 1 with 5 % HCl. The product was extracted with petrol/ethyl acetate (5:2) (3 x 10 ml), and the combined organic layers were dried over $MgSO_4$ and evaporated to give a crude product which was purified by column chromatography eluting with petrol/ethyl acetate (7:2) to give (*2R,3R,Z*)-2-docosyl-3-hydroxytetracont-21-enoic acid (**208**) (0.26 g, 81 %) as a white solid, m.p: 65-66 °C, $[\alpha]_D^{21}$ +3.43 (c = 0.97, $CHCl_3$) {Found *m/z* $[M + Na]^+$: 937.91, $C_{62}H_{122}NaO_3$ requires: 937.93 (MALDI)}. This showed δ_H (500 MHz, $CDCl_3$): 5.34-5.25 (2H, m), 3.70 (1H, dt, J = 8.2, 4.95 Hz), 2.43 (1H, td, J = 8.8, 5.35 Hz), 1.97 (4H, q, J = 6.65 Hz), 1.64-1.48 (16H, m), 1.21 (92H, br.s), 0.85 (6H, t, J = 6.6 Hz); δ_C: 175.14, 129.90, 72.17, 50.52, 35.56, 31.92, 29.77, 29.70, 29.66, 29.57, 29.48, 29.41, 29.35, 29.31, 27.31, 27.21, 25.71, 22.68, 14.10; ν_{max}/cm^{-1}: 3530, 2915, 2848, 2360, 1684, 1468, 1378, 1208, 965, 717.

Experiment 53: (*2R,3R*)-Methyl 3-(*tert*-butyldimethylsilyloxy)-2-docosyltetra-contanoate (335)



Palladium 10 % on carbon (0.18 g) was added to a stirred solution of (*E/Z*)-(*R*)-3-(*tert*-butyl-dimethylsilanyloxy)-octatriacont-20-enoic -2- tetracosanoic acid methyl ester **(333)** (0.16 g, 0.015 mmol) in IMS (10 ml) and (20 ml) THF. Hydrogenation was carried out for 1 hour. The solution was filtered over a bed of celite and the solvent was evaporated to give a crude product which was purified by column chromatography eluting with chloroform to give (*2R,3R*)-methyl 3-(*tert*-butyldimethylsilyloxy)-2-docosyl-tetra-contanoate (0.12 g, 75 %) as a white solid, m.p: 44-46 °C {Found *m/z* $[M + Na]^+$: 1068.19, $C_{69}H_{140}O_3SiNa$ requires: 1068.04 (MALDI)}, $[\alpha]_D^{20}$ + 4.65 (c = 1.01, $CHCl_3$). This showed δ_H (500 MHz, $CDCl_3$): 3.91 (1H, dt, J = 7.1, 4.55 Hz), 3.66 (3H, s), 2.55 (1H, ddd, J = 10.9, 7.15, 3.75 Hz), 1.59-1.55 (6H, m), 1.26 (108H, s), 0.88 (6H, t, J = 5.8 Hz), 0.86 (9H, s), 0.06 (3H, s), 0.02 (3H, s); δ_C: 17517, 73.22, 51.56, 51.22, 33.67, 31.92, 29.82, 29.70, 29.65, 28.58, 29.44, 29.36, 27.82, 27.50, 25.75, 23.66, 22.69, 17.96, 14.11; ν_{max}/cm^{-1}: 2917, 2849, 1740, 1463, 1376, 1165, 775, 719.

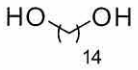
Experiment 54: Methyl (*2R,3R*)- 2-docosyl-3-hydroxytetracontanoate (336)



Methyl (*2R,3R*)-3-(*tert*-butyldimethylsilyloxy)-2-docosyltetracontanoate **(335)** (0.12 g, 0.11 mmol) was stirred in dry THF (5 ml) in a dry polyethylene vial under a nitrogen atmosphere at room temperature pyridine (0.1 ml) and HF.pyridine complex (0.6 ml) were added and the mixture was stirred for 18 hours at 40 °C. The reaction was diluted with chloroform and neutralized with sat. aq. $NaHCO_3$. The mixture was separated and the aqueous layer was re-extracted with chloroform (2x20 ml). The combined organic layers were washed with brine, dried and the solvent was evaporated. The product was purified with chromatography eluting with chloroform to obtain methyl (*2R,3R*)-2-docosyl-3-hydroxytetracontanoate **(336)** as a white solid (0.12 g, 83 %), m.p: 56-58 °C, $[\alpha]_D^{21}$ +13.4 (c = 0.93, $CHCl_3$) {Found *m/z* $[M + Na]^+$: 953.95, $C_{63}H_{126}O_3Na$ requires: 953.96 (MALDI)}. This showed δ_H (500 MHz, $CDCl_3$): 5.31-5.24 (2H, m), 3.86 (1H, dt, J = 7, 4.6Hz), 3.66 (3H, s), 2.48 (1H, ddd, J = 10.9, 7.1, 3.25Hz), 1.22 (113H, s), 0.83 (6H, t, J = 6.8 Hz); δ_C: 174.47, 72.31, 50.94,

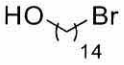
35.70, 34.92, 31.92, 29.70, 29.65, 29.35, 27.42, 25.76, 22.68, 14.09; ν_{max}/cm^{-1}: 3492, 2917, 2849, 1713, 1463, 1464, 1064.

Experiment 55: Tetradecane-1,14-diol (338)



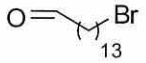
Tetradecaned inoic acid (8 g, 3.09 mmol) in THF (24 ml) was added drop wise over 15 min to a suspension of lithium aluminium hydride (3.55 g, 92.8 mmol) in THF (300 ml) at 0 °C. The mixture was allowed to reach room temperature and refluxed for 1 hour, when TLC show no starting material left, then cooled to 0 °C and sat. aq. sodium sulfate was added to the mixture until white precipitate formed. THF (50 ml) was added and the mixture was stirred at room temperature for 30 min and the mixture was dried over $MgSO_4$, filtered through a bed of silica gel and the solvent was evaporated. This showed δ_H (500 MHz, $CDCl_3$): 3.64 (4H, t, J = 6.6 Hz), 1.58 (4H, q, J = 6.65 Hz), 1.26 (22H, br s); δ_C: 67.67, 63.07, 32.79, 29.80, 29.58, 29.56, 29.53, 29.40, 29.13, 25.72; ν_{max}/cm^{-1}: 3467, 2918, 2849, 1751, 1493, 1050, 875. This was identical to the literature values.[241]

Experiment 56: 14-Bromotetradecan-1-ol (339)



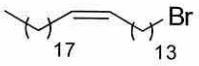
Tetradecane-1,14-diol (**338**) (5.33 g, 18.17 mmol) was dissolved in toluene (100 ml) and aqueous hydrobromic acid (25 ml, 37.25 g, 127.13 mmol, d = 1.49) was added and the mixture was refluxed for 18 hours. When TLC analysis indicated completion of the reaction the mixture was extracted with water (100 ml) and washed with sat. sodium hydrogen carbonate (3x50 ml). The combined organic layers were dried and evaporated to give a crude product which was purified via column chromatography eluting with petrol/ether (20:1, then 1:1) to give a yellow oil, 14-bromotetradecan-1-ol (5.5 g, 81 %) {Found *m/z* $[M + Na]^+$: 331.1011 $C_{14}H_{29}BrOK$ requires: 331.1038}. This showed δ_H (500 MHz, $CDCl_3$): 3.63 (2H, t, J = 6.6 Hz), 3.40 (2H, t, J = 6.9 Hz), 1.85 (2H, q, J = 6.9 Hz), 1.56 (2H, q, J = 6.6 Hz), 1.26 (21H, v b, s); δ_C: 63.04, 33.97, 32.82, 32.78, 29.56, 29.49, 29.39, 28.73, 28.15, 25.71; ν_{max}/cm^{-1}: 3444, 2917, 2849, 1609, 1471, 1451, 1118, 1050, 875.

Experiment 57: 14-Bromo-tetradecanal (340)



14-Bromotetradecan-1-ol (**339**) (2 g, 6.8 mmol) in dichloromethane (10 ml) was added at room temperature to a stirred solution of PCC (3.67 g, 17 mmol) in dichloromethane (70 ml). During the addition a black colour appeared. The reaction was stirred at room temperature for 2.5 hours, when the TLC showed the reaction was complete. The reaction mixture was poured into a mixture of (70 ml) petrol/ethyl acteate (1:1) and filtered through a bed of silica gel. The solvent was evaporated and the crude product was purified by column chromatography eluting with petrol/ethyl actate (5:1) to give a colourless oil of title compound (1.77g, 6.07mmol) {Found *m/z* $[M + Na]^+$: 313.1198 $C_{14}H_{27}BrONa$ requires: 313.1245}. This showed δ_H (500 MHz, $CDCl_3$): 9.72 (1H, t, J = 1.9 Hz), 3.36 (2H, t, J = 4.1 Hz), 2.40-2.37 (2H, m), 1.84-1.80 (2H, m), 1.59 (4H, q, J = 3.5 Hz), 1.3 (4H, v br s), 1.23 (12H, br s); δ_C: 202.75, 65.77, 60.26, 43.80, 33.89, 32.74, 29.45, 29.43, 29.40, 29.32, 29.30, 29.24, 29.05, 28.66, 28.07, 21.98; ν_{max}/cm^{-1}: 2925, 2854, 2716, 1727, 1464, 1409, 1390, 1256, 722, 644.

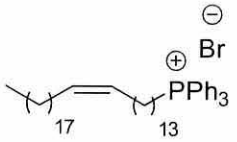
Experiment 58: (*Z*)-1-Bromo-dotriacont-14-ene (341)



Sodium bis(trimethylsilyl)amide (10.27 ml, 10 mmol) was added to a stirred solution of nonadecyltriphenylphosphonium bromide (**313**) (4.82 g, 7.9 mmol) at room temperature under a nitrogen atmosphere. The mixture was stirred for 20 min and then 14-bromotetradecanal (**340**) (1.77 g, 6 mmol) in dry THF was added. The mixture was stirred for 18 hours, when TLC showed no starting material was left, and then the reaction was quenched by addition of sat. aq. NH_4Cl (25 ml) and the product was extracted with petrol/ethyl acetate (10:1, 3x70 ml). The combined organic layers dried over $MgSO_4$, filtered and the solvent was evaporated. The product purified by column chromatography eluting by petrol to give a white solid, m.p 28-30 °C, (1.48 g, 2.73 mmol, 45 %) {Found *m/z* $[M + Na]^+$: 563.4172 $C_{33}H_{65}BrNa$ requires: 563.4167}. This showed δ_H (500 MHz, $CDCl_3$): 5.35 (2H, t, J = 4.75 Hz), 3.41 (2H, t, J = 6.95 Hz), 2.03(4H, q, J = 6.31 Hz), 1.86 (2H, pen, J = 6.95 Hz), 1.43 (2H, pent, J = 6.9Hz),

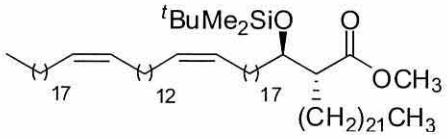
1.26 (50H, br s), 0.89 (3H t, J = 6.3Hz); δ_C: 129.89, 129.86, 33.92, 32.85, 31.93, 29.77, 29.71, 29.66, 29.62, 29.56, 29.45, 29.36, 29.31, 28.78, 28.19, 27.21, 22.69, 14.11; ν_{max}/cm^{-1}: 3004, 2923, 2853, 1465, 1368, 1354, 1257, 970, 720.

Experiment 59: ((*Z*)-Dotriacont-14-enyl)-triphenylphosphonium bromide (342)



(*Z*)-1-Bromo-dotriacont-14-ene (**341**) (1.46 g, 2.69 mmol) was added to a stirred solution of triphenylphosphine (1.06 g, 4.04 mmol) in toluene (20 ml). The reaction mixture was refluxed for 4 days. The solvent was evaporated and petrol (25 ml) was added, filtered, and again evaporated. The residue was treated with diethyl ether (20 ml) and stirred for 1 hour, and filtered to give the product, ((*Z*)-dotriacont-14-enyl)-triphenylphosphonium bromide (352) (1.5 g, 1.86 mmol, 70 %) {Found *m/z* $[M - H]^+$: 723.6048, $C_{51}H_{81}P$ requires: 723.6076}. This showed δ_H (500 MHz, $CDCl_3$): 7.70-7.34 (15H, m), 5.40-5.35 (2H, m), 3.41 (2H, t, J = 6.9 Hz), 2.02 (4H, q, J = 6.3 Hz), 1.86 (2H, q, J = 6.9 Hz), 1.43 (2H, q, J = 6.65 Hz), 1.26 (50H, s), 0.89 (3H, t, J = 6.6 Hz); δ_C: 133.20, 137.12 133.79, 133.64, 132.12, 132.04, 131.93, 131.91, 129.90, 129.87, 128.68, 128.53, 128.49, 128.44, 34.04, 32.83, 31.92, 29.76, br 29.69, 29.64, 29.61, 29.55, 29.43, 29.35, 29.30, 28.77, 28.17, 27.19.

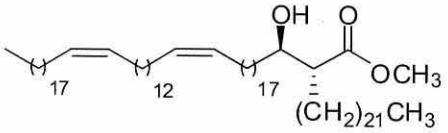
Experiment 60: (*20Z, 34Z*)-(*2R, 3R*)-3-(*tert*-Butyldimethylsilanyloxy)-nonatri-aconta-20,34-dienoic -2- tetracosanoic acid methyl ester (343)



Sodium bis(trimethylsilyl)amide, (0.42 ml, 0.42 mmol, 1.0 M in THF) was added to a solution of ((*Z*)-dotriacont-14-enyl)-triphenylphosphonium bromide (**342**) (0.26 g, 0.33 mmol) in (15 ml) of dry THF at room temperature under a nitrogen atmosphere. The mixture was stirred for 30 min and then 3-(*tert*-butyldimethylsilanyloxy)-21-hydroxy-heneicosanoic acid methyl ester (**330**) (0.2 g, 0.25 mmol) in dry THF (5 ml) at room temperature. The mixture was stirred for 18 hours, when TLC showed no starting material was left, and then the reaction quenched by addation of sat. aq. NH_4Cl (10 ml) and the product was extracted with petrol/ethyl acetate (10:1, 3x20

ml). The combined organic layer dried over $MgSO_4$, filtered and the solvent was evaporated. The crude was purified by column chromatography eluting solvent with petrol and then (40/1) petrol/ethyl acetate to give (0.1 g, 32 %), $[\alpha]_D^{24}$ -7.68 (c = 1.08, $CHCl_3$) {Found *m/z* $[M + H]^+$:1259.015, $C_{83}H_{163}O_3SiNa$ requires: 1260.2347 (MALDI)}. This showed δ_H (500 MHz, $CDCl_3$): 5.39-5.37(2H, m) *trans* isomer, 5.35 (4H, br t, J = 4.7 Hz) *cis* isomer, 3.91 (1H, dt, J = 6.9, 4.55 Hz), 3.66 (3H, s), 2.53 (1H, ddd, J = 11.05, 7.25, 3.8Hz), 2.06 (8H, q, J = 6.95Hz), 1.26 (126H, v br s), 0.88 (6H, t, J = 6.95 Hz), 0.86 (9H, s), 0.05 (3H, s), 0.02 (3H, s); δ_C: 175.14, 133.80, 133.64, 130.35, 129.88, 128.68, 128.49, 128.44, 73.21, 51.56, 51.21, 33.66, 32.84, 32.60, 31.92, 29.82, 29.77, 29.70, 29.58, 29.44, 29.36, 29.32, 29.17, 27.82, 28.49, 27.20, 25.75, 23.67, 22.68, 17.96, 14.11, -4.37, -4.94; ν_{max}/cm^{-1}: 2923, 2853, 1741, 1465, 1370, 1253, 1166, 1082, 836, 774, 724.

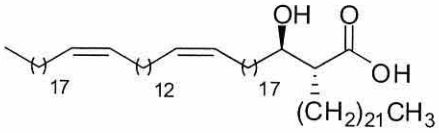
Experiment 61: (*21Z,35Z*)-(*2R,3R*)-3-(Hydroxy)-hentetraconta-21,35-dienoic-2-tetracosanoic acid methyl ester (344)



(*20Z,34Z*)-(*2R,3R*)-3-(*tert*-Butyl-dimethyl-silanyloxy)-nonatriaconta-20,34-dienoic-2-tetra-cosanoic acid methyl ester (**343**) (0.2 g, 0.16 mmol) was stirred in dry THF (5 ml) in a dry polyethylene vial under a nitrogen atmosphere at room temperature. Pyridine (0.1 ml) and HF.pyridine complex (0.6 ml) were added and the mixture was stirred for 18 hours at 40 °C. The reaction mixture was diluted with petrol/ethyl acetate (1:1, 10 ml) and neutralized with sat. aq. $NaHCO_3$. The mixture was separated and the aqueous layer was re-extracted with petrol/ethyl acetate (1:1, 2x 20 ml). The combined organic layers were washed with brine, dried and the solvent was evaporated. The crude product purified by chromatography over silica gel eluting with petrol/ethyl acetate 10:1 gave (*21Z,35Z*)-(*2R,3R*)-3-(hydroxy)-hentetraconta-21,35-dienoic-2-tetracosanoic acid (**344**), as a white solid (0.118 g, 67 %), m.p: 32-34 °C, $[\alpha]_D^{20}$ +22.85 (c = 0.21, $CHCl_3$) {Found *m/z* $[M + Na]^+$: 1146.1580, $C_{77}H_{150}O_3Na$ requires: 1146.1482}. This showed δ_H (500 MHz, $CDCl_3$): 5.39-5.32 (4H, m), 3.71 (3H, s), 3.66 (1H, dt, 8.15, 2.8 Hz), 2.44 (1H, ddd, J = 14.15, 10.4, 5 Hz), 2.02 (5H, q, J = 6.3 Hz), 1.61-1.59 (8H, m), 1.26 (122H, br s), 0.88 (6H, t, J = 6.6Hz); δ_C: 176.23,

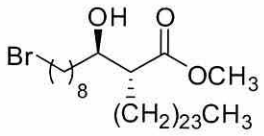
129.88, 72.29, 51.50, 50.92, 35.70, 32.60, 31.92, 29.77, 29.69, 29.57, 29.49, v br 29.42, 29.35, 29.32, 27.41, 27.20, 25.72, 22.68, 14.11; ν_{max}/cm^{-1}: 3429, 2917, 2849, 1721, 1639, 1464, 1374, 1201, 1174.

Experiment 62: (*21Z,35Z*)-(*2R,3R*)-3-(Hydroxy)-hentetraconta-21,35-dienoic-2-tetracosanoic acid (209)



Lithium hydroxide monohydrate (0.06 g, 1.57 mmol) was added to a stirred solution methyl ester (**344**) (0.11 g, 0.105 mmol) in THF (6 ml), methanol (0.6 ml) and water (0.6 ml) at room temperature. The mixture was stirred at 40 °C for 18 hours, when TLC showed no starting material was left. The reaction mixture was cooled down to room temperature and diluted with petrol/ethyl acetate (5:2, 10 ml) and acidified to pH = 1 with 5 % HCl. The product was extracted with petrol/ethyl acetate (5:2, 3x10 ml), and the combined organic layers were dried over $MgSO_4$ and evaporated to give a crude product which was purified by column chromatography eluting with petrol/ethyl acetate (7:2) to give (0.026 g, 81 %) as a white solid, m.p: 32-34 °C, $[\alpha]_D^{24}$ +2.7 (c = 0.55, $CHCl_3$) {Found *m/z* $[M + Na]^+$:1132.406, $C_{76}H_{148}O_3Na$ requires: 1132.132 (MALDI)}. This showed δ_H (500 MHz, $CDCl_3$): 5.37-5.33 (4H, m), 3.72 (1H, dt, J = 7.25, 4.1 Hz), 2.46 (1H, dt, 10.1, 5.35 Hz), 2.03-1.98(12H, m), 1.76-1.70 (1H, m), 1.65-1.59 (1H, m), 1.54-1.48 (4H, m), 1.26 (118H, br s), 0.88 (6H, t, J = 6.65Hz); δ_C: 179.64, 129.88, 72.13, 50.84, 35.49, 32.61, 31.92, 29.77, 29.70, 29.66, 29.62, 29.59, 29.58, 29.51, 29.42, 29.36, 29.32, 29.23, 29.18, 27.32, 27.21, 25.74, 25.71, 22.68, 14.11; ν_{max}/cm^{-1}: 3434, 2917, 2850, 1712, 1638, 1470, 1385, 1216, 471.

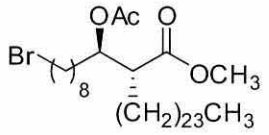
Experiment 63: (*R*)-Methyl 2-((*R*)-9-bromo-1-hydroxynonyl)hexacosanoate (378)



(*R*)-2-[(*R*)-9-Bromo-1-(*tert*-butyldimethylsilanyloxy)-non-3-enyl]-hexacosanoic acid methyl ester (**377**) (2.2 g, 2.94 mmol) was stirred in dry THF (15 ml) in a dry polyethylene vial under a nitrogen atmosphere at room temperature. Pyridine (0.2 ml)

and HF.Pyridine complex (2.5 ml) were added and the mixture was stirred for 17 hours at 45 °C, when the TLC showed reaction completed. The reaction was diluted with petrol/ethyl acetate (1:1, 50 ml) and neutralized by pouring into sat. aq. $NaHCO_3$ until no more carbon dioxide was liberated. The mixture was extracted and the aqueous layer was re-extracted with petrol/ethyl acetate (1:1, 2x100 ml). The combined organic layers were washed with brine, dried and evaporated to give a residue which was purified by column chromatography eluting with petrol/ethyl acetate (5:1) to give a semi solid, (*R*)-methyl 2-((*R*)-9-bromo-1-hydroxynonyl)hexacosanoate (1.7 g, 91 %), $[\alpha]_D^{20}$ +6.88 (c = 1.48, $CHCl_3$) {Found *m/z* $[M + Na]^+$: 653.6403, $C_{36}H_{71}BrO_3Na$ requires: 653.4484}. This showed δ_H (500 MHz, $CDCl_3$): 3.72 (3H, s), 3.66 (1H, dt, J = 4.1, 8.2 Hz), 3.41 (2H, t, J = 6.95 Hz), 2.44 (1H, dt, J = 9.15, 5.35 Hz), 1.85 (2H, pent., J = 6.95 Hz), 1.74-1.68 (1H, m), 1.61-1.57 (1H, m), 1.48-1.40 (6H, m), 1.26 (51H, br s), 0.88 (3H, t, J = 6.65 Hz); δ_C: 176.20, 76.74, 76.62, 72.26, 51.50, 50.96, 35.67, 33.94, 32.80, 31.92, 29.69, 29.66, 29.62, 29.56, 29.50, 29.42, 29.39, 29.35, 29.33 28.67, 28.13, 27.42, 25.68 2267, 14.09; ν_{max}/cm^{-1}: 3528, 2921, 2850, 1718, 1458, 1367, 1260, 1193, 1163.

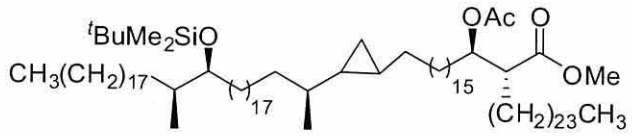
Experiment 64: (*R*)-Methyl 2-((*R*)-1-acetoxy-9-bromononyl)hexacosanoate (379)



A mixture of acetic anhydride (5 ml) and anhydrous pyridine (5 ml) was added to stirred solution of (*R*)-2-[(*R*)-9-bromo-1-(*tert*-butyl-dimethyl-silanyloxy)-non-3-enyl]-hexa-cosanoic acid methyl ester (**378**) (1.62 g, 2.56 mmol) in dry toluene (25 ml) at room temperature and the mixture was stirred at room temperature for 18 hours. The reaction mixture diluted with toluene (40 ml) and the solvent was evaporated under reduced pressure to give a solid. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (5:1) to give a semi solid, (*R*)-methyl 2-((*R*)-1-acetoxy-9-bromononyl)hexacosanoate (**379**) (1.6 g, 92 %), $[\alpha]_D^{23}$ -0.61 (c = 1.19, $CHCl_3$) {Found *m/z* $[M + Na]^+$: 695.5772, $C_{38}H_{73}BrO_4Na$ requires: 695.4589}. This showed δ_H (500 MHz, $CDCl_3$): 5.08 (1H, ddd, J = 11, 6.95, 2.85 Hz), 3.67 (3H, s), 3.39 (2H, t, J = 6.9 Hz), 2.61 (1H, ddd, J = 10.7, 6.6, 4.1 Hz), 1.84 (2H, pent., J = 6.9 Hz), 1.62-1.50 (4H, m), 141-1.38 (4H, m), 1.25(53H, v br s), 0.87 (3H, t, J = 6.9

Hz); δ_C: 173.60, 170.32, 51.52, 49.58, 33.89, 32.75, 31.90, 31.68, 29.67, 29.63, 29.61, 29.53, 29.44, 29.37, 29.34, 29.26, 29.21, 28.61, 28.09, 28.07, 27.44, 24.93, 22.66, 20.98, 14.08; ν_{max}/cm^{-1}: 2917, 2858, 1748, 1463, 1370, 1236, 1164, 1125, 1022, 721.

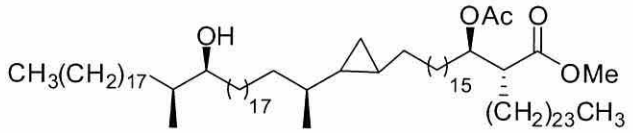
Experiment 68: (*R*)-2-((*R*)-1-Acetoxy-17-{(1*S*,2*R*)-2-[(1*S*,20*S*,21*S*)-20-(*tert*-butyldi-methylsilanyloxy)-1,21-dimethyl-nonatriacontyl]-cyclopropyl}-heptadecyl)-hexacosnoic acid methyl ester (350)



Dipotassium azodicarboxylate (2 g, 10.3 mmol) was added to a stirred solution of (*R*)-2-((*E*/*Z*)-(*R*)-1-acetoxy-17-{(1*S*,2*R*)-2-[(1*S*,20*S*,21*S*)-20-(*tert*-butyl-dimethyl-silanyl-oxy)-1,21-dimethylnonatriacontyl]-cyclopropyl}-heptadec-9-enyl)-hexacosnoic acid methyl ester (**383**) (1.6 g, 1.11 mmol) in THF (20 ml) and methanol (5 ml) at 5 °C. A solution of glacial acetic acid (2.5 ml) and THF (2.5 ml) was prepared and (0.2 ml) was added dropwise every 25 min at 5 °C and the mixture was stirred at room temperature. After two days the reaction mixture was added slowly in portions wise to a sat. aq. of $NaHCO_3$ and extracted with petrol/ethylacetate (10:1, 3 x 80 ml) and the combined organic layers were washed with water (50 ml) and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give a white semi-solid, (*R*)-2-((*R*)-1-acetoxy-17-{(1*S*,2*R*)-2-[(1*S*,20*S*,21*S*)-20-(*tert*-butyldimethyl-silanyl-oxy)-1,21-di-methyl-nonatri-acontyl]-cyclopropyl}-heptadecyl)-hexacosnoic acid methyl ester (**350**) (1.48 mg, 92 %), $[\alpha]_D^{22}$ + 3.05 (c = 1.04, $CHCl_3$) {Found *m/z* $[M + Na]^+$: 1474.5835, $C_{96}H_{190}NaO_5Si$ requires: 1474.4275}. This showed δ_H (500 MHz, $CDCl_3$): 5.09 (1H, ddd, J = 11.65, 7.9, 3.8 Hz), 3.68 (3H, s), 3.50 (1H, ddd, J = 9.45, 6.3, 2.8 Hz), 2.62 (1H, ddd, J = 10.7, 6.95, 4.1 Hz), 2.32-2.26 (1H, m), 2.03 (3H, s), 1.26 (147H, br s), 0.90 (3H, d, J = 6.95 Hz), 0.88 (9H, s), 0.87 (6H, t, J = 8.2 Hz), 0.8 (3H, d, J = 6.2 Hz), 0.70-0.62 (1H, m), 0.48-0.41 (1H, m, cyclopropane), 0.22-0.17 (2H, m), 0.16-0.09 (2H, m), 0.03 (3H, s), 0.02 (3H, s); δ_C: 173.63, 170.30, 74.08, 60.36, 51.50, 49.58, 38.12, 37.72, 37.42, 34.49, 33.53, 32.49, 31.93, 31.72, 30.07, 30.00, 29.89, 29.73, 29.71, 29.66, 29.57, 29.56, 29.44, 29.39, 29.36, 28.11, 27.71, 27.46, 27.26, 26.13, 28.11, 27.71, 27.46, 27.26, 26.13, 25.95, 25.91, 24.98, 22.69, 22.18, 20.99,

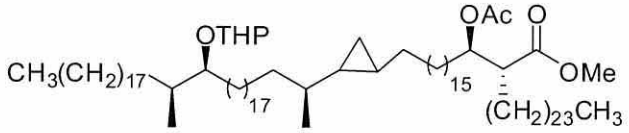
19.68, 18.62, 18.16, 14.40, 14.18, 14.10, 10.48, -4.21, -4.45; ν_{max}/cm^{-1}: 2926, 2846, 1742, 1455, 1371, 1234, 1167, 1023.

Experiment 69: (*R*)-2-((*R*)-1-Acetoxy-17-{(1*S*,2*R*)-2-[(1*S*,20*S*,21*S*)-20-hydroxy-1,21-di-methylnonatriacontyl]-cyclopropyl}-heptadecyl)-hexacosnoic acid methyl ester (355)



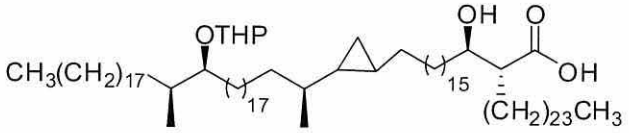
(*R*)-2-((*R*)-1-Acetoxy-17-{(1*S*,2*R*)-2-[(1*S*,20*S*,21*S*)-20-(*tert*-butyldimethylsilanyloxy)-1,21-dimethyl-nonatriacontyl]-cyclopropyl}-heptadecyl)-hexacosnoic acid methyl ester (**350**) (1.48 g, 1.02 mmol) was stirred in dry THF (15 ml) in a dry polyethylene vial under a nitrogen atmosphere at room temperature. Pyridine (0.2 ml) and HF. pyridine complex (2 ml) were added and the mixture was stirred for 17 hours at 45 °C. The reaction mixture was diluted with petrol/ethyl acetate (1:1, 25 ml) and neutralized with sat. aq. of $NaHCO_3$. The mixture was separated and the aqueous layer was re-extracted with petrol/ethylactate (1:1, 3 x 50 ml). The combined organic layers were washed with brine, dried and the solvent was evaporated to give a residue which was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give a semi solid, (*R*)-2-((*R*)-1-acetoxy-17-{(1*S*,2*R*)-2-[(1*S*,20*S*,21*S*)-20-hydroxy-1,21-dimethyl-nonatriacontyl]-cyclopropyl}-heptadecyl)-hexacosnoic acid methyl ester (**355**) (1.04 g, 76 %), $[\alpha]_D^{24}$ -1.25 (c = 0.95, $CHCl_3$), m.p.: 42-44 °C {Found *m/z* [M + Na]$^+$: 1360.3348, $C_{90}H_{176}NaO_5$ requires: 1360.3410}. This showed δ_H (500 MHz, $CDCl_3$): 5.09 (1H, ddd, J = 11.05, 8.2, 4.1 Hz), 3.68 (3H, s), 3.50 (1H, dt, J = 7.25, 4.1 Hz), 2.62 (1H, ddd, J = 10.7, 6.95, 4.4 Hz), 2.03 (3H, s), 1.47-1.39 (6H, m), 1.26 (144H, br s), 0.909 (3H, d, J = 6.9 Hz), 0.89 (3H, d, J = 6.95 Hz), 0.87 (6H, t, J = 7.55 Hz), 0.70-0.64 (1H, m), 0.48-0.42 (1H, m), 0.12-0.09 (3H, m); δ_C: 173.62, 170.30, 75.22, 74.12, 51.49, 49.61, 38.20, 38.10, 37.42, 34.55, 34.49, 33.39, 31.92, 31.76, 30.07, 29.96, 29.72, 29.69, 29.65, 29.58, 29.47, 29.39, 29.35, 28.13, 27.48, 27.42, 27.27, 26.15, 26.15, 25.01, 22.67, 20.99, 19.66, 18.61, 14.08, 13.59; ν_{max}/cm^{-1}: 3474, 2855, 1743, 1476, 1378, 1243, 1171, 1022.

