Synthesis of Single Enantiomers of Ketomycolic Acids

A thesis submitted to the Bangor University

for the degree of Doctor of Philosophy

by

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Table of Contents

Abbreviation

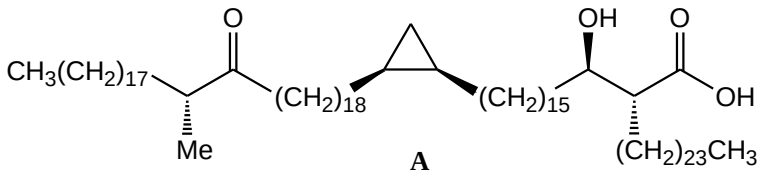
Ac	Acetyl
b	Broad
Bn	Benzyl
BuLi	Butyllithium
^{o}C	Degrees Celsius
cetrimide	Hexadecyltrimethylammonium bromide
m-CPBA	*m*-Chloroperbenzoic acid
CID	Collision Induced Dissociation
d	Doublet
DHP	2,3-Dihydro-2*H*-pyran
$(DHQD)_2PHAL$	Hydrochinidine 1,4-phthalazinediyl diether
$(DHQ)_2PHAL$	Dihydrochinine 1,4-phthalazinediyl diether
DIBAL-H	Diisobutylaluminium hydride
dil.	Diluted
DMAC	*N*,*N*-Dimethylacetamide
DMF	*N*,*N*-Dimethylformamide
Ether	Diethyl ether
EI	Electron Impact
GC	Gas Chromatography
h	hour (s)
HMPA	Hexamethylphosphorotriamide
HPLC	High Performance Liquid Chromatography
Hz	Hertz
IMS	Industrial Methylated Spirit
IR	Infra-Red
J	Coupling constant
LDA	Lithium *N*,*N*-diisopropylamide
m	Multiplet
m	*Meta*-
Me	Methyl
MeLi	Methyllithium

min	Minutes
mmol	millimols
mol eq.	Molar equivalents
m.p.	Melting Point
MALDI-TOF	Matrix Assisted Laser Desorption Ionization Time-Of-Flight
MS	Mass Spectroscopy
NBS	*N*-Bromosuccinimide
NMR	Nuclear Magnetic Resonance
Oxone	$2KHSO_5.KHSO_4.K_2SO_4$ ($KHSO_5$: potassium peroxomonosulfate)
PCC	Pyridinium Chlorochromate
Petrol	Petroleum Spirit (boiling point 40 to 60 ^oC)
Ph	Phenyl
ppm	parts per million
PPTS	Pyridinium *p*-toluenesulfonate
PTSA	*p*-Toluenesulfonic acid monohydrate
Pv	Pivaloyl
q	Quartet
R_f	Retardation factor
r.t.	Room temperature
s	Singlet
t	Triplet
TBAF	Tetra-n-butylammonium fluoride
THF	Tetrahydrofuran
THP	Tetrahydropyranyl
TLC	Thin-Layer Chromatography
TPBSH	2,4,6-Tri-isopropylbenzenesulphonyl hydrazide
v.br.	Very broad

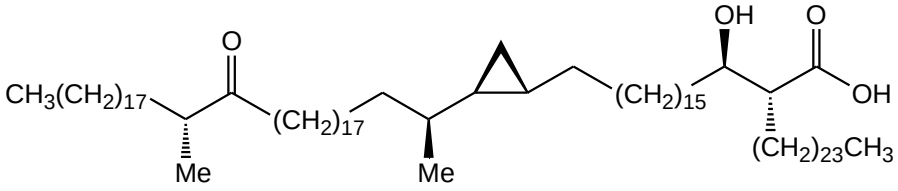
Abstract

Mycolic acids, long chain fatty acids often containing 70 to 90 carbons, are major constituents of the cell envelope of *M. tuberculosis* and other pathogenic mycobacteria. Their presence is thought to explain the characteristic resistance of these mycobacteria to most antibiotics and other chemotherapeutic agents.

The syntheses of single enantiomers of the following mycolic acid derivatives were achieved; *cis*-cyclopropane keto-mycolic acid (**A**), α-methyl-*trans*-cyclopropane keto-mycolic acid (**B**), *cis*-cyclopropane keto-meromycolaldehyde (**C**), α-methyl-*trans*-cyclopropane keto-meromycolaldehyde (**D**), α-methyl-*trans*-alkene keto-mycolic acid (**E**) and related hydroxy-mycolic acids. However, the synthesis of α'-mycolic acid (**F**) failed.



A



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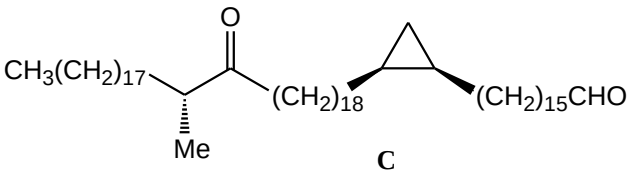

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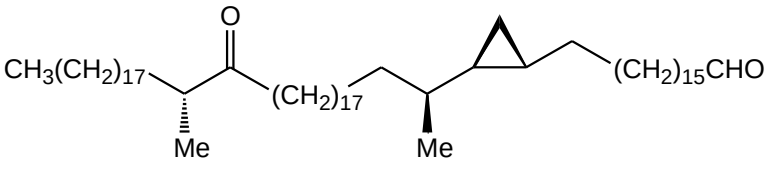

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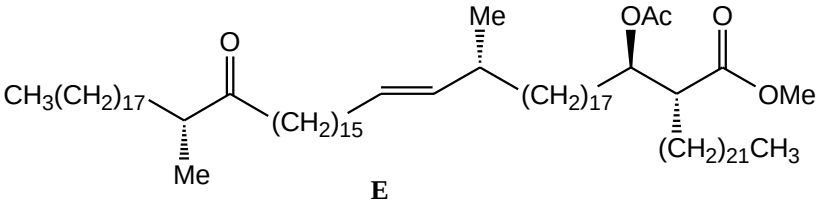

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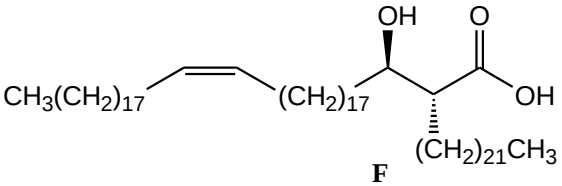


F

The synthesis of single enantiomers of mycolic acids will help to prove the stereochemistry of the natural ones, and tests of their biological activities may offer the development of new methods for the diagnosis and treatment of TB and related diseases.

1. Introduction

1.1 Tuberculosis and Related Diseases

1.1.1 Overview

Tuberculosis (TB) is a disease caused by an infection with the bacterium *Mycobacterium tuberculosis* and it has been present in humans since antiquity.[1] TB was identified as a single disease in the 1820s and named in 1839 by J.L. Schönlein.[2] During the 19th century, up to 25 % of deaths in Europe were caused by this disease.[3] The death toll began to fall as living standards improved at the start of the 20th century, and from the 1940s, effective medicines were developed.[4]

However, there are now more people in the world with TB than there were in 1950. Over one-third of the world's population now has the TB bacterium in their bodies and new infections are occurring at a rate of one per second. Each year about 2 million people die from this curable disease mainly in less developed countries.[5] One-third of the number of new TB cases occurs in South-east Asia, but the estimated incidence per capita is highest in sub-Saharan Africa.

Because HIV weakens the immune system, someone who is HIV/TB co-infected is many times more likely to become sick with TB than someone infected with TB who is HIV-negative.[6,7] TB is a leading cause of death among people who are HIV-positive, accounting for approximately 13 % of AIDS-related deaths worldwide.[8]

In addition, there has been a simultaneous increase in cases of drug-resistant tuberculosis, which is due to an ineffective administration of antibiotics and other chemotherapeutic agents.[9] Rates of multi-drug-resistant TB are high, especially in the former Soviet Union countries, and it is a significant threat for TB control efforts.[10] The World Health Organization estimates that up to 50 million people worldwide may be infected with drug resistant strains of tuberculosis.[11] Another factor that helps the spread of TB is the movement of people, travellers, refugees or displaced people.

The WHO declared TB a global health emergency in 1993, and the Stop TB Partnership proposed a Global Plan to Stop Tuberculosis which aims to save 14 million lives between 2006 and 2015.

Mycobacterium tuberculosis (MTB) is a small rod-like bacillus which can withstand weak disinfectants and can survive in a dry state for weeks. Normally, the bacteria can

only grow within a host organism, so in vitro culture of *M. tuberculosis* took a long time to develop, but is now a routine laboratory procedure.[12] MTB is identified microscopically by its staining characteristics: it retains certain stains after being treated with acidic solution and is thus classified as an "acid-fast bacillus" or AFB.[13]

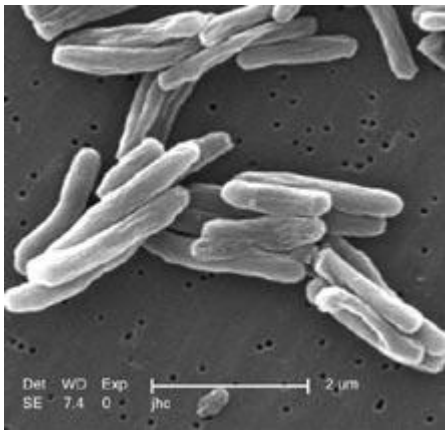


Figure 1: ***Scanning electron micrograph of M. tuberculosis***[14]

Apart from the pathogenic organisms of the *Mycobacterium tuberculosis* complex, there are several non-tuberculosis mycobacteria that may cause other human diseases. *Mycobacterium leprae* is the cause of leprosy, *Mycobacterium ulcerans* of Buruli ulcer and *M. avium paratuberculosis* of Johne's disease in animals and possibly of Crohn's disease in humans.[15,16,17,18,19] *M. avium intracellulare* complex and *M. kansasii* are the most common infective non-tuberculous mycobacteria found in immune deficient individuals,[20] but also rapid growing mycobacteria (*e.g.*, *M. fortuitum* and *M. chelonae*) can cause opportunistic diseases in HIV patients.

Mycobacterium marinum is the causative agent of fish tuberculosis, infecting more than 150 species of salt- and fresh-water fish.[21] The organism, first isolated in 1926 from a salt-water fish,[22] causes a systemic infection that ultimately leads to death of animals. *M. marinum* is a rare human disease but it can cause skin infections that occur as a result of contact with contaminated water, particularly that of fish tanks and swimming pools. This bacterium does not grow at normal body temperature. That is why it remains localized to the cooler skin surface. *M. marinum* is the most important agent of piscine mycobacteriosis. It is transmitted primarily through the consumption of contaminated feed, cannibalism of infected fish or ingestion of aquatic detritus.

1.1.2 The Mycobacterial cell envelope

Knowledge of the organisation and of the structure of the *M. tuberculosis* cell envelope is of fundamental importance because these are factors in the pathogenesis of this disease. The cell envelope of Mycobacterium ssp. is a rather complex structure, consisting of three major entities: the plasma membrane, the cell wall core and the outermost layer, also called the capsule in the case of pathogenic mycobacteria such as *M. tuberculosis*.[23] While the composition of the plasma membrane is similar to that of other living organisms, the cell wall core is composed of an *N*-glycolylated peptidoglycan linked to an heteropolysaccharide, D-arabino-D-galactan, which in turn is esterified by long chain (C_{60} to C_{90}) α-branched, β-hydroxylated fatty acids, the so called mycolic acids which will be explained in detail later.

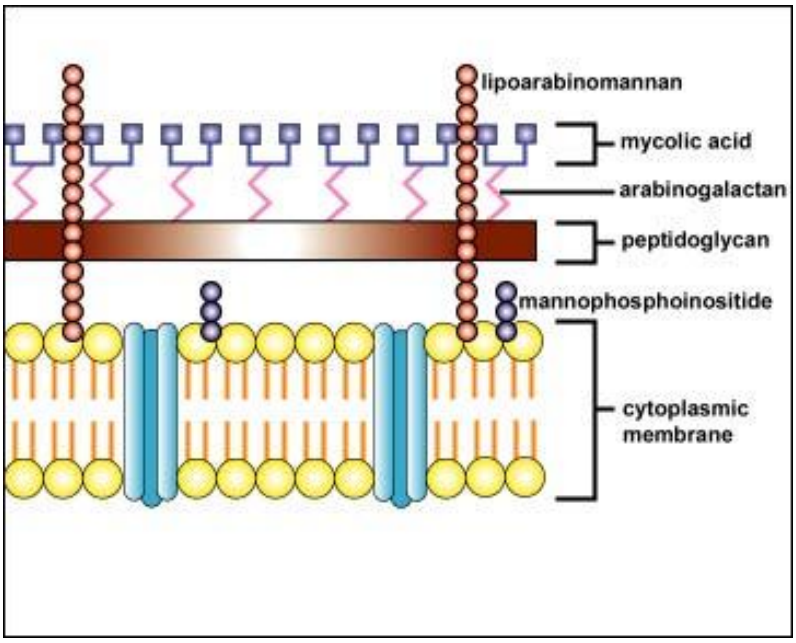


Figure 2: ***Schematic representation of the mycobacterial cell wall***[24]

The capsular material is rich in polysaccharides and protein, and its deeper compartment contains significant amounts of genus- and/or species-specific lipids that form the outer leaflet of an outer membrane whose inner leaflet is composed of mycolic acid residues.[25] The asymmetric permeability barrier confers on mycobacteria their characteristic resistance to solutes that include many antibiotics and therapeutic agents.[26,27]

The main constituents of the cell wall are mycolic acids and also there is a uniquely large number of different lipids: for example several different multi-methyl branched fatty acids;[28,29] lipomannan and lipoarabinomannans,[30,31] trehalose 6,6'-dimycolate and many others. The mycobacterial lipids, constituting up to 40 % of the dry weight of the cell envelope, have been the subject of numerous studies in order to determine their structure, biosynthesis and role in the virulence of the mycobacteria.[32,33,34]

A particularly interesting glycolipid obtained from tubercle bacilli is called "cord factor": a trehalose, or 1α, 1α'-diglycoside, esterified at both primary alcohol groups with mycolic acids (**Figure 3**)[35].

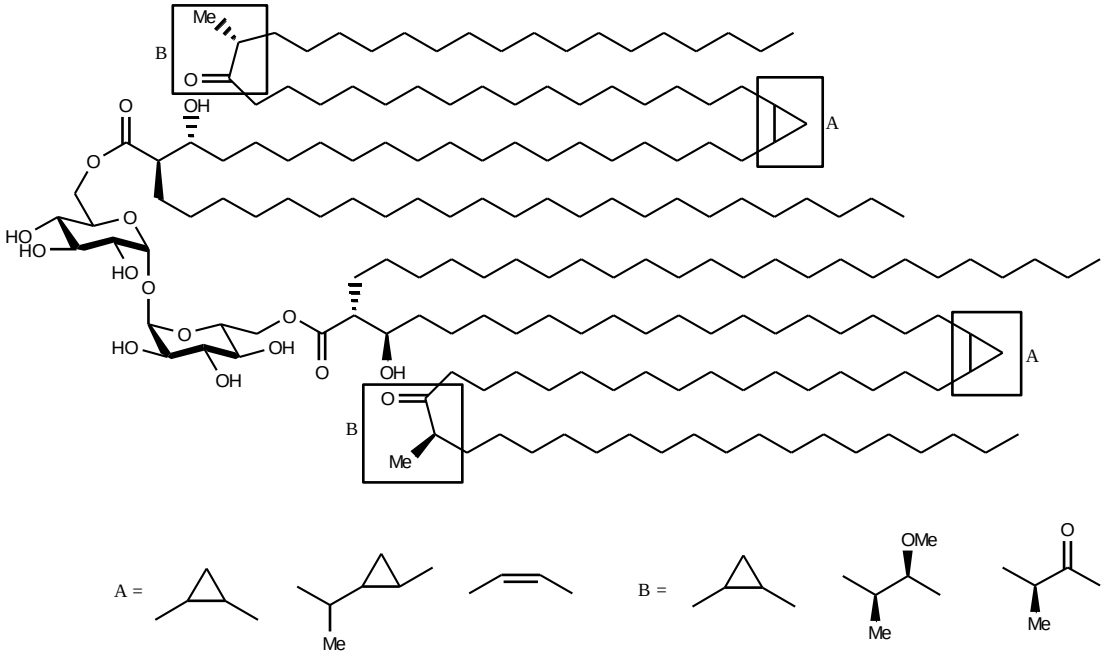


Figure 3: ***Cord factor types***

Cord factor is considered a "free lipid" since it can be liberated from the cell wall by extraction in an appropriate solvent, while the other mycolic acids, linked by covalent bonds to the arabinogalactan complex, can not be liberated so readily.

There is evidence that mycolic acids could be one of the antigenic means for its recognition.[36] In fact antibodies prepared against cord factors, showed greater reactivity for the types of mycolic acids contained in the glycolipids used as antigens, than for other kinds of mycolic acid.[37,38] The antibodies prepared with cord factors of *M. avium* and *M. tuberculosis* were able to distinguish between these two species by recognising their different mycolic acid subclasses.[39,40] Trehalose 6,6'-dimycolate has,

therefore, been studied for the serodiagnosis of several mycobacterial diseases giving diverse, non-optimal, results in specificity and selectivity.[41,42]

However, all these results were obtained with polyclonal antibodies using, as antigens, all the cord factors extracted from each organism. It might be interesting to verify if the use of monoclonal antibodies formed using only one type of antigen gives more successful results. Another reason for the current interest in mycolic acids as possible antigens is that the antibody response to mycolic acids is also preserved in HIV-seropositive patients.[43]

1.2 Mycolic Acids

1.2.1 Overview

Mycolic acids are major constituents of the cell envelope of *M. tuberculosis* and other mycobacteria, some of which are pathogenic to animals and humans.[44,45,46,47] First of all they are usually long chain high molecular mass (C_{70} – C_{90}), α-alkyl (C_{22} – C_{24}) branched β-hydroxy fatty acids, and contain different functionalities in the main chain (**Figure 4**).[23]

Mycolic acids consist of two characteristic parts; a saturated carboxylic acid part called corynomycolate and a long fatty alcohol part called a meromycolate chain, as shown in **Figure 4**. The structure of the corynomycolate portion is common to each mycolic acid, except for minor variation in the length of the chain in the α-position with respect to the carboxylic end. The meromycolate chain from pathogenic mycobacteria normally has two intra-chain groups, the distal and proximal groups, that vary. These can be classified into three types as shown in **Figure 4**. Mycolic acids are broadly separated into classes, according to the groups present in the meromycolate chain. The proximal or distal functional groups can be cyclopropanes, double bonds, an epoxy group, methoxy group, carbonyl group, or methyl group.[44] Two-dimensional TLC,[48,49,50] and subsequently GC[51] and HPLC,[52,53,54] in association with MS, IR, and NMR techniques, have permitted the identification of several kinds of mycolic acids present in each mycobacterium. Actually, HPLC patterns are characteristic for each mycobacterium and they have been used as a rapid diagnostic tool for speciating mycobacteria.[54]

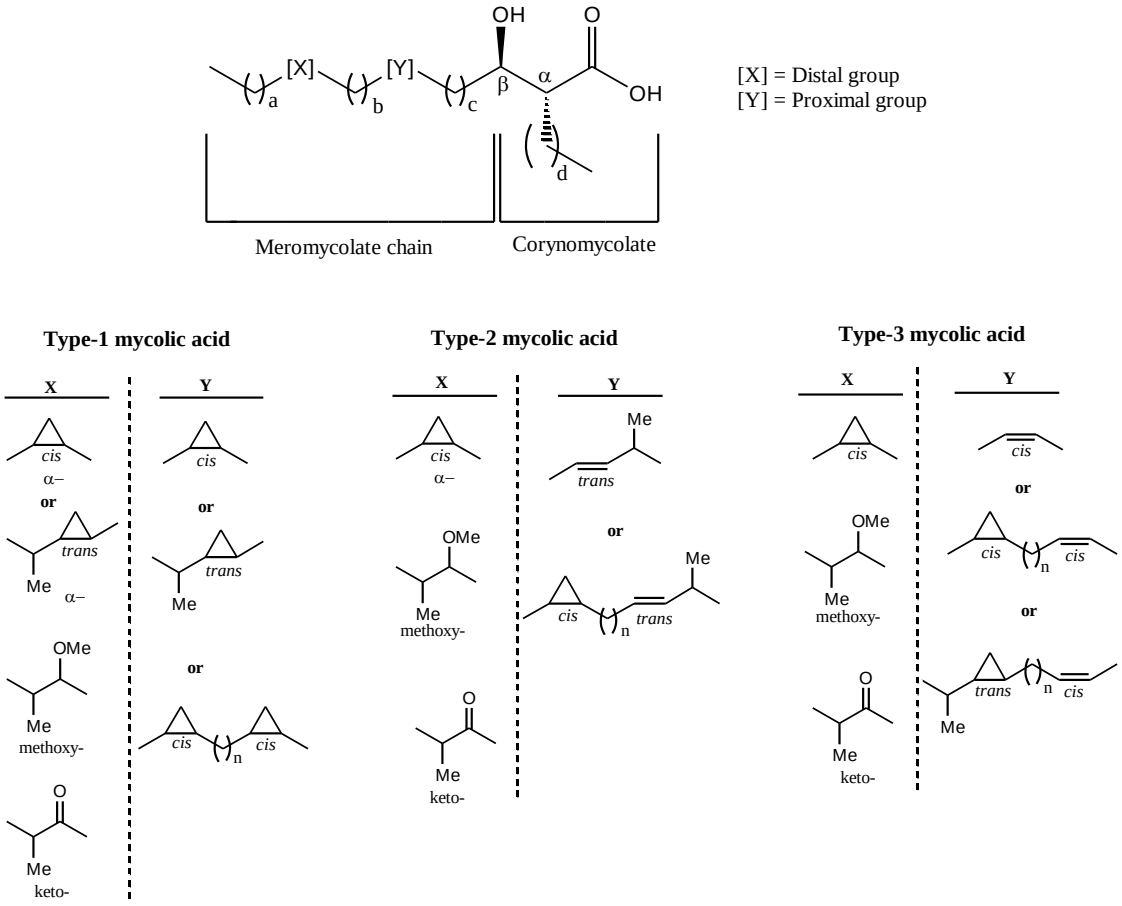


Figure 4: ***Generalized structures of major mycobacterial mycolic acids***

On the basis of the nature of the functional group in the meromycolate chains, mycolic acids from *M. tuberculosis* are categorized into three major groups: α-mycolic acid (**1**) with no oxygen-containing intra-chain groups, methoxy-mycolic acid (**2**) in which the distal group has a methoxy group and keto-mycolic acid (**3**) in which the distal group has a carbonyl group. Methoxy-mycolic acids and keto-mycolic acids have methyl branches next to the oxygenated functional group and natural mixtures have both *cis*-cyclopropane and α-methyl *trans*-cyclopropane rings, as shown in **Figure 5**.

The nature and the location of the groups in the meromycolate chains of mycolic acids from representative mycobacteria have been studied by extensive NMR and mass spectroscopic analyses by Watanabe et al.[46,47]

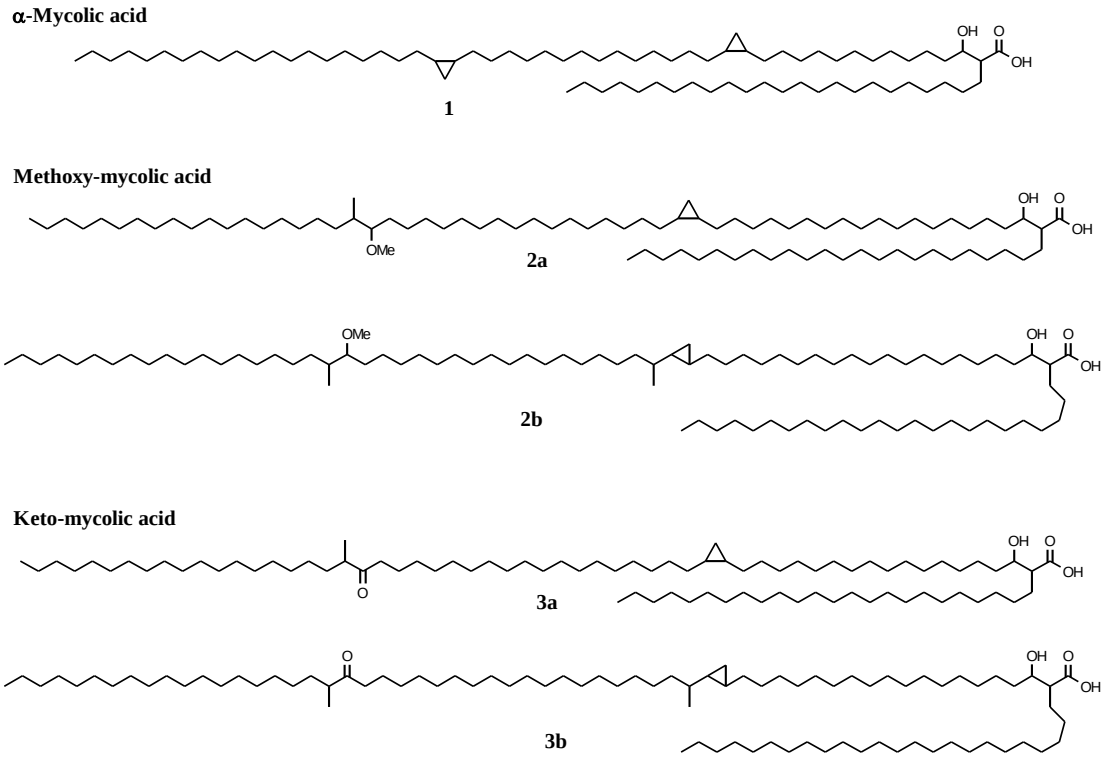


Figure 5: ***Major types of mycolic acids from M. tuberculosis***

As well as the occurrence of various chemical groups on the meromycolate chain, mycolic acids are also present in numerous mycobacteria in different sets. For example *M. smegmatis* contains α' and α-mycolic acids (**4, 5**) with either one or two double bonds, either in the *cis* or the *trans* configuration, whilst *M. fortuitum* contains epoxy-mycolic (**6**) acid with an epoxy ring. [55,56] Finally, more different oxygenated mycolic acids have been isolated in numerous mycobacteria; these include ω-carboxy-mycolic acid (**7**) from *M. phlei,*[57] ω-1-methoxy-mycolic acid (**8**) from *M. alvei*[58] and wax mycolic acid (**9**) (wax ester) from *M. aurum.*[59] Wax esters are the result of a Baeyer-Villiger type reaction on the keto group of the keto-mycolic acid.[60,61] There are also a lot of major or minor mycolic acids in combinations of the distal group and proximal group in different mycobacteria.

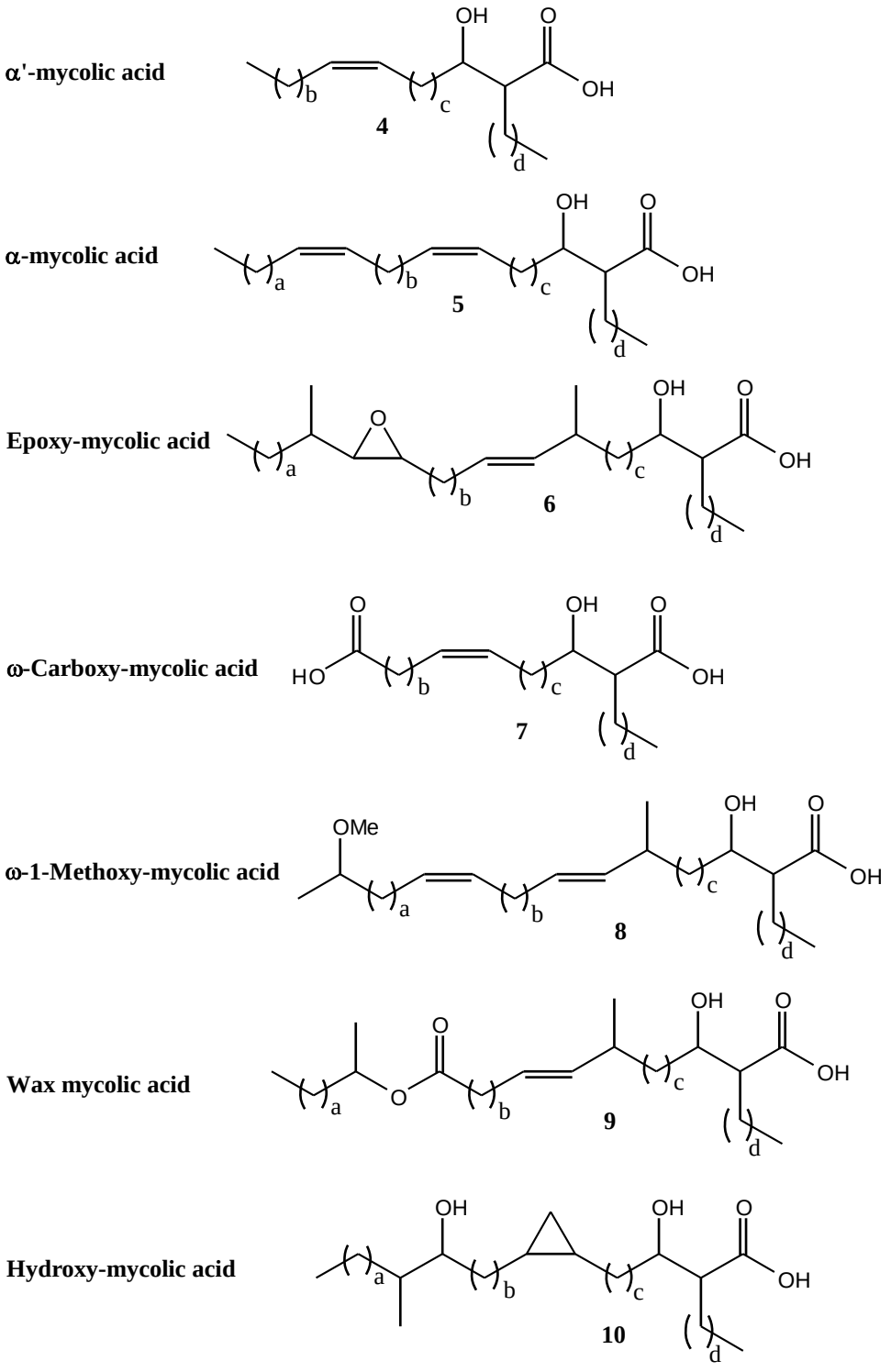


Figure 7: ***Mycolic acids from other mycobacteria***

E. Dubnau *et al* have reported the isolation of a gene cluster from the *M. bovis* bacillus Calmette-Guérin (BCG) which confers upon *M. smegmatis* the ability to introduce a keto-mycolic acid and a new type of mycolic acid, a hydroxy-mycolic acid (**10**)[66] as

shown in **Figure 7**. This is similar to all the oxygenated mycolic acids as it contains a methyl branch in the α position to the oxygenated function. A meticulous analysis of all the mycolic acids of *M. tuberculosis* H37Rv and *M. bovis* BCG showed that these organisms produce small amounts of the hydroxy-mycolic acids.[68] A mycolic acid with two hydroxyl groups was described several years ago in *M. tuberculosis* H37Ra, H37Rv and R1,[62] although its complete structure could not be resolved. Therefore hydroxy-mycolic acids probably exist in all species of the *M. tuberculosis* complex.

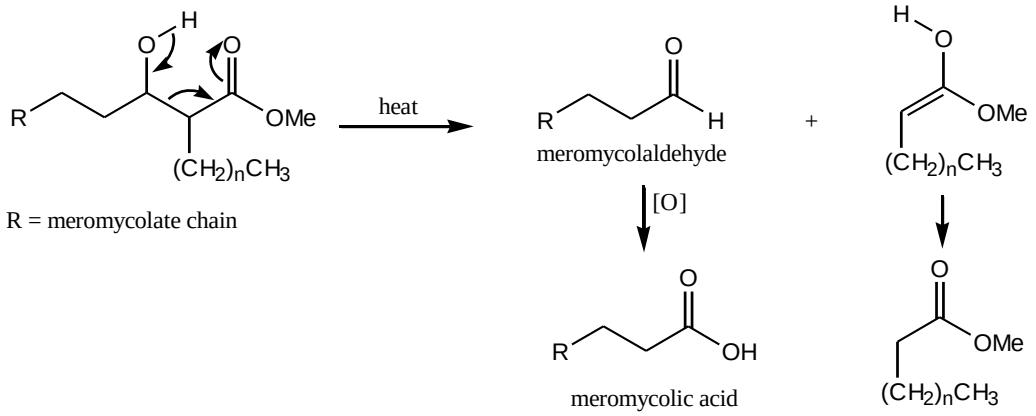
A recent review confirmed that the most widespread of the classes of mycolic acids are the α-mycolic acids, with either two double bonds or two cyclopropyl rings (**1**, **5**).[63] The next most widely distributed are keto-mycolic acids (**3**), which are found in both pathogenic and saprophytic mycobacteria, in both fast and slow growing species. α'-Mycolic acids (**4**) and wax esters (**9**) are also found extensively, while methoxy-mycolic acids (**2**) appear to be present only in pathogenic species that also produce keto-mycolic acids (**3**).[63,64] It would be interesting to understand the reasons for the existence of the methoxy-mycolic acids. In conclusion, ω-1-methoxy-mycolic acids (**8**) are specific to a very restricted number of mycobacteria.[63]

A study of ^{1}H NMR spectra showed that the distribution of functional groups in the total mycolic acid fraction from *M. tuberculosis* Aoyama B, showed a content of 6.0 % double bonds and a *cis* : *trans* ratio 1 : 1, with a ratio of *cis* : *trans* cyclopropane of 1 : 0.18 and α- : methoxy- : keto- mycolic acid ratio 1 : 0.74 : 0.25. The α-mycolic acid has only *cis* cyclopropane, methoxy-mycolic acid has a *cis* : *trans* cyclopropane ratio of 1 : 0.37 and keto-mycolic acid has a *cis* : *trans* cyclopropane ratio 1 : 3.3. Both the methoxy- and keto-mycolic acids contained significant amounts of *trans*-double bonds; methoxy- has 11.3 % and keto- has 18.6 % *trans* cyclopropane rings. Although the methoxy- and keto-mycolic acid ratios varied with the cultivation period and in different preparations, the sum of the methoxy-mycolate and the keto-mycolate content versus the α-mycolate content was almost always constant, implying a close relationship between the two oxygenated mycolates. However, the *trans*-cyclopropane ring content was much higher in the keto-mycolates than in the corresponding methoxy-mycolates and the same tendency was noted in the *trans*-double bond content.[46]

1.2.2 Chain length of mycolic acids

In all mycobacteria, there are not only different types of mycolic acids, but also different homologues for each of them. In *M. tuberculosis* alone, a family of over 500 individual mycolic acids with closely related chemical structures has been recognised.[63] These circumstances made the isolation of a single component and the determination of its real structure extremely difficult. However, mycolic acids have been the focus of constant study since their discovery. This is not only because they are unique to this type of organism but also because of their importance for the survival and virulence of the mycobacteria. In the past decades, electron impact (EI) mass spectrometry has been exhaustively used for the determination of the chain length of mycolic acids and the location of functional groups.[65,44,66,67,68] In particular, the positions the cyclopropane rings within mycolates were established.[69, 70] However, EI mass spectrometry studies on non-derivatized homologous mixtures of mycolic acids do not give reliable information on the location of double bonds, cyclopropane rings and methyl branches within these acids. Recently, MALDI-TOF mass spectrometry has provided a rapid and highly sensitive technique for analysis of mycolic acids and other lipids.[71] Examination by this technique demonstrated that the chain length of the various mycolates correlated with the growth rate of mycobacterial strains. Although slow growers, such as *M. tuberculosis* and *M. ulcerans*, produced a series of even carbon numbers (C_{74} – C_{82}) of α-mycolic acids, rapid growers synthesized both odd and even carbon numbers. In addition, the main chain of oxygenated (methoxy- and keto-) mycolic acids from slow growers were four to six carbon atoms longer than the corresponding α-mycolic acids, whereas rapid growers elaborated oxygenated homologues possessing the same chain lengths as their α-mycolic acids. This is in accordance with previous views.[20,72]

Watanabe *et al* used MALDI spectrometry to study mycolic acids (both major and minor components) present in 19 strain of the *M. tuberculosis* complex.[46] Combining this new methodology with collision-induced dissociation (CID) mass spectroscopy they succeeded in locating, precisely, the functional groups in the meromycolate moiety of different types of mycolic acids.[47] In this study, meromycolic acids were prepared from individual mycolate components by pyrolysis, followed by silver oxide oxidation (**Scheme 1**) and analysed.



Scheme 1: ***Preparation of meromycolic acids***

Since meromycolic acids possess a stable charged site, i.e. a carboxylate anion at one end of the chain, CID mass spectrometry, in which bond cleavage occurs independently of the charge, provides reliable information about the locations of functional groups, i.e. *cis* and *trans* double bonds, *cis* and *trans* cyclopropane rings, methoxy and keto groups, and methyl branches.

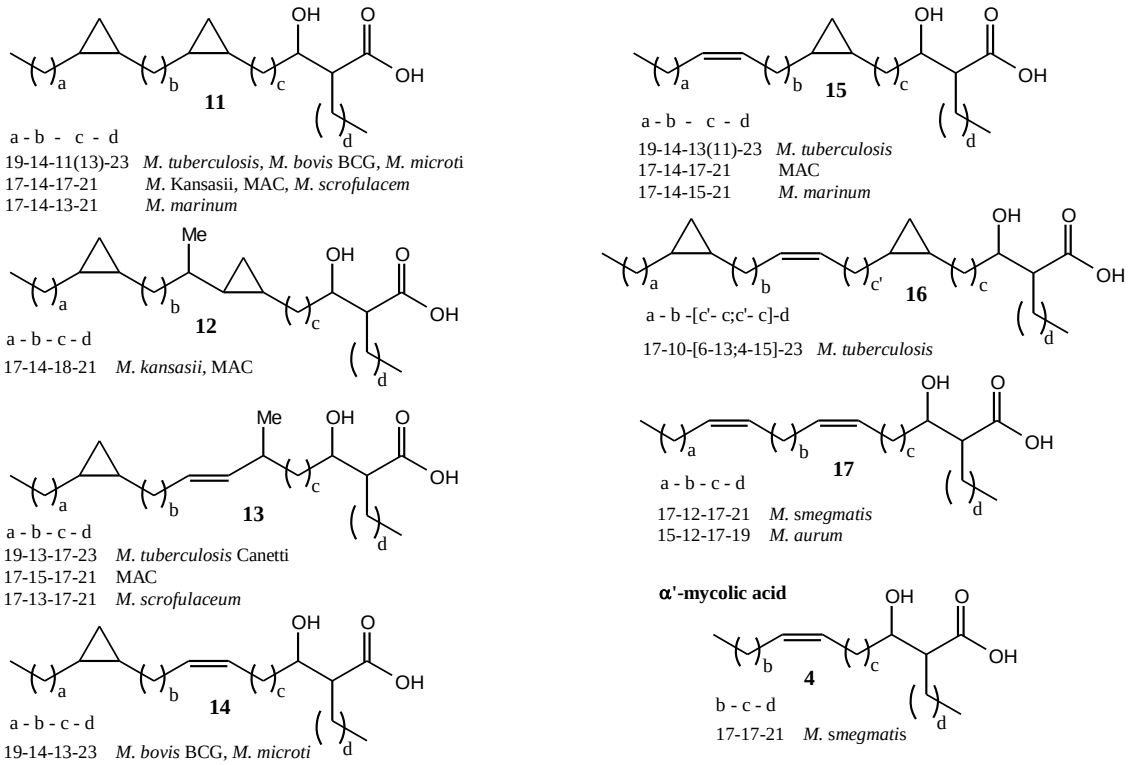
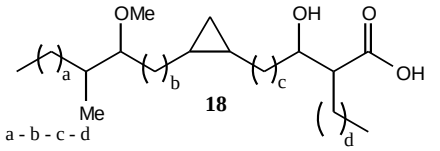


Figure 8: ***Structures and distribution of major and minor α-mycolic acids***[47,73]

The precise location of the functional groups in meromycolic acids were determined by CID mass spectrometry and, on the basis of these, *a-b-c* values for mycolic acid structures were elucidated and are shown in **Figure 8, Figure 9** and **Figure 10**. The methyl groups adjacent to the methoxy, the keto and the *trans*-cyclopropane in oxygenated mycolic acids such as (**18**, **19**, **25** and **26**) were confirmed to be on the distal side, whilst the methyl group adjacent to the *trans* double bond such as (**13**, **21** and **28**) was on the proximal side, the carboxyl end.

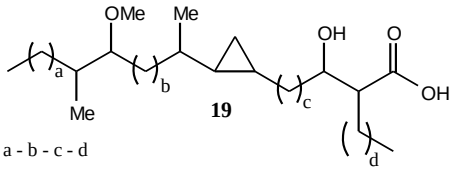
Moreover, analysing the location of the different functional groups, a close structural relationship was established between the methoxy (**2a, 2b**) and keto-mycolic acids (**3a, 3b**). Both of them contained significant amounts of *trans* double bonds and *cis* and *trans*-cyclopropane rings. Also, the total chain lengths of the major mycolates in each of these two classes of the same strains were apparently the same (**18**, **19**, **25** and **26**) as shown in **Figure 9** and **Figure 10**.



18

a - b - c - d

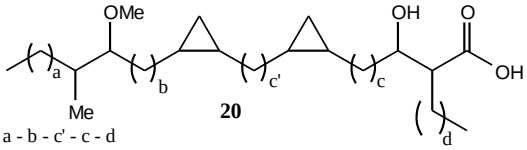
17-16-17-23 *M. tuberculosis*, *M. bovis* BCG, *M. microti*
15-14-19-23 *M. tuberculosis* H37Ra
17-16-19(17)-21 *M. kansasii*



19

a - b - c - d

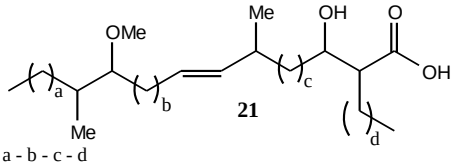
17-16-18-23 *M. tuberculosis*, *M. bovis*, *M. bovis* BCG, *M. microti*
17-16-22(20)-23 *M. tuberculosis Canetti*
17-16-18(20)-21 *M. kansasii*



20

a - b - c' - c - d

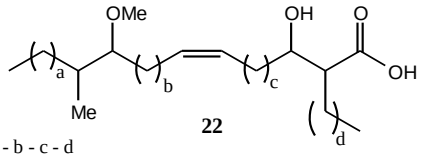
[15-10;17-8]-[12-11;10-13;8-15]-23 *M. tuberculosis* H37Ra
[15-12;17-10]-[10-11;8-13;6-15]-23 *M. tuberculosis* H37Ra



21

a - b - c - d

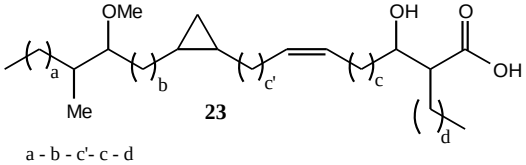
17-19-13-23 *M. tuberculosis*, *M. bovis*, *M. bovis* BCG
17-19-15-23 *M. tuberculosis* Canetti
17-15-17-21 *M. marinum*



22

a - b - c - d

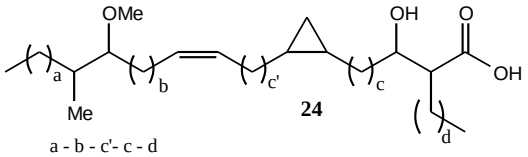
17-18-15-23 *M. tuberculosis*, *M. bovis*, *M. bovis* BCG, *M. microti*



23

a - b - c'- c - d

17-[14-4;12-6]-15-23 *M. bovis* BCG, *M. microti*
17-[14-2;12-4]-17-23 *M. bovis* BCG, *M. microti*



24

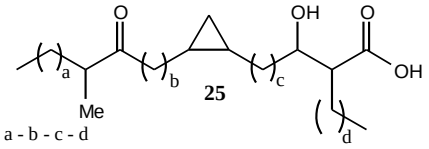
a - b - c'- c - d

[15-12;17-10]-6-15-23 *M. tuberculosis* H37Ra
[15-12;17-10]-6-17-23 *M. tuberculosis* H37Ra

Figure 9: ***Structures and distribution of major and minor methoxy-mycolic acids***[47]

Finally, through a detailed analysis of their structure, mycolic acids with one *cis* double bond (**22** and **29**) were structurally closely related to those with one *cis* cyclopropane ring (**18** and **25**), whereas the mycolic acids with one *trans* cyclopropane rings (**19** and **26**) were closely related to the corresponding mycolic acids with one *cis* cyclopropane ring (**18** and **25**).

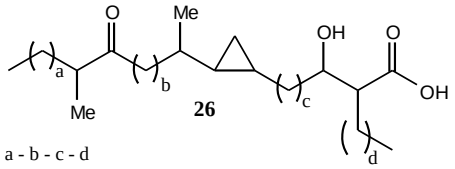
On the other hand, a simple and universal connection between major classes with a *trans*-cyclopropane mycolic acids (**19** and **26**) and the corresponding minor ones with a *trans*-mono olefinic mycolic acid (**21** and **28**) has not been found. Also new series of mycolic acids such as (**16**, **20**, **23**, **24**, **27**, **30** and **31**) have been found.



25

a - b - c - d

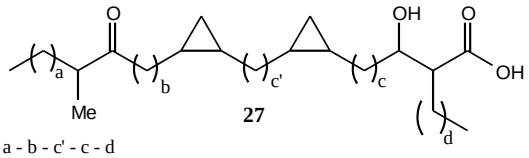
17-18-15-23 *M. tuberculosis, M. bovis, M. bovis* BCG, *M. microti*
15-16-19-23 *M. tuberculosis* H37Ra
17-20-17-21 *M. kansasii*
15-18-17-21 MAC
15-16-19-21 *M. scrofulaceum*



26

a - b - c - d

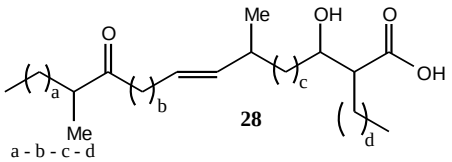
17-18-16-23 *M. tuberculosis, M. bovis, M. bovis* BCG, *M. microti*
15-18-18-21 *M. kansasii*
17-16-18-21 MAC
15-16-18-21 *M. scrofulaceum*



27

a - b - c' - c - d

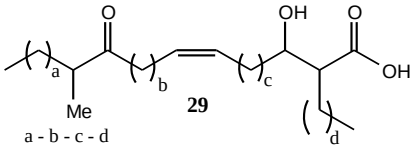
[15-10;17-8]-[12-13;10-15;8-17]-23 *M. tuberculosis* H37Ra
[15-12;17-10]-[10-13;8-15;6-17]-23 *M. tuberculosis* H37Ra



28

a - b - c - d

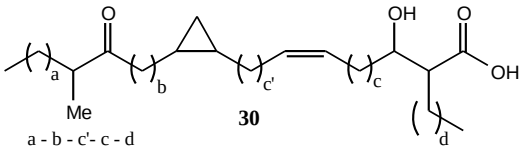
17-19-15(13)-23 *M. tuberculosis, M. bovis, M. bovis* BCG, *M. microti*
17-17-17-23 *M. tuberculosis Canetti*
17-17-17-21 MAC
15-17-17-21 *M. scrofulaceum*
17-15-17-21 *M. marinum*



29

a - b - c - d

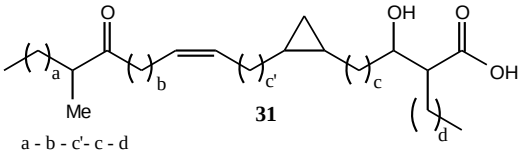
17-18-15-23 *M. tuberculosis, M. bovis* BCG
17-16-19-21 MAC



30

a - b - c'- c - d

17-[8-10;10-8;12-6;14-4]-15-23 *M. bovis* BCG



31

a - b - c'- c - d

[15-12;17-10]-6-17-23 *M. tuberculosis* H37Ra

Figure 10: ***Structures and distribution of major and minor keto-mycolic acids***[47]

1.2.3 Stereochemistry of mycolic acids

The absolute stereochemistry of the chiral centres in the mycolic acids has still not been completely determined. The two stereocentres in the α and β-positions relative to the carboxylic group (**38**, **Figure 11**) have been found to be both in the *R*-configuration for all mycolic acid examined, irrespective of the groups in the meromycolate chain.[74,75,76,77,78] The presence of the hydroxyl group and the relative configuration between it and the alkyl chain have been demonstrated to be capable of the altering the film molecular packing. The formation of a hydrogen bond between the hydroxyl group and the carboxylic group has a stabilising effect for the aligned conformation between the two long chains.[79,80]

Moreover, the absolute configuration of these two chiral centres is necessary for efficient recognition by T cells and the generation of an immune response by the host organism against pathogenic mycobacteria;[81] the same is also true for the antitumour properties of mycolic acid derivatives.[82]

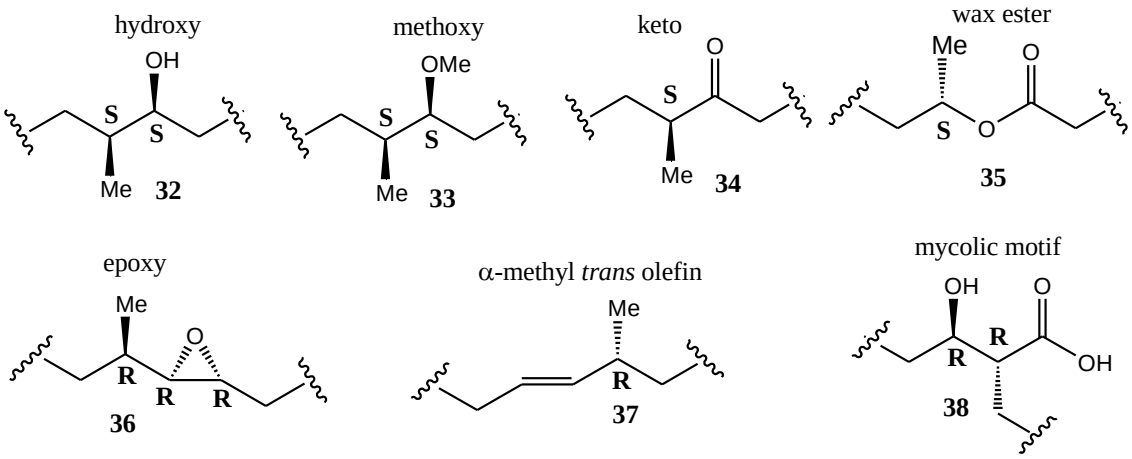


Figure 11: ***The strereochemistry of chiral centres of the mycolic acids***

Determination of absolute stereochemistry of the chiral centres in the meromycolate chain is particularly difficult and very little is known. Stereochemical studies on the different mycobacteria suggested that the methyl branch adjacent to the hydroxy (**32**), methoxy (**33**) and keto (**34**) groups in the mycolic acids is in the *S*-configuration. The hydroxyl (**32**) and the methoxy (**33**) at the distal group in the mycolic acids are also

calculated to be in the *S*-configuration (**Figure 11**).[74,75,66,78] This observation has been used to confirm that the two oxygenated species are biosynthetically related.

Moreover, the methyl branch of wax esters (**35**) apparently derived by enzymatic oxidation of keto-mycolic acids is also of *S*-stereochemistry.[83,84,85] In addition, it has been found that the three stereocentres of the α-methyl-*trans*-epoxy-mycolic acids (**36**) are in the *R*-configuration.[66,76,78] Furthermore, the methyl branch next to the *trans*-double bond (**37**) has been established as having the *R*-configuration in mycolic acids, *M. aurum*,[86] *M. marinum* and *M. ulcerans*.[76,78]

Mainly these configurations were deduced from a comparison of measured molecular rotations $([M]_D)^*$ with theoretical values. Theoretical values were calculated by adding the contribution of independent chiral centres estimated from the molecular rotation of α-mycolate and of corynomycolate or hentriacontane derivatives.[87] The stereochemical results have also been obtained by fragmenting mycolic acids into smaller sub-units which could be compared with known compounds.[76] The first method appears to be speculative, while the second, obtained by degradation of mycolic acids, could allow changes to the chiral centres. Therefore, more studies seem to be needed to obtain definitive results.

There are two more chiral groups in the mycolic acids; *cis*-cyclopropane and α-methyl-*trans*-cyclopropane. Little is known about the absolute stereochemistry of the *cis*-cyclopropane. Interestingly, however, a recent computational study of the binding of a model methoxymycolic acid glucose ester to the human MHC class I like molecule CD1b using a *R,R*-configuration for the α-methyl-β-methoxy unit shows a close fit to the shape of a non-functional natural mycolate.[88] The same paper reports a similar good fit for a model α-mycolic acid having a distal cyclopropane with as *S,R*-configuration.

However, a recent study determined the absolute configuration of the α-methyl-*trans*-cyclopropane.[89] The compound (**39**, **40** and **41**) were synthesised and optical rotation, the ^{1}H and ^{13}C NMR spectra were examined (**Figure 12**).

* The value equalling 1/100 of the product of the specific rotation of an optically active compound and its molecular weight.

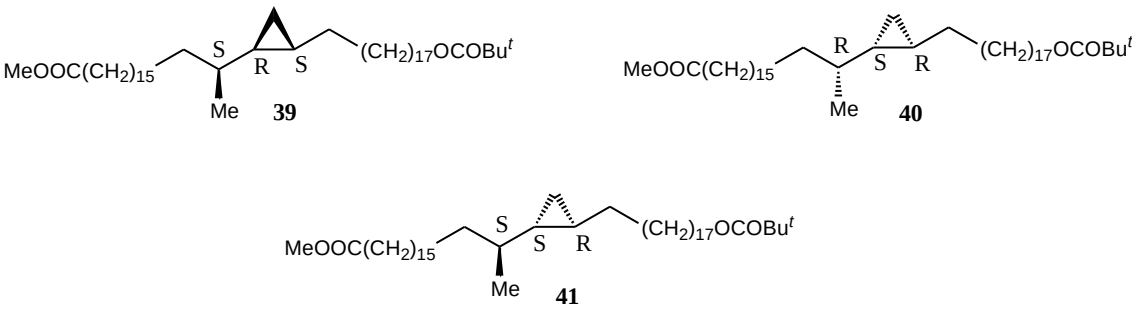


Figure 12: ***α-Methy-trans-cyclopropane wax esters in different stereochemistry***

The ^{1}H and ^{13}C NMR spectra of (**39** and **40**) were identical. However, they showed opposite specific rotation (+3.7 and -5.1, respectively). Because they are diastereomers to each other, the spectra for (**41**) were very similar to those for (**39** and **40**) but significant differences were seen in the high field regions in each case. Thus, although the cyclopropane regions of (**39** and **40**) were visually identical to those reported for mixtures of wax ester meromycolates, and indeed to the same region in methoxymethylmycolates containing an α-methyl-*trans*-cyclopropane subunit, the same region of (**41**) was clearly different. Thus, **Figure 13** shows the high field region of the ^{1}H NMR spectra of the three synthetic isomers, together with the same region for a natural wax ester in which there is a mixture of *cis*- and α-methyl-*trans*-cyclopropanes.

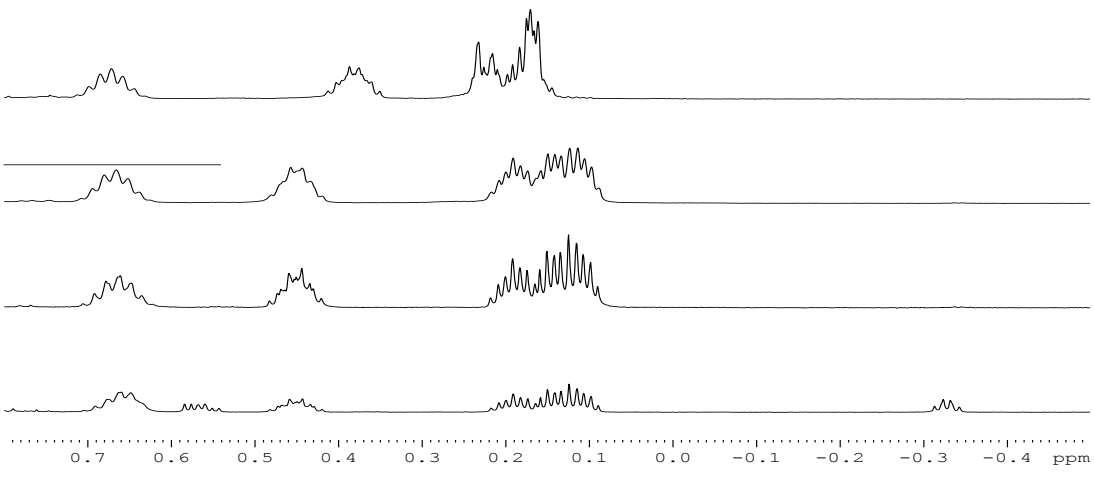
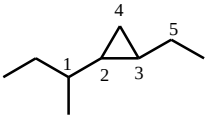


Figure 13: ***Cyclopropane region of ^{1}H NMR of (top to bottom) (i)* 41*; (ii)* 40*; (iii)* 39*; a natural sample of the dimethyl ester of an ω-carboxy mycolic acid extracted from M. avium;***[74] ***this sample contains mainly α-methyl-trans-cyclopropanes accompanied by some cis-cyclopropanes.***

Moreover, there were small but significant differences in the carbon NMR spectra between (**39/40**) and (**41**). Again the published carbon NMR shifts for either mycolic acids or wax esters containing the α-methyl *trans*-cyclopropane unit were identical to the compounds (**39** and **40**).

Thus, the relative stereochemistry of this unit is established. The close agreement between the rotation obtained for (**39**, $[\alpha]_D^{22}$ +3.7 (*c* 1.03, $CHCl_3$)) and that reported by Anderson for a natural mero-wax acid ($[\alpha]_D$ +5.3 in $CHCl_3$) suggests that the absolute stereochemistry of natural mycolic acid for the α-methyl-*trans*-cyclopropane unit is (*S,R,S*), though the Anderson product was a mixture obtained well before modern spectroscopic techniques were available.[83,84,85] It will be interesting to compare his results with the rotations of mero-wax acids from other bacteria when these become available (**Table 1**).

Table1: ***Selected ^{13}C NMR shift for α-methyl-trans-cyclopropane fragment of natural wax esters and mycolic acids compared to* 39/40 *and* 41**



1	2	3	4	Mycobacterium	Ref.
38.11	26.16	18.62	10.49	*M. tuberculosis*	[90]
38.13	26.14	18.62	10.50	*M. gordonae*	[91]
38.1	26.1	18.6	10.50	*M. avium-M. intracellulare complex*	[92]
38.11	26.13	18.61	10.48	**39** *SRS*	
38.11	26.11	18.60	10.47	**40** *RSR*	
38.05	26.11	17.34	11.81	**41** *SSR*	

1.2.4 Roles of different groups in the mycolic acid

The kinds of mycolic acids and their relative abundance depend upon growth conditions.[93,94,95] It has also been demonstrated that alterations in the proportion and structure of mycolic acids produce significant changes in the fluidity of the cell wall, changing the permeability and the virulence of the pathogenic mycobacterium.[96,97]

Analysing in more detail the role of each mycolic acids class in *M. tuberculosis*, the α-mycolates are predominant (about 50 % of the total).[47] One of the most interesting features of these lipids is the presence of cyclopropanes in the meromycolate chain. The necessity of this group for the pathogenesis of *M. tuberculosis* has been demonstrated.[98]

Cyclopropanation occurs in slow-growing pathogenic mycobacteria such as *M. tuberculosis*; it does not occur in environmental ones, like *M. smegmatis*.[99] Moreover, its formation in bacterial membranes should have a high energetic cost.[100] Many researchers have investigated the reasons for this modification in several fatty acids without finding a universally accepted answer. An hypothesis is that ring creation has an effect on resistance of bacteria under oxidative stress.[100] Cyclopropane fatty acids are less sensitive than unsaturated lipids to ozonolysis and other oxidative treatment.[101] Thus, it has been proposed that the cyclopropyl rings could also stabilise the mycolic acids of pathogenic mycobacteria in an oxidative environment.[102] Mycolic acids of *M. smegmatis* with an additional cyclopropane in the distal position appeared to be more stable to strongly oxidative situations.[102] However, cyclopropanes in the proximal position appear to have no significance against oxidants.[98,99]

Methoxy- and keto-mycolic acids, the oxygenated series, are also critical for the virulence of mycobacteria.[103, 104, 105] It has been determined that slow-growing pathogenic mycobacteria can manipulate the ratios between keto- and methoxy-mycolic acids in order to adapt better to the environment. Dubnau et al, studying a mutant unable to produce oxygenated mycolates, verified the importance of these compounds for the permeability and fluidity of the cell wall.[103]

Interestingly, in a monolayer prepared with keto-mycolic acids, the lipids do not experience the same conformational change observed for α-mycolic acids when a high surface pressure is applied to the monolayer.[106] This kind of mycolic acid could therefore play an important role in the determination of the permeability properties of the mycobacterial cell wall.[106] In particular, it has been observed that keto-mycolic acids have an essential role in the growth of the organism within the natural host cell.[104]

Conversely, no clear role has been attributed to methoxy-mycolic acids and recent studies demonstrated that the loss of these did not affect the cell wall permeability and

the resistance to antibiotics of *M. bovis* BCG even if it might influence its long-term growth *in vivo*.[107]

Cis-olefins produce "kinks" in the carbon chain of the lipids, yet the *trans*-types do not. In this way the latter allow mycolic acids to be more tightly packed making the cell wall less fluid.[63] *Trans*-cyclopropanes have similar functions and are fundamental for the maintenance of membrane viscosity. This is confirmed by the fact that, in some mycobacterial species, the levels of *trans*-compounds rise if the mycobacterium grows under higher temperatures.[96]

It has been demonstrated that clinical strains of *M. tuberculosis* have a higher *trans*-cyclopropanated oxygenated mycolic acid content than laboratory strains. This suggests that *in vivo* growth promotes either *trans*-cyclopropane formation, or the growth of sub-populations of *M. tuberculosis* with higher *trans*-cyclopropane content.[108] This evidence is consistent with the proposal that *trans*-mycolic acids modify the macroscopic qualities of the cell wall and that their production depends on growing conditions.[109]

Mutants over-producing *trans*-substituted mycolic acids have decreased vigour; they additionally have altered proportions of keto- to methoxy-mycolic acids.[108] It is important to note that these results suggest that the quantities of each of these sub-classes are interdependent. This could also lead to the hypothesis that these types of acids are biosynthetically related.

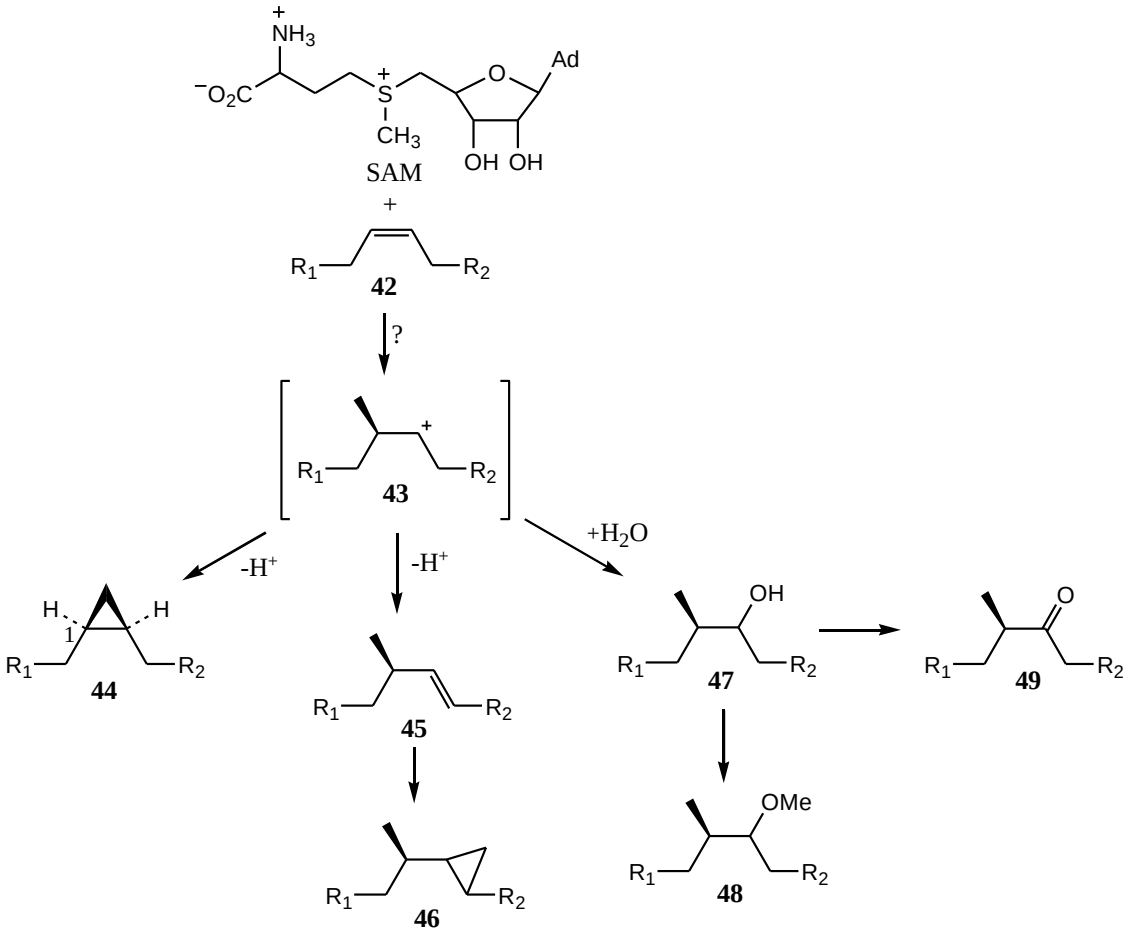
In conclusion, these results do not prove the necessity for mycobacterial virulence on one or another class of mycolic acids but they are the starting point in the evaluation of the role of each class for mycobacterial pathogensesis.[110]

However, an appropriate mycolate composition is a key prerequisite for mycobacterial growth and interference with this composition could be a valid strategy for the development of a new anti-mycobacterium treatment.[104] The role of each type of mycolic acid is difficult to understand because more than one mycolate functional group, or even different cellular molecules, can have similar effects on the fluidity of this membrane system.

The position of the different functional groups could also influence the macroscopic properties of the cell wall and in particular the characteristics of the mycolic acids packing.

1.2.5 A possible mechanism of formation of different functional groups

Much is now known about the enzymes that control the biosynthesis of mycolic acid,[108,111] and several hypotheses proposing different mechanisms for the processes of cyclopropanation, oxygenation and methylation have been put forward. Experiments in which bacteria are grown in the presence of labelled methionine indicate that the methyl group of methionine can become incorporated directly into mycolic acids. It has been shown that the bridging methylenes of the cyclopropane ring, the carbon of the methoxy functionality, and the methyl branches adjacent to *trans*-olefins, methoxy and keto moieties are all derived from methionine, presumably *via* S-adenosyl-*L*-methionine (SAM).[112,113,114,115] This process could be stabilised by the aromatic residues present in the active-site via π-cation interaction. Since it has been shown that cyclopropane fatty acids maintain the configuration of their substrate, the formation of the carbocation (**43**) has to be the slow step and then this rapidly transformed (**Scheme 2**).[116]



Scheme 2: ***The insertion of the functional groups***

While it had long been proposed that the meromycolyl cyclopropane groups were derived from double bonds, the first direct evidence for this idea came with the identification of a gene from *M. tuberculosis* capable of conferring upon *M. smegmatis* the ability to produce large amount of cyclopropane containing mycolic acids.[117]

Addition of a methyl group from SAM to Z-alkene (**42**) generates the carbenium ion shown which can then be deprotonated to form a *cis*-cyclopropane ring (**44**). The removal of a proton on the methylene group in the α-position yields the α-methyl-*trans*-olefin unit (**45**). The *trans*-olefin could be the substrate for a second SAM-dependent methylation to form the α-methyl-*trans*-cyclopropane unit (**46**) seen in the oxygenated mycolates of *M. tuberculosis*. The cation can also react with water to form the hydroxy-methyl-mycolate unit (**47**) precursor to methoxy- (**48**) and keto-mycolate unit (**49**) as shown in **Scheme 2**.[63,105]

However, labelling studies show that the methyl branches in the α-methyl-*trans*-cyclopropane of mycolic acids from *M. tuberculosis* are derived from 2-position of acetate units, whereas those from *M. smegmatis* are derived from C-1.[118] The *cma*A2 gene in virulent *M. tuberculosis* has been shown to be required for the synthesis of *trans*-cyclopropane in both keto- and methoxy-mycolate.[119]

It is important to note that this enzymatic route has a significant implication. Thus, the stereochemistry of at least one carbon for all the functional groups present in the meromycolic chain has always to be in the same configuration. Additionally, through a more accurate analysis of the stereochemistry of the chiral centres present in the meromycolate chain, important information about the biosynthesis of these compounds might also be acquired. In particular, following the theory firstly proposed by Jaureguiberry *et al*.[120] and considering the similarity of the structure of the active sites of some of the cyclopropane synthesis, it seems possible that all these enzymes share the same mechanism of action. It is possible that the C_1 addition to the double bonds would always happen on the same face of the olefin (**Scheme 2**).

1.2.6 A possible folded conformation of mycolic acids

It has been shown that the antibodies produced by animals infected with TB were able to distinguish between different types of mycolic acids.[39,40] Experiments with T cells gave more complex results, however, regarding recognition of the fine structure of the lipid moiety in cord factor.[88,121,122]

In particular Grant *et al*.[122] suggested a possible reason for the stronger recognition by T cell receptor of oxygenated mycolic acids. They suggested that keto- and methoxy-mycolic acids fold in a way that allows the three polar functions of the lipid chain to be in close proximity and to form a epitope, which is well recognized by this kind of receptor (**Figure 14**).

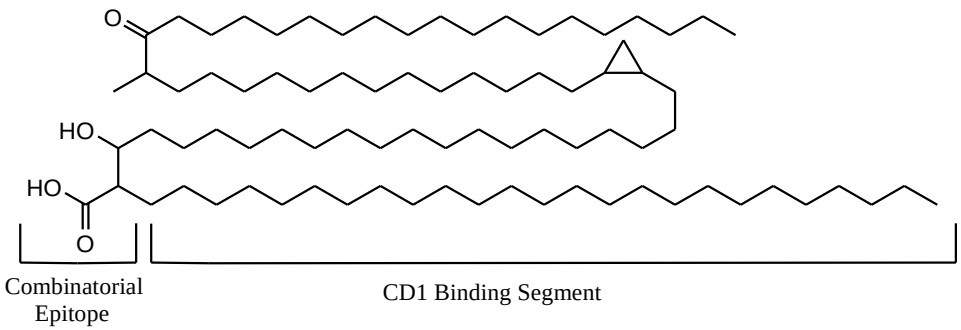


Figure 14: ***A possible folded conformation for keto-mycolic acid***

Hasegawa *et al*.[106, 123 , 124] also proposed that mycolic acids could exist in folded conformations, although this was in a completely different context, namely, when they are arranged in monolayer films. These conformations are thought to be particularly stable in keto-mycolic acids due to strong molecular interactive forces. In fact, it has been suggested that these lipids exist in a folded form even when high surface pressure is applied to the monolayer.

Finally, synthetic mycolic acids may be utilised for the preparation of a simple model of the multi-layer structure present in the *M. tuberculosis cell* wall. This method has already been used to determine the relationships between monolayer properties and the chemical structures of different natural types of mycolic acids.[124]

1.3 Asthma

Asthma is a chronic disease of the respiratory system in which the airway occasionally constricts and is lined with excessive amounts of mucus, often in response to one or more triggers. These acute episodes may be triggered by such things as exposure to an environmental stimulant, cold air, exercise or exertion, or emotional stress. In children, the most common triggers are viral illnesses such as those that cause the common cold. This airway narrowing causes symptoms such as wheezing, shortness of breath, chest tightness, and coughing, which respond to bronchodilators.[125]

The general approach to asthma treatment is acute rescue treatment, controller treatment and preventing of long-term complications. Over the last century, it has been recognized that asthma may be precipitated by certain environmental exposures and that eliminating these exposures may be of value in asthma treatment.[126]

Mycolic acids are distinctive components of the cell wall of *Mycobacterium tuberculosis*, the causative agent of tuberculosis, section **1.1.2**. A single application of mycolic acids into the wind-pipe of mice sensitised mice to an allergen. The persistent effect of the single treatment suggests a novel type of therapeutic compound that, by activating inherent immune regulatory mechanisms rather than blocking the consequences of inappropriate immune responses, promises a disease-modifying treatment of asthma. The '*Environment and Lifestyle Influence on Asthma*' briefly describes that early lifetime contacts with environmental micro-organisms are able to prime regulatory T cells (T_{reg}) and regulatory dendritic cells (DC_{reg}) that help control inappropriate inflammation and that are deficient in allergic humans.[127,128]

Recently, studies showed that a single intratracheal treatment of sensitized mice with mycolic acids prevented the onset of ovalbumin (OVA)-triggered allergic airway inflammation and promoted unresponsiveness to a secondary set of allergen exposures. Intratracheal (i.t.) instillation of mycolic acids mimics pathogen-associated host innate immune responses and renders OVA-sensitized mice tolerant for Th2-driven eosinophilic lung inflammation following OVA-aerosol challenge.[129, 130] Immune suppression coincides with the differentiation of professional airway phagocytes into foam cells, but is maintained after cells have disappeared and becomes even more pronounced with subsequent OVA-challenges. Thus, next to the innate foam cells that control the local immune suppression after mycolic acid uptake, a lasting allergen tolerance is generated, mediated by $CD25^{+}$ T_{reg}.[130]

1.4 Synthesis of mycolic acids

1.4.1 Aims

There are many practical reasons for the synthesis of mycolic acids. A single synthetic enantiomer of mycolic acids may help in understanding the physical and chemical properties of natural mycolic acids. The synthesis of these compounds will be important for the identification of the exact structure of natural mycolic acids. In particular, the configuration of the different chiral centres present in the meromycolate chain could be revealed through comparison between the natural compounds or their derivatives with the synthetic analogues. Additionally, through a more accurate analysis of the stereochemistry of the chiral centres present in the meromycolate chain, very important information about the biosynthesis of these compounds might also be acquired.

Currently, biological tests of mycolic acids for their effects in tuberculosis and asthma are of great interest. They could help to develop methods for detecting TB and other bacterial diseases. The functional groups in the meromycolate chain and the length of the α-unit have been recognized as significantly influencing the physical properties of monolayer films of mycolic acids. The preparation of different synthetic analogues could also help in the determination of the role of each particular feature of the acids in the regulation mechanisms of the drug permeability of the cell envelope.

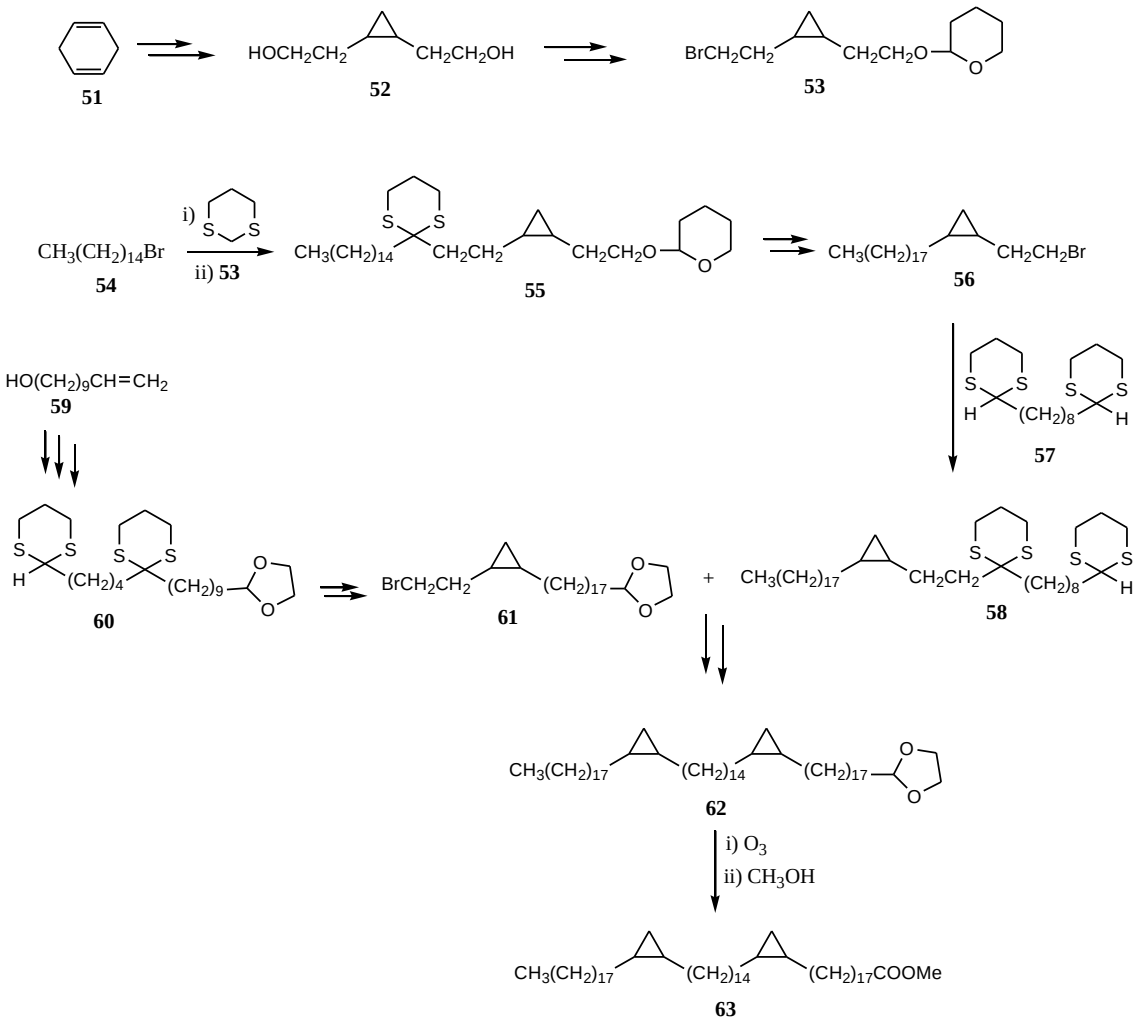
Another reason to produce synthetic mycolic acids is that antibodies produced against different synthetic mycolic acids which have similar but not equivalent structures, could be utilized for the identification of the structure and stereochemistry of the mycolic acids in mycobacteria. If only one type of these particular antibodies recognized the mycobacterium, the synthetic mycolic acid used as the antigen to produce these competitive antibodies, should have the same structure as the natural one. This type of analysis has already been utilized for a partial characterization of the structure of the cord factors and their mycolic acids.[81,131]

Finally, the functional groups in the meromycolate chain, and the length of the α-unit, have been recognised as significantly influencing the physical properties of monolayer films of mycolic acids. The preparation of different synthetic analogues could help in the determination of the role of each particular feature of the acids in the regulation mechanism of the drug permeability of the cell envelope.

1.4.2 Previous synthesis of meromycolic acids

In the synthesis of mycolic acids, one problem that has to be attended to is the synthesis of its major fragment, 'meromycolic acid'. One of the standard methods for characterisation of mycolic acids is thermolysis of the hydroxyacid functionality to give an aldehyde, or 'meromycolaldehyde'.[20,32,63, 132] This can be oxidised to the corresponding 'meromycolic acid' (**Scheme 1**).

Meromycolic acid was first synthesised by Gensler as a mixture of four stereoisomers containing two *cis*-cyclopropane rings (**63, Scheme 3**).[133]



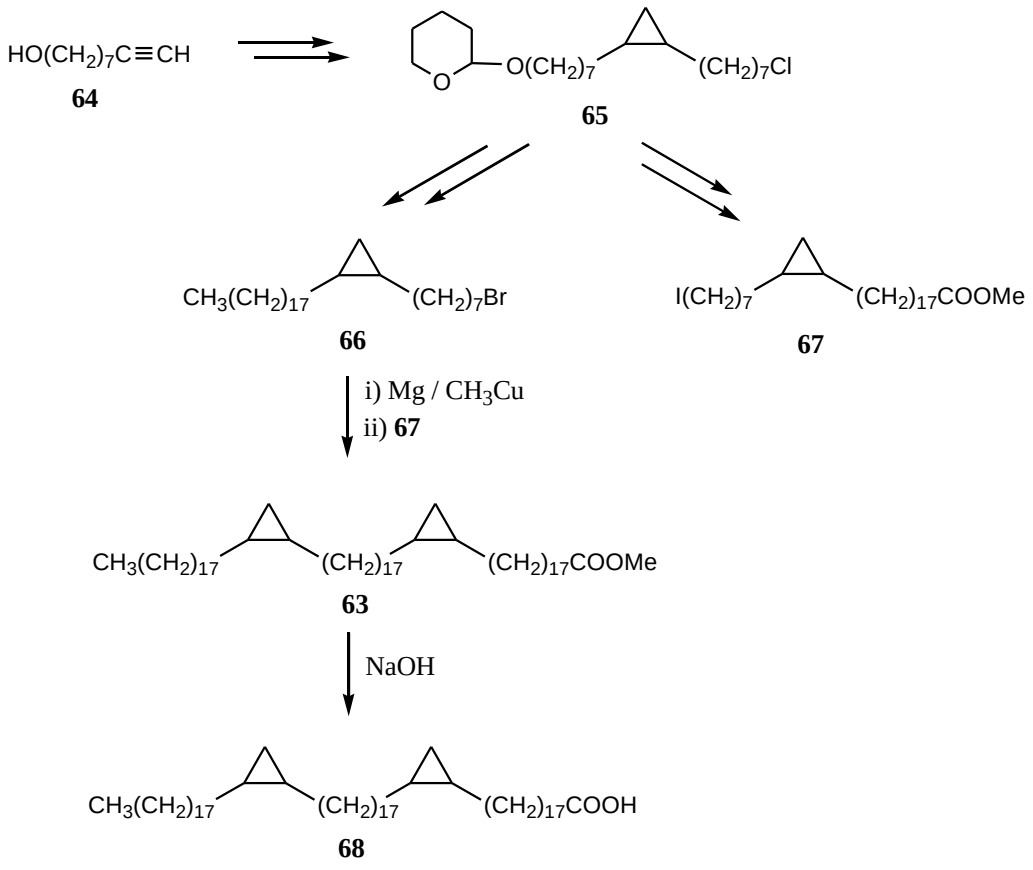
Scheme 3: ***The first synthesised meromycolic acid***

1,4-Cyclohexadiene (**51**) was used as starting material and after several steps the cyclopropane-containing bromide (**53**) was obtained as two pairs of enantiomers. The cyclopropane bromo compound (**56**) was formed by alkylation of pentadecyl bromide

(**54**) with (**53**), desulfurization with Raney nickel,[134] hydrolysis and bromination. The chain was extended to (**58**), by alkylation of (**56**) with (**57**). The synthesis was continued with the ozonolysis of 10-undecenol to 10-hydroxydecanal, which was converted to the protected compound (**60**) which in an alkylation reaction again with (**53**) gave the second intermediate (**61**).

Finally, the desired product (**63**) was obtained by coupling of these two intermediates, (**58**) and (**61**) followed by desulfurization and ozonolysis.

Subsequently, Gensler *et al*.[135] prepared the meromycolic acid (**68**) by another method which combined different fragments. This method is shorter and could be more easily scaled up (**Scheme 4**). To obtain the intermediate (**65**), the 1-hydroxy-8-nonyne (**64**) was used as a starting material and after four steps led to (**65**) from which another two intermediates, (**66**) and (**67**), were obtained.



Scheme 4: ***The second Gensler et al approach***

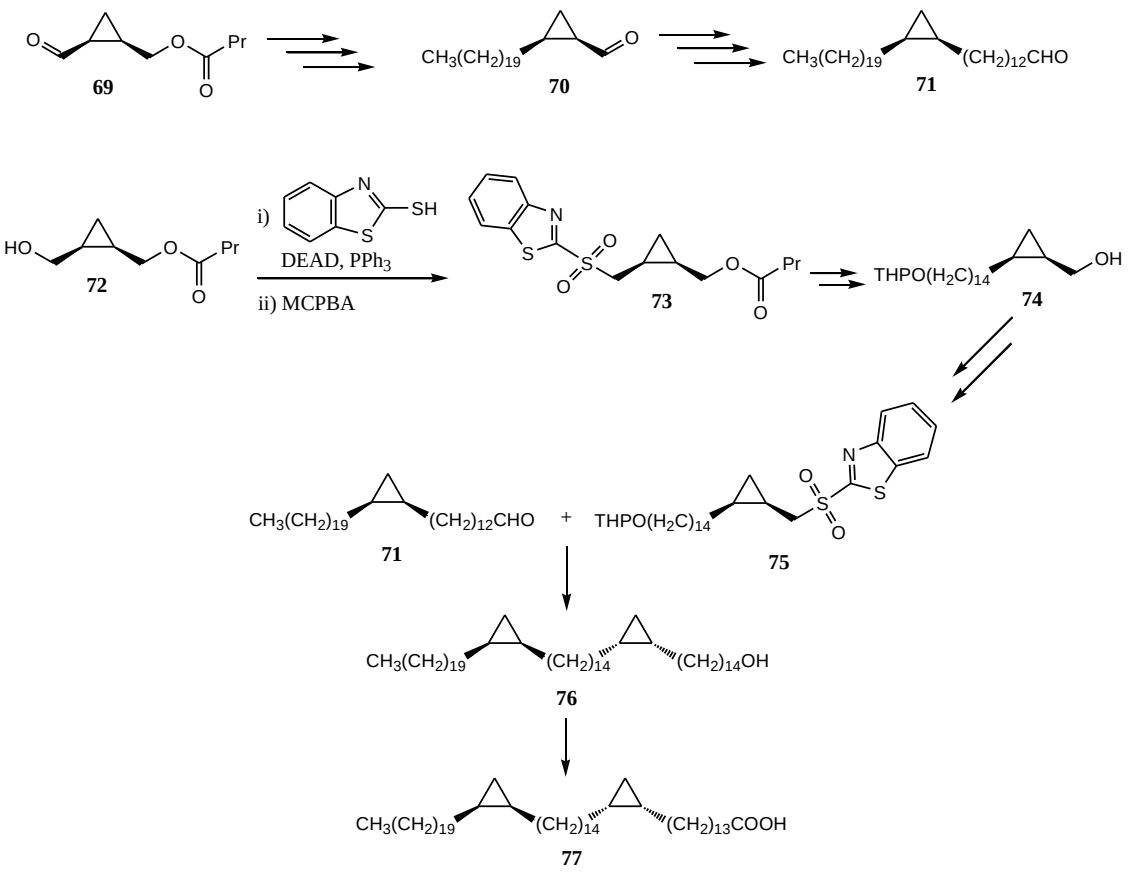
A Grignard reaction was used for the coupling in this method. The Grignard reagent was prepared from the alkyl bromide (**66**) and alkylated with the alkyl iodide (**67**). Finally, saponification gave the target mycolic acid (**68**).

This method is an improvement over the first approach but still presents some problems. The final coupling gives a very poor yield. In fact, the Grignard reagent not only gives the desired meromycolic acid (**68**) but also gives several other compounds, as seen through high-resolution mass spectroscopy. The required one is only a small part of the mixture. These results are possibly caused by the limited solubility of the reactants (**66** and **67**). The resulting heterogeneity reduces the efficiency of the reaction because the two compounds are not able to interact. Furthermore, Gensler's method does not have any control on the stereochemistry of chiral centres of the cyclopropyl groups.

The first synthesis of a single enantiomer of an analogue of meromycolic acid (**77**) was reported by Al Dulayymi *et al.*[136] There are two key steps in this method: firstly, the preparation of single enantiomers of cyclopropane building blocks followed by the coupling of these units.

The aldehyde (**69**) was prepared from the anhydride of cyclopropane-*cis*-1,2-dicarboxylic acid.[137] A Wittig reaction of this with nonadecyltriphenylphosphonium bromide and *n*-butyl lithium, and reduction with lithium aluminium hydride, led to the alcohol as mixture of *Z*- and *E*-isomers. Saturation of the alkene with di-imide, prepared *in situ* by reaction between hydrazine, sodium periodate and acetic acid, and oxidation of the alcohol led to aldehyde (**70**). Chain extension with a second Wittig reaction gave the aldehyde (**71**).

Other important features of this approach are the coupling reaction, which are used to link the different units in several stages, securing the final desired stereochemistry. First of all, the Julia reagent sulfone (**75**) was prepared and reacted with the aldehyde (**71**) to give a mixture of *E*- and *Z*-alkene. The subsequent deprotection of the alcohol group and the reduction of the derived compound with di-imide give the alcohol (**76**), ($[\alpha]_D$ +2.2 (c 0.055 g/10 ml; $CHCl_3$)). The alcohol (**76**) was oxidised under phase transfer conditions to the corresponding meromycolic acid (**77**, **Scheme 5**).



Scheme 5: ***The synthesis of a single enantiomer of meromycolic acid (Al Dulayymi et al.)***[136]

This method has some important advantages. The overall yield is better and the absolute stereochemistry of both cyclopropane rings is under control. It could be easily adapted for the synthesis of other homologous meromycolaldehydes because the different portions of the compounds are incorporated at different stage of the synthesis. It also allows preparation of the other stereoisomers by simple modification in the sequence of reaction, which controls the position of the substituents on the cyclopropyl groups. Therefore, it is particularly useful in order to obtain all the possible diastereoisomers of meromycolaldehydes.

In recent studies, it has been reported that various meromycolic acids which contain α-methyl-*trans*-cyclopropane unit, has been synthesised.[89,138] Mainly, the method used to prepare this compound (**Figure 15**) is similar to the method used to prepare α-meromycolic acid. The key route to prepare the α-methyl-*trans*-cyclopropane fragment will be discussed later.

The meromycolates (**79**, **80** and **81**) derived from ω-carboxy-mycolic acids represent an interesting synthetic target because they contain only one group of chiral centres.

This is the α-methyl-*trans*-cyclopropane group. It may allow the overall chirality of this part of the mycolic acid system and the absolute stereochemistry of this unit determined.

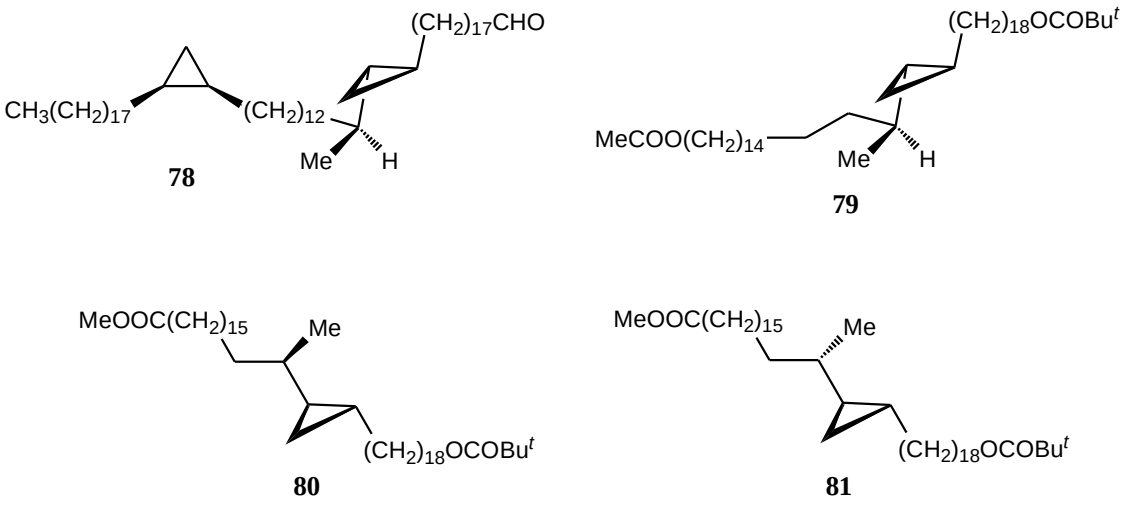
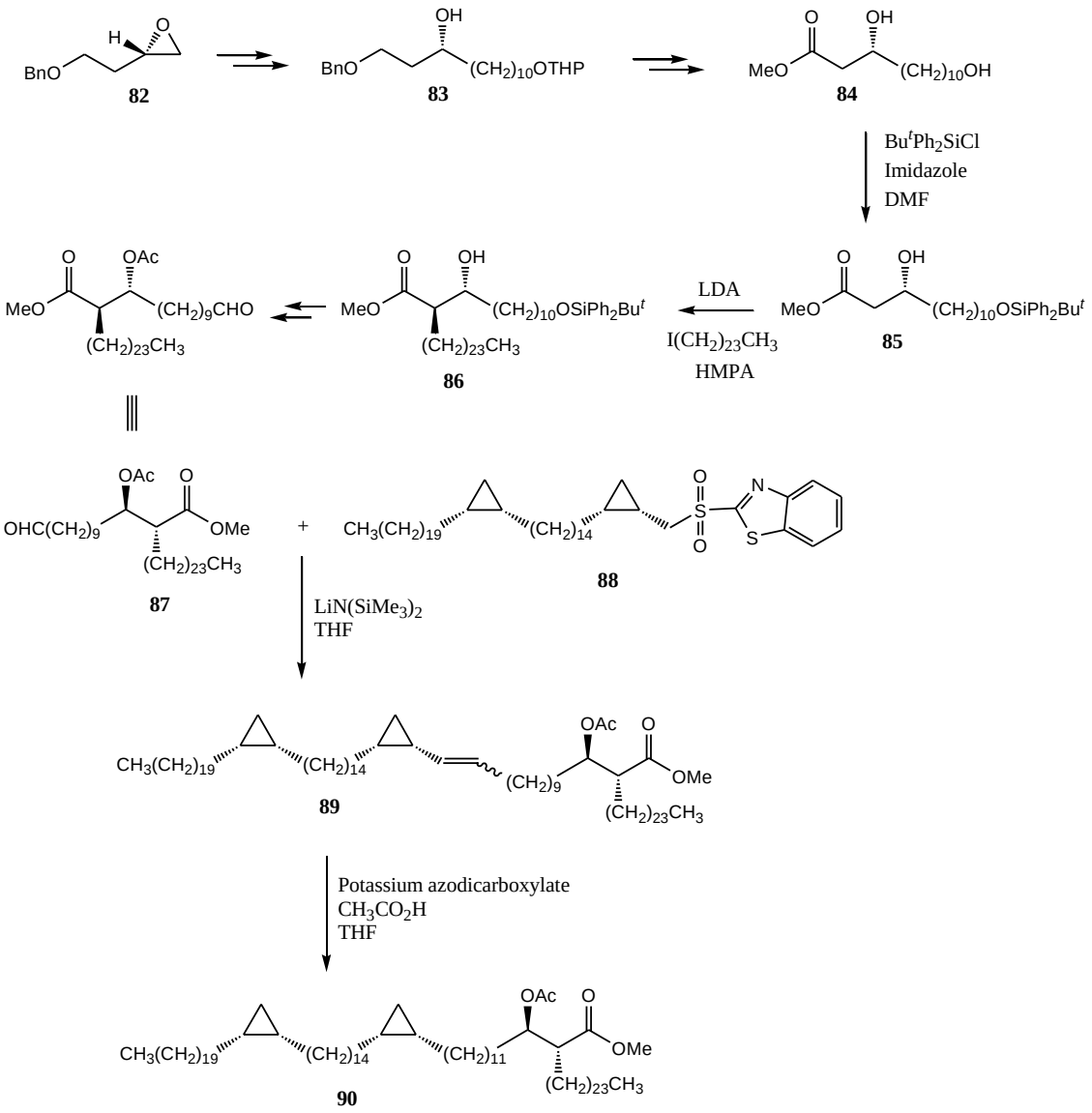


Figure 15: ***Meromycolic acids containing α-methyl-trans-cyclopropane***

1.4.3 Previous syntheses of whole mycolic acids

The first entire enantiomerically pure mycolic acid has been prepared by Al Dulayymi *et al*.[139] Ring opening of the epoxide (**82**)[140] with a Grignard reagent prepared from 9-bromononan-1-ol tetrahydropyranyl ether led to a single enantiomer of the mono-protected diol (**83**). This was transformed in four steps into the diol (**84**, **Scheme 6**). This diol was then protected at the primary alcohol group as a silyl ether (**85**) and then alkylated,[141] using 1-iodotetracosane to give the hydroxy ester (**86**). Protection of the secondary alcohol in (**86**) as the acetate, deprotection of the primary alcohol and oxidation led to the aldehyde (**87**). In the final and key stage of the synthesis, the single enantiomer of protected aldehyde (**87**) was coupled to the dicyclopropane. Treatment of the sulphone (**88**) with aldehyde (**87**) and base in a modified Julia reaction led to a mixture of *Z*- and *E*- alkenes (**89**). Hydrogenation with di-imide gave the desired protected mycolic acid (**90**),[142] $[\alpha]_D^{22}$ +4.2 (*c* 0.735, $CHCl_3$) (**Scheme 6**).[143]

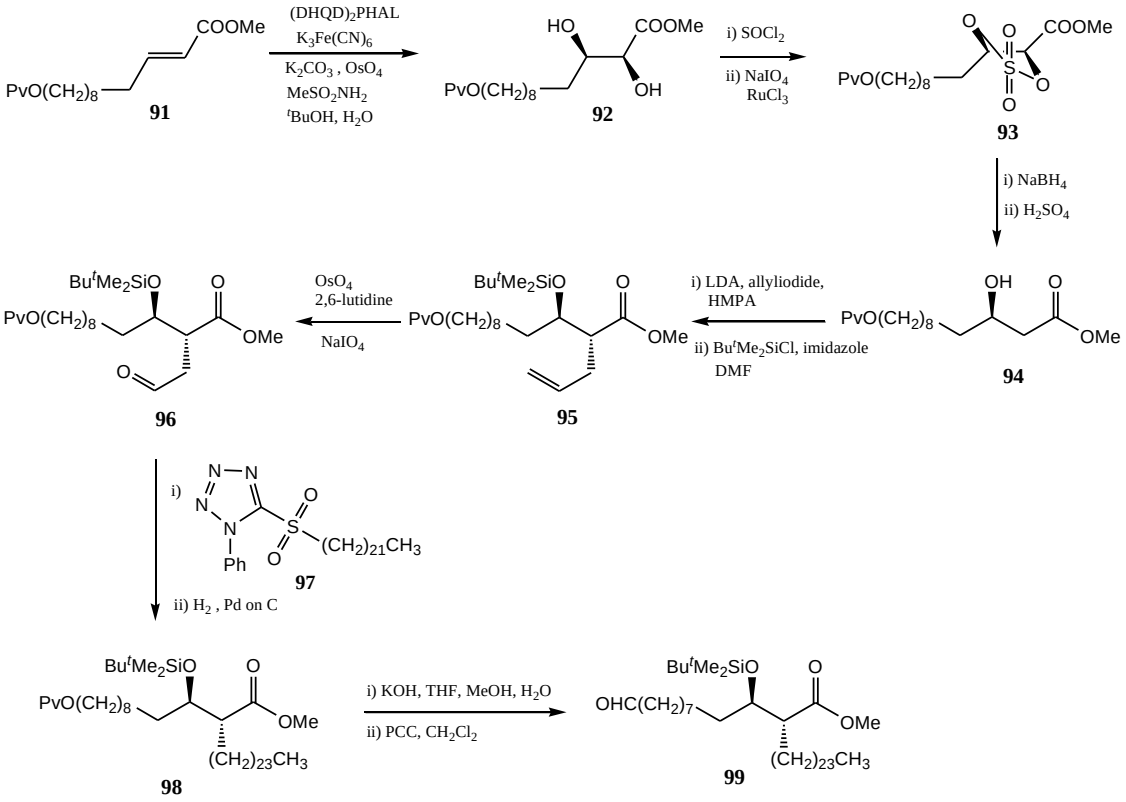


Scheme 6: ***A synthesis of an α-mycolic acid***[143]

The final protected compound shows basically identical ^{1}H and ^{13}C NMR spectra to those of a mixture of α-mycolic acids extracted from *M. tuberculosis* and then protected ($[\alpha]_D$ +3.7), which is a mixture of homologues in which (**90**) predominates. However, these data still do not allow the identification of the absolute configuration of the stereogenic carbon atoms in the mycolic acids, especially of those in the meromycolate chain, with certainty. In fact, the optical rotation probably would not show a significant change after the modification of the stereochemistries of the cyclopropyl rings. More tests to compare the natural and synthetic samples are needed.

The alkylation reaction of the β-hydroxy ester with a long chain iodide is the most difficult reaction of the synthesis of the mycolic acid. It gives low yield and it is a not always reproducible. Therefore, this reaction is of interest.

Recently, a slightly different method for the preparation of corynomycolate analogues has been reported.[144] In this method, a short chain allyl iodide was used as a alkylation agent and then chain extended using a Julia reaction. The *E*-α,β-unsaturated ester (**91**) was prepared from 1,10-decanediol in a four steps and transformed into the diol (**92**) using a Sharpless dihydroxylation.[145] The diol was converted into the sulfate (**93**) and then regioselectively reduced and hydrolysed to give the β-hydroxy ester (**94**) as shown in **Scheme 7**.



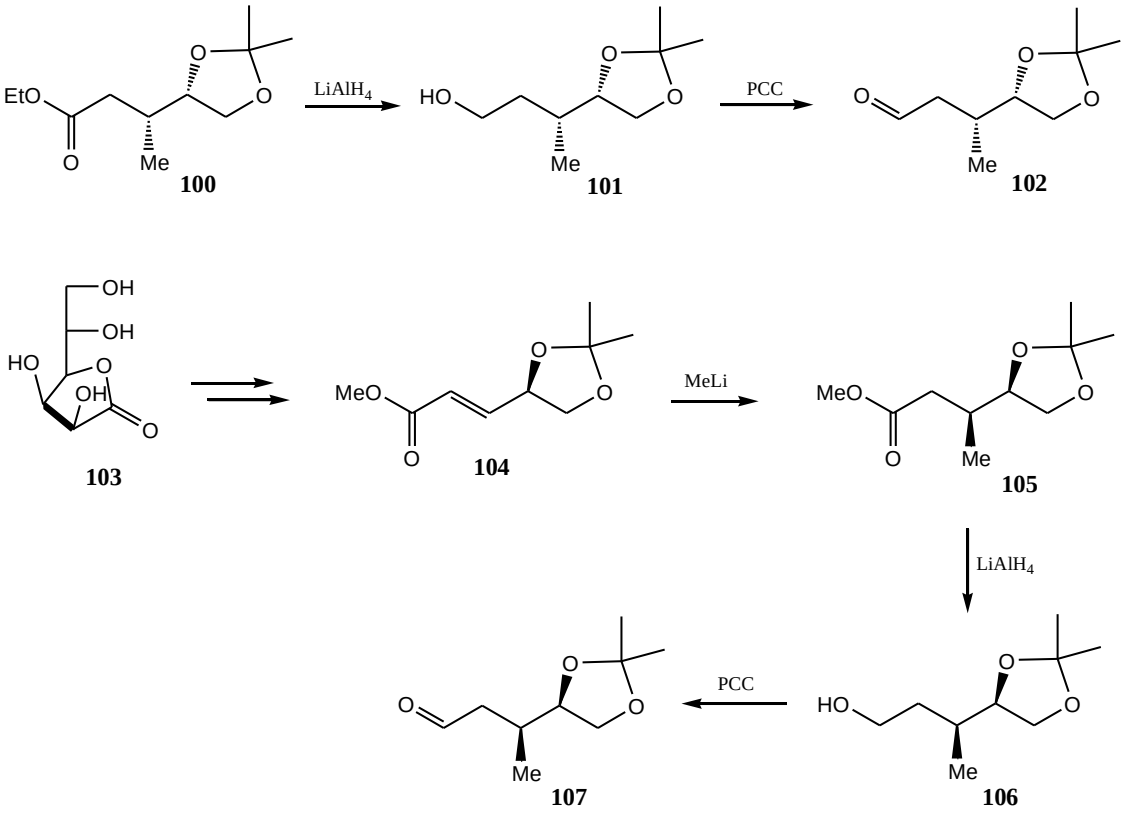
Scheme 7: ***An improved method to prepare the corynomycolic acid analoge***

Subsequently, a Fräter alkylation[146,147] with allyl iodide introduced an allyl chain at the α-position and the hydroxy group was protected to give (**95**, **Scheme 7**). The use of such a reactive electrophile resulted in a much more reliable reaction than that previously described,[139,143] which used a very long chain alkyl iodide. It also gave twice the best yield achieved with a long chain. Nonetheless, it was still very sensitive

to the precise conditions and sometimes produced a minor isomer apparently derived by allylation on oxygen.

The alkene (**95**) was converted to the corresponding aldehyde (**96**) by oxidation with OsO_4 – $NaIO_4$ and reacted with the sulphone (**97**) to give an unsaturated intermediate. Finally, reduction of this alkene allowed the formation of the desired long chain at the α-position (**98**, **Scheme 7**).

Most recently, in the synthesis of a series of mycolic acids, a route to the oxygenated methoxy-mycolic acids was reported.[148] The α-methyl-β-methoxy was first prepared from mannitol by a known sequence leading to the aldehyde (**102**).[149,150,151,152] In a similar way, the enantiomer (**107**) was prepared from L-gulono-1,4-lactone (**103**), derived by hydrogenation of ascorbic acid.[153]



Scheme 8: ***Preparation of the stereocentres of the α-methyl-β-methoxy units of methoxy mycolic acids***

The methoxy-mycolic acids (**108**, **109**, **110**, **111**, **112** and **113**, **Figure 16**) were prepared by a similar method to those previously described. The proton and carbon NMR spectra of (**108**) and (**109**) were essentially identical. Also the proton and carbon NMR spectra of the synthetic hydroxy ester (**110**) and (**112**) were identical to each

other and to those of a natural sample. The MALDI mass spectrum of (**112**) showed a molecular isotope ion pattern starting at 1290 that corresponded to that for the major component of the natural sample.

The specific rotations provided rather more insight into which of them corresponded to the natural compound. Thus the natural sample of the methyl ester showed an $[\alpha]_D^{22}$ of – 0.83 (the literature value reported in 1966 was – 0.1).[74] Compounds (**108** and **109**) showed $[\alpha]_D^{22}$ +7.2 and 7.7, respectively, the hydroxy esters (**110** and **111**) giving $[\alpha]_D^{22}$ +6.0 and +7.0 respectively. In contrast (**112)** and (**113**) gave – 1.0 and – 1.1, respectively. The specific rotations of these molecules are determined very largely by the hydroxy acid and methoxy methyl fragment stereochemistries and are affected to only a very small degree by the stereochemistry of the *cis*-cyclopropane. It seems extremely likely, therefore, that the methoxy methyl fragment is of *S,S*-stereochemistry. Currently, the biological properties of the synthetic methoxy-mycolic acids are being determined in order to establish firmly whether the stereochemistry is critical to biochemical effect and to establish the absolute stereochemistry present in the natural material.

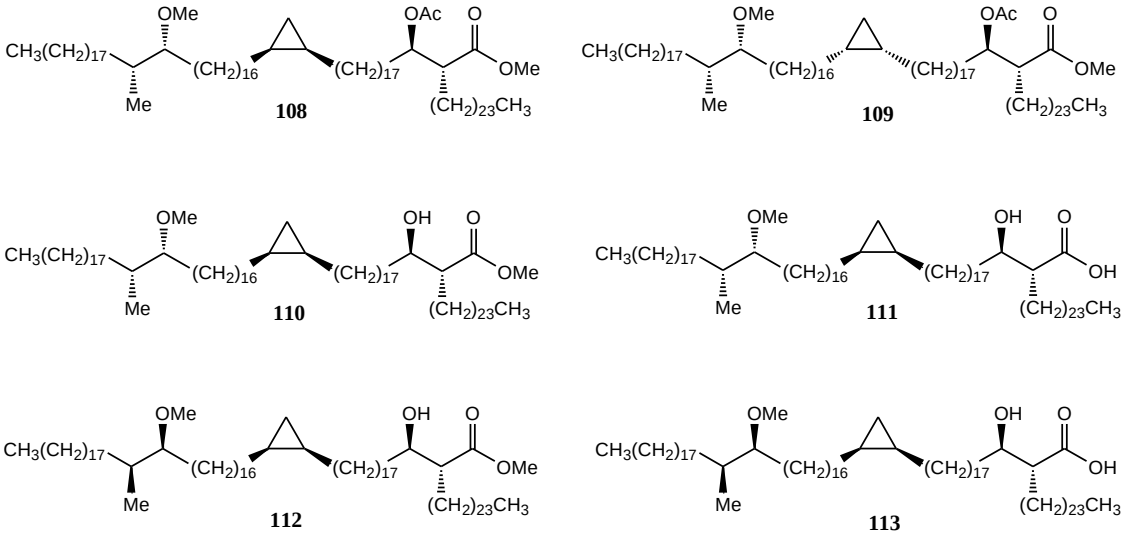


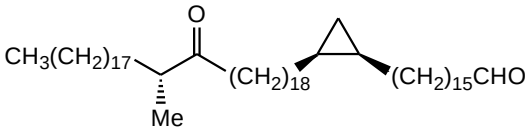
Figure 16: ***Methoxy-mycolic acids in different enantiomers***

2. Results and Discussion

2.1 Aim of the project

This project consists of two parts. The aim of the first part was to synthesise *cis*-cyclopropane and α-methyl-*trans*-cyclopropane keto-meromycolaldehydes (**114** and **115**), and *cis*-cyclopropane and α-methyl-*trans*-cyclopropane keto-mycolic acids (**116** and **117**) of *M. tuberculosis* and other mycobacteria. The aim of the second part was to synthesise α-methyl-*trans* olefin keto-mycolic acid (**118**) of *M. marinum* and α'-mycolic acid (**119**) of *M. smegmatis* (**Figure 17**). Synthetic mycolic acids will help to prove the stereochemistry of the natural mycolic acids, to be able to understand bacterial membranes and to develop methods for detecting tuberculosis and may offer possibilities for the treatment of asthma.

114



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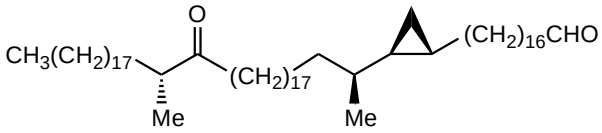

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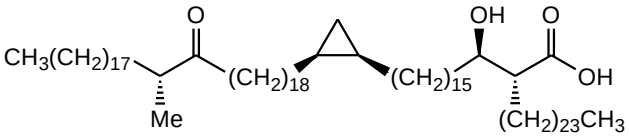

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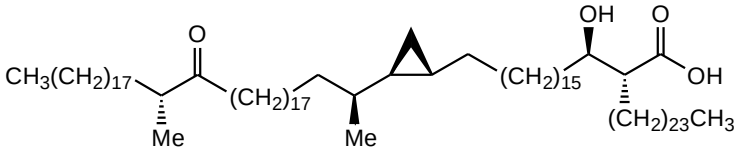

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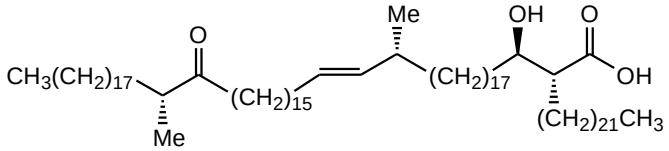

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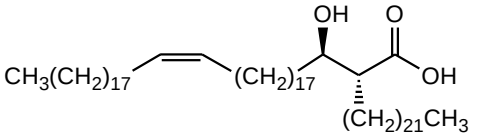
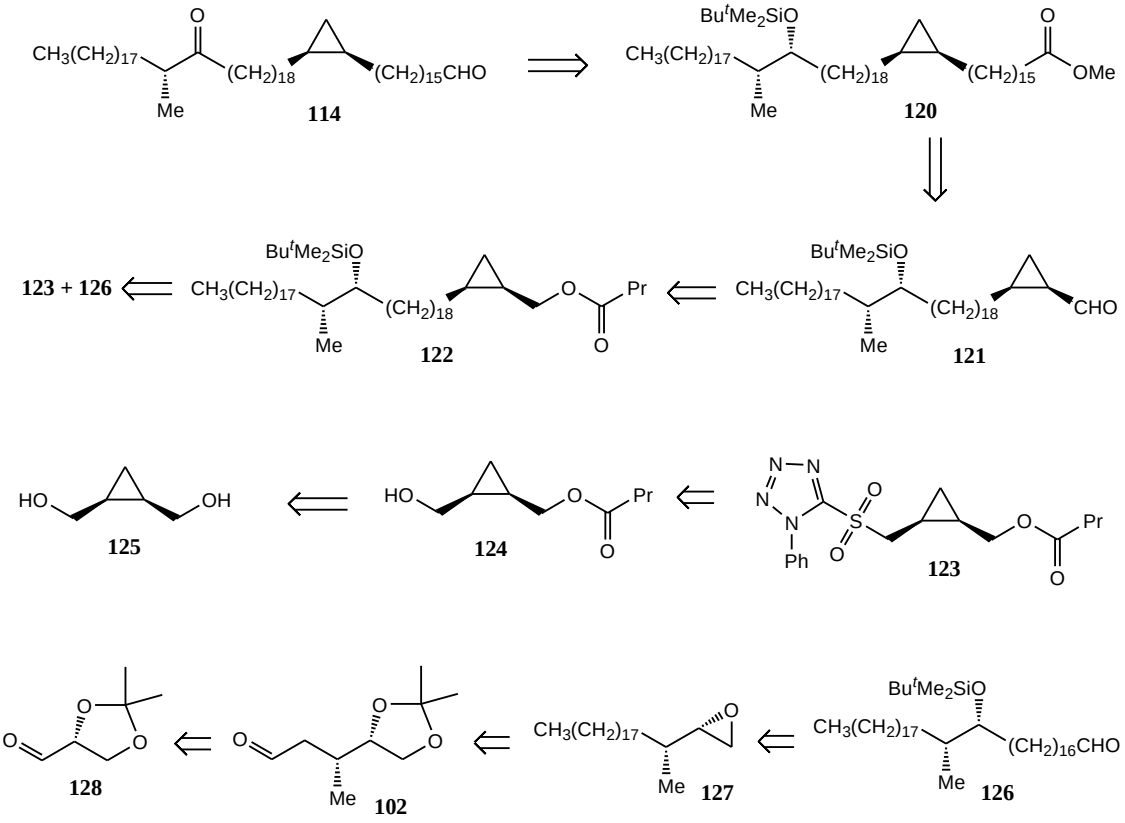


Figure 17: ***The target mycolic acids***

2.2 The synthesis of *cis*-cyclopropane keto-meromycolaldehyde

The method used to prepare the *cis*-cyclopropane keto-meromycolaldehyde (**114**) could be analysed as shown in the **Scheme 9**.

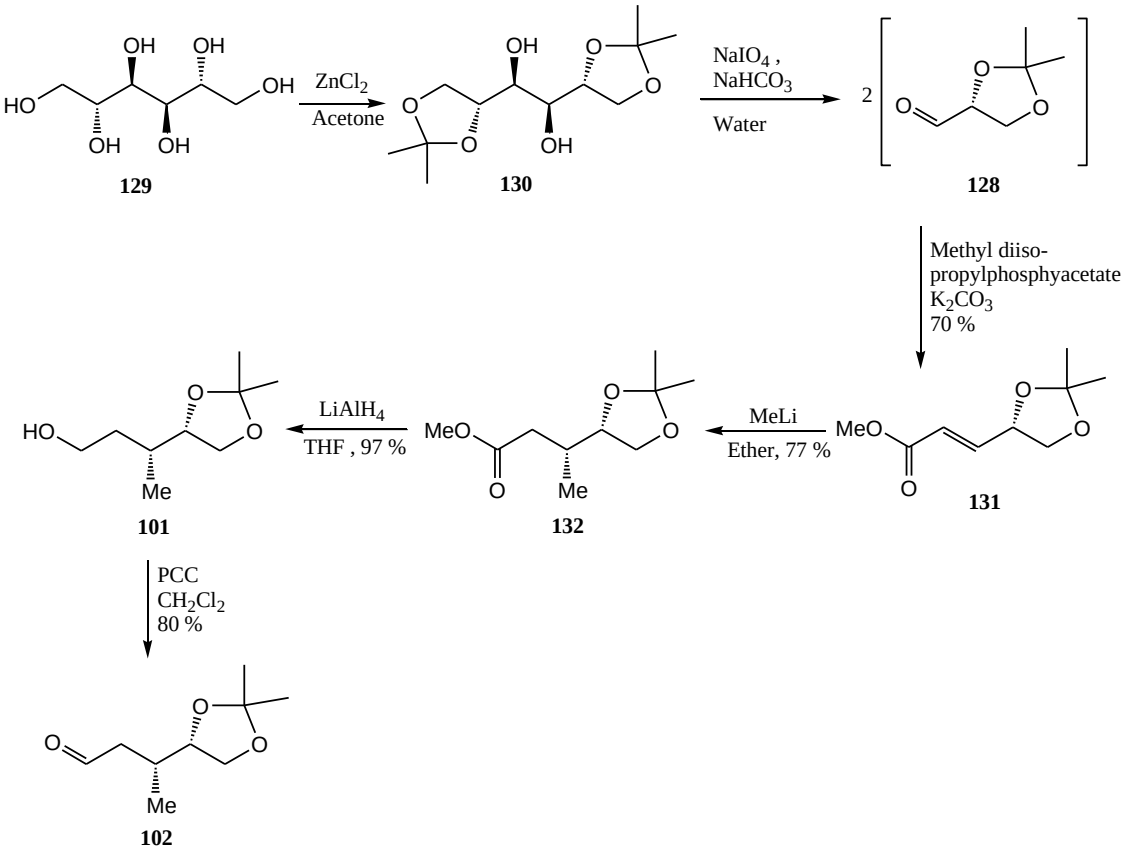


Scheme 9: ***The proposed preparation of cis-cyclopropane keto-meromycolaldehyde (114)***

2.2.1 Preparation of the α-methyl hydroxy unit

In the literature, the stereochemistry of the methyl adjacent to keto group was assigned as probably having *S*-configuration (**Section 1.2.3**). However, the biological activities of this group are not known. Therefore, D-mannitol (**129**), which is a commercially available and cheap polyhydroxy compound, was used as starting material, even though this would lead to the *R*-isomer. D-Mannitol was protected as 1,2:5,6-di-*O*-isopropylidene-D-mannitol (**130**) with zinc chloride and acetone using the literature method in 50 % yield.[154, 155] Oxidative cleavage of the diol (**130**) with sodium metaperiodate in aqueous sodium hydrogen carbonate gave the intermediate glyceraldehyde acetonide (**128**)[156] which, without isolation, was successively treated

with (diisopropoxy-phosphoryl)-acetic acid methyl ester (**133**, **Scheme 13**)[157] and aqueous potassium carbonate to obtain the *α,β*-unsaturated ester (**131**)[157,158] in 70 % yield *via* a Horner-Emmons reaction.[156,159,160] The product was of *E*-configuration. However, a very small amount (3 %) of *Z*-alkene was also found and separated by column chromatography (**Scheme 10**). The ^{1}H NMR spectrum of (**131**) showed the expected olefin signals as two double of doublets at δ 6.9 (*J* 5.7 and 15.8 Hz, vicinal and *trans* coupling constant) and 6.11 (*J* 1.6 and 15.8 Hz, allylic and *trans* coupling constant) respectively. The methyl on the ester appeared as a singlet at δ 3.75. The ^{13}C NMR showed a signal at δ 110.2 for the acetal carbon.

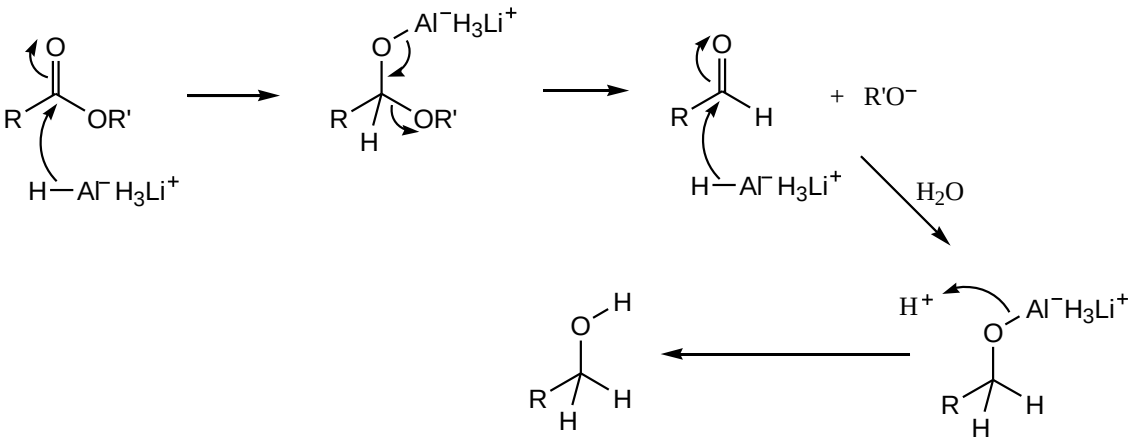


Scheme 10: ***Formation of the aldehyde (102)***

The diastereoselective conjugate addition of a methyl nucleophile to the *α,β*-unsaturated ester (**131**) was conducted as reported by Leonard and co-workers.[150] Thus, (**131**) was exposed to MeLi in diethyl ether at – 78 ^{o}C to give *syn*-adduct, (*R*)-3-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-butyric acid methyl ester (**132**) as a single diastereomer in 77 % yield. After attempting this reaction a few times it was realised

that it is important to keep the temperature below – 78 ^{o}C. The added methyl group appeared on the proton NMR spectra as a doublet at δ 0.98 (*J* 6.7 Hz). The carbon NMR spectrum showed signals at δ 173.0 for the carbonyl carbon and at δ 108.9 for the cyclic acetal carbon. The IR spectrum showed a broad signal at ν_{max} 1732 cm^{-1} for the C=O stretch.

The ester (**132**) was reduced to the corresponding primary alcohol (**101**) by lithium aluminium hydride ($LiAlH_4$) in dry THF, quenching with saturated aqueous Na_2SO_4, in a very good yield 97 %. The IR spectrum showed a broad stretch at ν_{max} 3426 cm^{-1} for the O–H group. The reaction proceeds via a tetrahedral metal-alkoxide. This species decomposes, displacing the alcohol component of the ester and forming an aldehyde. The aldehyde then reacts with another equivalent of $LiAlH_4$ forming another metal alkoxide complex, which, when quenched is protonated on the alkoxide oxygen, forming the alcohol product (**Scheme 11**).

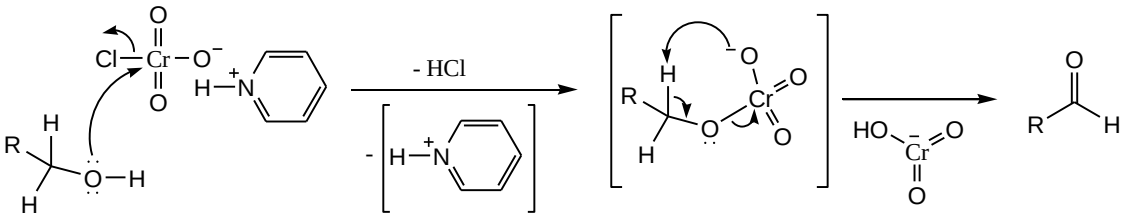


Scheme 11: ***The mechanism of LiAlH$_4$ reduction of an ester***

Finally, the alcohol (**101**) was oxidised to the desired aldehyde (**102**) with pyridinium chlorochromate (PCC) in dichloromethane. The ^{1}H NMR spectrum showed a triplet at δ 9.77 (*J* 1.9 Hz) for the aldehyde proton and in the ^{13}C NMR a signal appeared at δ 201.7 for the carbonyl carbon.

PCC is the ideal reagent for this particular oxidation of primary alcohols to aldehydes, as it performs the oxidation in pH neutral conditions, and will not further oxidise the aldehyde. Therefore, throughout this work, PCC was used to oxidise alcohols to the corresponding aldehydes. Alternative reagents such as chromic acid or potassium

permanganate require acid conditions, which could result deprotection of the acetal ring. Also aldehydes react rapidly with aqueous chromic acid to produce carboxylic acid. The mechanism of oxidation by PCC is believed to be as follows (**Scheme 12**) :



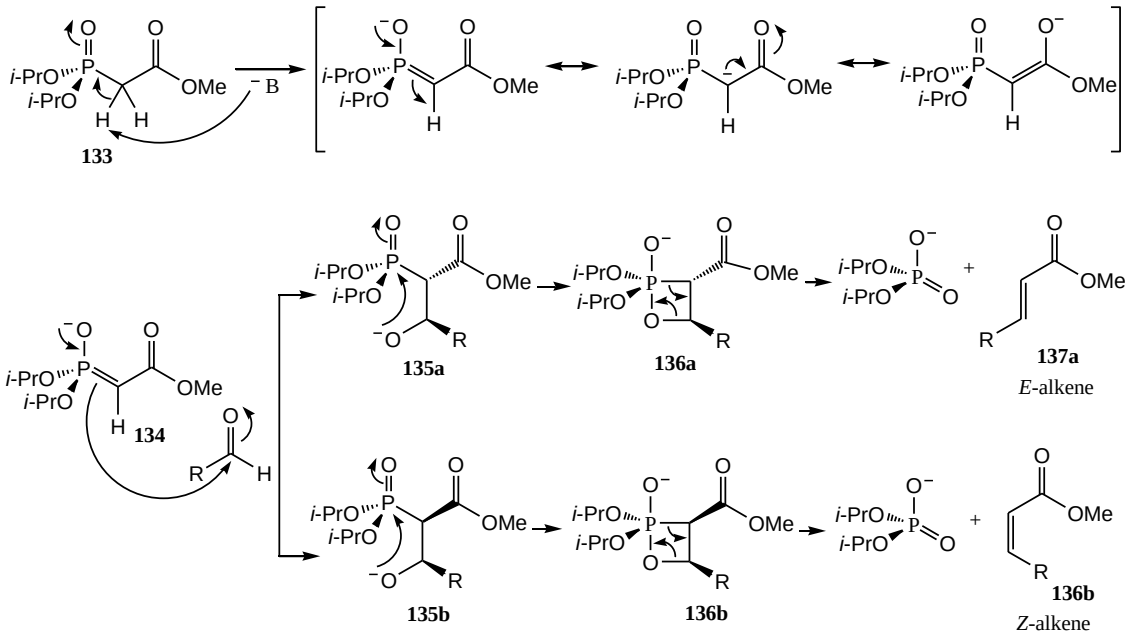
Scheme 12: ***The mechanism of oxidation by PCC***

Oxidation of the alcohol involves the formation of a chromate ester and an elimination reaction of it to generate the aldehyde. During the initial step of the oxidation one molar equivalent of HCl is formed and it is neutralised by the molar equivalent of pyridinium ions that is formed simultaneously. The consequence is that the chromium species formed at the end cannot initiate a further cycle of oxidation to the corresponding acid, giving the desired aldehyde.

2.2.2 The Horner-Wadsworth-Emmons reaction

The reaction of aldehydes or ketones with stabilized phosphorus ylides leads to olefins with excellent *E*-selectivity. The Horner-Wadsworth-Emmons reaction begins with the deprotonation of the phosphonate with a base to give the phosphonate carbanion (**134**). This carbanion is stable because of the resonance effect (**Scheme 13**). Nucleophilic addition of the carbanion onto an aldehyde produces the *anti* intermediate (**135a),** the ester group to the aldehyde R group, and the *syn* intermediate (**135b**). These intermediates can interconvert to the intermediates (**136a**) and (**136b**). Finally, the elimination of the (**136a)** and (**136b**) gives the desired compound in two configurations, *E*-alkene (**137a**) and the *Z*-alkene (**137b**), as shown in **Scheme 13**. BuLi, NaH and NaOMe are suitable bases for forming the ylide.

The Horner-Wadsworth-Emmons reaction favours the formation of *E*-alkenes. In general, the more equilibration amongst intermediates, the higher the selectivity for *E*-alkene formation.