Experiment 70: (*R*)-2-((*R*)-1-Acetoxy-17-{(1*S*,2*R*)-2-[(1*S*,20*S*,21*S*)-20-(tetrahydro-2H-pyran-2-yl)-1,21-dimethylnonatriacontyl]-cyclopropyl}-heptadecyl)-hexacosnoic acid methyl ester (384)



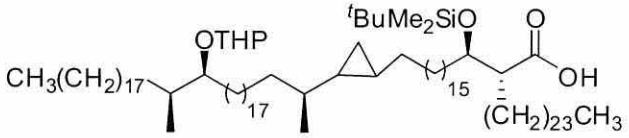
Pyridinium *p*-toluene sulfonate (0.1 g, 0.36 mmol) in dry dichloromethane (1 ml) was added to a stirred solution of (*R*)-2-((*R*)-1-acetoxy-17-{(1*S*,2*R*)-2-[(1*S*,20*S*,21*S*)-20-hydroxy-1,21-dimethyl-nonatriacontyl]-cyclopropyl}-heptadecyl)-hexacosnoic acid methyl ester (**355**) (1.04 g, 0.72 mmol) and freshly distilled dihydro-2H-pyran (0.12 ml, 1.4 mmol) in dry dichloromethane (5 ml) at room temperature under a nitrogen atmosphere. After stirring at room temperature for 1.5 hours, TLC showed no starting material was left then the reaction was quenched with a sat. aq. of $NaHCO_3$ (10 ml). The product was extracted with dichloromethane (3 x 50 ml) and the combined organic layers were dried and evaporated to give a residue which was purified by column chromatography, eluting with petrol/ethyl acetate (10:1) with a few drops of Et_3N to give a white semi-solid of title compound as a mixture of diastereoisomers (1.04g, 94 %) {Found *m/z* $[M + Na]^+$: 1443.6146, $C_{95}H_{184}O_6Na$ requires: 1444.4093}. This showed δ_H (500 MHz, $CDCl_3$): 5.09 (1H, ddd, J = 11.05, 8.2, 4.1 Hz), 4.65 (2H, td, J = 16.4, 3.45 Hz), 3.90-3.86 (1H, m), 3.68 (3H, s), 3.49-3.43 (2H, m), 2.62 (1H, ddd, J = 10.7, 6.95, 4.4 Hz), 2.03 (3H, s), 1.26 (158H, v br s), 0.90 (3H, d, J = 6.6 Hz), 0.89 (6H, t, J = 6.95 Hz), 0.87 (6H, t, J = 4.7 Hz), 0.85 (3H, d, J = 6.3 Hz), 0.7-0.64 (1H, m), 0.47-0.43 (1H, m), 0.22-0.09 (3H, m); δ_C: 173.87, 98.54, 97.84, 81.47, 80.94, 74.12, 51.49, 49.61, 38.09, 37.42, 36.48, 35.18, 34.49, 32.54, 32.10, 31.92, 31.75, 31.49, 31.31, 31.24, 30.07, 29.99, 29.96, 29.72, 29.70, 29.66, 29.57, 28.47, 29.39, 29.35, 28.13, 27.60, 27.55, 27.48, 27.27, 26.15, 25.77, 25.69, 25.63, 25.01, 22.67, 20.99, 20.08, 19.83, 19.65, 18.61, 15.16, 14.94, 14.08, 10.48; $\nu_{max}/cm^{-1}{}_x$: 2918, 2850, 1744, 1476, 1372, 1236, 1165, 1132, 1077, 1023, 721.

Experiment 71: (*R*)-2-{(*R*)-1-Hydroxy-17-[(1*S*,2*R*)-2-((1*S*,20*S*,21*S*)-20-(tetra-hydro-2H-pyran-2-yl)-1,21-dimethylnonatriacontyl)-cyclopropyl]-heptadecyl}-hexacosanoic acid (356)



Lithium hydroxide monohydrate (0.46 g, 1.1 mmol) was added to a stirred solution of (*R*)-2-((*R*)-1-acetoxy-17-{(1*S*,2*R*)-2-[(1*S*,20*S*,21*S*)-20-(tetrahydro-2H-pyran-2-yl)-1,21-dimethyl-nonatriacontyl]-cyclopropyl}-heptadecyl)-hexacosnoic acid methyl ester (**384**) (1.04 g, 0.738 mmol) in THF (10 ml), methanol (1 ml) and water (1.2 ml) at room temperature. The mixture was stirred at 45 °C for 18 hours, when TLC showed a small amount of starting material was left. It was cooled to room temperature and a mixture of petrol/ethyl acetate (10:1, 10 ml) and then neutralized the reaction mixture by HCl (5 %) until get (PH = 7). The mixture was extracted with petrol/ethyl acetate (5:2) (3x25 ml). The combined organic layers were washed with water (15 ml), dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (5:2) to give a semi-solid, (*R*)-2-{(*R*)-1-hydroxy-17-[(1*S*,2*R*)-2-((1*S*,20*S*,21*S*)-20-(tetrahydro-2H-pyran-2-yl)-1,21-dimethyl-nonatriacontyl)-cyclopropyl]-heptadecyl}-hexacosanoic acid (**356**) (1.0 g, 90 %) {Found *m/z* $[M + Na]^+$: 1388.6966, $C_{92}H_{180}O_5Na$ requires: 1388.3728}. This showed δ_H (500 MHz, $CDCl_3$): 4.64 (1H, td, J = 6.95, 3.45 Hz), 3.96-3.90 (1H, m), 3.73-3.71 (1H, m), 3.50-3.43 (2H, m), 2.46 (1H, dt, J = 10.1, 5.35 Hz), 1.85-1.80 (1H, m), 1.76-1.62 (3H, m), 1.53-1.51 (4H, m), 1.26 (158H, v br s), 0.89 (6H, t, J = 9.75 Hz), 0.85 (3H, d, J = 6.95 Hz), 0.71-0.64(1H, m, cyclopropane), 0.48-0.42 (1H, m, cyclopropane), 0.22-0.09 (3H, m); δ_C: 173.07, 98.54, 97.84, 81.47, 80.94, 74.12, 51.49, 49.61, 38.09, 37.42, 36.48, 35.18, 34.49, 32.54, 32.10, 31.92, 31.75, 31.49, 31.31, 31.24, 30.07, 29.99, 29.96, 29.72, 29.70, 29.66, 29.57, 28.47, 29.39, 29.35, 28.13, 27.60, 27.55, 27.48, 27.27, 26.15, 25.77, 25.69, 25.63, 25.01, 22.67, 20.08, 19.83, 19.65, 18.61, 15.16, 14.94, 14.08, 10.48; ν_{max}/cm^{-1}: 3519, 2917, 2850, 1682, 1469, 1377, 1259, 1214, 1131, 1077, 1024, 868, 719.

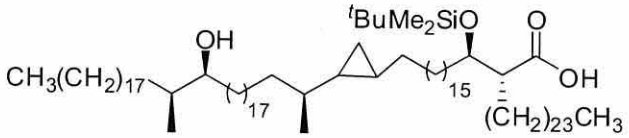
Experiment 72: (2*R*)-2-((1*R*)-1-(*tert*-Butyldimethylsilyloxy)-17-(2-((2*S*,21*S*,22*S*)-22-methyl-21-(tetrahydro-2H-pyran-2-yloxy)tetracontan-2-yl)cyclopropyl) heptadecyl)-hexacosanoic acid (385)



Imidazole (0.5 g, 0.73 mmol) was added to a stirred solution of (*R*)-2-{(*R*)-1-hydroxy-17-[(1*S*,2*R*)-2-((1*S*,20*S*,21*S*)-20-(tetrahydro-2H-pyran-2-yl)-1,21-dimethylnonatriacontyl)-cyclopropyl]-heptadecyl}-hexacosanoic acid (**356**) (0.95 g, 0.73 mmol) in dry DMF (5 ml) and dry toluene (10 ml) at room temperature followed by the addition of *tert*-butyl-dimethylsilylchloride (1.11 g, 0.73 mmol) and 4-dimethylaminopyridine (40 mg, 0.32 mmol). The reaction mixture was stirred at 70 °C for 18 hours. When TLC showed that no starting material was left, the solvent was removed under high vacuum and the residue was diluted with petrol/ethyl acetate (1:1) (150 ml). The organic layer was separated and the aqueous layer was re-extracted with petrol/ethyl acetate (10:1, 3x50 ml). The combined organic layers were washed with water, dried and evaporated to give a residue. The residue was dissolved in THF (20 ml), water (2 ml), and methanol (1.5 ml), to this was added potassium carbonate (0.5 g). The reaction mixture was stirred at 45 °C for 6 hours, and then TLC showed no starting material was left. The mixture was diluted with petrol/ethylacetate (10:1, 50 ml) and water (5 ml) then acidified with potassium hydrogen sulfate to a pH of 2. The organic layer was separated and the aqueous layer was re-extracted with petrol/ethyl acetate (10:1, 2x50 ml). The combined organic layers were washed with water, dried and evaporated to give a residue, which was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a white semi-solid of title compound (0.92 g, 84 %) {Found *m/z* $[M + Na]^+$: 1503.7209. $C_{98}H_{194}O_5SiNa$ requires: 1503.6715}. This showed δ_H (500 MHz, $CDCl_3$): 4.66 (1H, dt, J = 6.95, 3.5 Hz), 3.95-3.89 (1H, m), 3.84-3.81 (1H, m), 3.49-3.42 (1H, m), 2.53 (1H, ddd, J = 9.15, 6.3, 3.15 Hz), 1.84-1.80 (1H, m), 1.72-1.68 (3H, m), 1.57-1.53 (18H, br m), 1.26 (135H, br s), 0.93 (9H, s), 0.90 (3H, d, J = 6.6 Hz), 0.88 (3H, t, J = 2.85 Hz), 0.87 (3H, t, J = 2.85 Hz), 0.85 (3H, d, J = 6.65 Hz), 0.68-0.63 (1H, m), 0.48-0.41 (1H, m), 0.21-0.09 (3H, m), 0.15 (3H, s), 0.14 (3H, s); δ_C: 173.06, 73.71, 62.93, 62.76, 38.12, 37.41, 34.48, 31.97, 31.92, 31.38, 30.06, 29.70, 29.65, 29.62, 29.54, 29.46, 29.39, br 29.36, 27.40, 27.26,

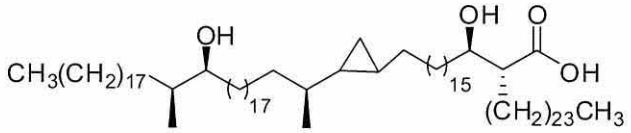
26.13, 25.71, 22.68, 22.30, 19.69, 18.62, 17.92, 14.11, 10.48, 9.77, -4.25, -4.87; ν_{max}/cm^{-1}: 2923, 2853, 1708, 1465, 1377, 1259, 1119, 1082, 1027, 835, 779, 728.

Experiment 73: (*R*)-2-{(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-17-[(1*S*,2*R*)-2-((1*S*,20*S*,21*S*)-20-hydroxy-1,21-dimethylnonatriacontyl) cyclopropyl]heptadecyl} hexacosanoic acid (357)



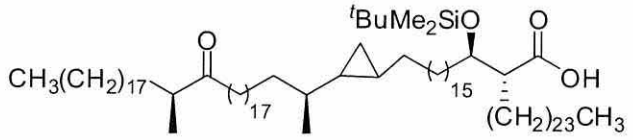
Pyridinium-*p*-toluenesulfonate (0.2 g, 0.79 mmol) was added to a stirred solution of (2*R*)-2-((1*R*)-1-(*tert*-butyldimethylsilyloxy)-17-(2-((2*S*,21*S*,22*S*)-22-methyl-21-(tetra-hydro-2H-pyran-2-yloxy)tetracontan-2-yl)cyclopropyl)heptadecyl)hexacosanoic acid (**385**) (0.9 g, 0.614 mmol) in THF (15 ml), methanol (2 ml), and water (0.2 ml) and stirred at 47 °C for 18 hours. When TLC showed that the reaction was almost complete sat. aq. solution of sodium bicarbonate (5 drops) was added and the product was extracted with petrol/ethyl acetate (5:1, 3x100 ml). The combined organic layers were dried over $MgSO_4$and evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give the title compound as a white semi-solid (**357**) (0.62 g, 0.44 mmol, 73 %) {Found *m/z* $[M + Na]^+$: 1417.5687, $C_{93}H_{186}O_4SiNa$ requires: 1418.5687}. This showed δ_H (500 MHz, $CDCl_3$): 3.82 (1H, ddd, J = 7.25, 5.05, 2.55 Hz), 3.50 (1H, dt, J = 6.9, 4.75 Hz), 2.53 (1H, ddd, J = 8.8, 6.2, 2.5 Hz), 1.73-1.68 (1H, m), 1.63-1.54 (6H, m), 1.26 (145H, v br s), 0.93 (9H, s), 0.909 (3H, d, J = 6.6 Hz), 0.902 (3H, d, J = 6.6 Hz), 0.87 (6H, t, J = 7.6 Hz), 0.69-0.63 (1H, m), 0.48-0.42 (1H, m), 0.21-0.09 (2H, m), 0.15 (3H, s), 0.14 (3H, s); δ_C: 172.31, 75.22, 73.72, 46.73, 38.15, 38.12, 37.42, 35.84, 34.48, 33.35, 31.92, 30.06, 29.95, 29.69, 29.65, 29.62, 29.54, 29.46, 29.39, 29.35, 27.40, 27.25, 26.27, 26.13, 25.71, 22.68, 19.69, 18.62, 17.92, 14.11, 13.57, 10.48, -4.24, -4.86; ν_{max}/cm^{-1}: 3485, 2856, 1743, 1471, 1379, 1244, 1171, 1020.

Experiment 74: (*R*)-2-{(*R*)-1-Hydroxy-17-[(1*S*,2*R*)-2-((1*S*,20*S*,21*S*)-20-hydroxy-1,21-dimethyl-nonatriacontyl)-cyclopropyl]-heptadecyl}-hexacosanoic acid (211)



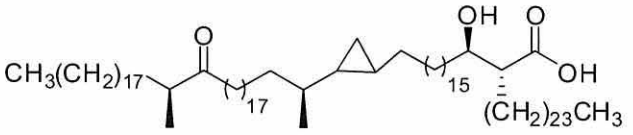
(*R*)-2-{(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-17-[(1*S*,2*R*)-2-((1*S*,20*S*,21*S*)-20-hydroxy-1,21-dimethyl-nonatriacontyl)-cyclopropyl]-heptadecyl}-hexacosanoic acid (**357**) (0.24 g, 0.1718 mmol) was dissolved in dry THF (5 ml) in a dry polyethylene vial under a nitrogen atmosphere at room temperature and stirred. Pyridine (0.1 ml) and HF.pyridine complex (0.6 ml) was added and the mixture was stirred for 17 hours at 45 °C, when TLC showed complete reaction. The reaction was diluted with petrol/ethyl acetate (5:2, 10 ml) and neutralized by pouring into sat. aq. $NaHCO_3$ until no more carbon dioxide was liberated. The mixture was extracted and the aqueous layer was re-extracted with petrol/ethyl acetate (1:1, 2 x 50 ml). The combined organic layers were washed with brine, dried and evaporated to give a residue which was purified by column chromatography eluting with petrol/ethyl acetate (5:2) to give a white solid, (*R*)-2-{(*R*)-1-hydroxy-17-[(1*S*,2*R*)-2-((1*S*,20*S*,21*S*)-20-hydroxy-1,21-dimethyl-nonatri-acontyl)-cyclo-propyl]-heptadecyl}-hexacosanoic acid (160 mg, 72 %), $[\alpha]_D^{16}$ -1.05 (c = 0.55, $CHCl_3$), m.p.: 51–53 °C {Found *m/z* $[M + Na]^+$: 1304.3206, $C_{87}H_{172}NaO_4$ requires: 1304.3148}. This showed δ_H (500 MHz, $CDCl_3$): 3.73–3.69 (1H, m), 3.54–3.51 (1H, m), 2.45 (1H, dt, J = 8.8, 5.4 Hz), 1.75–1.58 (1H, m), 1.63-1.60 (1H, m), 1.55–1.13 (150H, v. br. m), 0.90 (3H, d, J = 4.15 Hz), 0.88 (6H, t, J = 5.7 Hz), 0.87 (3H, d, J = 6.95 Hz), 0.69–0.63 (1H, m), 0.48–0.43 (1H, m), 0.22–0.16 (1H, m), 0.16-0.08 (2H, m); δ_C: 178.8, 75.5, 72.2, 50.8, 38.8, 38.1, 37.5, 35.3, 34.7, 34.6, 33.2, 31.8, 30.2, 30.01, 29.74, br 29.72, 29.67, 29.61, 29.55, 29.44, 29.37, 27.41, 27.32, 27.21, 26.31, 26.10, 25.71, 22.8, 19.72, 18.62, 14.20, 13.62, 10.53; ν_{max}/cm^{-1}: 3334, 2928, 2854, 1685, 1463, 1378, 1205.

Experiment 75: (*R*)-2-{(*R*)-17-[(1*S*,2*R*)-2-((1*S*,21*S*)-1,21-Dimethyl-20-oxo-nonatriacontyl)-cyclopropyl]-1-(*tert*-butyldimethylsilanyloxy)-heptadecyl}-hexacosanoic acid methyl ester (358)



(*R*)-2-{(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-17-[(1*S*,2*R*)-2-((1*S*,20*S*,21*S*)-20-hydroxy-1,21-dimethylnonatriacontyl)-cyclopropyl]-heptadecyl}-hexacosanoic acid **(357)** (0.53 g, 0.38 mmol) in dichloromethane (10 ml) was added at room temperature to a stirred solution of PCC (0.24 mg, 0.11 mmol) in dichloromethane (30 ml) at room temperature. During the addition a black colour appeared. The reaction mixture was stirred at room temperature for 2 hours, and then TLC showed the reaction was complete. The reaction mixture was poured into petrol/ethyl acetate (20 ml, 10:1), and filtered over a bed of silica gel. The solvent was evaporated and the crude product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give the title compound as a white semi-solid (0.47g, 88 %), {Found *m/z* [M + Na]$^+$: 1416.6186, $C_{93}H_{184}O_4SiNa$ requires: 1416.3964}, $[\alpha]_D^{21}$ + 6.33 (c = 0.71, $CHCl_3$). This showed δ_H (500 MHz, $CDCl_3$): 3.82 (1H, ddd, J = 7.9, 5.35, 2.55 Hz), 2.55-2.49 (1H, m), 2.41(1H, td, J = 7.25, 2.2 Hz), 1.55–1.49 (28H, v br, m), 1.26 (153H, br s), 1.06 (3H, d, J = 6.9 Hz), 0.94 (9H, s), 0.90 (3H, d, J = 6.2 Hz), 0.90 (6H, t, J = 6.95 Hz), 0.48-0.42 (1H, m), 0.21-0.16 (1H, m), 0.16 (3H, s), 0.15 (3H, s), 0.13-0.09 (1H, m); δ_C: 170.99, 38.90, 31.92, 29.73, 29.70, 29.66, 29.54, 29.50, 29.39, 29.36, 25.72, 22.68, 14.10, -4.85, -5.49; ν_{max}/cm^{-1}: 2943, 2857, 1689, 1468, 1372, 1209.

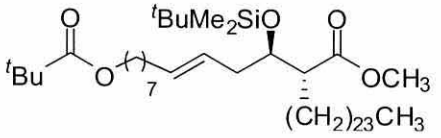
Experiment 76: (*R*)-2-{(*R*)-17-[(1*S*,2*R*)-2-((1*S*,21*S*)-1,21-Dimethyl-20-oxononatriacontyl)-cyclopropyl]-1-hydroxyheptadecyl}-hexacosanoic acid (212)



(*R*)-2-{(*R*)-17-[(1*S*,2*R*)-2-((1*S*,21*S*)-1,21-Dimethyl-20-oxo-nonatriacontyl)-cyclopropyl]-1-(*tert*-butyl-dimethyl-silanyloxy)-heptadecyl}-hexacosanoic acid methyl ester **(358)** (0.21 g, 0.152 mmol) was stirred in dry THF (5 ml) in a dry polyethylene

vial under a nitrogen atmosphere at room temperature and stirred. Pyridine (0.1 ml) and HF. Pyridine complex (0.6 ml) were added and the mixture was stirred for 17 hours at 45 °C. The reaction mixture was diluted with petrol/ethylacetate (5:2, 10 ml) and neutralized sat. aq. of $NaHCO_3$. The combined organic layers were washed with brine, dried and the solvent was evaporated. The residue was purified by column chromatography eluting with petrol/ethyl acetate (7:3) to give a white solid, (*R*)-2-{(*R*)-17-[(1*S*,2*R*)-2-((1*S*,21*S*)-1,21-dimethyl-20-oxo-nonatriacontyl)-cyclopropyl]-1-hydroxyheptadecyl}hexacosanoic acid (0.16 g, 84 %, $[\alpha]_D^{19}$ +10.55 (c = 0.54, $CHCl_3$), m.p.: 67 – 68 °C {Found *m/z* $[M + Na]^+$: 1302.2938, $C_{87}H_{170}NaO_4$ requires: 1302.2991}. This showed δ_H (500 MHz, $CDCl_3$): 3.71 (1H, dt, J = 7.9, 4.75 Hz), 2.38-2.34 (1H, m), 2.35-2.32 (1H, m), 2.29 (2H, td, J = 9.45, 1.9 Hz), 1.77-1.70 (1H, m), 1.66-1.59 (2H, m), 1.56-1.46 (5H, m), 1.26 (142, br s), 1.06 (2H, d, J = 6.95 Hz), 0.90 (2H, d, J = 6.95 Hz), 0.88 (6H, t, J = 6.65 Hz), 0.69-0.64 (1H, m), 0.47-0.42 (1H, m), 0.21-0.17 (1H, m), 0.16-0.08 (2H, m); δ_C: 215.48, 179.78, 72.11, 50.86, 46.33, 41.14, 38.12, 37.42, 35.49, 34.48, 33.03, 31.92, 30.06, 29.70, 29.65, 29.60, 29.52, 29.49, 29.47, 29.42, 29.36, 29.33, 27.32, 27.25, 26.12, 25.72, 23.72, 22.68, 19.69, 18.62, 16.34, 14.10, 10.48; ν_{max}/cm^{-1}: 2922, 2853, 1716, 1685, 1470, 1037.

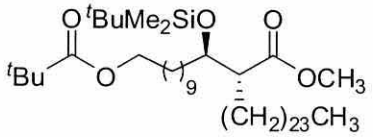
Experiment 77: (*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-11-(2,2-dimethyl-propionyloxy)-undec-3-enyl]-hexacosanoic acid methyl ester (407)



Lithium bis(trimethyl silyl)amide (14.41ml, 15.2mmol) was added to a stirred solution of (*R*)-2-[(*R*)-1-(*tert*-butyl-dimethyl-silanyloxy)-3-oxo-propyl]-tetracosanoic acid methyl ester (**373**) (4.05 g, 6.78 mmol) and 8-((1-phenyl-1H-tetrazol-5-yl)sulfonyl)octyl pivalate (**406**) (4.30 g, 0.678 mmol) in dry THF (100 ml) at -10 °C. The reaction turned bright yellow and was left to reach room temperature and stirred for one hour under a nitrogen atmosphere; when TLC showed no starting material was left the reaction quenched by addtion of sat. aq. NH_4Cl. The product was extracted with petrol/ethyl acetate (5/2, 3x150 ml) dried over $MgSO_4$, filtered and evaporated. The crude product was purified by column chromotography eluting with petrol/ethyl acetate (20/1) to obtain the title compound (**407**) (5.03 g, 0.064 mmol, 93 %) {Found

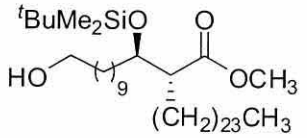
m/z [M + Na]$^+$: 818.2535, $C_{49}H_{98}O_5SiNa$ requires: 817.7081 (MALDI)}. This showed δ_H (500 MHz, $CDCl_3$): 5.43-5.36 (2H, m), 4.02 (2H, t, J = 6.3Hz), 3.87 (1H, dt, J = 7.9, 3.45 Hz), 3.61 (3H, s), 2.49 (1H, ddd, J = 11.35, 8.2, 4.4 Hz), 2.29-2.12 (2H, m), 1.58 (2H, q, J = 6Hz), 1.22 (55H, br s), 1.16 (12H, s), 0.84 (3H, t, J = 6 Hz), 0.8 3(9H, s), 0.01 (3H, s), -0.01(3H, s); δ_C: 174.95, 174.76, 133.46, 131.84, 124.87, 124.41, 64.31, 60.21, 51.42, 51.26, 38.61, 37.27, 32.61, 31.88, 31.85, br 29.63, 29.58, 29.50, 29.47, 29.44, 29.42, 29.39, 29.28, 29.18, 29.10, 29.04, 28.99, 28.56, 27.78, 27.64, 27.52, 27.11, 25.83, 25.64, 22.60, 17.86, 17.84, 14.09, -4.37, -4.39; ν_{max}/cm^{-1}: 3438, 2924, 2854, 1732, 1463, 1363, 1284, 1254, 1158, 1078, 1005, 972, 939, 836, 776, 721.

Experiment 78: (*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-11-(2,2-dimethyl-propionyloxy)-undecyl]-hexacosanoic acid methyl ester (408)



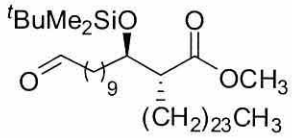
Palladium 10 % on carbon (1 g) was added to a stirred solution of (*R*)-2-[(*R*)-1-(*tert*-butyl-di-methyl-silanyloxy)-11-(2,2-dimethyl-propionyloxy)-undec-3-enyl]-hexacosanoic acid methyl ester (**407**) (4.5 g, 7.52 mmol) in IMS (50 ml) and THF (15 ml). Hydrogenation was carried out for 3 hours. The solution was filtered over a bed of celite and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a white semi-solid (4.3 g, 95 %), $[\alpha]_D^{28}$ -3.2 (c = 1.64, $CHCl_3$) {Found *m/z* [M + Na]$^+$: 805.6534 $C_{41}H_{82}O_5SiK$ requires: 805.6507}. This showed δ_H (500 MHz, $CDCl_3$): 4.03 (2H, t, J = 6.95 Hz), 3.90 (1H, dt, J = 6.3, 4.4 Hz), 3.64 (3H, s), 2.51 (1H, ddd, J = 10.7, 6.9, 3.45 Hz), 1.60 (2H, q, J = 6.6 Hz), 1.24 (46H, s), 1.18 (9H, s), 0.88 (3H, t, J = 6.6 Hz), 0.85 (9H, s), 0.03 (3H, s), 0.01 (3H, s); δ_C: 178.51, 175.02, 64.38, 60.29, 51.53, 51.13, 38.66, 33.64, 31.90, 29.79, 29.67, 29.63, 29.55, 29.51, 29.46, 29.42, 29.34, 29.20, 28.59, 27.80, 27.45, 27.16, 25.89, 25.79, 25.71, 23.70, 22.65, 20.95, 14.15, -4.41, -4.97; ν_{max}/cm^{-1}: 2925, 2854, 1732, 1463, 1362, 1284, 1254, 1156, 1078, 1005, 836, 775.

Experiment 79: (*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-11-hydroxyundecyl]-hexacosanoic acid methyl ester (409)



(*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-11-(2,2-dimethylpropionyloxy)-undecyl]-hexa-cosanoic acid methyl ester (**408**) (4.24 g, 5.33 mmol) was added to a stirred solution of potassium hydroxide (4.48 g, 79.96 mmol) dissolved in a mixture of THF:MeOH:H_2O (10:10:1, 150 ml). The mixture was refluxed at 70 °C and monitored by TLC, after 3 hours, the TLC showed no starting material was left and the reaction was quenched with water and extracted with ethyl acetate (3x300 ml), dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (5:2) to give a semi-solid compound, (*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilanyloxy)-11-hydroxy-undecyl]-hexacosanoic acid methyl ester (**409**) (3.18 g, 4.47 mmol, 83 %), $[\alpha]_D^{24}$ - 4.46 (c = 1.06, $CHCl_3$) {Found $[M + H]^+$: (710.6684, $C_{44}H_{89}O_4Si$ requires: 710.6608}. This showed δ_H (500 MHz, $CDCl_3$): 3.91(1H, dt, J = 9.8, 4.4 Hz), 3.65 (3H, s), 3.63 (2H, t, J = 7.9 Hz), 2.52 (1H, ddd, J = 10.75, 7.25, 3.8 Hz), 1.8-1.54 (8H, m), 1.25 (57H, s), 0.89 (3H, t, J = 10.75 Hz), 0.86 (9H, s), 0.04 (3H, s), 0.02 (3H, s); δ_C: 175.11, 63.03, 51.57, 51.20, 33.65, 32.82, 31.91, 29.79, 29.69, 29.64, 29.56, 29.53, 29.49, 29.43, 29.41, 29.34, 27.82, 27.46, 25.74, 23.70, 22.67, 17.95, 14.09, -4.39, -4.94; ν_{max}/cm^{-1}: 3368, 2925, 2853, 1741, 1464, 1361, 1195, 1166, 1072, 938, 836, 720.

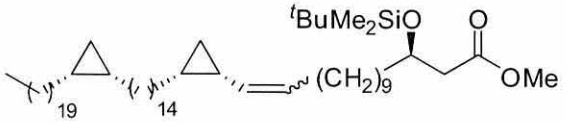
Experiment 80: (*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-11-oxoundecyl] hexa-cosanoic acid methyl ester (410)



(*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-11-hydroxyundecyl]-hexacosanoic acid methyl ester (**409**) (2.59 g, 3.64 mmol) in dichloromethane (20 ml) was added solution of PCC (1.96 g, 0.91 mmol) in dichloromethane (40 ml). During the addition a black colour appeared. The mixture was stirred at room temperature for 3 hours, when TLC showed the reaction was complete, then poured into petrol/ethyl acetate (5:1, 30

ml), and filtered through a bed of silica gel. The solvent was evaporated and the crude product was purified by column chromatography eluting with petrol/ether (5:1) to give the product as colourless oil (2.34 g, 90 %), $[\alpha]_D^{28}$ -3.85 (c =1.13, $CHCl_3$) {Found *m/z* $[M + Na]^+$: 731.63; $C_{44}H_{88}O_4SiNa$ requires: 731.63}. This showed δ_H (500 MHz, $CDCl_3$): 9.74 (1H, br, t, 1.9 Hz), 3.92-3.89 (1H, m), 3.64 (3H, s), 2.52-2.49 (2H, m), 2.42 (2H, dt, J = 1.59, 7.35 Hz), 2.36 (2H, t, J = 7.2 Hz), 1.45-1.35 (59H, br s), 0.87 (3H, t, J = 6.95 Hz), 0.85 (9H, s), 0.04 (3H, s), 0.02 (3H, s); δ_C: 202.85, 175.12, 73.16, 51.59, 51.24, 43.87, 33.94, 31.92, 29.76, 29.68, 29.55, 29.48, 29.45, 29.36, 29.32, 29.17, 29.16, 27.83, 27.47, 25.74, 24.67, 23.72, 22.68, 22.09, 17.96, 14.02, -4.38, -4.96; ν_{max}/cm^{-1}: 2925, 2857, 1742, 1464.

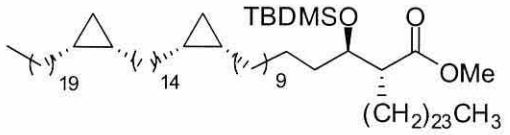
Experiment 81: (*R*)-2-((*E*)-(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-12-{(1*S*,2*R*)-2-[14-((1*S*,2*R*)-2-eicosylcyclopropyl)-tetradecyl]-cyclopropyl}-dodec-11-enyl)-hexacosanoic acid methyl ester (412)



Lithium bis(trimethyl silyl)amide (4.6 ml, 4.87 mmol) was added to a stirred solution of (*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilanyloxy)-11-oxoundecyl] hexacosanoic acid methyl ester (**410**) (2.22 g, 3.13mmol) and 5-(((*1S, 2R*)-2-(14-((*1S, 2R*)-2-eicosylcyclopropyl) tetradecyl) cyclo-propyl) methylsulfonyl)-1-phenyl-1H-tetrazole (**411**) (2.93 g, 3.75 mmol) in dry THF (80 ml) at -10 °C. The reaction turned bright yellow and was left to reach room temperature and stirred for one hour under a nitrogen atmosphere, when TLC showed no starting material was left, then quenched by addition of sat. aq. NH_4Cl. The product was extracted with petrol/ethyl acetate (10/1, 3x150 ml), dried over $MgSO_4$, filtered and evaporated. The crude prodect was purified by column chromotography over silica gel, eluting solvent with petrol/ethyl acetate (20/1) to obtain the title compound (3.08 g, 2.43 mmol, 80 %). This showed ^{1}HNMR (500MHz, TMS, $CDCl_3$): 5.54-5.38 (1H, m), 5.20-5.01 (1H, m), 3.86 (1H, dt, J = 6.95, 4.75 Hz), 3.61 (3H, s), 2.53 (1H, ddd, J = 10.7, 6.95, 3.8 Hz), 2.15 (1H, q, J = 6.95 Hz), 2.00 (1H, q, J = 6.9 Hz), 1.55-1.51 (6H, br m), 1.26 (122H, br s), 0.89 (6H, t, J = 6.6 Hz), 0.87 (9H, s), 0.66-0.64 (4H, m), 0.56 (2H, dt, J = 7.9, 3.8 Hz), 0.05 (3H, s), 0.02 (3H, s), -0.32 (2H, q, J = 5.35); δ_C: 130.45, 129.51, 73.23, 51.60, 51.19,

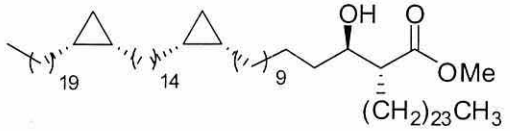
33.71, 31.92, 30.22, 29.85, 29.80, 29.70, 29.65, 29.58, 29.45, 29.35, 28.72, 27.84, 27.50, 25.76, 23.76, 22.68, 18.45, 18.27, 17.97, 15.78, 14.10, 10.91, -4.37, -4.92; ν_{max}/cm^{-1}: 2920, 2851, 1736, 1637, 1558, 1493, 1451, 1403, 1050, 874.

Experimnet 82: (*R*)-2-((*R*)-1-(*tert*-Butyldimethylsilanyloxy)-12-{(1*S*,2*R*)-2-[14-((1*S*,2*R*)-2-eicosyl-cyclopropyl)-tetradecyl]-cyclopropyl}-dodecyl)-hexacosanoic acid methyl ester (413)



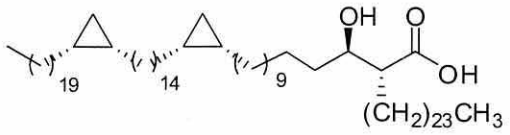
Dipotassium azodicarboxylate (6.0 g, 30.92 mmol) was added to a stirred solution of (*R*)-2-((*E*)-(*R*)-1-(*tert*-butyldimethylsilanyloxy)-12-{(1*S*,2*R*)-2-[14-((1*S*,2*R*)-2-eicosyl-cyclo-propyl)-tetradecyl]-cyclopropyl}-dodec-11-enyl)-hexacosanoic acid methyl ester (**412**) (3.08 g, 2.37 mmol) in THF (100 ml) and methanol (10 ml) at 5 °C. A solution of glacial acetic acid (5 ml) and THF (5 ml) was prepared and (0.5 ml) was added dropwise every 25 min at 5 °C and the reaction mixture was stirred at room temperature. After two days the reaction mixture was added slowly in potions to a sat. aq. $NaHCO_3$ and extracted with petrol/ethyl acetate (5:1, 3x100 ml,) and the combined organic layers were washed with brine (50 ml) and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give the title compound a white solid, $[\alpha]_D^{24}$ –4.43 (c = 1.24 in $CHCl_3$) {Found *m/z* $[M + Na]^+$: 1288.2854, $C_{44}H_{88}O_4SiNa$ requires: 1288.2660 (MALDI)}. This showed δ_H (500 MHz, $CDCl_3$): 3.91 (1H, dt, J=7.25, 4.7 Hz), 3.66 (3H, s), 2.53 (1H, ddd, J = 11.05, 7.25, 3.8 Hz), 1.38-1.35 (15H, m), 1.26 (134H, br s), 1.15-1.12 (5H, m), 0.89 (6H, t, J = 6.9 Hz), 0.87 (9H, s), 0.67-0.64 (4H, m), 0.58 (2H, dt, J = 3.75, 7.85 Hz), 0.05 (3H, s), 0.02 (3H, s), -0.32 (2H, q, J = 5.35); δ_C: 175.12, 51.58, 51.20, 33.69, 31.92, 30.22, br 29.83, 29.70, 29.66, 29.61, 29.58, 29.57, 29.44, 29.36, 28.72, 27.83, 27.50, 25.76, 22.68, 17.97, 15.78, 14.19, 10.91, -4.37, -4.92; ν_{max}/cm^{-1}: 2922, 2852, 1743, 1464, 1361, 1254, 1193, 1166, 1074, 1021, 836, 775, 720.

Experiment 83: (*R*)-2-((*R*)-1-Hydroxy-12-{(1*S*,2*R*)-2-[14-((1*S*,2*R*)-2-eicosylcyclo-propyl)-tetradecyl]-cyclopropyl}-dodecyl)-hexacosanoic acid methyl ester (414)



(*R*)-2-((*R*)-1-(*tert*-Butyldimethylsilanyloxy)-12-{(1*S*,2*R*)-2-[14-((1*S*,2*R*)-2-eicosyl-cyclopropyl)-tetradecyl]-cyclopropyl}-dodecyl)-hexacosanoic acid methyl ester (**413**) (3.08 g, 2.43 mmol) was stirred in dry THF (25 ml) in a dry polyethylene vial under a nitrogen atmosphere at room temperature. Pyridine (1 ml) and HF. pyridine complex (4.5 ml) were added and the mixture was stirred for 18 hours at 40 °C. The reaction mixture was diluted with petrol/ ethyl acetate (1:1, 10 ml) and neutralized with sat.aq. $NaHCO_3$. The mixture was separated and the aqueous layer was re-extracted with petrol/ethyl acetate (1:1, 2x 20 ml). The combined organic layers were washed with brine, dried and the solvent was evaporated. The crude product purified with column chromatography eluting with petrol/ethyl acetate (10:1) gave (*R*)-2-((*R*)-1-hydroxy-12-{(1*S*,2*R*)-2-[14-((1*S*,2*R*)-2-eicosylcyclopropyl)-tetradecyl]-cyclopropyl}-dodecyl)-hexacosanoic acid methyl ester (**414**) as a white solid (2.01 g, 83 %), m.p: 46 - 48 °C, $[\alpha]_D^{20}$ +2.265 (c = 1.8, $CHCl_3$) {Found *m/z* $[M + Na]^+$: 1174.98; $C_{79}H_{154}O_3Na$ requires: 1175.08}, This showed δ_H (500 MHz, $CDCl_3$): 3.71 (3H, s), 3.67 (1H, ddd, J = 10.4, 5.7, 2.55 Hz), 2.43 (1H, dt, J = 5.35, 9.45 Hz), 1.74-1.67 (1H, m), 1.26 (134H, s), 0.88 (6H, t, J = 6.95 Hz), 0.67-0.64 (4H, m), 0.57 (2H, ddd, 12.25, 8.2, 4.1Hz), -0.32 (2H, q, J = 5.4 Hz); δ_C: 176.22, 72.31, 51.49, 50.94, 35.71, 31.92, 30.22, 29.70, 29.66, 29.63, 29.61, br 29.56, 29.50, 29.42, 29.36, 28.72, 27.42, 25.73, 22.68, 15.78, 14.10, 10.91; ν_{max}/cm^{-1}: 3521, 2917, 2850, 1709, 1465, 1366, 1195, 1165, 1137, 1062, 1018, 720.

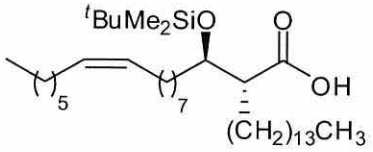
Experiment 84: (*R*)-2-((*R*)-1-Hydroxy-12-{(1*S*,2*R*)-2-[14-((1*S*,2*R*)-2-eicosyl-cyclo-propyl)-tetradecyl]-cyclopropyl}-dodecyl)-hexacosanoic acid (213)



Lithium hydroxide monohydrate (1.06 g, 25.25 mmol) was added to a stirred solution of methyl ester (**414**) (1.94 g, 1.68 mmol) in THF (20 ml), methanol (1.5 ml) and water (1 ml) at room temperature. The mixture was stirred at 40 °C for 18 hours,

when TLC showed no starting material was left. The reaction mixture was cooled down to room temperature and diluted with petrol/ethyl acetate (5:2, 50 ml) and acidified to pH = 1 with 5 % HCl. The product was extracted with petrol/ethyl acetate (5:2, 3 x 150 ml), and the combined organic layers were dried over $MgSO_4$ and evaporated to give a crude product which was purified by column chromatography eluting with petrol/ethyl acetate (5:2) to give the title compound (**213**) (95 %), $[\alpha]_D^{24}$ -4.43 (c = 1.24, $CHCl_3$) {Found *m/z* $[M + Na]^+$: 1160.1612, $C_{78}H_{152}O_3Na$ requires: 1160.1741}. This showed δ_H (500 MHz, $CDCl_3$): 3.75-3.71 (1H, m), 2.48 (1H, dt, J = 10.1, 5.4 Hz), 1.64-1.49 (16H, m), 1.26 (120H, br s), 0.88 (6H, t, J = 6.65 Hz), 0.67-0.64 (4H, m), 0.57 (2H, dt, J = 8.2, 4.1 Hz), -0.32 (2H, q, J = 5.05 Hz); δ_C: 177.05, 72.17, 49.93, 35.56, 31.94, 30.24, 29.72, 29.59, 29.51, 29.43, 29.37, 28.74, 27.33, 25.73, 22.70, 15.79, 14.14, 10.94; ν_{max}/cm^{-1}:3517, 3288, 2917, 2850, 2332, 1710, 1620, 1559, 1470, 1366, 1260, 1234, 1196, 1166, 1137, 1063, 960, 889, 844, 775.

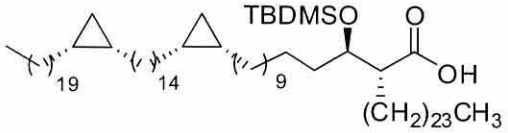
Experiment 85: (*Z*)-(*2R,3R*)-3-(*tert*-Butyldimethylsilanyloxy)-2-tetradecyl-nonadec-11-enoic acid (417)



Imidazole (0.28 g, 4.14 mmol) was added to a stirred solution of (*Z*)-(*2R,3R*)-3-(hydroxy)-2-tetradecyl-nonadec-11-enoic acid methyl ester (**207**) (0.21 g, 0.414 mmol) in dry DMF (3 ml) and dry toluene (4 ml) at room temperature followed by the addition of *tert*-butyldimethylsilylchloride (0.93 g, 6.22 mmol) and 4-dimethylaminopyridine (0.05 g, 0.414 mmol). The reaction mixture was stirred at 70 °C for 24 hours. When TLC showed that no starting material was left, the solvent was removed under high vacuum and the residue was diluted with petrol/ethyl acetate 10:1 (50 ml) and water (10 ml). The organic layer was separated and the aqueous layer was re-extracted with petrol/ethyl acetate (2x30 ml). The combined organic layers were washed with water, dried and evaporated to give a colourless oil residue. The residue was dissolved in THF (10 ml), water (2 ml), and methanol (2 ml), and to this was added potassium carbonate (0.20 g, 0.0014 mmol). The reaction mixture was stirred at 45 °C for 18 hours, and then TLC showed no starting material was left. The

mixture was diluted with petrol/ethyl acetate 10:1, (20 ml) and water (2 ml) then acidified with potassium hydrogen sulfate to pH 2. The organic layer was separated and the aqueous layer was re-extracted with petrol/ethyl acetate (10:1, 2x20 ml). The combined organic layers were washed with water, dried and evaporated to give a residue, which was purified by column chromatography eluting with petrol/ethyl acetate 10:1 to give the title compound as colourless oil (0.200 g, 90 %), $[\alpha]_D^{22}$ 6.55 (c = 0.61, $CHCl_3$) {Found *m/z* $[M + Na]^+$: 631.6075, $C_{38}H_{76}NaO_3Si$ requires: 631.5461}. This showed δ_H (500MHz, $CDCl_3$): 5.35 (2H, t, J = 5.65 Hz), 3.86 (1H, dt, J = 9.8, 5.95 Hz), 2.53 (1H, dt, J = 9.7, 4.7 Hz), 2.01 (4H, q, J = 7.14 Hz), 1.63-1.59 (1H, m), 1.54-1.51 (2H, m), 1.45-1.43 (1H, m), 1.25 (43H, br s), 0.90 (9H, s), 0.89 (6H, t, J = 7.5 Hz), 0.11 (3H, s), 0.09 (3H, s); δ_C: 177.70, 129.96, 129.77, 73.53, 50.75, 31.90, 29.73, 29.71, 29.69, 29.65, 29.64, 29.56, 29.42, 29.36, 28.98, 25.73, 5.68, 25.63, 24.45, 22.68, 22.65, 18.11, 17.95, 14.10, -4.32, -4.95; ν_{max}/cm^{-1}: 3434, 2926, 2855, 1708, 1638, 1463, 1361, 1254, 1070, 836, 775.

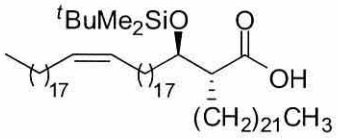
Experiment 86: (*R*)-2-((*R*)-1-(*tert*-Butyldimethylsilanyloxy)-12-{(1*S*,2*R*)-2-[14-((1*S*,2*R*)-2-eicosyl-cyclopropyl)-tetradecyl]-cyclopropyl}-dodecyl)-hexacosanoic acid (418)



Imidazole (0.73 g, 10.8 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-eicosylcyclopropyl)-tetradecyl]-cyclo-propyl}dodecyl)-hexa-cosanoic acid (**213**) (1.23 g, 1.08 mmol) in dry DMF (10 ml) and dry toluene (13 ml) at room temperature followed by the addition of *tert*-butyldimethylsilylchloride (2.44 g, 16.2 mmol) and 4-dimethylaminopyridine (0.13 g, 10.8 mmol). The reaction mixture was stirred at 70 °C for 24 hours. When TLC showed that no starting material was left, the solvent was removed under reduced vacuum and the residue was diluted with petrol/ethyl acetate 10:1 (50 ml) and water (25 ml). The organic layer was separated and the aqueous layer was re-extracted with petrol/ethyl acetate (5:1, 2x40 ml). The combined organic layers were washed with water, dried and evaporated to give a colourless oil residue. The residue was dissolved in THF (20 ml), water (2 ml), and methanol (2 ml), to this was added

potassium carbonate (0.40 g, 2.89 mmol). The reaction mixture was stirred at 45 °C overnight, and then TLC showed no starting material was left. The mixture was diluted with petrol/ethyl acetate (10:1, 20 ml) and water (2 ml) then acidified with $KHSO_3$ to pH 2. The organic layer was separated and the aqueous layer was re-extracted with petrol/ethyl acetate (5/1, 2x50 ml). The combined organic layers were washed with water, dried and evaporated to give a residue, which was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give the title compound as colourless oil (1.16 g, 86 %), $[\alpha]_D^{24}$ +1.64 (c = 1.76, $CHCl_3$) {Found *m/z* $[M + Na]^+$: 1275.3342; $C_{84}H_{166}O_3Si_1Na$ requires: 1275.3243}. This showed δ_H (500 MHz, $CDCl_3$): 3.83 (1H, ddd, J = 7.9, 5.05, 2.55 Hz), 2.53 (1H, ddd, J = 9.15, 6.35, 2.85 Hz), 1.75-1.48 (12H, m), 1.26 (129H, br s), 0.93 (9H, s), 0.89 (6H, t, 6.65 Hz), 0.67- 0.64 (4H, m), 0.57 (2H, dt, J = 8.2, 4.1 Hz), 0.15 (3H, s), 0.14 (3H, s), -0.31(2H, q, J = 5.4 Hz); δ_C: 173.44, 50.03, 35.87, 31.92, 30.22, 29.69, 29.65, 29.62, 29.58, 29.54, 29.46, 29.39, 29.35, 28.72, 27.41, 25.72, 25.18, 22.67, 17.93, 15.79, 14.09, 10.92, -4.23, -4.85; ν_{max}/cm^{-1}: 2924, 2853, 1709, 1464, 1255, 1073, 835, 775, 720.

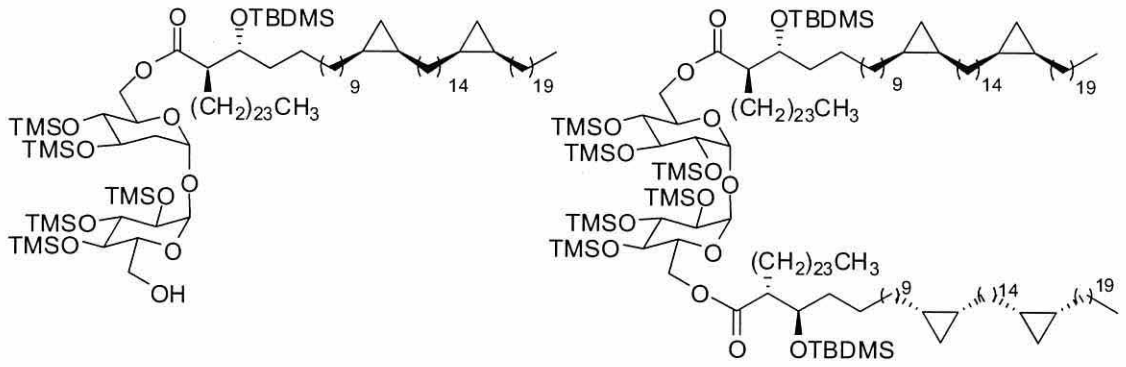
Experiment 87: (*2R, Z*)-2-Docosyl-3-(*R*)-3-(*tert*-butyldimethylsilanyloxy)-21-enoic acid (419)



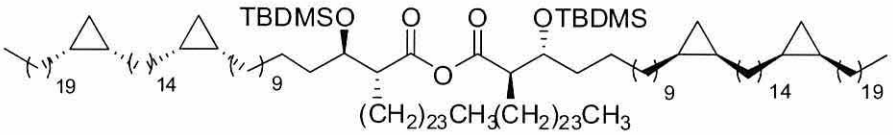
Imidazole (0.1 g, 1.56 mmol) was added to a stirred solution of (*2R, 3R, Z*)-2-docosyl-3-hydroxytetracont-21-enoic acid (**208**) (0.14 g, 0.15 mmol) in dry DMF (3 ml) and dry toluene (4 ml) at room temperature and followed by the addition of *tert*-butyl-dimethylsilylchloride (0.35 g, 2.3 mmol) and 4-dimethylaminopyridine (0.01 g, 0.15 mmol). The reaction mixture was stirred at 70 °C for 24 hours and at room temperature for another 18 hours, when TLC showed that no starting material was left, the solvent was removed under high vacuum and the residue was diluted with petrol/ethyl acetate 10:1 (50 ml) and water (10 ml). The organic layer was separated and the aqueous layer was re-extracted with petrol/ethyl acetate (10:1, 2x30 ml). The combined organic layers were washed with water, dried and evaporated to give a colourless oil residue. The residue was dissolved in THF (10 ml), water (2 ml), and methanol (2 ml), and to this was added potassium carbonate (0.20 g). The reaction

mixture was stirred at 45 °C for 18 hours, and then TLC showed no starting material was left. The mixture was diluted with petrol/ethyl acetate 10:1, (20 ml) and water (2 ml) then acidified with potassium hydrogen sulfate to pH 2. The organic layer was separated and the aqueous layer was re-extracted with petrol/ethyl acetate (10:1, 2x20 ml). The combined organic layers were washed with water, dried and evaporated to give a residue, which was purified by column chromatography eluting with petrol/ethyl acetate 10:1 to give the title compound as colourless oil (**419**) (0.200 g, 85 %), $[\alpha]_D^{22}$ 6.55 (c = 0.61, $CHCl_3$) {Found *m/z* $[M + Na]^+$: 1029.9102 $C_{68}H_{136}O_3Si$; requires: 1029.8911}. This showed δ_H (500 MHz, $CDCl_3$): 5.35 (2H, br t, J = 4.7 Hz), 3.85 (1H, dt, J = 7.25, 5.05 Hz), 2.53 (1H, ddd, J = 9.15, 5.35, 3.75 Hz), 2.02 (4H, v br q, J = 6.6 Hz), 1.72-1.64 (2H, m), 1.58-1.52 (4H, m), 1.23 (101H, br s), 0.92 (9H, s), 0.87 (6H, t, J = 3.85 Hz), 0.02 (6H, br s); δ_C: 129.89, 73.64, 50.29, 41.35, 35.47, 31.93, 29.77, 29.71, 29.66, 29.63, 29.58, 29.55, 29.49, 29.44, 29.41, 29.36, 29.33, 29.31, 27.45, 27.21, 25.72, 25.68, 25.64, 24.91, 22.69, 22.61, 18.11, 17.93, 14.11, -4.26, -4.90; ν_{max}/cm^{-1}: 3433, 2919, 2851, 1706, 1638, 1467, 1254, 1070, 835, 760, 720.

Experiment 88: 6,6'-Bis-O-(*R*)-2-((*R*)-1-(*tert*-Butyldimethylsilanyloxy)-12-{(1*S*,2*R*)-2-[14-((*1S,2R*)-2-eicosylcyclopropyl)-tetradecyl]-cyclopropyl}-dodecyl)-hexacosanoic-2,3,4,2',3',4'-hexakis-O-(trimethylsilyl)-α,α'-trehalose (421) and 6-O-(*R*)-2-((*R*)-1-(*tert*-butyldimethylsilanyloxy)-12-{(1*R*,2*S*)-2-[14-((1*R*,2*S*)-2-eicosylcyclopropyl)-tetradecyl]-cyclopropyl}-dodecyl)-hexacosanoic-2,3,4,2',3',4'-hexakis-O-(trimethylsilyl)-α,α'-trehalose (422)



1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (100 mg, 0.51 mmol) and 4-dimethylaminopyridine (55 mg, 0.45 mmol) were added to a stirred solution of (*R*)-2-((*R*)-1-(*tert*-butyldimethylsilanyloxy)-12-{(1*S*,2*R*)-2-[14-((1*S*,2*R*)-2-eicosylcyclo-propyl)-tetradecyl]-cyclopropyl}dodecyl)-hexacosanoic acid (**418**) (0.202 g, 0.16 mmol), [6-(6-hydroxymethyl-3,4,5-tris-trimethylsilanyl-oxytetrahydro-pyran-2-yloxy)-3,4,5-tris-trimethyl-silanyloxytetrahydropyran-2-yl]methanol **(180)** (50 mg, 0.064 mmol) and powdered 4 A° molecular sieves in dry dichloromethane (4 ml) at room temperature under a nitrogen atmosphere. The mixture was stirred for 6 days at room temperature, when TLC showed no starting material was left then diluted with dichloromethane (5 ml) and filtered. The filtrate was evaporated under reduced pressure to give a residue, which was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a first fraction (**(a)** (*R*)-2-((*R*)-1-(*tert*-butyl-dimethylsilanyloxy)-12-{(1*S*,2*R*)-2-[14-((1*S*,2*R*)-2-eicosylcyclopropyl)tetradecyl]-cyclopropyl}-dodecyl)-hexacosanoic anhydride (**420**) (0.036 g), a second fraction **(b)** (0.094 g) as a colourless thick oil and a third fraction **(c)** compound (0.068 g, 70 %), and a third fraction (**c**) (0.05 g) as colourless thick oil.



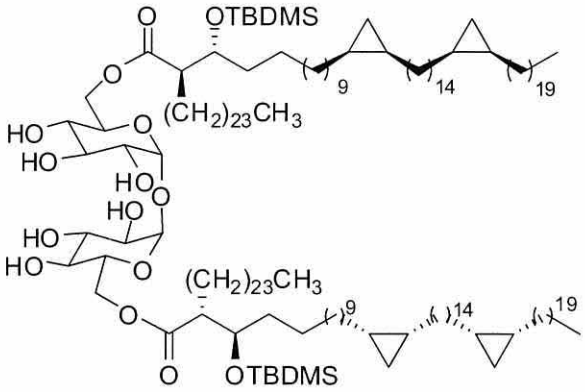
Compound (**420**) {Found *m/z* $[M + Na]^+$: 2509.59, $C_{168}H_{330}O_5Si_2Na$ requires: 2509.64}. This showed δ_H (500 MHz, $CDCl_3$): δ 3.92 (2H, dt, J = 11.35, 5.7Hz), 2.60 (2H, dt, 11.05, 5.05 Hz), 1.38–1.33 (30H, br m), 1.17 (238H, v br s), 0.89 (12H, t, J = 3.75 Hz), 0.87 (18H, s), 0.66-0.64 (8H, m), 0.58 (4H, dt, J = 7.85, 4.1 Hz), 0.08 (6H, s), 0.07 (3H, s), 0.05 (3H, s), -0.31 (4H, q, J = 5.4 Hz); ν_{max}/cm^{-1}: 2963, 2856, 1815, 1745, 1466, 1369, 1254, 1093, 902, 831, 774, 732.

Compound **(b),** 6,6'-Bis-O-(*R*)-2-((*R*)-1-(*tert*-Butyl-dimethyl-silanyloxy)-12-{(1*S*,2*R*)-2-[14-((*1S,2R*)-2-eicosylcyclopropyl)-tetradecyl]-cyclopropyl}-dodecyl)-hexacosanoic-2,3,4,2',3',4'-hexakis-O-(trimethylsilyl)-α,α'-trehalose, $[\alpha]_D^{24}$ +15.68 (c = 0.51, $CHCl_3$) {Found *m/z* $[M + Na]^+$: 3266.32, $C_{198}H_{398}O_{15}Si_8Na$ requires: 3266.93}. This showed δ_H (500 MHz, $CDCl_3$): 4.86 (2H, d, J = 2.85 Hz), 4.37 (2H, d, J = 10.4 Hz), 4.04 (2H, d, J = 12.6 Hz), 3.95 (4H, br q, J = 5.05 Hz), 3.90 (2H, t, J = 8.8 Hz), 3.52 (2H, t, J = 9.15 Hz), 3.39 (2H, dd, J = 9.5, 2.85 Hz), 2.55 (2H, dt, J =

9.8, 4.75 Hz), 1.54-1.13 (268H, m), 0.89 (12H, t, J = 4.75 Hz), 0.88 (18H, s), 0.60-0.56 (8H, m), 0.57 (4H, dt, J = 8.5, 3.8 Hz), 0.166 (18H, s), 0.15 (18H, s), 0.14 (18H, s), 0.07 (12H, s), -0.31 (4H, q, J = 5.35 Hz); δ_C: 173.84, 94.83, 73.55, 73.18, 72.82, 71.83, 70.73, 67.70, 64.69, 62.39, 51.85, 42.97, 33.46, 31.94, 30.24, 30.04, 29.81, 29.75, 29.72, 29.68, 29.53, 29.39, 28.74, 25.98, 25.93, 25.89, 25.84, 22.70, 18.03, 15.78, 14.12, 10.92, 1.10, 0.95, 0.16, -4.43, -4.50, -4.64; ν_{max}/cm^{-1}: 2922, 2851, 1741, 1460, 1250, 1164, 1110, 1075, 1006, 897, 872, 841, 747.

Compound **(c),** 6-O-(*R*)-2-((*R*)-1-(*tert*-Butyl-dimethyl-silanyloxy)-12-{(1*R*,2*S*)-2-[14-((1*R*,2*S*)-2-eicosylcyclopropyl)-tetradecyl]-cyclopropyl}-dodecyl)-hexacosanoic-2,3,4,2',3',4'-hexakis-O-(trimethylsilyl)-α,α'-trehalose, $[\alpha]_D^{24}$ +48.79 (c = 0.58, $CHCl_3$) {Found *m/z* $[M + Na]^+$: 2032.137, $C_{114}H_{234}O_{13}Si_7Na$ requires: 2032.66}. This showed δ_H (500 MHz, $CDCl_3$): 4.91 (1H, d, J = 3.15 Hz), 4.85 (1H, d, J = 3.15 Hz), 4.35 (1H, dd, J = 2.2, 11.65 Hz), 4.07 (1H, dd, J = 4.1, 11.95 Hz), 3.99 (1H, dt, J = 6, 2.2 Hz), 3.96-3.94 (1H, m), 3.91 (2H, dt, J = 2.2, 6.6 Hz), 3.84 (1H, dt, J = 6.6, 3.45 Hz), 3.72-3.67 (2H, m), 3.49 (2H, dt, J = 9.15, 5.7 Hz), 3.42 (1H, dd, J = 3.15, 9.45 Hz), 3.39 (1H, dd, J = 3.15, 9.45 Hz), 2.55 (1H, ddd, J = 9.45, 5.56, 3.45 Hz), 1.62-160 (4H, m), 1.38-1.14 (137H, v br s), 0.88 (6H, t, J = 6.95 Hz), 0.88 (9H ,s), 0.67-0.64 (4H, m), 0.57 (2H, dt, J = 4.1, 8.2Hz), 0.17 (9H, s), 0.16 (9H, s), 0.156 (9H, s), 0.151 (9H, s), 0.150 (9H, s), 0.12 (9H, s), 0.06(3H, s) , 0.05(3H, s), -0.32 (2H, q, J = 5 Hz); δ_C: 174.08, 94.50, 94.38, 73.43, 73.35, 72.87, 72.81, 72.75, 71.98, 71.41, 70.74, 62.45, 61.66, 51.82, 33.42, 31.92, 30.22, 29.82, 29.78, 29.70, 29.66, 29.54, 29.36, 28.72, 28.10, 26.41, 25.82, 24.85, 22.69, 18.01, 15.77, 14.11, 10.91, 1.05, 1.00, 0.92, 0.84, 0.17, 0.04, -4.48, -4.69; ν_{max}/cm^{-1}: 3056, 2922, 2851, 1741, 1459, 1379, 1250, 1164, 1075, 1005, 964, 841, 747, 719.

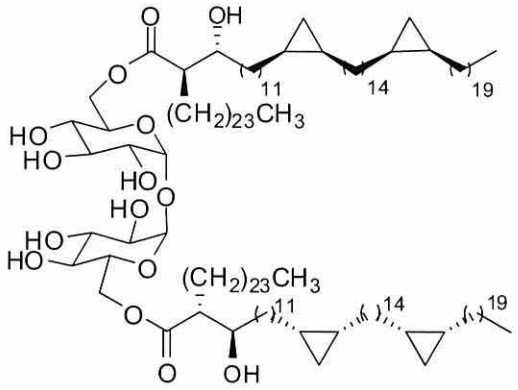
Experiment 89: 6,6'-Bis-O-(*R*)-2-((*R*)-1-(*tert*-Butyldimethylsilanyloxy)-12-{(1*S*,2*R*)-2-[14-((1*S*,2*R*)-2-eicosylcyclopropyl)-tetradecyl]-cyclopropyl}-dodecyl)-hexacosanoic-α,α'-trehalose (423)



Tetrabutylammonium fluoride (0.2 ml, 0.2 mmol) was added to a stirred solution of 6,6'-bis-O-(*R*)-2-((*R*)-1-(*tert*-butyl-dimethyl-silanyloxy)-12-{(1*S*,2*R*)-2-[14-((1S,2R)-2-eicosyl-cyclo-propyl)-tetradecyl]-cyclopropyl}-dodecyl)-hexacosanoic-2,3,4,2',3',4',-hexakis-O-(tri-methylsilyl)-α,α'-trehalose (**421**) (0.11 g, 0.035 mmol) in dry THF (5 ml) at 5 °C under nitrogen atmosphere. The mixture was allowed to reach room temperature and stirred for 1 hour when TLC showed no starting material was left. The reaction was cooled to 5 °C and quenched with sat.aq. sodium bicarbonate (3 ml) then diluted with $CHCl_3$ (50 ml). The organic layer was separated and the aqueous layer was re-extracted with $CHCl_3$ (2 x 50 ml). The combined organic layers were washed with brine (50 ml), dried and evaporated to give a residue, which was purified by column chromatography eluting with $CHCl_3$/MeOH 0.85:0.15 to give the title compoumd (0.051 g, 53 %) as a colourless thick oil, $[\alpha]_D^{26}$ +15.49 (c = 3.2 g, $CHCl_3$) {Found *m/z* $[M + Na]^+$: 2833.8709, $C_{180}H_{350}O_{15}Si_2Na$ requires: 2833.8628}. This showed δ_H (500 MHz, $CDCl_3$+ few drops of CD_3OD): 4.99 (2H, d, J = 3.45 Hz), 4.22 (2H, br dd, J = 13.2, 3.5 Hz), 4.17 (2H, d, J = 11 Hz), 3.88 (2H, br d, J = 9.45 Hz), 3.82 (2H, br q, J = 5.4 Hz), 3.71 (2H, d, J = 6.3 Hz), 3.39 (2H, dd, J = 9.45, 3.45 Hz), 3.25 (2H, d, J = 9.15 Hz), 2.46 (2H, ddd, J = 10.1, 6.35, 3.5 Hz), 1.16 (274 H, br s), 0.79 (12H, t, J = 4.1 Hz), 0.77 (18H, s), 0.56-0.55 (8H, m), 0.47 (4H, dt, J = 8.2, 4.1 Hz), -0.04 (6H, s), -0.06 (6H, s), -0.41 (4H, q, J = 5.05 Hz); δ_C: 175.08, 93.37, 73.10, 70.18, 69.79, 62.82, 51.53, 49.30, 49.12, 48.95, 48.61, 48.44, 33.42, 31.73, 30.05, 30.01, 29.50, 29.39, 29.16, 28.52, 27.58, 25.63, 25.53, 22.48, 17.75,

15.57, 13.83, 10.68, 10.66, -4.72, -5.14; ν_{max}/cm^{-1}: 3381, 2927, 2857, 2366, 1742, 1468, 1257, 1076, 833, 772, 722.