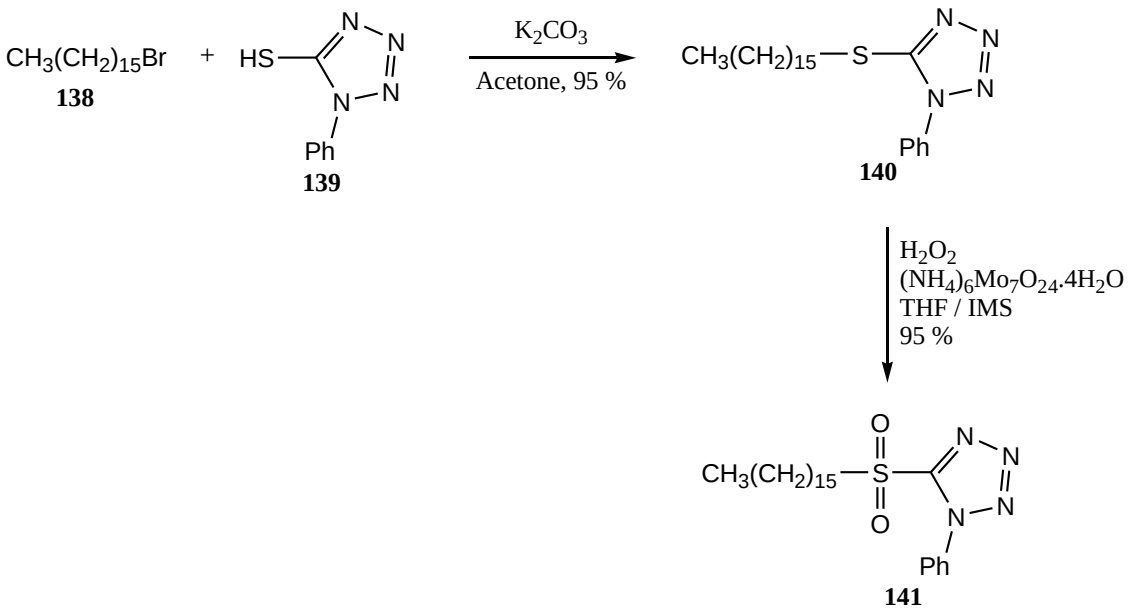
Scheme 13: ***Mechanism of the Horner-Wadsworth-Emmons Reaction***

2.2.3 Preparation of 5-(hexadecane-1-sulfonyl)-1-phenyl-1*H*-tetrazole (141)

In this work, the modified Julia olefination (**Section 2.2.4**) was used for coupling. For this, a sulfone was needed. Reaction of a bromo compound with 1-phenyl-1*H*-tetrazole-5-thiol (**139**) in the presence of potassium carbonate and acetone gave a sulfane such as (**140**) in excellent yield. The oxidation of the sulfane with hydrogen peroxide and ammonium molybdate (VI) tetrahydrate in the presence of THF and IMS gave a sulfone such as (**141**).

Firstly, the 1-phenyl-1*H*-tetrazole-5-thiol (**139**) was reacted with 1-bromohexadecane (**138**) to form 5-hexadecylsulfanyl-1-phenyl-1*H*-tetrazole (**140**). The crude product was purified by re-crystallization in a mixture of acetone and methanol to give a white solid. This was then oxidised to corresponding sulfone (**141**) using excess of hydrogen peroxide and ammonium molybdate (VI) tetrahydrate. It was purified by re-crystallisation from methanol (**Scheme 14**).[148]

The ^{1}H NMR spectrum of compound (**140**) showed a multiplet at δ 7.61–7.55 for the five aromatic protons, a triplet at δ 3.42 (*J* 7.6 Hz) for the -CH_2- bonded to the S atom and a triplet at δ 0.89 (*J* 6.9 Hz) for the terminal methyl. The ^{13}C NMR showed a signal at δ 154.5 for the carbon in the tetrazole ring, four signals for the aromatic carbons at δ 133.8–123.9 and signals at δ 33.4 for the long chain carbon bonded to the S atom and at δ 14.1 for the terminal carbon.



Scheme 14: ***Preparation of the sulfone (141)***

Interestingly, the proton NMR spectrum of the sulfone (**141**) showed a multiplet at δ 7.73–7.69 (two proton) and another multiplet at δ 7.66–7.59 (three proton) for the aromatic protons. This confirmed that the sulfone had formed, since on the sulfane the five aromatic protons appear together. An anomalous signal appeared at δ 3.74 for the two protons (H_A and $H_{A'}$) next to the sulfonyl group. These displayed the typical pattern for an AA'BB' system, where the two substituents on the C–C bond mean that A and A' and B and B' respectively are not magnetically equivalent (**Figure 18**). This system was seen throughout this work and also confirmed the success of the reaction.

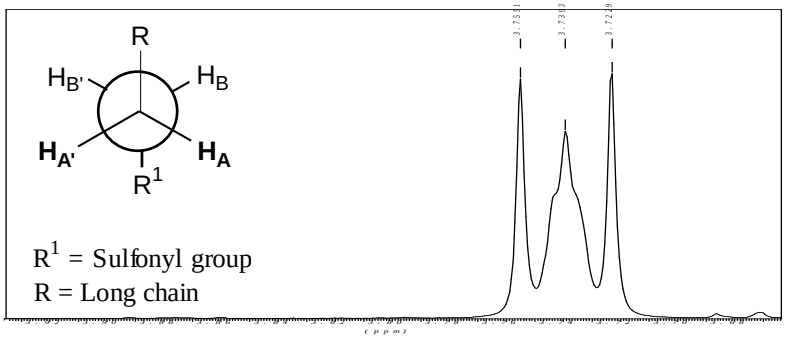
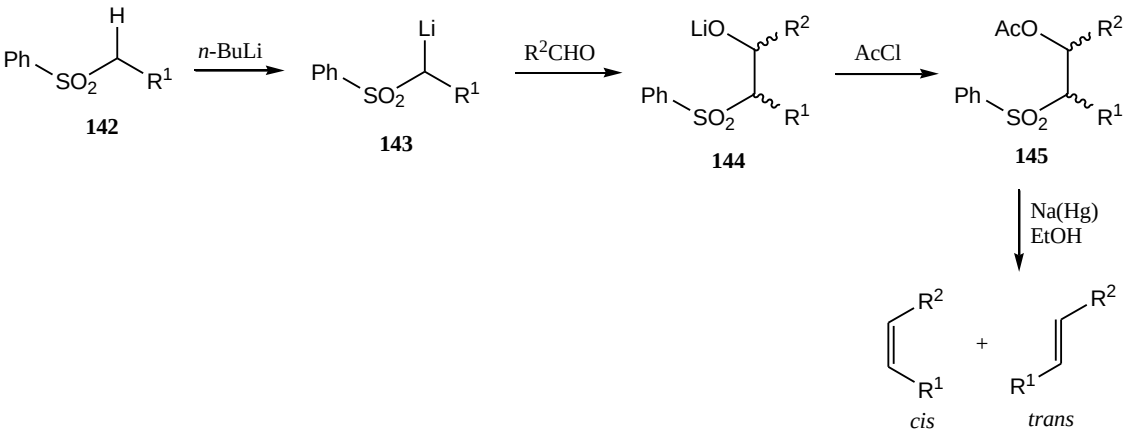


Figure 18: ***The characteristic signal of the protons (H_A and $H_{A'}$) adjacent to a sulfonyl group***

2.2.4 Overview of the modified Julia olefination

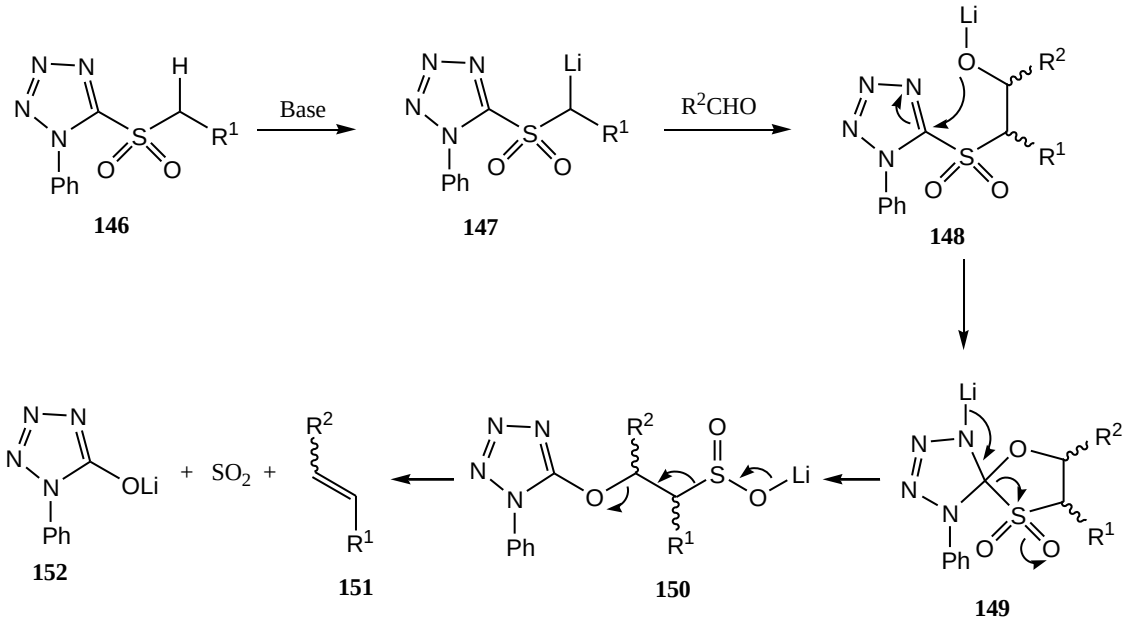
Formation of an alkene with a phenylsulfone and aldehyde was first published by Marc Julia and Jean-Marc Paris and called classical Julia olefination.[161] The method was later significantly developed by Lythgoe and Kocienski[162,163,164,165] and has since found pivotal use in the synthesis of many natural product molecules.[166] The method can be summarised as following: metallation of a phenylsulfone (**142**), addition of the metallate (**143**) to an aldehyde, acylation of the resulting β-alkoxysulfone (**144**) and reductive elimination of the β-acyloxysulfone (**145**) with a single electron donor to afford alkene products (**Scheme 15**).



Scheme 15: ***Mechanism of the classical Julia olefination***

An alternative olefination to the classical Julia olefination was found by Sylvestre Julia and co-workers. They replaced the phenylsulfones, traditionally used in the classical Julia olefination, with certain heteroarylsulfones, in a so-called 'modified Julia olefination'. [167] The presence of an electrophilic imine-like moiety within the heterocycle opens a new mechanistic pathway which is responsible for the transformed reactivity. The addition of a metallated sulfone (**147**) to an aldehyde gives β-alkoxysulfone (**148**). However, this β-alkoxysulfone is inherently unstable and it therefore readily undergoes a Smiles rearrangement.[168] The rearrangement occurs *via* a putative spirocyclic intermediate (**149**) and results in transfer of the heterocycle from sulphur to oxygen to yield sulfinate salt (**150**). Spontaneous elimination of sulphur

dioxide and lithium 1-phenyl-1*H*-tetrazolone (**152**) from (**150**) leads to the desired alkene (**151**) as a mixture of *E*- and *Z*-isomers (**Scheme 16**).



Scheme 16: ***Mechanism of the modified Julia olefination***

For the modified Julia olefination, four heterocyclic activators have been identified which provide useful levels of stereoselectivity in certain scenarios: benzothiazol-2-yl (**BT**), pyridin-2-yl (**PYR**), 1-phenyl-1*H*-tetrazol-5-yl (**PT**) and 1-*tert*-butyl-1*H*-tetrazol-5-yl (**TBT**) (**Figure 19**).

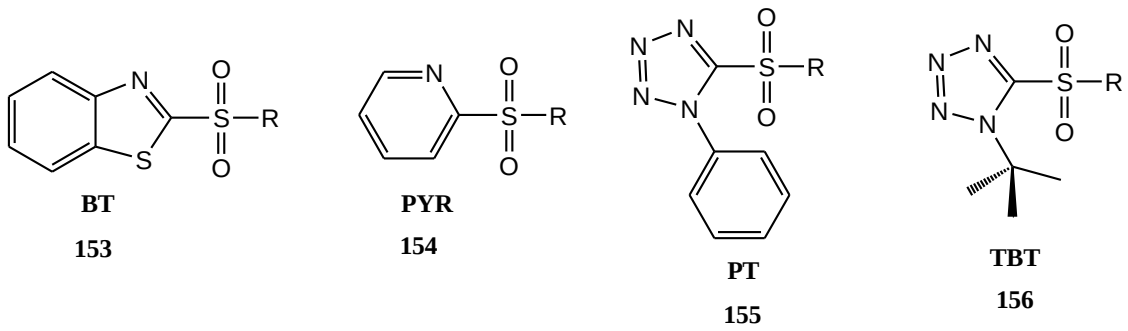
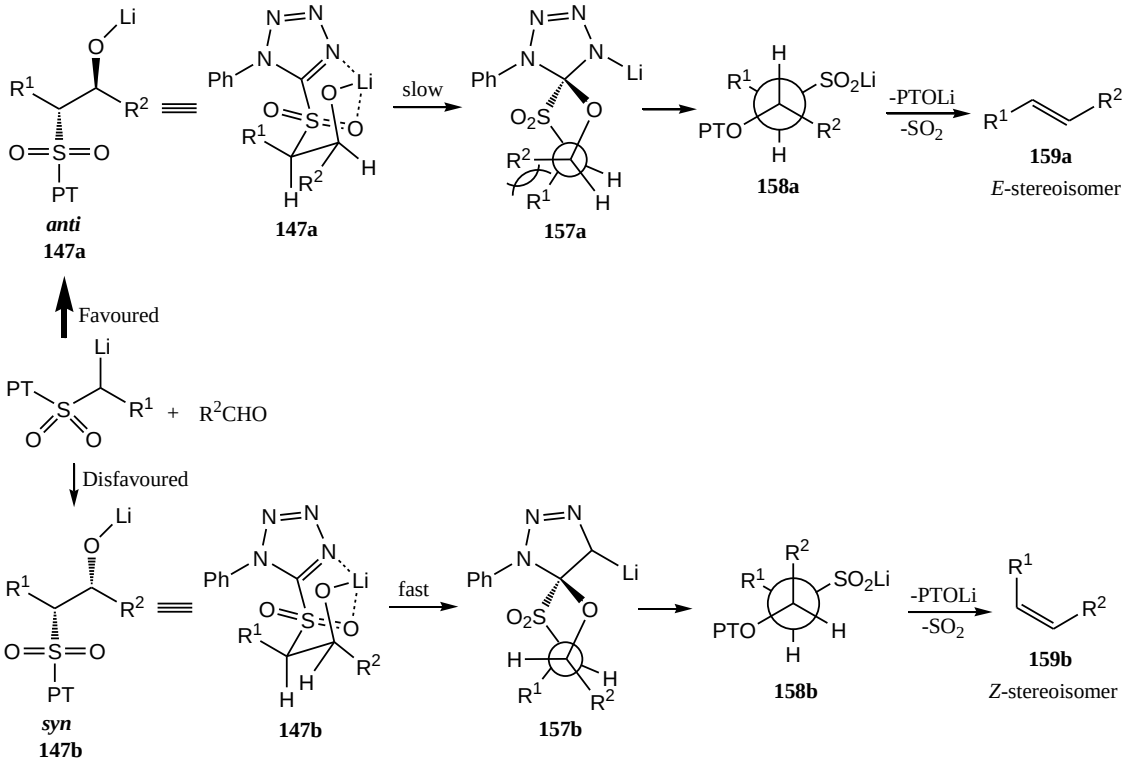


Figure 19: ***Four heterocyclic sulfones for the modified Julia olefination***

In this work, the 1-phenyl-1*H*-tetrazol-5-yl sulfonyl reagent (**155**) was chosen because it was easy to prepare from commercially available materials, and it has not shown problems with self condensation.[169,170] The modified Julia olefination was in time

called the Julia reaction. The modified Julia olefination is widely used in synthetic organic chemistry, specially in natural product synthesis.[171]

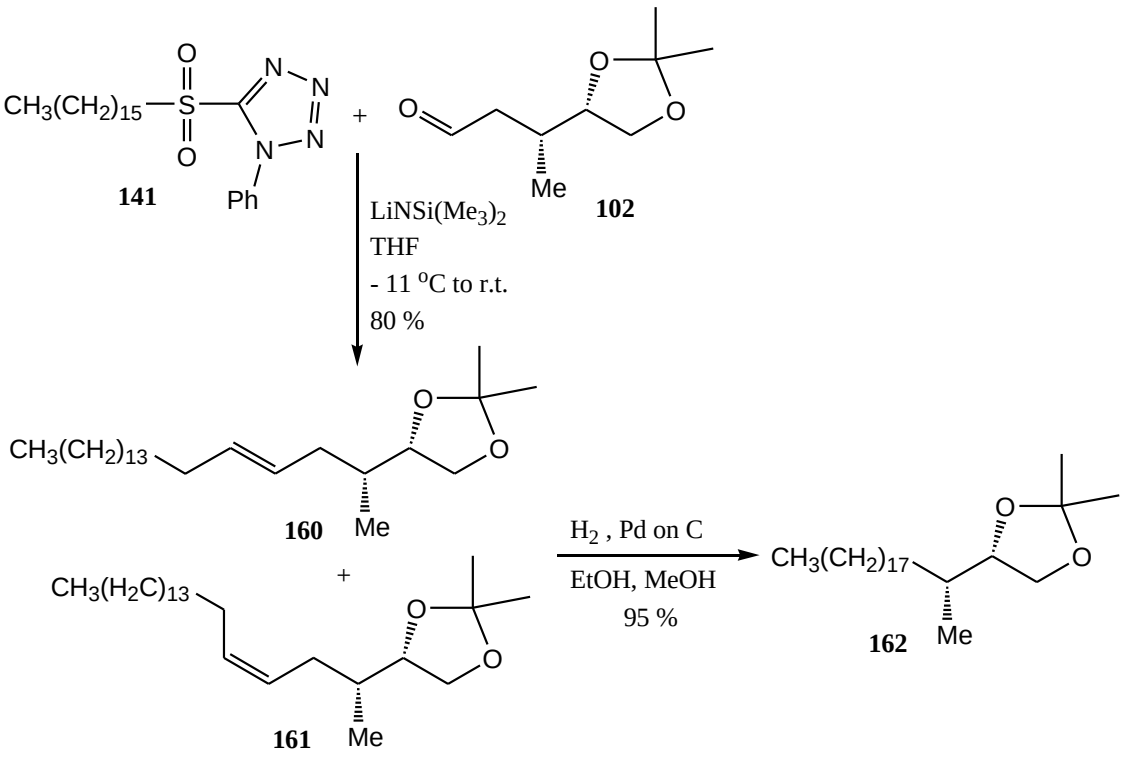
The Julia olefination gives mainly *E*-alkenes and it is often almost impossible to separate these from minor *Z*-isomer by column chromatography. The reasons for the major formation of the *E*-alkenes among the mixture of product obtained from the modified Julia olefination have been investigated.[169,172] The geometrical selectivities are a consequence of poor diastereocontrol in the initial nucleophilic addition event and the anti addition is favoured. The *E* : *Z* ratio of the olefins accurately reflects the *anti* : *syn* ratio of the intermediate β-alkoxysulfones (**147a** and **147b**) (**Scheme 17**). Analysing the mechanism of the Julia olefination, Blakemore asserted that using this kind of heterocyclic sulfone, there is a kinetically controlled addition between the two reagents aldehyde (**102**) and sulfone (**141**), which leads to the formation of the *anti*-β-alkoxysulfone (**147a).**[169] The subsequent two stages of the reaction, the Smiles rearrangement and the elimination are stereospecific, producing the *E*-alkene (**159a**). Conversely, the *syn*-β-alkoxysulfone (**147b**) is formed in smaller amounts and therefore the minor *Z*-alkene (**159b**) is produced as shown in **Scheme 17.**



Scheme 17: ***Reasons for the stereoselectivity of the modified Julia olefination***

2.2.5 The Julia reaction between (102) and (141)

The sulfone (**141**) was dissolved in THF, in which both reagents, aldehyde and sulfone are very soluble, and the aldehyde (**102**) was added. The coupling reaction was started by addition of a non-nucleophilic strong base such as lithium bis(trimethylsilyl) amide at – 10 ^{o}C. Subsequently, it was allowed to reach room temperature and stirred for 2 hours to complete the reaction. The reaction appeared to be very straightforward, giving the desired alkene with a 80 % yield. The product was a mixture of *E*-(**160**) and *Z*-(**161**) stereoisomers in a ratio of 2.3 : 1 (**Scheme 18**). The ^{1}H NMR spectrum of (**160** and **161**) showed two multiplets at δ 5.47–5.40 and at δ 5.37–5.31 respectively for the two alkene protons, and a multiplet at δ 1.33–1.26 for the long chain protons. The ^{13}C NMR spectrum showed two signals at δ 132.7 and 127.5 for the olefinic carbons of the major *E*-isomer and another two signals at δ 131.7 and 127.1 for the olefinic carbons of the minor *Z*-isomer. The cyclic acetal carbon appeared at δ 108.5 and two signals appeared at δ 80.0 and 67.8 for the carbons adjacent to oxygen atoms.



Scheme 18: ***The Julia reaction between (102) and (141)***

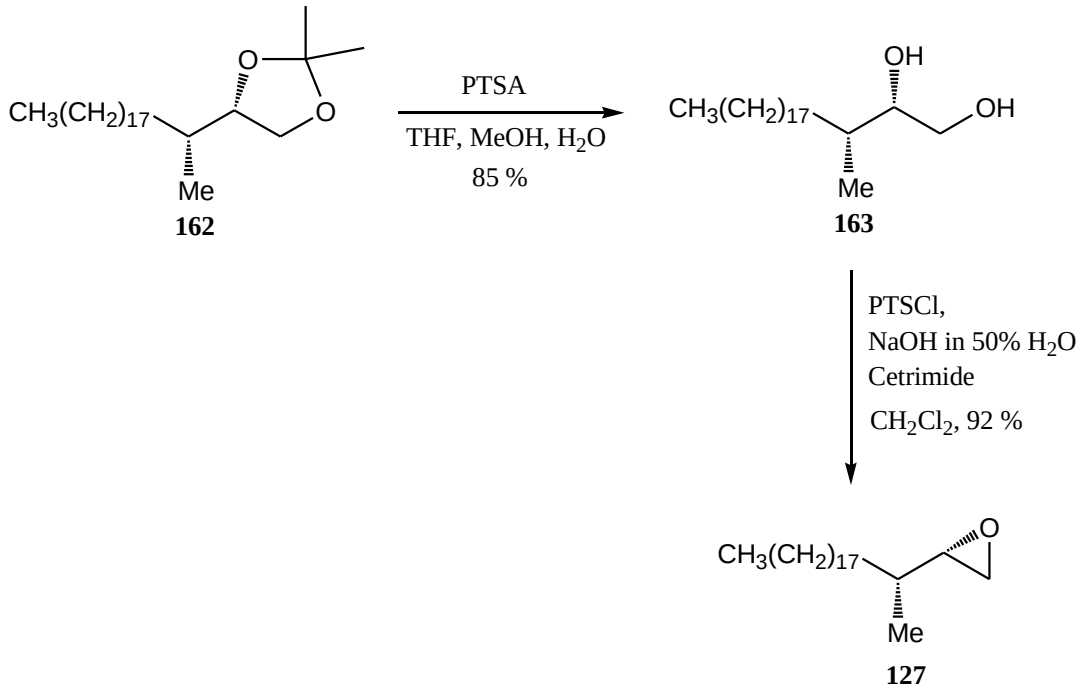
The alkenes (**160** and **161**) were then saturated by hydrogenation in ethanol and methanol using Pd (10 %) on carbon as a catalyst and hydrogen gas as shown in

Scheme 18. The hydrogenation sometimes proceeded in a straightforward way but sometimes took a long time and it was even necessary to repeat the hydrogenation. The proton and carbon NMR spectra showed that there were no signals at the olefinic region, which proved that the hydrogenation had been completed.

2.2.6 Preparation of (*S*)-2-((*R*)-1-methylnonadecyl)-oxirane (127)

The cyclic acetal (**162**) was deprotected with *p*-toluenesulfonic acid monohydrate in THF, methanol and water, and refluxed for 2 hours to give (*S*)-2-((*R*)-1-methyl-nonadecyl)-oxirane (**163**) in 85 % yield (**Scheme 19**).[173] The ^{1}H NMR spectrum showed the expected signals which included a multiplet at δ 3.69–3.65 (one proton) and another multiplet at δ 3.61–3.53 (two proton) for the three proton next to the hydroxy group. Other characteristic signals appeared as a broad multiplet at δ 1.43–1.26 for the long chain, a doublet at δ 0.93 (*J* 7.0 Hz) for the vicinal coupling of the α-methyl and a triplet at δ 0.89 (*J* 7.0 Hz) for the terminal methyl. The ^{13}C NMR spectrum showed two signals at δ 75.8 and 65.2 for the carbons next to the hydroxy groups, signals between 35.7 and 22.7 for the long chain and two signals at δ 14.6 and 14.1 for the two methyl groups. In the IR spectrum, a broad peak appeared at 3283 cm^{-1} for the O–H stretching. The optical rotation of the diol (**163**) was $[\alpha]_D^{23}$ = + 12.7 (*c* 0.79, $CHCl_3$).

The diol (**163**) was converted stereospecifically into the epoxide (**127**) *via* the monotosylate. The reaction was carried out under phase transfer conditions using (*n*-hexadecyl) trimethyl ammonium bromide, 50 % aq. sodium hydroxide and *p*-toluenesulphonyl chloride in the presence of dichloromethane. It was completed at room temperature in 45 minutes in 92 % yield (**Scheme 19**). The proton NMR spectrum showed that the protons next to the oxygen atom were shifted upfield and appeared as two doublets of doublets at δ 2.77 (*J* 4.1, 5.0 Hz) and 2.54 (*J* 2.6, 4.8 Hz), and a multiplet at δ 2.71–2.67. The geminal and vicinal coupling constants were smaller than normal. In the ^{13}C NMR spectrum, the carbons next to oxygen atom also shifted upfield and appeared at δ 57.2 and 47.1. The remaining signals for the proton and carbon NMR were similar to those of the diol (**163**). In the IR spectrum, the broad peak of the diol for the hydroxy groups disappeared. The optical rotation became very small, $[\alpha]_D^{23}$ = + 0.6 (*c* 0.84, $CHCl_3$).



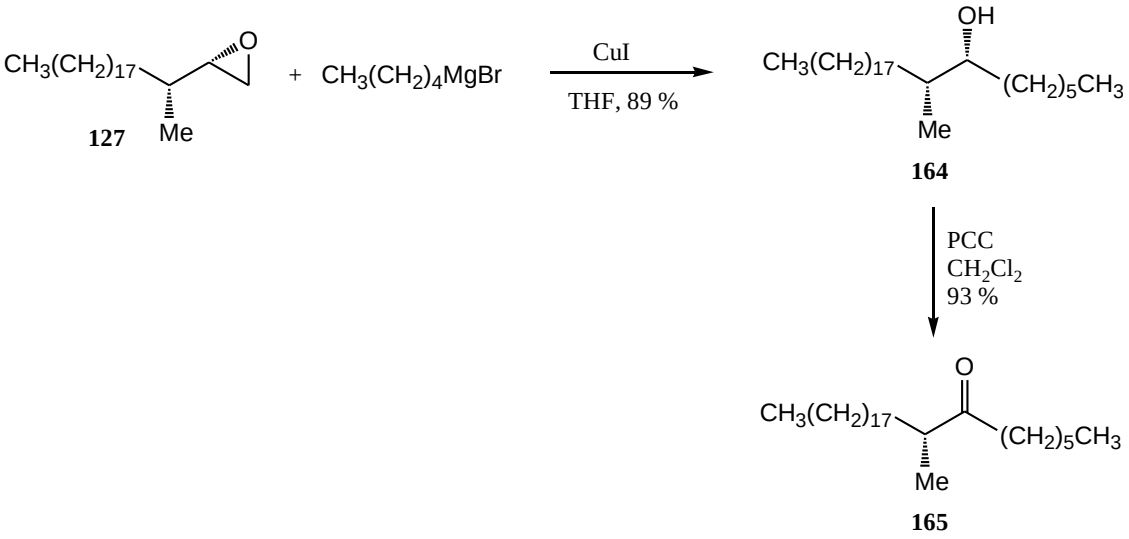
Scheme 19: ***Formation of the epoxide (127)***

2.2.7 Preparation of a model α-methyl keto unit

Preparation of a model α-methyl keto unit at this step from the epoxide (**127**) was very important. This helped in understanding the NMR spectra and also the optical rotation, because the spectrum of the simple molecule would help the analysis of the more complicated mycolic acid.

Addition of a Grignard reagent to an epoxide gives a secondary alcohol as shown in **Scheme 20**. Preparation of the Grignard reagent is quite tricky and it needs the flask and solvents to be very dry and under an inert gas such as nitrogen. The Grignard reagent was prepared from 1-bromopentane. 1-Bromopentane in dry THF was added slowly by a syringe to metallic magnesium in dry THF whilst the flask was heated by a heat gun then refluxed for one hour to generate the Grignard reagent. This reagent then added to copper (I) iodide[174] in dry THF and at – 30 ^{o}C and stirred for 30 minutes. The epoxide (**127**) was then added and stirred at first – 30 ^{o}C and then at room temperature for 18 hours. The yield was quite high, 89 % (**Scheme 20**). The proton NMR spectrum of (**164**) showed a multiplet at δ 3.51–3.49 for the proton adjacent to the hydroxy group and other signals similar to those for diol (**163**) explained above. The IR showed a broad peak at 3360 cm^{-1} for the O–H stretch. The optical rotation of the alcohol (**164**) was $[\alpha]_D^{24}$ = + 12.9 (*c* 0.84, $CHCl_3$), which was similar to that reported in the literature

for a related compound, (15*R*,16*R*)-15-methyl-hentriacontan-16-ol ($[\alpha]_D$ = + 9.3 in $CHCl_3$).[175]



Scheme 20: ***Preparation of the α-methyl keto unit***

Finally, the alcohol (**164**) was oxidised to the desired α-methyl keto compound (**165**). The oxidation was carried out in dichloromethane using PCC. The ^{1}H NMR spectrum showed a sextet at δ 2.51 (*J* 6.9 Hz) for the proton adjacent to keto and methyl groups. The appearance of the sextet could be explained if the hydrogen has the same coupling constant with methyl hydrogens and two -CH_2- hydrogens. However, the signals for the two protons next to keto groups were complicated. Each proton appeared as a doublet of triplets at δ 2.44 (*J* 17.0, 7.6 Hz) and 2.40 (*J* 17.0, 7.3 Hz) respectively. Importantly, the oxidation did not cause racemisation of the ketone as shown in **Figure 20**. The long chain protons appeared as a multiplet at δ 1.37–1.26, the methyl protons next to keto as a doublet at δ 1.05 (*J* 6.9 Hz) and the two terminal methyls as a triplet at δ 0.89 (*J* 6.9 Hz). The ^{13}C NMR spectrum showed a signal at δ 215.2 for the keto carbon, and two signals at δ 46.3 and 41.1 for the carbons next to carbonyl carbons. The long chain carbons appeared between 33.1 and 22.5 and the methyl carbons at δ 16.4, 14.1 and 14.0. The specific rotation of the (*R*)-8-Methyl-hexacosan-7-one (**165**) was measured as $[\alpha]_D^{22}$ = – 12.1 (*c* 1.24, $CHCl_3$). In the literature, related compounds have close optical rotations to (**165**); (*R*)-15-methyl-hentriacontan-16-one has $[\alpha]_D$ = – 9.5 in $CHCl_3$[175] and (10*R*)-10-methyl-octadecan-9-one has $[\alpha]_D$ = – 13.2 in $CHCl_3$.[176]

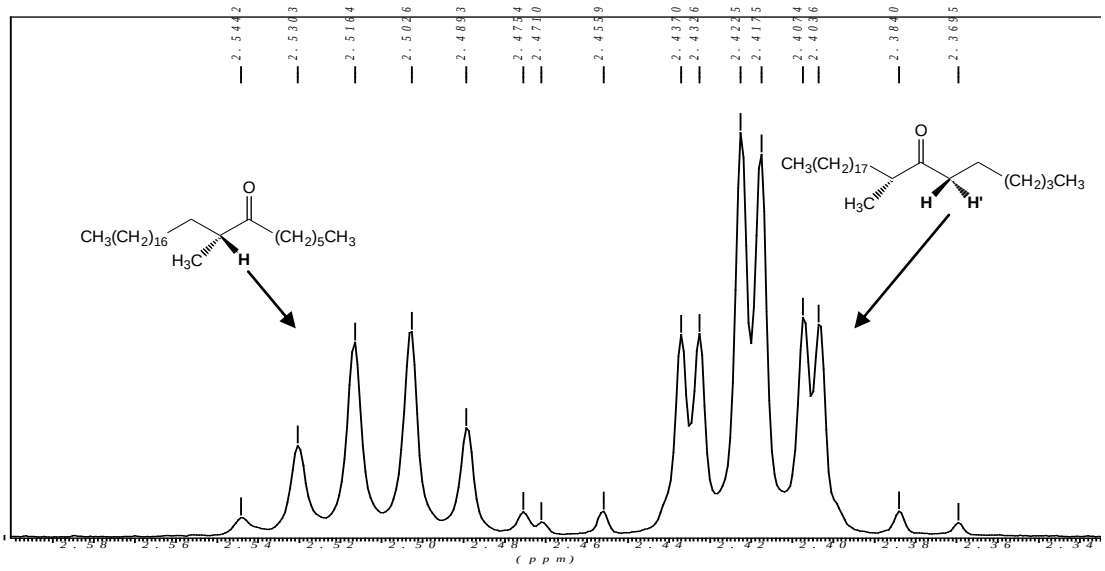


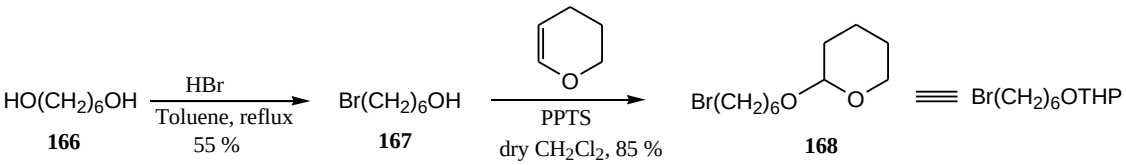
Figure 20: ***The ^{1}H NMR spectrum of the protons next to keto group***

2.2.8 The Grignard reaction

2.2.8a Preparation of protected 6-bromohexan-1-ol

As mentioned previously, the Grignard reaction is widely used in synthetic organic chemistry to form carbon carbon single bonds. Nucleophilic additions of a Grignard regent to an epoxide give a stereoselective product. This addition is one of the key steps for the preparation of both the methoxy- and keto-mycolic acids.

Bifunctional diols, HO–$(CH_2)_n$–OH, were used in this work to extend chain lengths as they are inexpensive, are available in a variety of different chain length and they can be easily regioselectively modified. Moreover, other kinds of bifunctional chain, X–$(CH_2)_n$–Y, are not so easily accessible from commercial sources. A Grignard reagent was needed for the reaction of the epoxide (**127**) and six carbon chain length 1,6-hexanediol (**166**) was chosen, as preparation of long chain Grignard reagent is very difficult.[177] Diols could be easily mono-brominated with 48 % HBr with a well known literature method.[178,179] 1,6-Hexanediol was mono-brominated with 48 % HBr by refluxing in toluene for 18 hours to give 6-bromo-hexan-1-ol (**167**) in 55 % yield. The NMR spectrum matched the literature values.[180]



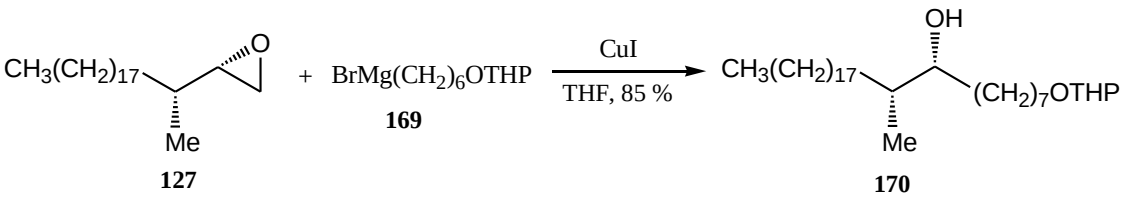
166 **167** **168**

Scheme 21: ***Preparation of a chain***

The hydroxy group of the 6-bromo-hexan-1-ol (**167**) was protected with 3,4-dihydro-2*H*-pyran to give 2-(6-bromo-hexyloxy)-tetrahydro-pyran (**168**) in 85 % yield. Pyridinium-p-toluene-sulfonate was used as a catalyst and dry dichloromethane as solvent (**Scheme 21**). The proton NMR spectrum showed a multiplet at δ 4.59–4.57 for the acetal proton and proved the protection had occurred. The two protons on the ring next to oxygen appeared a multiplet at δ 3.89–3.85, and a doublet of triplets at δ 3.75 (*J* 6.6, 9.5 Hz). The two protons next to bromine appeared as a triplet at δ 3.43 (*J* 6.6 Hz). The other signals were as expected on comparison of the starting alcohol. The ^{13}C NMR spectrum showed a signal at δ 98.9 for the acetal carbon and two signals at δ 67.4 and 62.4 for the carbons bonded to oxygen.

2.2.8b Addition of the Grignard reagent to the epoxide

A Grignard reagent from (**168**) was prepared as previously described in section **2.2.7** and the same method was used for the addition of this reagent to the epoxide (**127**). Reaction of 6-(tetrahydropyran-2-yloxy)hexyl magnesium bromide (**169**) (3 mol eq.) with epoxide (**127**) in dry THF in the presence of a catalytic amount of cupper (I) iodide gave the alcohol (**170**). The best yield was found to be 85 % after repeating the reaction several times (**Scheme 22**). Preparation of this single enantiomer of α-methyl alcohol is an important achievement for the synthesis of mycolic acids.



127 **169** **170**

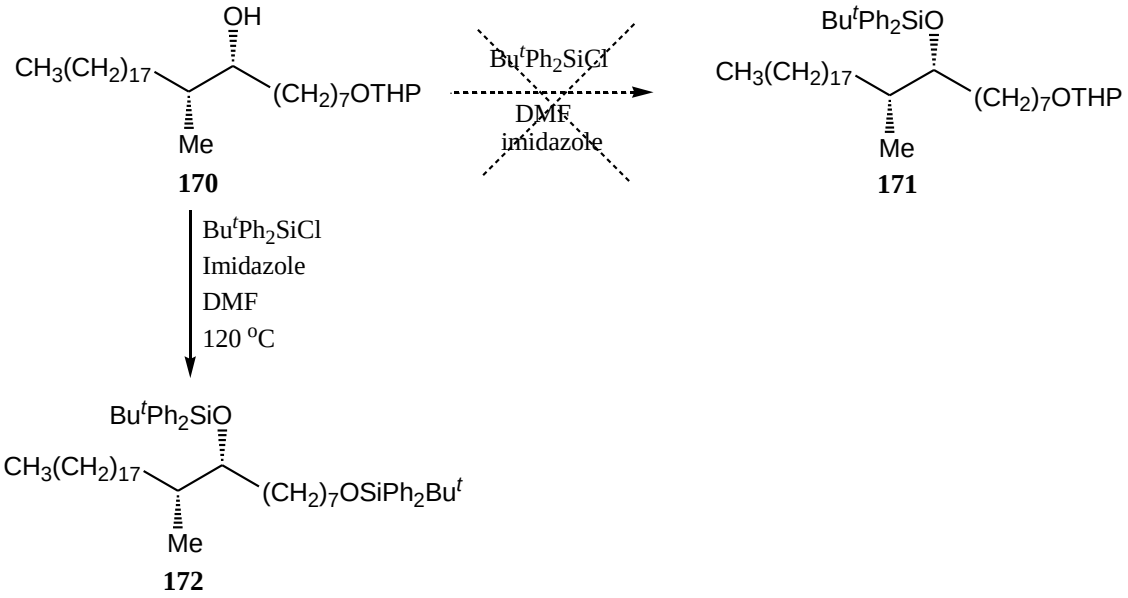
Scheme 22: ***Addition of the Grignard reagent to epoxide***

In the IR spectrum of the alcohol (**170**), it was possible to observe the signal for the O–H stretch at 3450 cm^{-1}. In its ^{1}H NMR spectrum, the protecting group's protons on the ring adjacent to oxygen appeared a doublet of doublets at δ 4.58 (*J* 2.9, 4.1 Hz), a multiplet at δ 3.90–3.85, and another multiplet at δ 3.51–3.49. The protons on the chain next to oxygen appeared as a doublet of triplets at δ 3.74 showing a vicinal coupling (7.0 Hz) and a geminal coupling (9.8 Hz), and as a doublet of triplets at δ 3.39 (*J* 6.6 (vicinal), 9.8 (geminal) Hz). The proton adjacent to the secondary hydroxyl group appeared as multiplet at δ 3.51–3.49. The long chain's protons appeared at δ 1.61–1.26 and the proton adjacent to the methyl appeared as a multiplet at δ 1.17–1.13. The α-methyl protons appeared as doublet at δ 0.87 (*J* 7.0 Hz) and the terminal methyl protons appeared at δ 0.89 (*J* 6.7 Hz). The ^{13}C NMR spectrum showed four signals at δ 98.9 (acetal carbon), 75.2 (hydroxy carbon), 67.7($O\underline{C}H_2$-) and 62.4($O\underline{C}H_2$-) for the carbons adjacent to oxygen. The terminal methyl carbon appeared at δ 14.1 and the α-methyl carbon at 13.6. The specific rotation of (**170**) was $[\alpha]_D^{24}$ + 10.9 (*c* 1.42, $CHCl_3$), as expected in comparison of the model secondary alcohol (**164**).

2.2.9 Protecting the secondary alcohol with *tert*-butyl-dimethylsilylchloride

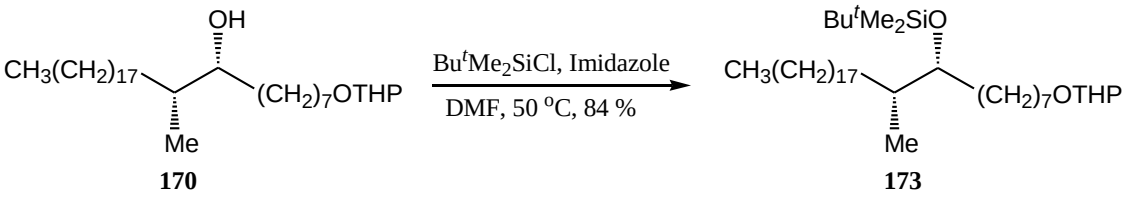
For the synthesis of the keto-mycolic acid, the alcohol had to be protected at this stage and deprotected after the whole chain had been prepared and oxidised to the keto group. The secondary alcohol (**170**) was protected as a silyl ether since this is not sensitive to base. *tert*-Butyldiphenylsilyl ethers are more stable than *tert*-butyldimethylsilyl ethers. Therefore, initially, it was attempted to protect with *tert*-butyldiphenylsilyl chloride. There is a well known literature method to protect an alcohol to a silyl ether. This involves stirring an alcohol with a silyl chloride and imidazole in dry DMF. The alcohol (**170**) was stirred with *tert*-butyldiphenylsilylchloride (1.4 mol eq.) and imidazole (2.5 mol eq.) in dry DMF at room temperature for 18 hours, but there was no product. So the mixture was stirred at 50 ^{o}C for 24 hours, but still there was no product. Further *tert*-butyldiphenylsilylchloride (1 mol eq.) and imidazole (1.5 mol eq.) were added and stirred at 120 ^{o}C for 24 hours. Unfortunately, the desired protected compound (**171**) was not obtained. However, (**172**), in which the THP protected oxygen was also

protected as a silyl ether was obtained as well as the starting secondary alcohol (**170**) (**Scheme 23**).



Scheme 23: ***An attempted reaction to protect secondary alcohol with Bu^{t}Ph$_2$SiCl***

So it was decided to protect as a *tert*-butyldimethylsilyl ether. The alcohol (**170**) was stirred with *tert*-butyldimethylsilyl-chloride (1.3 mol eq.) and imidazole (2.5 mol eq.) in dry DMF at room temperature for 18 hours and then for a further 5 hours at 50 ^{o}C to complete the protection. After column chromatography, *tert*-butyl-dimethyl-{(1*R*,2*R*)-2-methyl-1-[7-(tetrahydropyran-2-yloxy)-heptyl]-eicosyloxy}silane (**173**) was obtained in 84 % yield and also there was 11 % the unprotected alcohol (**170)** (**Scheme 24**). The yield was satisfactory and the synthesis was continued to next step. The *tert*-butyl and the two methyls on the silyl ether appeared in the proton NMR spectrum as singlets at δ 0.89, 0.03 and 0.02 respectively. The silyl carbons in the ^{13}C NMR spectrum appeared as follows: a signal for the methyl carbons on the *tert*-butyl group at δ 26.0, a signal for the quaternary carbon at δ 18.2 and two signals for the methyls bonded to silicon at δ - 4.2 and - 4.4. The IR spectrum showed no peak around 3400 cm^{-1} for an O–H stretch. All these data proved that the alcohol was protected.

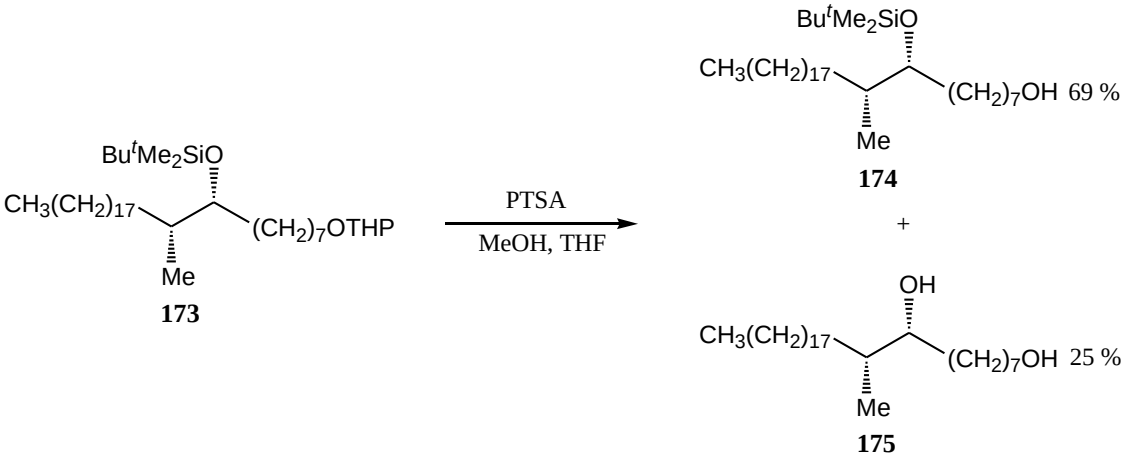


Scheme 24: ***Protecting the secondary alcohol with Bu^{t}Me$_2$SiCl***

2.2.10 Deprotection of the tetrahydropyranyloxy group

The next task was the deprotection of the terminal hydroxyl group which had been protected with the tetrahydropyranyloxy (THP) group and at the same time the Bu^{t}Me$_2$Si ether, protected to the secondary alcohol, had to remain. As generally known, the THP ether is much more acid sensitive than the silyl ether and also the THP group is on a primary alcohol. So this deprotection was not expected to be difficult.

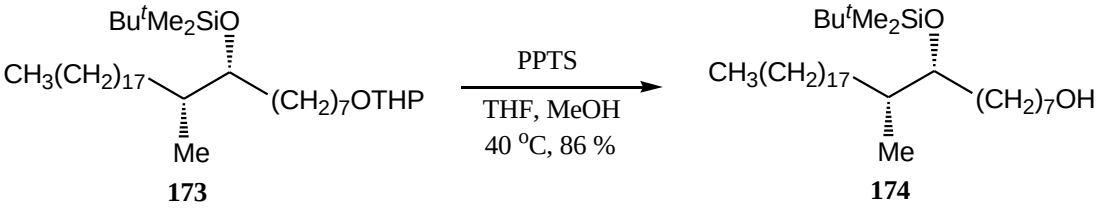
Initially, the protected compound (**173**) was treated with *p*-toluenesulphonic acid monohydrate (0.05 mol eq.) in the presence of methanol and THF. After stirring for 2 hour at room temperature it was worked up and columned. There was target product (**174**) in 69 % yield, together with diol (**175**), in which both silyl and THP ethers had been deprotected (**Scheme 25**).



Scheme 25: ***An attempted deprotection with PTSA***

So a second attempt was carried out using pyridinium *p*-toluenesulfonate (PPTS), which is less acidic than the PTAS. Treatment of the protected compound (**173**) with PPTS (0.5 mol eq.) in the presence of methanol and THF at room temperature gave no desired alcohol, but on stirring at 40 ^{o}C for 2.5 hours there was 86 % of desired alcohol (**174**) (**Scheme 26**) as well as 8 % both protected (**173**) and 3 % both deprotected diol (**175**). The proton NMR spectrum showed that there was no signal for the THP group, but there was a quartet at δ 3.65 (*J* 6.5 Hz) for the newly formed primary alcohols and a doublet of triplets at δ 3.5 (*J* 3.5, 6.0 Hz) for the C<u>H</u> next to the silyl protected oxygen. The corresponding carbons appeared in the carbon NMR at δ 75.9 and 63.1 (-<u>C</u>H$_2$OH). The signals for the silyl ether were still present. The IR showed a peak at

3321 for O–H stretching. The optical rotation of (**174**), $[\alpha]_D^{23}$ = + 12.9 (*c* 1.39, $CHCl_3$), showed the stereochemistry was unchanged in comparison with the model product (**164**) which had $[\alpha]_D^{24}$ = + 12.9 (*c* 0.84, $CHCl_3$).



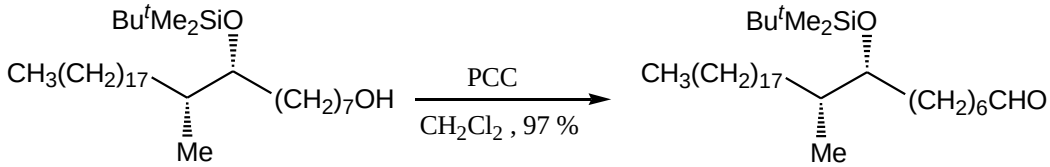
173 **174**

Scheme 26: ***Deprotection of the THP group***

2.2.10 Oxidation of the alcohol

Finally, the primary alcohol (**174**) was oxidised to the corresponding aldehyde (**176**) with PCC (2.5 mol eq.) in the presence of dichloromethane. The reaction was carried out at room temperature for 2 hours and purified by column chromatography immediately (**Scheme 27**). The aldehyde was used on the same day to avoid the possibility of polymerisation.

The ^{1}H NMR spectrum of (**176**) showed a triplet at δ 9.77 (*J* 1.6 Hz) for the aldehyde proton and the ^{13}C NMR spectrum showed a signal at δ 202.9 for the aldehyde carbon. In the IR spectrum, the hydroxyl peak had disappeared and a broad peak appeared at 1731 cm^{-1} for the aldehyde's C=O stretching. All this confirmed that the aldehyde had formed.



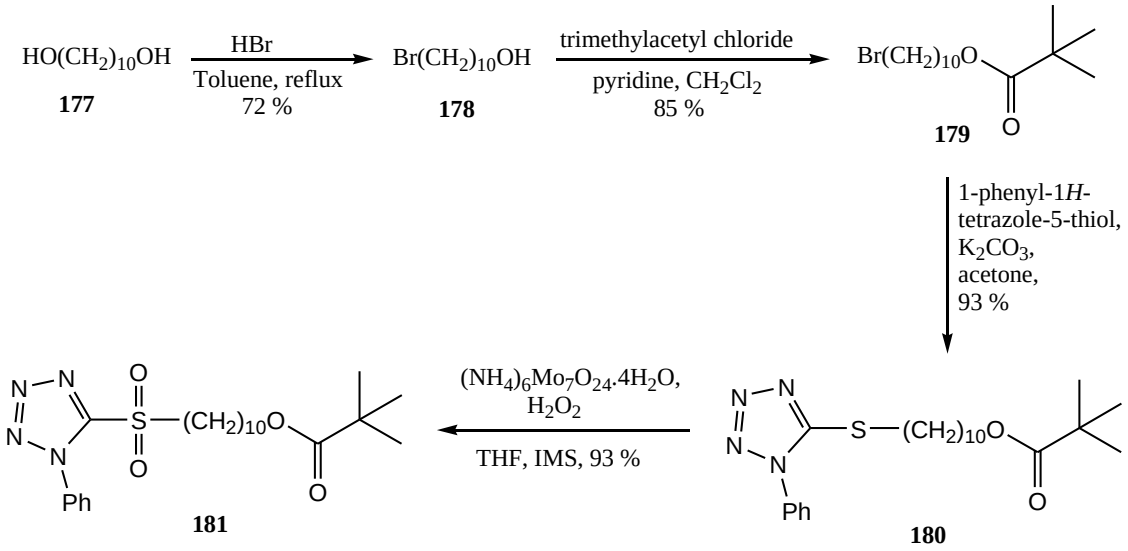
174 **176**

Scheme 27: ***Oxidation of the alcohol***

2.2.11 Preparation of C_{10} sulfone

The chain length of the aldehyde (**176**) is too short to couple to cyclopropane ring. In the next step it was necessary to add ten more carbons to prepare the exact length of the natural chain. The Julia reaction was used to extend the chain rather than Wittig coupling, because preparation of a long chain Wittig salt is difficult and also the yield is lower than that in the Julia reaction.

The sulfone (**181**) was prepared starting from 1,10-decanediol (**177**). The diol was monobrominated to 10-bromo-decan-1-ol (**178**) with 48 % HBr refluxing in toluene. The hydroxy group was protected with trimethylacetyl chloride. This protection is very common and deprotection is easy with strong base such as KOH and $LiAlH_4$. Understanding the proton and carbon NMR spectra is also easy compared to the THP protecting group. The monoalcohol (**178**) was protected with trimethylacetyl chloride in the presence of pyridine and 4-dimethylaminopyridine (DMAP), to catalyse the addition of the alcohol anion to the carboxylic group (**Scheme 28**).[181]



Scheme 28: ***Preparation of C_{10} sulfone***

The ^{1}H NMR spectrum of the protected compound (**179**) showed a triplet at δ 4.05 (*J* 6.6 Hz) for the protons adjacent to the carbonyl group, and a triplet at δ 3.41 (*J* 7.0 Hz) for the protons adjacent to the bromine group. The *tert*-butyl group protons appeared at δ 1.20 as a singlet. The ^{13}C NMR spectrum showed a signal at δ 178.6 for the carbonyl carbon, and a signal at δ 38.7 for the quaternary carbon of the protecting group, and a

signal at δ 27.2 for the *tert*-butyl methyl carbons. The IR showed a peak at 1729 cm^{-1} for the carbonyl group C=O stretch.

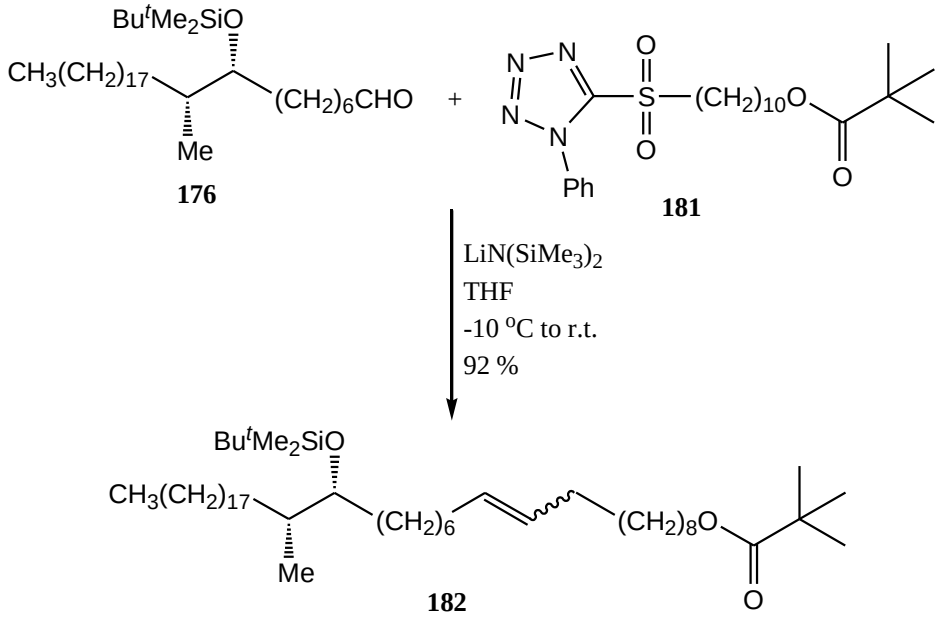
The protected bromo compound (**179**) was reacted with 1-phenyl-1*H*-tetrazole-5-thiole in acetone to give the sulfane (**180**) which was purified by column chromatography (**Scheme 28**). The proton NMR spectrum showed a multiplet at δ 7.61–7.54 for the phenyl group protons, and the carbon NMR spectrum showed five signals in the aromatic region. One signal was seen at δ 154.5 for the tetrazole ring carbon, and another four signals at δ 133.8, 130.0, 129.7 and 123.8 for the phenyl group carbons.

Finally, the sulfane (**180**) was oxidised to the desired sulfone (**181**) as previously discussed in section **2.2.3**. The sulfone was purified by column chromatography. The characteristic signal of the protons adjacent to the sulfonyl group appeared as a multiplet at δ 3.75–3.72. The required starting material, the sulfone (**181**), was thus obtained in four steps with an overall yield of 55 % (**Scheme 28**).

2.2.12 Extension of the chain

2.2.12a The Julia reaction

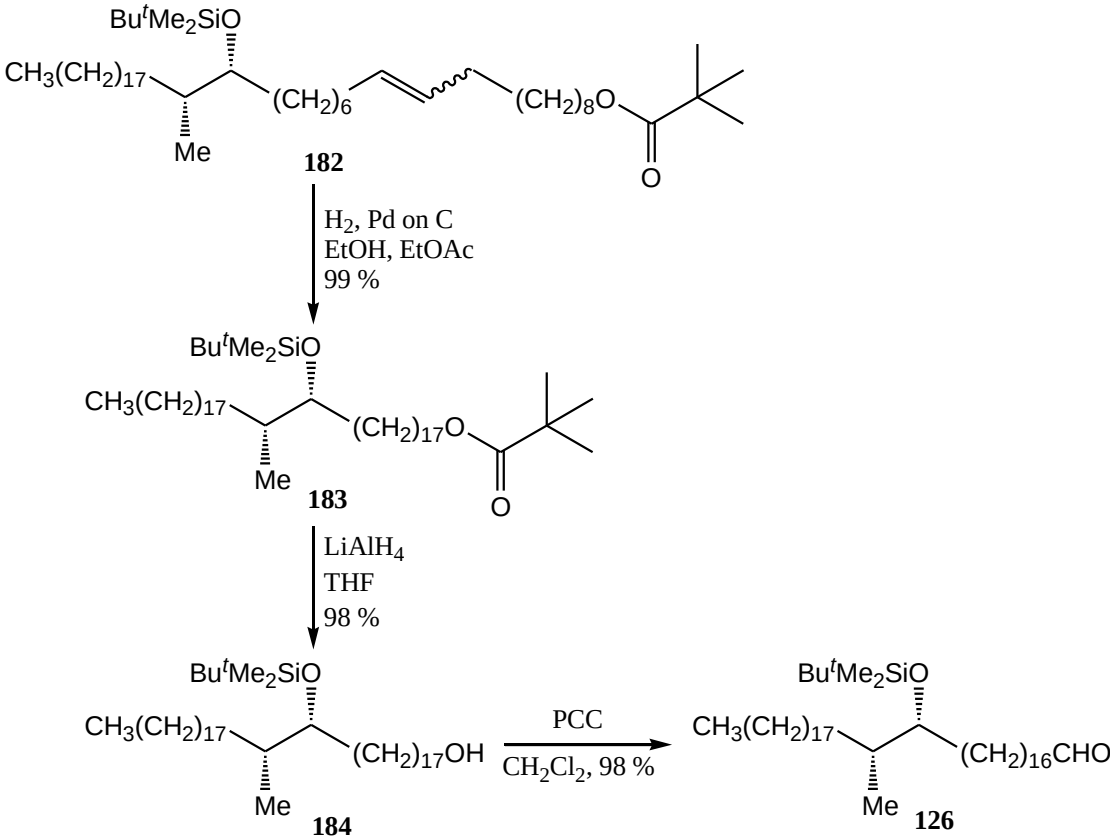
The chain was extended using the Julia coupling reaction as explained in section **2.2.5**. The reaction of the aldehyde (**176**) with the sulfone (**181**) in the presence of lithium bis(trimethylsilyl)amide in dry THF gave the olefin (**182**) in very good yield (92 %) (**Scheme 29**). The product was a mixture of *E*- and *Z*-stereoismers in a ratio 2.3:1.



Scheme 29: ***The Julia reaction***

2.2.12b Hydrogenation, deprotection and oxidation

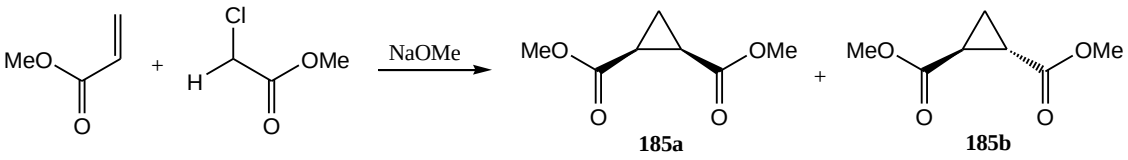
Hydrogenation of the mixture of alkenes in ethanol and ethyl acetate using Pd (10 %) on carbon as a catalyst gave the saturated compound (**183**) in excellent yield (99 %). The hydrogenated ester (**183**) was deprotected in the presence of $LiAlH_4$ in dry THF to the primary alcohol (**184**) in 98 % yield. The IR spectrum showed a broad peak at 3330 cm^{-1} for the O–H stretch. Finally, the alcohol (**184**) was oxidised to the corresponding aldehyde (**126**) with PCC in dichloromethane in excellent yield (98 %) for a further Julia reaction (**Scheme 30**). From ester to aldehyde, all compounds gave a similar molecular rotations which for the ester (**183**) was + 36.3 and for the aldehyde (**126**) was + 39.3. This means that during the reactions, the stereochemistry of the chiral centres did not change. The proton NMR spectrum of the aldehyde (**126**) showed a triplet at δ 9.77 (*J* 1.6 Hz) for the aldehyde proton, and a very broad multiplet at δ 1.50–1.13 for the long chain protons. The α-methyl protons to the silyl protected oxygen appeared as a doublet at δ 0.80 (*J* 6.7 Hz), and the two methyl bonded to silicon as singlets at δ 0.03 and 0.02. All these data proved the formation of the aldehyde.



Scheme 30: ***Extension of the chain***

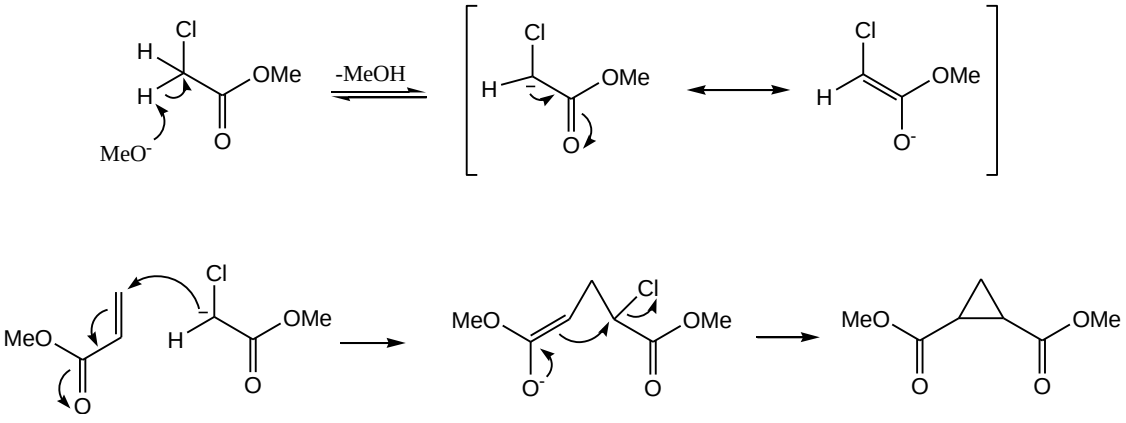
2.2.13 Formation of the cyclopropane by Michael Induced Ring Cyclisation (MIRC)

The cyclopropane ring is a common unit in a large number of natural products and most types of mycolic acid also include it. In this work, a single enantiomer of the *cis*-cyclopropane was required. A modified version of the procedure described by McCoy in 1958[182] was used to form the initial cyclopropane diester (**185a**) (**Scheme 31**). Sodium methoxide was added to a stirred mixture of methyl acrylate and methyl chloroacetate to form *cis*-cyclopropane (**185a**) and *trans*-cyclopropane (**185b**). This is an exothermic reaction and it is important to keep temperature below 30 ^{o}C. At higher temperatures, some polymerised product is also formed. It is possible to separate the *cis*-cyclopropane-1,2-dicarboxylic acid dimethyl ester (**185a**) from the *trans*-diastereomer (**185b**). The crude product was first vacuum distilled and then subjected to flash chromatography. It was necessary to column two or three times, monitoring by GLC. The desired pure *cis*-cyclopropane was obtained in 24 % yield. This yield is low but the two starting materials are inexpensive. The NMR spectra of the (**185a**) and **(185b**) matched the literature values and a very detailed analysis by I. O. Roberts.[183]



Scheme 31: ***The MIRC reaction***

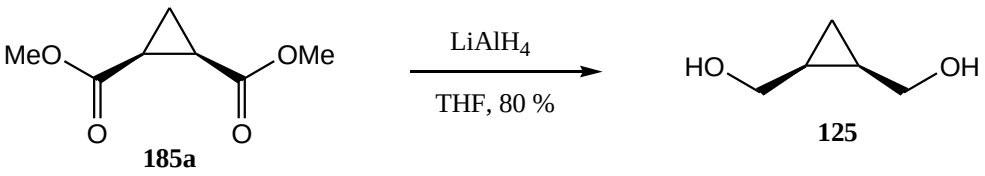
The mechanism of the Michael induced ring cyclisation is as shown in **Scheme 32**. The α-proton to carbonyl group in the methyl chloroacetate is acidic and this proton is removed by the base (NaOMe in this case) to form a resonance-stabilised carbanion. This undergoes a Michael type addition to the methyl acrylate, triggering the ring closure.