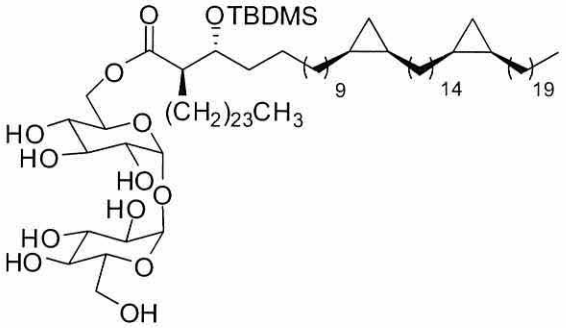
Experiment 90: 6,6'-Bis-O-(*R*)-2-((*R*)-1-hydroxy-12-{(1*S*,2*R*)-2-[14-((1*S*,2*R*)-2-eicosyl-cyclopropyl)-tetradecyl]-cyclopropyl}-dodecyl)-hexacosanoic-α,α'-trehalose (214)



A dry polyethylene vial equipped with an acid proof rubber septum was charged with 6,6'-Bis-O-(*R*)-2-((*R*)-1-(*tert*-Butyldimethylsilanyloxy)-12-{(1*S*,2*R*)-2-[14-((1*S*,2*R*)-2-eicosyl-cyclopropyl)-tetradecyl]-cyclopropyl}-dodecyl)-hexacosanoic-α,α'-trehalose (**424**) (0.1 g, 0.27 mmol) and pyridine (0.1 ml) in dry THF (10 ml) and stirred at room temperture under a nitrogen atmosphere. Hydrogen fluoride-pyridine complex (~70 % hydrogen fluoride, 0.4ml) at 5 °C was added. The mixture was stirred at 43 °C for 17 hours, when TLC showed no starting material was left, and then neutralized by pouring slowly into sat. aq. sodium bicarbonate until no more CO_2 was liberated. The product was extracted with chloroform (3x50 ml), then the combined organic layers were dried, evaporated to give a residue which was purified by chromatography eluting with $CHCl_3$/MeOH 10:1 to give the title compound (0.04g, 61 %) as a syrup, $[\alpha]_D^{27}$ +32.65 (c = 0.47, $CHCl_3$)} {Found *m/z* $[M + Na]^+$: 2603.0467; $C_{168}H_{322}O_{15}Na$ requires: 2603.4331}. This showed δ_H (500 MHz, $CDCl_3$ + few drops of CD_3OD): 4.88 (2H, d, J = 3.15 Hz), 4.42 (2H, br d, J = 10.75), 4.01 (2H, t, J = 8.85 Hz), 3.91 (2H, dd, J = 11.35, 6.65 Hz), 3.61 (2H, t, J = 9.15 Hz), 3.51-3.48 (2H, m), 3.33 (2H, dd, J = 9.45, 3.15 Hz), 3.12 (2H, br t, J = 9.45 Hz), 2.25 (2H, ddd, J = 12, 7.9, 4.75 Hz), 1.41-1.09 (276H, m), 0.71 (12H, t, J = 1.6 Hz), 0.49-0.47 (8H, m), 0.39 (4H, dt, J = 7.9, 4.1 Hz), -0.49 (4H, br q, J = 5.05 Hz); δ_C: 175.08, 93.37, 72.63, 73.10, 71.56, 70.18, 69.79, 62.82, 51.53, 49.30, 49.12, 48.95, 48.78, 48.61, 48.44, 48.27, 33.42, 31.73, 30.05, 30.01, 29.50, br 29.39, 29.16, 28.52, 27.58, 25.63, 25.53, 22.48, 17.75,

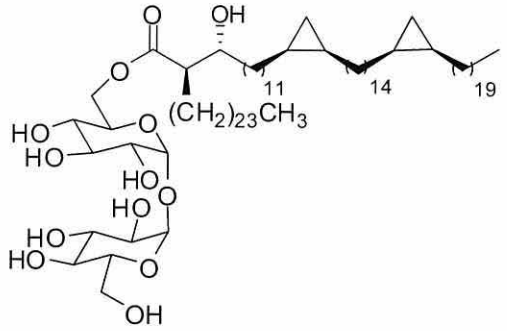
15.57, 13.83, 10.68, 10.66, -4.72, -5.14; ν_{max}/cm^{-1}: 3389, 2929, 2858, 2357, 1743, 14689, 1256, 1074, 835, 771, 720.

Experiment 91: 6-O-(*R*)-2-((*R*)-1-(*tert*-Butyldimethylsilanyloxy)-12-{(1*S*,2*R*)-2-[14-((1*S*,2*R*)-2-eicosyl-cyclopropyl)-tetradecyl]-cyclopropyl}-dodecyl)-hexacosanoic-α,α'-trehalose (424)



Tetrabutylammonium fluoride (0.5 ml, 0.502 mmol, 1M) was added to a stirred solution of 6-O-(*R*)-2-((*R*)-1-(*tert*-butyl-dimethyl-silanyloxy)-12-{(1*R*,2*S*)-2-[14-((1*R*,2*S*)-2-eicosyl-cyclopropyl)-tetradecyl]-cyclopropyl}-dodecyl)-hexacosanoic-2,3,4,2',3',4',-hexakis-O-(tri-methylsilyl)-α,α'-trehalose (**422**) (0.1264 g, 0.0628 mmol) in dry THF (3 ml) at 5 °C under a nitrogen atmosphere. The mixture was allowed to reach room temperature and stirred for 1 hour, when TLC showed no starting material was left. It was worked up as described above and purified by column chromatography eluting with $CHCl_3$/MeOH (8.5:1.5) to give the title compound (0.4 g, 40 %) as a colourless syrup, $[\alpha]_D^{26}$ +43.94 (c = 0.57, $CHCl_3$)} {[Found *m/z* {M + Na]}$^+$: 1599.30, $C_{96}H_{186}O_{13}SiNa$ requires: 1599.60}. This showed δ_H (500 MHz, $CDCl_3$): 4.93 (2H, d, J = 3.5 Hz), 4.15 (1H, d, J = 2.8 Hz), 3.81 (1H, br dt, J = 6, 2.8 Hz), 3.77 (1H, q, J = 6 Hz), 3.68 (1H, d, J = 2.85 Hz), 3.65-3.61 (3H, m), 3.55-3.50 (1H, m), 3.35 (1H, dt, J = 6.95, 3.15 Hz), 3.23-3.17 (2H, m), 2.90-2.86 (2H, m), 2.40 (1H, ddd, J = 10.4, 6.65, 3.8 Hz), 1.59-1.52 (2H, m), 1.21-0.96 (139H, m), 0.83 (3H, t, J = 7.55 Hz), 0.72 (3H, t, J = 3.75 Hz), 0.70 (9H, s), 0.51-0.49 (4H, m), 0.39 (2H, dt, J = 8.2, 4.1 Hz), -0.10 (3H, s), -0.12 (3H, s), -0.48 (2H, q, J = 5.05 Hz); δ_C: 175.02, 93.52, 73.07, 72.97, 72.64, 72.02, 71.51, 70.60, 70.12, 69.80, 62.62, 61.80, 52.15, 51.53, 49.05, 48.88, 48.71, 48.55, 48.38, 48.22, 33.38, 31.70, 29.98, 29.47, 29.13, 28.50, 27.54, 26.78, 25.49, 25.04, 23.99, 22.45, 19.77, 15.54, 13.80, 13.19, 10.64, -4.77, -5.17; ν_{max}/cm^{-1}: 3423, 2919, 2851, 1729, 1646, 1468, 1253, 1077, 991, 835.

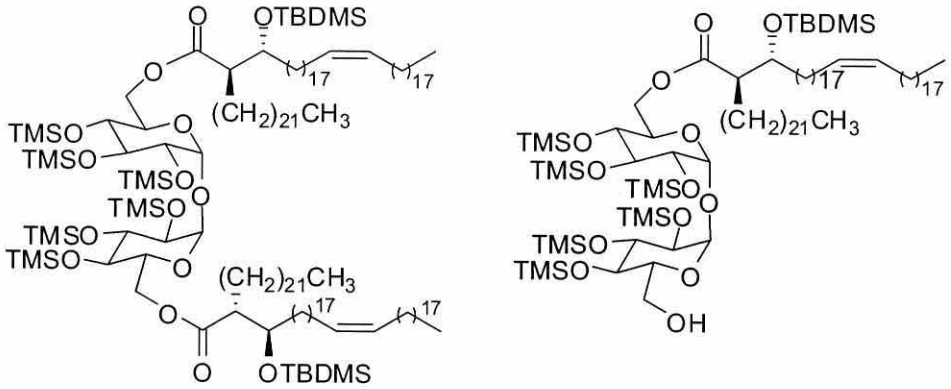
Experiment 92: 6-O-(*R*)-2-((*R*)-1-Hydroxy-12-{(1*S*,2*R*)-2-[14-((1*S*,2*R*)-2-eicosyl-cyclopropyl)-tetradecyl]-cyclopropyl}-dodecyl)-hexacosanoic-α,α'-trehalose (214)



A dry polyethylene vial equipped with a rubber septum was charged with 6-O-(*R*)-2-((*R*)-1-(*tert*-butyldimethylsilanyloxy)-12-{(1*S*,2*R*)-2-[14-((1*S*,2*R*)-2-eicosyl-cyclopropyl)-*tetra*-decyl]-cyclopropyl}-dodecyl)-hexacosanoic-α,α'-trehalose (**424**) (0.040 g, 0.022 mmol) and pyridine (0.05 ml) in dry tetrahydrofuran (4 ml) and stirred at room temperture under a nitrogen atmosphere. Hydrogen fluoride-pyridine complex (~70 % hydrogen fluoride, 0.4 ml) was added. The mixture was stirred at 43 °C for 17 hours, when TLC showed no starting material, then neutralized by pouring slowly into sat. aq. sodium bicarbonate until no more CO_2 was liberated. The product was extracted with chloroform (3 x 50 ml), then the combined organic layers were dried, evaporated to give a residue which was purified by chromatography eluting with $CHCl_3$/MeOH (10:1 then 1:1) to give crude of the title compound (0.014 g, 32 %) as a syrup. The crude product was purified by column chromatography eluting chloroform/MeOH (8.5:1.5), $[\alpha]_D^{26}$ +58.46 (c = 0.53, $CHCl_3$) {Found *m/z* $[M + Na]^+$: 1484.1478; $C_{90}H_{172}O_{13}Na$ requires: 1484.2695}. This showed δ_H (500 MHz, $CDCl_3$ + few drops of CD_3OD): 4.99 (1H, d, J = 3.45 Hz), 4.96 (1H, d, J = 3.45 Hz), 4.52 (1H, br d, J = 10.75 Hz), 4.07 (1H, t, J = 7.25Hz), 3.97 (1H, dd, J = 11.7, 7.25 Hz), 3.57-3.54 (3H, m), 3.45 (1H, dd, J = 9.75, 3.45 Hz), 3.39 (1H, dd, J = 9.8, 3.8 Hz), 3.21 (2H, q, J = 1.6 Hz), 3.21-3.15 (2H, m), 2.32 (1H, ddd, J = 9.45, 7.9, 4.1 Hz), 1.15 (138H, v br s), 1.03 (6H, q, J = 6 Hz), 0.93 (2H, t, J = 5.65 Hz), 0.77 (3H, t, J = 6.3 Hz), 0.56-0.0.52 (4H, m), 0.45 (2H, dt, J = 8.2, 3.8 Hz), -0.43 (2H, q, J = 5.05 Hz); δ_C: 175.32, 94.30, 72.62, 72.54, 72.43, 72.16, 71.45, 71.25, 70.91, 70.71, 69.84, 63.93, 61.86, 52.36, 49.21, 49.03, 48.87, 48.70, 48.52, 48.36, 48.18, 34.54, 31.68, 30.00, 29.96, br 2955, 29.44, 29.31, 29.20, 29.11, 28.48, 27.09, 25.04, 22.43, 15.54, 13.76, 10.62; ν_{max}/cm^{-1}: 3369, 2920, 2851, 1730, 1466, 1150, 1118, 1025, 997, 725.

Experiment 93: (*2S,2'S,3S,3'S,21Z,21'Z*)-((*2R,2'R,3R,3'R,4S,4'S,5R,5'R,6R,6'R*)-6,6'-Oxybis(3,4,5-tris((trimethylsilyl)oxy)tetrahydro-2H-pyran-6,2-diyl))bis(methylene) bis(3-((tert-butyldimethylsilyl)oxy)-2-tetracosyltetracont-21-enoate) (426) and

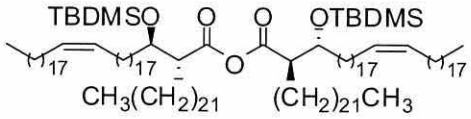
(*2S,3S,Z*)-((*2R,3S,4S,5R,6R*)-3,4,5-Trihydroxy-6-(((*2R,3R,4S,5S,6R*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)tetrahydro-2H-pyran-2-yl)methyl 3-((tert-butyldimethylsilyl)oxy)-2-docosyltetracont-21-enoate (427)



1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (79 mg, 0.413 mmol) and 4-dimethylaminopyridine (44 mg, 0.36 mmol) were added to a stirred solution of (*2R, Z*)-2-docosyl-3-(*R*)-3-(*tert*-butyldimethylsilanyloxy)-21-enoic acid (**419**) (0.133 g, 0.129 mmol), [6-(6-hydroxymethyl-3,4,5-tris-trimethylsilanyl-oxytetrahydropyran-2-yloxy) -3,4,5-tris-trimethyl-silanyloxytetrahydropyran-2-yl]methanol (**180**) (40 mg, 0.05 mmol) and powdered 4 A° molecular sieves in dry dichloromethane (3 ml) at room temperature under a nitrogen atmosphere. The mixture was stirred for 6 days at room temperature, when TLC showed no starting material, and then diluted with dichloromethane (5 ml) and filtered. The filtrate was evaporated under reduced pressure to give a residue, which was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give three fractions: (**(a)** anhydride (**425**) (0.023 g), **(b)** compound (**426**) TDM (0.0542 g, 39 %) as a colourless thick oil and **(c)** compound (**437**) TMM (0.0526 g, 89 %).

Compound (a) (*2R,3R,Z*)-3-((*tert*-Butyldimethylsilyl)oxy)-2-docosyltetracont-21-enoic anhydride (430) {Found *m/z* $[M + Na]^+$: 2063.0595, $C_{136}H_{270}O_5Si2Na$ requires: 2063.0309 (MALDI)} showed δ_H (500 MHz, $CDCl_3$): 5.35 (4H, t, J = 4.7 Hz), 3.96 (2H, dd, J = 11.05, 6.6 Hz), 2.55 (2H, ddd, J = 13.6, 9.8, 3.8 Hz), 2.02 (8H, q, J = 6.3

Hz), 1.26 (212H, s), 0.88 (12H, 7.25 Hz), 0.87 (18H, s), 0.07 (6H, s), 0.02 (6H, s); δ_C: 169.48, 129.89, 52.83, 31.92, 29.74, 29.70, 29.66, 29.60, 29.57, 29.45, 29.36, 29.32, 27.22, 25.82, 25.69, 25.64, 22.69, 18.11, 14.11, 1.01, -4.45, -4.72.; ν_{max}/cm^{-1}: 2914, 2865, 1843, 1746, 1468, 1364, 1246, 1059, 901, 836, 771, 739.

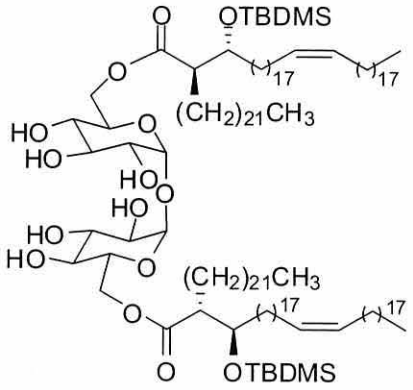


Compound (b) (*2S,2'S,3S,3'S,21Z,21'Z*)-((*2R,2'R,3R,3'R,4S,4'S,5R,5'R,6R,6'R*)-6,6'-oxy-bis-(3,4,5-tris((trimethylsilyl)oxy)tetrahydro-2H-pyran-6,2-diyl))bis(methylene) bis(3-((*tert*-butyl-dimethylsilyl)oxy)-2-tetracosyltetracont-21-enoate), $[\alpha]_D^{22}$ +93.44 (c = 0.42, $CHCl_3$) {Found *m/z* $[M + Na]^+$: 2819.8630, $C_{98}H_{204}O_{13}Si_7Na$ requires: 2819.3737 (MALDI)}. This showed δ_H (500 MHz, $CDCl_3$): 5.20 (4H, t, J = 4.75 Hz), 4.69 (2H, d, J = 2.85 Hz), 4.20 (2H, br d, J = 9.75 Hz), 3.85 (4H, dt, J = 11.7, 3.15 Hz), 3.79-3.72 (4H, m), 3.36 (2H, t, J = 8.85 Hz), 3.21 (2H, dd, J = 9.15, 2.85 Hz), 2.39 (2H, ddd, J = 14.15, 10.05, 4.7 Hz), 1.86 (8H, q, J = 6.3 Hz), 1.15-1.06 (78H, m), 0.72 (18H, s), 0.72 (12H, t, J = 3.8 Hz), 0.00 (18H, s), -0.01 (18H, s), -0.02 (18H, s), -0.10 (12H, br s); δ_C: 173.82, 129.89, 94.83, 73.53, 73.41, 72.81, 71.81, 70.72, 62.38, 51.85, 41.35, 33.43, 31.92, 29.85, 29.79, br 29.70, 29.61, 29.57, 29.51, 29.36, 29.32, 29.05, 28.13, 27.66, 27.22, 26.22, 25.93, 25.83, 25.69, 25.17, 22.69, 22.61, 20.44, 19.42, 18.02, 14.31, 14.11, 11.42, 1.09, 0.94, 0.15, -4.51, -4.64; ν_{max}/cm^{-1} : 2924, 2854, 1746, 1607, 1493, 1452, 1403, 1251, 1076, 1050, 686, 825.

Compound (c) (*2S,3S,Z*)-((*2R,3S,4S,5R,6R*)-3,4,5-trihydroxy-6-(((2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)tetrahydro-2H-pyran-2yl)methyl 3-((tert-butyldimethylsilyl)oxy)-2-docosyltetracont-21-enoate, $[\alpha]_D^{21}$ +46.58 (c 0.41, $CHCl_3$) {Found *m/z* $[M + Na]^+$: 1808.8608, $C_{98}H_{204}O_{13}Si_7Na$ requires: 1808.3584 (MALDI)} showed δ_H (500 MHz, $CDCl_3$): 5.33 (2H, t, J = 4.7 Hz), 4.91 (1H, d, J= 3.15 Hz), 4.84 (1H, d, J = 2.8 Hz), 4.35 (1H, dd, J = 11.95, 2.2 Hz), 4.06 (1H, dd, J = 11.95, 7.6 Hz), 3.99 (1H, dt, J = 6.3, 2.5 Hz), 3.96-3.92 (1H, m), 3.89 (2H, dd, J = 9.15, 2.55 Hz), 3.84 (1H, td, J = 6.65, 3.5Hz), 3.72-3.65 (2H, m), 3.48 (2H, dt, J = 8.85, 5.7 Hz), 3.43 (1H, dd, J = 9.15, 3.15 Hz), 3.40 (1H, dd, J = 9.15, 2.85 Hz), 2.55 (1H, ddd, J = 9.1, 5.35, 3.15 Hz), 2.02 (4H, br.q, J = 6.6 Hz), 1.26 (116H, s), 0.88 (6H, t, J = 6 Hz), 0.87 (9H, s), 0.17 (9H, s), 0.159 (9H, s), 0.155 (9H,

s), 0.14 (9H, s), 0.12 (9H, s), 0.052 (3H, s), 0.05 (3H, s); δ_C: 173.91, 129.89, 94.51, 94.39, 72.87, 72.81, 72.75, 71.97, 71.42, 70.74, 62.45, 61.66, 51.82, 41.35, 33.42, 31.92, 29.72, 29.70, 29.59, 29.56, 29.36, 29.31, 29.05, 28.11, 27.66, 27.22, 26.38, 25.82, 24.86, 22.68, 22.61, 20.44, 18.02, 14.31, 14.11, 1.05, 1.007, 0.92, 0.84, 0.17, 0.04, -4.48, -4.68; ν_{max}/cm^{-1}: 3609, 2925, 2854, 1744, 1493, 1607, 1493, 1453, 1403, 1251, 1076, 1050, 1006, 874, 747, 686.

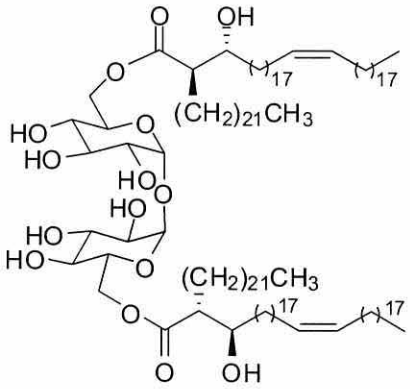
Experiment 94: (*2S,2'S,3S,3'S,21Z,21'Z*)-((*2R,2'R,3S,3'S,4S,4'S,5R,5'R,6R,6'R*)-6,6'-Oxybis(3,4,5-trihydroxytetrahydro-2H-pyran-6,2-diyl))bis(methylene) bis(3-((tert-butyldimethylsilyl)oxy)-2-tetracosyltetracont-21-enoate) (428)



Tetrabutylammonium fluoride (0.15 ml, 0.15 mmol, 1M) was added to a stirred solution of (*2S,2'S,3S,3'S,21Z,21'Z*)-((*2R,2'R,3R,3'R,4S,4'S,5R,5'R,6R,6'R*)-6,6'-oxybis (3,4,5-tris ((tri-methylsilyl)oxy)tetrahydro-2H-pyran-6,2-diyl))bis(methylene) bis(3-((*tert*-butyldimethyl-silyl)oxy)-2-tetracosyltetracont-21-enoate) (**426**) (0.052 g, 0.018 mmol) in dry THF (2ml) at 5 °C under a nitrogen atmosphere. The mixture was allowed to reach room temperature and stirred for 1 hour when TLC showed no starting material. The reaction was cooled to 5 °C and quenched with sat. aq. sodium bicarbonate (3 ml) then diluted with $CHCl_3$ (50 ml). The organic layer was separated and the aqueous layer was re-extracted with $CHCl_3$ (2x50 ml). The combined organic layers were washed with brine (50 ml), dried and evaporated to give a residue, which was purified by column chromatography eluting with $CHCl_3$/MeOH (8.5:1.5) to give the title compound (0.026 g, 62 %) as a colourless thick oil, $[\alpha]_D^{26}$ +43.75 (c = 0.4, $CHCl_3$) {Found *m/z* $[M + H]^+$: 2387.503, $C_{148}H_{288}O_{15}Si_2Na$ requires: 2387.1366}. This showed δ_H (500 MHz, $CDCl_3$): 5.28 (4H, t, J = 4.75 Hz), 4.97 (2H, d, J = 3.15 Hz), 4.65 (2H, br d, J = 11 Hz), 4.20 (2H, t, J = 9.1 5Hz), 3.92 (2H, dd, J = 10.7, 6.6 Hz), 3.74 (2H, t, J = 8.8 Hz), 3.64-3.60 (4H, m), 3.45 (2H, dd, J = 9.15, 2.85 Hz), 2.36

(1H, ddd, J = 12.6, 8.2, 5.05 Hz), 1.96 (4H, q, J = 6 Hz), 1.20 (244H, vbr s), 0.85 (9H, s), 0.82 (6H, t, J = 6.3 Hz), 0.01 (6H br s); δ_C: 175.41, 129.75, 94.89, 72.51, 71.27, 71.12, 69.79, 64.36, 52.20, 49.92, 49.57, 49.22, 49.06, 48.89, 48.55, 34.60, 31.78, 29.55, 29.46, 29.42, 29.32, 29.22, 29.16, 27.16, 27.07, 27.05, 25.73, 25.43, 25.07, 22.54, 18.24, 13.92, -3.97, -6.00; ν_{max}/cm^{-1}: 3376, 2917, 2849, 1735, 1466, 1076, 836.

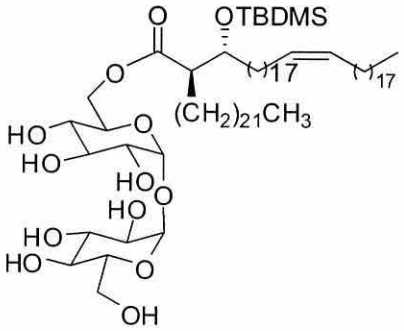
Experiment 95: ***(2S,2'S,3S,3'S,21Z,21'Z)-((2R,2'R,3S,3'S,4S,4'S,5R,5'R,6R,6'R)-6,6'-Oxybis(3,4,5-trihydroxytetrahydro-2H-pyran-6,2-diyl))bis(methylene) bis(3-hydroxy-2-tetracosyltetracont-21-enoate) (216)***



A dry polyethylene vial equipped with an acid proof rubber septum was charged with *(2S,2'S,3S,3'S,21Z,21'Z)*-((*2R,2'R,3S,3'S,4S,4'S,5R,5'R,6R,6'R*)-6,6'-oxybis(3,4,5-trihydroxy-tetrahydro-2H-pyran-6,2-diyl))bis(methylene) bis(3-((*tert*-butyldimethyl-silyl)-oxy)-2-tetra-cosyltetracont-21-enoate) (**428**) (0.028 g, 0.01 mmol) and pyridine (50 µl) in dry THF (4 ml) and stirred at r.t under argon. Hydrogen fluoride-pyridine complex (~70 % hydrogen fluoride, 0.2 ml) at 5 °C was added. The mixture was stirred at 43 °C for 17 hours, when TLC showed no starting material was left, and then neutralized by pouring slowly into sat. aq. sodium bicarbonate until no more CO_2 was liberated. The product was extracted with chloroform (3x50 ml), then the combined organic layers were dried, evaporated to give a residue which was purified by chromatography eluting with $CHCl_3$/MeOH (8.5:1.5) to give the title compound (0.022 g, 88 %) as a syrup, $[\alpha]_D^{27}$ +32.65, (c = 0.47, $CHCl_3$) {Found *m/z* $[M + Na]^+$: 2158.3899, $C_{136}H_{262}O_{15}Na$ requires: 2158.6328}. This showed δ_H (500 MHz, $CDCl_3$+ few drops of CD_3OD): 5.24 (4H, t, J = 4.4Hz), 4.93 (2H, d, J = 3.45Hz), 4.48 (2H, br d, J = 10.4 Hz), 4.06-3.97 (4H, m), 3.56 (2H, dt, J = 7.9, 3.15 Hz), 3.39 (2H, dd, J = 9.75, 3.8 Hz), 3.26 (2H, J = 1.55), 3.17 (2H, t, J = 9.45), 2.33-2.29 (2H, m), 1.91 (6H, br q, J = 6.65 Hz), 1.15 (222H, vbr s), 0.77 (12H, t, J = 6.6 Hz); δ_C: 175.50, 129.85,

95.05, 72.47, 71.45, 71.19, 69.87, 66.95, 64.54, 52.20, 50.07, 49.72, 49.55, 49.38, 49.21, 49.03, 48.87, 48.69, 39.25, 38.95, 38.68, 38.32, 37.63, 37.37, 37.32, 37.29, 37.17, 36.97, 36.86, 36.53, 35.22, 34.64, 34.30, 33.58, 33.36, 33.29, 33.09, 32.93, 32.63, 32.50, 32.36, 32.24, 32.14, 32.10, 31.81, 30.03, 29.91, 29.58, 29.50, 29.45, 29.35, 29.31, 29.25, 29.20, 28.61, 27.92, 27.86, 27.30, 27.19, 27.08, 26.96, 26.79, 26.62, 26.35, 26.28, 25.79, 25.09, 24.68, 24.34, 23.29, 22.94, 22.70, 22.57, 22.51, 22.47, 20.02, 19.59, 19.45, 19.08, 14.26, 13.96, 11.25; ν_{max}/cm^{-1}: 3392, 2920, 2850, 1716, 1466, 1376, 1078.

Experiment 96: (*2S,3S,Z*)-((*2R,3S,4S,5R,6R*)-3,4,5-Trihydroxy-6-(((*2R,3R,4S,5S,6R*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)tetrahydro-2H-pyran-2-yl)methyl 3-((tert-butyldimethylsilyl)oxy)-2-docosyltetracont-21-enoate (217)



Tetrabutylammonium fluoride (0.227 ml, 0.227 mmol, 1M) was added to a stirred solution of (*2S,3S,Z*)-((*2R,3S,4S,5R,6R*)-3,4,5-trihydroxy-6-(((*2R,3R,4S,5S,6R*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)tetrahydro-2H-pyran-2-yl)methyl 3-((tert-butyl-dimethylsilyl)oxy)-2-docosyltetracont-21-enoate (**427**) (0.0284 g, 0.29 mmol) in dry THF (5 ml) at 5 °C under a nitrogen atmosphere. The mixture was allowed to reach room temperature and stirred for 3 hr when TLC showed no starting material. The reaction was cooled to 5 °C and quenched with sat. aq. sodium bicarbonate (3 ml) then diluted with $CHCl_3$ (50 ml). The organic layer was separated and the aqueous layer was re-extracted with $CHCl_3$ (2x50 ml). The combined organic layers were washed with brine (50 ml), dried and evaporated to give a residue, which was purified by column chromatography eluting with $CHCl_3$/MeOH (8.5:1.5) to give the title compound (11 mg, 53 %) as a colourless thick oil, $[\alpha]_D^{26}$ +7.01 (c = 0.51, $CHCl_3$) {Found *m/z* $[M + Na]^+$: 1484.1478, $C_{80}H_{156}O_{13}SiNa$ required 1484.2798}. This showed δ_H (500 MHz, $CDCl_3$): 5.26 (2H, t, J = 4.7 Hz), 5.007 (1H,

d, J = 3.75 Hz), 4.46 (1H, d, J = 1.25 Hz), 4.25 (1H, dd, J = 11.7, 3.8 Hz), 4.16 (1H, br d, J = 12.3 Hz), 3.90-3.86 (1H, m), 3.70 (2H, t, J = 6.3 Hz), 3.39 (1H, d, J = 8.2 Hz), 3.33(1H, d, J = 8.55 Hz), 3.2 (6H, t, J = 12.5 Hz), 2.53-2.49 (1H, m), 2.35-2.31 (1H, m), 2.24 (1H, t, J = 7.9 Hz), 1.96 (9H, d, J = 6 Hz), 1.82 (6H, q, J = 3.45 Hz), 1.24-1.20 (87H, m), 0.87 (6H, t, J = 5.7 Hz, 0,83 (9H, s), 0.81 (13H, s), 0.00 (3H, s), -0.02 (3H, s); δ_C: 173.49, 129.82, 67.90, 54.10, 53.75, 53.64, 51.79, 51.38, 50.97, 49.36, 49.19, 49.02, 48.85, 48.68, 31.83, 29.67, 29.60, 29.50, 29.27, 29.21, 27.10, 25.65, 25.46, 22.58, 17.87, 13.96, -5.01, -5.56; ν_{max}/cm^{-1}: 3378, 2916, 2844, 1741, 1468, 1075, 839.

References:

1 B. M. Rothschild, L. D. Martin, G. Lev, H. Bercovier, G. K. Bar-Gal, C. Greenblatt, H. Donoghue, M. Spigelman and D. Brittain, *Clini. Infecti. Dise.,* 2001, 33, 305-311 (DOI:10.1086/321886).

2 I. Hershkovitz, H. Donoghue, D. E. Minnikin, G. S. Besra, O. Y. Lee, A. M. Gernaey, E. Galili, V. Eshed, C. L. Greenblatt, E. Lemma, G. K. Bar-Gal and M. Spigelman, *Plos One,* 2008, 3, 3426.

3 K. Sepkowitz, J. Raffalli, L. Riley, T. Kiehn and D. Armstrong, *Clin. Microbiol. Rev.,* 1995, 8, 180-199.

4 R. R. Trail, *the History of Medicine,* 1970, 14, 166.

5 WHO, *http://www.who.int/mediacentre/factsheets/fs104/en/index.html,* 2010, Fact sheet N°104.

6 C. Bonah, *Studies in History and Philosophy of Science Part C: Studies in History and Philosophy of Biological and Biomedical Sciences,* 2005, 36, 696-721 (DOI:DOI: 10.1016/j.shpsc.2005.09.003).

7 T. Schaberg, *Europ. Resp. J.,* 1996, 9, 866-867.

8 G. A. W. Rook, G. Seah and A. Ustianowski, *Europ. Resp. J.,* 2001, 17, 537-557.

9 K. Lönnroth, K. G. Castro, J. M. Chakaya, L. S. Chauhan, K. Floyd, P. Glaziou and M. C. Raviglione, *The Lancet,* 2010, 375, 1814-1829 (DOI:DOI: 10.1016/S0140-6736(10)60483-7).

10 D. Butler, *Nature,* 2000, 403, 692-692.

11 D. A. Siegele and R. Kolter, *J. Bacteriol.,* 1992, 174, 345-348.

12 J. M. Grange, *Tubercle Lung Dis.,* 1992, 73, 249-251 (DOI:DOI: 10.1016/0962-8479(92)90128-7).

13 R. Balasubramanian, S. Sivasubramanian, V. K. Vijayan, R. Ramachandran, M. S. Jawahar, C. N. Paramasivan, N. Selvakumar and P. R. Somasundaram, *Tubercle,* 1990, 71, 253-258 (DOI:DOI: 10.1016/0041-3879(90)90037-9).

14 G. Marshall, J. W. S. Blacklock, C. Cameron, N, B, Capon, R. Cruickshank, J. H. Gaddum, F. R. G. Heaf, A. B Hill, L. E. Houghton, J. C. Hoyle, H. Raistrick, J. G. Scadding, W. H. Tytler, G. S. Wilson, P.D. Hart, *BMJ,* 1948, 30, 769.

15 WHO,
http://www.who.int/tb/publications/global_report/2009/pdf/key_points_en.pdf.

16 WHO, *Guidelines for the programmatic management of drug-resistant tuberculosis*, 2006.

17 M. E. Villarino, L. J. Geiter, P. M. Simone, *Public Health Reports,* 1992, 107, 616.

18 M. Scalcini, G. Carré, M. Jean-Baptiste, E. Hershfield, S. Parker, J. Wolfe, K. Nelz, R. Long, *Am Rev Respir Dis.,* 1990, 142, 508.

19 R. L. Cowie, *Tubercle,* 1990, 71, 39-42 (DOI:DOI: 10.1016/0041-3879(90)90059-H).

20 M. Hawken, P. Nunn, P. Godfrey-Faussett, K. P. W. J. McAdam, J. Morris, J. Odhiambo, W. Githui, C. Gilks, M. Hawken, S. Gathua, P. Nunn, M. Hawken, R. Brindle and B. Batchelor, *The Lancet,* 1993, 342, 332-337 (DOI:DOI: 10.1016/0140-6736(93)91474-Z).

21 WHO, *Global Tuberculosis Control*, 2009.

22 P. J. Brennan and H. Nikaido, *Annu. Rev. Biochem.,* 1995, 64, 29-63 (DOI:10.1146/annurev.bi.64.070195.000333).

23 A. Thayer, *Chem. Eng. News,* 2007, 85, 21-32 (DOI:10.1021/cen-v085n039.p021).

24 R. J. Anderson, *J. Biol. Chem. 1927, 74, 525-535.*

25 R. J. Anderson and E. Chargaff, *J. Biol. Chem. 1929 83, 169-175.*

26 F. H. Stodola, A. Lesuk and R. J. Anderson, *J. Biol. Chem.,* 1938, 126, 505-513.

27 R. J. Anderson and M. M. Creighton, *J. Biol. Chem.,* 1939, 129, 57-63.

28 M. A. Abdallah, N. van Pittius, P. A. D. Champion, J. Cox, J. Luirink, C. M. J. E. Vandenbroucke-Grauls, B. J. Appelmelk and W. Bitter, *Nat. Rev. Microbiol.,* 2007, 5, 883.

29 A. C. Vollmer and T. K. Van Dyk, *Adv. Microb. Physiol.*, 2004, 49, 131-74.

30 I. Vergne, J. Chua, S. B. Singh and V. Deretic, *Annu. Rev. Cell Dev. Biol,* 2004, 20, 367.

31 M. Daffé and G. Etienne, *Tuber. Lung Dis.,* 1999, 79, 153-169.

32 L. A. Davidson, P. Draper and D. E. Minnikin, *J. Gen. Microbiol.,* 1982, 128, 823-828.

33 D. E. Minnikin and N. Polgar, *Chem. Commun. (London),* 1966, , 648-649.

34 D. E. Minnikin, L.Kremer, L. G. Dover, G. S. Besra, *Chemistry & Biology,* 2002, 9, 545-553.

35 D. E. Minnikin and N. Polgar, *Tetrahedron Lett.,* 1966, 7, 2643-2647 (DOI:DOI: 10.1016/S0040-4039(01)84131-9).

36 M. Watanabe, Y. Aoyagi, H. Mitome, T. Fujita, H. Naoki, M. Ridell, D. E. Minnikin, *Microbiology,* 2002, 148, 1881-1902.

37 R. Slayden, R. Lee, J. Armour, A. Cooper, I. Orme, P. Brennan and G. Besra, *Antimicrob. Agents Chemother.,* 1996, 40, 2813-2819.

37 b M. Senn,T . Ioneda, J. Pudles, E. Lederer, *Eur. J. Biochem.* 1967, **1,** 353-356

38 C. E. Barry III, R. E. Lee, K. Mdluli, A. E. Sampson, B. G. Schroeder, R. A. Slayden and Y. Yuan, *Prog. Lipid Res.,* 1998, 37, 143-179 (DOI: 10.1016/S0163-7827(98)00008-3).

39 M. Watanabe, Y. Aoyagi, M. Ridell and D. E. Minnikin, *Microbiology,* 2001, 147, 1825-1837.

40 D. E. Minnikin, S. M. Minnikin, G. Dobson, M. Goodfellow, F. Portaels, D. B. Van and D. Sesardic, *J. Gen. Microbiol.,* 1983, 129, 889-891.

41 E. Rafidinarivo, M. Lanéelle, H. Montrozier, P. Valero-Guillén, J. Astola, M. Luquin, J. Promé and M. Daffé, *J. Lipid Res,* 2009, 50, 477-490 (DOI:10.1194/jlr.M800384-JLR200).

42 R. Toubiana, J. Berlan, H. Sato and M. Strain, *J. Bacteriol.,* 1979, 139, 205-211.

43 A. Quémard, M. Lanéelle, H. Marrakchi, D. Prome, E. Dubnau and M. Daffé, *Europ. J. Biochem.,* 1997, 250, 758-763 (DOI:10.1111/j.1432-1033.1997.00758.x).

44 G. O. Guerrant, M. A. Lambert and C. W. Moss, *J. Clini. Microbiol.,* 1981, 13, 899-907.

45 D. E. Minnikin and N. Polgar, *Chem. Commun. (London),* 1967, 1172-1174.

46 D. E. Minnikin and N. Polgar, *Chem. Commun. (London),* 1967, 916-918.

47 C. P. Asselineau, C. S. Lacave, H. L. Montrozier and J. Promé, *Eur. J. Biochem.,* 1970, 14, 406-410.

48 C. Lacave, M. Lanéelle, M. Daffé, H. Montrozier, M. Rols and C. Asselineau, *Eur. J. Biochem.,* 1987, 163, 369-378 (DOI:10.1111/j.1432-1033.1987.tb10809.x).

49 M. Daffé, M. Lanéelle and P. L. V. Guillen, *Europ. J.Biochem.,* 1988, 177, 339-344 (DOI:10.1111/j.1432-1033.1988.tb14380.x).

50 C. A. M. R. Almog, *Biophys. J.,* 1 December 1996, 71, 3311-3319.

51 E. Durand, M. Gillois, J. Tocanne and G. Lanéelle, *Europ. J. Biochem.,* 1979, 94, 109-118 (DOI:10.1111/j.1432-1033.1979.tb12877.x).

52 M. Villeneuve, M. Kawai, M. Watanabe, Y. Aoyagi, Y. Hitotsuyanagi, K. Takeya, H. Gouda, S. Hirono, D. E. Minnikin and H. Nakahara, *Biochimica et Biophysica Acta (BBA) - Biomembranes,* 2007, 1768, 2343 (DOI:10.1016/j.bbamem.2007.08.003).

53 M. Villeneuve, M. Kawai, M. Watanabe, Y. Aoyagi, Y. Hitotsuyanagi, K. Takeya, H. Gouda, S. Hirono, D. E. Minnikin and H. Nakahara, *Biochimica et Biophysica Acta (BBA) - Biomembranes,* 2007, 1768, 1717-1726 (DOI:10.1016/j.bbamem.2007.04.003).

54 M. Villeneuve, M. Kawai, H. Kanashima, M. Watanabe, D. E. Minnikin and H. Nakahara, *Biochimica et Biophysica Acta (BBA) - Biomembranes,* 2005, 1715, 71-80 (DOI:10.1016/j.bbamem.2005.07.005).

55 R. Watanabe, Y. C. Yoo, K. Hata, M. Mitobe, Y. Koike, M. Nishizawa, D. M. Garcia, Y. Nobuchi, H. Imagawa, H. Yamada and I. Azuma, *Vaccine,* 1999, 17, 1484-1492 (DOI: 10.1016/S0264-410X(98)00367-3).

56 E. Dubnau, M. Lanéelle, S. Soares, A. Benichou, T. Vaz, D. Prome, J. Prome, M. Daffé and A. Quémard, *Mol. Microbiol.,* 1997, 23, 313-322.

57 M. Daffé, M. A. Lanéelle and C. Lacave, *Res. Microbiol.,* 1991, 142, 397-403 (DOI: 10.1016/0923-2508(91)90109-N).

58 J. R. Al Dulayymi, M. S. Baird, E. Roberts and D. E. Minnikin, *Tetrahedron,* 2006, 62, 11867-11880 (DOI:DOI: 10.1016/j.tet.2006.09.019).

59 A. Quémard, C. Lacave and G. Lanéelle, *Antimicrob. Agents Chemother.,* 1991, 35, 1035-1039.

60 R. J. Kinsella, D. A. Fitzpatrick, C. J. Creevey and J. O. McInerney, *Proc. Natl. Acad. Sci. U. S. A.,* 2003, 100, 10320-10325.

61 S. T. Cole, R. Brosch, J. Parkhill, T. Garnier, C. Churcher, D. Harris, S. V. Gordon, K. Eiglmeier, S. Gas, C. E. Barry III, F. Tekaia, K. Badcock, D. Basham, D. Brown, T. Chillingworth, R. Connor, R. Davies, K. Devlin, T. Feltwell, S. Gentles, N. Hamlin, S. Holroyd, T. Hornsby, K. Jagels, A. Krogh, J. McLean, S. Moule, L. Murphy, K. Oliver, J. Osborne, M. A. Quail, M. -. Rajandream, J. Rogers, S. Rutter, K. Seeger, J. Skelton, R. Squares, S. Squares, J. E. Sulston, K. Taylor and B. G. Whitehead S.and B. G. Barrell, *Nature,* 1998, 393, 537-544 (DOI:10.1038/31159).

62 K. Bloch, *Methods Enzymol.* 1975, 35, 84-90.

63 D. N. Brindley, S. Matsumura and K. Bloch, *Nature,* 1969, 244, 666-669 (DOI:doi:10.1038/224666a0).

64 G. S. Besra and L. Kremer, *Expert Opin. Investig. Drugs,* 2002, 11, 1033-1049 (DOI:10.1517/13543784.11.8.1033).

65 S. J. Senior, P. A. Illarionov, S. S. Gurcha, I. B. Campbell, M. L. Schaeffer, D. E. Minnikin and G. S. Besra, *Bioorg. Med. Chem. Lett.,* 2003, 13, 3685-3688 (DOI:DOI: 10.1016/j.bmcl.2003.08.015).

66 S. J. Senior, P. A. Illarionov, S. S. Gurcha, I. B. Campbell, M. L. Schaeffer, D. E. Minnikin and G. S. Besra, *Bioorg. Med. Chem. Lett.,* 2004, 14, 373-376 (DOI:DOI: 10.1016/j.bmcl.2003.10.061).

67 C. W. Hing, L. Gaohua, Y. Zhang, Charles O. Rock and Jie-Zheng, *J. Biol.Chem.,* 2002, 277, 15874.

68 C. Vilcheze, H. R. Morbidoni, T. R. Weisbrod, H. Iwamoto, M. Kuo, J. C. Sacchettini and W. R. Jacobs Jr., *J. Bacteriol.,* 2000, 182, 4059-4067 (DOI:10.1128/JB.182.14.4059-4067.2000).

69 P. E. Kolattukudy, N. D. Fernandes, A. K. Azad, A. M. Fitzmaurice and T. D. Sirakova, *Mol. Microbiol.,* 1997, 24, 263-270.

70 M. Gastambide-Odier and E. and Lederer, *Nature,* 1959, 184, 1563-1564 (DOI:10.1038/1841563b0).

71 N. Qureshi, K. Takayama and H. K. Schnoes, *J.Biol Chem.,* 1980, 255, 182-189.

72 J. Liu and H. Nikaido, *Proc. Natl. Acad. Sci. U. S. A.,* 1999, 96, 4011-4016.

73 D. Portevin, C. de Sousa-D'Auria, C. Houssin, C. Grimaldi, M. Chami, M. Daffé and C. Guilhot, *Proc. Natl. Acad. Sci. U. S. A.,* 2004, 101, 314-319.

74 G. S. Besra, T. Sievert, R. E. Lee, R. A. Slayden, P. J. Brennan and K. Takayama, *Proceedings of the National Academy of Sciences,* 1994, 91, 12735-12739.

75 G. D. Coxon, J. R. Al Dulayymi, C. Morehouse, P. J. Brennan, G. S. Besra, M. S. Baird and D. E. Minnikin, *Chem. Phys. Lipids,* 2004, 127, 35-46 (DOI: 10.1016/j.chemphyslip.2003.09.001).

76 W. R. Butler and J. O. Kilburn, *J Clinical Microbiology,* 1988, 26, 50.

77 C. Huang, C. V. Smith, M. S. Glickman, W. R. Jacobs and J. C. Sacchettini, *J. Biol. Chem.,* 2002, 277, 11559-11569.

78 G. Jaureguiberry, M. Lenfant, B. C. Das and E. Lederer, *Tetrahedron,* 1966, 22, 27-32 (DOI:DOI: 10.1016/S0040-4020(01)82166-9).

79 D. W. Grogan and J. E. Cronan, *Microbiol Mol Biol Reviews,* 1997, 61, 429.

80 Y. Yuan, D. C. Crane, J. M. Musser, S. Sreevatsan and C. E. Barry, *J. Biol. Chem.*, 1997, 272, 10041-10049.

81 M. S. Glickman, J. S. Cox and W. R. Jacobs Jr., *Mol. Cell,* 2000, 5, 717-727 (DOI: 10.1016/S1097-2765(00)80250-6).

82 M. S. Glickman, S. M. Cahill and W. R. Jacobs, *J.Biol. Chem.,* 2001, 276, 2228-2233 (DOI:10.1074/jbc.C000652200).

83 D. Barkan, Z. Liu, J. C. Sacchettini and M. S. Glickman, *Chem. Biol.,* 2009, 16, 499-509 (DOI: 10.1016/j.chembiol.2009.04.001).

84 W. J. Gensler, J. P. Marshall, J. J. Langone and J. C. Chen, *J. Org. Chem.,* 1977, 42, 118-125 (DOI:10.1021/jo00421a024).

85 W. J. Gensler, R. S. Prasad, A. P. Chaudhuri and I. Alam, *J. Org. Chem.,* 1979, 44, 3643-3652 (DOI:10.1021/jo01335a006).

86 J. R. Al Dulayymi, M. S. Baird and E. Roberts, *Chem. Commun.,* 2003, , 228-229.

87 D. Grandjean, P. Pale and J. Chuche, *Tetrahedron,* 1991, 47, 1215-1230 (DOI: 10.1016/S0040-4020(01)86378-X).

88 G. D. Coxon, S. Knobl, E. Roberts, M. S. Baird, J. R. Al Dulayymi, G. S. Besra, P. J. Brennan and D. E. Minnikin, *Tetrahedron Lett.,* 1999, 40, 6689-6692 (DOI: 10.1016/S0040-4039(99)01378-7).

89 G. D. Coxon, J. R. Al-Dulayymi, M. S. Baird, S. Knobl, E. Roberts and D. E. Minnikin, *Tetrahedron: Asymmetry,* 2003, 14, 1211-1222 (DOI: 10.1016/S0957-4166(03)00165-4).

90 J. R. Al Dulayymi, M. S. Baird and E. Roberts, *Tetrahedron Lett.,* 2000, 41, 7107-7110 (DOI: 10.1016/S0040-4039(00)01147-3).

91 J. R. Al Dulayymi, M. S. Baird and E. Roberts, *Tetrahedron,* 2005, 61, 11939-11951 (DOI: 10.1016/j.tet.2005.09.056).

92 T. Morikawa, H. Sasaki, R. Hanai, A. Shibuya and T. Taguchi, *J. Org. Chem.,* 1994, 59, 97-103 (DOI:10.1021/jo00080a017).

93 G. Koza, Ph. D Thesis, Bangor University, *Synthesis of single enantiomers of ketomycolic acids*, 2007.

94 J. R. Al-Dulayymi, M. S. Baird, H. Mohammed, E. Roberts and W. Clegg, *Tetrahedron,* 2006, 62, 4851-4862 (DOI:10.1016/j.tet.2006.03.007).

95 S. Rodríguez, K. T. Schroeder, M. M. Kayser and J. D. Stewart, *J. Org. Chem.,* 2000, 65, 2586-2587 (DOI:10.1021/jo9917036).

96 D. Kalaitzakis, J. D. Rozzell, S. Kambourakis and I. Smonou, *Org. Lett.,* 2005, 7, 4799-4801 (DOI:10.1021/ol051166d).

97 X. Wu, Y. Wang, J. Ju, C. Chen, N. Liu and Y. Chen, *Tetrahedron: Asymmetry,* 2009, 20, 2504-2509 (DOI: 10.1016/j.tetasy.2009.10.036).

98 A. Forni, I. Moretti, F. Prati and G. Torre, *Tetrahedron,* 1994, 50, 11995-12000 (DOI: 10.1016/S0040-4020(01)89310-8).

99 D. J. Ager and S. A. Laneman, *Tetrahedron: Asymmetry,* 1997, 8, 3327-3355 (DOI:DOI: 10.1016/S0957-4166(97)00455-2).

100 L. Horner, H. Siegel and H. Büthe, *Angewandte Chemie International Edition in English,* 1968, 7, 942-942 (DOI:10.1002/anie.196809422).

101 W. S. Knowles and M. J. Sabacky, *Chem. Commun. (London),* 1968, , 1445-1446.

102 R. Noyori, *Tetrahedron,* 1994, 50, 4259-4292 (DOI: 10.1016/S0040-4020(01)89365-0).

103 J. P. Genêt, C. Pinel, V. Ratovelomanana-Vidal, S. Mallart, X. Pfister, L. Bischoff, M. C. C. De Andrade, S. Darses, C. Galopin and J. A. Laffitte, *Tetrahedron: Asymmetry,* 1994, 5, 675-690 (DOI: 10.1016/0957-4166(94)80030-8).

104 M. Murata, T. Morimoto, K. Achiwa, *Synlett,* 1991, 827-829.

105 T. Hayashi, Y. Matsumoto and Y. Ito, *J. Am. Chem. Soc.,* 1989, 111, 3426-3428 (DOI:10.1021/ja00191a049).

106 T. Hayashi, Y. Matsumoto and Y. Ito, *J. Am. Chem. Soc.,* 1988, 110, 5579-5581 (DOI:10.1021/ja00224a057).

107 S. Akutagawa, *Applied Catalysis A: General,* 1995, 128, 171-207 (DOI: 10.1016/0926-860X(95)00097-6).

108 R. Noyori and H. Takaya, *Acc. Chem. Res.,* 1990, 23, 345-350 (DOI:10.1021/ar00178a005).

109 L. Shao, H. Kawano, M. Saburi and Y. Uchida, *Tetrahedron,* 1993, 49, 1997-2010 (DOI: 10.1016/S0040-4020(01)86300-6).

110 R. Noyori, M. Tokunaga, M. Kitamura, *Bull.Chem. Soci.Japan,* 68, 36-55.

111 A. Mezzetti, A. Tschumper and G. Consiglio, *J. Chem. Soc. , Dalton Trans.,* 1995, , 49-56.

112 L. P. C. Nielsen, C. P. Stevenson, D. G. Blackmond and E. N. Jacobsen, *J. Am. Chem. Soc.,* 2004, 126, 1360-1362 (DOI:10.1021/ja038590z).

113 S. E. Schaus, B. D. Brandes, J. F. Larrow, M. Tokunaga, K. B. Hansen, A. E. Gould, M. E. Furrow and E. N. Jacobsen, *J. Am. Chem. Soc.,* 2002, 124, 1307-1315 (DOI:10.1021/ja016737l).

114 P. Gupta and P. Kumar, *Europ.J.Org. Chem,* 2008, 2008, 1195-1202 (DOI:10.1002/ejoc.200700868).

115 K. Mori, Y. Shikichi, S. Shankar and J. Y. Yew, *Tetrahedron,* 2010, 66, 7161-7168 (DOI: 10.1016/j.tet.2010.06.080).

116 J. A. Frick, J. B. Klassen, A. Bathe, J. M. Abramson and H. Rapoport, *Synthesis,* 1992, , 621-623.

117 G. Koza, C. Theunissen, J. R. Al Dulayymi and M. S. Baird, *Tetrahedron,* 2009, 65, 10214-10229 (DOI:10.1016/j.tet.2009.09.099).

118 H. Bloch, *J.Exp.Med.,* 1950, 91, 197-218 (DOI:10.1084/jem.91.2.197).

119 H. Bloch and H. Noll, *J.Exp.Med.,* 1953, 97, 1-16 (DOI:10.1084/jem.97.1.1).

120 S. Attorri, S. Dunbar and and Clarridge, J. E. III, *J. Clin. Microbiol.,* 2000, 38, 1426-1429.

121 M. B. Goren, *Bacteriol. Rev.,* 1972, 36, 33-34.

122 N. Andreu, I. Gibert, M. Luquin, P. E. Kolattukudy and T. Sirakova, *J. Clin. Microbiol.,* 2004, 42, 1379-1380 (DOI:10.1128/JCM.42.3.1379-1380.2004).

123 H. Noll, H. Bloch, J. Asselineau and E. Lederer, *Biochim. Biophys. Acta,* 1956, 20, 299-309 (DOI: 10.1016/0006-3002(56)90289-X).

124 H. Bloch, J. Defaye, E. Lederer and H. Noll, *Biochim. Biophys. Acta,* 1957, 23, 312-321 (DOI: 10.1016/0006-3002(57)90333-5).

125 P. Rapp, H. Bock, V. Wrayand F. Wagner, *J. Gen.Microbiol.,* 1979, 115, 491-503 (DOI:10.1099/00221287-115-2-491).

126 A. Bekierkunst, *J. Bacteriol.,* 1968, 96, 958-961.

127 R. Toubiana, B. C. Das, J. Defaye, B. Mompon and M. Toubiana, *Carbohydr. Res.,* 1975, 44, 308-312 (DOI: 10.1016/S0008-6215(00)84175-0).

128 Y. Fujita, T. Naka, T. Doi and I. Yano, *Microbiology,* 2005, 151, 1443-1452 (DOI:10.1099/mic.0.27791-0).

129 M. Kato and J. Maeda, *Infect. Immun.,* 1974, 9, 8-14.

130 G. Puzo, G. Tissié, H. Aurelle, C. Lacave and J. Promé, *Europ. J.Biochem.,* 1979, 98, 99-105 (DOI:10.1111/j.1432-1033.1979.tb13166.x).

131 M. Kai, Y. Fujita, Y. Maeda, N. Nakata, S. Izumi, I. Yano and M. Makino, *FEBS Lett.,* 2007, 581, 3345-3350 (DOI: 10.1016/j.febslet.2007.06.029).

132 A. K. Datta, K. Takayama, M. A. Nashed and L. Anderson, *Carbohydr. Res.,* 1991, 218, 95-109 (DOI: 10.1016/0008-6215(91)84089-W).

133 A. K. Datta and K. Takayama, *Carbohydr. Res.,* 1993, 245, 151-158 (DOI: 10.1016/0008-6215(93)80068-P).

134 K. Takayama and E. L. Armstrong, *J. Bacteriol.,* 1977, 130, 569-570.

135 K. Takayama and E. L. Armstrong, *Biochemistry (N. Y.),* 1976, 15, 441-447 (DOI:10.1021/bi00647a032).

136 J. Carlsson, *Appl. Environ. Microbiol.,* 1973, 25, 287-289.

137 A. Bekierkunst, E. Yarkoni, I. Flechner, S. Morecki, E. Vilkas and E. Lederer, *Infect. Immun.,* 1971, 4, 256-263.

138 M. Artman, A. Bekierkunst and I. Goldenberg, *Arch. Biochem. Biophys.,* 1964, 105, 80-85 (DOI: 10.1016/0003-9861(64)90237-1).

139 M. Kato, *Arch. Biochem. Biophys.,* 1970, 140, 379-390 (DOI: 10.1016/0003-9861(70)90079-2).

140 N. Rastogi and H. L. David, *Biochimie,* 1988, 70, 1101-1120 (DOI: 10.1016/0300-9084(88)90272-6).

141 A. Bekierkunst, I. S. Levij, E. Yarkoni, E. Vilkas, A. Adam and E. Lederer, *J. Bacteriol.,* 1969, 100, 95-102.

142 R. L. Breton, *The synthesis of cord factor analogs*, PH. D, Thesis, University of Ottawa, 1990.

143 F. Numata, K. Nishimura, H. Ishida, S. Ukei, Y. Tone, C. Ishihara, I. Saiki, I. Sekikawa and I. Azuma, *Chemi. Pharma. Bull.,* 1985, 33, 4544-4555.

144 G. Birch and A. C. Richardson, *Carbohydr. Res.,* 1968, 8, 411-415 (DOI: 10.1016/S0008-6215(00)81525-6).

145 J. Polonsky, E. Soler and J. Varenne, *Carbohydr. Res.,* 1978, 65, 295-300 (DOI: 10.1016/S0008-6215(00)84324-4).

146 S. Hanessian, M. M. Ponpipom and P. Lavallee, *Carbohydr. Res.,* 1972, 24, 45-56 (DOI: 10.1016/S0008-6215(00)82258-2).

147 S. Hanessian and P. Lavallée, *Carbohydr. Res.,* 1973, 28, 303-311 (DOI: 10.1016/S0008-6215(00)82785-8).

148 J. Tocanne, *Carbohydr. Res.,* 1975, 44, 301-307 (DOI: 10.1016/S0008-6215(00)84174-9).

149 A. Liav, H. M. Flowers and M. B. Goren, *Carbohydr. Res.,* 1984, 133, 53-58 (DOI: 10.1016/0008-6215(84)85182-4).

150 A. Liav and M. B. Goren, *Carbohydr. Res.,* 1980, 81, c1-c3 (DOI: 10.1016/S0008-6215(00)85692-X).

151 M. B. Goren and K. Jiang, *Carbohydr. Res.,* 1980, 79, 225-234 (DOI: 10.1016/S0008-6215(00)83834-3).

152 T. Iwashige and H. Saeki, *Chemi. Pharma. Bull.,* 1967, 15, 1803-1806.

153 K. Yoyshimoto, T. Wakamiya and Y. Nishikawa, *Chemi. Pharma. Bull.,* 1982, 30, 1169.

154 M. Kates and D. J. Hanahan, *Handbook of lipid research*, Plenum Press, New York, NY u.s.a., 1990.

155 S. Bottle and I. D. Jenkins, *J. Chem. Soc. , Chem. Commun.,* 1984, 385-385.

156 I. D. Jenkins and M. B. Goren, *Chem. Phys. Lipids,* 1986, 41, 225-235 (DOI: 10.1016/0009-3084(86)90024-1).

157 D. A. Johnson, *Carbohydr. Res.,* 1992, 237, 313-318 (DOI: 10.1016/S0008-6215(92)84254-P).

158 J. R. Al Dulayymi, M. S. Baird, M. Maza-Iglesias, S. V. Beken and J. Grooten, *Tetrahedron Lett.,* 2009, 50, 3702-3705 (DOI: 10.1016/j.tetlet.2009.03.213).

159 E. Lederer, *J. Med. Chem.,* 1980, 23, 819-825 (DOI:10.1021/jm00182a001).

160 H. H. Baer and R. L. Breton, *Carbohydr. Res.,* 1991, 209, 181-189 (DOI: 10.1016/0008-6215(91)80155-G).

161 H. H. Baer, R. L. Breton and Y. Shen, *Carbohydr. Res.,* 1990, 200, 377-389 (DOI: 10.1016/0008-6215(90)84204-8).

162 K. Kaneda, S. Imaizumi, S. Mizuno, T. Baba, M. Tsukamura and I. Yano, *J.Gen. Microbiol.,* 1988, 134, 2213-2229 (DOI:10.1099/00221287-134-8-2213).

163 G. R. Gray, M. Y. H. Wong and S. J. Danielson, *Prog. Lipid Res.,* 1982, 21, 91-107 (DOI:10.1016/0163-7827(82)90001-7).

164 T. Baba, K. Kaneda, E. Kusunose, M. Kusunose and I. Yano, *Lipids,* 1988, 23, 1132-1138 (DOI: 10.1007/BF02535279).

165 M. Y. Wong and G. R. Gray, *J. Biol.Chem.,* 1979, 254, 5741-5744.

166 H. C. Huang, J. K. Rehmann and G. R. Gray, *J. Org. Chem.,* 1982, 47, 4018-4023 (DOI:10.1021/jo00142a003).

167 Y. Nakamura, S. Takeuchi, S. Zhang, K. Okumura and Y. Ohgo, *Tetrahedron Lett.,* 2002, 43, 3053-3056 (DOI: 10.1016/S0040-4039(02)00407-0).

168 A. I. Vogel, B. S. Furniss and A. I. Vogel, *Vogel's Textbook of practical organic chemistry*, Longman Scientific & Technical, London.

169 M. Nishizawa, H. Yamamoto, H. Imagawa, V. Barbier- Chassefière, E. Petit, I. Azuma and D. Papy-Garcia, *J. Org. Chem.,* 2007, 72, 1627-1633 (DOI:10.1021/jo062018j).

170 G. Fráter, U. Müller and W. Günther, *Tetrahedron,* 1984, 40, 1269-1277 (DOI: 10.1016/S0040-4020(01)82413-3).

171 G. Fráter, U. Müller and F. Schröder, *Tetrahedron: Asymmetry,* 2004, 15, 3967-3972 (DOI:DOI: 10.1016/j.tetasy.2004.11.003).

172 G. Fráter, *Tetrahedron Lett.,* 1981, 22, 425-428 (DOI:DOI: 10.1016/0040-4039(81)80116-5).

173 J. D. Morrison and J. W. Scott, *Asymmetric synthesis*, Academic Press, New York, 1983.

174 R. W. Dugger, J. L. Ralbovsky, D. Bryant, J. Commander, S. S. Massett, N. A. Sage and J. R. Selvidio, *Tetrahedron Lett.,* 1992, 33, 6763-6766 (DOI: 10.1016/S0040-4039(00)61770-7).

175 G. Toschi, *Synthetic Approaches to Single Enantiomers of Mycolic Acids, Ph. D University of Bangor*, 2004.

176 K. Jarowicki and P. Kocienski, *J. Chem. Soc. , Perkin Trans. 1,* 1998, , 4005-4037.

177 E. J. Corey and A. Venkateswarlu, *J. Am. Chem. Soc.,* 1972, 94, 6190-6191 (DOI:10.1021/ja00772a043).

178 W. Yu, Y. Mei, Y. Kang, Z. Hua and Z. Jin, *Org. Lett.,* 2004, 6, 3217-3219 (DOI:10.1021/ol0400342).

179 M. Julia and J. Paris, *Tetrahedron Lett.,* 1973, 14, 4833-4836 (DOI: 10.1016/S0040-4039(01)87348-2).

180 P. J. Kocienski, B. Lythgoe and S. Ruston, *J. Chem. Soc. , Perkin Trans. 1,* 1978, 829-834.

181 P. J. Kocienski, B. Lythgoe and D. A. Roberts, *J. Chem. Soc. , Perkin Trans. 1,* 1978, 834-837.

182 P. J. Kocienski, B. Lythgoe and S. Ruston, *J. Chem. Soc. , Perkin Trans. 1,* 1979, 1290-1293.

183 P. J. Kocienski, B. Lythgoe and I. Waterhouse, *J. Chem. Soc. , Perkin Trans. 1,* 1980, 1045-1050.

184 G. E. Keck, K. A. Savin and M. A. Weglarz, *J. Org. Chem.,* 1995, 60, 3194-3204 (DOI:10.1021/jo00115a041).

185 P.R. Blakemore, W. J. K. P. Colea and Morleyb A., *Synlett,* 1998, 1, 26-28.

186 P. R. Blakemore, *J. Chem. Soc. , Perkin Trans. 1,* 2002, 2563-2585.

187 J. B. Baudin, G. Hareau, S. A. Julia and O. Ruel, *Tetrahedron Lett.,* 1991, 32, 1175-1178 (DOI: 10.1016/S0040-4039(00)92037-9).

188 A. B. Charette, C. Berthelette and D. St-Martin, *Tetrahedron Lett.,* 2001, 42, 5149-5153 (DOI: 10.1016/S0040-4039(01)00941-8).

189 A. B. Charette, C. Berthelette and D. St-Martin, *Tetrahedron Lett.,* 2001, 42, 6619-6619 (DOI: 10.1016/S0040-4039(01)01357-0).

190 A. B. Charette and H. Lebel, *J. Am. Chem. Soc.,* 1996, 118, 10327-10328 (DOI:10.1021/ja9619420).

191 P. Liu and E. N. Jacobsen, *J. Am. Chem. Soc.,* 2001, 123, 10772-10773 (DOI:10.1021/ja016893s).

192 J. Pospíšil and I.E. Markó, *Org. Lett.,* 2006, 8, 5983-5986 (DOI:10.1021/ol062433y).

193 K. N. Campbell and L. T. Eby, *J. Am. Chem. Soc.,* 1941, 63, 216-219 (DOI:10.1021/ja01846a050).

194 K. Ahmad and F. M. Strong, *J. Am. Chem. Soc.,* 1948, 70, 1699-1700 (DOI:10.1021/ja01185a007).

195 J. Li, R. Hua and T. Liu, *J. Org. Chem.,* 2010, 75, 2966-2970 (DOI:10.1021/jo100247a).

196 C. A. Brown, *J. Org. Chem.,* 1970, 35, 1900-1904 (DOI:10.1021/jo00831a039).

197 C. A. Brown and V. K. Ahuja, *J. Org. Chem.,* 1973, 38, 2226-2230 (DOI:10.1021/jo00952a024).

198 M. Eckhardt and G. C. Fu, *J. Am. Chem. Soc.,* 2003, 125, 13642-13643 (DOI:10.1021/ja038177r).

199 W. Zhang, X. Zhang and J. Li, *J. Org. Chem.,* 2010, 75, 5259-5264 (DOI:10.1021/jo1010284).

200 G. W. Kabalka, M. Yao and S. Borella, *Org. Lett.,* 2006, 8, 879-881 (DOI:10.1021/ol052957i).

201 Zhang Y., Xiong Q., Yang G., Li M. and ZHANG J., *Analytical Sciences,* 2007, 23, 911.

202 T. H. Vaughn, G. F. Hennion, R. R. Vogt and J. A. Nieuwland, *J. Org. Chem.,* 1937, 02, 1-22 (DOI:10.1021/jo01224a001).