Scheme 32: ***The mechanism of the MIRC reaction***

2.2.14 Reduction of the diester by $LiAlH_4$

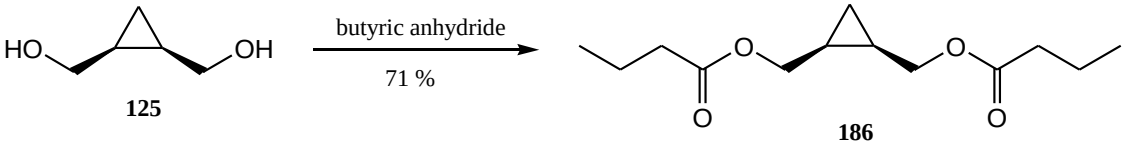
The separated *cis*-cyclopropane *bis*-methyl ester (**185a**) was reduced to the corresponding diol (**125**) using $LiAlH_4$ in THF (**Scheme 33**). The mixture was refluxed for 1 hour in THF and quenched with saturated aqueous Na_2SO_4 at room temperature to yield 80 % (*cis*-2-hydroxymethylcyclopropyl)-methanol. The crude diol (**125**) gave a clear NMR spectrum and therefore it was not columned.[184]



Scheme 33: ***The reduction of the diester (185a) to diol (125)***

2.2.15 Protection of the diol

The *cis*-cyclopropane diol (**125**) was protected with butyric anhydride to give *cis*-1,2-bis(butyryloxymethyl)cyclopropane (**186**),[184] because a single enantiomer of the monoester (**124**) can be obtained from this diester in quantitative yield. The diol and butyric anhydride (2.2 mol eq.) were refluxed for 1 hour and then worked up to give the pure diester in 71 % yield (**Scheme 34**).



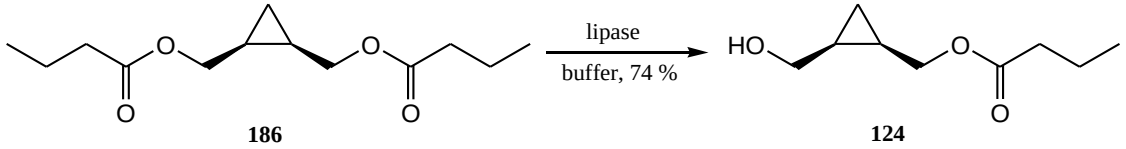
125 **186**

Scheme 34: ***Protection of the diol***

2.2.16 Enzymatic hydrolysis of the cyclopropyl dibutyrate

This reaction is the key step in the synthesis, as the symmetrical meso-diester (**186**) is converted into a single enantiomer of monoester (**124**) (**Scheme 35**). Grandjean *et al*[184] first reported the synthesis of an optically pure *cis*-cyclopropane monoester *via* an enzymatic desymmetrisation and this procedure was used in this work. The crude lipase, extracted from pig pancreas (PPL), was dissolved in ethylene glycol and water under nitrogen and cooled to 3 ^{o}C. The dibutyrate (**186**) was added at pH 6.8. When hydrolysis began, the pH was lowered due to the formation of butyric acid. An aqueous solution of sodium hydroxide was added by syringe to bring it back to pH 6.5. The completion of hydrolysis took five hours and then work up gave the single enantiomer, (1*R*,2*S*)-2-hydroxymethylcyclopropylmethyl butyrate (**124**) (**Scheme 35**).

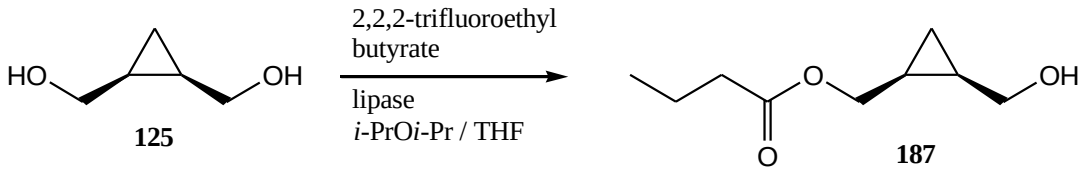
The propyl group in the proton NMR spectrum of (**124**) appeared as a triplet at δ 2.31 (*J* 7.6 Hz) for the two protons next to the carbonyl group, a sextet at δ 1.65 (*J* 7.4 Hz) for the middle -CH_2- and a triplet at δ 0.95 (*J* 7.4 Hz) for the terminal methyl. The two protons adjacent to the carbonyl oxygen appeared as doublet of doublets at δ 4.48 (*J* 5.7, 12.0 Hz) and 3.85 (*J* 5.4, 12.0 Hz). The two protons adjacent to the hydroxy group appeared as doublets of doublet at δ 3.81 (*J* 9.8, 12.0 Hz) and 3.40 (*J* 9.2, 12.0 Hz). Finally, the cyclopropane protons appeared as a multiplet at δ 1.37–1.24 (for two –CH-), as a doublet of triplets at δ 0.85 (for the *cis* proton of -CH_2-, *J* 5.1, 8.5 Hz) and a broad quartet at δ 0.22 (for the *trans* proton of -CH_2-, *J* 5.4 Hz). The ^{13}C NMR spectrum showed a signal at δ 173.6 for the carbonyl carbon and two signals at δ 64.4 and 62.5 for the two carbons next to oxygen. The IR showed a broad peak at 3417 cm^{-1} for the O–H stretch.



186 **124**

Scheme 35: ***Enzymatic hydrolysis of the dibutyrate to monobutyrate***

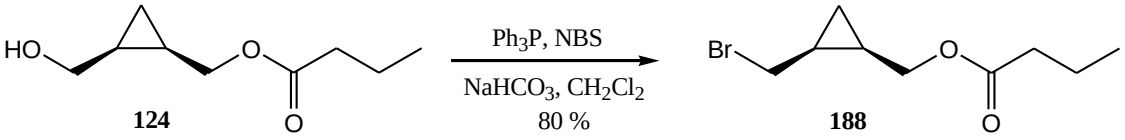
Determination of the absolute stereochemistry of the cyclopropane unit is possible by the optical rotation. Literature values of the (1*R*,2*S*)-2-hydroxymethylcyclopropyl-methyl butyrate (**124**) are +18.2[184] and +19.8[185], and that found in this work was +18.9 (in $CHCl_3$). The other enantiomer of the cyclopropane unit, (1*S*,2*R*)-2-hydroxymethyl-cyclopropyl-methyl butyrate (**187**), could be prepared as shown in **Scheme 36** and the optical rotation of this compound was – 18.1.[185]



Scheme 36: ***The enzyme catalysed transesterification***

2.2.17 Bromination of the alcohol (124)

The alcohol was converted to the corresponding butyric acid (1*R*,2*S*)-2-bromomethyl-cyclopropylmethyl ester (**188**) for the preparation of a sulfane for the next reaction. The conversion was done using *N*-bromosuccinimide and triphenylphosphine in dichloromethane. Sodium bicarbonate was added to the mixture for neutralisation of any acid formed. After work up, 80 % bromo-compound was isolated (**Scheme 37**).



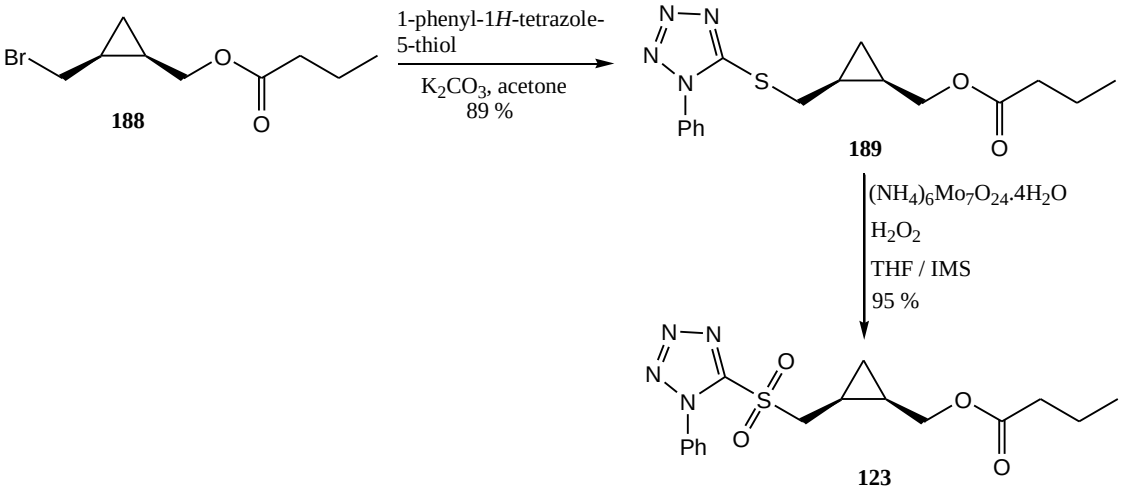
Scheme 37: ***Bromination of the alcohol with NBS***

The ^1H NMR spectrum of the bromo *cis*-cyclopropane (**188**) showed two doublets of doublets at δ 3.53 (*J* 7.6, 10.4 Hz) and at δ 3.40 (*J* 8.2, 10.7 Hz) for the two protons adjacent to bromine. The cyclopropane ring protons appeared as a multiplet at δ 1.56–1.45 (for the two -CH- protons), a doublet of triplets at δ 1.03 (for the one of the $-CH_2-$ proton, *J* 5.4, 8.5 Hz) and a broad quartet at δ 0.38 (for the other $-CH_2-$ proton, *J* 5.4 Hz). The carbon adjacent to bromine appeared at δ 34.0 in the carbon NMR spectrum. The specific rotation was $[\alpha]_D^{23} = -10.6$ (*c* 0.81, $CHCl_3$).

2.2.18 Preparation of the cyclopropyl sulfone

The cyclopropyl group was joined to the long chain by a Julia reaction. As discussed before, a sulfone and an aldehyde are required. The butyric acid (1*R*,2*S*)-2-bromomethyl-cyclopropylmethyl ester (**188**) was converted to the sulfide (**189**) with 1-phenyl-1H-tetrazole-5-thiol and potassium carbonate in acetone with 89 % yield.[148] The ^{1}H NMR spectrum showed two doublets of doublets at δ 3.58 (*J* 7.7, 13.4 Hz) and 3.42 (*J* 8.0, 13.4 Hz) for the protons next to the sulfur atom.

Finally, the sulfide was oxidised with hydrogen peroxide to the desired butyric acid (1*R*,2*S*)-2-(1-phenyl-1*H*-tetrazole-5-sulfonylmethyl)cyclopropylmethyl ester (**123**)[148] with 95 % yield by using the method discussed in section **2.2.3** (**Scheme 38**).



Scheme 38: ***Preparation of the cyclopropyl sulfone***

Interestingly, in the proton NMR spectrum the two protons adjacent to the sulfonyl group appeared as two doublets of doublets at δ 4.05 (*J* 5.1, 14.8 Hz), and 3.67 (*J* 8.5, 14.8 Hz). However, normally the protons adjacent to the sulfonyl group in the straight chain such as (**141**, p 40) appeared as a multiplet. The ^{1}H NMR spectrum of (**123**) was as shown in **Table 2**. The optical rotation of this sulfone was high, $[\alpha]_{D}^{23}$ = + 52.7 (*c* 1.45, $CHCl_3$).

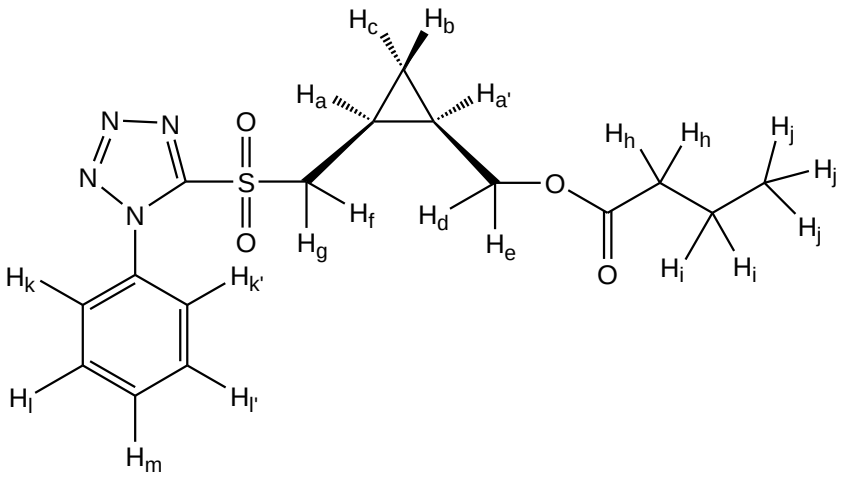


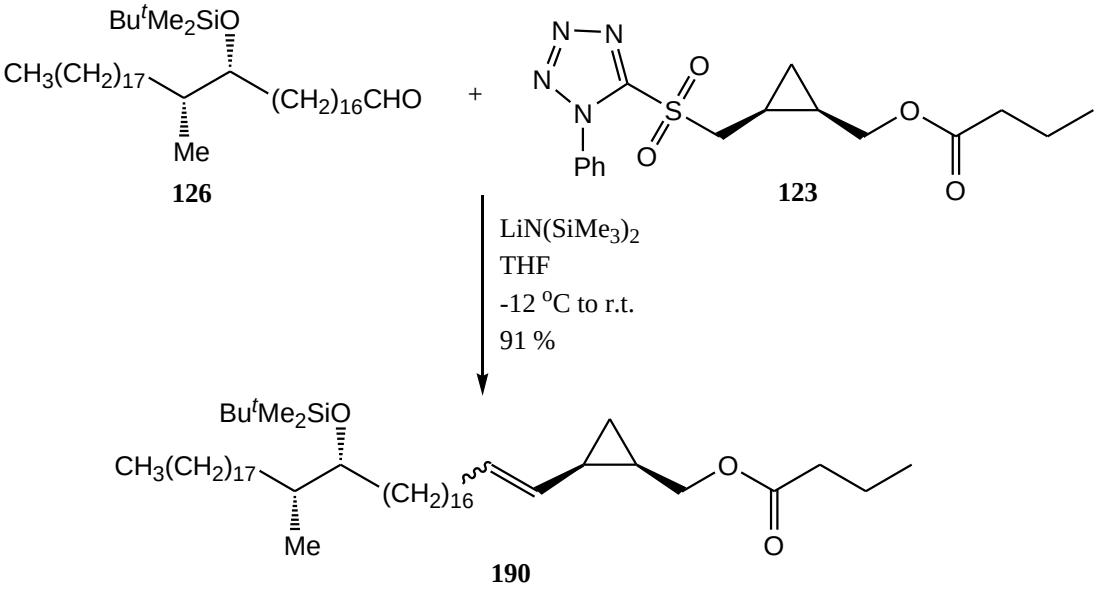
Figure 21: ***Structure of butyric acid (1R,2S)-2-(1-phenyl-1H-tetrazole-5-sulfonylmethyl)cyclopropylmethyl ester (123)***

Table 2: ***The ^{1}H NMR data for (123)***

δ (ppm)	Multiplicity	*J* geminal (Hz)	*J* vicinal (Hz)	Assignment
7.71	m			H_k and $H_{k'}$
7.63	m			H_l, $H_{l'}$ and H_m
4.38	dd	12.3	5.4	H_e
4.05	dd	14.8	5.1	H_g
3.91	dd	12.3	8.2	H_d
3.67	dd	14.8	8.5	H_f
2.31	t		7.4	H_h
1.66	sext		7.4	H_i
1.48	m			H_a and $H_{a'}$
1.03	dt	6.0	8.5	H_c
0.96	t		7.4	H_j
0.60	br.q	6.0	6.0	H_b

2.2.19 The Julia reaction between (123) and (126)

The (18*R*,19*R*)-18-(*tert*-butyldimethylsilanyloxy)-19-methyl-heptatriacontanal (**126**) and butyric acid (1*R*,2*S*)-2-(1-phenyl-1*H*-tetrazole-5-sulfonylmethyl)-cyclopropyl-methyl ester (**123**) were coupled by Julia reaction to give the olefin (**190**) (91 %). The product was a mixture of *E*- and *Z*-stereoisomers in a ratio 1.6:1 (**Scheme 39**).



Scheme 39: ***The Julia reaction***

The ^{1}H NMR spectrum showed a doublet of triplets at δ 5.57 (*J* 15.2, 7.0 Hz) and a doublet of doublets at δ 5.22 (*J* 15.2, 7.6 Hz) for the olefinic protons in the *E*-stereoisomer. The high coupling constant (15.2 Hz) confirmed the formation of the *E*-isomer. A doublet of triplets at δ 5.47 (*J* 11.0, 7.3 Hz) and a broad triplet at δ 5.04 (*J* 10.4 Hz) appeared for the olefinic protons in the *Z*-stereoisomer. The ^{13}C spectrum showed two signals at δ 132.3 and 127.3 for the *E*-isomer and two signals at δ 132.2 and 127.4 for the *Z*-isomer as shown in **Figure 22**.

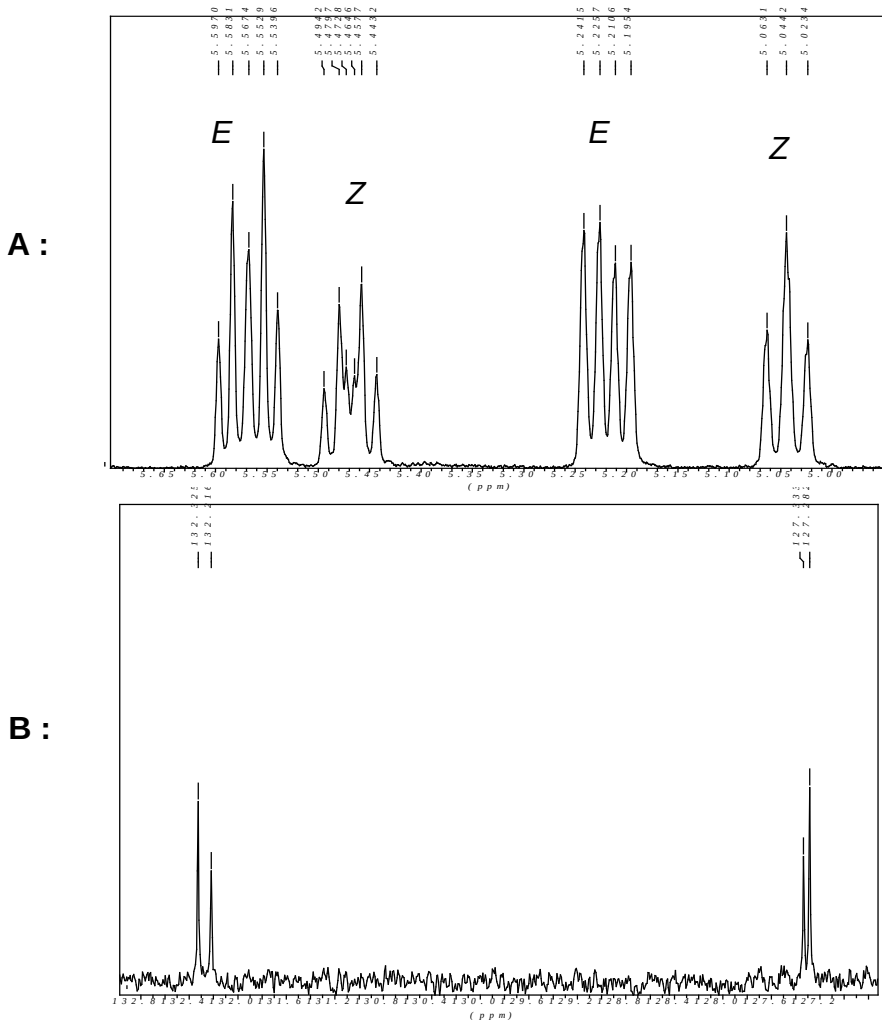


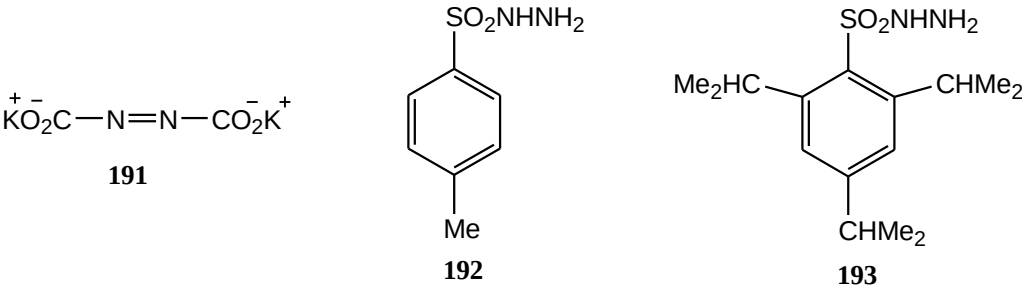
Figure 22: ***A : the ^{1}H NMR , B : the ^{13}C NMR of the alkene (189) for the olefinic region***

2.2.20 Hydrogenation of the alkene (190) by di-imide

Hydrogenation of the alkene product formed by Julia reaction and containing a cyclopropane ring requires milder conditions than the catalytic hydrogenation using palladium on carbon and hydrogen gas, because this can result in hydrogenation of the cyclopropane as well as the alkene. Di-imide (HN=NH) is a milder hydrogenation agent, reported first time by Corey in 1929, during a reinvestigation of work done 24 years previously on the formation of the hydrazone of glyceryl oleate. It was shown that under certain conditions, the oleate was hydrogenated to stearate, though they did not realise that the species responsible for the hydrogenation was in fact di-imide.[186]

Di-imide has been generated by a number of different methods. The most widely used have been the oxidation of hydrazine,[186, 187 , 188] the decarboxylation of potassium azodicarboxylate (**191**),[186,187,188] the decomposition of benzenesulphonyl[188] or *p*-

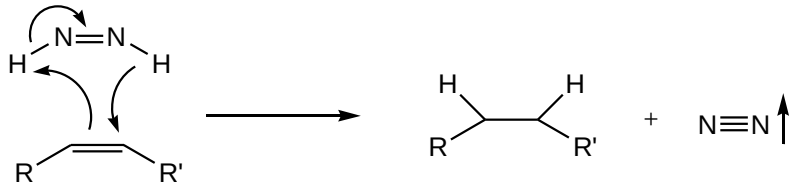
toluene-sulphonyl hydrazide (**192**).[187] Moreover, 2,4,6-tri-isopropylbenzenesulphonyl hydrazide (TPBSH) (**193**) was reported by N. J. Cusack *et al*[189] as a convenient source of di-imide which was considerably more labile to heat than *p*-toluene-sulphonyl hydrazide (**192**).



191 **192** **193**

Figure 23: ***Some di-imide sources***

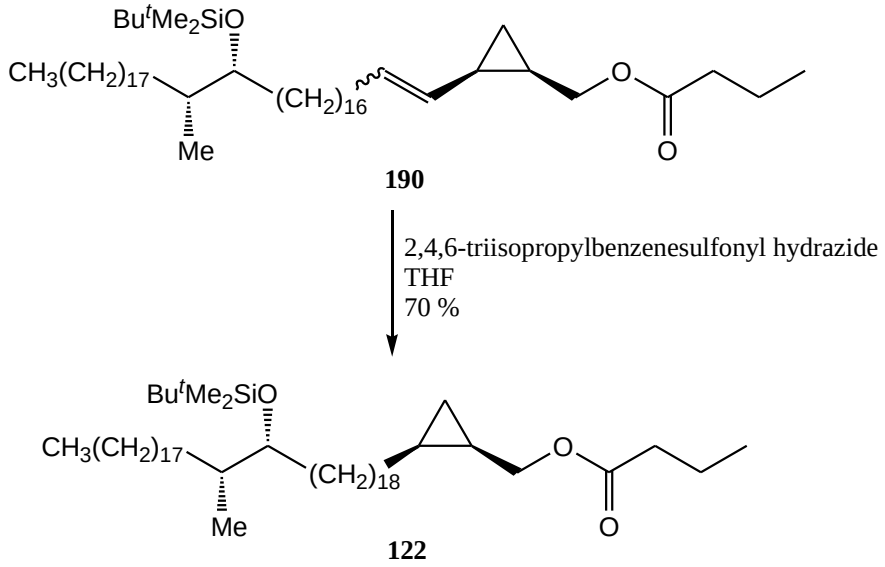
Hydrogenation of an alkene by di-imide proceeds via the following mechanism (**Scheme 40**):



Scheme 40: ***The mechanism of hydrogenation by di-imide***

For the hydrogenation of the unsaturated ester (**190**), di-imide was generated from TPBSH which on heating decomposed to di-imide. The unsaturated ester was dissolved in THF and an excess of TPBSH (4.5 mol eq.) was added and heated for 27 hours. After work up, the NMR spectrum showed there was still a small amount of unsaturated ester which was eliminated with potassium permanganate and cetrimide in dichloromethane and water to give the saturated ester (**122**) in 70 % yield (**Scheme 41**). The NMR spectrum showed no signals in the olefinic region and the proton adjacent to the silyl protected oxygen appeared at δ 3.51 as a doublet of triplets (*J* 3.5, 6.3 Hz), and the *tert*-butyl group bonded to silyl at δ 0.89 as a singlet. There was a doublet of triplets at δ 0.75 (*J* 5.1, 8.5 Hz) and a broad quartet at δ 0.01 (*J* 5.1 Hz) for the cyclopropane ring protons. The methyl α- to the silyl protected oxygen appeared as a

doublet at δ 0.81 (*J* 6.9 Hz). The IR spectrum showed a broad peak at 1739 cm^{-1} for the C=O stretch.



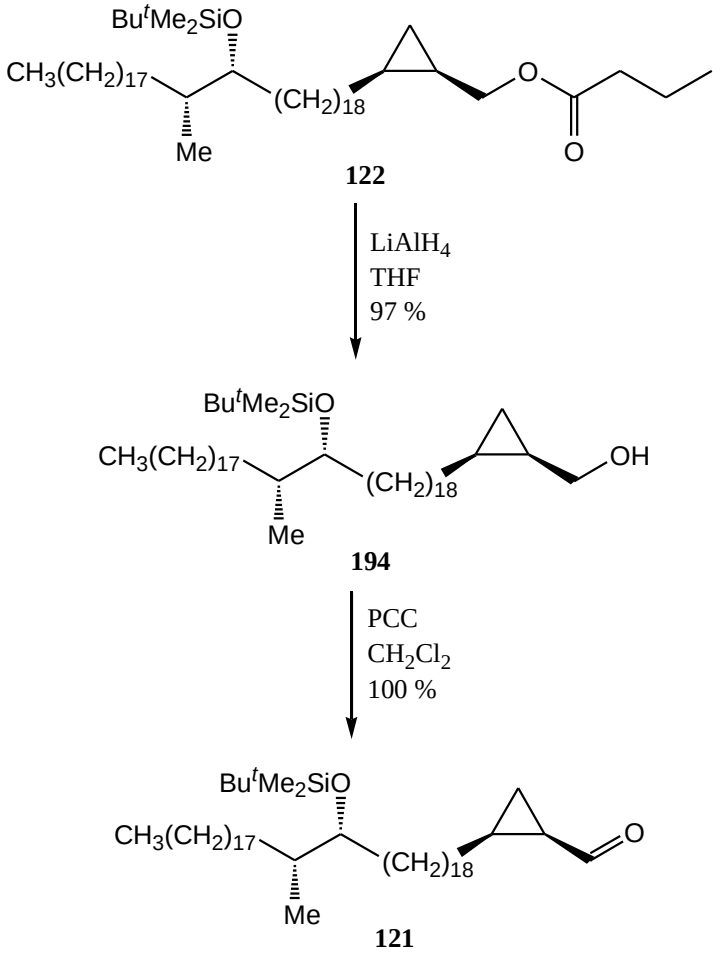
Scheme 41: ***Hydrogenation of a cyclopropyl olefin***

2.2.21 Reduction and oxidation

The saturated ester (**122**) was reduced to the {(1*R*,2*S*)-2-[(19*R*,20*R*)-19-(*tert*-butyl-dimethyl-silanyloxy)-20-methyl-octatriacontyl]-cyclopropyl}-methanol (**194**) by $LiAlH_4$ in THF in 97 % yield (**Scheme 42**). In the proton NMR spectrum, the ester protons disappeared and two multiplets appeared at δ 3.67–3.64 and 3.61–3.57 for the protons adjacent to hydroxy group. The two cyclopropane protons appeared as a doublet of triplets at δ 0.71 (*J* 4.4, 8.2 Hz) and a broad quartet δ - 0.03 (*J* 5.4 Hz). The two methyl protons bonded to silicon appeared as a singlet at δ 0.04 and 0.03. The IR spectrum showed a broad peak at 3346 cm^{-1} for the O–H stretch.

Finally, the alcohol (**194**) was oxidised to (1*R*,2*S*)-2-[(19*R*,20*R*)-19-(*tert*-butyldimethylsilanyloxy)-20-methyl-octatriacontyl]-cyclopropanecarbaldehyde (**121**) for the another Julia reaction. PCC was dissolved with dichloromethane and the alcohol was added slowly by pipette and stirred for three hours to yield the aldehyde (**121**) in 100 % yield (**Scheme 42**). The small quantity of the alcohol (0.9 g, 1.2 mmol) meant that it became practical to use an even greater excess of dichloromethane without being too wasteful. This resulted in an easier work up due to the black material

formed during the reaction staying very mobile, and consequently becoming more granular on addition of the diethyl ether at the end of the reaction. This gave a better yield.



Scheme 42: ***Reduction and oxidation***

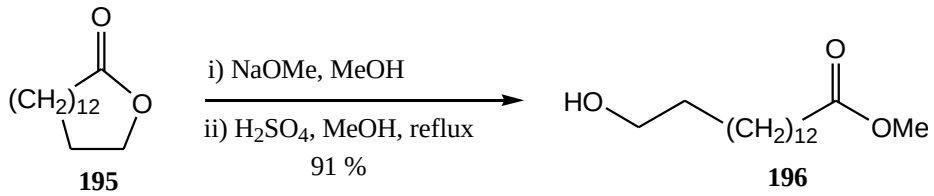
The ^{1}H NMR spectrum of (**121**) showed a doublet at δ 9.36 with a vicinal coupling (*J* 5.7 Hz) for the aldehyde proton and a very broad multiplet at δ 1.50–1.18 for the long chain protons. The cyclopropane protons were shifted far downfield in comparison to the alcohol starting material by the deshielding effect of the carbonyl group. The proton adjacent to the carbonyl group appeared as a complicated doublet of doublets of triplets at δ 1.87 (*J* 8.2, 5.7, 5.4 Hz). However, the remaining cyclopropane protons were obscured by the long chain protons. The ^{13}C NMR spectrum showed a signal at δ 201.7 for the aldehyde carbon and a signal at δ 75.9 for the carbon bonded to the silyl protected oxygen and the other signals were as expected.

2.2.22 Final extension of the side chain

2.2.22a Ring opening of lactone for preparation of the C_{14} chain

To complete the final coupling reaction for the synthesis of the meromycolaldehyde, the fifteen carbon chain sulfone had to be prepared. Commercially available and inexpensive *ω*-pentadecalactone (**195**) was chosen as a starting material. The lactone's ring was opened by reaction of sodium methoxide which was freshly prepared by addition of small piece of sodium to HPLC grade methanol. *ω*-Pentadecalactone was added to sodium methoxide solution and stirred at 80 ^{o}C for 3 hours. The solution was acidified with aq. HCl (1N) and worked up. The proton NMR spectrum showed the product was a mixture of the desired methyl ester and the carboxylic acid. Therefore, the acid was esterified in methanol and a catalytic amount of H_2SO_4 by refluxing for 90 minutes to give 15-hydroxypentadecanoic acid methyl ester (**196**) in 91 % yield (**Scheme 43**).[190]

The ^{1}H NMR spectrum of (**196**) showed a singlet at δ 3.67 for the ester methyl protons, a triplet at δ 3.64 (*J* 6.6 Hz) for the two protons next to the hydroxy group, and a triplet at δ 2.31 (*J* 7.6 Hz) for the two protons next to the carbonyl group. The long chain protons appeared as a multiplet at δ 1.36–1.26. The ^{13}C NMR spectrum showed a carbonyl signal at δ 174.3, a signal at δ 63.1 for the carbon next to the hydroxy group, and a signal at δ 51.4 for the ester's methyl carbon. The IR spectrum showed a broad peak at 3298 cm^{-1} for the O–H stretch and another broad peak at 1742 cm^{-1} for the C=O stretch.



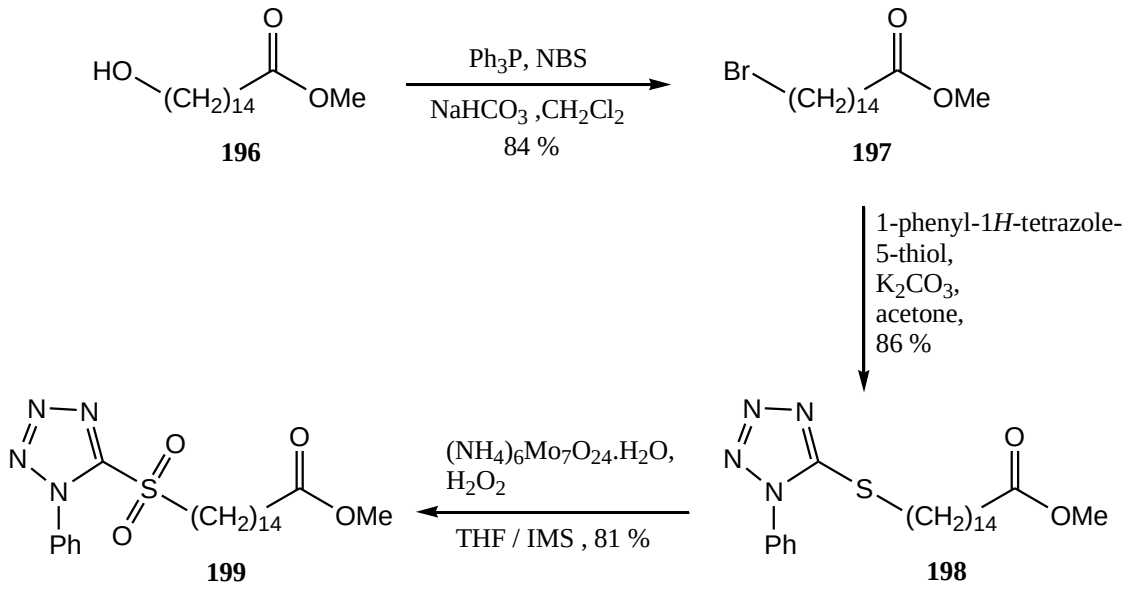
195 **196**

Scheme 43: ***Ring opening of the lactone***

2.2.22b Preparation of the sulfone

As discussed previously (p 60), conversion of the hydroxy ester (**196**) to 15-bromopenta-decanoic acid methyl ester (**197**) was achieved using triphenylphosphine and *N*-bromosuccinimide as shown in **Scheme 44**.[190] The protons next to bromine appeared in the proton NMR spectrum as a triplet at δ 3.41 (*J* 6.6 Hz) which shifted upfield in comparison to the alcohol.

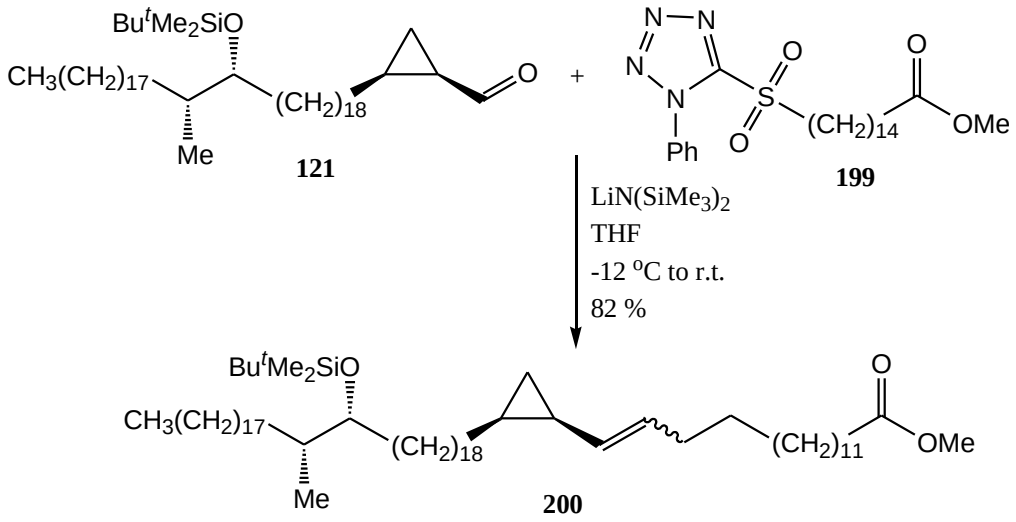
The bromo ester (**197**) was converted into 15-(1-phenyl-1*H*-tetrazol-5-ylsulfanyl)-pentadecanoic acid methyl ester (**198**) in 86 % yield (**Scheme 44**). In the proton NMR spectrum, the phenyl protons appeared as a multiplet at δ 7.60–7.7.56 and the two protons next to the sulfanyl group appeared as a triplet at δ 3.39 (*J* 7.3 Hz). Finally, the sulfane was oxidised to the corresponding sulfone (**199**) with hydrogen peroxide in 81 % yield. There were two pieces of evidence for formation of the sulfone; the phenyl protons appeared as two different multiplets, one having two protons and the other having three protons, while the phenyl protons appeared as only one multiplet for the sulfane. The second piece of evidence was the appearance of the protons adjacent to sulphur. These appear as a clear triplet adjacent to a sulfanyl group, but as a multiplet for the sulfonyl group which forms an AA'BB' system. The ^{1}H NMR spectrum of the sulfone (**199**) showed two multiplet at δ 7.70–7.69 (2H) and 7.64–7.58 (3H) for the phenyl protons and a multiplet at δ 3.75–3.72 for the two protons adjacent to the sulfonyl group.



Scheme 44: ***Preparation of the sulfone (199)***

2.2.23 Final Julia reaction for the preparation of the keto-meromycolaldehyde

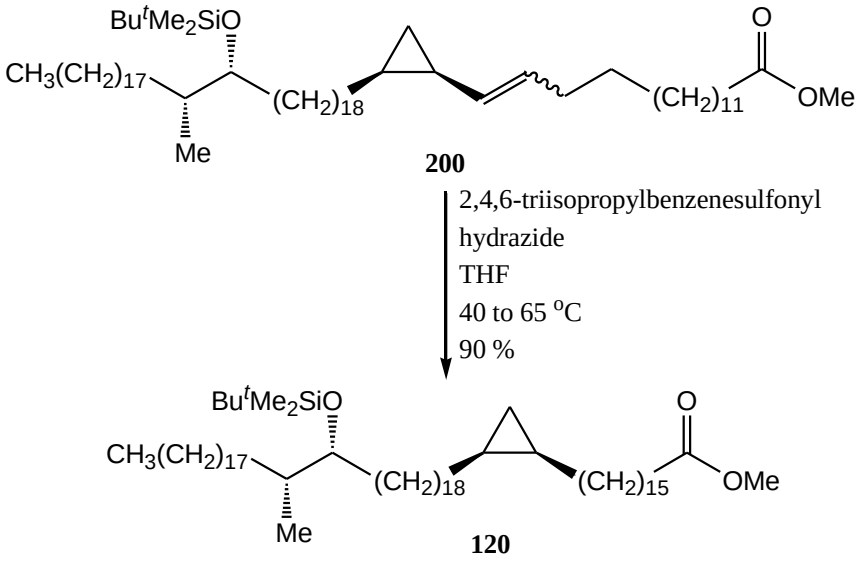
The Julia reaction between the aldehyde (**121**) and the sulfone (**199**) gave the final chain extension as a mixture of *E*- and *Z*-stereoisomers in ratio 5:1 of the desired compound (**200**) in 82 % yield (**Scheme 45**). The ^{1}H NMR spectrum showed a doublet of triplets at δ 5.52 (*J* 15.2, 6.9 Hz) and a doublet of doublets at δ 5.18 (*J* 15.2, 8.5 Hz) for the *trans* olefin protons of the olefin, and a doublet of triplets at δ 5.41 (*J* 10.4, 7.5 Hz) and a multiplet at δ 5.07–5.02 for the *cis* olefin protons.



Scheme 45: ***Final Julia reaction for the keto-meromycolaldehyde***

2.2.24 Hydrogenation of the olefin (200) by di-imide

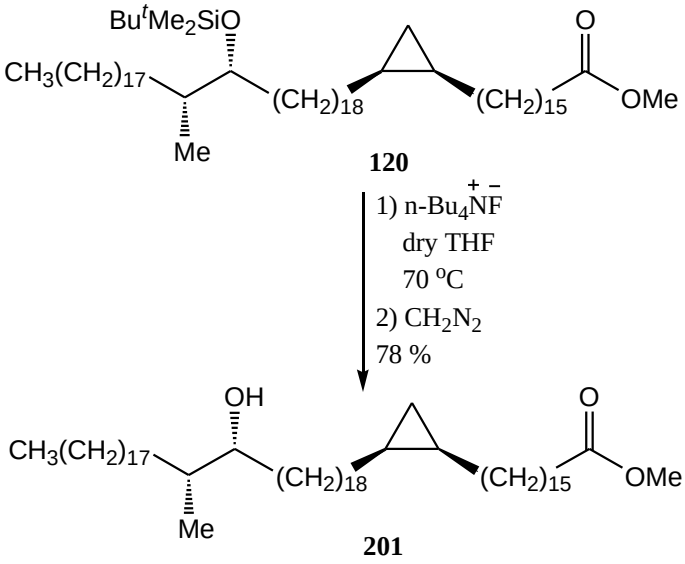
The olefin (**200**) was hydrogenated with di-imide using TPBSH. The olefin was dissolved in THF and excess of TPBSH (7 mol eq.) was added and stirred at 65 ^{o}C for 18 hours. The NMR spectrum showed there was still starting material and further TPBSH (4.3 mol eq.) was added and stirred at 40 ^{o}C for another 18 hours, but the NMR showed there was still small amount of the olefin which was separated by column chromatography on a mixture of silver nitrate (1 g) and silica (30 g) to give the hydrogenated product (**120**) in 79 % yield (**Scheme 46**). The ^{1}H NMR spectrum of (**120**) showed a multiplet at δ 0.67–0.64 (two protons), a broad doublets of triplet at δ 0.56 (*J* 4.1, 8.2 Hz) and a broad quartet at δ - 0.32 (*J* 5.4 Hz) for the cyclopropane ring protons.



Scheme 46: ***Hydrogenation of the olefin***

2.2.25 Desilylation

The next step, deprotection of the silyl group, was not an easy reaction as it was secondary and had a long chain bulky group on both sides. Tetra-n-butylammonium fluoride (TBAF), a mild common deprotection reagent, was used to deprotect the *tert*-butyldimethyl silyl group. The TBAF was added to a stirred solution of the silyl ether in dry THF and stirred for 3 hours. TLC showed there was no product, so the mixture was stirred at 50 ^{o}C for 6 hours, but there was still no product. Further TBAF was added and stirred at 70 ^{o}C for 18 hours and there was small amount of product so further TBAF was added and stirred for 45 hours and the reaction was then worked up. After column chromatography, the desired secondary alcohol methyl ester (**201**) was obtained in 49 % yield and the corresponding secondary alcohol carboxylic acid also obtained in 29 % yield which was converted into the desired methyl ester by diazomethane (**Scheme 47**). The *tert*-butyl and the two methyls disappeared on the NMR spectrum. The IR spectrum showed peaks at 3511 cm^{-1} for the O–H stretch and at 1726 cm^{-1} for the C=O stretch.



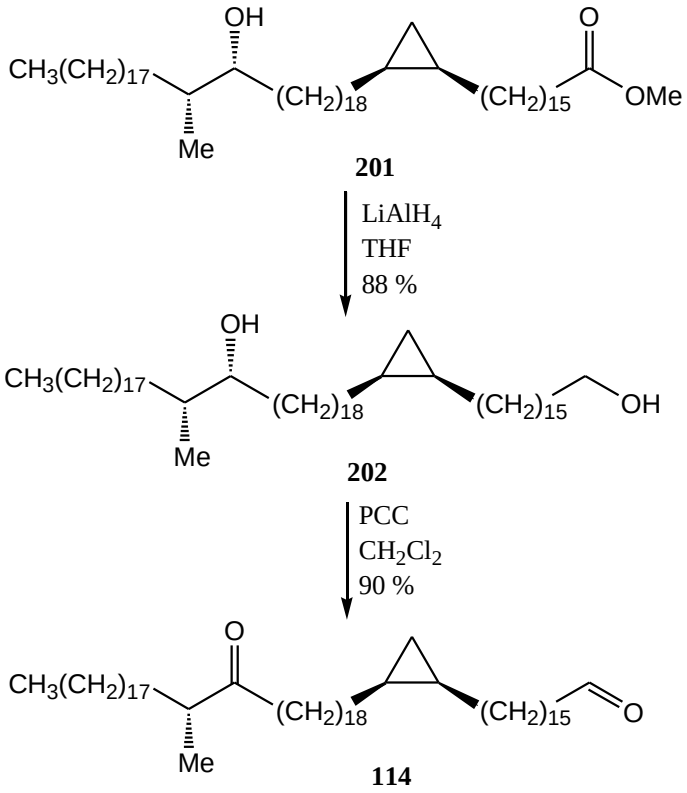
Scheme 47: ***Desilylation of the Bu^{t}Me$_2$Si group***

2.2.26 Reduction and oxidation

The hydroxy methyl ester (**201**) was reduced to the corresponding diol (**202**) in 88 % yield using the procedure described before (p 37). The white solid diol was not soluble in dichloromethane and the silica bed for filtration was washed with hot chloroform to get pure product (**Scheme 48**). The diol was also dissolved in hot $CDCl_3$ to run the NMR spectra. The ^{1}H NMR spectrum showed a multiplet at δ 3.66 for the two protons adjacent to primary alcohol and another multiplet at δ 3.50 for the proton adjacent to secondary alcohol and the protons of the methyl for the ester group had disappeared. The ^{13}C NMR spectrum showed a signal at δ 75.3 for the carbon next to the secondary alcohol and a signal at δ 63.1 for the carbon next to the primary alcohol. The IR spectrum showed a peak at 3345 cm^{-1} for the O–H stretch and no peak for the C=O stretch. The specific rotation of the diol (**202**) was $[\alpha]_D^{35}$ = + 5.2 (*c* 0.575, $CHCl_3$).

Finally, the diol was oxidised to target keto-meromycolaldehyde (**114**) with PCC (**Scheme 48**). The reaction temperature was kept at 40 ^{o}C, because of the solubility of the diol, and worked up to give a white solid in 90 % yield. Selected NMR spectrum data are shown in **Figure 24** and **Table 3**. The long chain protons and carbons which appeared as a very broad multiplet at δ 1.66–1.12 (for protons) and at δ 33.0–22.1 (for carbons). The IR showed two peaks at 1716 and 1699 cm^{-1} for the two C=O stretches. The molecular rotation of (**114**) was – 40.5 which was as expected if compared to the

model ketone (**165**) mentioned in section **2.2.7**, for which it was – 47.7. This means the cyclopropane group does not make a big contribution to the molecular rotation.



Scheme 48: ***Final reduction and oxidation to keto-meromycolaldehyde***

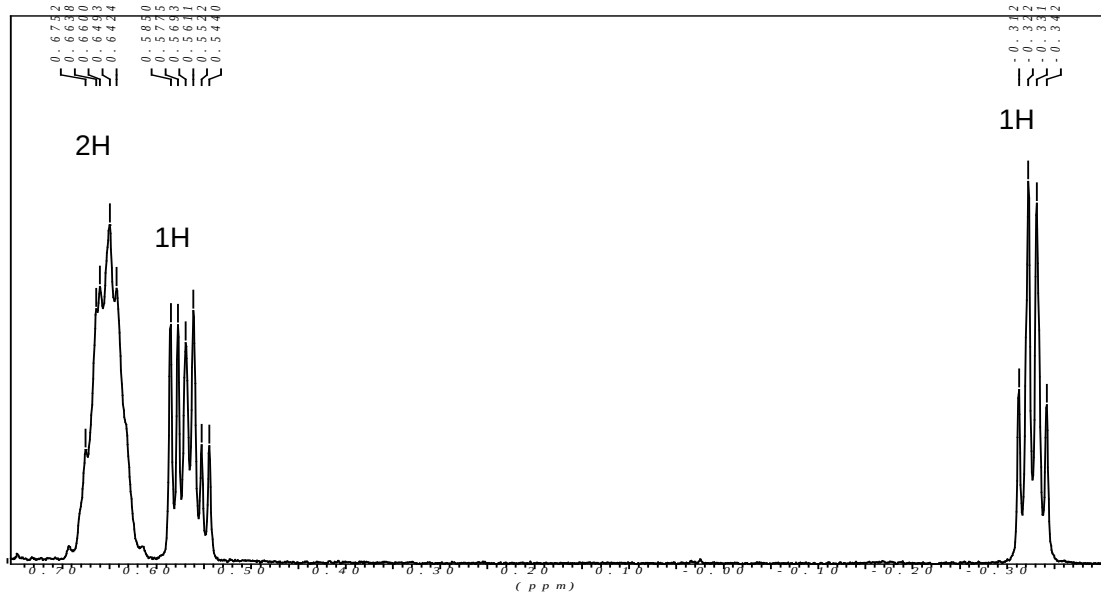
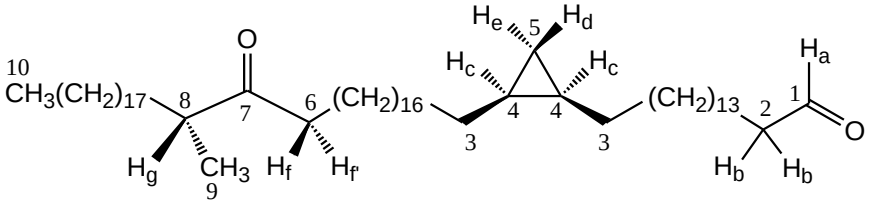


Figure 24: ***The signals of the four cyclopropane protons of the keto-meromycolaldehyde***

Table 3: ***The ^{1}H and ^{13}C NMR data of the keto-meromycolaldehyde***



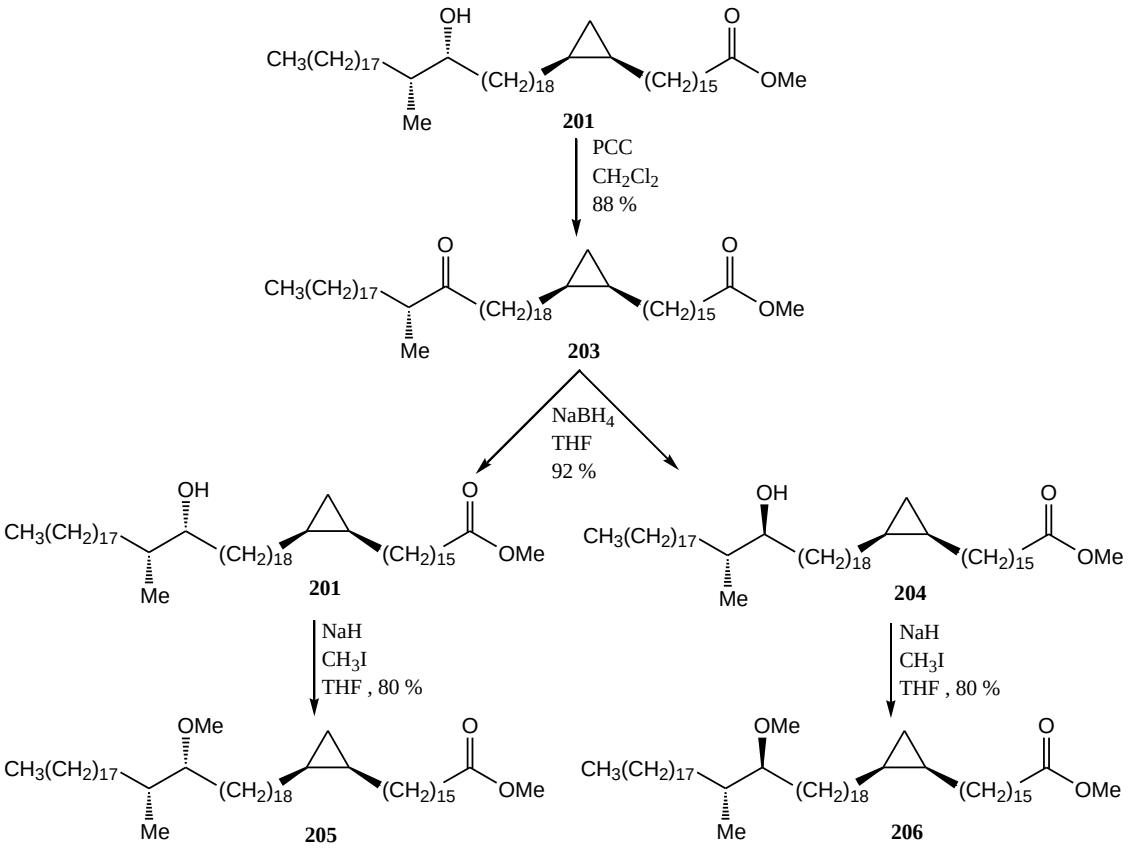
Proton	δ	Multiplicity	*J*	Carbon	^{13}C NMR
H_a	9.77	t	1.9	1	202.9
H_b	2.42	dt	1.9, 7.3	2	43.9
H_c	0.65	m		3	29.0
H_e	0.56	br.dt	4.1, 8.2	4	15.8
H_d	-0.33	br.q	5.4	5	10.9
H_f	2.46-2.40	m		6	41.1
$H_{f'}$	2.44-2.36	m		7	215.2
H_g	2.51	sext	6.9	8	46.3
9-CH_3	1.05	d	6.9	9	16.4
10-CH_3	0.89	t	7.0	10	14.1

The synthesis of the keto-meromycolaldehyde might be help to of establish the relative stereochemistry of the stereocenters on the meromycolate chain in comparison to the specific rotation and NMR spectra of natural keto-meromycolaldehyde. However this has not been yet sourced as a pure compound.

2.2.27 Preparation of methoxy-meromycolic acid

Another model was used to find out the relative stereochemistry of the methyl and methoxy groups in methoxymycolates. Thus, the secondary alcohol (**201**) was oxidised to ketone (**203**) with PCC in 90 % yield. Reduction of this with sodium borohydride led to an inseparable mixture of alcohols (**201**) and (**204**) (**Scheme 49**). These two alcohols could be distinguished by their NMR spectra. The hydrogens adjacent to the alcohol appeared as multiplets at δ 3.50 and 3.43, and the attached carbon signals

appeared at δ 76.1 or 75.2, respectively.[†,191] The alcohol mixture was methylated using sodium hydride and iodomethane in dry THF stirring 18 hours at room temperature to give the *R*-methoxy (**205**) and *S*-methoxy (**206**) in 80 % yield. The ^{1}H NMR spectrum of the mixture showed the two isomers, giving distinct patterns for the methoxy groups as singlets at δ 3.36 and 3.35, and for the β-methyl-group as doublets at δ 0.87 and 0.85. Pairs of signals for the two isomers were also seen in the ^{13}C NMR spectrum at δ 85.5 and 85.4 for the secondary carbons bonded to oxygen, and at δ 57.7 and 57.3 for the methoxy carbons. These two carbons appeared at δ 85.5 and 57.7 for the synthetic methoxy mycolic acid (**109**), and at δ 85.4 and 57.7 for (**112**).[148] Moreover, these two carbons appeared at δ 85.5 and 57.7 for the natural methoxy mycolic acid.[177] The *syn*-methoxy compound (**205**) was identical to the pattern for a natural methoxy mycolic acid (**Scheme 49**).

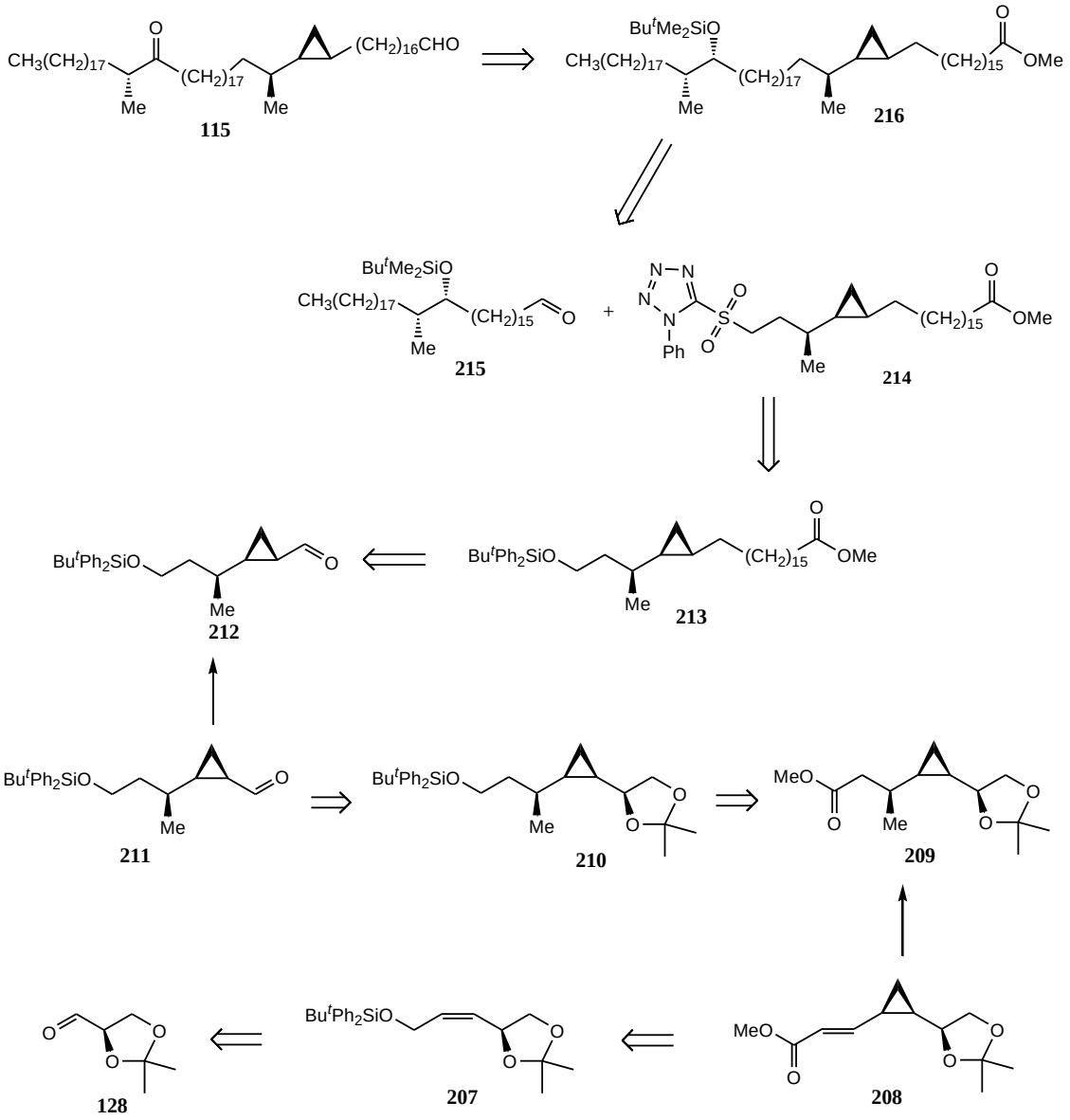


Scheme 49: ***Preparation of a mixture of methoxy-meromycolic acid***

[†] The reduction of the natural homologous mixtures of keto- to hydroxymycolic acids has been described; but no NMR data are provided to indicate whether a mixture of epimers was observed (ref. 191)

2.3 Synthesis of α-methyl *trans*-cyclopropane keto-meromycol-aldehyde

The α-methyl *trans*-cyclopropane keto-meromycolaldehyde (**115**) was prepared by a similar method to that used to prepare *cis*-cyclopropane keto-meromycolaldehyde (**114**) as previously discussed (p 35 to 73). The key stage to synthesis of this was the preparation of the intermediate single enantiomer of the α-methyl *trans*-aldehyde (**212**). The reactions are summarized in **Scheme 50**.

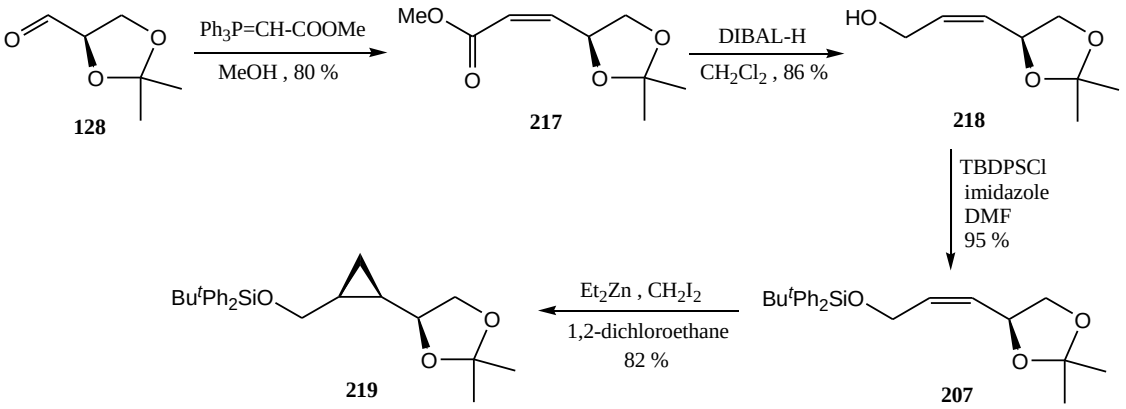


Scheme 50: ***Diagram of the preparation of α-methyl trans-cyclopropane keto-meromycolaldehyde***

2.3.1 Preparation of the silyl protected cyclopropane acetal

tert-Butyl-[(1*S*,2*R*)-2-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-cyclopropylmethoxy]-diphenyl-silane (**219**) was obtained using literature method (**Scheme 51**).[192,193] The Wittig reaction between the well known (*R*)-glyceraldehyde acetonide (**128**)[156] and methyl (triphenylphosphoranylidene)acetate[194] in methanol at 0 ^{o}C gave a mixture of alkenes as Z/E-stereoisomers. The mixture was primarily the Z-isomer and separated by column chromatography to give the (*Z*)-3-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-acrylic acid methyl ester (**217**). The methyl ester was then reduced to the corresponding alcohol (**218**) with DIBAL-H (in hexanes, 1M) in dry dichloromethane. The reaction was stirred at – 70 to – 55 ^{o}C for 2 hours and worked up to give the desired product in 86 % yield. Subsequent protection of the hydroxy group with *tert*-butyldiphenylchlorosilane and imidazole in dry DMF gave the protected alkene (**207**) in a very good yield of 95 % (**Scheme 51**).