203 J. Clayden, N. Greeves, S. Warren and J. Wothers, *Organic Chemistry*,Oxford University Press, 2004.

204 M. D. Bartberger, J. M. Fukuto and K. N. Houk, *Proc.Nati.Acad.Scie.,* 2001, 98, 2194-2198 (DOI:10.1073/pnas.041481598).

205 J. D. Bradshaw, D. Solooki, C. A. Tessier and W. J. Youngs, *J. Am. Chem. Soc.,* 1994, 116, 3177-3179 (DOI:10.1021/ja00087a001).

206 B. M. Trost and Y. Shi, *J. Am. Chem. Soc.,* 1991, 113, 701-703 (DOI:10.1021/ja00002a064).

207 C. A. Brown and A. Yamashita, *J. Am. Chem. Soc.,* 1975, 97, 891-892 (DOI:10.1021/ja00837a034).

208 S. R. Macaulay, *J. Org. Chem.,* 1980, 45, 734-735 (DOI:10.1021/jo01292a042).

209 T. Kimmel and D. Becker, *J. Org. Chem.,* 1984, 49, 2494-2496 (DOI:10.1021/jo00187a038).

210 C. D. Blue and D. J. Nelson, *J. Org. Chem.,* 1983, 48, 4538-4542 (DOI:10.1021/jo00172a019).

211 G. Westman, O. Wennerström and I. Raston, *Tetrahedron,* 1993, 49, 483-488 (DOI: 10.1016/S0040-4020(01)80315-X).

212 B. E. Maryanoff and A. B. Reitz, *Chem. Rev.,* 1989, 89, 863-927 (DOI:10.1021/cr00094a007).

213 R. J. Anderson and C. A. Henrick, *J. Am. Chem. Soc.,* 1975, 97, 4327-4334 (DOI:10.1021/ja00848a032).

214 A. B. Reitz, S. O. Nortey, A. D. Jordan, M. S. Mutter and B. E. Maryanoff, *J. Org. Chem.,* 1986, 51, 3302-3308 (DOI:10.1021/jo00367a010).

215 B. E. Maryanoff, A. B. Reitz and B. Duhl-Emswiler, *J. Am. Chem. Soc.,* 1985, 107, 217-226 (DOI:10.1021/ja00287a040).

216 R. F. Newton, D. P. Reynolds, C. F. Webb and S. M. Roberts, *J. Chem. Soc. , Perkin Trans. 1,* 1981, 2055-2058.

217 A. K. L. Yuen and C. A. Hutton, *Tetrahedron Lett.,* 2005, 46, 7899-7903 (DOI: 10.1016/j.tetlet.2005.09.101).

218 R. M. Silverstein, F. X. Webster and D. J. Kiemle, *spectrometric identification of organic compounds, John Wily*, 2005.

219 Y. Uenishi, T. Takii, I. Yano and M. Sunagawa, *J. Microbiol. Methods,* 2009, 77, 320-322 (DOI:10.1016/j.mimet.2009.03.006).

220 N. D. Fernandes and P. E. Kolattukudy, *Gene,* 1996, 170, 95-99 (DOI:DOI: 10.1016/0378-1119(95)00842-X).

221 Y. Yuan, R. E. Lee, G. S. Besra, J. T. Belisle and C. E. Barry, *Proce.Nati.Acade. Scie.,* 1995, 92, 6630-6634.

222 C. Theunissen, . Unpublish Ph. D, Thesis, University of Bangor.

223 G. Koza, R. Rowles, C. Theunissen, J. R. Al-Dulayymi and M. S. Baird, *Tetrahedron Lett.,* 2009, 50, 7259-7262 (DOI: 10.1016/j.tetlet.2009.10.009).

224 P. J. Kocienski, *Protecting groups*, G. Thieme, Stuttgart; New York, 1994.

225 J. R. Al Dulayymi, M. S. Baird, E. Roberts, M. Deysel and J. Verschoor, *Tetrahedron,* 2007, 63, 2571-2592 (DOI: 10.1016/j.tet.2007.01.007).

226 F. Yoneda, K. Suzuki and Y. Nitta, *J. Org. Chem.,* 1967, 32, 727-729 (DOI:10.1021/jo01278a049).

227 D. I. Schuster and C. W. Kim, *J. Org. Chem.,* 1975, 40, 505-510 (DOI:10.1021/jo00892a029).

228 J.H. Harris, *PARA-SEMIDINE REARRANGEMENT*, MSc, Thesis, Texas Technological College, 1968.

229 M. Nakatsuka, J. A. Ragan, T. Sammakia, D. B. Smith, D. E. Uehling and S. L. Schreiber, *J. Am. Chem. Soc.,* 1990, 112, 5583-5601 (DOI:10.1021/ja00170a024).

230 M. Miyashita, A. Yoshikoshi and P. A. Grieco, *J. Org. Chem.,* 1977, 42, 3772-3774 (DOI:10.1021/jo00443a038).

231 K. M. George, Y. Yuan, D. R. Sherman and C. E. Barry, *J. Biol. Chem.,* 1995, 270, 27292-27298.

232 Y. Yuan, D. D. Crane and C. Barry 3, *J. Bacteriol.,* 1996, 178, 4484-4492.

233 M. Maza-Iglesias, *Synthesis of Cord Factors*, Ph. D, Thesis, University of Bangor, 2011.

234 M. K. Dhaon, R. K. Olsen and K. Ramasamy, *J. Org. Chem.,* 1982, 47, 1962-1965 (DOI:10.1021/jo00349a028).

235 M. Beukes, Y. Lemmer, M. Deysel, J. R. Al Dulayymi, M. S. Baird, G. Koza, M. M. Iglesias, R. R. Rowles, C. Theunissen, J. Grooten, G. Toschi, V. V. Roberts, L. Pilcher, S. Van Wyngaardt, N. Mathebula, M. Balogun, A. C. Stoltz and J. A. Verschoor, *Chem. Phys. Lipids,* 2010, 163, 800-808 (DOI:10.1016/j.chemphyslip.2010.09.006).

236 Y. Benadie, M. Deysel, D. G. R. Siko, V. V. Roberts, S. Van Wyngaardt, S. T. Thanyani, G. Sekanka, A. M. C. Ten Bokum, L. A. Collett, J. Grooten, M. S. Baird and J. A. Verschoor, *Chem. Phys. Lipids,* 2008, 152, 95-103 (DOI:DOI: 10.1016/j.chemphyslip.2008.01.004).

237 G. Berretta and G. D. Coxon, *Tetrahedron Lett.,* 2012, 53, 214-216 (DOI:10.1016/j.tetlet.2011.11.021).

238 M. Degueil-Castaing, L. Moutet and B. Maillard, *J. Org. Chem.,* 2000, 65, 3961-3965 (DOI:10.1021/jo9918495).

239 W. Oppolzer, R. N. Radinov and E. El-Sayed, *J. Org. Chem.,* 2001, 66, 4766-4770 (DOI:10.1021/jo000463n).

240 M. Guillemineau, S. Singh, M. Grossutti and F. Auzanneau, *Carbohydr. Res.,* 2010, 345, 2723-2730 (DOI:10.1016/j.carres.2010.09.035).

241 Database of NMR spectra, *NMR Predictor*, http://www.nmrdb.org/predictor.

242 T. Hasegawa and R. M. Leblanc, *Biochimica et Biophysica Acta (BBA) - Biomembranes,* 2003, 1617, 89-95 (DOI: 10.1016/j.bbamem.2003.09.008).

243 G. Toschi and M. S. Baird, *Tetrahedron,* 2006, 62, 3221-3227 (DOI: 10.1016/j.tet.2006.01.070).

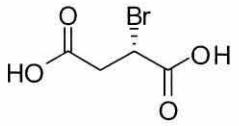
244 G. Koza and M. S. Baird, *Tetrahedron Lett.,* 2007, 48, 2165-2169 (DOI: 10.1016/j.tetlet.2007.01.100).

245 R. Schubert-Rowles, Ph. D Thesis, Bangor University, *The synthesis of mycolic acids*, 2010.

Appendix A

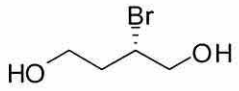
The following experiments were repeats of earlier ones used to produce some key intermediates used in the thesis.

Experiment 1: (*S*)-(-)-Bromosuccinic acid (139)



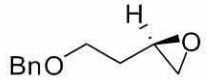
L-Aspartic acid (**137**) (50.03 g, 0.380 mol) and potassium bromide (201.06 g, 1.69 mol) in H_2SO_4 (2.5 M, 1L) was cooled to -5 °C and a solution of sodium nitrite (46.68 g, 680 mmol) in water was added slowly without allow the temperature to exceed 0°C. The colour of the solution became dark brown and it was stirred for 2 hours at -5 °C before being extracted with ethyl acetate (4x500 ml). The combined organic extracts were dried over $MgSO_4$ and filtered to yield the product in the form of a white powder (69.22 g , 351.15 mmol, 93.5 %). physical properties same as the literature.[242]

Experiment 2: (*S*)-2-Bromo-1, 4-butanediol (140)



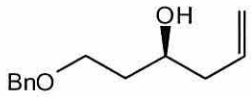
Borane tetrahydrofuran (800 ml, 1M, 800 mmol) was added to (*S*)-(-)-bromosuccinic acid (**139**) (52.46 g, 260 mmol) in dry THF (400 ml) at 0 °C over a period of 1 hour. The reaction mixture was stirred for 4 hours at room temperature. When the reaction was complete, the mixture was re-cooled and quenched by adding THF/water (100 ml, 1:1) drop wise. Calcined K_2CO_3 (160 g) was added and the mixture was stirred and then filtered. The solid residue was washed with ether (3x100 ml) and the filtrates were combined and evaporated to yield an oil and borate salts. The oil was re-dissolved in ether (2x200 ml) before being filtered to remove any borate salts present and then dried over $MgSO_4$. The crude product was concentrated to give a colourless oil before being purified by chromatography over silica gel, eluting with petrol/ethyl acetate (1:2) to obtain a colourless oil (41.66 g, 246.59 mmol, 92 %), $[\alpha]_D^{24}$ -32.07 (c = 1.61, $CHCl_3$) (lit: $[\alpha]_D^{24}$-31.9 (c = 15.2, $CHCl_3$)) .[116] This showed δ_H (500 MHz, $CDCl_3$): 4.27-4.17 (2H, m), 3.83-3.77 (4H, m), 2.13-2.09 (3H, m); δ_C: 66.96, 59.87, 54.39, 37.73; ν_{max}/cm^{-1}: 3343, 2936, 2887, 1421, 1371, 1258, 1166, 1055, 955, 895.

Experiment 3: (*R*)-(2-Benzyloxyethyl)oxirane (141)



Sodium hydride (22.82 g, 60 % dispersion in oil, 571 mmol) was washed with petrol (3x100 ml) and suspended in dry THF (250 ml). Bromodiol **(140)** (32 g, 190 mmol) in dry THF (20 ml) was added over a 5 min. period at -10 °C. The mixture was stirred for 25 min. before adding benzyl bromide (46.28 ml, 190 mmol) followed by *tetra*-butyl ammonium iodide (7.45 g, 11 mmol). The mixture was stirred at -10 °C for 5 min. before the cooling bath was removed and the reaction mixture was allowed to warm to room temperature. The mixture was stirred at room temperature for two hours before cooling to -10 °C and being quenched by addition of sat. aq. NH_4Cl (200 ml). The aqueous layer was extracted with ethyl acetate (3x200 ml), dried over $MgSO_4$, filtered and the solvent was evaporated. The crude product was purified by column chromatography over silica gel, eluting with petrol/ether (5:1) to yield a colourless oil (34.99 g, 196 mmol, 91 %),[116] $[\alpha]_D^{24}$ +17.58 (c = 0.98, $CHCl_3$) (lit: $[\alpha]_D^{24}$ +15.0 (c= 3.37, CH_2Cl_2)). [116] This showed δ_H (500 MHz, $CDCl_3$): 7.29-7.27 (5H, m), 4.45(2H, s), 3.57-3.52(2H, m), 3.00-2.97 (1H, m), 2.68(1H, t, J=5.05 Hz), 2.43(1H, dd, J=5.05, 2.5 Hz), 1.85-1.8(1H, m), 1.73-1.66 (1H, m); δ_C: 138.10, 128.14, 127.34, 125.97, 74.58, 49.75, 47.60, 32.73; ν_{max}/cm^{-1}:3031, 2923, 2861, 1495, 1454, 1362, 1259, 1206, 1104, 1028, 909, 833, 738, 698.

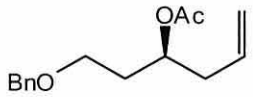
Experiment 4: (*S*)-1-Benzyloxy-hex-5-en-3-ol (142)



Copper iodide (3.2 g, 16.84 mmol) was dissolved in dry THF (300 ml) at room temperature under a nitrogen atmosphere and cooled to –75 °C. Vinylmagnesium bromide (112.2 ml, 112.2 mmol, 1M in THF) was added between –75 °C to –50 °C and the mixture was stirred at –50 °C to –40 °C for 30 min. The reaction mixture was re-cooled to –75 °C and a solution of the (*R*)-(2-benzyloxyethyl)oxirane **(141)** (10 g, 56.14 mmol) in dry THF (100 ml) was added between –75 °C and – 40 °C and the reaction was stirred at – 40 °C to – 30 °C for 1 hour then at – 20 °C for 15 min. Sat. aq. ammonium chloride (400 ml) was added. The mixture was then extracted with ethyl acetate (3x300 ml) and the combined organic layers were washed with water,

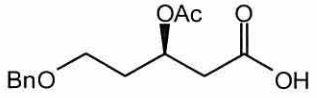
dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (2:1) to give a colourless oil, (*S*)-1-benzyloxy-hex-5-en-3-ol (10.99 g, 53.31 mmol, 95 %),[117] $[\alpha]_D^{27}$ -3.93 (c = 1.05, $CHCl_3$) (lit: $[\alpha]_D^{24}$ – 5.3 (c = 1.2, $CHCl_3$)) {Found *m/z* $[M + Na]^+$: 229.1194,$C_{13}H_{18}NaO_2$ requires : 229.1199}. This showed δ_H (500 MHz, $CDCl_3$): 7.34-7.33 (5H, m), 5.85 (1H, ddt, J=14.2, 10.4, 6.95 Hz), 5.13-5.08 (2H, m), 4.51 (2H, br s), 3.89 (1H, pent., J=6.6 Hz), 3.72-3.68 (1H, m), 3.66-3.61(1H, m), 3.04 (1H,br ,s), 2.25 (2H , t, J = 6.6 Hz), 1.81-1.71 (2H, m); δ_C: 137.84, 134.70, 128.17, 128.24, 127.43, 127.39, 117.16, 72.98, 68.47, 60.10, 41.74, 35.79; ν_{max}/cm^{-1}: 3449 br, 3067, 3030, 2860, 1951, 1640, 1496, 1097, 737, 698.

Experiment 5: Acetic acid (*S*)-1-(2-benzyloxy-ethyl)-but-3-enyl ester (143)



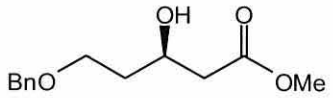
Acetic anhydride (21.3 ml) and then anhydrous pyridine (21.3 ml) were added to a stirred solution of the (*S*)-1-benzyloxy-hex-5-en-3-ol **(142)** (9.3g, 45.11 mmol) in dry toluene (180 ml) at room temperature and the mixture was left stirring for 18 hours. The reaction mixture was diluted with toluene (100 ml) and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ether (6:1) to give a colourless oil, acetic acid (*S*)-1-(2-benzyloxy-ethyl)-but-3-enyl ester (9.7 g, 390.08 mmol, 87 %),$[\alpha]_D^{30}$ +34.89 (c = 1.69, $CHCl_3$) (lit: $[\alpha]_D^{23}$= + 49.0 (c = 1.13, $CHCl_3$))[93] {Found *m/z* $[M + H]^+$: 249.1485,$C_{15}H_{21}O_3$ requires: 249.1485}; This showed δ_H (500 MHz, $CDCl_3$): 7.37-7.33 (5H, m), 5.81 (1H, ddt, J = 14.2, 10.5, 6.95 Hz), 5.14-5.07 (3H, m), 4.49 (2H, d, J = 3.8 Hz), 3.54-3.47 (2H, m), 2.40-2.30 (2H, m), 1.99 (3H, s), 1.92-1.84 (2H, m); δ_C: 170.38, 138.20, 133.37, 128.20, 127.54, 127.51, 117.68, 72.85, 70.58, 66.37, 38.72, 33.61, 21.12; ν_{max}/cm^{-1}: 3066, 3031, 2923, 2861, 1737, 1643, 1497,1454, 1436, 1372, 1240 , 1100, 1026, 997, 918, 790, 698, 605.

Experiment 6: (*R*)-3-Acetoxy-5-benzyloxy-pentanoic acid (144)



Acetic acid (*S*)-1-(2-benzyloxy-ethyl)-but-3-enyl ester **(143)** (8.88 g, 35.78 mmol) was dissolved in dry DMF (450 ml) and oxone (87.99 g, 90.96 mmol) then OsO_4 2.5 % in 2-methyl-2-propanol (4.48 ml, 0.72 mmol) was added at 10 °C. The mixture was allowed to reach 32 °C and stirred 3 hours. The mixture was quenched with water (3 L) and extracted with ethyl acetate (1x500 ml then 2x250 ml). The combined organic layers were washed with water (700 ml), dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (1:1 then 1:2) to give a colourless oil, (*R*)-3-acetoxy-5-benzyloxy-pentanoic acid (8.54 g, 95 %),[117] $[\alpha]_D^{24}$ +13.39 (c = 1.68, $CHCl_3$) (lit: $[\alpha]_D^{22}$ +15.2 (c = 0.89, $CHCl_3$))[93] {Found *m/z* $[M + H]^+$: 267.1216, $C_{14}H_{19}O_5$ requires: 267.1227}. This showed δ_H (500 MHz, $CDCl_3$): 7.35-7.28 (5H, m), 5.35 (1H, pent., J=6.3 Hz), 4.47 (2H, s), 3.55-3.48 (2H, m), 2.68 (1H, d, J = 1.25 Hz), 2.67 (1H, d, J= 1.9 Hz), 1.99 (3H,s), 1.96 (2H, br q, J = 6.25 Hz); δ_C: 175.64, 170.4, 137.93, 128.29, 127.64, 127.61, 73.88, 68.56, 66.09, 38.83, 35.86; ν_{max}/cm^{-1}: 3031, 2931, 2869, 1740, 1680, 1496, 1454, 1414, 1374, 1241, 1176, 1101, 1046, 944, 741, 699.

Experiment 7: (*R*)-5-Benzyloxy-3-hydroxy-pentanoic acid methyl ester (138)



Concentrated H_2SO_4 (70 drops) was added to a stirred solution of (*R*)-3-acetoxy-5-benzyloxy-pentanoic acid **(144)** (10.5 g, 41.9 mmol) in methanol (300 ml) and refluxed for 3.5 hours. When TLC showed that the reaction was complete, the methanol was evaporated and the remaining solution was dissolved in ethyl acetate (250 ml) and sat. aq. $NaHCO_3$ (200 ml). The mixture was extracted and the aq. layer re-extracted with ethyl acetate (2x150 ml) and the combined organic layers were dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (3:2) to give a colourless oil, (*R*)-5-benzyloxy-3-hydroxypentanoic acid methyl ester (7.02 g, 28.06 mmol, 70.2 %), $[\alpha]_D^{28}$ -9.15 (c = 1.58, $CHCl_3$) (lit: $[\alpha]_D^{26}$ –12.2 (c = 1.23, $CHCl_3$)[117] {Found *m/z* $[M + H]^+$: 261.1085,$C_{13}H_{18}NaO_4$ requires: 261.1097}. This showed δ_H (500 MHz, $CDCl_3$):7.35-7.28(5H, m), 4.51(2H, br.s), 4.24 (1H, pent. J= 6.3 Hz), 3.69 (5H, br. s (OCH_3/OCH_2)), 2.5 (2H, d, J=6.3 Hz,), 1.82-1.78(2H, m); δ_C: 172.53, 137.85, 128.19,

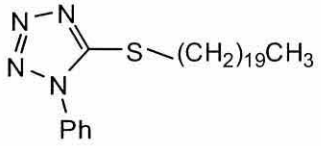
127.45, 127.42, 72.97, 67.64, 66.89, 66.60, 51.40, 41.30; ν_{max}/cm^{-1}: 3459, 3030, 2951, 2863, 1737, 1454, 1438, 1168, 1099, 739, 699.

Experiment 8: 8-Bromooctan-1-ol

$Br(CH_2)_8OH$

1,8-Octanediol (25.00 g, 171.0 mmol) was dissolved in toluene (300 ml) and aqueous hydrobromic acid (30 ml, 44.7 g, 552.4 mmol) was added and the mixture was refluxed for 18 hours, the reaction mixture was monitoring with TLC. The mixture was then cooled to room temperature, the organic layer was separated and the solvent was removed. The organic layer, a brown oil, was dissolved in dichloromethane (500 ml) and washed with sat. aq. sodium hydrogen carbonate (3x50 ml). The aqueous layer was re-extracted with dichloromethane (3x100 ml). The combined organic layers were dried and evaporated to give a crude product which was purified via column chromatography eluting with petrol/ethyl acetate (20:1 then 5:1) to give a yellow oil, 8-bromooctan-1-ol (29.02 g, 81 %), The NMR spectra of the compound obtained were identical to these reported.[243]

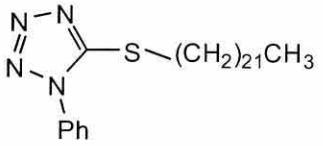
Experiment 9: 5-Docosylsulfanyl-1-phenyl-1*H*-tetrazole



1-Phenyl-1*H*-tetrazole-5-thiol (7.76 g, 43.57 mmol), 1-bromoicosane (15 g, 41.49 mmol), anhydrous potassium carbonate (8.6 g, 62.24 mmol) and acetone (600 ml) were mixed. The mixture was vigorously stirred and refluxed at 60 °C for 15 hours. When TLC indicated complete removal of the thiol, the inorganic salts were filtered off and washed well with acetone. The acetone solution was evaporated to a small bulk and dissolved in dichloromethane (300 ml). The solution was washed with water (250 ml) and the aqueous layer was re-extracted with dichloromethane (2x150 ml). The combined organic phases were washed with water (150 ml), dried and the solvent was evaporated. The crude product was recrystallised from acetone (250 ml) and methanol (2500 ml) to give a white solid, 5-(docosylthio)-1-phenyl-1H-tetrazole (34.5 g, 54.12 mmol, 88 %), m.p. 60-62 °C (lit: m.p. 62–64 °C) [244] {Found *m/z* $[M + Na]^+$: 459.3500, $C_{27}H_{46}N_4S$ requires: 459.3516}. This showed δ_H (500 MHz, $CDCl_3$): 7.75-7.58 (5H, m), 3.39 (2H, t , J= 7.2 Hz), 1.68 (2H, pent., J= 7.25 Hz), 1.44 (2H, pent., J

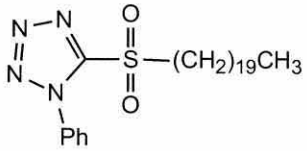
= 7.6 Hz), 1.34-1.22 (32H, br s), 0.89 (3H, t, J= 6.25 Hz,); δ_C: 154.6, 133.82, 130.22, 129.81, 123.92, 33.43, 31.92, 29.67, 29.64, 29.63, 29.55, 29.43, 29.31, 29.12, 28.67, 22.72, 14.15. ν_{max}/cm^{-1}: 2916, 1523, 1472, 1395, 898, 768, 688.

Experiment 10: 5-Docosylsulfanyl-1-phenyl-1*H*-tetrazole



1-Phenyl-1*H*-tetrazole-5-thiol (7.76 g, 43.5 mmol), 1-bromodocosane (25 g, 34.58 mmol), anhydrous potassium carbonate (15 g, 41.498 mmol) and acetone (600 ml) were mixed. The mixture was vigorously stirred and refluxed at 60 °C for 15 hours. When TLC indicated complete removal of the thiol, the inorganic salts were filtered off and washed well with acetone. The acetone solution was evaporated to a small bulk and dissolved in dichloromethane (250 ml). The solution was washed with water (200 ml) and the aqueous layer was re-extracted with dichloromethane (2x150 ml). The combined organic phases were washed with water (200 ml), dried and the solvent was evaporated. The crude product was recrystallised from acetone (90 ml) and methanol (180 ml) to give a white solid, 5-docosylsulfanyl-1-phenyl-1*H*-tetrazole (17.0 g, 37.05 mmol, 89 %), m.p. 66-68 °C (lit : m.p.: 70-72 °C)[222] {Found *m/z* [M + H]$^+$: 487.3837; $C_{29}H_{51}N_4S^+$ requires: 487.3829 This showed δ_H (500 MHz, $CDCl_3$): 7.75-7.62 (5H, m), 3.40 (2H, t , J= 7.2 Hz), 1.76 (2H, pent., J= 7.25 Hz), 1.44 (2H, pent., J= 7.25 Hz), 1.34-1.22 (36H, br), 0.89 (3H, t, J= 6.95 Hz,); δ_C: 154.5, 133.8, 130.12, 129.8, 123.9, 33.4, 31.9, 29.68, 29.65, 29.62, 29.54, 29.44, 29.3, 29.09, 28.65, 22.7, 14.1; ν_{max}/cm^{-1}: 2917, 1501, 1471, 1390, 892, 763, 686.

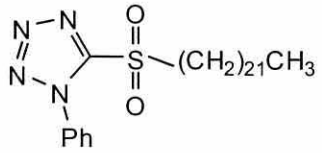
Experiment 11: 5-(Docosane-1-sulfonyl)-1-phenyl-1*H*-tetrazole



3-Chloroperoxy benzoic acid (9.08 g, 377 mmol) in dichloromethane (100 ml) was added to a solution of 5-docosylsulfanyl-1-phenyl-1*H*-tetrazole (16.82 g, 36.6 mmol) and $NaHCO_3$ (13.86 g, 164.0 mmol) in dichloromethane (100 ml) at 0 °C. The reaction mixture was stirred at room temperature overnight before being quenched with a sodium hydroxide solution (200 ml, 5 %) and extracted with dichloromethane

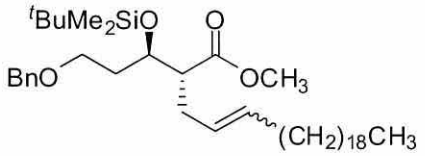
(3 x 200ml). The combined organic layers were dried over $MgSO_4$, filtered and evaporated to give a powder. The crude product was purified by recrystallisation from methanol/ acetone (1:1), to give 5-(docosane-1-sulfonyl)-1-phenyl-1*H*-tetrazole (14.96 g, 30.48 mmol, 83 %),[244] m.p. 65-67-58°C (lit: m.p.: 69–70 °C)[93] {Found *m/z* [M + Na]$^+$: 491.3403, $C_{27}H_{46}O_2S$ requires: 491.3414}. This showed δ_H (500 MHz, $CDCl_3$): 7.72-7.70 (2H, m), 7.64-7.60 (3H, m), 3.74 (2H, destoted t, J=7.91 Hz), 1.96 (2H, br, pent., J = 7.8 Hz), 1.50 (2H, br, pent., J = 7 Hz), 1.36-1.26 (32 H, m), 0.89 (3H, t, J = 6.95 Hz); δ_C: 153.46, 133.45, 131.45, 129.72, 125.08, 56.04, 31.93, 29.7, 29.63, 29.57, 29.46, 29.36, 29.19, 28.9, 28.15, 22.69, 21.95, 14.12; ν_{max}/cm^{-1}: 2913, 2846, 1493, 1461, 1337, 1148, 763, 686.

Experiment 12: 5-(Docosane-1-sulfonyl)-1-phenyl-1*H*-tetrazole



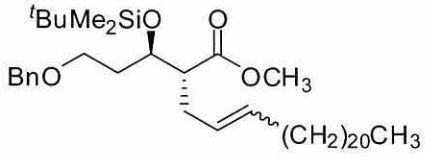
3-Chloroperbenzoic acid (14.2 g, 82.24mmol) in dichloromethane (50 ml) was added to a solution of 5-docosylsulfanyl-1-phenyl-1*H*-tetrazole (20 g, 41.12 mmol) in dichloromethane (250 ml) and $NaHCO_3$ (15.54 g, 185.04 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 18 hours. The reaction was quenched with NaOH solution (200 ml, 5 %). The organic layer was separated and the aqueous layer was re-extracted with dichloromethane (2 x 200 ml). The combined organic layers were dried and evaporated to give a white solid which was re-crystallised from acetone/methanol (200 ml, 1:1) to give a white solid, 5-(docosane-1-sulfonyl)-1-phenyl-1*H*-tetrazole (18.1 g, 85 %),[117] m.p. 55–57 °C (lit: m.p.: 56–59 °C) {Found *m/z* [M + H]$^+$: 519.3706; $C_{29}H_{51}N_4O_2S$ requires: 519.3727}. This showed δ_H (500 MHz, $CDCl_3$): 7.72-7.70 (2H, m), 7.62-7.58 (3H, m), 3.73 (2H, t, J = 6.2 Hz), 1.95-186 (2H, m), 1.55-1.50 (2H, m), 1.43–1.24 (36H, m, chain), 0.88 (3H, t, J = 7.25 Hz); δ_C: 153.54, 133.09, 131.36,129.62, 125.07, 56.04, 31.92, 29.67, 29.65, 29.63, 29.55, 29.41, 29.32, 29.11, 28.83, 28.13, 22.66, 21.90, 14.09; δ_C: 62.38, 33.70, 32.59, 32.45, 29.26, 29.18, 29.15, 28.51, 27.92, 25.53; ν_{max}/cm^{-1}: 2922, 1506, 1476, 1393, 895, 764, 687 ν_{max}: 3019, 2925, 2853, 1496,1344, 1153 cm^{-1}.

Experiment 13: (*E/Z*) 2-[3-Benzyloxy-1-(*tert*-butyldimethylsilanyloxy)propyl]-tetracos-4-enoic acid methyl ester



Lithium bis(trimethyl silyl)amide (16.44 ml, 17.4 mmol) was added to a stirred solution of 5-benzyloxy-3-(*tert*-butyldimethyl-silanyloxy)-2-(2-oxoethyl)pentanoic acid methyl ester (3.82 g, 9.68 mmol) and 5-(docosane-1-sulfonyl)-1-phenyl-1*H*-tetrazole in dry THF at -10 °C. The reaction turned bright yellow and was left to reach room temperature and stirred for one hour under a nitrogen atmosphere. When TLC showed no starting material, the reaction was quenched by adding a sat. aq. NH_4Cl. The product was extracted with petrol/ether (1/2) (3x150 ml) dried over $MgSO_4$, filtered and evaporated and the crude product purified by column chromatography over silica gel, eluting with petrol/ether (20/1) to yield the titled compound (4.92 g, 7.41 mmol, 79 %). {Found *m/z* $[M+H]^+$: 659.5409, $C_{41}H_{75}O_4Si$ requires: 659.5426]. This showed ^{1}HNMR (500MHz, TMS, $CDCl_3$): δ= 7.23-7.2 (5H, m), 5.4-5.32 (1H, m), 5.27-5.21 (1H, m), 4.43 (2H, s), 4.09 (1H,dt, J=11.65, 5.65Hz), 3.58 (3H, s), 3.55-3.52 (2H, m), 2.61-2.54-2.49 (1H, m), 2.2 (2H, m), 1.99-1.75 (2H, m), 1.22 (36H, s),0.85-0.81 (12H,m), 0.00(6H,s). δ_C: 173.98, 138.47, 132.81, 128.29, 127.81, 127.54, 72.88, 70.31, 66.51, 66.26 , 52.44 ,52.13, 51.25, 51.21, 33.61,31.25, 32.51, 31.92, 30.4, 29.96, 29.65, 29.59, 25.5, 22.6 , 17.9, -4.59, -.4.85;[93] ν_{max}/cm^{-1}:2923, 2852, 1738, 1466, 1362, 1254, 1168, 1106.

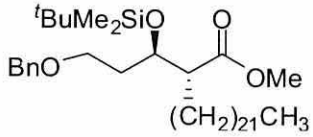
Experiment 14: (*E/Z*)-(*R*)-2-[(*R*)-3-Benzyloxy-1-(*tert*-butyl-dimethyl-silanyloxy)-propyl]-hexacos-4-enoic acid methyl ester



Lithium bis(trimethyl silyl)amide (25.84 ml, 27.39 mmol) was added to a stirred solution of 5-benzyloxy-3-(tert-butyl-dimethyl-silanyloxy)-2-(2-oxo-ethyl)-pentanoic acid methyl ester (6 g, 15.2 mmol) and 5-(docosane-1-sulfonyl)-1-phenyl-1*H*-tetrazole (9.46 g, 18.2 mmol) in dry THF (150 ml) at -10 °C. The reaction turned bright yellow and was left to reach room temperature and stirred for one hour under a

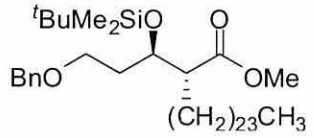
nitrogen atmosphere when TLC show no starting material, then quenched by addition of sat. NH_4Cl. The product was extracted with petrol/ether (1:2, 3x150 ml), dried over $MgSO_4$, filtered and evaporated; the crude prodect purified by column chromotography over silica gel, eluting with petrol/ether (20:1) to give the title compound (4.92 g, 7.41mmol, 65 %). This showed ^{1}HNMR (500MHz, TMS, $CDCl_3$): 7.30-7.20 (5H, m), 5.40-5.32 (1H, m), 5.28-5.18 (1H, m), 4.43 (2H, s), 3.57 (3H, s), 3.52 (1H, dt, J=9.75, 6.3Hz), 2.58 (1H, ddd, J=12.6, 7.9, 6Hz), 2.36-2.20 (2H, m), 1.89 (2H, q, J=6.3Hz), 1.78 (2H, pent., J=5.05Hz), 1.21 (40H, br s), 0.84 (3H, t, J=6.65Hz), 0.81 (9H, s), 0.00 (6H, br s); δ_C: 173.85, 128.19, 127.43, 127.34, 126.80, 66.09, 52.15, 51.16, 33.62, 32.45, 31.86, 30.35, 29.64, 29.60, 29.47, 29.41, 29.30, 29.02, 25.64, 22.61, 17.85, 14.09, -4.68, -4.97;[222] ν_{max}/cm^{-1} : 2935, 2856, 1746, 1466, 1365, 1252, 1102, 1052, 839.

Experiment 15: (*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-3-ethoxypropyl]-tetra-cosanoic acid methyl ester



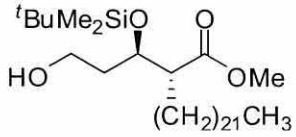
Palladium 10 % on carbon (2.5 g) was added to a stirred solution of (*E/Z*)-(*R*)-2-[(*R*)-3-benzyloxy-1-(*tert*-butyldimethylsilanyloxy)propyl]-tetracosanoic acid methyl ester (5.5 g, 8.34 mmol) in IMS (80 ml) and THF (80 ml). Hydrogenation was carried out for 3 hours. The solution was filtered over a bed of celite and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ether (2:1) to give a colourless oil (4.96 g, 7.52 mmol, 90 %), the title compound (4.96 g, 90 %), $[\alpha]_D^{23}$ –5.42 (c = 1.13, $CHCl_3$) (lit: $[\alpha]_D^{23}$ – 5.4 (c = 1.13, $CHCl_3$))[93] {Found *m/z* $[M + H]^+$: 661.5570, $C_{41}H_{76}O_4Si$ requires: 661.5586}. This showed δ_H (500MHz, $CDCl_3$): 7.33-7.31 (4H, m), 7.28-7.25 (1H, m), 4.44 (2H, br.s), 4.13-4.06 (1H, m), 3.65 (3H, s), 3.59-3.54 (2H, m), 2.52 (1H, ddd, J= 10.4, 6.6, 3.8 Hz), 1.82 (2H, q, J= 6.6 Hz), 1.64-1.51 (2H, m), 1.43-1.15 (39H, br.m), 0.85 (3H, t, J= 7 Hz), 0.82 (9H, s), 0.05 (3H, s), 0.04 (3H, s); δ_C: 174.65, 138.46, 128.25, 127.5, 127.41, 72.86, 70.71, 66.12, 52.00, 51.19, 33.64, 31.92, 29.69, 29.66, 29.65, 29.63, 29.56, 29.43, 29.35, 27.86, 27.22, 25.7, 22.7, 17.9, 14.08, - 4.62, - 4.93; ν_{max}/cm^{-1}: 2928, 2856, 1738, 1665, 1361, 1254, 1192, 1168, 1102.

Experiment 16: (*R*)-2-[(*R*)-3-Benzyloxy-1-(*tert*-butyldimethylsilanyloxy)propyl]-hexacosanoic acid methyl ester



Palladium 10 % on carbon (2.5 g) was added to a stirred solution of (*E*/*Z*)-(*R*)-2-[(*R*)-3-benzyloxy-1-(*tert*-butyldimethylsilanyloxy)propyl]-hexacos-4-enoic acid methyl ester (6.43 g, 9.36 mmol) in THF (80 ml) and IMS (80 ml). Hydrogenation was carried out for 3 hours. The solution was filtered over a bed of celite and the solvent was evaporated to give a crude product which was purified by column chromatography eluting with petrol/ether (20:1) to give a colourless oil, (*R*)-2-[(*R*)-3-benzyloxy-1-(*tert*-butyldimethylsilanyl-oxy)propyl]-hexacosanoic acid methyl ester (6.30 g, 98 %), $[\alpha]_D^{28}$ –6.20 (c = 0.79, $CHCl_3$) (lit: $[\alpha]_D^{28}$ – 6.20 (c = 0.79, $CHCl_3$))[222] {Found *m/z* $[M + H]^+$: 661.5570, $C_{43}H_{80}O_3Si$ requires: 661.5586}. This showed δ_H (500MHz, $CDCl_3$): 7.37–7.28-7.23 (5H, m), 4.55 (2H, s), 4.15 (1H, q, J = 5.1 Hz), 3.67 (3H, s), 3.55–3.52 (2H, m), 2.58 (1H, ddd, J = 3.8, 6.6, 10.4 Hz), 1.86 (2H, q, J = 6.65 Hz), 1.66–1.14 (45H, m, v.br., chain), 0.87 (3H, t, J = 7.25 Hz), 0.85 (9H, s), 0.05 (3H, s), 0.03 (3H, s); δ_C: 174.76, 138.52, 128.31, 127.62, 127.53, 72.92, 70.72, 66.21, 52.23, 51.31, 33.71, 31.92, 29.72, 29.63, 29.55, 29.43, 29.36, 27.92, 27.21, 25.72, 22.73, 17.91, 14.12, - 4.63, - 4.92; ν_{max}/cm^{-1}: 2926, 2853, 1742, 1662, 1463, 1365, 1257, 1198, 1163, 1104.

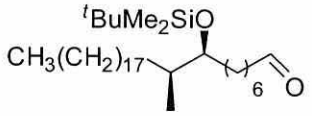
Experiment 17: (*R*)-2-[(*R*)-1-(*tert*-Butyl-dimethylsilanyloxy)-3-hydroxypropyl]-tetra-cosanoic acid methyl ester (145)



Palladium 10 % on carbon (2.5 g) was added to a stirred solution of 2-[3-benzyloxy-1-(*tert*-butyl-dimethylsilanyloxy)-propyl]-tetracosanoic acid methyl ester (4.96 g, 7.52 mmol) in IMS (100 ml) and THF (20 ml). Hydrogenation was carried out for 3 days. The solution was filtered over a bed of celite and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ether (2:1) to give a white solid (4.77 g, 90 %), m.p. 35-37 °C, $[\alpha]_D^{28}$ -1.76 (c = 0.81, $CHCl_3$) (lit:

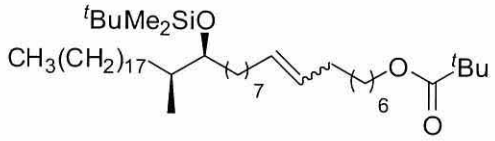
$[\alpha]_D^{22}$ – 8.3 (c = 0.4, C_6H_6))[93] {Found *m/z* $[M + H]^+$: 571.5101, $C_{34}H_{71}O_4Si$ requires: 571.5116}. This showed δ_H (500MHz, $CDCl_3$): 4.29 (1H, td, J = 6, 4.4 Hz), 3.70-3.66 (2H, m) 3.56 (3H, s), 2.66 (1H, ddd, J = 10.5, 6.65, 3.8 Hz), 1.98 (1H, br, s), 1.69-1.65 (2H, m), 1.44-1.39 (1H, m), 1.38-1.32 (1H, m), 1.21-1.08 (40H, m), 0.78 - 0.72 (12H, including a singlet at δ 0.77), 0.00 (3H, s), -0.04 (3H, s); δ_C: 174.66, 72.01, 59.47, 51.39, 35.23, 31.92, 29.69, 29.66, 29.64, 29.61, 29.56, 29.55, 29.43, 29.34, 27.85, 27.16, 25.68, 22.67, 17.84, 14.08, -4.35, -4.99; ν_{max}/cm^{-1}: 3435, 2926, 2854, 1739, 1470, 1443, 1258, 1174, 1093, 836.

Experiment 18: (8*S*,9*S*)-8-(*tert*-Butyldimethylsilanyloxy)-9-methylheptacosanal (360)



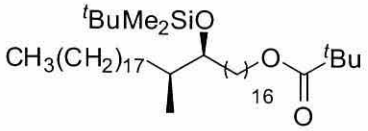
(8*S*,9*S*)-8-(*tert*-Butyldimethylsilanyloxy)-9-methylheptacosan-1-ol (**359**) (5.5 g, 10.17 mmol) in dichloromethane (20 ml) was added at room temperature to a stirred solution of PCC (5.48 g, 25.43 mmol) in dichloromethane (200 ml). During the addition a black colour appeared. The reaction was stirred at room temperature for 2 hours, when TLC showed the reaction was complete, the reaction mixture was poured in to petrol/ethyl acetate (5:1) and filtered through a bed of silica gel. The solvent was evaporated and the crude product purified by coloumn chromatography eluting petrol/ethyl acetate (10/1) to give (*8S,9S*)-8-(*tert*-butyldimethylsilanyloxy)-9-methylheptacosanal (**360**) (5.35 g, 97 %), $[\alpha]_D^{25}$ –8.44 (c = 1.01, $CHCl_3$) (lit: $[\alpha]_D^{25}$ – 8.02 (c = 1.71, $CHCl_3$)[223] {Found *m/z* $[M + Na]^+$: 561.5037, $C_{34}H_{70}NaO_2Si$ requires: 561.5020}. This showed δ_H (500 MHz, $CDCl_3$): 9.76 (1H, t, J = 1.65 Hz), 3.52-3.48 (1H, m), 2.54 (1H, dt, J = 6.2, 3.54 Hz), 1.57 (2H, quintet, J = 7.5 Hz), 1.49–1.34 (43H, v br m), 1.09–1.02 (1H, m), 0.92–0.87 (12H, s and t, J = 6.95 Hz), 0.81 (3H, d, J = 6.65 Hz), 0.04 (3H, s), 0.02 (3H, s); δ_C: 202.9, 75.95, 63.15, 37.75, 33.54, 32.81, 32.41, 31.91, 30.12, 29.95, 29.95, 29.67, 29.54, 29.42, 27.75, 26.54, 25.95, 25.77, 22.72, 18.21, 14.54, 14.15, - 4.21, - 4.4; ν_{max}/cm^{-1}: 3328, 2927, 2858, 1467, 1374, 1255, 1052.

Experiment 19: (*16S,17S*)-16-(*tert*-Butyldimethylsilyloxy)-17-methylpentatriacont -7-enyl pivalate (362)



Lithium bis(trimethylsilyl)amide (11.35 ml, 8.7 mmol, 1.06 M in THF) was added to a stirred solution of (8*S*,9*S*)-8-(*tert*-butyldimethylsilanyloxy)-9-methylheptacosanal (**360**) (3.62 g, 6.7 mmol) and 9-((1-phenyl-1H-tetrazol-5-yl)sulfonyl)nonyl pivalate (**361**) (3.8 g, 6.7 mmol) in dry THF (50 ml) at -5 °C. The reaction turned bright yellow and was left to reach room temperature, and stirred for one hour under a nitrogen atmosphere when TLC showed no starting material was left. The reaction was quenched by addition of sat.aq. NH_4Cl (50 ml). The product was extracted with petrol/ethyl acetate (5:1, 3x150 ml). The combined organic layers were dried over $MgSO_4$, filtered and evaporated. The crude product purified with column chromatography eluting petrol/ethyl acetate (20:1), to give 2,2-dimethylpropionic acid (*E/Z*)-(18S,19S)-18-(*tert*-butyldimethylsilanyloxy)-19-methyl-heptatriacont-10-enyl ester (**360**) (4.05 g, 5.39 mmol). The product was hydrogenated without characterisation.

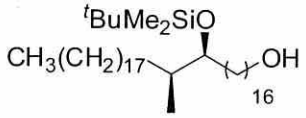
Experiment 20: 2,2-Dimethylpropionic acid (17*S*,18*S*)-17-(*tert*-butyldimethyl-silanyl-oxy)-18-methylhexatriacontyl ester (363)



Palladium 10 % on carbon (0.5 g) was added to a stirred solution of the (16S,17S)-16-(*tert*-butyldimethylsilyloxy)-17-methylpentatriacont-7-enyl pivalate (**362**) (4.05 g, 5.39 mmol) in IMS (80 ml) and THF (20 ml). Hydrogenation was carried out 1 hour. The solution was filtered over a bed of celite and the solvent was evaporated to give a crude product which was purified by column chromatography eluting petrol/ethyl acetate(20:1), to obtain a colourless oil, 2,2-dimethylpropionic acid (17*S*,18*S*)-17-(*tert*-butyldimethyl-silanyloxy)-18-methyl-hexatriacontyl ester (3.95 g, 97 %), $[\alpha]_D^{25}$ – 5.42 (c = 1.23, $CHCl_3$) (lit: $[\alpha]_D^{28}$ - 7.01 (c 1.61, $CHCl_3$)[245] {Found *m/z* $[M + Na]^+$: 773.7168, $C_{48}H_{98}NaO_3Si$ requires: 773.7177}. This showed δ_H (500 MHz, $CDCl_3$):

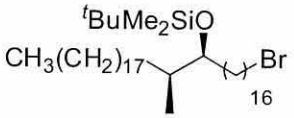
4.05 (2H, t, J = 6.6 Hz), 3.50 (1H, dt, J = 6.3, 3.5 Hz), 1.62 (2H, q, J = 7.55 Hz), 1.25 (63H, br s), 1.20 (9H, s), 0.89 (9H, s),0.87 (3H, t, J = 6.95 Hz), 0.81 (3H, d, J = 6.65 Hz), 0.03 (3H, s), 0.02 (3H, s); δ_C: 178.63, 75.87, 64.45, 60.37, 38.71, 37.72, 33.53, 32.48, 31.92, 30.00, 29.88, 29.69, 29.65, 29.57, 29.52, 29.35, 29.23, 28.61, 27.70, 27.19, 25.95, 25.91, 22.68, 22.60, 21.02, 18.17, 14.19, -4.19, -4.43; ν_{max}/cm^{-1}: 2924, 2853, 1732, 1462, 1153, 835, 772.

Experiment 21: (*17S,18S*)-17-(*tert*-Butyldimethylsilanyloxy)-18-methyl-hexatri-acontan-1-ol (364)



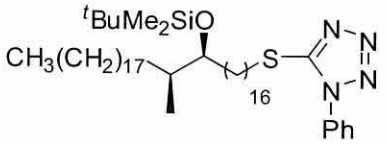
2,2-Dimethylpropionic acid (17*S*,18*S*)-17-(*tert*-butyldimethyl-silanyloxy)-18-methyl-hexa-triacontyl ester (**363**) (3.95 g, 5.25 mmol) in THF (10 ml) was added dropwise over 5 min to a suspension of lithium aluminium hydride (0.4 g, 10.5 mmol) in THF (40 ml) at 0 °C. The reaction mixture was allowed to reach room temperature and refluxed for 1 hour, when TLC showed the no starting material was left, then cool to 0 °C and sat. aq. sodium sulfate dehydrate was added until a white preciptate formed. THF (50 ml) was added and the mixture was stirred at room temperature for 30 min, filtered through a bed of silica gel and the solvent was evaporated. The crude product was purified by column chromatography eluting petrol/ethyl acetate (5:2) to give a colourless oil, (17*S*,18*S*)-17-(*tert*-butyldimethylsilanyloxy)-18-methyl-hexatriacontan-1-ol (**364**) (3.32 g, 95 %), $[\alpha]_D^{24}$ –5.4 (c = 1.01, $CHCl_3$) (lit: $[\alpha]_D^{21}$ -2.07 (c = 0.743)) {Found *m/z* $[M + Na]^+$: 689.6622, $C_{43}H_{90}NaO_2Si$ requires: 689.6602}. This showed δ_H (500 MHz, $CDCl_3$): 3.64 (2H, t, J = 6.65 Hz), 3.50 (1H, dt, J = 6.2, 3.45 Hz), 1.57 (2H, q, J =7.25 Hz), 1.26 (64H, s), 0.89 (9H, s), 0.87 (3H, t, J = 6.9 Hz), 0.80 (3H, d, J= 7.6 Hz), 0.03 (3H, s), 0.009 (3H, s); δ_C: 75.87, 63.08, 60.37, 37.72, 33.53, 32.82, 32.48, 31.92, 30.00, 29.88, 29.69, 29.63, 29.61, 29.44, 29.36, 27.70, 25.95, 25.92, 25.74, 22.68, 21.02, 18.17, 14.18, -0.4.19, -4.43; ν_{max}/cm^{-1}: 3323, 2924, 2853, 1464, 1378, 1252, 1057, 835, 772, 720.

Experiment 22: [(1*S*,2*S*)-1-(16-Bromo-hexadecyl)-2-methyl-eicosyloxy]-*tert*-butyldi-methylsilane (365)



N-Bromosuccinimide (1.14 g, 6.4 mmol) was added in portions over 15 min. to a stirred solution of (17*S*,18*S*)-17-(*tert*-butyldimethylsilanyloxy)-18-methyl-hexatri-acontan-1-ol (**364**) (3.3 g, 4.9 mmol) and triphenylphosphine (1.62 g, 6.1 mmol) in dichloromethane (70 ml) at 0 °C. The reaction mixture was stirred at room temperature for one hour then TLC showed no starting material was left. The reaction was quenched with sat. aq. sodium meta-bisulfate (50 ml). The organic layer was separated and the aqueous layer was re-extracted with dichloromethane (2x30 ml). The combined organic layers were washed with water, dried and evaporated to give a residue. The residue was treated with petrol (150 ml) and refluxed for 30 min, then the triphenylphosphine oxide was filtered off and washed with ether (50 ml). The filtrate was evaporated and the residue was purified by column chromatography eluting with petrol/ether (20:1) to give a colourless oil of the title compound (3.1 g, 86 %),[93] $[\alpha]_D^{23}$–5.2 (c = 1.08, $CHCl_3$) (lit: $[\alpha]_D^{25}$ -6.78 (c = 0.953, $CHCl_3$)).[245] This showed δ_H (500 MHz, $CDCl_3$): 3.51 (1H, dt, J = 6.3, 3.5 Hz), 3.41 (2H, t, J = 6.95 Hz), 1.86 (2H, q, J = 6.95 Hz), 1.27(63H, br s), 0.90 (9H, s), 0.87 (3H, t, J = 6.25 Hz), 0.81 (3H, d, J =7.2 Hz), 0.04 (3H, s), 0.03 (3H, s); δ_C: 133.81, 133.65, 128.68, 128.49, 128.44, 75.88, 41.36, 37.74, 33.94, 33.56, 32.86, 32.51, 31.94, 30.01, 29.90, 29.71, 29.69, 29.68, 29.65, 29.56, 29.46, 29.37, 29.07, 28.79, 28.20, 27.72, 27.67, 25.97, 25.93, 22.70, 22.61, 20.45, 19.43, 18.18, 14.31, 14.11, -4.1, -4.42; ν_{max}/cm^{-1}: 2924, 2852, 1462, 1377, 1251, 1071, 835, 772, 720.

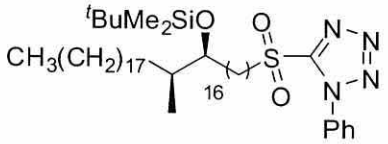
Experiment 23: 5-[(*17S,18S*)-17-(*tert*-Butyldimethylsilanyloxy)-18-methyl-hexa-triacontylsulfanyl]-1-phenyl-1*H*-tetrazole (366)



[(*1S,2S*)-1-(16-Bromo-hexadecyl)-2-methyl-icosyloxy]-*tert*-butyldimethylsilane (**365**) (3.1 g, 4.25 mmol),1-phenyl-1H-tetrazol-5-thiol (0.9 g, 5.1 mmol), potassium carbonate (0.99 g, 7.23 mmol) and acetone (50 ml) were mixed at room temperature.

The mixture was vigorously stirred for 18 hours at room temperature. When TLC indicated complete reaction of the thiol, the inorganic salts were filtered off and washed well with acetone. The acetone was evaporated and the residue was diluted with CH_2Cl_2 (50 ml) and water (30 ml). The organic layer was separated and the aqueous layer was re-extracted with the same solvent (2x30 ml). The combined organic layers were dried and evaporated to give a pale yellow viscous oil which was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a colourless viscous oil, 5-[(17*S*,18*S*)-17-(*tert*-butyl-dimethyl-silanyloxy)-18-methyl-hexatriacontylsulfanyl]-1-phenyl-1*H*-tetrazole (**366**) (3.14 g, 89 %), $[\alpha]_D^{22}$ –4.08 (c = 1.25, $CHCl_3$) (lit: $[\alpha]_D^{24}$ -7.03 (c = 1.68, $CHCl_3$))[245] {Found *m/z* $[M + Na]^+$: 849.6838, $C_{50}H_{94}N_4SSiONa$ requires: 850.4592}. This showed δ_H (500 MHz, $CDCl_3$): 7.60-7.53 (5H, m), 3.50 (1H, dt, J = 6.3, 3.45 Hz), 3.39 (2H, t, J = 7.55 Hz), 1.82 (2H, q, 7.25 Hz), 1.50-1.41 (6H, m), 1.26 (67H, br s), 0.88 (9H, s), 0.87 (3H, t, J = 6.2 Hz), 0.81 (3H, d, J = 7.25 Hz), 0.03 (3H, s), 0.02 (3H, s); δ_C: 154.47, 133.78, 130.00, 129.72, 123.382, 75.74, 60.34, 37.71, 33.52, 33.37, 32.47, 31.91, 29.98, 29.87, 29.69, 29.54, 29.43, 29.34, 29.07, 29.02, 28.64, 27.69, 27.64, 25.95, 25.91, 22.67, 22.59, 21.00, 19.41, 18.15, 14.29, 14.17, -4.21, -4.45; ν_{max}/cm^{-1}: 2926, 2855, 1599, 1501, 1464, 1386, 1250, 1073, 835, 773.

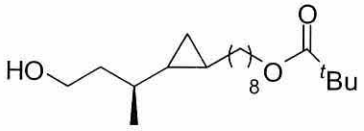
Experiment 24: 5-[(*17S,18S*)-17-(*tert*-Butyldimethylsilanyloxy)-18-methyl-hexa-triacontylsulfon- yl]-1-phenyl-1*H*-tetrazole (352)



A solution of ammonium heptamolybdate (VI) tetrahydrate (2.11 g, 1.7 mmol) in an ice cold hydrogen peroxide (35 %) (w/w) (21 ml) was added to a stirred solution of 5-[(*17S,18S*)-17-(*tert*-butyl-dimethyl-silanyloxy)-18-methyl-hexatriacontylsulfanyl]-1-phenyl-1*H*-tetrazole (**366**) (3.14 g, 3.79 mmol) in methylated spirt (50 ml) and THF (20 ml) at 5 °C and stirred for 2 hours. A further solution of ammonium heptamolybdate (VI) tetrahydrate (1.05 g, 0.85 mmole) in ice cold H_2O_2 (35 % w/w, 42 ml) was added at room temperature, stirred for 16 hours., then poured into 250 ml of water and extracted with dichloromethane (3 × 25 ml). The combined organic phases were washed with water (2 × 30 ml), dried and evaporated to give a residue

which was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give a white solid, 5-[(*17S,18S*)-17-(*tert*-butyldimethylsilanyloxy)-18-methyl-hexatriacontylsulfonyl]-1-phenyl-1*H*-tetrazole (**352**) (3.17 g, 97 %), $[\alpha]_D^{22}$ – 3.74 (c = 1.15, $CHCl_3$) (lit: -6.23 (c = 1.23, (CHC_3))[245] {Found *m/z* $[M + Na]^+$: 881.6722, $C_{50}H_{94}N_4O_3SSiNa$ requires: 882.458}. This showed δ_H (500 MHz, $CDCl_3$): 7.71-7.69 (2H, m), 7.63-7.60(3H, m), 3.73(2H, t, J = 7.85 Hz), 3.50 (1H, dt, J = 3.45 Hz), 1.98-1.92 (3H, m), 1.51-1.42(4H, pent., J = 6.95Hz), 1.26 (58H, br s), 0.88-0.84(12H, br m, including terminate CH_3), 0.80 (3H, d, J = 7.52 Hz), 0.03 (3H, s), 0.02 (3H, s); δ_C: 153.49, 133.06, 131.40, 129.67, 125.05, 56.00, 37.71, 33.52, 32.47, 31.90, 29.98, 29.87, 29.68, 29.63, 29.56, 29.44, 29.34, 29.18, 28.88, 28.13, 25.94, 25.91, 22.66, 20.99, 18.15, 14.16, 14.08, -4.21, -4.45; ν_{max}/cm^{-1}: 2924, 2852, 1498, 1462, 1342, 1251, 1152, 1074, 835, 772, 687.

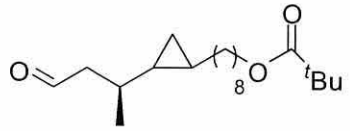
Experiment 25: 2,2-Dimethyl-propionic acid 8-[(1*S*,2*R*)-2-((*S*)-3-hydroxy-1-methyl- propyl)-cyclopropyl]-octyl ester (368)



Tetra-n-butylammonium fluoride (1.55 ml, 5.3mmol, 1M sol. in THF) was added to a stirred solution of (**367**) 8-{2-[(2*S*)-4-[(*tert*-butyldiphenylsilyl)oxy]butan-2-yl]cyclo-propyl}octyl 2,2-dimethylpropanoate (2.33 g, 4.12 mmol) in dry tetrahydrofuran (25 ml) at 0 °C under a nitrogen atmosphere. The mixture was allowed to reach room temperature and stirred for 18 hours. When TLC showed no starting material, the mixture was diluted with petrol/ethyl acetate (1:1, 25 ml), cooled to 5 °C and quenched with sat. aq. ammonium chloride (10 ml) then extracted. The water layer was re-extracted with petrol/ethyl acetate (1:1, 2x25 ml), the combined organic layers were washed with brine (75 ml), dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (5:1) to give 2,2-dimethyl-propionic acid 8-[2-(3-hydroxy-1-methyl-propyl)-cyclopropyl]-octyl ester (**368**) (1.02 g, 76 %), $[\alpha]_D^{25}$ +7.61 (c = 1.31, $CHCl_3$) ($[\alpha]_D^{22}$ + 5.67 (c = 1.21, $CHCl_3$))[245] {Found *m/z* $[M + Na]^+$: 349.2699, $C_{20}H_{38}O_3Na$ requires: 349.5139}. This showed δ_H (500 MHz, $CDCl_3$): 4.04 (2H, t, J = 6.65 Hz), 3.75-3.67 (2H, m), 1.70 (1H, sext., J = 6.85 Hz), 1.62 (3H, q, J = 6.55 Hz), 1.54 (1H, q, J = 6.95 Hz), 1.45 (1H, q, J

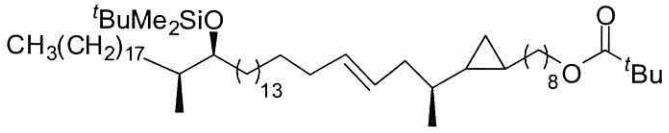
= 6.65 Hz), 1.38 (1H, q, J = 7.25Hz), 1.34-1.27 (11H, br m), 1.18 (9H, s), 0.94 (3H, d, J = 6.65 Hz), 0.7-0.64 (1H, m), 0.34-0.27 (1H, m), 0.08-0.03 (2H, m); δ_C: 178.68, 64.42, 40.33, 38.69, 34.93, 34.29, 29.50, 29.45, 29.42, 29.15, 28.54, 27.16, 26.53, 25.88, 25.83, 19.78, 18.65, 10.58; ν_{max}/cm^{-1}: 3386, 2927, 2856, 1731, 1480, 1461, 1367, 1286, 1158, 1055.

Experiment 26: 2,2-Dimethylpropionic acid 8-[(1*S*,2*R*)-2-((*S*)-1-methyl-3-oxopropyl)-cyclopropyl]-octyl ester (369)



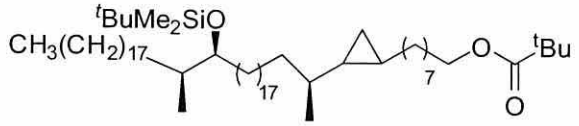
2,2-Dimethylpropionic acid 8-[(1*S*,2*R*)-2-((*S*)-3-hydroxy-1-methylpropyl)-cyclopropyl]-octyl ester (**368**) (1.02 g, 3.12 mmol) in dichloromethane (5 ml) was added at room temperature to a stirred solution of PCC (1.68 g, 7.8 mmol) in dichloromethane (20 ml). During the addition a black colour appeard. The reaction was stirred at room temperature for 2.5 hours, when TLC showed the reaction was complete, the reaction mixture was poured into petrol/ethyl acetate (1:1, 20 ml) and filtered through a bed of silica gel. 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, 2,2-dimethyl-propionic acid 8-[(1*S*,2*R*)-2-((*S*)-1-methyl-3-oxopropyl)-cyclopropyl]-octyl ester (**369**) (0.8 g, 80 %), $[\alpha]_D^{24}$ +20.93 (c = 1.12, $CHCl_3$) (lit: $[\alpha]_D^{26}$ +15.41 (c = 0.98, $CHCl_3$)[245] {Found *m/z* $[M + Na]^+$: 347.2535, $C_{20}H_{36}O_3Na$ requires: 324.498}. This showed δ_H (500 MHz, $CDCl_3$): 9.78 (1H, dd, J = 2.2, 2 Hz), 4.04 (2H, t, 6.6 Hz), 2.52 (1H, ddt, J = 6.3, 2.9,1.95 Hz), 2.38 (1H, ddt, 9.44, 7.55,1.9 Hz), 1.61 (2H, q, J = 6.6 Hz), 1.33-1.28 (13H, m), 1.19 (9H, s),1.03 (3H, d, J = 6.6 Hz), 0.49 (1H, dt, J = 1.9, 6.3 Hz), 0.34-0.31 (1H, m), 0.30-0.26 (1H, m), 0.25-0.2 (1H, m); δ_C: 202.87, 178.62, 64.42, 51.42, 38.71, 34.04. 33.87, 29.53, 29.47, 29.43, 29.20, 28.59, 25.88, 25.58, 19.95, 18.78, 11.39, -0.02; ν_{max}/cm^{-1}: 2922, 2853, 1729.