The Simmons-Smith reaction is the most widely used method for the stereoselective cyclopropanation of olefins[195,196] and its application to asymmetric reaction has been well studied. Finally, the alkene (**207**) was cyclopropanated according to the method of Taguchi *et al.*[192] which used this reaction. Reaction of the alkene (**207**) with diethyl zinc (3 mol eq.) and diiodomethane (6 mol eq.) in 1,2-dichloroethane at – 28 to 0 ^{o}C for 4 hours gave the single diastereomer of cyclopropane (**219**) in 82 % yield as shown in **Scheme 51**. The diastereomeric excess of the cyclopropanated product (**219**) was determined as ≅ 100 %.[192] The ^{1}H and ^{13}C NMR spectrum of the cyclopropanated product showed only a single diastereomer. The detail of the NMR spectra of this compound were as in the literature.[192,193]

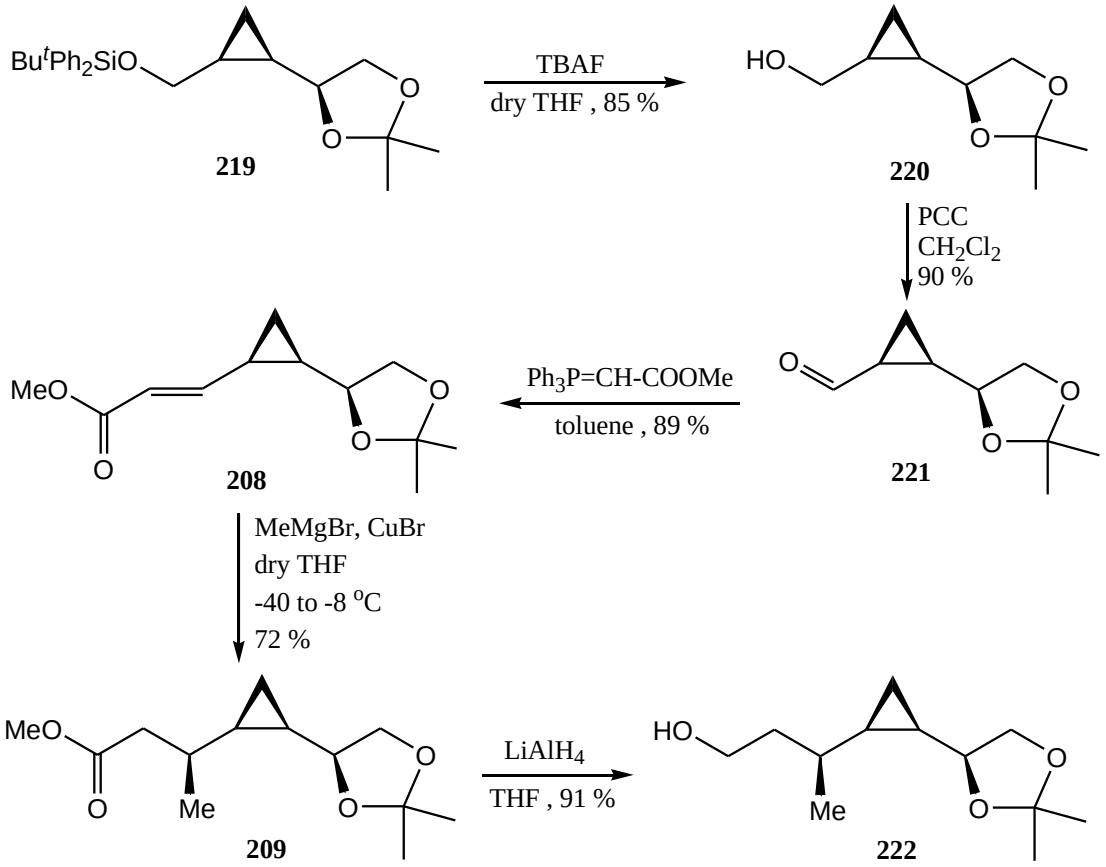


Scheme 51: ***Preparation of the single enantiomer of the cyclopropane***

2.3.2 Preparation of the α-methyl cyclopropane

The *tert*-butyldiphenylsilane group of (**219**) was desilylated to the corresponding alcohol (**220**) with tetra-n-butylammonium fluoride. TBAF was added to a stirred solution of the protected compound (**219**) in dry THF and stirred 18 hours to give the alcohol (**220**) in 85 % yield.[138] The alcohol was oxidised to the corresponding aldehyde (**221**) with PCC as previously described method, and treated with methyl (triphenyl-phosphoranylidene)acetate[194] in toluene at room temperature for 18 hours to give a mixture of alkene *E*- and *Z*-isomers in a ratio 2:1.[138]

Michael addition of a methyl to this α,β-unsaturated ester is a crucially important step to prepare the α-methyl *trans*-cyclopropane unit of the mycolic acid. A study showed that addition of methyl magnesium bromide in the presence of copper bromide to the *E*-isomer (**208**) led to a single alkylated product (**209**). A trace of a minor compound, probably the isomer with the opposite methyl group stereochemistry, was also observed (1:15). Moreover, when the mixture of the *E*- and *Z*-alkene was used the same product (**209**) was obtained (**Scheme 52**).[138]

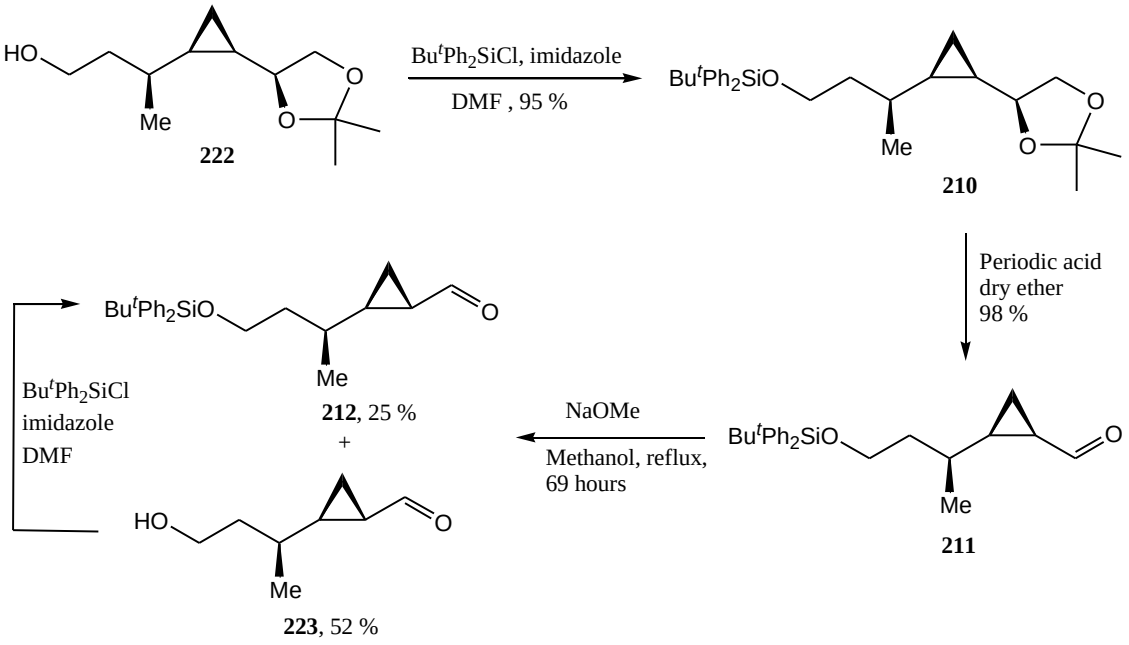


Scheme 52: ***Preparation of the α-methyl cyclopropane***

Methyl magnesium bromide (3 mol eq.) was added to a suspension of copper bromide (1.5 mol eq.) in dry THF at – 40 ^{o}C and stirred for 30 minutes. The α,β-unsaturated ester (**208**) in *E*- and *Z*-isomer was added and stirred to reach the temperature to – 8 ^{o}C for two hour, and worked up to give the desired methylated product (**209**). The ester of (**209**) was reduced to corresponding alcohol (**222**) with $LiAlH_4$. The absolute configuration of this alcohol (**222**) was confirmed by X-ray crystallography of the corresponding 3,5-dinitrobenzoate, based on the known configuration of (**219**). NMR spectrum and specific rotation of all these compounds were in agreement with the literature values.[138]

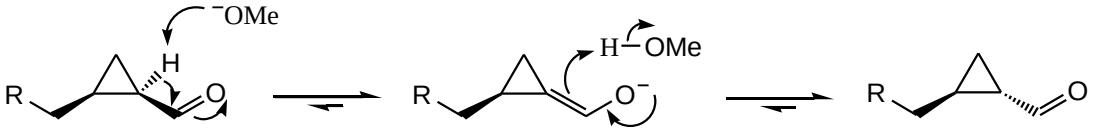
2.3.3 Preparation of α-methyl *trans*-cyclopropane aldehyde

The primary alcohol was protected as a silyl ether using *tert*-butyldiphenylchlorosilane to give the product (**210**), because the silyl ether is stable against acids and the next step needs acid for the conversion of the acetal group into an aldehyde. Periodic acid (2.5 mol eq.) was added to a stirred solution of the compound (**210**) in dry ether and stirred at room temperature for 17 hours to effect both removal of the isopropylidene group and oxidative cleavage of the resultant diol to provide the cyclopropane *cis*-aldehyde (**211**) in excellent yield (**Scheme 53**).[193]



Scheme 53: ***Preparation of α-methyl trans-cyclopropane aldehyde***

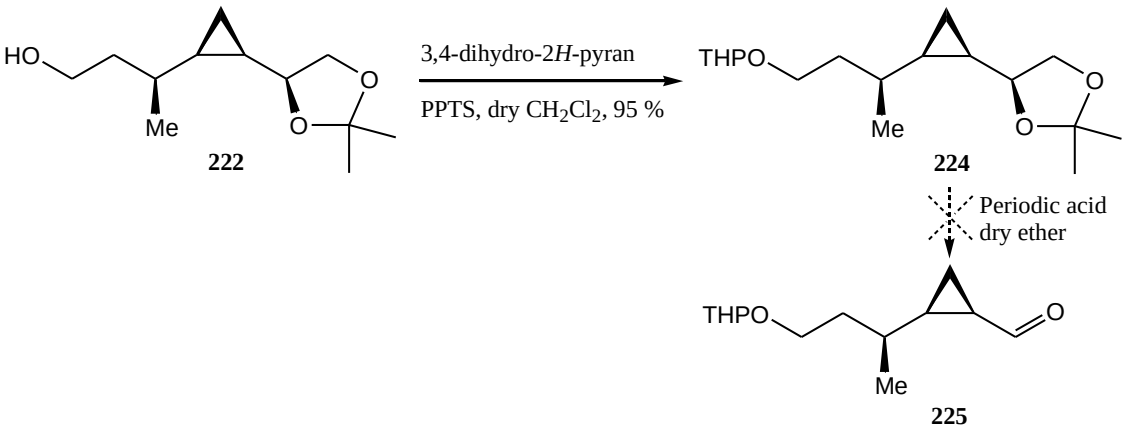
The *trans*-cyclopropane aldehyde (**212**) was formed from the *cis*-cyclopropane aldehyde (**211**) by epimerisation. The *cis*-aldehyde was refluxed with NaOMe (1.1 mol eq.) in dilute methanol for 69 hours to form the thermodynamically more favoured *trans*-compounds (**212**) in 25 % yield and (**223**) in 52 % yield (**Scheme 53**), by the following mechanism (**Scheme 54**):



Scheme 54: ***The mechanism of the epimerisation reaction***

The alcohol (**223**) was silylated to desired *trans*-aldehyde (**212**) in 60 % yield. The overall yield of this epimerisation was 56 % which after eleven steps have been completed is quite low. Therefore, another attempt has been made for the formation of the *trans*-aldehyde. The alcohol (**222**) was protected with 3,4-dihydro-2*H*-pyran in dry dichloromethane to give the protected compound (**224**) in 95 % yield (**Scheme 55**). However, removal of the acetal group and oxidative cleavage of the resultant diol, retaining the protected THP group to form the *cis*-aldehyde (**225**) with periodic acid did not succeed.

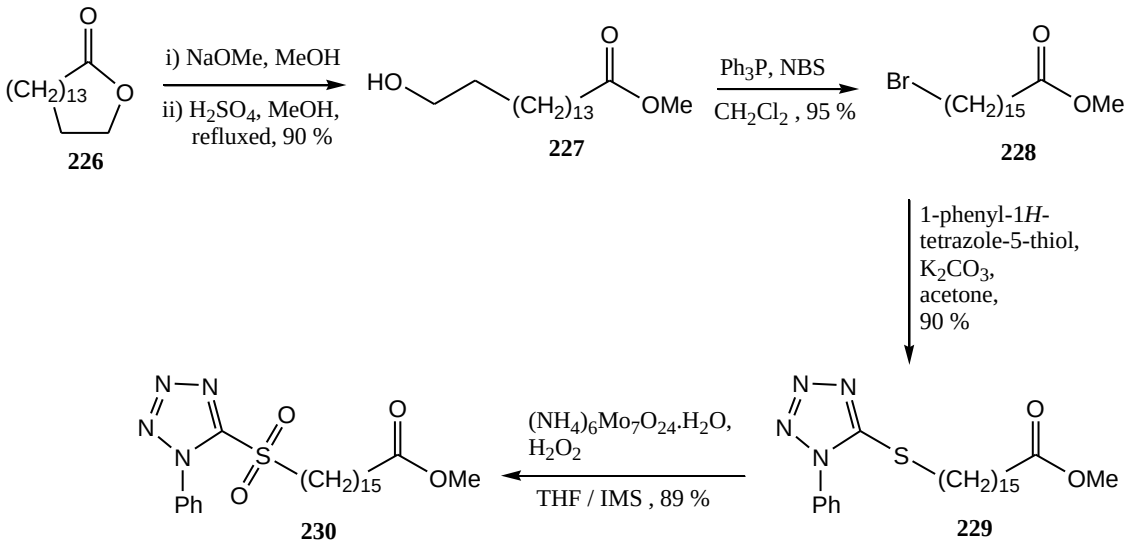
The aldehyde proton of the *cis*-aldehyde (**211**) appeared in the proton NMR spectrum as a doublet at δ 9.32 (*J* 6 Hz) and the *trans*-aldehyde (**212**) as a doublet at δ 9.00 (*J* 5.4 Hz)[89]. Moreover, the specific rotation of *cis*-aldehyde was + 3.7, and *trans*-aldehyde was + 22.9.



Scheme 55: ***An attempt to prepare THP protected cyclopropane aldehyde***

2.3.4 Preparation of a sulfone for side chain extension

The sulfone (**230**) was prepared for the side chain extension by a Julia reaction. 16-Hexadecanolide (**226**) was used as a starting material and reacted with NaOMe in methanol to give the product (**227**). The alcohol was brominated with NBS and PPh_3 in dichloromethane to give the bromo ester (**228**), followed by reaction with 1-phenyl-1*H*-tetrazole-5-thiol to form the sulfane (**229**). Finally, the sulfane was oxidised to the corresponding sulfone (**230**) with hydrogen peroxide in 89 % yield as shown in **Scheme 56**. All these reactions from the lactone to the sulfone were discussed in detail in the section **2.2.22** for the lower homologue.

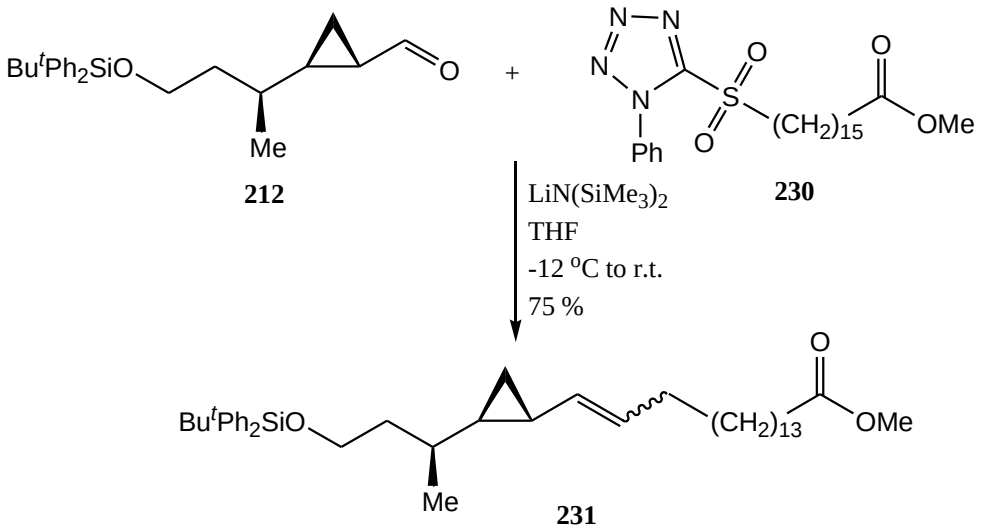


Scheme 56: ***Preparation of the sulfone (230)***

2.3.5 The Julia reaction

A coupling reaction between the *trans*-(1*S*,2*R*)-2-[(*S*)-3-(*tert*-butyl-diphenyl-silanyloxy)-1-methyl-propyl]-cyclopropanecarbaldehyde (**212**) and the 16-(1-phenyl-1*H*-tetrazole-5-sulfonyl)-hexadecanoic acid methyl ester (**230**) using the Julia reaction gave an alkene (**231**) in a 75 % yield. The product was a mixture of *E*- and *Z*-isomers in a ratio 3.7:1 (**Scheme 57**). The ^{1}H NMR spectrum showed a doublet of triplets at δ 5.35 (*J* 15.2, 6.6 Hz) and a doublet of doublets at δ 4.94 (*J* 15.2, 8.5 Hz) for the olefinic protons of the *E*-isomer, and a doublet of triplets at δ 5.24 (*J* 10.7, 7.3 Hz) and a broad triplet at δ 4.72 (*J* 10.7 Hz) for the olefinic protons of the *Z*-isomer. The

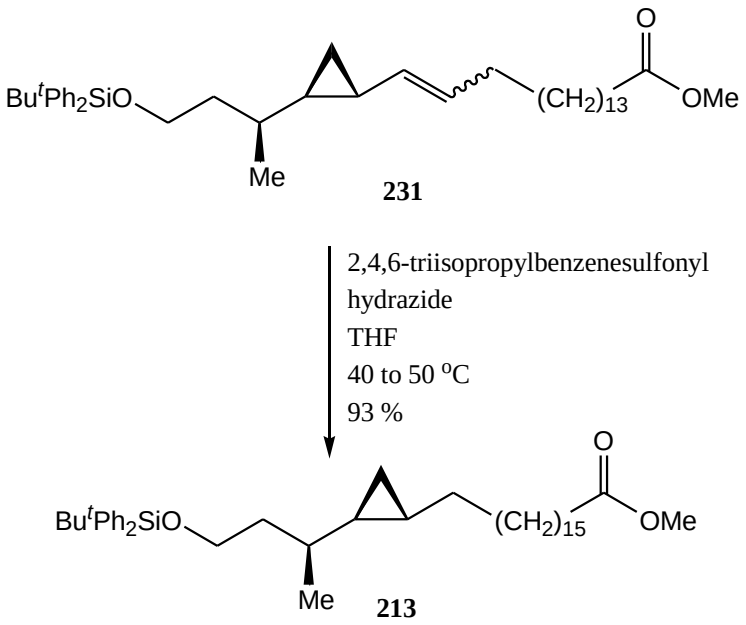
protected group silyl ether appeared a multiplet at δ 7.68–7.66 (4H) and 7.44–7.35 (6Hm) for the phenyl protons and a singlet at δ 0.95 for the *tert*-butyl group protons.



Scheme 57: ***A Julia reaction***

2.3.6 Hydrogenation of the olefin

Catalytic hydrogenation of the product of the Julia reactions using palladium on carbon and hydrogen could result in hydrogenation of the *trans*-cyclopropane as well as the alkene as discussed in section **2.2.20** for the *cis*-cyclopropane. A milder method of hydrogenation, di-imide was used. The olefin (**231**) was dissolved in THF and the TPBSH (4 mol eq.) was added and stirred at 40 ^{o}C for 18 hours. Further TPBSH (1.6 mol eq.) was added and stirred at 50 ^{o}C for another 18 hours and work up gave a hydrogenated product as a colourless oil in 93 % yield (**Scheme 58**). The NMR spectrum of (**213**) showed no olefinic signals. The *trans*-cyclopropane protons in the proton NMR spectrum appeared as multiplets at δ 0.47–0.40 (1H) and 0.18–0.08 (3H). The *trans*-cyclopropane signals in the carbon NMR spectrum appeared at δ 26.9 for the carbon the long chain methyl ester side, 18.6 for the carbon the α-methyl side, and 10.5 for the -CH_2-. The long chain carbons signals appeared around at δ 29.

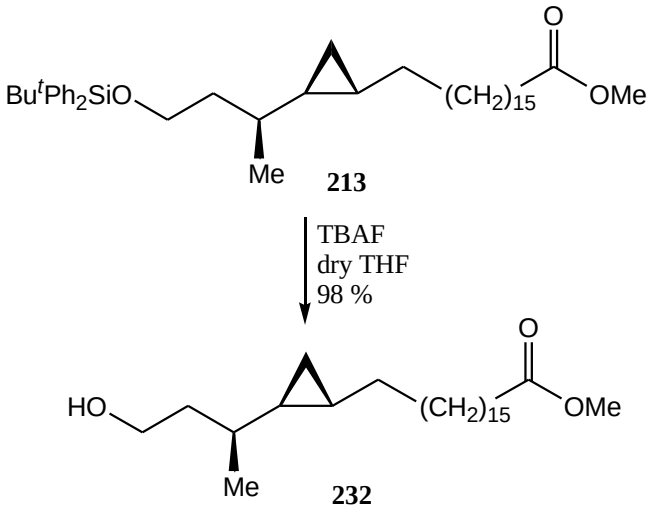


Scheme 58: ***Hydrogenation of the olefin with TPBSH***

2.3.7 Desilylation

The sulfone (**214**) had to be prepared for the coupling reaction of the α-methyl side of the *trans*-cyclopropane with the long chain. First of all, the *tert*-butyldiphenylsilyl group of the compound (**213**) was desilylated to the corresponding alcohol (**232**). Tetra-n-butylammonium fluoride (1.3 mol eq.) was added to a stirred solution of the protected compound (**213**) and stirred at room temperature for 18 hours and work up gave a white solid alcohol (**232**) in 98 % yield (**Scheme 59**). Physically, the product became a solid instead of the oily starting material, due to the hydrogen bonding of the alcohol.

The protected silyl ether group clearly disappeared on the NMR spectrum and the ^{1}H NMR spectrum of (**232**) showed a multiplet at δ 3.79–3.69 for the two protons adjacent to the hydroxyl group, and a doublet at δ 0.96 (*J* 6.6 Hz) for the methyl protons at the α position to the *trans*-cyclopropane. The ^{13}C NMR spectrum showed a signal at δ 61.4 for the carbon next to hydroxyl group and a signal at δ 19.8 for the methyl carbon α to the ring. The IR spectrum showed a broad peak at 3386 cm^{-1} for the O–H stretch. The optical rotation was $[\alpha]_D^{21}$ = + 13.5 (*c* 0.99, $CHCl_3$). The NMR spectra and optical rotation showed that no epimerisation had happened.

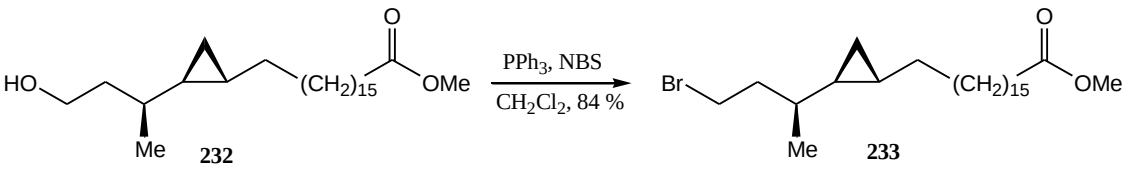


Scheme 59: ***The desilylation of the silyl ether***

2.3.8 Bromination of the alcohol

The alcohol (**232**) was brominated to give the bromo product (**233**) for the preparation the sulfane (**234**) (**Scheme 61**). *N*-bromosuccinimide (1.27 mol. eq.) was added to a stirred solution of the alcohol and triphenylphosphine in dichloromethane (1.15 mol eq.) at 0 to 4 ^{o}C and stirred at room temperature. The reaction was monitored by TLC, where the product was obvious, having a much large R_f value than the alcohol. After one hour the reaction was worked up to give a white solid in 84 % yield as shown in **Scheme 60**.

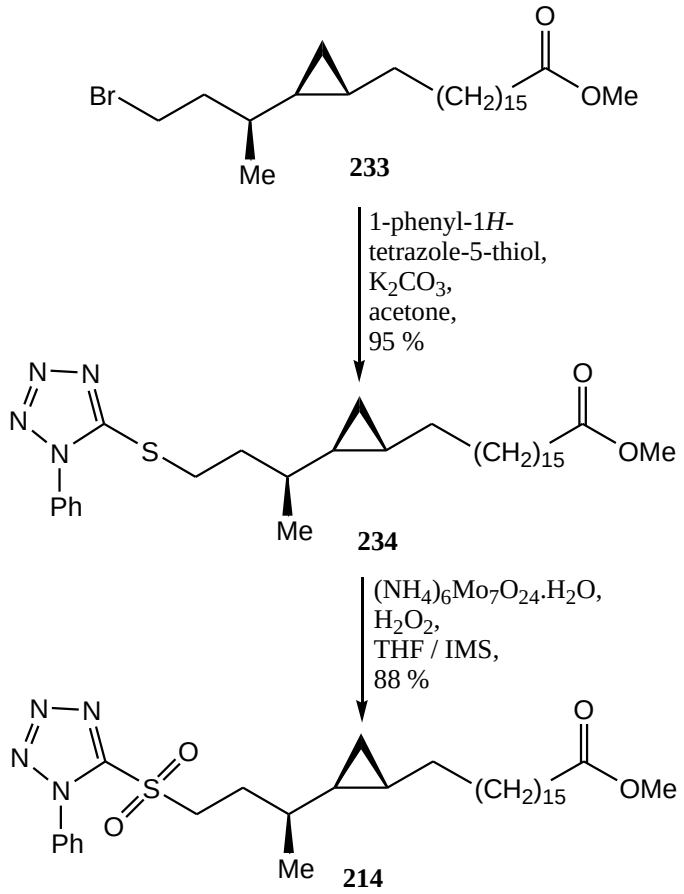
The methylene-bromide group protons appeared as a multiplet on the proton NMR spectrum, albeit shifted slightly upfield to δ 3.56–3.46, whereas the alcohol showed a multiplet at δ 3.79–3.69. The methylene-bromide carbon on the carbon NMR spectrum shifted more upfield to δ 32.5, whereas the alcohol showed a signal at δ 61.4.



Scheme 60: ***Bromination of the alcohol***

2.3.9 Preparation of the sulfone

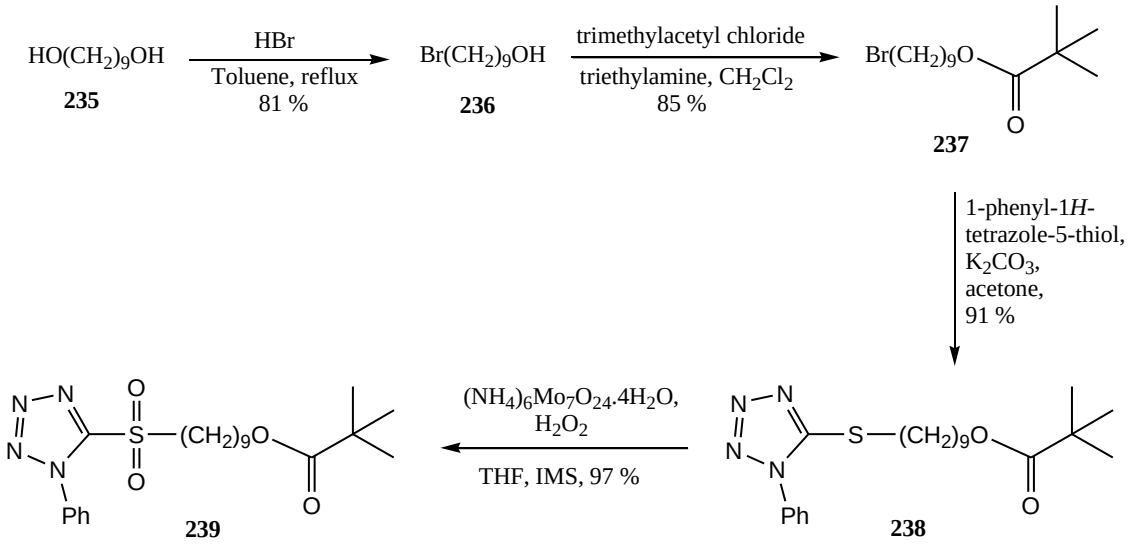
The bromo-compound (**233**) was treated with 1-phenyl-1*H*-tetrazole-5-thiol (1 mol eq.) in the presence of potassium carbonate in acetone at room temperature for 18 hours. After work up, the sulfane (**234**) was obtained in a very good yield (95 %). The ^{1}H NMR spectrum of the sulfane (**234**) showed a multiplet at δ 7.60–7.54 for the aromatic protons, and a multiplet at δ 3.53–3.44 for the two protons next to the S atom. The subsequent oxidation of the sulfane with hydrogen peroxide in the presence of ammonium molybdate tetrahydrate gave the desired sulfone (**214**) as shown in **Scheme 61**. The chain carbon bonded to the sulfonyl group (**214**) appeared on the carbon NMR spectrum shifted downfield to δ 54.4, whereas that bonded to the sulfanyl group (**243**) appeared at δ 37.6. The specific rotation of the sulfane (**234**) was $[\alpha]_D^{25}$ = + 10.3 (*c* 1.18, $CHCl_3$), and it changed for the sulfone (**214**) to $[\alpha]_D^{26}$ = + 3.6 (*c* 1.04, $CHCl_3$).



Scheme 61: ***Preparation of the sulfone***

2.3.10 Preparation of C_9 sulfone

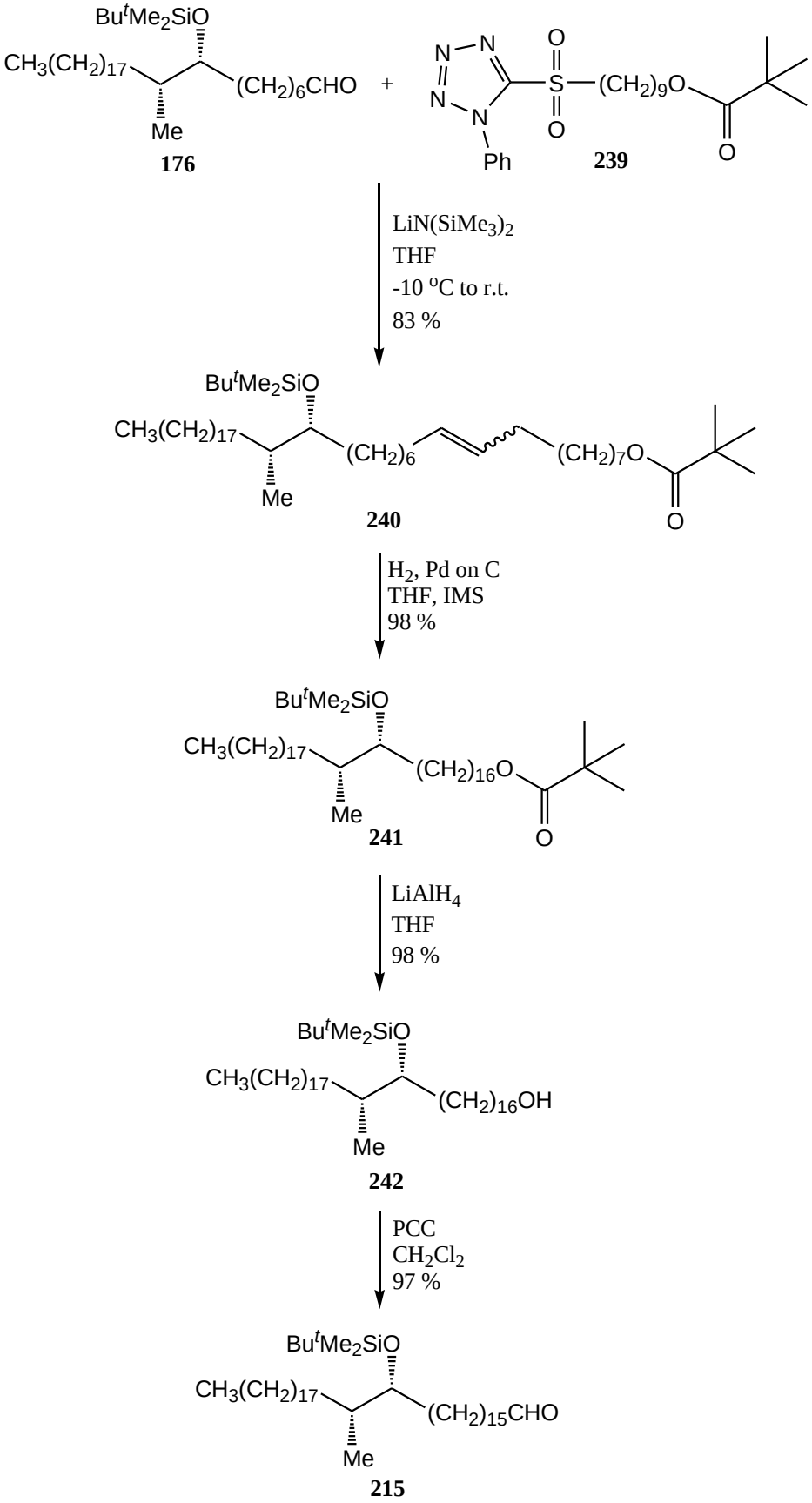
Nine more carbon were added to the side chain of aldehyde (**176**) (**Scheme 27**) for the preparation of the meromycolate chain of the α-methyl *trans*-cyclopropane by a Julia reaction. Therefore, the sulfone (**239**) was prepared starting from 1,9-nonanediol. This was monobrominated to (**236**) with HBr by refluxing in toluene. The alcohol was protected with trimethylacetyl chloride, followed by reaction with 1-phenyl-1*H*-tetrazole-5-thiol to give the sulfane (**238**). Finally, oxidation of the sulfane with hydrogen peroxide gave the desired sulfone (**239**) (**Scheme 62**). The detail of these reactions was explained in section **2.2.11** for the homologous C_{10} sulfone.



Scheme 62: ***Preparation of the sulfone 239***

2.3.11 The chain extension

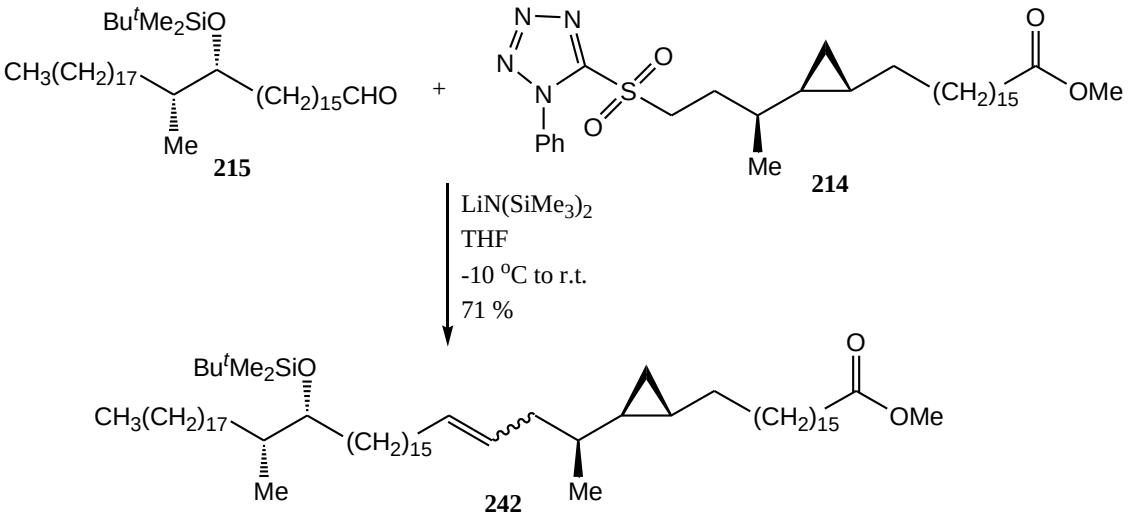
Reaction of the aldehyde (**176**) with sulfone (**239**) and base in a Julia reaction gave the alkene (**240**) as *E*- and *Z*-isomers in a ratio 2.1:1; hydrogenation with hydrogen gas and Pd on C as a catalyst led to the saturated product (**241**). Terminal deprotection with $LiAlH_4$ gave the primary alcohol (**242**) which was oxidised with PCC to the corresponding aldehyde (**215**) (**Scheme 63**). Details of these reactions were again explained for the homologue in section **2.2.12**. This aldehyde (**215**) was used for a coupling reaction with the α-methyl *trans*-cyclopropane sulfone (**214**).



Scheme 63: ***A chain extension***

2.3.12 Final Julia reaction for the *trans*-cyclopropane meromycolaldehyde

A Julia reaction of the aldehyde (**215**) with 17-{(1*S*,2*R*)-2-[(*S*)-1-methyl-3-(1-phenyl-1*H*-tetrazole-5-sulfonyl)-propyl]-cyclopropyl}-heptadecanoic acid methyl ester (**214**) using lithium bis(trimethylsilyl) amide as base gave a final coupled alkene product (**242**) as a mixture of *E*- and *Z*-stereoisomers in a ratio 2:1 in 71 % yield (**Scheme 64**). The ^{1}H NMR spectrum showed a multiplet at 5.45–5.37 for the two protons of both isomers. However, it was easy to see both isomers in the ^{13}C NMR spectrum in which there were four signals at 131.4, 130.4, 128.9 and 128.4.

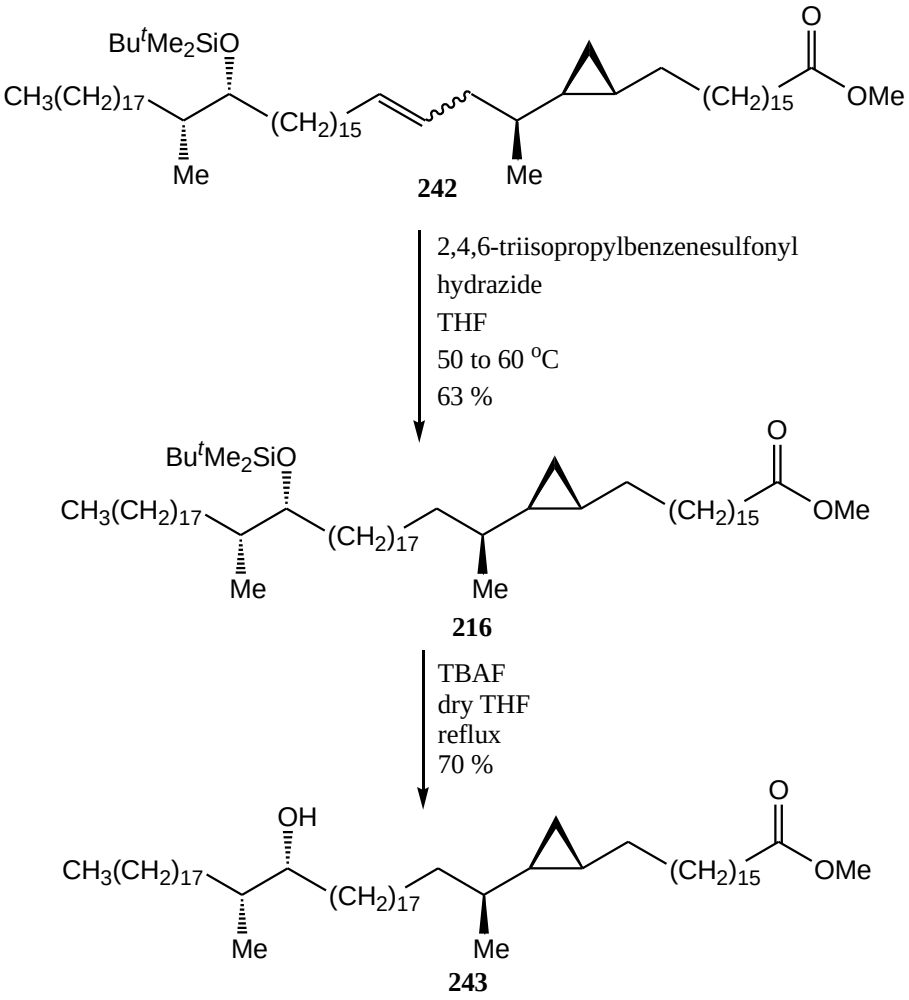


Scheme 64: ***A coupling reaction based on the Julia reaction***

2.3.13 Hydrogenation and Desilylation

The alkene (**242**) was hydrogenated with di-imide using TPBSH. The alkene was dissolved in THF and TPBSH (4 mol eq.) was added and stirred at 40 °C for 18 hours, further TPBSH (1.6 mol eq.) was added and stirred at 50 °C for 23 hours, but NMR showed that there was still alkene present. Further TPBSH (1.6 mol eq.) was added and stirred at 60 °C another 23 hours and the mixture was worked up to give the saturated product (**216**) in 63 % yield (**Scheme 65**). The yield of this method was not particularly high; there appeared to be a balance between the amount of TPBSH and the reaction temperature to give the highest yield. The NMR spectrum showed that there were no signals in the olefinic region.

Another difficult reaction was the deprotection of the secondary silyl ether. TBAF was used as a deprotection agent, and added to a stirred solution of the silyl ether (**216**) in THF and refluxed for 18 hours. Further TBAF was added and refluxed another 23 hours after which the NMR spectrum showed that deprotection was almost complete. Work up gave a white solid, the secondary alcohol (**243**) in 70 % yield as shown in **Scheme 65**. The ^{1}H NMR spectrum of (**243**) showed no signals for the protected silyl group and a singlet at δ 3.67 for the methyl protons of the methyl ester. The proton adjacent to secondary alcohol appeared as a multiplet at δ 3.51–3.49 and the two protons next to methyl ester appeared as a triplet at δ 2.31 (*J* 7.6 Hz). The IR spectrum showed a peak at 3521 cm^{-1} for the O–H stretch.

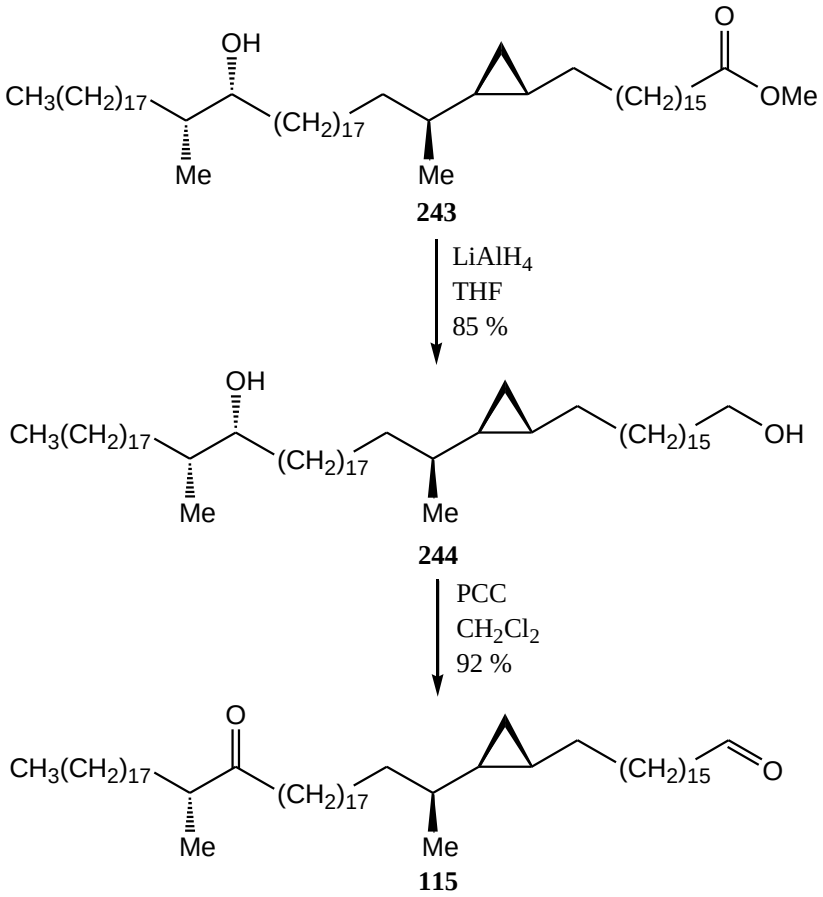


Scheme 65: ***Hydrogenation and desilylation***

2.3.14 Reduction and oxidation

The methyl ester of compound (**243**) was reduced to the corresponding alcohol (**244**) with $LiAlH_4$ (**Scheme 66**). The product was a white solid and was not soluble in chloroform at room temperature. It was gently heated to dissolve for the preparation of an NMR sample and measurement of the specific rotation. The proton NMR spectrum showed a triplet at δ 3.64 (*J* 6.6 Hz) for two protons next to the primary alcohol; the carbon NMR spectrum showed two signals at δ 75.2 for the carbon bonded to the secondary alcohol, and 63.0 for the carbon bonded to the primary alcohol.

Finally, the diol (**243**) was oxidised to the target α-methyl *trans*-cyclopropane meromycolaldehyde (**115**) with PCC. The reaction temperature was kept below 40 ^{o}C to dissolve the diol and, after work up, column chromatography produced pure (**115**) as a white solid in 92 % yield. The molecular rotation was – 16.0 which was lower than the *cis*-cyclopropane meromycolaldehyde (**114**) (– 40.5). The NMR spectrum of the α-methyl *trans*-cyclopropane unit is tabulated in **Table 4** and the appearance of the cyclopropane ring protons and the proton next to methyl was as shown in **Figure 25**.



Scheme 66: ***Preparation of the target α-methyl trans-cyclopropane meromycolaldehyde***

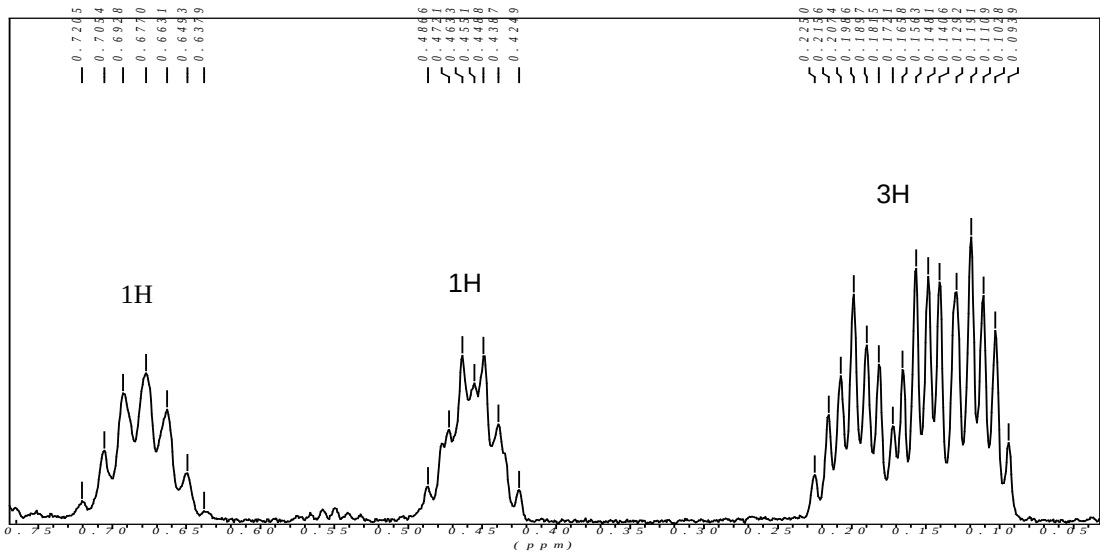
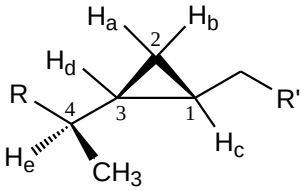


Figure 25: ***Appearance of the trans-cyclopropane ring on the proton NMR spectrum***

Table 4: ***The NMR data of the α-methyl trans-cyclopropane unit***

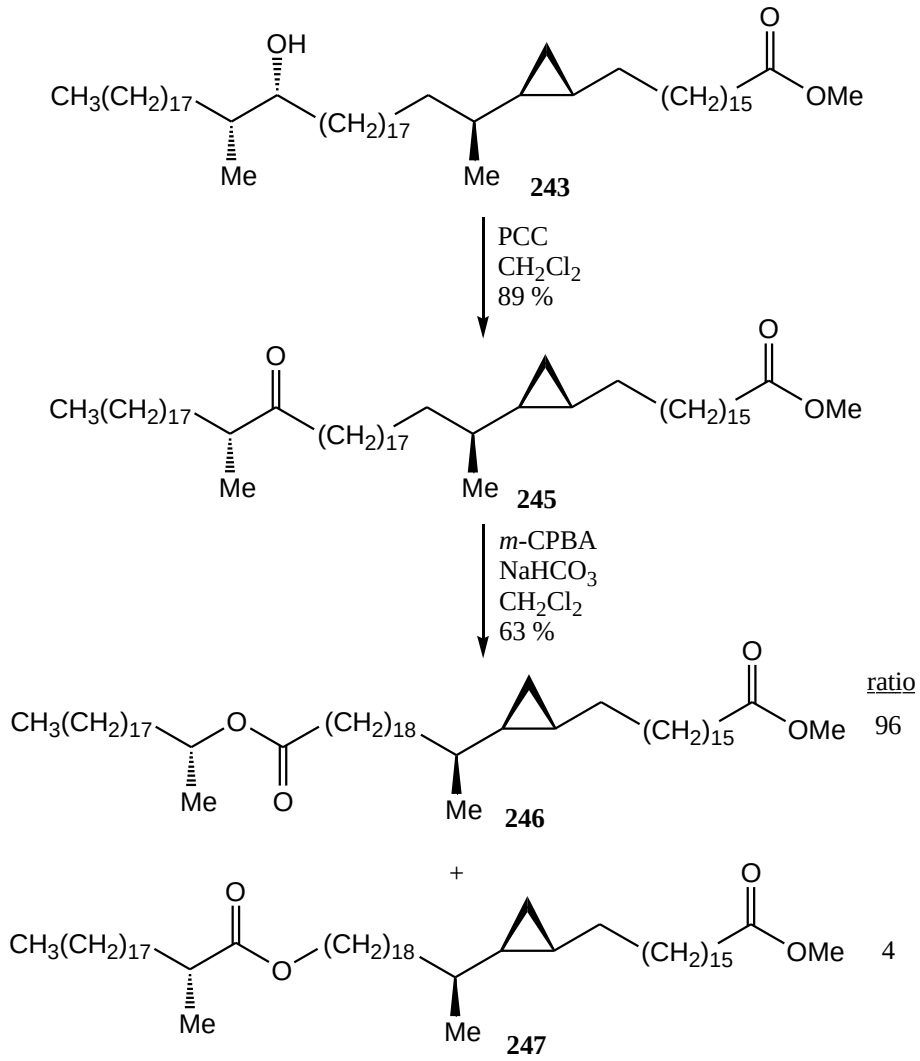


Proton	δ	Multiplicity	Carbon	^{13}C NMR
H_a and H_b	0.17-0.09	m	1	26.2
H_c	0.23-0.18	m	2	10.5
H_d	0.49-0.43	m	3	18.6
H_e	0.72-0.65	m	4	38.1
CH_3	0.91	d (*J* 6.6)	CH_3	19.7

2.3.15 Baeyer-Villiger oxidation

The absolute stereochemistry of the α-methyl adjacent to the ketone in a keto-mycolic acid has not been determined by an experimental method yet. Therefore experiments were done to find out the stereochemistry the methyl next to ketone. Baeyer-Villiger oxidation of a ketone gives an ester and saponification of this ester gives an alcohol and a carboxylic acid, importantly during these reactions stereochemistry of chiral centres remain.[197,198] Applying this method to both the synthetic and natural keto-mycolic acid and comparison of the specific rotations of the α-methyl alcohol would determine the absolute stereochemistry.

The secondary alcohol (**243**) was oxidised to the ketone (**245**) with PCC in dichloromethane in 89 % yield (**Scheme 67**), followed by oxidation with *m*-chloroperbenzoic acid. The best method found was that described by White *et al*.[199]



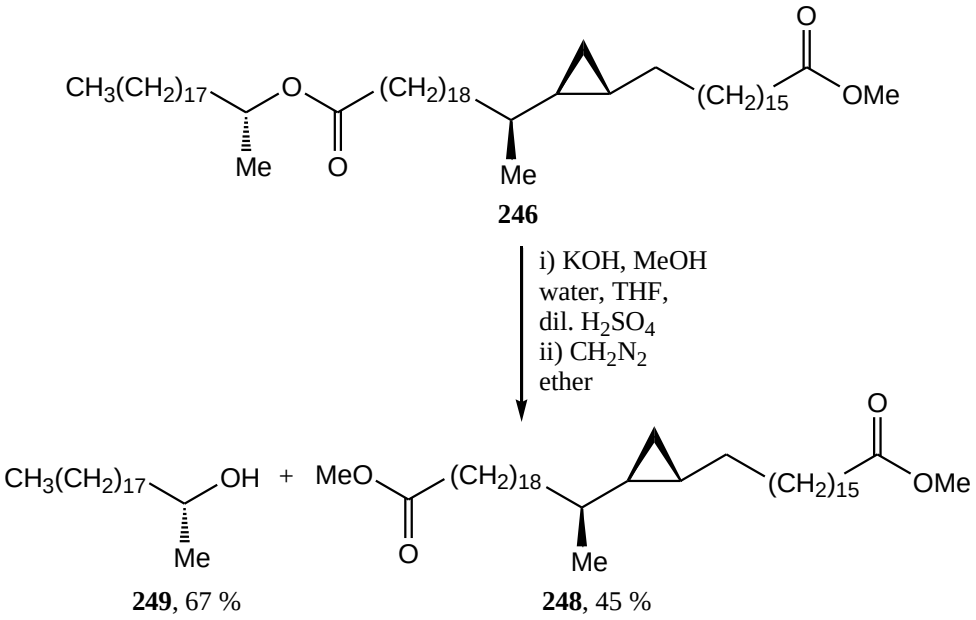
Scheme 67: The Baeyer-Villiger oxidation

The ketone (**245**) was treated with *m*-chloroperbenzoic acid (3 mol eq.) and sodium hydrogen carbonate (4.7 mol eq.) in dry dichloromethane at room temperature for 4 days and refluxed at 45 ^{o}C for 24 hours. The mixture was worked up and purified by column chromatography to give the esters (**246**) and (**247**) in a ratio 96:4 as determined by proton NMR (**Scheme 67**). There was 20 % the starting ketone, but that was not a problem in continuing to the next step. The observation of a downfield signal of (**246**) as a sextet at δ 4.91 (*J* 6.0 Hz) in the ^{1}H NMR spectrum for the proton in the α-position confirmed the formation of the ester, and there was a very weak signal at δ 4.06 for the other isomer, ester (**247**). The ^{13}C NMR spectrum showed two signals at δ 174.3 and 173.6 for the two carbonyl carbons and two signals at δ 70.7 for the α carbon and 51.4 for the methyl carbon of the methyl ester. There were no clear signals for the minor isomer (**247**).

2.3.16 Hydrolysis of the ester

The ester (**246**) was added to a stirred solution of potassium hydroxide (15 mol eq.) in a mixture of THF (7.5 ml), methanol (7.5 ml) and water (1 ml) and refluxed at 75 ^{o}C for 4 hours. The mixture was cooled down, filtered through a sinter funnel and washed with ether. The liquid layer was washed with water, dried, the solvent evaporated and the residue purified by column chromatography to give the (*R*)-icosan-2-ol (**249**) as a white solid in 67 % yield. The precipitate was dissolved in hot water and diluted with a mixture of petrol and ethyl acetate, then acidified with dil. H_2SO_4 and worked up. Re-esterification of the carboxylic with excess of diazomethane in ether gave (*S*)-20-[(1*R*,2*S*)-2-(16-methoxycarbonyl-hexadecyl)-cyclopropyl]-henicosanoic acid methyl ester (**248**) in 45 % yield (**Scheme 68**).

The ^{1}H NMR spectrum of (*R*)-icosan-2-ol (**249**) showed a sextet at δ 3.80 (*J* 6.3 Hz) for the proton at the α position, a doublet at δ 1.20 (*J* 6.0 Hz) for the α-methyl, and a triplet at δ 0.89 (*J* 6.6 Hz) for the terminal methyl. The specific rotation of the alcohol was $[\alpha]_D^{23} = -3.8$ (*c* 0.47, $CHCl_3$) and literature value also was – 3.8.[200] The ^{1}H NMR spectrum of the wax-ester (**248**) showed a singlet at δ 3.67 for the methyl protons of the esters, a doublet at δ 0.90 (*J* 6.6 Hz) for the methyl protons α to the cyclopropane ring and multiplets at δ 0.48–0.42 and 0.22–0.09 for the cyclopropane ring protons.



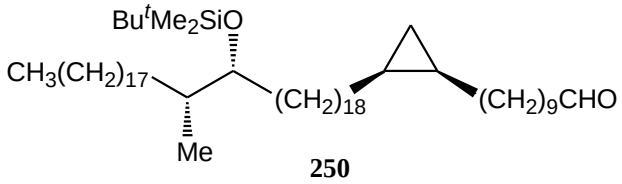
Scheme 68: ***The saponification reaction***

The specific rotation of the synthetic wax-ester (**248**) was $[\alpha]_D^{23} = +3.3$ (*c* 0.6, $CHCl_3$) and the literature value of a mixture of homologous natural wax-esters (**39**) was + 3.7.[89] The comparison of the specific rotation of the alcohol (**249**) with the literature established both that no epimerization had occurred in oxidation of the secondary alcohol to produce the ketone and that, at least in this model, the supposed enzymatic Baeyer-Villiger oxidation could be reproduced chemically and occurred with the retention of stereochemistry at the migrating centre. (*S*)-Eicosan-2-ol was isolated from saponification of the natural wax-ester by Pangborn and Anderson[201] for which a specific rotation was measured as + 3.5, and after further re-crystallisation the rotation was + 4.2. Considering of these results, the stereochemistry of the methyl α to ketone of the natural keto-mycolic acid should be (*S*) and the enzymatic oxidation of natural keto-mycolic acid also occurs with retention of stereochemistry.

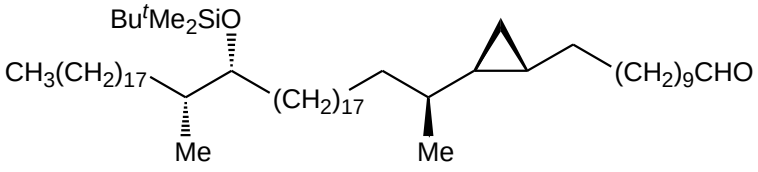
The oxidation of a natural keto-mycolic acid was examined using the method described above. The reaction mixture was refluxed 4 days. However, the proton NMR spectrum showed that the desired ester formed in very small yield and it was not enough to continue hydrolysis. This reaction might work on a natural meromycolic acid, but this remains to be tested.

2.4 The synthesis of intermediates for keto-mycolic acids

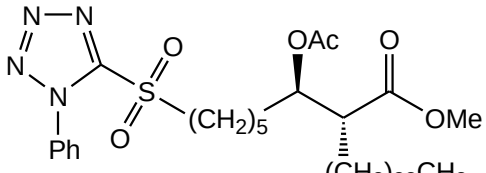
The main purpose of this project was the synthesis of *cis*-cyclopropane and α-methyl *trans*-cyclopropane keto-mycolic acids from *M. tuberculosis*. For the synthesis of these two mycolic acids (**116** and **117**) (p 34), the intermediate aldehydes (**250**) for the *cis*-cyclopropane and (**251**) for the α-methyl *trans*-cyclopropane were prepared and coupled respectively with the intermediate sulfone corynomycolate moiety (**252**) (**Scheme 69**). Detail of the preparation of these intermediates will be discussed in the next steps.



250



251

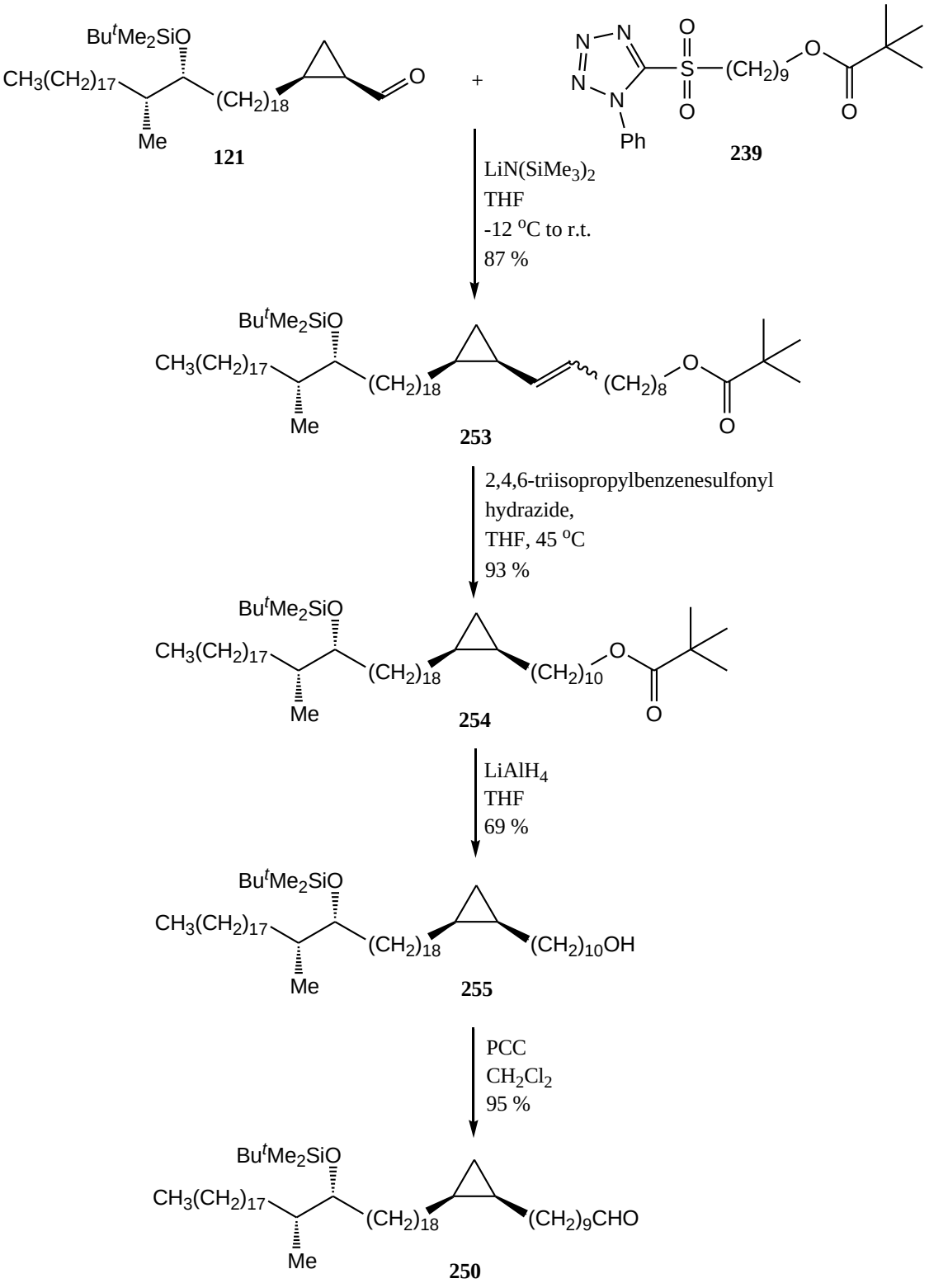


252

Scheme 69: ***Intermediates for the synthesis of keto-mycolic acids***

2.4.1 Preparation of the *cis*-cyclopropane intermediate

The aldehyde (**121**) (p 67) was coupled with the sulfone (**239**) (p 86) in THF using lithium bis(trimethylsilyl) amide as base to give the alkene (**253**) as a mixture of *E*- and *Z*-stereoisomers in a ratio 5.9:1 in 87 % yield (**Scheme 70**). The ^{1}H NMR spectrum showed a doublet of triplets at δ 5.52 (*J* 6.6, 15.4 Hz) and a doublet of doublets at δ 5.18 (*J* 8.5, 15.2 Hz) for the *E*-isomer and a doublet of triplets at δ 5.40 (*J* 7.3, 11.1 Hz) and a multiplet at δ 5.06–5.02 for the *Z*-isomer.



Scheme 70: ***The preparation of the intermediate (250)***

The alkene (**253**) was hydrogenated with TPBSH. A mixture of alkene and TPBSH (3.5 mol eq) in THF was stirred at 45 ^{o}C for 24 hours and further TPBSH (1.2 mol eq.)

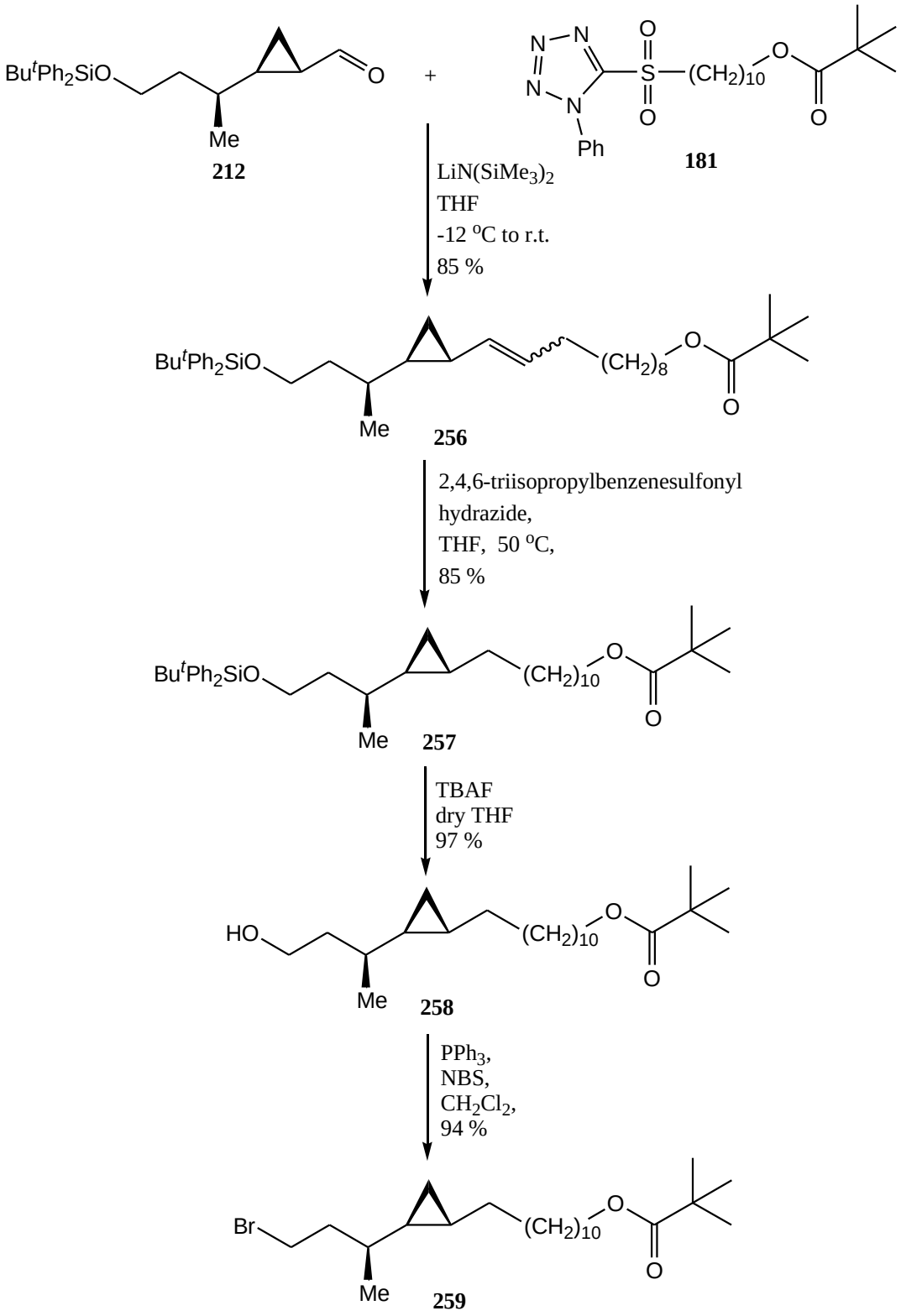
was added and stirred for another 24 hours. The mixture was worked up and purified to give a saturated oil (**254**) in a 93 % yield. The NMR spectra showed that all the functional groups, cyclopropane ring and protected silyl ether and pivalate, remained.
The pivalate group was deprotected with $LiAlH_4$ to give the alcohol (**255**). The proton NMR spectrum showed a triplet at δ 3.65 (*J* 6.6 Hz) for the protons adjacent to the hydroxyl group and the carbon NMR spectrum showed a signal at δ 63.1 for the carbon next to the hydroxyl group. The IR spectrum showed a broad peak at 3332 cm^{-1} for the O–H stretch.
Finally, the alcohol was oxidised to the desired aldehyde (**250**) with PCC. The aldehyde proton appeared as a triplet at δ 9.77 (*J* 1.9 Hz) in the proton NMR spectrum and the aldehyde carbon appeared at δ 202.9 in the carbon NMR spectrum. The molecular rotation was + 44.6.

2.4.2 Preparation of the α-methyl-*trans*-cyclopropane intermediate

Preparation of the intermediate aldehyde (**251**) can be summarised in three steps: synthesis of the bromo compound (**259**), conversion of it to the sulfone (**261**) and coupling of this sulfone with the aldehyde (**215**) to give the intermediate aldehyde (**251**).

2.4.2a Preparation of the bromo compound (259)

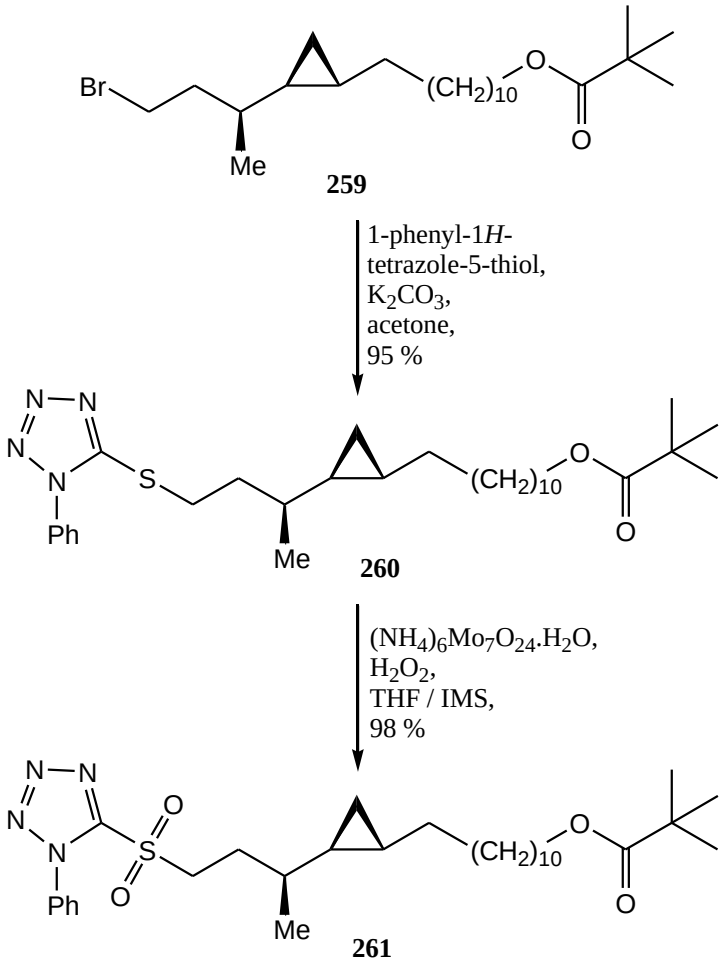
The α-methyl-*trans*-cyclopropane aldehyde (**212**) (p 79) was coupled with the sulfone (**181**) (p 55) using the Julia reaction to obtain an alkene (**256**) in 85 % yield as a mixture of *E*- and *Z*-isomers in a ratio 3.6:1 and then it was hydrogenated with TPBSH. A mixture of the alkene (**256**) and TPBSH (3.4 mol eq.) was stirred at 50 ^{o}C for 40 hours. There was still some olefin left; therefore the mixture was treated with potassium permanganate and columned to get rid of the olefin. The saturated compound (**257**) was treated with tetra n-butyl ammonium fluoride to desilylate the *tert*-butyldiphenylsilyl group to obtain the corresponding alcohol (**258**) in a 97 % yield. Finally, the alcohol was brominated to the bromo compound (**259**) with NBS in a 94 % yield as shown in **Scheme 71**. The NMR spectra of these compounds were similar to the compounds discussed in section **2.3.5** to **2.3.8** (p 81 – 84).



Scheme 71: ***Preparation of the intermediate (259)***

2.4.2b Preparation of the sulfone (261)

The bromo compound (**259**) was converted into the desired sulfone (**261**); it was first reacted with 1-phenyl-1*H*-tetrazole-5-thiol to obtain the sulfane (**260**) and then oxidised with hydrogen peroxide (**Scheme 72**); the details of similar reactions were discussed in section **2.3.9** (p 85). The protons next to the sulfonyl group in (**261**) appeared in the ^{1}H NMR spectrum as a doublet of doublets of doublets at δ 3.87 (*J* 5.1, 11.1, 14.2 Hz) and at δ 3.79 (*J* 5.4, 11.1, 14.5 Hz). However, the protons next to sulfanyl group in (**260**) appeared as a multiplet at δ 3.53–3.44. The ^{13}C NMR spectra showed a signal at δ 36.5 for the carbon next to sulfanyl group and a signal at δ 54.5 for the sulfonyl group. Also the specific rotation of the sulfane was $[\alpha]_D^{25}$ = + 11.7 (*c* 1.28, $CHCl_3$) and it was $[\alpha]_D^{22}$ = + 2.4 (*c* 1.24, $CHCl_3$) for the sulfone.



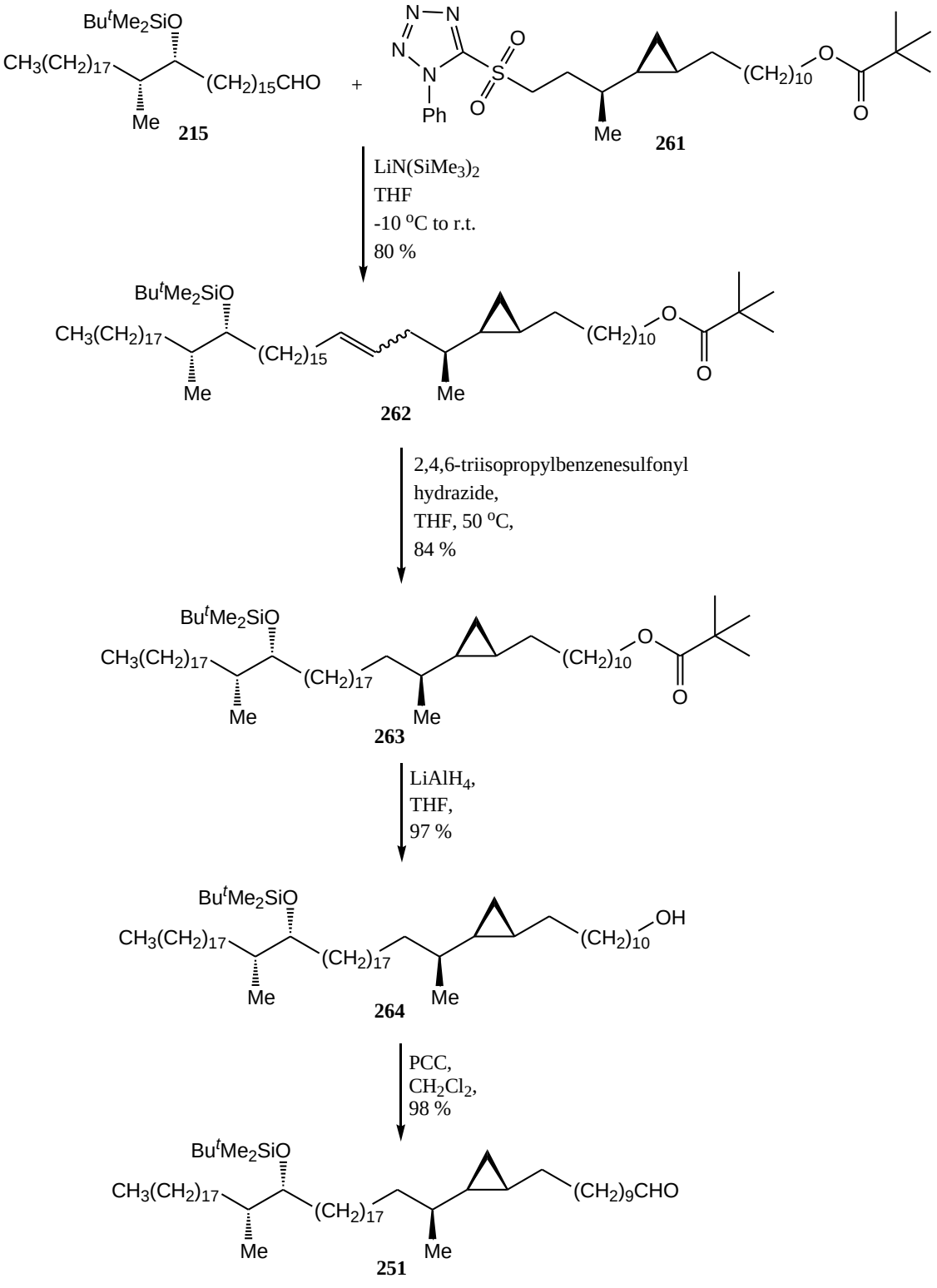
Scheme 72: ***Preparation of the sulfone (261)***

2.4.2c Preparation of the intermediate aldehyde (251)

The previously synthesised aldehyde (**215**) (p 87) was coupled with the sulfone (**261**) in a Julia reaction to give the alkene (**262**) as *E*- and *Z*-isomers in a ratio 3:1 with 80 % yield. The alkene protons appeared in the proton NMR spectrum as a multiplet at δ 5.45–5.37 and the alkene carbons appeared in the carbon NMR spectrum at δ 131.4 (*trans*), 130.4 (*cis*), 128.9 (*trans*) and 128.4 (*cis*). The alkene (**262**) was subsequently hydrogenated with TPBSH. A mixture of alkene and TPBSH (3.7 mol eq.) in THF was stirred at 50 ^{o}C for 40 hours, worked up and treated with potassium permanganate to separate any unsaturated compound by column chromatography. The saturated product (**263**) was obtained with 84 % yield (**Scheme 73**). The NMR spectra of (**263**) showed no signals in the olefinic region. The ^{1}H NMR spectrum showed a triplet at δ 4.05 (*J* 6.6 Hz) for the protons next to the pivalate group, a singlet at δ 1.21 for the protons of the *tert*-butyl on the pivalate group, and a singlet at δ 0.89 for the protons of the *tert*-butyl on the silyl protection group.

The pivalate group was deprotected with $LiAlH_4$ to give the corresponding alcohol (**264**) with 97 % yield (**Scheme 73**). The ^{1}H NMR spectrum showed no signal for the *tert*-butyl of the pivalate group and a triplet at δ 3.65 (*J* 6.6 Hz) for the protons adjacent to the hydroxyl group. The carbon adjacent to the hydroxyl group in the ^{13}C NMR spectrum appeared at δ 63.1.

Finally, the desired aldehyde (**251**), which was one of the final intermediates for the coupling reaction for the synthesis of the α-methyl-*trans*-cyclopropane keto-mycolic acid, was obtained by oxidation of the alcohol (**264**) with PCC in dichloromethane with 98 % yield as a colourless oil (**Scheme 73**). The ^{1}H NMR spectrum of the aldehyde (**251**) showed a triplet at δ 9.77 (*J* 1.9 Hz) for the aldehyde proton and a doublet of triplets at δ 2.42 (*J* 1.9, 7.3 Hz) for the protons adjacent to the aldehyde carbonyl group. The carbonyl carbon in the ^{13}C NMR spectrum appeared at δ 202.9. IR spectroscopy showed the distinctive C=O band of an aldehyde at 1731 cm^{-1}. The molecular rotation was + 53.7.



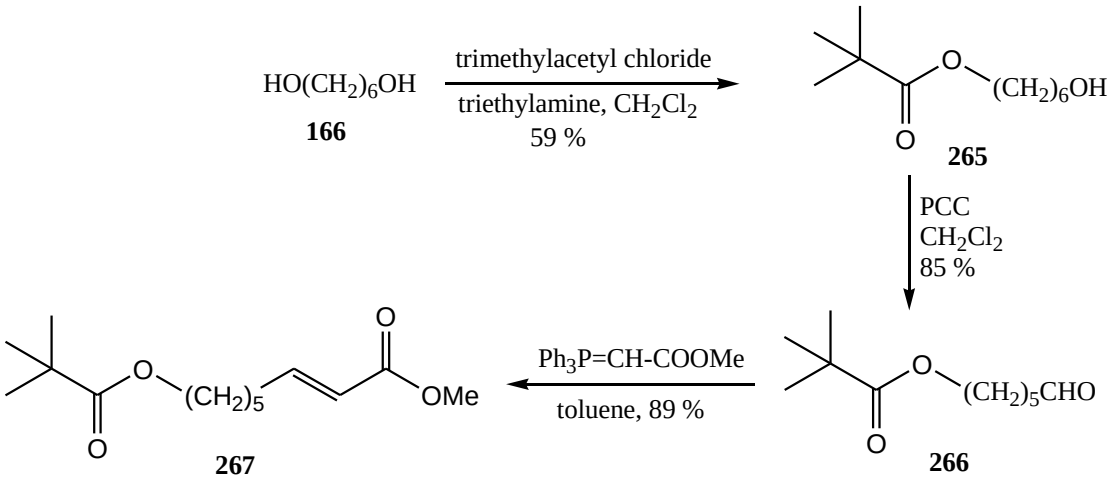
Scheme 73: ***Preparation of the intermediate aldehyde (251)***

2.4.3 Preparation of the intermediate corynomycolate moiety

Recently, two methods have been published for the synthesis of single enantiomers of the corynomycolate part of the mycolic acids. Basically, the methods were based on the preparation of a β-hydroxy methyl ester and alkylation of it at the α-position. The first one[139] was direct long chain alkylation which is not repeatable and reliable. The second one[144] was short chain allylation then extension to the desired chain, which is repeatable. In this work the second method was chosen. The preparation of the intermediate sulfone corynomycolate moiety (**252**), can be summarised as synthesis of α,β-unsaturated ester, conversion of it into β-hydroxy ester, alkylation and oxidation of it to the desired sulfone.

2.4.3a Preparation of the α,β-unsaturated ester

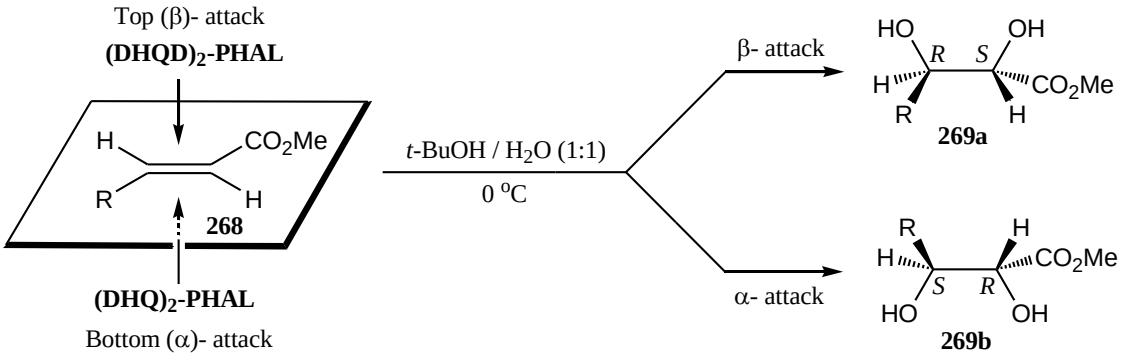
Reaction of 1,6-hexanediol (**166**) with trimethylacetyl chloride (1.1 mol eq.) gave the mono-protected 2,2-dimethyl-propionic acid 6-hydroxy-hexyl ester (**265**)[202] in a 59 % yield and some doubly protected compound. The alcohol was oxidised to the aldehyde (**266**) [203] with PCC and subsequently treated with (methoxycarbonyl-methylene) triphenylphosphorane in toluene at room temperature to give the Wittig product, α,β-unsaturated ester (**267**) mainly as the *E*-isomer (**Scheme 74**). The small amount of the *Z*-isomer was separated by column chromatography. The ^{1}H NMR spectrum showed a doublet of triplets at δ 6.96 (*J* 7.0, 15.8 Hz) and a doublet at δ 5.82 (*J* 15.8 Hz), the high coupling constant proving the formation of the *E*-isomer.



Scheme 74: ***Preparation of the α,β-unsaturated ester (267)***

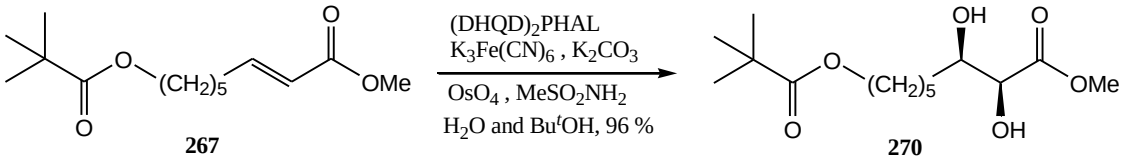
2.4.3b Sharpless dihydroxylation

Asymmetric dihydroxylations of olefins were widely studied by Sharpless and co-workers.[145,204,205] Catalytic of OsO_4 is added as oxidant, because the transformation is performed in the presence of another stoichiometric reoxidant such as potassium ferricyanide.[206] In order to obtain an enantioselective dihydroxylation, Sharpless *et al*. proposed the use of one of two diastereomeric ligands, either $(DHQD)_2PHAL$ or $(DHQ)_2PHAL$, which bond the osmium atom at the reactive species controlling the stereoselectivity of the reaction.[205] These PHAL derivatives have an ideal structure for catalysing the reaction, providing both high ligand acceleration and enantioselectivity.[205] Each of these dimers has a binding pocket that stabilises the transition state of the reaction but also forces the attack on the olefin onto only one of the two faces. In particular, $(DHQD)_2PHAL$ attacks from the top face of *trans*-alkenes (**268**) to give the (2*S*,3*R*)-dihydroxy compound (**269a**), while $(DHQ)_2PHAL$ attacks from the bottom face to produce the other enantiomer (**269b**) as shown in **Scheme 75**.



Scheme 75: ***The stereoselectivity of the dihydroxylation***

The dihydroxylation of the (*E*)-α,β-unsaturated ester (**267**) with osmium tetroxide (0.04 mol eq.) and potassium ferricyanide (3 mol eq.) as co-oxidant in the presence of $(DHQD)_2PHAL$ (0.01 mol eq.) as chiral ligand in a mixture of Bu^tOH and H_2O (1:1) at 2 ^oC gave the diol (**270**) in excellent yield 96 % (**Scheme 76**). Methanesulfonamide (1 mol eq.) was also added, since it favoured the hydrolysis of the osmium (VI) glycolate intermediate.[204] Without the $MeSO_2NH_2$, it would be necessary to increase the temperature which would ultimately produce a decrease in the stereoselectivity of the reaction.[207]



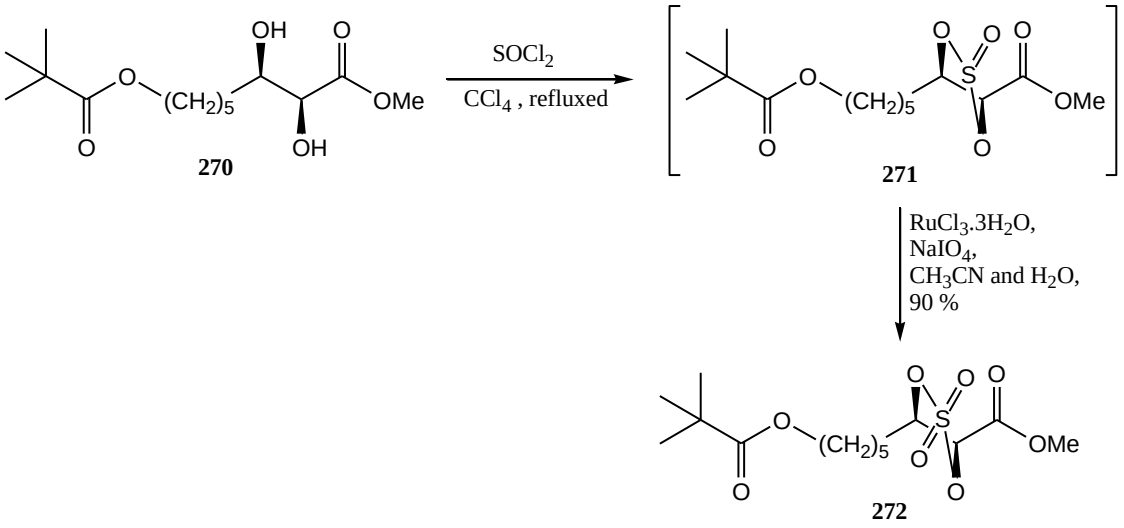
Scheme 76: ***The Sharpless dihydroxylation***

The protons of the diol (**270**) adjacent to the hydroxyl groups appeared in the ^{1}H NMR spectrum as a broad singlet at δ 4.10 for the α-position and a multiplet at δ 3.90–3.87 for the β-position. The ^{13}C NMR spectrum showed two signals at δ 73.1 and 72.3 for the carbons bonded the hydroxyl group. The specific rotation was measured as $[\alpha]_{D}^{25}$ = + 13.5 (*c* 1.245, $CHCl_3$) which can be compared to a known compound having the same functional group which was reported as + 11.5.[144]

2.4.3c The formation of the cyclic sulfate

The diol (**270**) was converted to the cyclic sulfate (**272**) *via* the cyclic sulfite (**271**, **Scheme 77**). The conversion consisted of two reactions: first, the formation of the cyclic sulfite using thionyl chloride and secondly, the oxidation with sodium (meta)periodate (1.5 mol eq.) and in the presence of a catalytic amount of ruthenium trichloride hydrate.[208]

The diol (**270**) was treated with thionyl chloride (2.2 mol eq.) and refluxed in CCl_4 to form the intermediate cyclic sulfite (**271**). During the reaction, HCl was produced. Since the cyclic sulfite was not stable under acidic conditions, the acid was removed by refluxing the reaction under a strong flow of nitrogen. The reaction mixture was cooled to room temperature, followed by addition of sodium (meta)periodate and ruthenium trichloride hydrate to complete the oxidation to form the sulfate (**272**) as shown in **Scheme 77**. After these two steps, the cyclic sulfate was isolated as a colourless oil in 90 % yield.

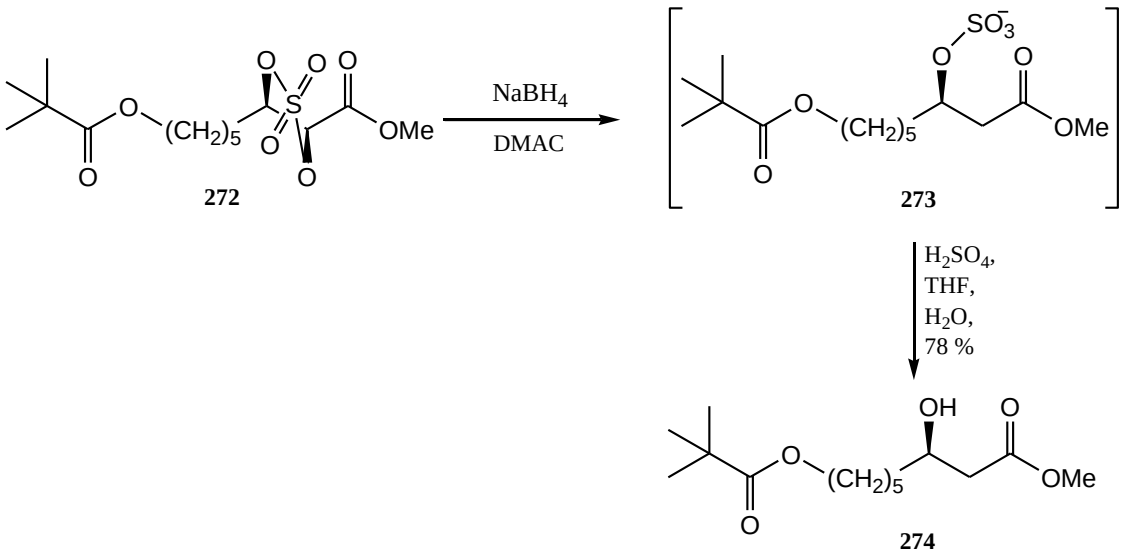


Scheme 77: ***The formation of the cyclic sulfate***

The ^{1}H NMR spectrum possessed an unusual group of signals for the protons of this ring. The proton in the β-position appeared as a multiplet at δ 4.97–4.93, while the proton at the α-carbon with respect to the carboxylic group appeared as a broad doublet at δ 4.88. The ^{13}C NMR spectrum showed two signals at δ 83.8 and 79.7 for the ring carbons. The IR spectrum showed two intense peaks at 1398 and 1212 cm^{-1} characteristic of the sulfate ring.[209] The optical rotation of the cyclic sulfate was $[\alpha]_D^{24}$ = + 39.4 (*c* 1.18, $CHCl_3$).

2.4.3d The reduction of the cyclic sulfate

The next step was the regioselective reductive ring opening of the cyclic sulfate (**272**) to the desired β-hydroxy ester (**274**). Nucleophilic addition of hydride at the α-position of the cyclic sulfate gave an intermediate (**273**), which was hydrolysied in strongly acid conditions (**Scheme 78**).[208,210] The cyclic sulfate (**272**) was treated with sodium borohydride (1 mol eq.) in *N*,*N*-dimethylacetamide at 0 ^{o}C for 30 minutes and the solvent was removed to give the intermediate sulfonic ester (**273**) which was not isolated, but was directly transformed into the β-hydroxy ester (**274**) by dissolving the reaction mixture with THF (300 ml), water (0.47 ml) and H_2SO_4 (1.3 ml, ~ 10 %) and stirring for one hour (**Scheme 78**). The yield was 78 %.



Scheme 78: ***The reduction of the cyclic sulfate***

The ^{1}H NMR spectrum of the (**274**) showed two doublets of doublets at δ 2.52 (*J* 3.2, 16.4 Hz) and 2.42 (*J* 9.2, 16.4 Hz) for the two protons on the α-position of the carboxylic group. The small coupling constants were for vicinal couplings with the neighbouring protons at the chiral centre equivalent to 3.2 Hz for the "*cis*" coupling and 9.2 Hz for the "*trans*" coupling. The large coupling constants were for the geminal coupling as shown in **Figure 26**. The IR spectrum showed a broad peak at 3510 cm^{-1} for the O–H stretch. The specific rotation was measured as $[\alpha]_{D}^{24}$ = – 12.3 (*c* 1.34, $CHCl_3$) which it was very similar to the literature value of – 11.6.[144]

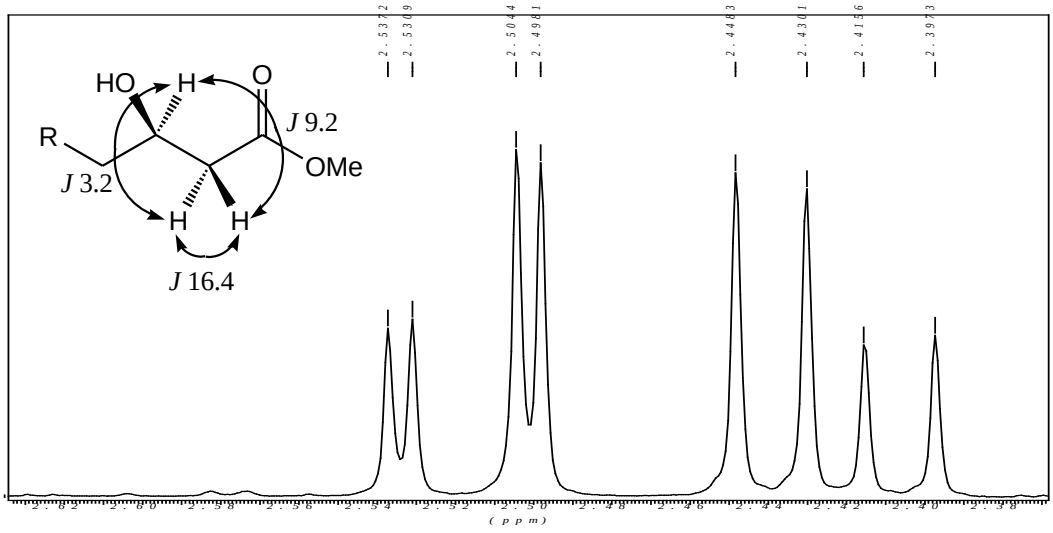
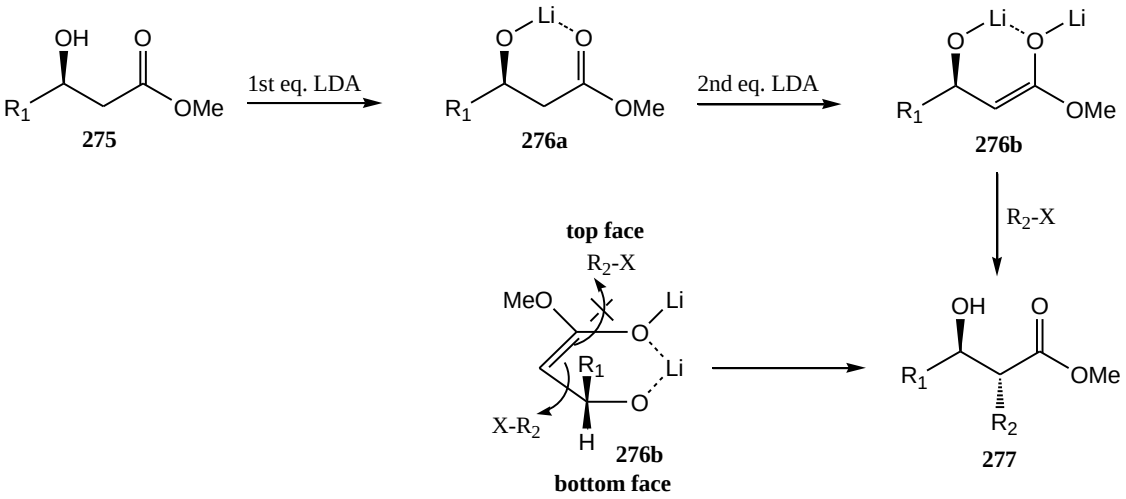


Figure 26: ***Appearance of the protons adjacent to the carboxylic group in the ^{1}H NMR***

2.4.3e The Fräter alkylation

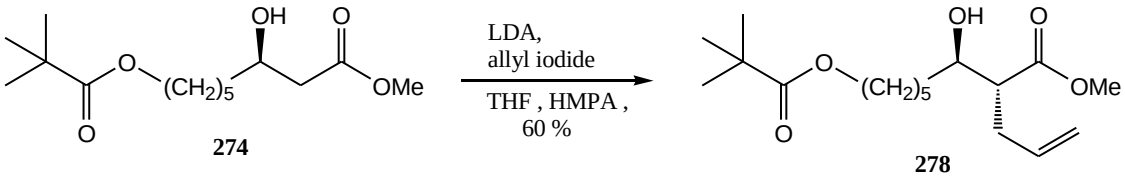
In the preparation of the intermediate sulfone corynomycolate moiety (**252**) for the synthesis of mycolic acids, the key step was the *anti*-alkylation of the β-hydroxy ester (**274**). The widely utilized asymmetric alkylation method is based on the use of the Fräter alkylation.[146,147] This was chosen for the insertion of the alkyl chain at the α-position of the β-hydroxy ester, since it gives great diastereoselectivity (95:5).[146] First of all, (**274**) was asymmetric ally alkylated with a short chain then this was extended to the required natural mycolic acid chain, because direct insertion of the necessary long chain (24 carbons) almost impossible. Treatment of such a compound (**275**) with LDA (2 mol eq.) forms the stable chelated *Z*-enolate (**276b**). In fact, the geometry of the enolate is not so important. However, the formation of a chelated species is fundamental (**Scheme 79**).[211] The steric effect on the top face of the six-membered ring intermediate (**276b**) allows a good level of diastereoselective addition from the bottom face to form the *anti*-alkylated product (**277**, **Scheme 79**).



Scheme 79: ***The formation of the intermediate enolate (276b) and the mechanism of the anti-addition***

To make this alkylation reaction work was not very easy. After attempting many times at different temperature and stirring times, and also preparation of LDA with different methods, sometimes it gave no product and sometimes low yield (45 – 55 %). The best yield (60 %) was found with the following method: n-BuLi (19.6 mmol, 2.15 mol eq.) was added to diisopropylamine (19.6 mmol) at – 78 ^{o}C and allowed to reach + 15 ^{o}C. It was then cooled to – 65 ^{o}C and (*R*)-8-(2,2-dimethyl-propionyloxy)-3-hydroxy-

octanoic acid methyl ester (**274**) (9.12 mmol) was added. The mixture was allowed to warm – 3 ^{o}C over 2 hours, stirred at 0 ^{o}C for 10 minutes, then cooled to – 55 ^{o}C and 1-iodoprop-2-ene (11.86 mmol) and HMPA (27.4 mmol, 3 mol eq.) in dry THF were added. The reaction was allowed to reach – 8 ^{o}C over 2 hours and then work up. The *anti*-alkylated product (**278**) was obtained in a great selectivity such that the minor *syn*-diastereoisomer did not appear in the ^{13}C NMR spectrum. Also 11 % of the β-hydroxy ester (**274**) was recovered (**Scheme 80**).



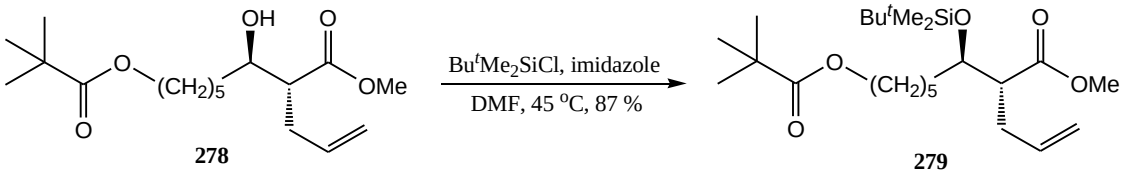
Scheme 80: ***The Fräter alkylation of the β-hydroxy ester (274)***

The ^{1}H NMR spectrum showed a doublet of doublets of doublets at δ 2.54 (*J* 5.1, 6.3, 7.9 Hz) for the proton in the α-position, a multiplet at δ 2.50–2.50 for the -CH_2- group protons adjacent to the alkene group, a doublet of doublets of triplets at δ 5.76 (*J* 17.0 Hz "*trans* coupling", 10.1 Hz "*cis* coupling", 7.0 Hz "vicinal coupling") for the alkene proton adjacent to -CH_2- group, a multiplet at δ 5.13–5.04 for the terminal alkene protons, and a multiplet at δ 3.71–3.68 for the proton adjacent to the hydroxyl group. The ^{13}C NMR spectrum showed two carbonyl carbon signals at δ 178.6, 175.3 (methyl ester), two olefinic signals at 134.8, 117.2 (terminal carbon), one signal at δ 71.6 for the carbon adjacent to hydroxyl group, one signal at δ 51.6 for the methyl carbon on the ester, and one signal at δ 50.5 for the α-carbon.

The specific rotation of the (2*R*,3*R*)-2-allyl-8-(2,2-dimethyl-propionyloxy)-3-hydroxy-octanoic acid methyl ester (**278**) was $[\alpha]_D^{26}$ = + 4.0 (c 1.08, $CHCl_3$) and in the literature the specific rotation of (2*R*,3*R*)-2-allyl-8-(2,2-dimethyl-propionyloxy)-3-hydroxydodecanoic acid methyl ester[144] was reported as + 3.1 and that for (2*R*,3*R*)-3-hydroxy-2-tetradecyl-octodecanoic acid methyl aster[212] also was reported as + 5.7. These data showed that the α-alkylated β-hydroxy ester was the correct stereoisomer.

2.4.3f Protection of the secondary alcohol

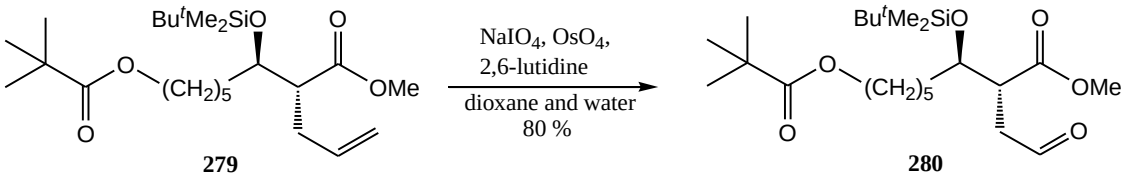
The secondary alcohol was protected with a *tert*-butyldimethylsilyl group in order to complete the chain extension at the α-position. A mixture of the secondary alcohol (**278**) with *tert*-butyldimethylchlorosilane and imidazole was stirred in DMF at 45 ^{o}C for 18 hours to give (**279**) in 87 % yield (**Scheme 81**). The protecting group protons appeared in the proton NMR spectrum as a singlet at δ 0.87 for the *tert*-butyl group and two singlets at δ 0.06 and 0.04 for the two methyls. The specific rotation of the protected product was $[\alpha]_{D}^{24} = -13.7$ (*c* 1.06, $CHCl_3$).



Scheme 81: ***The protection of the secondary alcohol***

2.4.3g Oxidation of the olefin to an aldehyde

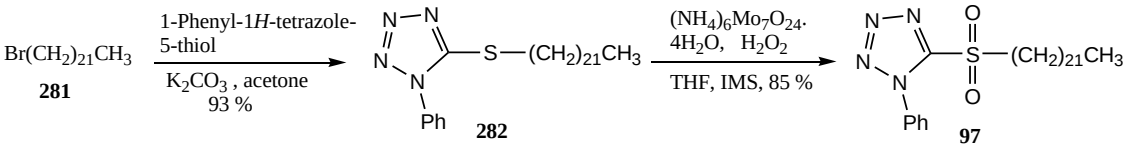
The olefin (**279**) was oxidised to the aldehyde (**280**) for a Julia coupling reaction. It has been reported that the yield of the classic oxidative cleavage of olefins by OsO_4–$NaIO_4$ was dramatically improved with addition of 2,6-lutidine, since this base can suppress side reactions.[213] A mixture of the alkene (**279**) (7.01 mmol), 2,6-lutidine (14.02 mmol), OsO_4 (0.14 mmol) and $NaIO_4$ (28.04) was stirred in 1,4-dioxane – water (3:1) at 25 ^{o}C for 2 hours to obtain the aldehyde (**280**) in 80 % yield. The ^{1}H NMR spectrum showed a broad singlet at δ 9.82 for the aldehyde proton and the ^{13}C NMR spectrum showed a signal at δ 200.5 for the carbonyl carbon.



Scheme 82: ***Oxidation of the olefin to aldehyde***

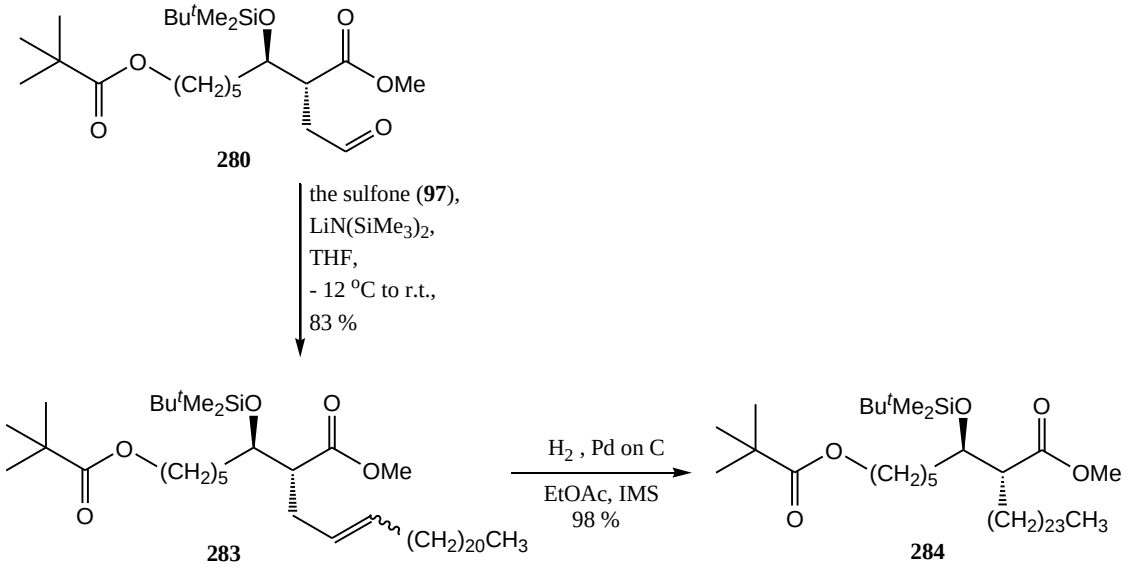
2.4.3h The chain extension

The sulfone (**97**) was prepared for the chain extension of the aldehyde (**280**). 1-Bromodocosane (**281**) was used as starting material and the method explained in section **2.2.3** (p 39) was followed. The sulfone (**97**) could not been purified by re-crystallisation, since there was some compound present which was not completely oxidised. It was purified by column chromatography and the column was gently warmed because of the solubility of the sulfone (**Scheme 83**).



Scheme 83: ***The preparation of the sulfone (97)***

The aldehyde (**280**) was coupled with the sulfone (**97**) to give the olefin (**283**) in 83 % yield as a mixture of *E*- and *Z*-isomers in a ratio 2.1:1 (**Scheme 84**). This was followed by hydrogenation of the olefin with hydrogen gas and Pd on carbon as a catalyst in ethyl acetate and IMS to give the saturated product (**284**) in 98 % yield which had the desired long chain at the α-position (**Scheme 84**). The saturated product gave clear NMR spectra and a specific rotation of – 5.0 (*c* 1.24, $CHCl_3$).

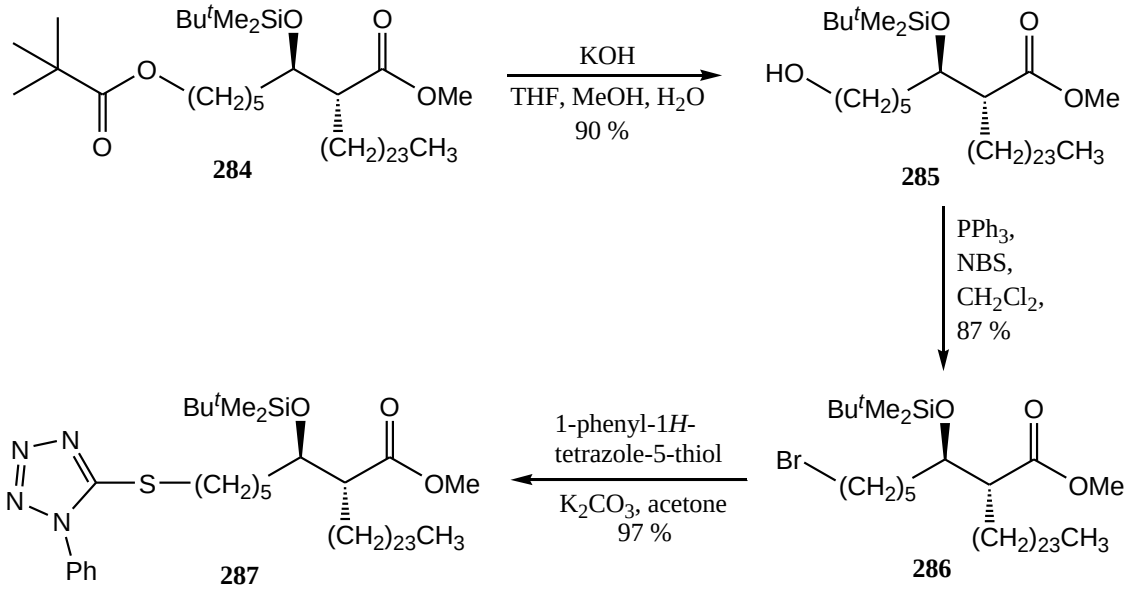


Scheme 84: ***The chain extension***

2.4.3i Preparation of the sulfane (287)

The pivalate group of (**284**) was selectively deprotected with potassium hydroxide (15 mol eq.) by refluxing at 70 ^{o}C for 3 hours in a mixture of THF : MeOH : H_2O (10:10:1) to give the corresponding alcohol (**285**) in 90 % yield (**Scheme 85**). Fortunately, under these conditions the methyl ester did not hydrolyse to free acid. The hydroxyl group of the alcohol was converted into the bromide (**286**) in 87 % yield using *N*-bromosuccinimide (1.27 mol eq.) in the presence of triphenylphosphine (1.15 mol eq.) in dichloromethane as shown in **Scheme 85**. Comparing the ^{1}H NMR spectra of the staring material alcohol (**285**) and the brominated product (**286**), the most significant change was the shift to a higher field of the triplet from 3.65 (*J* 6.7 Hz, -C$\underline{H}_2$OH) to 3.41 (J 6.9 Hz, -C$\underline{H}_2$Br), which was indicative of the success of the reaction.

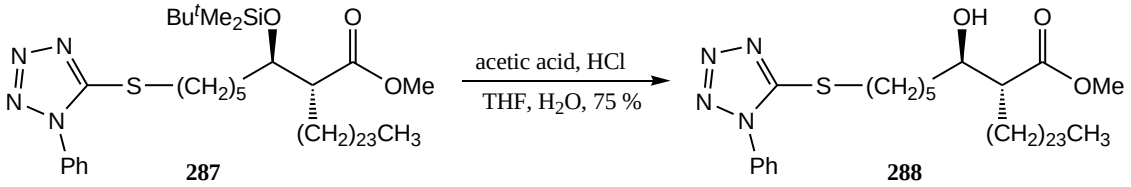
Finally, the bromo compound (**286**) was reacted with 1-phenyl-1H-tetrazole-5-thiol (1 mol eq.) in the presence of potassium carbonate (2.1 mol eq.) in acetone to give the desired methyl ester (**287**) in an excellent yield (97 %). The proton NMR spectrum showed a multiplet at δ 7.61–7.54 for the aromatic proton of the sulfane (**287**). The specific rotations of the alcohol (**285**), the bromo compound (**286**) and the sulfane (**287**) were – 4.9, – 5.0 and – 4.5 respectively, which showed that stereochemistry of the stereocentres remained during the reactions.



Scheme 85: ***Preparation of the sulfane (287)***

2.4.3j Deprotection of the silyl ether group

The *tert*-butyldimethylsilyl group at this step had to be changed to an acetyl group, since the silyl ether is already present on the intermediates (**250** and **251**, p 95). Removing of this silyl group was not so easy, since it is sterically hindered. Removal failed with tetra n-butyl ammonium fluoride and formic acid. However, successful desilylation was obtained with a literature method.[214] The silyl compound (**287**) was dissolved in THF and a mixture of acetic acid, water, THF and HCl (2N) was added at room temperature and stirred for 18 hours to obtain the secondary alcohol (**288**) as a white solid with 75 % yield (**Scheme 86**). Interestingly, the specific rotation changed from negative 4.5 (silyl) to positive 6.4 (alcohol).

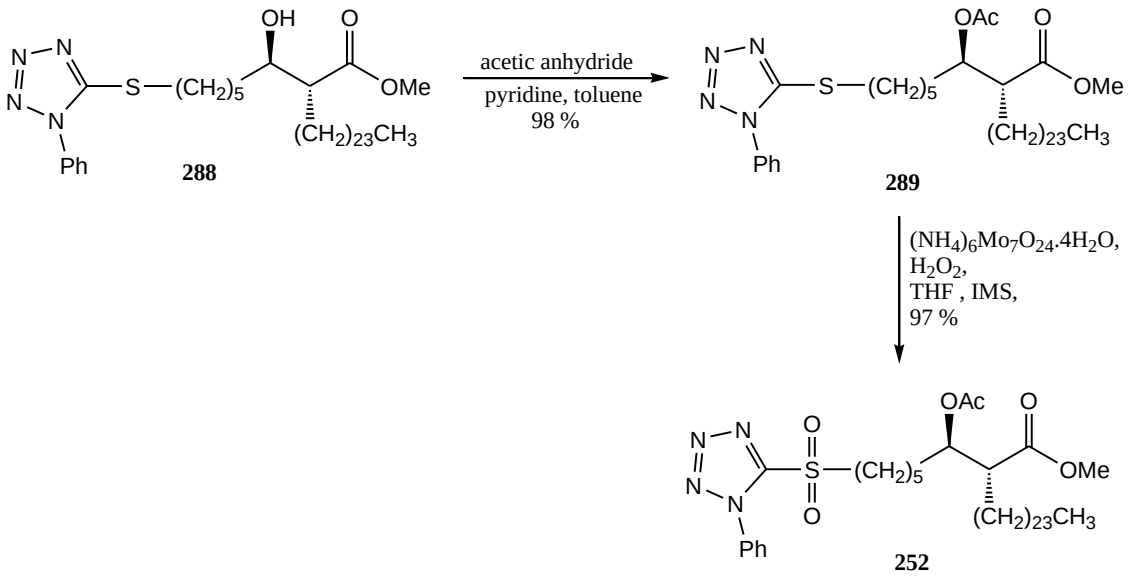


287 **288**

Scheme 86: ***The deprotection of the silyl ether group***

2.4.3k Preparation of the intermediate sulfone

The secondary alcohol (**288**) was protected as the acetate (**289**) with excess acetic anhydride in pyridine and toluene with an excellent yield of 98 % (**Scheme 87**). In particular, in the ^{1}H NMR, the proton adjacent acetoxy group spectrum shifted downfield to δ 5.08 and appeared as a doublet of doublets of doublets (*J* 3.8, 6.8, 8.4 Hz) while the proton adjacent to the hydroxyl group (**288**) appeared at δ 3.65 as a multiplet. The methyl protons on the acetyl group also appeared as a singlet at δ 2.03. Finally, this acetylated sulfane (**289**) was oxidised to desired intermediate sulfone (**252**) with an excellent yield 97 % (**Scheme 87**), using hydrogen peroxide by the method explained in section **2.2.3** (p 39). The ^{1}H NMR spectrum showed a multiplet at δ 3.75–3.72 for the protons next to the sulfonyl group and a doublet of doublets of doublets at δ 2.61 (*J* 4.3, 6.8, 10.7 Hz) for the proton on the α-position of the ester. The molecular rotation of the sulfone (**252**) was + 68.4.

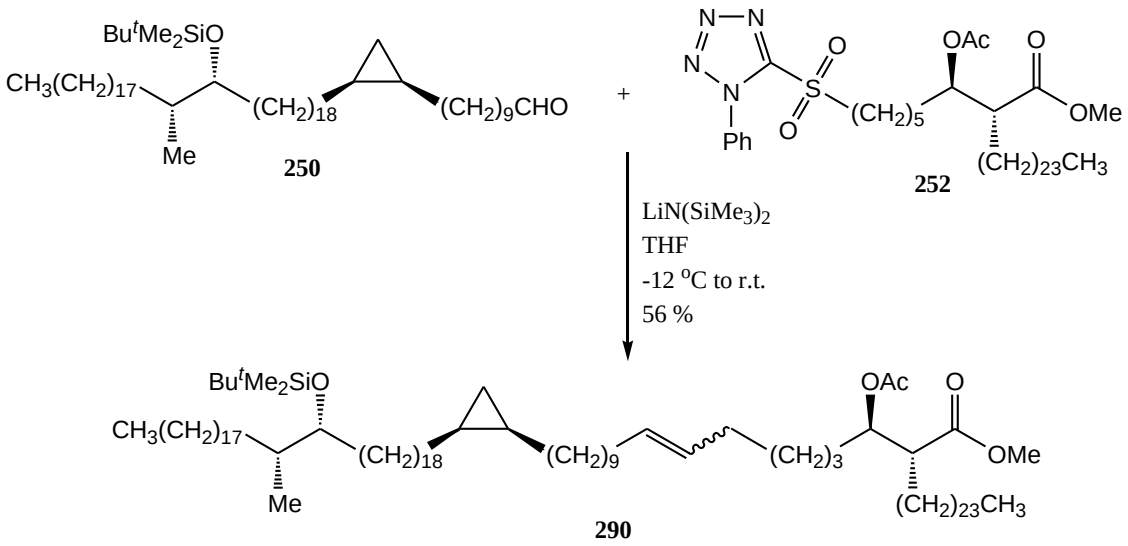


Scheme 87: ***The preparation of the intermediate sulfone (252)***

2.5 The synthesis of *cis*-cyclopropane keto-mycolic acids

2.5.1 The final coupling reaction

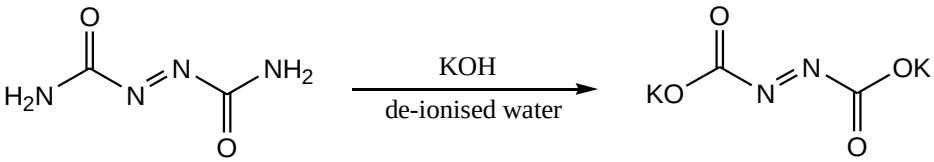
Reaction of the aldehyde (**250**) with the sulfone (**252**) gave the final coupling to form the whole structure of the *cis*-cyclopropane keto-mycolic acid with 56 % yield. The product (**290**) was a mixture of *E*- and *Z*-stereoisomers in a ratio 2:1 (**Scheme 88**).



Scheme 88: ***Final coupling reaction for the cis-cyclopropane keto-mycolic acid***

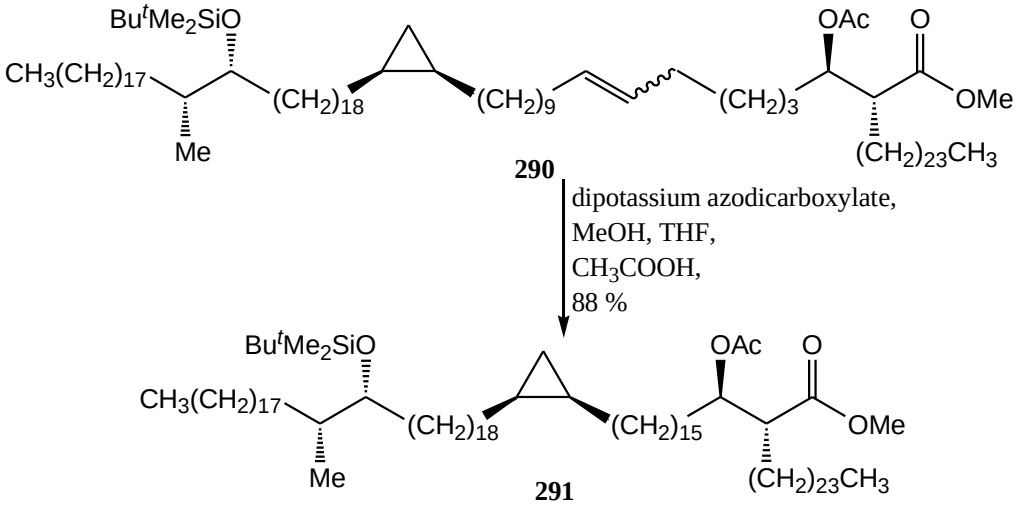
2.5.2 Hydrogenation of the alkene with dipotassium azodicarboxylate

The hydrogenation of this alkene was performed using di-imide formed from dipotassium azodicarboxylate, rather than from 2,4,6-tri-isopropylbenzenesulphonyl hydrazide (TPBSH) (**193**), because, this gives a better yield and cleaner product. The dipotassium azodicarboxylate, a thermally unstable compound, was prepared by reaction of azodicarbonamide with potassium hydroxide at 0 ^{o}C in de-ionised water (**Scheme 89**). To avoid decomposition of the compound upon working up, the product (a bright yellow solid) was washed with water, re-crystallised from ice water and freezer cooled solvents and stored in a sealed container in a freezer.



Scheme 89: ***The formation of dipotassium azodicarboxylate***

The alkene (**290**) was dissolved in THF and MeOH, and dipotassium azodicarboxylate was added. A solution of acetic acid in THF was added at 5 ^{o}C to this stirred mixture and after two hours more acetic acid solution was added and stirred for 2 days at room temperature. The NMR spectra showed that there was still unreacted alkene, therefore the reaction was repeated to complete the hydrogenation. The saturated product (**291**) was obtained as a white solid with 88 % yield (**Scheme 90**). The molecular rotation of the (**291**) was + 110.5 which corresponds to the sum of those of the aldehyde (**250**) (+ 44.6) and the sulfone (**252**) (+ 68.4).

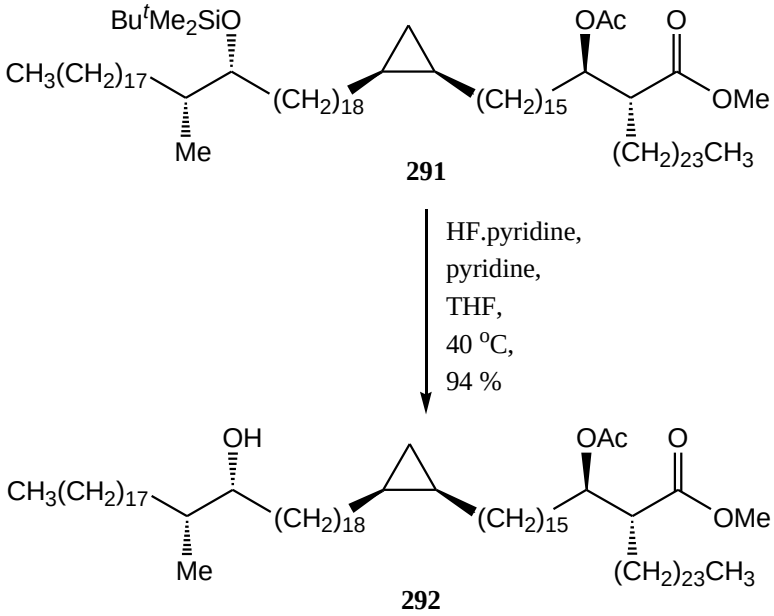


Scheme 90: ***Hydrogenation with dipotassium azodicarboxylate***

2.5.3 Desilylation

The *tert*-butyldimethylsilyl group of the silyl ether (**291**) was removed to give corresponding secondary alcohol (**292**). This deprotection failed with tetra n-butyl ammonium fluoride even at 80 ^{o}C and there also was some elimination product at the α,β-position of the ester. The method used in section **2.4.3j** (p 112) could not be used because of the reactivity of the cyclopropane ring to acid. However, there is another literature method using HF.pyridine, but handling of this reagent is more difficult than n-TBAF. After many steps, the silyl ether product had been obtained in very small amount (300 mg), so a very small amount of HF.pyridine was needed. An attempt at room temperature gave almost no product, but at 40 ^{o}C it gave the corresponding alcohol in an excellent yield.

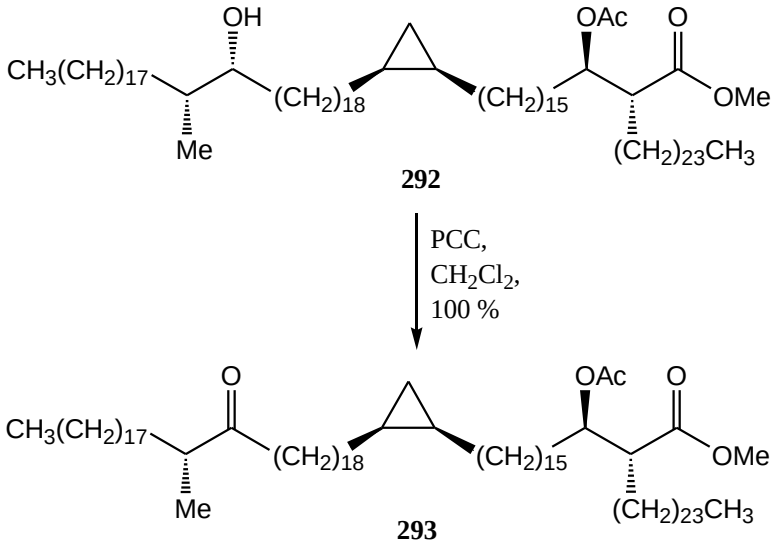
The silyl ether was dissolved with THF in a polyethylene vial followed by addition of pyridine and HF.pyridine. Stirring of the mixture at 40 ^{o}C for 17 hours gave the alcohol (**292**) in 94 % yield as a solid (**Scheme 91**). The NMR spectra did not show any signals for the *tert*-butyldimethylsilyl group and other signals were as expected. The IR spectrum showed a broad peak at 3449 cm^{-1} for the O–H stretch. The molecular rotation (+ 120) confirmed that, during the reaction, the stereochemistry of the stereocenter remained unchanged. Compound (**292**) represents the first example of a synthetic (protected) hydroxy-mycolic acid. These have been reported in the literature,[66,68] but they are uncommon.