Experiment 27: 8-(2-((*2S,21S,22S,E,Z*)-21-(*tert*-Butyldimethylsilyloxy)-22-methyl-tetracont-4-en-2-yl)cyclopropyl)octyl pivalate (370)



Lithium bis(trimethylsilyl)amide (3.6 ml, 35.89 mmol, 1.06 M) was added to a stirred solution of 5-[(17*S*,18*S*)-17-(*tert*-butyldimethylsilanyloxy)-18-methyl-hexatri-acontyl-sulfonyl]-1-phenyl-1*H*-tetrazole (**352**) (2.33 g, 2.716 mmol) and 2,2-dimethyl-propionic acid 8-[(1*S*,2*R*)-2-((*S*)-1-methyl-3-oxopropyl)-cyclopropyl]-octyl ester (**369**) (0.8 g, 2.47 mmol) in dry THF (30 ml) at -5 °C. The reaction turned bright yellow and was left to reach room temperature and stirred for one hour under a nitrogen atmosphere when the TLC showed no starting material was left and the reaction was quenched by addition of sat.aq. ammonium chloride (15 ml). The product was extracted with petrol/ethyl acetate (10:1, 3x10 ml). The combined organic layers were washed with brine (20 ml), dried and evaporated to give an oil, which was purified via column chromatography eluting with petrol/ethyl acetate (20:1) to give the title compound (2.01 g, 85 %) {Found *m/z* $[M + Na]^+$: 980.0336, $C_{63}H_{124}O_3SiNa$ requires: 979.9217}. This showed δ_H (500 MHz, $CDCl_3$): 5.39-5.36 (2H, m), 4.02 (2H, t, J = 6.6 Hz), 3.48 (1H, dt, J = 6.3, 3.9 Hz), 2.15-2.08 (1H, m), 1.99-1.8 (4H, m), 1.58 (4H, q, J = 6.95 Hz), 1.23 (93H, br s), 1.2 (3H, d, J = 7.25 Hz), 0.80 (3H, d, H = 7.12 Hz), 0.46-0.41 (1H, m), 0.26-0.18 (1H, m), 0.17-0.14 (1H, m), 0.13-0.09 (1H, m), 0.00 (3H, s), -0.03 (3H, s); δ_C: 178.61, 131.42, 130.42, 128.85, 128.40, 64.45, 60.37, 41.36, 40.35, 38.84, 38.72, 37.74, 36.08, 34.72, 34.67, 34.52, 34.39, 33.55, 32.68, 32.51, 31.94, 31.59, 30.01, 29.90, 29.71, 29.61, 29.56, 29.51, 29.36, 29.26, 29.22, 29.06, 28.64, 27.72, 27.29, 27.20, 26.92, 25.96, 25.93, 25.81, 25.71, 25.28, 22.69, 22.65, 22.61, 20.69, 19.28, 19.21, 18.75, 18.54, 18.17, 14.41, 14.19, 14.11, 11.42, 10.80, 10.73, -4.19, -4.43; ν_{max}/cm^{-1}: 2924, 2853, 1732, 1462, 1366, 1283, 1252, 1154, 1070, 967, 835, 772, 720. [245]

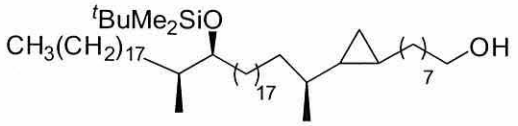
Experiment 28: 2,2-Dimethyl-propionic acid 8-{(1*S*,2*R*)-2-[(1*S*,20*S*,21*S*)-20-(*tert*-butyl-dimethylsilanyloxy)-1,21-dimethyl-nonatriacontyl]-cyclopropyl}-octyl ester (371)



Dipotassium azodicarboxylate (5 g, 25.77 mmol) was added to a stirred solution of 8-(2-((*2S,21S,22S,E,Z*)-21-(*tert*-butyldimethylsilyloxy)-22-methyltetracont-4-en-2-yl)cyclo-propyl) octyl pivalate (**370**) (2.01 g, 2.1 mmol) in THF (30 ml) and methanol (5 ml) at 10 °C. A solution of glacial acetic acid (1 ml) and THF (2 ml) was prepared

and (0.2 ml) was added dropwise every 25 min. at 5 °C and the mixture was stirred at room temperature. After two days the reaction mixture was added slowly in portions to sat. aq. sodium hydrogen carbonate (15 ml) and extracted with petrol/ethyl acetate (10:1, 3x25 ml). The combined organic layers were washed with water (10 ml), dried and evaporated to give thick oil which slowly solidified. The residue was purified via column chromatography eluting in petrol/ethyl acetate (20:1) to give a colourless oil, 2,2-dimethyl-propionic acid 8-{(1*S*,2*R*)-2-[(1*S*,20*S*,21*S*)-20-(*tert*-butyldimethyl-silanyloxy)-1,21-dimethyl-nonatriacontyl] -cyclopropyl}-octyl ester (**371**) (1.8 g, 92 %) {Found *m/z* $[M + Na]^+$: 981.9379, $C_{63}H_{126}O_3SiNa$ requires: 982.7582}. This showed δ_H (500 MHz, $CDCl_3$): 4.05 (2H, t, J = 6.6 Hz), 3.5 (1H, dt, J = 3.45, 5.95 Hz), 1.62 (2H, q, J = 6.65 Hz), 1.26 (83H, br s), 1.20 (9H, s), 0.908 (3H, d, J = 6.95 Hz), 0.9 (3H, t, J = 3.15 Hz), 0.89 (9H, br s), 0.79 (3H, d, J = 6.6 Hz), 0.68-0.61 (1H, m), 0.45-.039 (1H, m), 0.18-0.06 (3H, m), 0.03 (3H, s), 0.02 (3H, s); δ_C: 178.57, 75.86, 64.43, 60.34, 38.70, 38.11, 37.72, 37.43, 34.47, 33.55, 32.51, 31.94, 30.08, 30.01, 29.90, 29.71, 29.60, 56, 29.49, 29.37, 29.26, 28.64, 27.72, 27.28, 27.20, 26.91, 26.14, 25.96, 25.93, 22.69, 21.00, 19.67, 18.59, 18.16, 14.41, 14.19, 14.11, 10.49, -4.19, -4.43; ν_{max}/cm^{-1}: 2926, 2855, 1741, 1469, 1287, 1254, 1152, 1077.[245]

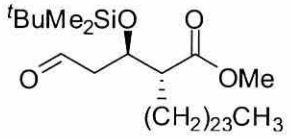
Experiment 29: 8-{(1*S*,2*R*)-2-[(1*S*,20*S*,21*S*)-20-(*tert*-Butyldimethylsilanyloxy)-1,21-dimethyl-nonatriacontyl]-cyclopropyl}-octan-1-ol (372)



2,2-Dimethyl-propionic acid 8-{(1*S*,2*R*)-2-[(1*S*,20*S*,21*S*)-20-(*tert*-butyldimethyl-silanyl-oxy)-1,21-dimethyl-nonatriacontyl]-cyclopropyl}-octyl ester (**371**) (1.8 g, 1.9 mmol) in THF (8 ml) was added dropwise over 15 min to a suspension of lithium aluminium hydride (0.14 g, 3.8 mmol) in THF (30ml) at -10 °C. The mixture was allowed to reach room temperature and refluxed for 1 hour, when TLC showed no starting material was left, then cooled to -10 °C and sat. aq. sodium sulfate was added until a white precipitate formed. The resultant mixture was stirred for 30 min and then filtered through a bed of silica gel and the solvent evaporated. The product was purified via column chromatography eluting with petrol/ethyl acetate (5:1) to give a colourless oil of 8-{(1*S*,2*R*)-2-[(1*S*,20*S*,21*S*)-20-(*tert*-butyl-dimethyl-silanyloxy)-1,21-di-methyl-nonatriacontyl]-cyclopropyl}-octan-1-ol (**372**) (1.03 g, 1.19 mmol, 82 %),

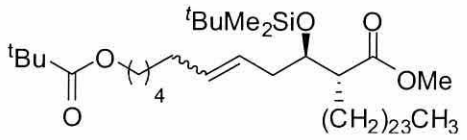
$[\alpha]_D^{22}$ - 0.81 (c = 1.69, $CHCl_3$) (lit: $[\alpha]_D^{28}$-1.73 (c= 0.97. $CHCl_3$)).[245] This showed δ_H (500 MHz, $CDCl_3$): 3.64 (2H, t, J = 6.65 Hz), 3.50 (1H, ddd, J = 3.45, 6, 9.45 Hz), 1.57 (2H, q, J = 6.65Hz), 1.26 (84H, br s), 0.91 (3H, d, J = 6.25 Hz), 0.90 (3H, t, J = 3.45Hz), 0.89 (9H, s), 0.81(3H, d, J = 6.6Hz), 0.66 (1H, q, J=6.9 Hz), 0.46-0.42 (1H, m) 0.22-0.1 (1H, m), 0.16-0.13 (1H, m), 0.12-0.09 (1H, m), 0.03 (3H, s), 0.02 (3H, s); δ_C: 75.87, 63.08, 60.37, 38.12, 37.73, 37.43, 34.47, 33.55, 32.83, 32.50, 31.94, 30.09, 30.01, 29.90, 29.74, 29.71, 29.67, 29.66, 29.62, 29.53, 29.46, 29.37, 27.72, 27.28, 26.92, 26.15, 25.96, 25.93, 25.76, 22.70, 22.65, 19.67, 18.60, 18.17, 14.41, 14.19, 14.11, 10.49, -4.19, -4.43; ν_{max}/cm^{-1}: 3329, 2926, 2851, 1468, 1254, 1056.

Experiment 30: (*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-3-oxopropyl]-hexacosanoic acid methyl ester (373)



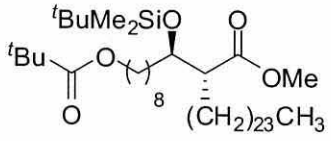
(*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-3-hydroxypropyl]-hexacosanoic acid methyl ester (**146**) (4.39 g, 7.32 mmol) in dichloromethane (20 ml) was added at room temperature to a stirred solution of PCC (3.94 g, 18.32 mmol) in dichloromethane (300 ml). The mixture was stirred at room temperature for 2.5 hours, when TLC showed the reaction was complete, thenpoured into (70 ml) petrol/ethyl acetate (1:1) and filtered through a bed of silica gel. The solvent was evaporated and the crude product was purified by column chromatography eluting with petrol and ether (4:1) to give a colourless oil, (*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilanyloxy)-3-oxopropyl]-hexacosanoic acid methyl ester (4.05 g, 6.78 mmol, 92 %), $[\alpha]_D^{28}$ –3.95 (c = 1.12, $CHCl_3$) (lit: $[\alpha]_D^{28}$ –4.42 (c = 1.29, $CHCl_3$))[222] {Found *m/z* $[M + Na]^+$: 591.4774, $C_{36}H_{72}O_4Si$ requires: 591.4778}. This showed δ_H (500 MHz, $CDCl_3$): 9.83 (1H, dd, J = 1.8, 2.9 Hz), 4.43 (1H, dt, J = 4.8, 6.2 Hz), 3.69 (3H, s), 2.68 (1H, ddd, J = 1.6, 4.5, 6.3 Hz), 2.64 (1H, ddd, J = 2.7, 6.6, 8.8 Hz), 2.54 (1H, ddd, J = 4.3, 6.3, 10.2 Hz), 1.26-1.61 (46H, v br m), 0.90 (3H, t, J 6.8 Hz), 0.87 (9H, s), 0.08 (3H, s), 0.07 (3H, s); δ_C: 201.29, 174.13, 68.67, 52.26, 51.5, 48.12, 31.78, 29.72, 29.67, 29.61, 29.53, 29.4, 29.3, 29.2, 27.7, 27.1, 25.3, 22.6, 17.8, 14.1, - 4.5, - 4.7; ν_{max}/cm^{-1}: 2926, 2851, 1742, 1439, 1365, 1254, 1197, 1162, 1096.

Experiment 31: 2-[1-(*tert*-Butyldimethylsilanyloxy)-9-(2,2-dimethylpropionyloxy) -nonyl]-hexacosanoic acid methyl ester (374)



Lithium bis(trimethylsilyl)amide (1.06 M in THF, 7.52 ml, 7.9 mmol) was added to a stirred solution of (*R*)-2-[(*R*)-1-(*tert*-butyl-dimethyl-silanyloxy)-3-oxo-propyl]-tetracosanoic acid methyl ester (**373**) (2.44 g, 4.09 mmol) and 2,2-dimethyl-propionic acid 6-(1-phenyl-1H-tetrazole-5-sulfonyl)-hexyl ester (**306**) (2.09 g, 5.31 mmol) in dry THF (50 ml) at -5 °C. The reaction turned bright yellow and was left to reach room temperature and stirred for one hour under a nitrogen atmosphere, when TLC showed no starting material was left, the reaction quenched by addition of sat. aq. NH_4Cl (10 ml). The product was extracted with petrol/ethyl acetate (5:1,3x150 ml). The combined organic layers were dried over $MgSO_4$, filtered and evaporated, to give a crude product which was purified by column chromotography over silica gel, eluting with petrol/ethyl acetate (20:1) to give a colourless oil, (2.33 g, 75 %) {Found *m/z* $[M + Na]^+$: 787.7613 $C_{47}H_{92}O_5SiNa$ requires: 787.6611}. This showed δ_H (500 MHz, $CDCl_3$): 5.47-5.43 (2H, m), 4.04 (2H, t, J = 6.6 Hz), 3.94-3.87 (1H, m), 3.65 (3H, s), 2.52 (1H, ddd, J = 11.2, 7.45, 3.65 Hz), 2.33-2.23 (2H, m), 2.04 (3H, s), 1.62 (3H, t, J = 6.9 Hz), 1.25 (49H, br s), 1.19 (8H, s), 0.87 (3H, t, J = 6.95 Hz), 0.85 (9H, s), 0.04 (3H,s), 0.01 (3H,s); δ_C: 178.56, 175.06, 133.25, 131.61, 125.20, 124.75, 64.34, 64.28, 60.34, 51.49, 51.33, 38.69, 37.29, 32.57, 31.94, 31.90, 29.68, 29.63, 29.56, 29.48, 29.41, 29.34, 29.19, 29.03, 28.53, 28.45, 27.81, 27.70, 27.68, 27.59, 27.43, 27.17, 25.70, 25.63, 25.46, 22.66, 21.00, 1762, 17.90, 14.08, -4.29, -4.32; ν_{max}/cm^{-1}: 2957, 2869, 1723, 1595, 1498, 1480, 1461, 1398, 1341, 1285, 1153, 1043, 1015, 764, 689, 627.

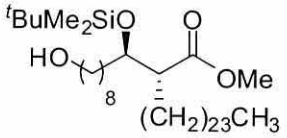
Experiment 32: 2-[1-(*tert*-Butyldimethylsilanyloxy)-9-(2,2-dimethylpropionyloxy) -nonyl]-hexacosanoic acid methy l ester (375)



Palladium 10 % on carbon (0.5 g) was added to a stirred solution of the 2-[1-(*tert*-butyl-dimethyl-silanyloxy)-9-(2,2-dimethyl-propionyloxy)-nonyl]-hexacosanoic acid

methyl ester (**374**) (2.33 g, 3.57 mmol) in IMS (60 ml) and THF (80 ml). Hydrogenation was carried out for one hour. The solution was filtered over a bed of celite and the solvent was evaporated to give pure colourless oil, 2-[1-(*tert*-butyl-dimethyl-silanyloxy)-9-(2,2-dimethyl-propionyl-oxy)-nonyl]-hexacosanoic acid methyl ester (**375**) (2.33 g, 99 %), $[\alpha]_D^{25}$ -3.52 (c = 1.87, $CHCl_3$)(lit: $[\alpha]_D^{22}$ -5.01 (c = 0.92, $CHCl_3$))[233] {Found *m/z* $[M + Na]^+$: 789.8859, $C_{47}H_{94}O_5SiNa$ requires: 789.6782}. This showed δ_H (500 MHz, $CDCl_3$): 4.04 (2H, t, J = 6.65 Hz), 3.91-3.88 (1H, m), 3.65 (3H), 2.52 (1H, ddd, J = 10.7, 6.4, 3.45 Hz), 1.61 (2H, q, J = 6.6 Hz), 1.25 (58H, v br s), 1.19 (9H, s), 0.87 (3H, t, J = 6.9 Hz), 0.86 (9H, s), 0.04 (3H, s), 0.01 (3H,s); δ_C: 178.58, 175.05, 73.16, 64.41, 51.56, 51.19, 38.69, 33.63, 31.91, 29.73, 29.69, 29.63, 29.56, 29.44, 29.34, 29.18, 28.61, 27.83, 27.44, 27.18, 25.88, 25.73, 23.72, 22.67, 21.00, 17.95, 14.09, -4.09, -4.9; ν_{max}/cm^{-1}: 2923, 2856, 1732, 1464, 1371, 1284, 1250, 1157, 1049, 1005, 938, 836, 775, 721.

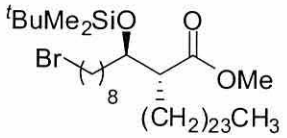
Experiment 33: (*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-non-3-enyl]-9-hydroxyl-hexacosanoic acid methyl ester (376)



2-[1-(*tert*-Butyl-dimethyl-silanyloxy)-9-(2,2-dimethyl-propionyloxy)-nonyl]-hexa-cosanoic acid methyl ester (**375**) (2.33 g, 3.03 mmol) was added to a stirred solution of potassium hydroxide (2.55 g, 45.4 mmol) dissolved in THF: MeOH: H_2O (10:10:1, 21 ml). The mixture was refluxed at 70 °C and monitored by TLC. After 3 hours, the TLC showed no starting material was left and the reaction was cooled down, quenched with water and extracted with ethyl acetate (3x100 ml). The combined organic layers were dried and the solvent was evaporated. The product was purified by column chromatography eluting with petrol/ethyl acetate (5:2) to give a semi-solid, (*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilanyloxy)-non-3-enyl]-9-hydroxyhexacosanoic acid methyl ester (**375**) (1.91 g, 92 %), $[\alpha]_D^{20}$ -5.34 (c 1.97) (lit: $[\alpha]_D^{25}$-6.43 (c = 1.54, $CHCl_3$))[233] {Found *m/z* $[M + Na]^+$: 705.7938, $C_{42}H_{86}O_4SiNa$ requires: 705.6193}. This showed δ_H (500 MHz, $CDCl_3$): 3.91 (1H, dt, J = 6.95, 4.75 Hz), 3.65 (3H, s), 3.64 (2H, t, J = 6.6 Hz), 2.52 (1H, ddd, J = 3.75, 7.25, 11 Hz), 1.56 (2H, q, J = 6.6 Hz), 1.47 (2H, q, J = 3.75 Hz), 1.43-1.39 (2H, m), 1.25 (55H, br s), 0.88 (3H, t, J =

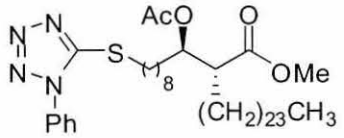
6.95 Hz), 0.86 (9H, s), 0.0 (3H, s), 0.02 (3H, s); δ_C: 175.12, 73.20, 63.05, 60.39, 51.60, 51.23, 33.65, 32.80, 31.93, 29.76, 29.70, 29.66, 29.59, 29.52, 29.46, 29.36, 27.85, 27.47, 25.75, 25.71, 23.72, 22.64, 17.98, 14.12, -4.36, -4.92; ν_{max}/cm^{-1}: 3357, 2924, 2854, 2738, 2709, 2683, 1741, 1464, 1436, 1406, 1361, 1253, 1195, 1167, 1070, 836, 775, 721, 662.

Experiment 34: (*R*)-2-[(*R*)-9-Bromo-1-(*tert*-butyldimethylsilanyloxy)-non-3-enyl]-hexa-cosanoic acid methyl ester (377)



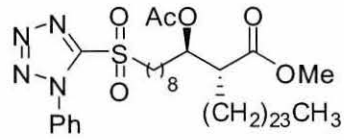
N-Bromosuccinimide (0.74 g, 4.18 mmol) was added in portions over 15 min. to a stirred solution of (*R*)-2-[(*R*)-9-hydroxy-1-(*tert*-butyldimethylsilanyloxy)-non-3-enyl]-hexa-cosanoic acid methyl ester (**376**) (2.2 g, 3.2 mmol) and triphenylphosphine (1.05 g, 4.02 mmol) in dichloromethane (70 ml) at 0 °C. The reaction mixture was stirred at room temperature for one hour when TLC showed no starting material was left. The reaction was quenched with sat. aq. sodium meta-bisulfate (50 ml). The organic layer was separated and the aqueous layer was re-extracted with dichloromethane (2x30 ml). The combined organic layers were washed with water, dried and evaporated to give a residue. The residue was treated with a mixture of petrol (150 ml) and refluxed for 30 min. then the triphenylphosphine oxide was filtered off and washed with ether (50 ml). The filtrate was evaporated and the residue was purified by column chromatography eluting with petrol/ether (20:1) to give a colourless oil, (*R*)-2-[(*R*)-9-bromo-1-(tert-butyl-dimethyl-silanyloxy)-non-3-enyl]-hexacosanoic acid methyl ester (**377**) (2.2 g, 90 %), $[\alpha]_D^{20}$ –1.95 (c = 1.12, $CHCl_3$) ($[\alpha]_D^{26}$ – 2.1 (c = 1.35, $CHCl_3$))[222] {Found *m/z* $[M + Na]^+$: 767.5371 $C_{42}H_{85}BrNaO_3Si$ requires: 767.5344}. This showed δ_H (500 MHz, $CDCl_3$): 3.91(1H, dt, J = 6.9, 5.05 Hz), 3.66 (3H, s), 3.40 (2H, t, J = 6.95), 2.52 (1H, ddd, J = 11, 7.25, 3.75 Hz), 1.85 (2H, pent., J = 6.95 Hz), 1.58-1.47 (4H, m), 1.26 (54H, s), 0.88 (3H, t, J = 6.65 Hz), 0.86 (9H, s), 0.04(3H, s), 0.02(3H, s); δ_C: 175.08, 73.17, 51.57, 51.21, 33.93, 33.63, 32.82, 31.92, 31.58, 29.7, 29.65, 29.58, 29.44, 29.36, 28.69, 28.13, 27.83, 27.83, 27.46, 25.75, 23.69, 22.68, 17.97, -437, -4.93; ν_{max}/cm^{-1}: 2923, 2857, 1742, 1461, 1433, 1367, 1257, 1191, 1162, 1063, 1015, 932, 835, 812, 774, 722, 665.

Experiment 35: (*R*)-2-[(*R*)-1-Acetoxy-9-(5-phenyl-5*H*-tetrazol-1-ylsulfanyl)-nonyl]-hexacosanoic acid methyl ester (380)



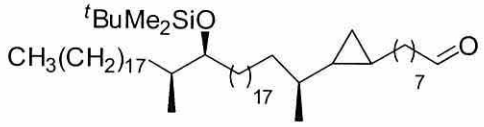
(*R*)-Methyl 2-((*R*)-1-acetoxy-9-bromononyl)hexacosanoate (**379**) (1.6 g, 2.37 mmol), 1-phenyl-1H-tetrazol-5-thiol (0.44 g, 2.49 mmol), potassium carbonate (0.49 g, 3.56 mmol) and acetone (60 ml) were mixed. The mixture was vigorously stirred for 18 hours at room temperature. When TLC indicated complete removal of the thiol, the inorganic salts were filtered off and washed well with acetone. The acetone solution was evaporated and dissolved in a CH_2Cl_2 (50 ml) and water (50 ml). The organic layer was separated and the aqueous layer was re-extracted with the same solvent (2x50 ml). The combined organic layers were dried and evaporated to give a pale yellow viscous oil which was purified by column chromatography eluting with petrol/ethyl acetate (10:1 then 5:1) to give a colourless viscous oil of the title compound (**380**), (1.62 g, 88 %), $[\alpha]_D^{22}$ +10.2 (c = 1.4, $CHCl_3$) (lit: $[\alpha]_D^{17}$ +6.6 (c = 1.20, $CHCl_3$))[222] {Found *m/z* $[M + Na]^+$: 793.5641, $C_{45}H_{78}N_4NaO_4S$ requires: 793.5636}. This showed δ_H (500 MHz, $CDCl_3$): 7.58-7.53 (5H, m), 5.1-5.06 (1H, ddd, J = 10.7, 8.2, 3.75 Hz), 3.67 (3H, s), 3.39 (2H, t, J = 7.25 Hz), 2.61 (1H, ddd, J = 11.05, 6.95, 4.4 Hz), 2.03 (3H, s), 1.81(2H, q, J = 7.6 Hz), 1.62-1.48(4H, m), 1.43 (4H, q, 5.65 Hz), 1.23 (50H, br s), 0.83 (3H, t, J = 6.6 Hz); δ_C: 173.61, 170.32, 154.46, 133.77, 130.03, 129.73, 123.84, 74.01, 51.51, 49.61, 33.32, 31.90, 29.67, 29.63, 29.61, 29.54, 29.46, 29.39, 29.33, 29.28, 29.23, 29.06, 28.90, 28.56, 28.10, 27.46, 24.99, 22.66, 20.99, 14.08; ν_{max}/cm^{-1}: 2924, 2849, 2360, 1741, 1597, 1499, 1460, 1384, 1236, 1164, 1086, 1016, 760, 721, 693.

Experiment 36: (*R*)-2-[(*R*)-1-Acetoxy-9-(5-phenyl-5*H*-tetrazol-1-sulfonyl)-nonyl]-hexacosanoic acid methyl ester (381)



A solution of ammonium molybdate (VI) tetrahydrate (1.15 g, 0.93 mmol) in 35 % H_2O_2 (10 ml), prepared and cooled in an ice bath, was added to a stirred solution of (*R*)-2-[(*R*)-1-acetoxy-9-(5-phenyl-5*H*-tetrazol-1-ylsulfanyl)-nonyl]-hexacosanoic acid methyl ester (**380**) (1.6 g, 2.07 mmol) in THF (10 ml) and IMS (20 ml) at 10 °C and stirred at room temperature for 2 hours. A further solution of ammonium molybdate (VI) tetrahydrate (1.15 g, 0.93 mmol) in 35 % H_2O_2 (20 ml) was added and the mixture was stirred at room temperature for 18 hours. The mixture was poured into water (50 ml) and extracted with dichloromethane (1x75 ml, 3x50 ml). The combined organic phases were washed with water (100 ml), dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ethyl acetate (5:1) to give a yellow oil, (*R*)-2-[(*R*)-1-acetoxy-9-(5-phenyl-5*H*-tetrazol-1-sulfonyl)-nonyl]-hexacosanoic acid methyl ester (**381**), (1.5 g, 90 %), $[\alpha]_D^{20}$ +12.3 (c = 1.69, $CHCl_3$) ($[\alpha]_D^{17}$ + 7.2 (c = 0.47, $CHCl_3$))[222] {Found *m/z* $[M + Na]^+$: 825.5551, $C_{45}H_{78}N_4NaO_6S$ requires: 825.5534}. This showed δ_H (500 MHz, $CDCl_3$): 7.70-7.68 (2H, m), 7.63-7.58 (3H, m), 5.08 (1H, ddd, J = 10.7, 6.95, 3.8 Hz), 3.72 (2H, t, J = 7.9 Hz), 3.67 (3H, s), 2.61 (1H, ddd, J = 10.75, 6.65, 4.1 Hz), 2.03 (3H, s), 1.97-1.91 (2H, m), 1.54-1.40 (5H, m), 1.25 (53H, br s), 0.87 (3H, t, J = 6.9 Hz); δ_C: 173.57, 170.32, 153.45, 133.02, 131.41, 129.67, 125.03, 73.93, 55.93, 51.52, 49.60, 31.89, 31.67, 29.66, 29.61, 29.60, 29.53, 29.44, 29.37, 29.32, 29.18, 28.98, 28.75, 28.09, 28.02, 24.93, 22.65, 21.90, 20.98, 14.08; ν_{max}/cm^{-1}: 2919, 2851, 1742, 1499, 1468, 1343, 1239, 1153, 1023, 765, 689.

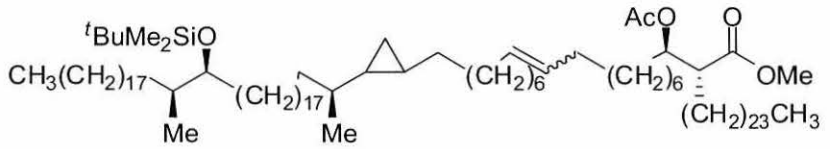
Experiment 37: 8-{(*1S,2R*)-2-[(1*S*,20*S*,21*S*)-20-(*tert*-Butyldimethylsilanyloxy)-1,21-dimethyl-nonatriacontyl]-cyclopropyl}-octanal (382)



8-{(1*S*,2*R*)-2-[(1*S*,20*S*,21*S*)-20-(*tert*-Butyldimethylsilanyloxy)-1,21-di-methyl-non-atriacontyl]-cyclopropyl}-octan-1-ol (**372**) (1.35 g, 1.56 mmol) in dichloromethane (10 ml) was added to a stirred solution of PCC (0.84 g, 3.91 mmol) in dichloromethane (50 ml). The reaction mixture was stirred at room temperature for 2 hours, when TLC showed that the reaction was complete, then poured into (20 ml) petrol/ethyl acetate (10:1) and filtered through a bed of silica gel. The solvent was

evaporated anr the crude product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give a colourless oil of 8-{(1*S*,2*R*)-2-[(1*S*,20*S*,21*S*)-20-(*tert*-butyl-dimethyl-silanyloxy)-1,21-dimethyl-nonatri-acontyl]-cyclopropyl}-octanal (**382**) (1.2 g, 88 %), $[\alpha]_D^{25}$ –0.71 (c = 0.52, $CHCl_3$) (lit: $[\alpha]_D^{25}$ –0.71 (*c* 0.52, $CHCl_3$))[222] {Found *m/z* $[M + Na]^+$: 895.8627, $C_{58}H_{116}NaO_2Si$ requires: 895.8637}. This showed δ_H (500 MHz, $CDCl_3$): 9.77 (1H, t, J = 1.9 Hz), 3.52–3.49 (1H, m), 2.43 (2H, dt, J = 1.9, 7.3 Hz), 1.64 (2H, br pent., J = 7.3 Hz), 1.58–1.01 (82H, m, v.br. chain), 0.91–0.88 (15H, m, including a singlet and a triplet, J = 7.3 Hz), 0.80 (3H, d, J = 7.0 Hz), 0.70–0.65 (1H, m), 0.22–0.09 (3H, m), 0.04 (3H, s), 0.03 (3H, s); δ_C: 202.9, 75.9, 43.9, 38.1, 37.7, 37.4, 34.4, 33.5, 32.5, 31.9, 30.1, 30.0, 29.9, 29.72, br 29.70, 29.65, 29.60, 29.5, 29.4, 29.35, 29.3, 29.2, 27.7, 27.3, 26.1, 26.0, 25.9, 22.7, 22.1, 19.7, 18.6, 18.2, 14.4, 14.1, 10.5, -4.2, -4.4; ν_{max}/cm^{-1}: 2926, 2844, 1742, 1455, 1365, 1259, 1075.

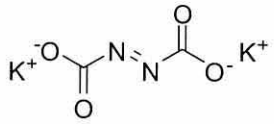
Experiment 38: (*R*)-2-((*E*/*Z*)-(*R*)-1-Acetoxy-17-{(1*S*,2*R*)-2-[(1*S*,20*S*,21*S*)-20-(*tert*-butyl-dimethyl-silanyloxy)-1,21-dimethyl-nonatriacontyl]-cyclopropyl}-heptadec-9-enyl)-hexacosnoic acid methyl ester (383)



Lithium bis(trimethylsilyl)amide (1.06 M in THF, 2.05 ml, 2.1 mmol) was added to a stirred solution of 8-{(*1S,2R*)-2-[(1*S*,20*S*,21*S*)-20-(*tert*-butyl-dimethyl-silanyloxy)-1,21-dimethyl-nonatriacontyl]-cyclopropyl}-octanal **(382)** (1.2 g, 1.3 mmol) and (*R*)-2-[(*R*)-1-Acetoxy-9-(5-phenyl-5*H*-tetrazol-1-sulfonyl)-nonyl]-hexacosanoic acid methyl ester (**381**) (1.34 g, 1.67 mmol) in dry THF (25 ml) at - 5 °C. The reaction turned bright yellow and was left to reach room temperature and stirred for one hour under a nitrogen atmosphere, when TLC showed no starting material was left. The reaction was quenched by addition of sat. aq. NH_4Cl (10 ml). The product was extracted with petrol/ethyl acetate (10:1, 3 x 50 ml). The combined organic layers were dried over $MgSO_4$, filtered and evaporated, to give a crude product whish was purified by column chromotography over silica gel, eluting with petrol/ethyl acetate (10:1) to give a colourless oil, (1.67 g, 83 %). This showed δ_H (500 MHz, $CDCl_3$): 5.39-5.35 (2H, m), 5.09 (1H, ddd, J = 11.05, 7.55, 3.8 Hz), 3.68 (3H, s), 3.50 (1H, dt,

J = 6.3, 3.45 Hz), 2.62 (1H, ddd, J = 10.75, 6.65, 4.1 Hz), 2.03 (3H, s), 1.69-1.49 (8H, m), 1.26 (143H, v br s), 0.90-0.84 (18H, m including singlet at δ = 0.89 for *t*-butyl), 0.80 (3H, d, J = 6.6 Hz), 0.70-0.65 (1H, m), 0.48-0.44 (1H, m), 0.21-0.1 (3H, m), 0.04 (3H, s), 0.03 (3H, s).; δ_C: 173.63, 170.31, 130.43, 130.22, 129.76, 75.86, 74.09, 51.51, 49.61, 31.93, 30.08, 30.00, 29.89, 29.72, br 29.70, 29.66, 29.57, 29.47, 29.41, 29.36, 27.71, 25.97, 22.69, 14.41, 14.10, -4.19, -4.43; ν_{max}/cm^{-1}: 2934, 2826, 1745, 1456, 1362, 1226, 1165, 1021, 966. [222]

Experiment 39: Di-potassium azodicarboxylate



Azodicarbonamide (7.5 g, 64 mmol) was slowly added in small portions to a vigorously stirred solution of potassium hydroxide (15 g, 260 mmol) in distilled water (15 ml) at 0 °C in an ice-salt water bath, maintaining the temperature below 5 °C. The resultant bright yellow solution was stirred at 0-5 °C for 45 min, during which a bright yellow precipitate formed. The precipitate was filtered on a sintered funnel and washed with ice-cold methanol (60 ml). The yellow precipitate was dissolved in water (40 ml) and then filtered through into pre-cooled IMS (60 ml, -20 °C) to give a yellow precipitate. The precipitate was then filtered again through a sinter funnel and washed with cold methanol (50 ml, -20 °C), followed by cold petrol (50 ml, -20 °C) and the solid dried on the vacuum. The solid was then transferred to a pre-cooled round bottomed flask under nitrogen. The flask was then stored in the freezer.[226]