Scheme 91: ***Desilylation***

2.5.4 Oxidation of the secondary alcohol to ketone

This was the last step for the formation of the keto-mycolic acid. Oxidation of the hydroxy-mycolic acid (**292**) with PCC gave the target *cis*-cyclopropane keto-mycolic acid (**293**) as a white solid in 100 % yield (**Scheme 92**). The ^{1}H NMR spectrum showed that the cyclopropane protons of the synthetic mycolic acid (**293**) gave identical signals to natural keto-mycolic acid, appearing at δ 0.68–0.64, 0.57 and –0.32; this *cis*-cyclopropane keto-mycolic acid (**293**) was the major homologue of the minor component in a mixture of natural keto-mycolic acid isolated from *M. tuberculosis*.[90] The major component of that natural mixture was α-methyl-*trans*-cyclopropane keto-mycolic acid. A comparison of detailed NMR spectra will be made with natural sample in section **2.7** (p 119). The IR spectrum showed the peak for the O–H stretch of the alcohol (**292**) had disappeared and there was a peak at 1708 cm^{-1} for the C=O stretch of the ketone. The optical rotation of this keto-mycolic acid (**293**) was measured as $[\alpha]_D^{20}$ + 3.0 (*c* 0.7, $CHCl_3$), while it was $[\alpha]_D^{21}$ + 9.3 (*c* 0.95, $CHCl_3$) for the hydroxy-mycolic acid (**292**). The reduction in the specific rotation is consistent with the change in sign of the rotation in the model compound (**164**) on oxidation to the ketone (**165**) (this system having only one set of chiral centres). The optical rotation of the alcohol (**164**) was + 12.9 and the ketone (**165**) – 12.1 (p 46 and 47).



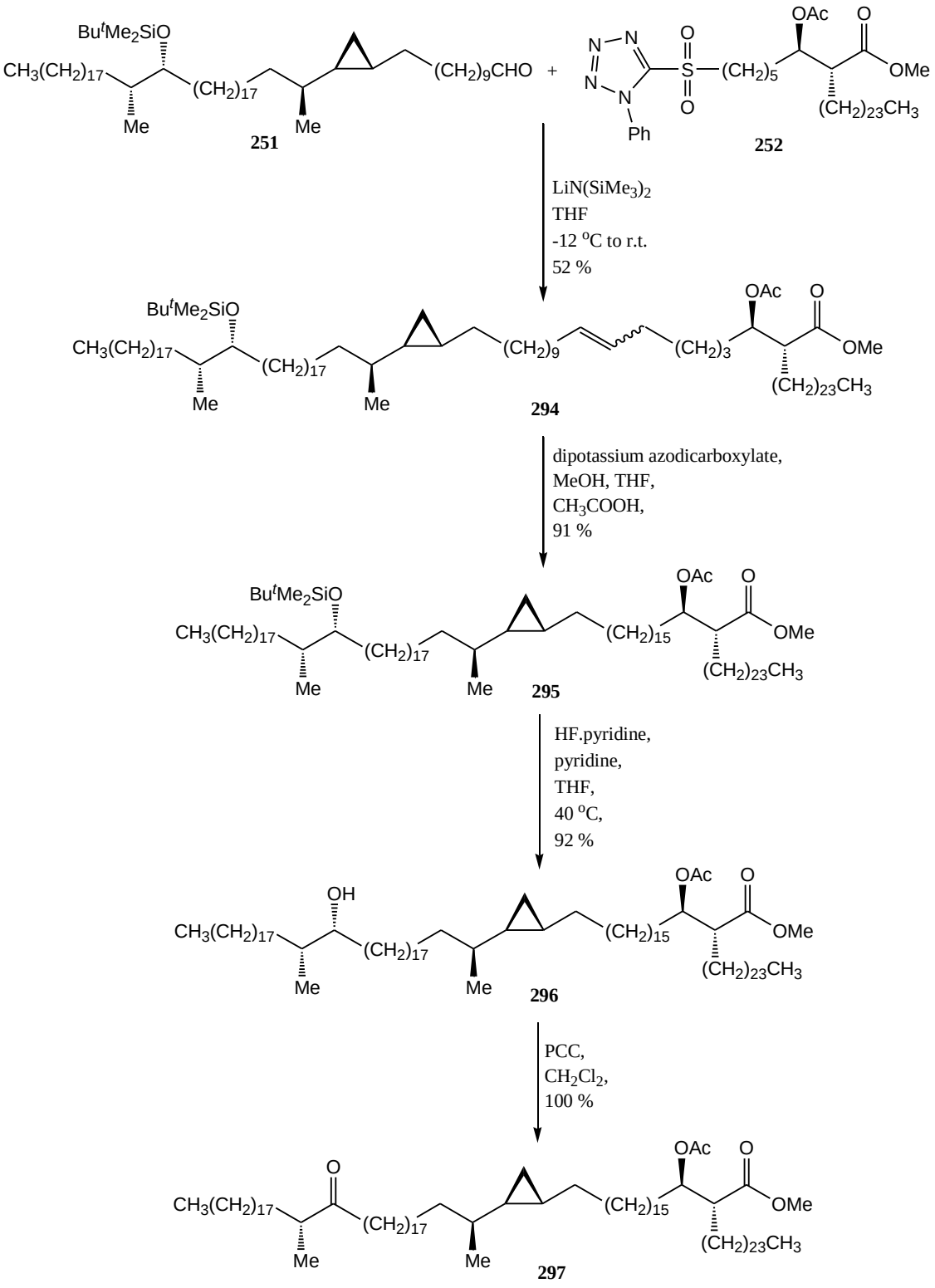
Scheme 92: ***Oxidation of the secondary alcohol to aimed keto-mycolic acid***

2.6 The synthesis of α-methyl-*trans*-cyclopropane keto-mycolic acid

The above methods (p 113) were also used for the synthesis of α-methyl-*trans*-cyclopropane keto-mycolic acid (**297**). Reaction of aldehyde (**251**) with the sulfone (**252**) in the presence of lithium bis(trimethylsilyl) amide gave the corresponding alkene (**294**) as a mixture of *E* and *Z*-isomers in ratio 2:1 with 52 % yield. The alkene (**294**) was then hydrogenated with dipotassium azodicarboxylate and acetic acid in THF and MeOH to give the saturated compound (**295**) as a white solid in 91 % yield (**Scheme 93**). The molecular rotation of this compound (**295**) was found to be + 126 which agrees well with the sum of the aldehyde (+ 53.7) and the sulfone (+ 68.4). These data showed that during the reaction no epimerisation occurred. The ^{1}H NMR spectrum was as expected; there was a doublet of doublets of doublets at δ 5.10 (*J* 4.2, 6.9, 8.1 Hz) for the proton adjacent to the acetoxy group and two singlets at δ 0.04 and 0.03 for the two methyl groups on the silyl ether.

The silyl protected group of (**295**) was removed by stirring it with HF.pyridine in pyridine and THF at 40 ^{o}C for 18 hours to give the corresponding secondary alcohol (**296**) as a white solid with 92 % yield. This secondary alcohol is also an example of a hydroxy mycolic acid. The silyl group protons had disappeared on the proton NMR spectrum and there was a very broad signal at δ 1.67–1.14 for the long chain protons. The proton adjacent to hydroxyl group appeared at δ 3.5 as a multiplet. The specific rotation of this secondary alcohol (**296**) was measured as $[\alpha]_{D}^{21}$ = + 10.0 (*c* 0.83, $CHCl_3$).

Eventually, the target α-methyl-*trans*-cyclopropane keto-mycolic acid (**297**) was obtained by oxidation of the secondary alcohol (**296**) using PCC as oxidant with 100 % yield (**Scheme 93**). The specific rotation of this keto-mycolic acid was $[\alpha]_{D}^{20}$ = + 3.1 (*c* 0.96, $CHCl_3$). The β-hydroxy group of a mixture of natural keto-mycolic acid methyl esters, isolated from *M. tuberculosis*,[90] was acetylated and the optical rotation was measured as $[\alpha]_{D}^{24}$ = + 4.4 (*c* 1.06, $CHCl_3$). Comparison of these two results showed the configuration of the methyl next to ketone could be *S*-isomer rather than *R*-isomer, as the optical rotations are not so close. To find out the absolute stereochemistry of this methyl and to compare biological activities, the *S*-isomer of the keto-mycolic acid is currently being synthesised. The detailed NMR spectra with comparison of natural sample will be explained later (p 119).



Scheme 93: ***Final steps to synthesis of the α-methyl trans-cyclopropane keto-mycolic acid***

2.7 The NMR spectra of the keto-mycolic acids

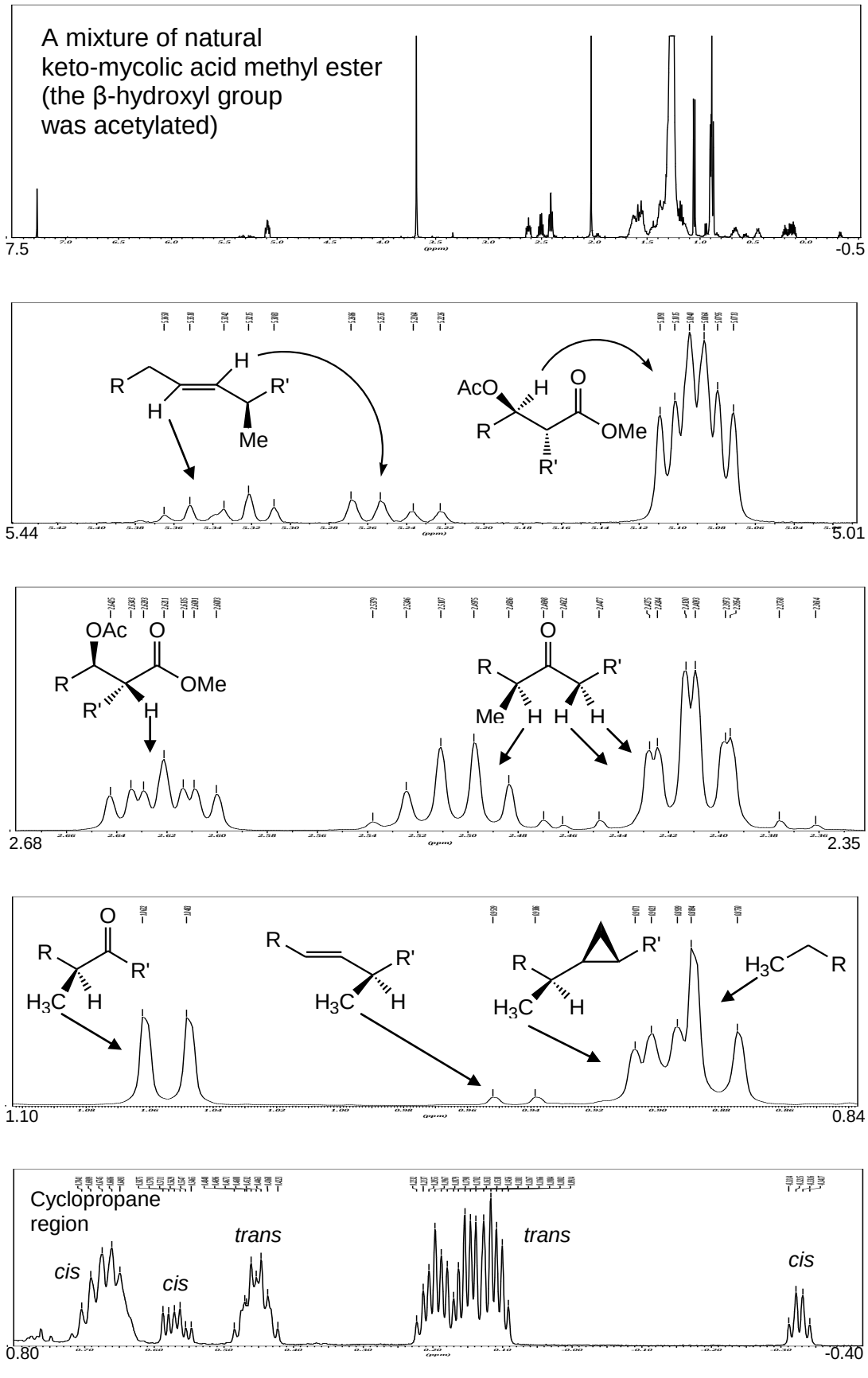


Figure 27: ***The ^{1}H NMR spectrum of a natural mixture of keto-mycolic acids***

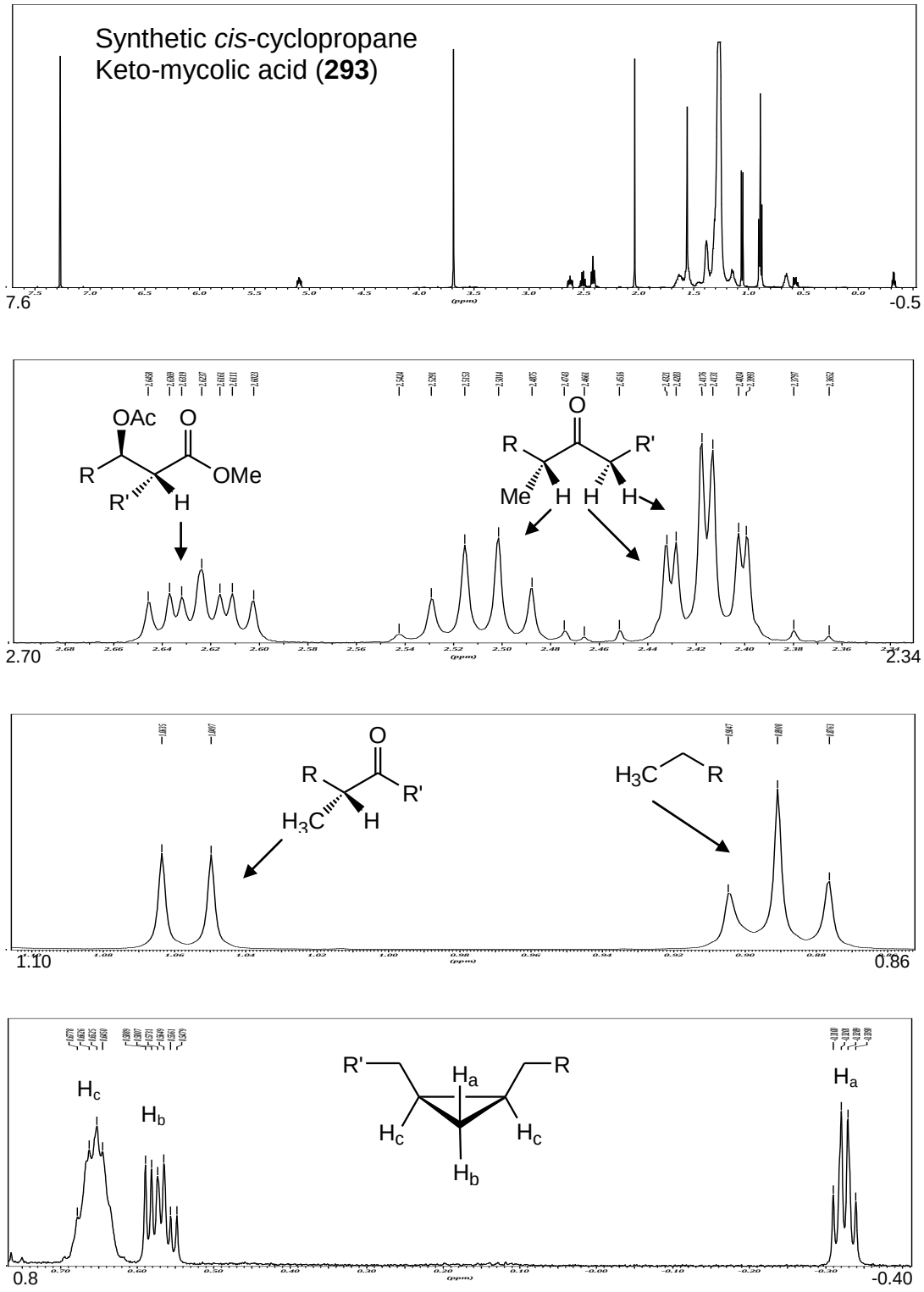


Figure 28: ***The [1]H NMR spectrum of the synthetic cis-cyclopropane keto-mycolic acid***

By comparing **Figure 27** and **Figure 28**, the ^{1}H NMR spectra showed the signals for the synthetic *cis*-cyclopropane keto-mycolic acid to be identical to those of the minor component of the natural keto-mycolic acids isolated from *M. tuberculosis*.[90]

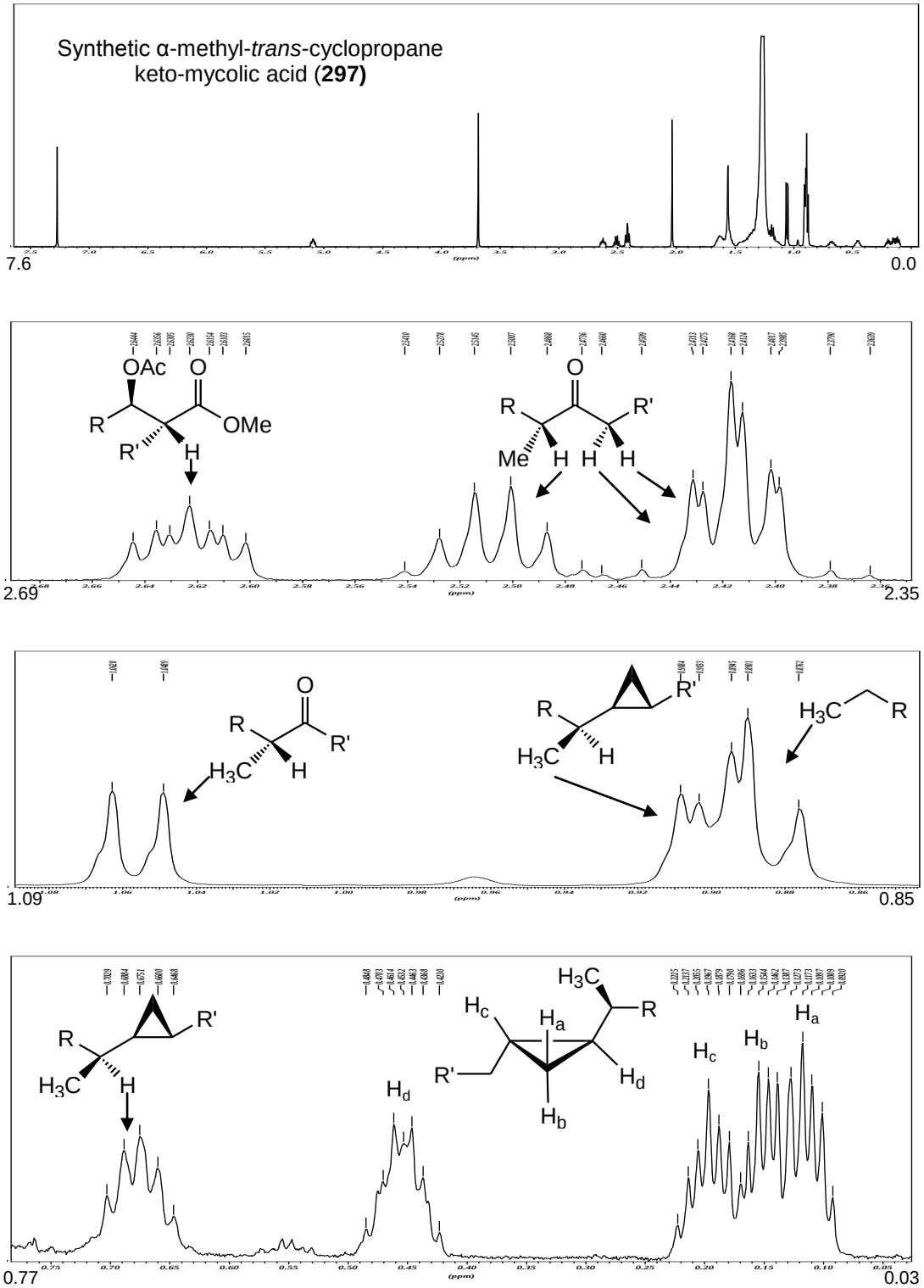
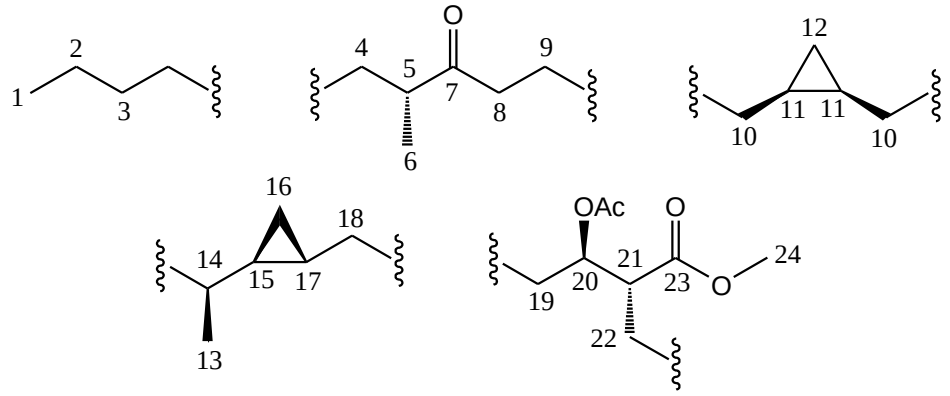


Figure 29: ***The ^{1}H NMR spectrum of the synthetic α-methyl-trans-cyclopropane keto-mycolic acid***

By comparing **Figure 27** and **Figure 29**, the ^{1}H NMR spectra showed the signals for the synthetic α-methyl-*trans*-cyclopropane keto-mycolic acid were identical to those of the major component of the natural keto-mycolic acids isolated from *M. tuberculosis*.[90]

Table 5: ***Chemical shifts of the ^{13}C NMR spectra of selected carbons from synthetic and natural keto-mycolic acids***



Carbon No	Synthetic (ppm)	Natural (ppm)	Carbon No	Synthetic (ppm)	Natural (ppm)
1	14.09	14.09	13	19.66	19.66
2	22.68	22.68	14	38.10	38.10
3	31.93	31.93	15	18.61	18.61
4	33.05	33.05	16	10.48	10.48
5	46.33	46.33	17	26.13	26.14
6	16.35	16.35	18	27.26	27.27
7	215.08	215.03	19	31.74	31.75
8	41.13	41.13	20	74.11	74.11
9	23.74	23.74	21	49.60	49.61
10	28.73	28.73	22	25.00	25.00
11	15.78	15.79	23	170.30	170.29
12	10.93	10.93	24	51.49	51.49

Note: The long chain carbons mainly appeared between 30 and 28.

Table 5 also showed that the synthetic keto-mycolic acids (**293** and **297**) gave identical signals to the natural keto-mycolic acids.

2.8 MALDI-TOF mass spectroscopy of the keto-mycolic acids

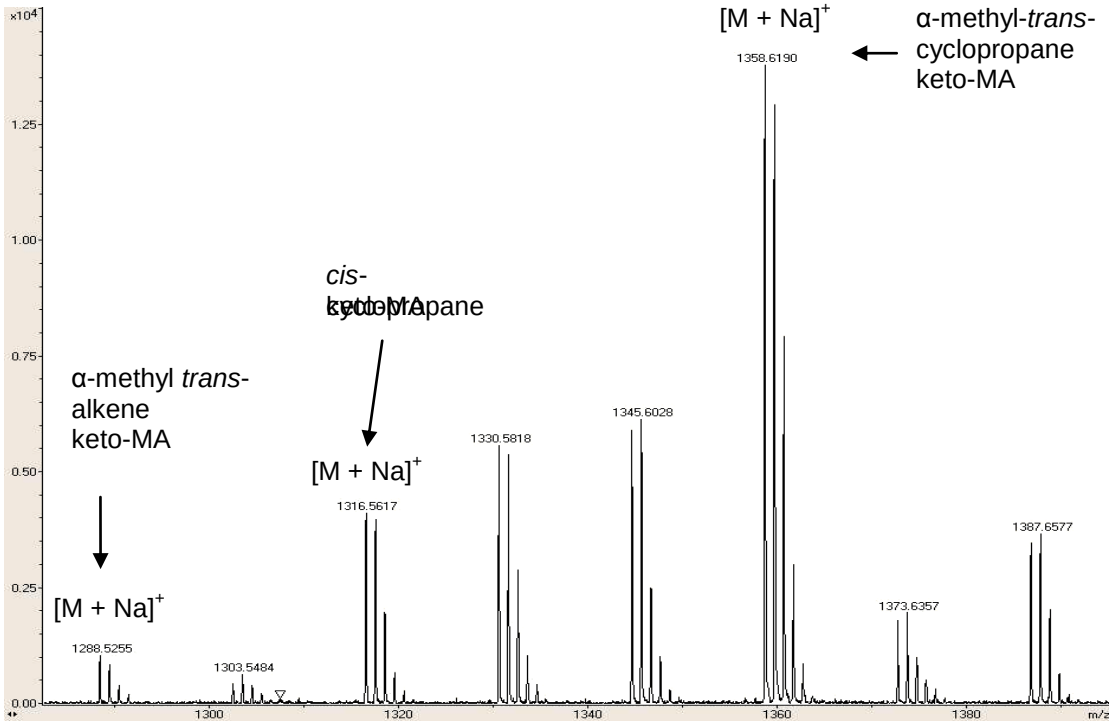


Figure 30: ***MALDI-TOF mass spectroscopy of a mixture of natural keto-mycolic acid***

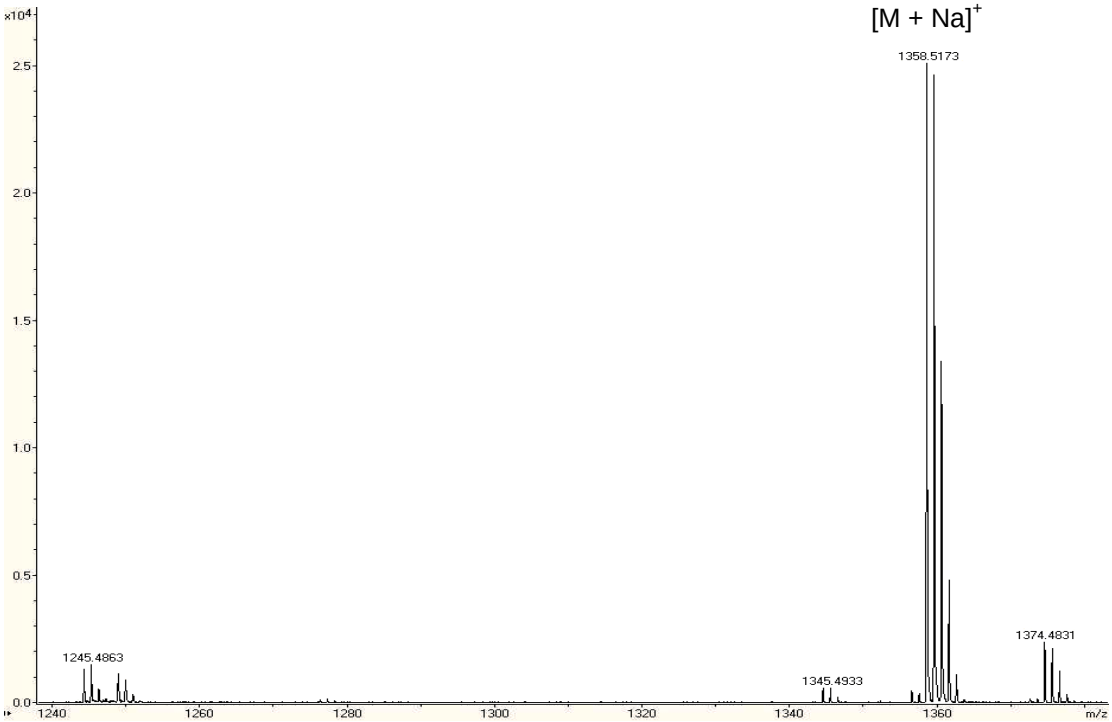


Figure 31: ***MALDI-TOF mass spectroscopy of the α-methyl-trans-cyclopropane keto-mycolic acid***

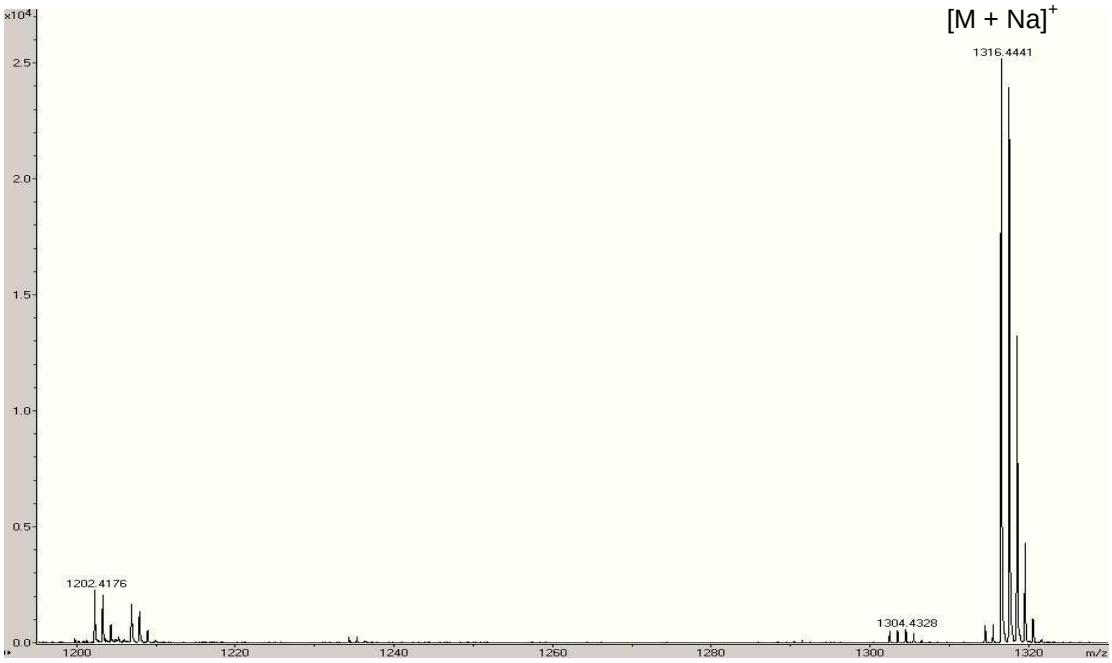
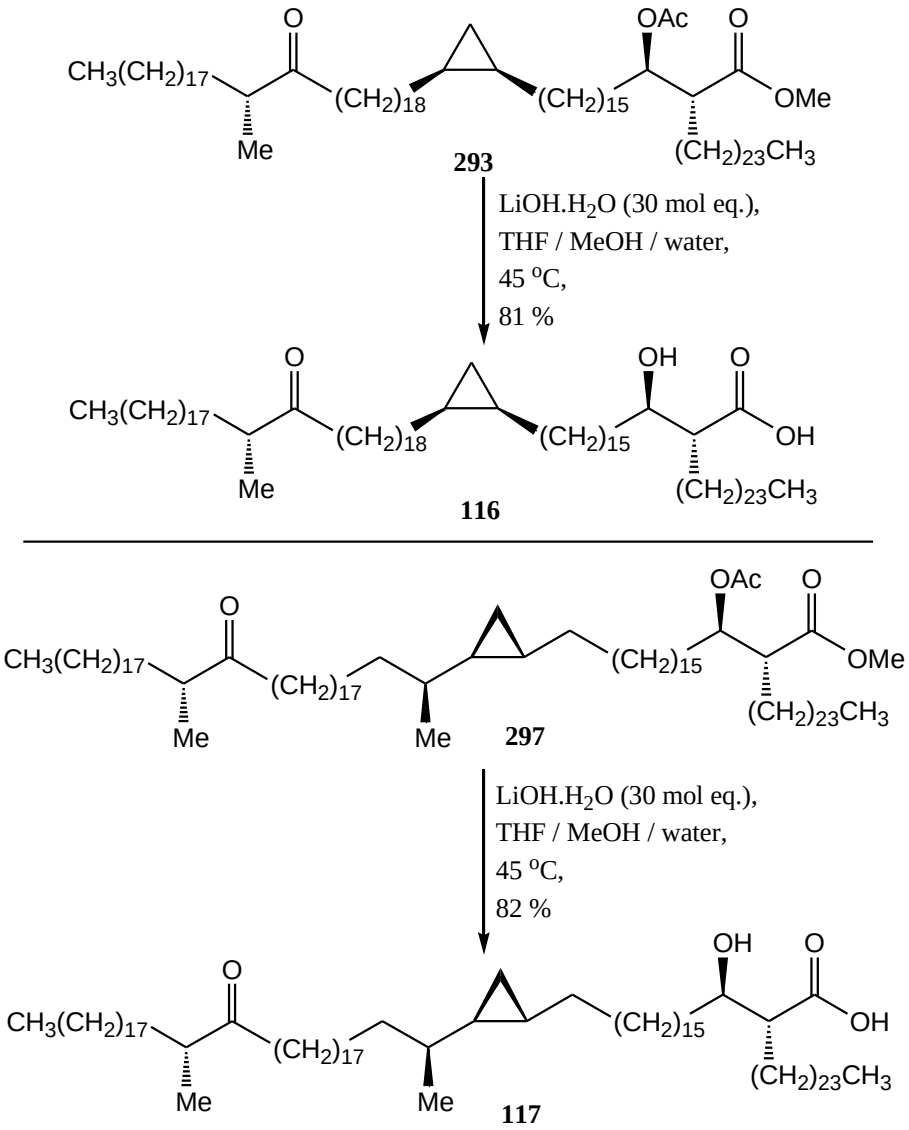


Figure 32: ***MALDI-TOF mass spectroscopy of the cis-cyclopropane keto-mycolic acid***

The synthetic *cis*-cyclopropane keto-mycolic acid (**293**), the α-methyl-*trans*-cyclopropane keto-mycolic acid (**297**), and a natural mixture of keto-mycolic acid were examined with a MALDI-TOF mass spectroscopy.[215] **Figure 30** shows the natural mixture, **Figure 31** shows the result for (**297**) and **Figure 32** shows that for (**293**). **Figure 30** shows that the natural keto-mycolic acid consists of a series of homologues and different functional group on the proximal position. From the NMR spectra, it is known that there are α-methyl-*trans*-alkene (very minor), *cis*-cyclopropane (minor) and α-methyl-*trans*-cyclopropane (major) at the proximal group. In comparison of the synthetic mycolic acids with the natural one, **Figure 32** showed a molecular ion $[M + Na]^+$ at 1316.4441 for the *cis*-cyclopropane keto-mycolic acid (**293**), which the **Figure 30** showed a molecular ion $[M + Na]^+$ at 1316.5615 as a minor peak. Therefore, the synthesised *cis*-cyclopropane keto-mycolic acid was identical to one minor component of the natural mixture. Moreover, the **Figure 31** showed a molecular ion $[M + Na]^+$ at 1358.5173 for the α-methyl-*trans*-cyclopropane keto-mycolic acid (**297**), which the **Figure 30** showed a molecular ion $[M + Na]^+$ at 1358.6190 as a major peak. Therefore, the synthesised α-methyl-*trans*-cyclopropane keto-mycolic acid was identical to one major component of the natural mixture.

2.9 The hydrolysis of the mycolic acids

The hydrolysis of the mycolic acid methyl ester to free acid was necessary for biological testing. The acetate and methyl ester groups were deprotected using excess of LiOH in THF, MeOH and water. The mixture was stirred at 45 ^{o}C for 18 hours to obtain free hydroxy acid (**Scheme 94**). The specific rotation of protected *cis*-cyclopropane keto-mycolic acid (**293**) was $[\alpha]_{D}^{20}$ + 3.0 (*c* 0.7, $CHCl_3$) and its corresponding free hydroxy acid (**116**) changed to $[\alpha]_{D}^{26}$ + 4.4 (*c* 1.02, $CHCl_3$). Moreover, the specific rotation of protected α-methyl-*trans*-cyclopropane keto-mycolic acid (**297**) was $[\alpha]_{D}^{20}$ + 3.1 (*c* 0.96, $CHCl_3$) and its corresponding free hydroxy acid (**117**) also changed to $[\alpha]_{D}^{26}$ + 5.3 (*c* 0.96, $CHCl_3$). From these changes, it is presumed

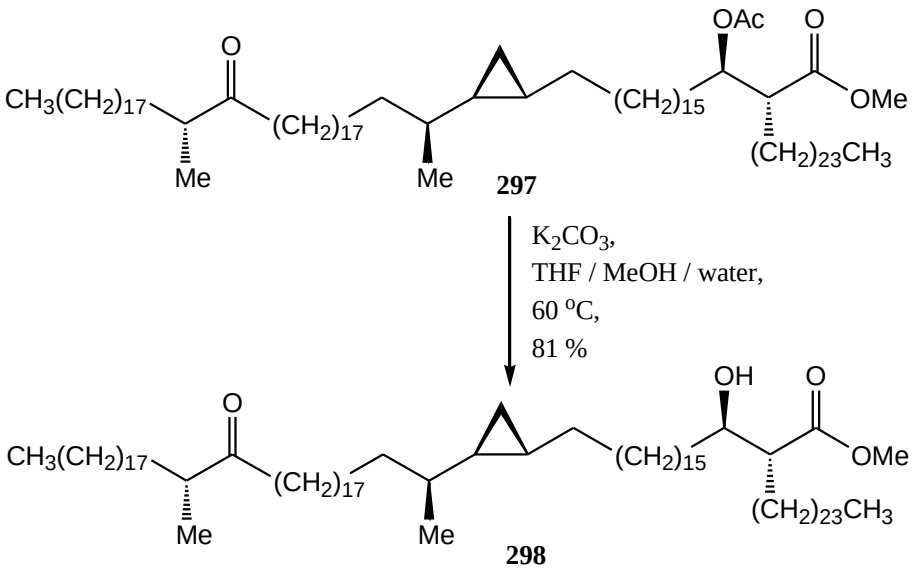


Scheme 94: ***Hydrolysis of the mycolic acid to free acid with LiOH***

to have epimerised because the compounds have the methyl adjacent to the ketone. There is no evidence as to whether the epimerisation is partial or complete and also the proton in the α-position to carboxylic group, which is slightly acidic, might be epimerised in a very small percentage.

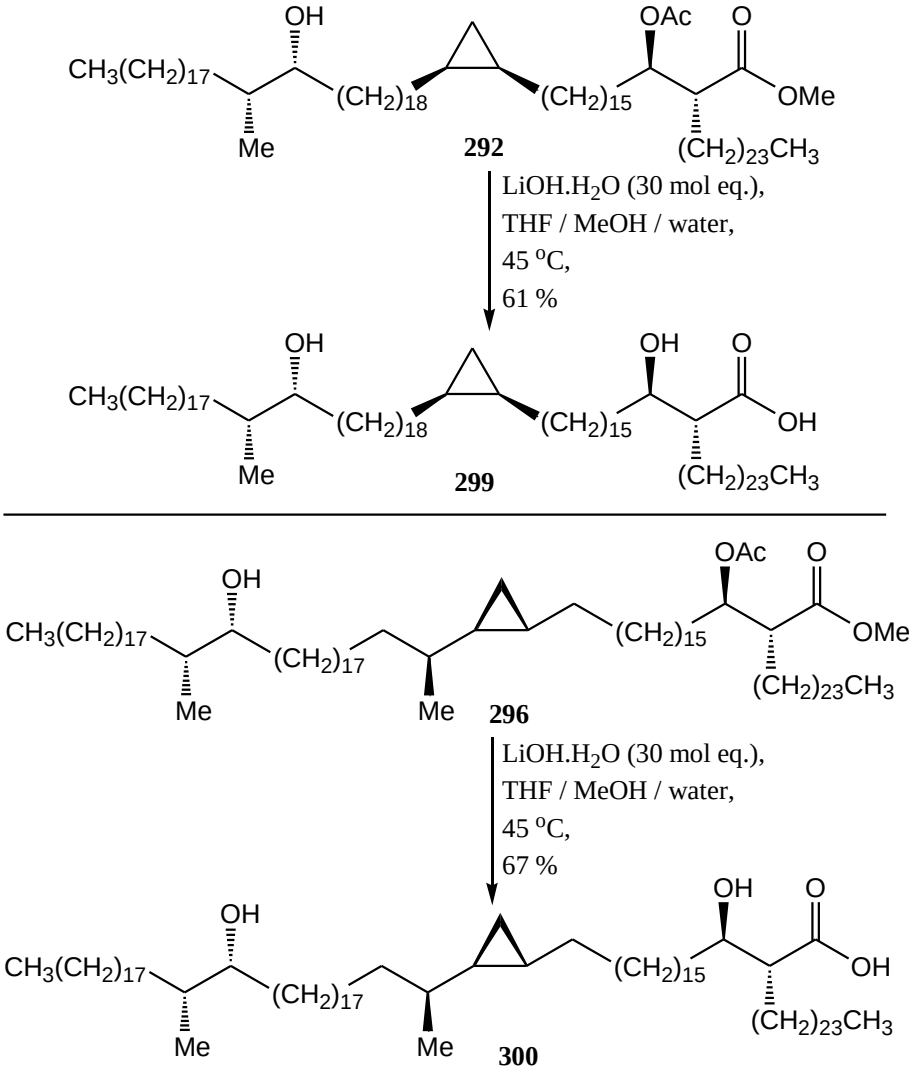
Another method was attempted to obtain free hydroxy mycolic acid without epimerisation. Sodium carbonate, which is a weak base, was used instead of lithium hydroxide, which is a strong base. Excess of K_2CO_3 was added to a solution of the protected compound (**297**) in THF, MeOH and water and stirred at 60 ^oC for 18 hours. TLC indicated that the acetyl group was deprotected, but the methyl on the ester remained. The reaction was worked up to obtain the mycolic acid (**298**) in 81 % yield (**Scheme 95**). The NMR spectra showed no signals for the acetyl group and proton NMR showed a singlet at δ 3.72 for the methyl protons of the ester. The specific rotation, $[\alpha]_D^{24}$ = + 3.0 (*c* 1.0, $CHCl_3$), confirmed that epimerisation did not occur during the reaction. However, the target free acid could not obtain.

In the literature,[216] there is a mild method for the hydrolysis of the ester: enzymatic hydrolysis. A model compound was treated with lipase (PPL, type II, crude) and lipase (from candida rugosa) in different buffer solution, but unfortunately no desired free acid was obtained. Hydrolysis of the keto-mycolic acids without any epimerisation could not be achieved in this work and it was left for future work.



Scheme 95: ***Hydrolysis of the mycolic acid to free hydroxy methyl ester with Na_2CO_3***

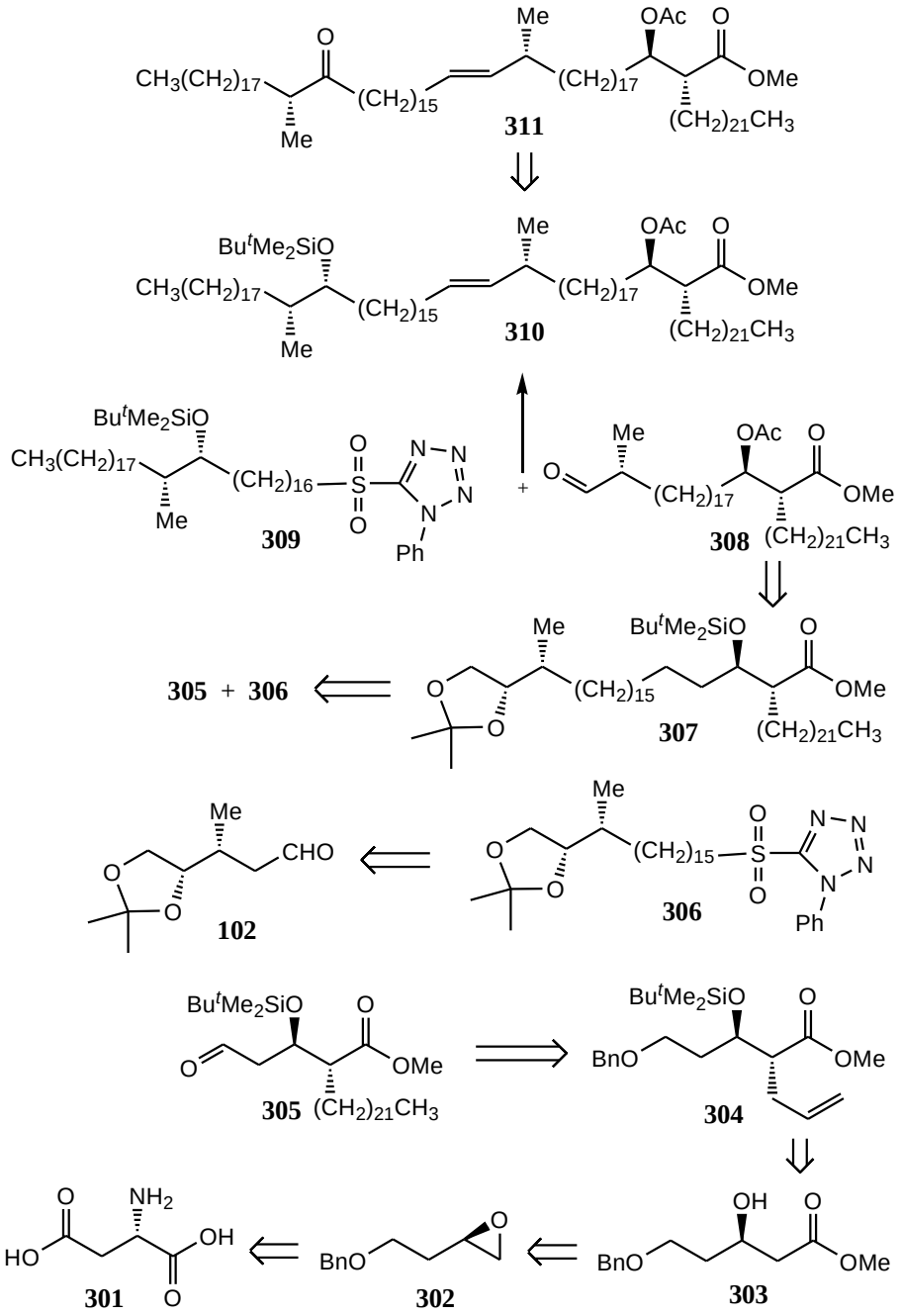
The protected hydroxy-mycolic acids (**292** and **296**) were also deprotected using LiOH to obtain the free hydroxy-mycolic acids (**299** and **300**) as shown in **Scheme 96**. The α-proton of the free acids (**299**) and (**300**) in the ^{1}H NMR spectrum appeared as a doublet of triplets at δ 2.46 (*J* 8.8, 5.4 Hz), while they appeared as a doublet of doublets of doublets for the protected compounds (**292**) and (**296**). The specific rotation of the deprotected hydroxy-mycolic acid (**299**) was $[\alpha]_D^{25}$ + 9.1 (*c* 0.6, $CHCl_3$) and protected hydroxy-mycolic acid (**292**) was $[\alpha]_D^{21}$ + 9.3 (c 0.95, $CHCl_3$), and also the $[\alpha]_D^{26}$ was + 11.4 (*c* 0.60, $CHCl_3$) for the deprotected hydroxy-mycolic acid (**300**), while the protected one (**296**) gave $[\alpha]_D^{21}$ + 10.0 (*c* 0.83, $CHCl_3$). These data showed that during the hydrolysis, the methyl adjacent to the hydroxyl group did not epimerise.



Scheme 96: ***Hydrolysis of the hydroxy-mycolic acids***

2.10 The synthesis of α-methyl-*trans*-alkene keto-mycolic acid

The α-methyl-*trans*-alkene keto-mycolic acid (**311**) was prepared by a similar method to that used to prepare the keto-mycolic acids (**293**) (p 116) and (**297**) (118). The corynomycolate moiety was prepared by a different method which started from L-aspartic acid. Stereoselective formation of the *trans*-alkene was the key stage which was achieved by a Julia reaction using potassium bis (trimethylsilyl) amide in 1,2-dimethoxyethane. The reactions are summarized in **Scheme 97**.



Scheme 97: ***Diagram of the preparation of α-methyl-trans-alkene keto-mycolic acid***

2.10.1 Preparation of the intermediate corynomycolate moiety

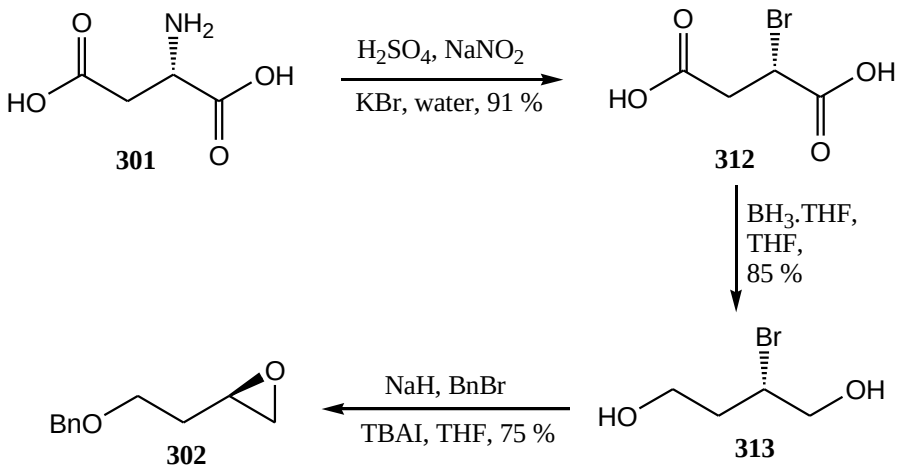
Preparation of the intermediate corynomycolate part is probably the most difficult part for the synthesis of the mycolic acids. In the previously discussed method (**Section 2.4.3**) (p 102), preparation of a large amount of β-hydroxy ester (**274**) was difficult. Moreover, the yield of *anti*-alkylation of the β-hydroxy ester was low. Therefore, a new process was carried out to obtain the β-hydroxy ester. In this process, the chiral centre was present in the initial starting material, naturally occurring L-aspartic acid (**301**), while the previous one had been obtained through asymmetric synthesis.

2.10.1a The preparation of a chiral epoxide

(2-Benzyloxyethyl)oxirane have been widely utilised as a chiral building block in the synthesis of numerous biologically important compounds.[140] Frick *et al*. developed a method for preparation of (*R*)-(2-benzyloxyethyl)oxirane (**302**) starting from commercially available L-aspartic acid (**301**) in 3 steps with 65 % overall yield and more than 99 % enantiomeric purity.[140] This method was chosen for the synthesis of the epoxide (**302**).

The first step was the conversion of the α-amino acid (**301**) into α-bromo succinic acid (**312**) with retention of configuration. The product (**312**) was readily formed from the amino acid (**301**) by treatment with potassium bromide (4.5 mol eq.) in the presence of sodium nitrite (1.8 mol eq.) in an aqueous solution of sulphuric acid (2.5 M) with 91 % yield (**Scheme 98**). For the success of the reaction, the concentration of sulphuric acid was critical. If this was too high, the salt precipitated making the reaction impossible to accomplish.

The second step was the reduction of the diacid (**312**) into the diol (**313**) using BH_3.THF (3 mol eq.) in THF. The reagent was added to a stirred solution of the diacid (**312**) in THF at 0 ^oC and then stirred at room temperature for 5 hours to obtain the diol (**313**) in 85 % yield (**Scheme 98**). The borane is a mild reducing reagent which was able to reduce the two carboxylic acids into diols, while leaving the bromine group unchanged.

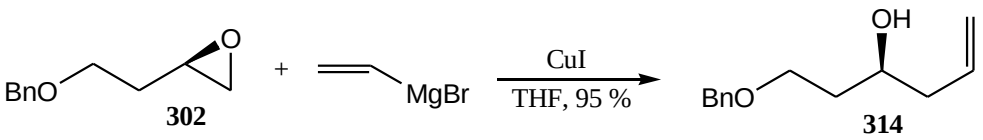


Scheme 98: ***Formation of the chiral epoxide***

The third and final step was the formation of the epoxide. The (*R*)-(2-Benzyloxyethyl)-oxirane (**302**) was obtained from the diol (**313**) by treatment first with sodium hydride (3 mol eq.) in THF, followed by addition of benzyl bromide (1.1 mol eq.) in order to obtain the simultaneous protection of the other hydroxyl group. The reaction was conducted in THF using tetra-n-butylammonium iodide (0.1 mol eq.), which is a standard catalyst for the benzylation of an alcohol in an organic solvent. The purified epoxide (**302**) showed the same NMR spectra as reported in the literature.[140] The specific rotation was measured as $[\alpha]_D^{23}$ = + 17.8 (*c* 1.42, $CHCl_3$), while the same epoxide was reported as showing $[\alpha]_D^{22}$ = + 15.0 (*c* 3.37, CH_2Cl_2)[140] and as + 16.9 (*c* 2.51, $CHCl_3$).[217]

2.10.1b The ring opening of the epoxide

The target of this work was the synthesis of the β-hydroxy ester (**303**) from the epoxide (**302**). To achieve this, the next step was the regioselective ring opening of the epoxide by vinylmagnesium bromide. Nucleophilic attack of a Grignard reagent to an epoxide is favoured at the less substituted carbon, since this is the more accessible position. The reaction was started at – 75 ^oC in the presence of catalytic copper (I) iodide in THF and then allowed to reach – 20 ^oC to obtain unsaturated alcohol (**314**)[218,219] with an excellent yield of 95 % (**Scheme 99**).



Scheme 99: ***Grignard reaction of the epoxide***

The ^{1}H NMR spectrum of compound (**314**) showed a multiplet at δ 7.38–7.28 for the five aromatic protons and a broad triplet at δ 4.54 (*J* 12.0 Hz) for the two benzyl protons. The alkene proton next to -CH_2- appeared as a clear doublet of doublets of triplets at δ 5.85 (*J* 17.4 (*trans*), 10.1 (*cis*), 7.3 (vicinal) Hz) as shown in **Figure 33**, and the two terminal alkene protons appeared as a multiplet at δ 5.14–5.10. The proton next to the hydroxyl group appeared as a multiplet at δ 3.91–3.87 and one of the two protons next to the benzyloxy group appeared as a doublet of triplets at δ 3.73 (*J* 9.5 (geminal), 5.4 (vicinal) Hz) and the other one as a doublet of doublets of doublets at δ 3.66 (*J* 9.5 (geminal), 7.3 (vicinal), 5.4 (vicinal) Hz). The ^{13}C NMR spectrum showed four aromatic signals at δ 138.0, 128.4, 127.7 and 127.6; two olefinic signals at δ 134.9 and 117.6 (terminal carbon); one signal at δ 70.3 for the carbon next to the hydroxyl group; two signals at δ 73.3 for Ph$\underline{C}H_2$O- and at δ 68.9 for -O$\underline{C}H_2CH_2$-. IR showed a broad peak at 3425 cm^{-1} for the O–H stretch. All these data confirmed the success of the reaction. Moreover, the specific rotation, $[\alpha]_D^{24}$ = – 5.3 (*c* 1.2, $CHCl_3$), of the product also confirmed that the reaction was stereocontrolled.

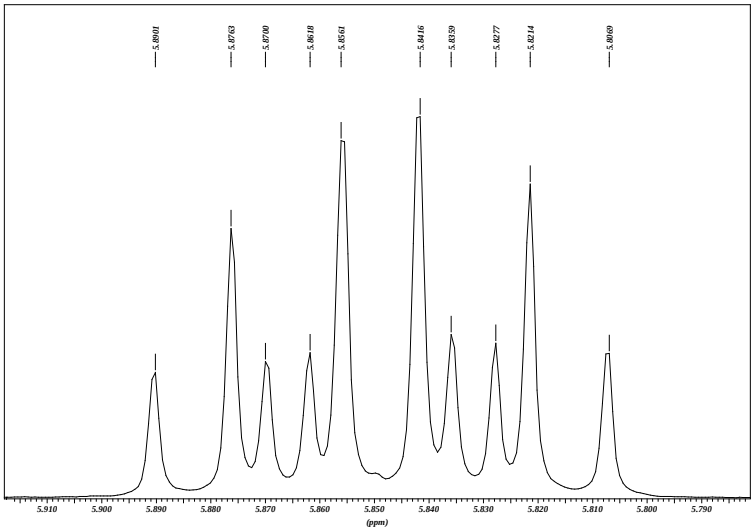
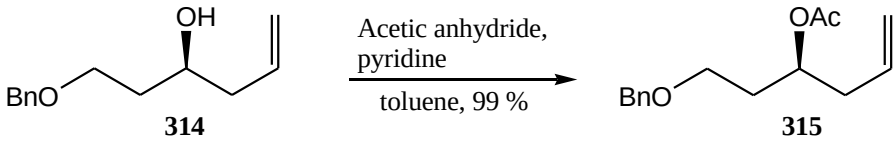


Figure 33: ***The signals of the alkene proton adjacent to CH_2***

2.10.1c Acetylation of the alcohol

The alkene group of (**314**) was to be oxidised to a carboxylic acid. Therefore, the hydroxyl group was protected to prevent further oxidation. The acetyl protecting group was chosen as it is cheap and easy to handle. The alcohol (**314**) was treated with acetic anhydride and anhydrous pyridine in toluene to give the protected compound (**315**) with an excellent yield (99 %) (**Scheme 100**). The acetylation was proved by the proton NMR spectrum which showed a singlet at δ 2.00 for the acetyl protons. The proton next to acetoxy group was shifted 5.10 and was not clearly identified, because it was hidden among the signals of the two terminal alkene protons. The specific rotation was quite high; $[\alpha]_D^{23} = +49.0$ (*c* 1.13, $CHCl_3$).



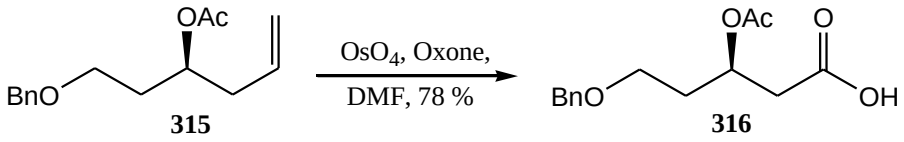
314 **315**

Scheme 100: ***Acetylation of the hydroxyl group***

2.10.1d Oxidative cleavage of the alkene to carboxylic acid

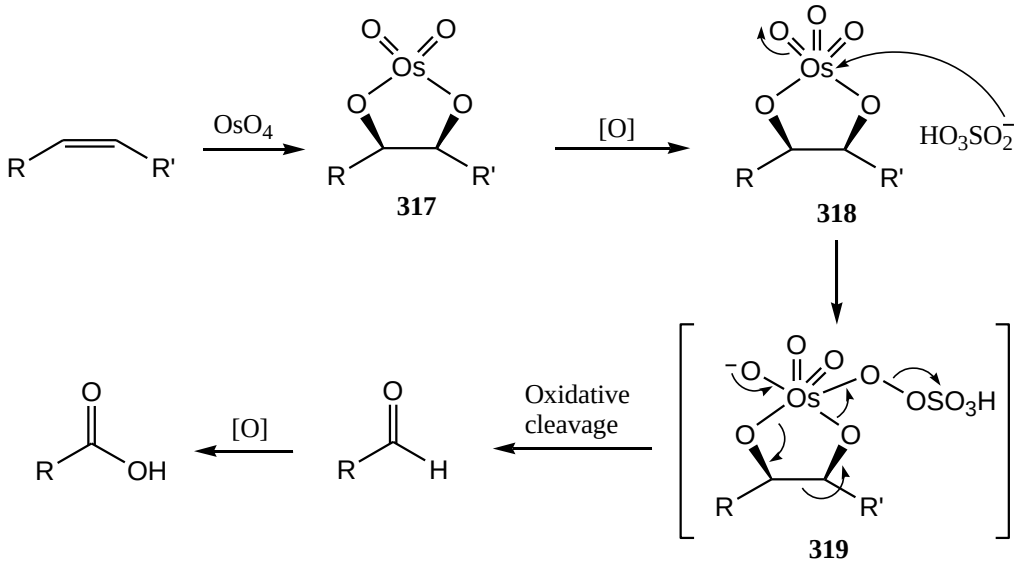
The target of this step was conversion of the alkene group to a methyl ester. There is a literature method for this direct conversion.[220] The ozonolysis of an alkene in methanolic sodium hydroxide in dichloromethane as co-solvent leads directly to methyl ester. This method was applied to the alkene (**314**). Unfortunately, after attempting it three times the desired methyl ester could not be obtained. Recently, another method was published for the oxidative cleavage of alkenes to a carboxylic acid.[221] This method is a mild alternative to ozonolysis. Oxone is used as the co-oxidant for oxidative cleavage of olefins with OsO_4 in DMF to give the carboxylic acids. Fortunately, this method was successful. The alkene (**315**) was treated with oxone (4 mol eq.) and OsO_4 (0.01 mol eq.) in DMF at 10 ^oC and the temperature was allowed to reach 32 ^oC to give the desired carboxylic acid (**316**) as shown in **Scheme 101**. The two protons at the α-position to the carboxylic acid appeared in the ^1H NMR spectrum as two doublets of doublets at δ 2.71 (*J* 5.7, 15.8 Hz) and 2.69 (*J* 6.9, 16.1 Hz), and the proton next to acetoxy group appeared as a quintet at δ 5.36 (*J* 6.3 Hz). The ^{13}C NMR spectrum showed a signal at δ 175.4 for the carboxylic acid carbon. The

IR spectrum showed a peak at 1740 cm^{-1} for the C=O stretch of the acetyl group and another at 1680 cm^{-1} for the C=O stretch of the newly formed carboxylic acid.



Scheme 101: ***Oxidative cleavage of the alkene to carboxylic acid***

The mechanism of the oxidative cleavage of olefins with OsO_4 and oxone is not certain, but Borhan *et al.*[221] proposed one. Osmate (**317**) is oxidized by oxone to form (**318**), which is subsequently attacked by the same oxone to yield intermediate (**319**). Fragmentation of (**319**) re-generates OsO_4 and produces two aldehydes, which can undergo further oxidation to yield carboxylic acids (**Scheme 102**).

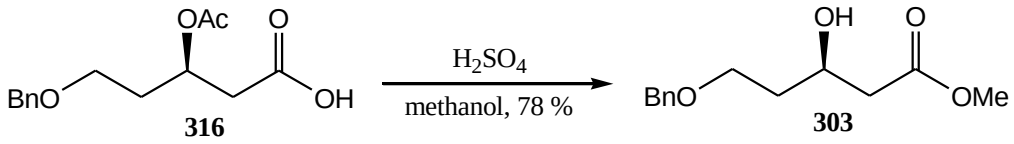


Scheme 102: ***Proposed mechanism of the oxidative cleavage of alkenes with OsO_4 and oxone***

2.10.1e Esterification of the carboxylic acid

Finally, the desired β-hydroxy methyl ester (**303**) was obtained from the acid (**316**) in one step. Refluxing the β-protected carboxylic acid (**316**) in methanol (300 ml) in the presence of con.H_2SO_4 (70 drops) gave the β-hydroxy methyl ester (**303**) with 78 %

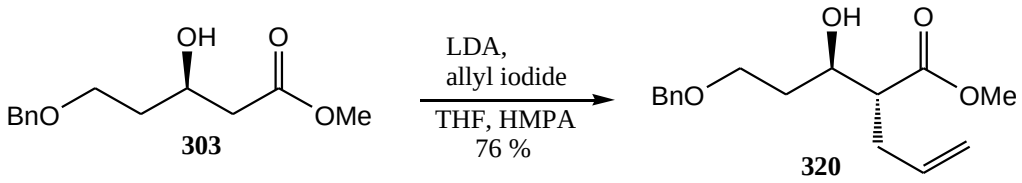
yield (**Scheme 103**). In the ^{1}H NMR spectrum, the two protons at the α-position appeared as a doublet at δ 2.52 (*J* 6.3 Hz). They were expected to show two doublets of doublet as in compound (**274**) (p 106), but because these two protons coincidentally have the same chemical shift the geminal coupling did not appear. The specific rotation of the methyl ester was $[\alpha]_D^{26} = -12.2$ (*c* 1.23, $CHCl_3$) and it was the same as the other β-hydroxy methyl ester (**274**) (– 12.3) (p 106). In the literature, it was reported as – 11.5.[141]



Scheme 103: ***Formation of the β-hydroxy methyl ester (303)***

2.10.1f The Fräter alkylation

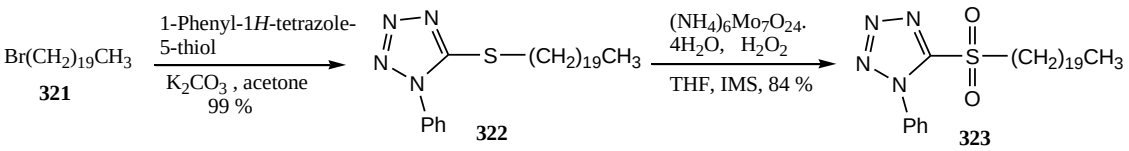
Treatment of the β-hydroxy methyl ester (**303**) with LDA (2.15 mol eq.) in THF, followed by a solution of allyl iodide (1.5 mol eq.) in HMPA (2 mol eq.) and THF led to the *anti*-alkylated product (**320**) (**Scheme 104**). The best yield of 76 % was greater than the yield (60 %) found for (**278**) (p 108). The ^{1}H NMR spectrum showed a doublet of doublets of triplets at δ 5.75 (*J* 17.0 (*trans*), 10.1 (*cis*), 6.9 (*vicinal*) Hz) for the alkene proton next to -CH_2- and a multiplet at δ 5.12–5.03 for the terminal alkene protons. The ^{13}C NMR spectrum showed a signal at δ 134.9 for the carbon –$\underline{C}H=CH_2$ and another signal at δ 117.1 for the –$CH=\underline{C}H_2$. The specific rotation of (**320**) was $[\alpha]_D^{21} = -6.9$ (*c* 1.09, $CHCl_3$), while the enantiomer of it, methyl (2*S*, 3*R*)-2-allyl-4-(*tert*-butyldimethylsilyloxyl)-3-hydroxylbutanoate, was reported as showing $[\alpha]_D^{20} = +6.5$ (*c* 1.0, CH_2Cl_2).[222]



Scheme 104: ***The Fräter alkylation***

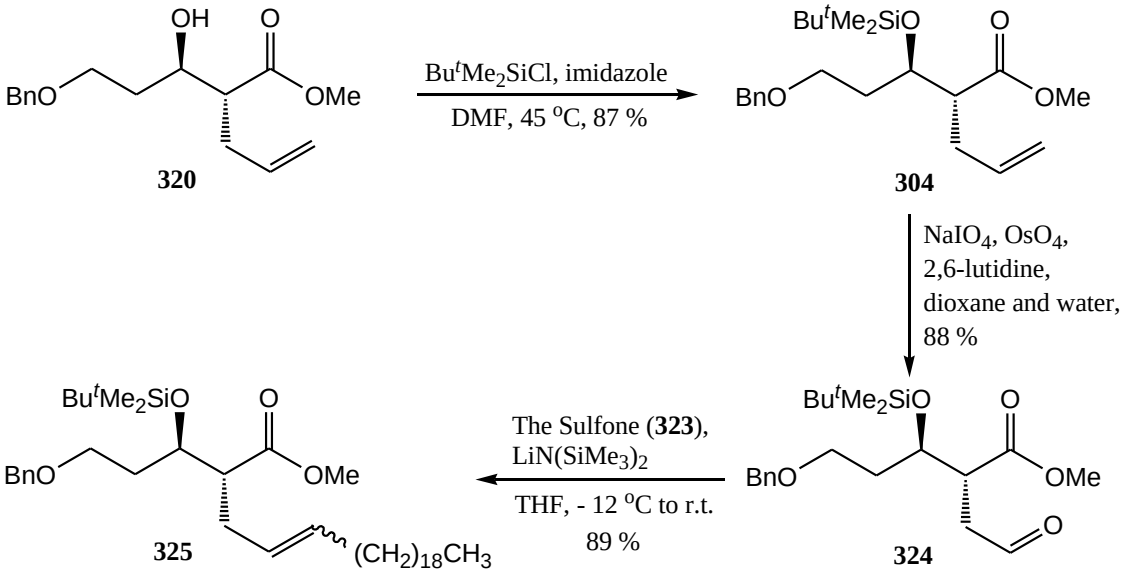
2.10.1g The chain extension

To complete the chain extension of the corynomycolate moiety at the α-position, the sulfone (**323**) was prepared for a coupling reaction (**Scheme 105**) by the same method mentioned in section **2.4.3h** (p 110) starting from 1-bromoeicosane (**321**).



Scheme 105: ***Preparation of the sulfone (323)***

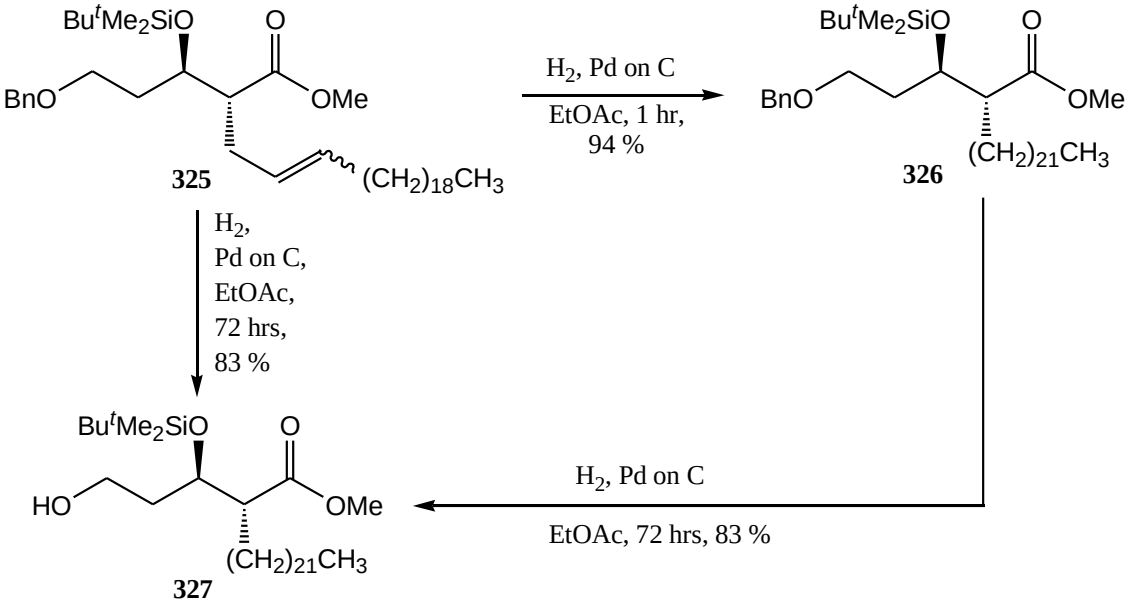
The secondary alcohol (**320**) was protected with a *tert*-butyldimethylsilyl group at 45 ^{o}C to give the product (**304**), followed by oxidative cleavage of olefin (**304**) with OsO_4–$NaIO_4$ and 2,6-lutidine in dioxane-water to give the aldehyde (**324**) in 88 % yield (**Scheme 106**). In the ^{1}H NMR spectrum, the aldehyde proton appeared as a singlet at δ 9.81 and in the carbon NMR spectrum, the aldehyde carbon appeared at δ 200.4. Coupling reaction of the aldehyde (**324**) with the sulfone (**323**) in the presence of lithium bis(trimethylsilyl) amide in THF gave the chain extended alkene compound (**325**) in 89 % yield as a mixture of *E*- and *Z*-isomers in a ratio 2:1 (**Scheme 106**).



Scheme 106: ***The chain extension***

2.10.1h Hydrogenation and debenzylation

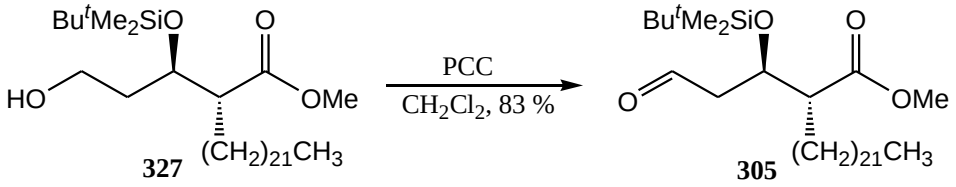
To complete the chain extension, the alkene (**325**) was hydrogenated by hydrogen gas using Pd on C as a catalyst in ethyl acetate as shown in **Figure 107**. With a reaction time of one hour, the hydrogenated benzyl compound (**326**) was obtained, but, if the reaction time was 3 days, the saturated and debenzylated compound (**327**) was obtained in 83 % yield. The alcohol (**327**) did not show clear NMR spectra in $CDCl_3$; there were some extra signals. However, the spectrum was clear in C_6D_6. In the proton NMR, there was a doublet of triplets at δ 4.24 (*J* 4.1, 6.6 Hz) for the proton adjacent to the *tert*-butyldimethylsilanyloxy group and a doublet of doublets of doublets at δ 2.79 (*J* 3.8, 6.3, 10.1 Hz) for the proton at the α-position. In the carbon NMR, there were signals at δ 71.7 for the carbon next to the silyl protecting group and δ 59.0 for the carbon next to the hydroxyl group. The specific rotation of this alcohol (**327**) was also measured in benzene, $[\alpha]_D^{22} = -8.3$ (*c* 0.4, C_6H_6), since it was not stable in $CHCl_3$.



Scheme 107: ***Hydrogenation and debenzylation***

2.10.1i Oxidation of the alcohol

The alcohol (**327**) was oxidised to corresponding aldehyde (**305**) as shown in **Figure 107** for a coupling reaction to extend the chain. The NMR spectrum was as expected and the specific rotation was measured as $[\alpha]_D^{26} = -5.0$ (*c* 1.23, $CHCl_3$).

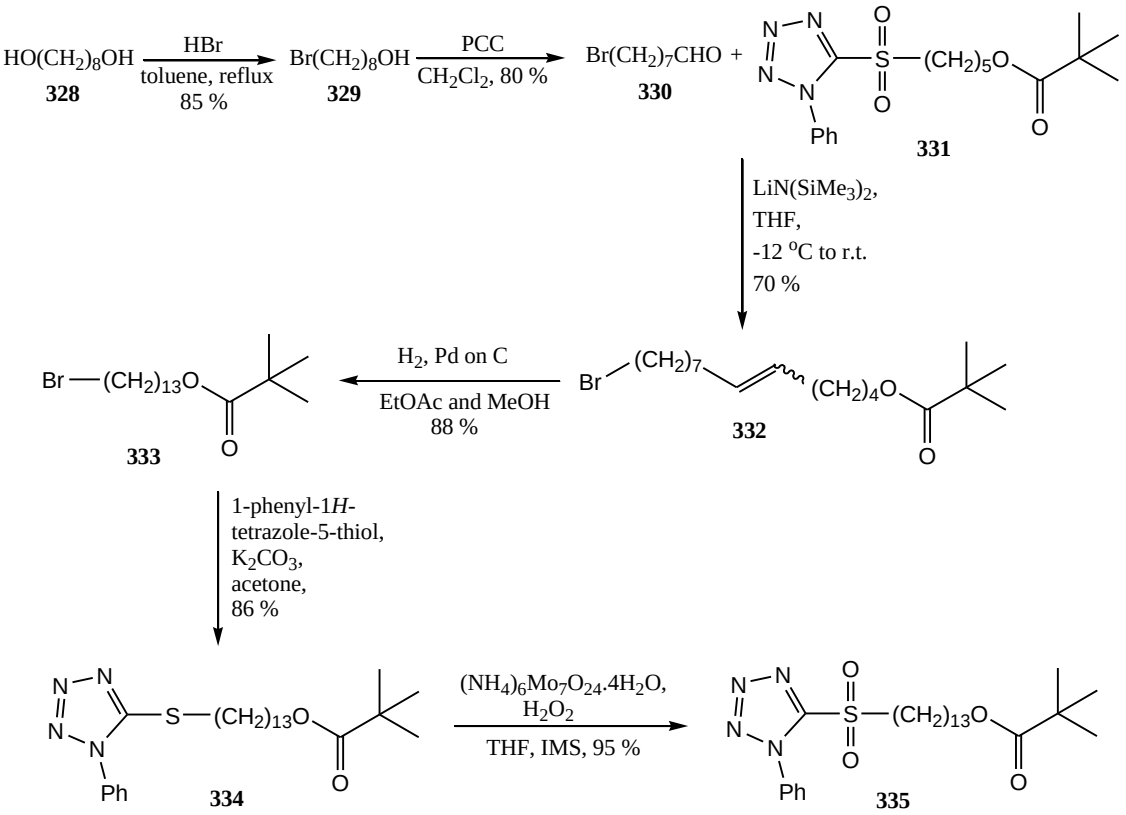


327 **305**

Scheme 107: ***Oxidation of the alcohol***

2.10.2 Preparation of a thirteen carbon chain sulfone

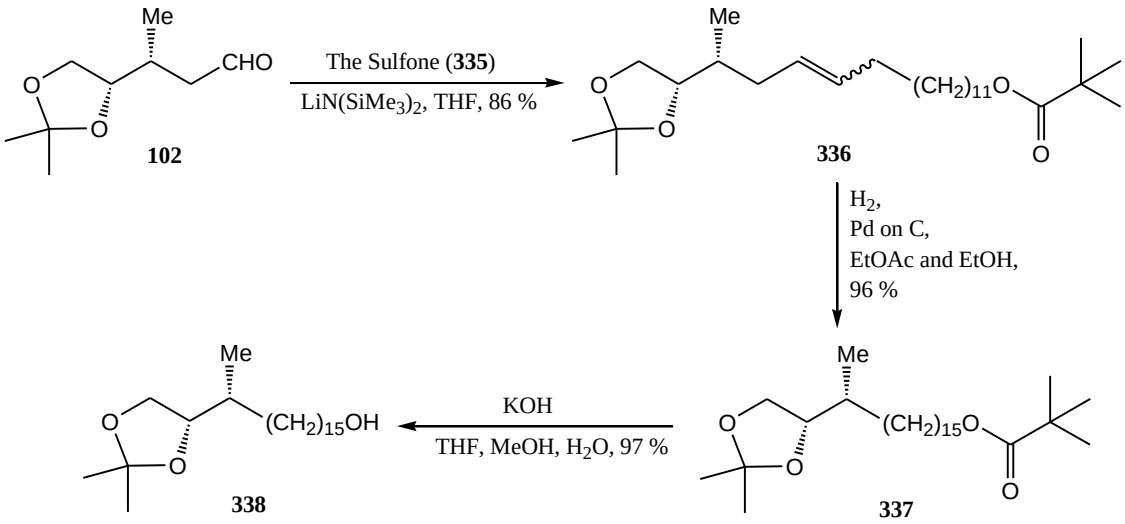
For the extension of chain, it was necessary to prepare a thirteen carbon chain which is not available from common sources. The Julia coupling reaction is one of methods for chain extension, and this was chosen. An eight carbons aldehyde was coupled with a five carbons sulfone to complete the chain. 1,8-Octanediol (**328**) was monobrominated to 8-bromo-octan-1-ol (**329**) with HBr in toluene, followed by oxidation of the alcohol to the corresponding aldehyde (**330**) using PCC in dichloromethane. The aldehyde (**330**) was then coupled with the sulfone (**331**), prepared by the literature method,[89] in the presence of lithium bis(trimethylsilyl) amide in THF to give the alkene (**332**) as a mixture of *E* and *Z*-isomers in 70 % yield (**Scheme 108**). The alkene (**332**) was hydrogenated to give the product (**333**) to complete formation of the thirteen carbon chain (**Scheme 108**). The bromo compound (**333**) was reacted with 1-phenyl-1*H*-tetrazole-5-thiol to give the sulfane (**334**). Finally, the sulfane (**334**) was oxidised with hydrogen peroxide to the corresponding sulfone (**335**) in 95 % yield (**Scheme 108**). The characteristic sulfone protons in the ^{1}H NMR spectrum appeared as two multiplets at δ 7.71–7.68 and 7.64–7.27 for the aromatic protons, and another multiplet at δ 3.74–3.71 for the two protons next to the sulfonyl group. The other protons gave signals as follows; the two protons next to pivalate ester appeared as triplet at δ 4.04 (*J* 6.6 Hz) and the pivalate ester protons appeared as a singlet at δ 1.19. The long chain protons appeared between δ 1.52–1.26.



Scheme 108: ***Preparation of thirteen carbon chain sulfone***

2.10.3 The chain extension

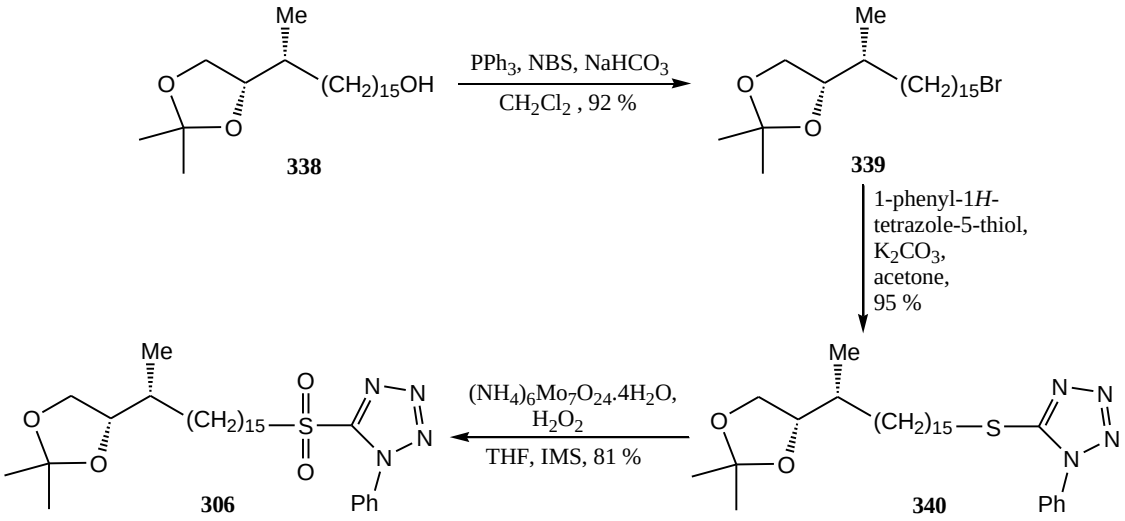
The aldehyde (**102**), prepared as discussed in section **2.2.1** (p 36), was used the source of the α-methyl *trans*-alkene unit. Simply, this aldehyde chain was extended and converted to an intermediate sulfone for a coupling reaction with the early mentioned aldehyde (**305**) (p 137). A Julia reaction of the aldehyde (**102**) with sulfone (**335**) gave the alkene (**336**) as a mixture of *E* and *Z*-isomers in 86 % yield, followed by hydrogenation with hydrogen gas in the catalyst of Pd on C in ethyl acetate and ethanol. Removal of the protecting pivalate ester of the product (**337**) with potassium hydroxide (15 mol eq.) in THF / MeOH / water (10:10:1) led to the alcohol (**338**) in an excellent yield, 97 % (**Scheme 109**). The ^{1}H NMR spectrum in C_6D_6 showed a triplet at δ 3.41 (*J* 6.6 Hz) for the two protons adjacent to the hydroxyl group. The IR spectrum showed a broad peak at 3395 cm^{-1} for the O–H stretch.



Scheme 109: ***A chain extension of the aldehyde (102)***

2.10.4 Preparation of the intermediate sulfone

The alcohol (**338**) was converted into the bromo compound (**339**) using NBS and PPh_3 in dichloromethane, while $NaHCO_3$ was added to the reaction mixture to neutralise the acidity since the acetal protecting group is very acid sensitive. The bromo compound (**339**) was then reacted with 1-phenyl-1*H*-tetrazole-5-thiol to form the sulfane (**340**). Finally, the sulfane was oxidised to the corresponding sulfone (**306**) with hydrogen peroxide as shown in **Scheme 110**. The overall yield of this conversion was 70 %.

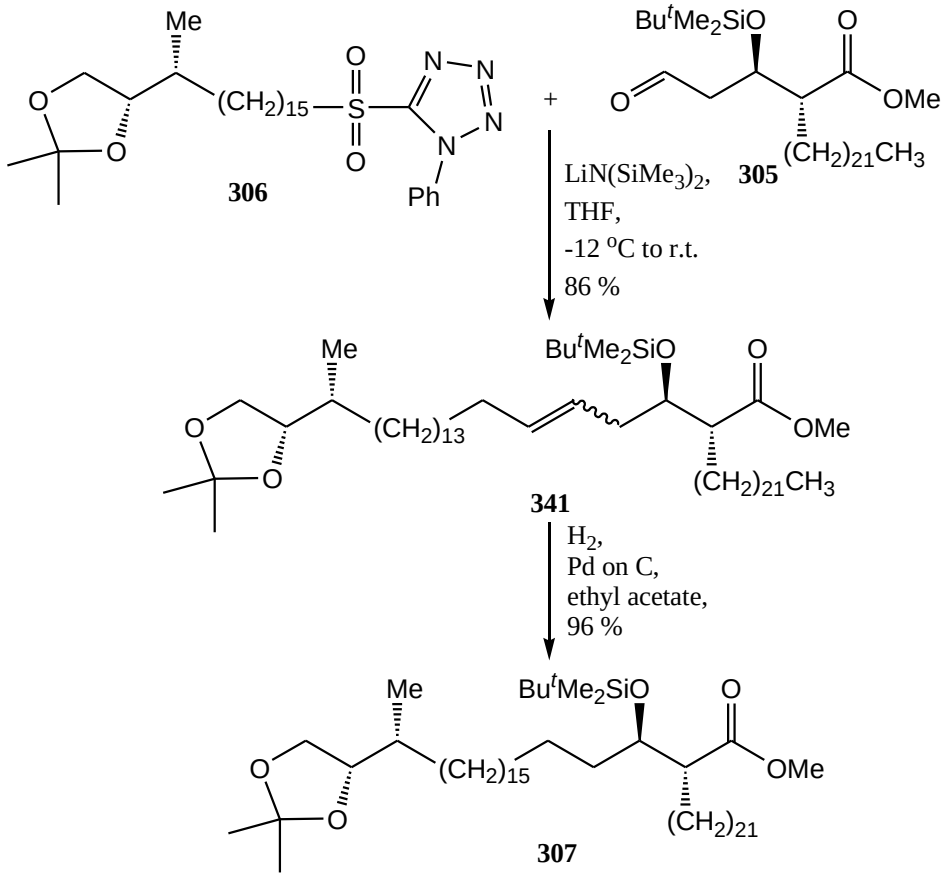


Scheme 110: ***Preparation of the sulfone (306)***

The protecting acetal group remained during the oxidation, which was confirmed by the NMR spectra. The ^{1}H NMR spectrum of the sulfone (**306**) showed a multiplet at δ 3.75–3.72 for the two protons next to the sulfonyl group, a doublet at δ 0.96 (*J* 6.6 Hz) for the chiral methyl protons and two singlet at δ 1.41 and 1.35 for the two methyls on the acetal protected group. The acetal carbon signal appeared in the carbon NMR spectrum at δ 108.5.

2.10.5 The coupling reaction

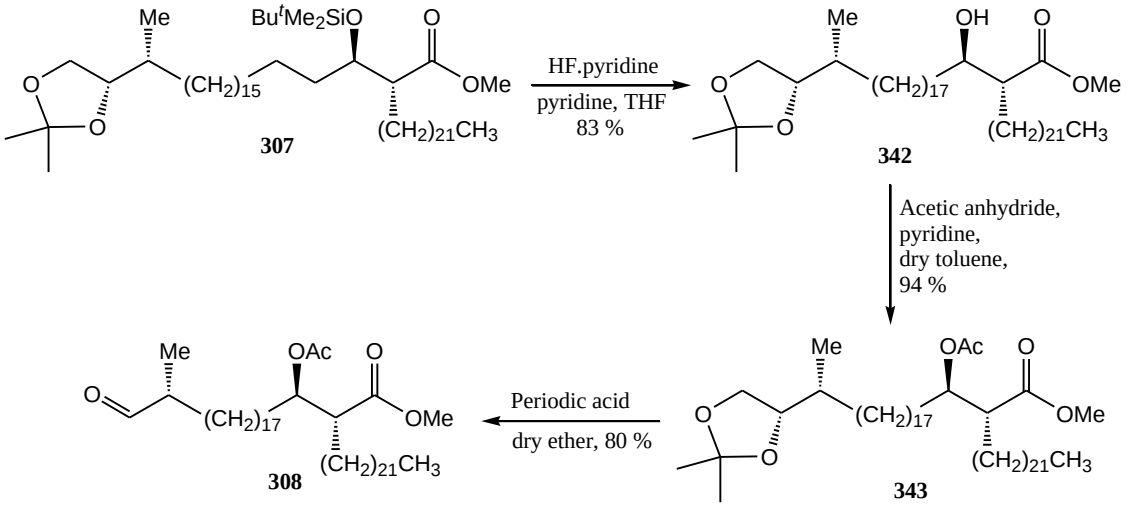
This step involved the coupling of the corynomycolate moiety with the long chain containing the methyl group which would finally be next to the *trans*-alkene unit. The aldehyde (**305**), was coupled with the sulfone (**306**) in the presence of lithium bis(trimethylsilyl) amide in THF to give the alkene (**341**) as a mixture of *E* and *Z*-isomers in ratio 2.2:1 in 70 % yield (**Scheme 111**). The alkene was then hydrogenated with hydrogen gas using Pd on C as a catalyst in ethyl acetate to give the saturated product (**307**) in 96 % yield. The NMR spectra were as expected.



Scheme 111: ***The coupling reaction***

2.10.6 Preparation of the intermediate aldehyde

One of intermediates for formation of the α-methyl-*trans*-alkene unit was α-methyl aldehyde (**308**). This aldehyde was prepared by further reaction of product (**307**). The silyl protecting group at this step had to be changed to an acetyl group, since the silyl ether is already present on the intermediate (**309**) (p 142). The strong deprotection reagent HF.pyridine complex was applied to remove the sterically hindered *tert*-butyldimethylsilyl. The product, secondary alcohol (**342**), was obtained in 83 % yield and there was a very small amount of product deprotected at the cyclic acetal group. The IR of (**342**) showed a peak at 3520 cm^{-1} to confirm the formation of the O–H bond. The alcohol was protected as an acetoxy group (**343**) using acetic anhydride and anhydrous pyridine in dry toluene in 94 % yield. Finally, oxidative cleavage of the cyclic acetal group (**343**) using periodic acid in dry ether led to the desired aldehyde (**308**) in 80 % yield (**Scheme 112**).

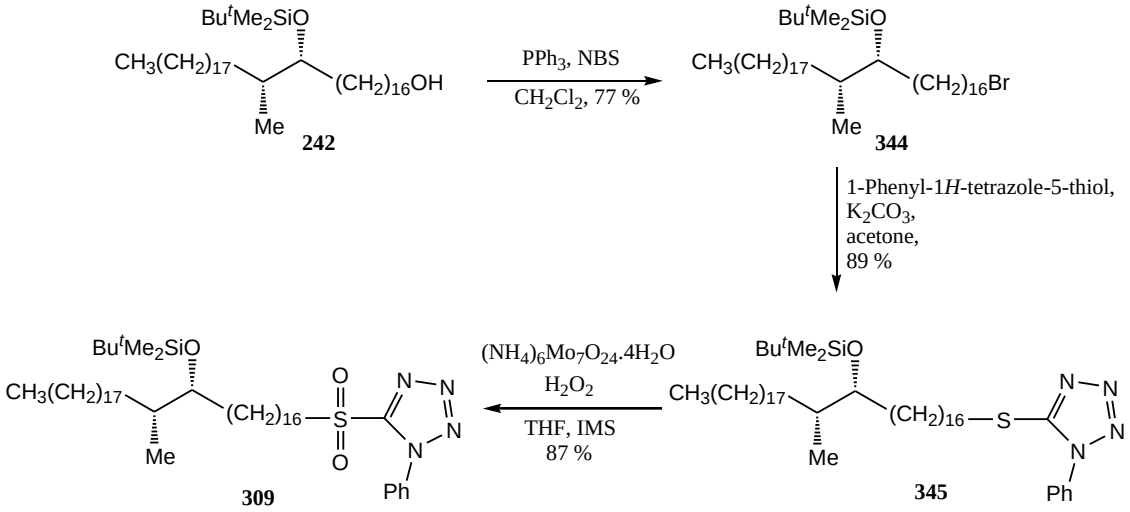


Scheme 112: ***The synthesis of α-methyl aldehyde***

There was no sign in the NMR spectra of epimerisation of the methyl next to the aldehyde. The ^{1}H NMR spectrum showed a doublet at δ 9.62 (*J* 1.9 Hz) for the aldehyde proton, a double sextet at δ 2.33 (*J* 1.9, 7.0 Hz) for the proton next the aldehyde proton and a doublet at δ 1.09 (*J* 7.0 Hz) for the methyl protons at the α-position to the aldehyde. The ^{13}C NMR spectra showed a signal at δ 205.4 for the carbonyl carbon of the aldehyde, a signal at δ 46.3 for the carbon next to the aldehyde and a signal at δ 13.3 for the carbon of methyl at the α-position to the aldehyde.

2.10.7 Preparation of the intermediate sulfone

Another intermediate for formation of the α-methyl-*trans*-alkene unit was the sulfone (**309**). The alcohol (**242**) (p 87) was the right chain length to start for preparation of this sulfone.[47] This alcohol was converted to bromo compound (**344**) with NBS and PPh_3 in dichloromethane, followed by the reaction with 1-phenyl-1*H*-tetrazole-5-thiol to form the sulfane (**345**). The sulfane was then oxidised to the desired sulfone (**309**) with hydrogen peroxide as shown in **Scheme 113**. The overall yield of this conversion was 60 %. The molecular rotation confirmed that during those reactions the stereochemistry of the chiral centres remained. Since the molecular rotation of the alcohol (**242**) was calculated as + 39, and it was calculated for the sulfone (**309**) as + 35.

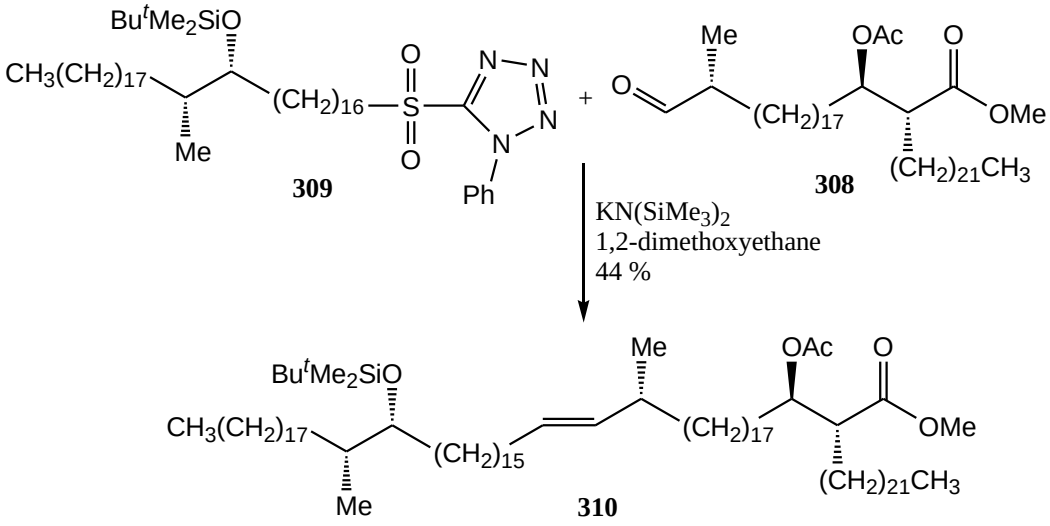


Scheme 113: ***Preparation of the intermediate sulfone (309)***

2.10.9 Final coupling reaction to form *trans*-alkene

Studies showed that the stereoselectivity and yield of the modified Julia olefination is sensitive to the base used to deprotonate the sulfone and solvent polarity.[223,224] A noteworthy new development revealed that 1-phenyl-1*H*-tetrazole sulfones (**155**) (p 42) can give great stereoselectivity (*E*-isomers) with potassium bis (trimethylsilyl) amide as base and 1,2-dimethoxyethane as solvent.[170] Recent studies supported this discovery that the stereoselectivity could lead only to *E*-isomer especially if the sulfone or aldehyde was α-substituted.[225,226]

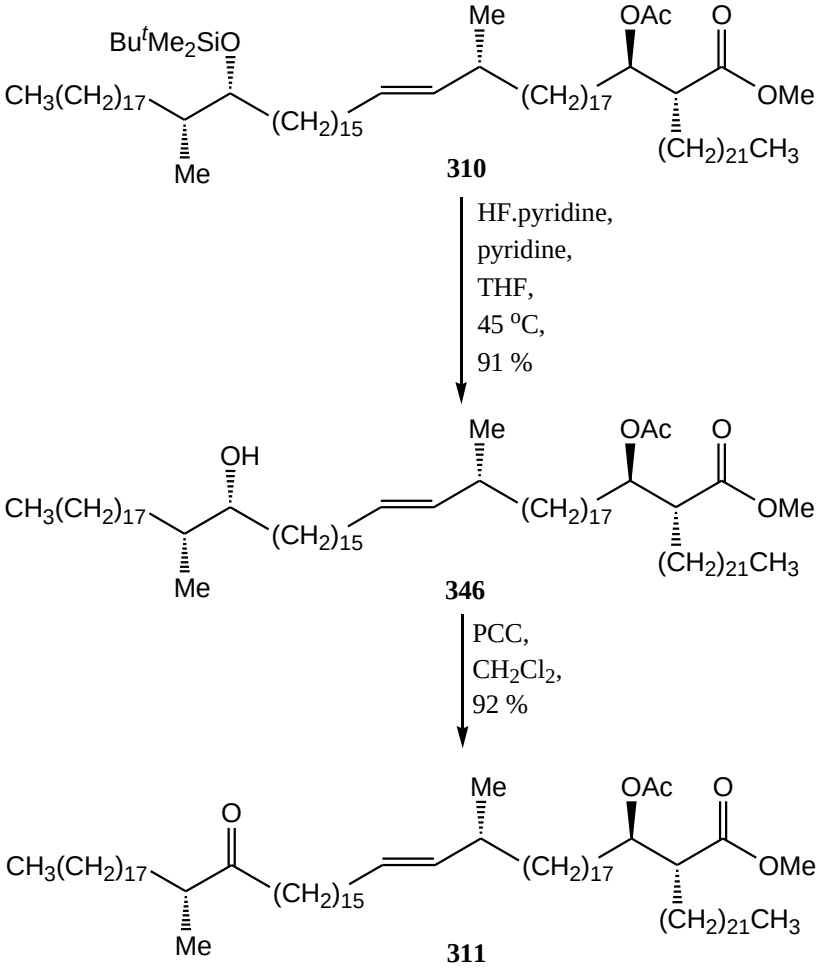
A coupling reaction between the aldehyde (**308**) and the sulfone (**309**) using potassium bis(trimethylsilyl)amide in 1,2-dimethoxyethane gave the desired alkene (**310**) as only *E*-diastereomer in 44 % yield and this coupling gave the whole structure of the α-methyl-*trans*-alkene mycolic acid (**Scheme 114**). The stereoselectivity as *E*-isomer was confirmed by NMR. The ^{1}H NMR spectrum showed a triplet of doublets at δ 5.33 (*J* 6.6, 15.1 Hz) and a doublet of doublets at δ 5.24 (*J* 7.6, 15.1 Hz) for the alkene protons. The coupling constant of 15.1 Hz between the olefinic hydrogen atoms confirmed the formation of the *trans*-alkene. The ^{13}C NMR spectrum showed only two signals at the olefinic region at δ 136.5 and 128.4 for the *trans*-alkene carbons. The molecular rotation confirmed that during the reaction no epimerisation occurred; the molecular rotation of the product (**310**) was found to be + 60 and it was so close to sum of the aldehyde (**308**) (+ 24) and sulfone (**309**) (+ 35). Moreover, in the literature,[225] an α-methyl substituted aldehyde was coupled with the sulfone under the same conditions as explained above without any epimerisation.



Scheme 114: ***A coupling reaction to form trans-alkene***

2.10.10 Final steps to achieve the α-methyl-*trans*-alkene keto-mycolic acid

The *tert*-butyldimethylsilyl protecting group of the compound (**310**) was finally removed with HF.pyridine in pyridine and THF at 45 ^{o}C to give the uncommon α-methyl-*trans*-alkene hydroxy-mycolic acid (**346**) in very good yield 91 %. The specific rotation of this hydroxy-mycolic acid was measured as $[\alpha]_D^{24} = +4.9$ (*c* 0.85, $CHCl_3$). Finally, this hydroxy-mycolic acid (**346**) was oxidised with PCC in dichloromethane to give the target α-methyl-*trans*-alkene keto-mycolic acid (**311**) in very good yield (92 %) (**Scheme 115**). The specific rotation of this molecule was measured as $[\alpha]_D^{24} = -2.4$ (*c* 0.55, $CHCl_3$). The *trans*-alkene mycolic acids mainly occur in the *Mycobacterium marinum*,[78,227] but to obtain a single enantiomer is very difficult and at present there is not a natural sample to compare the specific rotation. It is believed that during hydrolysis of the methyl ester and acetyl protecting group, as discussed in section **2.9** (p 125), the α-methyl to the ketone would epimerise with strong base. Therefore, this keto-mycolic acid (**311**) has not been hydrolysied yet.



Scheme 115: ***Final reactions to achieve the target mycolic acid (311)***

The proton NMR spectrum (**Figure 34**) of the synthetic α-methyl-*trans*-alkene keto-mycolic acid (**311**) was identical to a very minor component in a natural mixture of keto-mycolic acids isolated from *M. tuberculosis* (**Figure 27**) (p 119). Also, the carbon NMR spectrum showed identical signals in the alkene region; the synthetic one gave signals at δ 136.43 and 128.39, and the natural one at δ 136.47 and 128.41. The MALDI-TOF molecular ion ($[M + Na]^+$: 1288.3) of (**311**) was identical to one component of the natural mixture ($[M + Na]^+$: 1288.5255, **Figure 30**) (p 123).

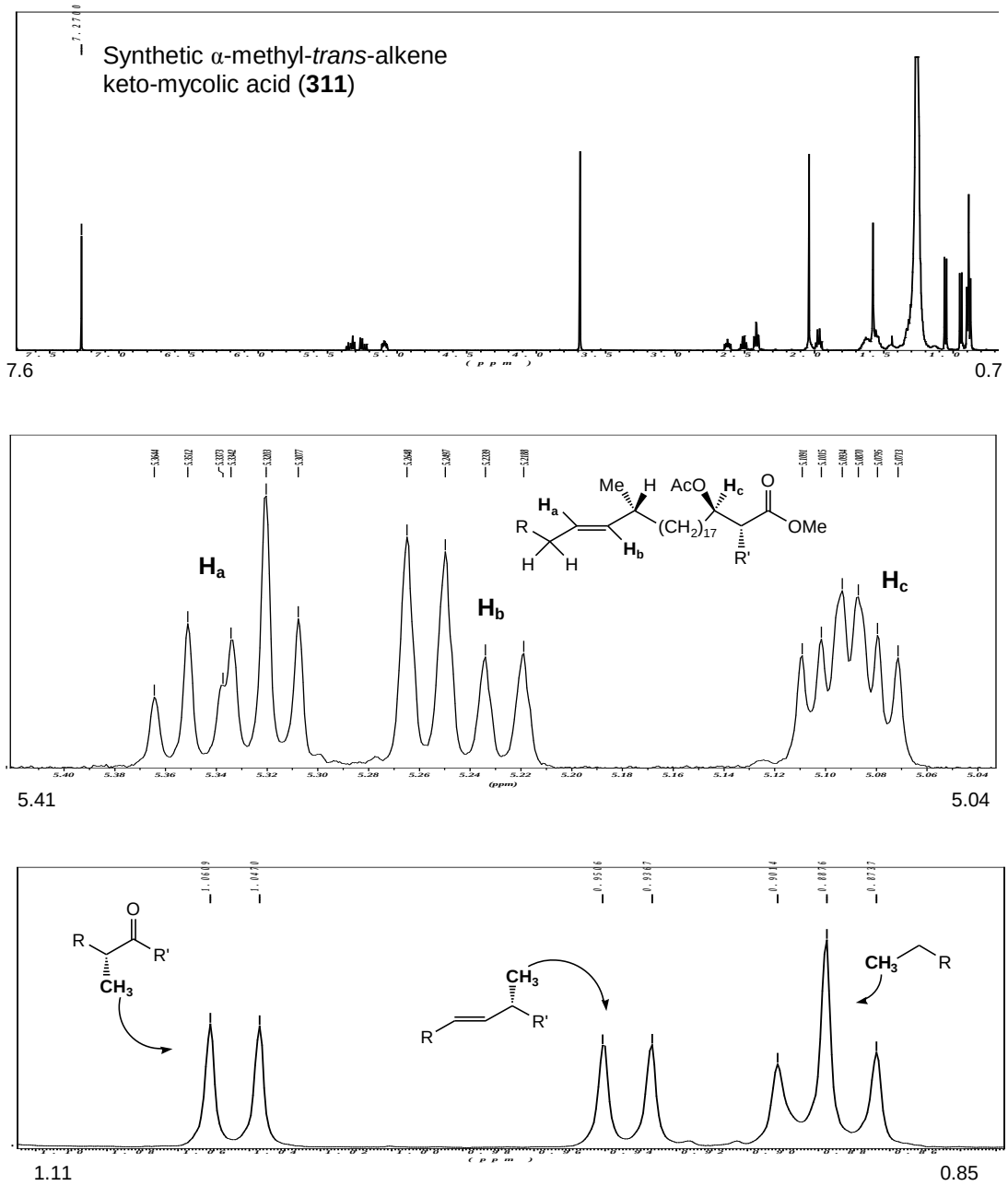
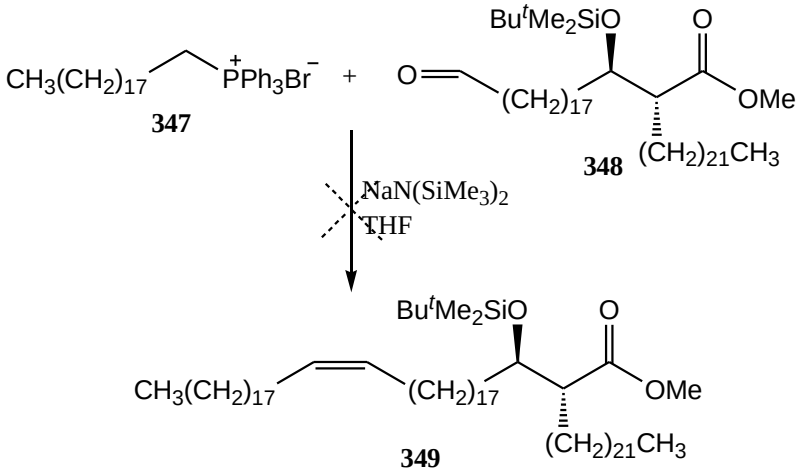


Figure 34: ***The ^{1}H NMR spectrum of the synthetic α-methyl-trans-alkene keto-mycolic acid***

2.10.11 An attempt to synthesise the α'-mycolic acid (119)

The aim of one part of this study was the synthesis of the α'-mycolic acid (**119**) (p 34) present in *Mycobacterium smegmatis*. The crucially important part of this synthesis was formation of the *Z*-alkene isomer. There is one well known literature method, coupling of an aldehyde with a phosphonium salt in the presence of sodium bis(trimethylsilyl)amide that gives the Z-stereoisomer with great selectivity.

The nonadecyltriphenylphosphonium (**347**) was prepared from the literature method[143] and the aldehyde (**348**) was prepared from the chain extension of the aldehyde (**305**) (p 137). A coupling reaction between these two compounds was tried. Unfortunately, the reaction did not work well. Only once was the desired *Z*-alkene (**349**) obtained in 19 % yield. The stereoselectivity was really good and the carbon NMR spectrum showed only one signal in the olefinic regain at δ 129.9 for the *cis*-carbons. However, the amount of (**349**) was not enough to continue to the free acid (**119**) (p 34) and then carry out biological tests. The above process was not repeatable and even the best yield was very low. Therefore it remains to find the right conditions to form the Z-alkene efficiently and this must be future work.



Scheme 116: ***An attempted way to formation of the Z-isomer***

3. Conclusions

The aim of this work was total synthesis of the keto-mycolic acids present in *Mycobacterium tuberculosis* and in other mycobacteria. Throughout the work, to test biological activities and to compare the specific rotation with natural sample to find out the absolute stereochemistry, the following mycolic acids were successfully synthesised;

1. *cis*-Cyclopropane keto-meromycolaldehyde (**114**).
2. α-Methyl-*trans*-cyclopropane keto-meromycolaldehyde (**115**).
3. *cis*-Cyclopropane keto-mycolic acid (**116**).
4. *cis*-Cyclopropane hydroxy-mycolic acid (**299**).
5. α-Methyl-*trans*-cyclopropane keto-mycolic acid (**117**).
6. α-Methyl-*trans*-cyclopropane hydroxy-mycolic acid (**300**).
7. α-Methyl-*trans*-alkene keto-mycolic acid (**311**).
8. α-Methyl-*trans*-alkene hydroxy-mycolic acid (**346**)

The Julia olefination was found to be the best way for chain extension in the synthesis of mycolic acids. In this study, a 1-phenyl-1*H*-tetrazole-5-yl sulfone was coupled with an aldehyde using lithium bis(trimethylsilyl)amide in THF to give an alkene, followed by hydrogenation to complete the chain extension. Hydrogen gas was used for hydrogenation for products that did not contain a cyclopropane ring and di-imide was used for hydrogenation that of intermediates contain a cyclopropane ring. D-Mannitol was used as starting material to prepare the meromycolate chain with the chiral hydroxyl group on the distal functional group coming from it. An enantioselective addition of MeLi to the α,β-unsaturated ester (**131**) gave the chiral methyl at the α-position of the ketone and hydroxyl group. The hydroxyl group was protected as *tert*-butyldimethylsilyl ether and at the last steps it was removed by HF.pyridine, followed by oxidation to ketone with PCC. The *cis*-cyclopropane ring at the proximal position was prepared from methyl acrylate and methyl chloroacetate. The α-methyl-*trans*-cyclopropane was prepared in 12 steps starting from (*R*)-glyceraldehyde acetonide (**128**). The α-methyl-*trans*-alkene was prepared from a stereoselective coupling reaction between the α-methyl aldehyde (**308**) and the sulfone (**309**). However, a stereoselective formation of *Z*-alkene for the α'-mycolic acid failed.

The corynomycolate part was prepared from an enantioselective Fräter alkylation of an β-hydroxy ester which this ester was prepared from two ways. The first way was the Sharpless dihydroxylation of an α,β-unsaturated ester and then removal of the hydroxyl group at the α-position. The second way was the formation of the (*R*)-(2-benzyloxyethyl)-oxirane starting from L-aspartic acid, regioselective addition of vinylmagnesium bromide, oxidative cleavage of the alkene group to carboxylic acid, and esterification of the acid.

In future work, it is important that keto-mycolic acids with the stereochemistry of the methyl α- to the keto group in the *S*-configuration are synthesised. This requires an effective hydrolysis of protected keto-mycolic acids without epimerisation adjacent to the carbonyl group. There are literature examples where this has been reported for natural mycolic acids. This work is underway. Also methoxy-mycolic acids containing an α-methyl *trans*-alkene should be synthesised for testing of their biological activities. In terms of alkene containing mycolic acids, further much is required to prepare the *cis*-isomers in acceptable yield.

4. Experimental section

4.1 General considerations

Starting materials and reagents were purchased from Lancaster Synthesis Ltd., Aldrich Chemical Co. Ltd., or Avocado Chemical Co. Ltd and used without further purification unless otherwise stated. Diethyl ether, THF and 1,2-dimethoxyethane were dried over sodium wire and benzophenone under a nitrogen atmosphere, while dichloromethane, diisopropylamine and HMPA were dried over calcium hydride. Toluene was dried over sodium wire. Organic solutions were dried over anhydrous magnesium sulphate and bulk solvents were removed at 14 mmHg residual traces of solvent were finally removed at 0.1 mmHg. Petrol was of boiling 40–60 ^{o}C. Reactions ranges under inert conditions were carried out under a balloon of nitrogen or argon. All glassware used in anhydrous reactions was dried at 250 ^{o}C. Reactions carried out at low temperatures were cooled using a bath of IMS with liquid nitrogen.

Column chromatography was conducted under medium pressure using silica gel from DBH (particle size 33-70 μm). Thin layer chromatography was performed using Merck silica gel 60 F_{254} plates, separated components were detected using variously UV light, I_2 and phosphomolybdic acid solution in IMS followed by charring. Gas liquid chromatography was carried out on a Perkin-Elmer Model 8410 on a capillary column (15 m x 0.53 mm).

NMR spectra were recorded on Bruker Avance 500 spectrometer in $CDCl_3$ if not differently indicated. All chemical shifts are quoted in δ relative to the trace resonance of protonated chloroform (δ 7.27 ppm), and $CDCl_3$ (δ 77.0 ppm). Where signs are given in carbon spectra, + = CH_2, - = CH, CH_3 and no sign is quaternary if not differently indicated. In some molecules containing long carbon chain, it was not possible to identify a signal for each individual carbon. Infra-red spectra were carried out on a Perkin-Elmer 1600 series FTIR spectrometer as liquid films or KBr disc (solid). Melting points were measured using a Gallenkamp melting point apparatus. Optical rotations were measured as solutions in chloroform of known concentration using a Polar 2001 automatic polarimeter. Accurate mass spectra were recorded on a Bruker MicroTOF time of flight mass spectrometer with ESI or APCI source. Elemental analysis was performed with a Carlo-Erba Model 1106 CHN analyser with a precision of 0.2 % for each element.

Preparation of 2,4,6-triisopropyl-benzenesulphonyl hydrazide

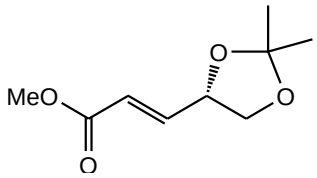
2,4,6-Triisopropyl-benzenesulphonyl chloride (24.3 g, 80.2 mmol) was dissolved in THF (60 ml) and cooled to – 10 ^{o}C. Hydrazine hydrate (8.2 g, 163 mmol) was slowly added by maintaining the solution in the temperature range – 4 ^{o}C to – 10 ^{o}C with constant stirring. The mixture was kept in the temperature range for a further 2 h. Water (3 ml) was added to dissolve the solid. The mixture separated into two phases; the aqueous phase was separated and the organic phase washed with brine (2 x 20 ml). The organic phase was dried at 0 ^{o}C for 30 min., filtered and washed with diethyl ether. The solvent was evaporated at 10 ^{o}C to a white solid, 2,4,6-triisopropyl-benzenesulphonyl hydrazide (21.5 g, 90 %). This was sealed and stored in a freezer.[189]

Preparation of di-potassium azodicarboxylate

Azodicarbonamide (7.5 g, 64 mmol) was slowly added in small portions to a vigorously stirred solution of KOH (15 g, 260 mmol) in de-ionised water (15 ml) at 0 ^{0}C on a salted ice-water bath, maintaining the temperature below 5 ^{o}C. The bright yellow solution was stirred at 0–5 ^{0}C for a further 45 min., during which time a thick bright yellow precipitate of di-potassium salt formed. The precipitate was filtered into a sintered funnel and washed with ice-cold methanol (60 ml). The yellow precipitate was dissolved in water (40 ml) on the sintered glass funnel at 18 ^{o}C. The yellow solution was sucked through the sinter by vacuum into pre-cooled (- 20 ^{0}C) methylated spirit (60 ml) giving a yellow precipitate. The yellow precipitate was again filtered through a sinter funnel and washed with cold (- 20 ^{o}C) methanol (50 ml), followed by cold (- 20 ^{0}C) petrol (50 ml). The solid was dried by vacuum and powdered with a spatula before being transferred under nitrogen into a pre-cooled round bottomed flask. The flask was sealed and stored in a freezer.[177]

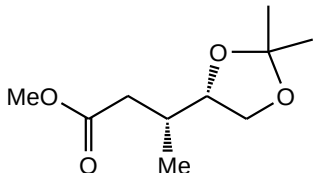
4.2 Experiments

Experiment 1: (*E*)-3-((*S*)-2,2-Dimethyl-[1,3]dioxolan-4-yl)-acrylic acid methyl ester (131)



A solution of $NaIO_4$ (19.6 g, 91.6 mmol) in water (100 ml) was added dropwise to a stirred solution of 1,2:5,6-O-isopropylidene-D-mannitol (**130**) (20 g, 76.3 mmol) in 5% $NaHCO_3$ (200 ml) at 0 ^oC and stirring was continued for 1 h at r.t. The mixture was cooled to 0 ^oC and (diisopropoxy phosphoryl)-acetic acid methyl ester (40 g, 168.1 mmol) was added with stirring followed by a 6M solution of aq. K_2CO_3 (260 ml) at 0–4 ^oC. The reaction was allowed to reach r.t. and stirring was continued for 20 hrs at r.t. The mixture was extracted with CH_2Cl_2 (3 x 300 ml), the combined organic extracts were dried and the solvent was evaporated. The crude product was purified and separated by column chromatography eluting with petrol / ethyl acetate (5:0.5) to give a colourless oil, (*E*)-3-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-acrylic acid methyl ester (**131**)[150] (19.9 g, 70 %), $[\alpha]_D^{24}$ = + 40.4 (*c* 1.09, $CHCl_3$). This showed ν_{max}/cm^{-1}: 2988, 1728, 1663, 1063, 845; δ_H: 6.90 (1H, dd, *J* 5.7, 15.8 Hz), 6.11 (1H, dd, *J* 1.6, 15.8 Hz), 4.67 (1H, dq, *J* 1.6, 7.0 Hz), 4.19 (1H, dd, *J* 6.6, 8.2 Hz), 3.75 (3H, s), 3.68 (1H, dd, *J* 7.0, 8.2 Hz), 1.45 (3H, s), 1.41 (3H, s); δ_C: 166.4, 145.0(+), 121.9(+), 110.2, 74.9(+), 68.7(-), 51.6(+), 26.4(+), 25.7(+).

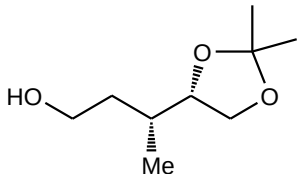
Experiment 2: (*R*)-3-((*S*)-2,2-Dimethyl-[1,3]dioxolan-4-yl)-butyric acid methyl ester (132)



(*E*)-3-((*S*)-2,2-Dimethyl-[1,3]dioxolan-4-yl)-acrylic acid methyl ester (**131**) (10 g, 53.76 mmol) was dissolved in dry ether (300 ml) and stirred under argon. It was cooled to -78 ^oC and MeLi (77 ml, 107.5 mmol, 1.4M) was added at -78 ^oC. The mixture was maintained at -78 ^oC for 2.5 hrs, and then allowed to gradually warm up to -60 ^oC when water (10 ml) was added. After 5 min, sat. aq. NH_4Cl (60 ml) was

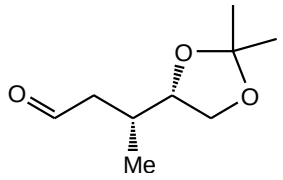
added, whereupon the temperature rose to -40 ^{o}C. The cooling bath was removed and the temperature of the mixture brought to 0 ^{o}C by addition of water (100 ml). The aqueous layer was extracted with ether (100 ml). The combined organic phases were washed with brine (2 x 100 ml), dried and the solvent was evaporated. The crude product was purified and separated by column chromatography eluting with petrol / ether (2:1) to give a colourless oil, (*R*)-3-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-butyric acid methyl ester (**132**)[150] (8.4 g, 77 %), $[\alpha]_D^{23}$ = + 8.6 (*c* 1.05, $CHCl_3$), {Found $(M + Na)^+$: 225.1092, $C_{10}H_{18}NaO_4$ requires: 225.1097}. This showed ν_{max}/cm^{-1}: 2984, 2980, 1732, 1437, 1370, 1012, 858, 795; δ_H: 4.05–3.98 (2H, m), 3.67 (3H, s), 3.63 (1H, br.t, *J* 7.1 Hz), 2.41 (1H, dd, *J* 4.7, 14.8 Hz), 2.26–2.19 (1H, m), 2.14 (1H, dd, *J* 8.5, 14.8 Hz), 1.40 (3H, s), 1.33 (3H, s), 0.98 (3H, d, *J* 6.7 Hz); δ_C: 173.0, 108.9, 78.6(+), 66.6(-), 51.5(+), 37.2(-), 32.9(+), 26.3(+), 25.2(+), 15.3(+) [+ = CH, CH_3, - = CH_2].

Experiment 3: (*R*)-3-((*S*)-2,2-Dimethyl-[1,3]dioxolan-4-yl)-butan-1-ol (101)



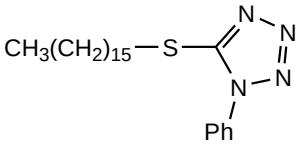
A solution of the methyl ester (**132**) (8.08 g, 40 mmol) in dry THF (30 ml) was added to a stirred solution of $LiAlH_4$ (1.7 g, 44 mmol) in dry THF (150 ml) at -20 ^{o}C and the mixture was refluxed for 1 h. Sat. aq. of sodium sulphate was added at -20 ^{o}C until a white precipitate had formed and then stirred for 30 min. The mixture was filtered through a bed of silica, washed with THF and the solvent evaporated. The crude product was dissolved in a mixture of ether (75 ml), petrol (75 ml) and triethylamine (2 drops). The solution was dried by stirring for 1 h over anhydrous potassium carbonate, filtered and the solvent was evaporated to give a colourless oil, (*R*)-3-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-butan-1-ol (**101**)[151] (6.76 g, 97 %), $[\alpha]_D^{25}$ = + 19.2 (*c* 1.12, $CHCl_3$). This showed ν_{max}/cm^{-1}: 3426 (b), 2984, 2835, 1457, 1062, 861; δ_H: 4.02–3.97 (2H, m), 3.75–3.70 (1H, m), 3.65–3.61 (2H, m), 2.18 (1H, t, *J* 4.8 Hz), 1.84–1.80 (1H, m), 1.66–1.60 (1H, m), 1.42–1.38 (1H, m), 1.40 (3H, s), 1.33 (3H, s), 0.96 (3H, *J* 6.7 Hz); δ_C: 108.7, 79.6(-), 67.2(+), 60.3(+), 35.6(+), 32.7(-), 26.4(-), 25.3(-), 15.1(-).

Experiment 4: (*R*)-3-((*S*)-2,2-Dimethyl-[1,3]dioxolan-4-yl)-butyraldehyde (102)



A solution of the primary alcohol (**101**) (6.1 g, 35.1 mmol) in CH_2Cl_2 (30 ml) was added to a stirred solution of PCC (16 g, 74.2 mmol) in CH_2Cl_2 (140 ml). Addition was done portion and portion, and during the addition a black colour appeared. The reaction was stirred for 30 min, then refluxed for 30 min. The reaction was cooled, diluted first with ether (250 ml) and then petrol (100 ml). The solution was filtered through a bed of silica and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol / ether (2:1) to give a colourless oil, (*R*)-3-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-butyraldehyde (**102**)[151] (4.8 g, 80 %), $[\alpha]_D^{23}$ = + 8.3 (*c* 1.44, $CHCl_3$). This showed ν_{max}/cm^{-1}: 2985, 1725, 1371, 1215, 1066, 859; δ_H: 9.77 (1H, t, *J* 1.9 Hz), 4.09–4.05 (1H, m), 3.98 (1H, dd, *J* 6.6, 8.2 Hz), 3.64 (1H, dd, *J* 7.3, 8.2 Hz), 2.56 (1H, ddd, *J* 1.6, 5.4, 16.7 Hz), 2.42–2.34 (1H, m), 2.27 (1H, ddd, *J* 1.9, 8.2, 16.7 Hz), 1.41 (3H, s), 1.35 (3H, s), 1.00 (3H, d, *J* 6.9 Hz); δ_C: 201.7(-), 109.1, 78.6(-), 66.3(+), 46.6(+), 30.4(-), 26.2(-), 25.1(-), 15.5(-).

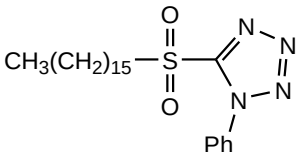
Experiment 5: 5-Hexadecylsulfanyl-1-phenyl-1*H*-tetrazole (140)



1-Phenyl-1*H*-tetrazole-5-thiol (8.4 g, 47 mmol), 1-bromohexadecane (16.2 g, 53 mmol), anhydrous potassium carbonate (15.2 g, 110 mmol) and acetone (165 ml) were mixed. The mixture was vigorously stirred and refluxed for 2.5 hrs 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 CH_2Cl_2 (150 ml). The solution was washed with water (300 ml) and the aqueous layer was re-extracted with CH_2Cl_2 (2 x 25 ml). The combined organic phases were washed with water (300 ml), dried and the solvent was evaporated to give a solid. This was dissolved in acetone (50 ml) and diluted with methanol (100 ml). The mixture was left at ambient temperature for 1 h and then at 0 ^oC for 1 h. A white solid crystallised out; this was filtered off and washed with cold acetone / methanol (1:2) to

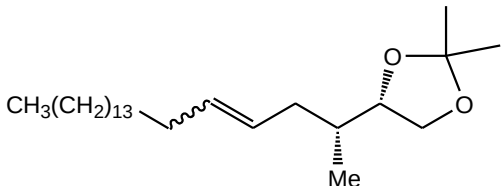
give a white solid, 5-hexadecylsulfanyl-1-phenyl-1*H*-tetrazole (**140**)[148] (18 g , 95%), m.p.: 48–50 ^{o}C, {Found: C, 68.29; H, 9.36; N, 13.99. $C_{23}H_{38}N_4S$ requires: C, 68.61; H, 9.51; N, 13.91}. This showed ν_{max}/cm^{-1}: 2917 (b), 1596, 1501, 1472, 1390, 1252, 1091, 759; δ_H: 7.61–7.55 (5H, m), 3.42 (2H, t, *J* 7.6 Hz), 1.84 (2H, quintet, *J* 7.6 Hz), 1.46 (2H, quintet, *J* 6.6 Hz), 1.36–1.26 (24H, m), 0.89 (3H, t, *J* 6.9 Hz); δ_C: 154.5, 133.8, 130.1(-), 129.8(-), 123.9(-), 33.4(+), 31.9(+), 29.68(+), 29.65(+), 29.62(+), 29.54(+), 29.44(+), 29.3(+), 29.09(+), 29.03(+), 28.7(+), 22.7(+), 14.1(-).

Experiment 6: 5-(Hexadecane-1-sulfonyl)-1-phenyl-1*H*-tetrazole (141)



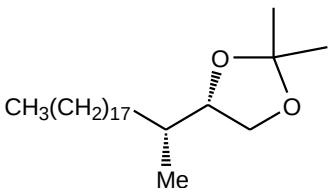
A solution of ammonium molybdate (VI) tetrahydrate (23.7 g, 19.2 mmol) in 35 % H_2O_2 (53 ml), prepared and cooled in an ice bath, was added to a stirred solution of 5-hexadecylsulfanyl-1-phenyl-1*H*-tetrazole (**140**) (17 g, 42.2 mmol) in THF (180 ml) and IMS (360 ml) at 12 ^{o}C and stirred at 15-20 ^{o}C for 2 hrs. A further solution of ammonium molybdate (VI) tetrahydrate (9 g, 7.3 mmol) in 35% H_2O_2 (23 ml) was added and the mixture was stirred at r.t for 18 hrs. The mixture was poured into 3 L of water and extracted with CH_2Cl_2 (3 x 200 ml). The combined organic phases were washed with water (2 x 300 ml), dried and the solvent was evaporated. The residue was dissolved in methanol (200 ml) and the mixture was left at ambient temperature for 1 h and then at 0 ^{o}C for 1 h. A white solid crystallised out; this was filtered off and washed with cold methanol to give a white solid, 5-(hexadecane-1-sulfonyl)-1-phenyl-1*H*-tetrazole (**141**)[148] (16.97 g, 93 %), m.p.: 65–67 ^{o}C, {Found: C, 63.64; H, 8.99; N, 12.65. $C_{23}H_{38}N_4O_2S$ requires: C, 63.56; H, 8.81; N, 12.89}. This showed ν_{max}/cm^{-1}: 2918 (b), 1594, 1470, 1343, 1154, 770; δ_H: 7.73–7.69 (2H, m), 7.66–7.59 (3H, m), 3.76–3.72 (2H, m), 1.99–1.93 (2H, m), 1.53–1.47 (2H, m), 1.36–1.26 (24H, m), 0.89 (3H, t, *J* 6.6 Hz); δ_C: 153.5, 133.1, 131.5(-), 129.7(-), 125.1(-), 57.0(+), 31.9(+), 29.69(+), 29.67(+), 29.64(+), 29.59(+), 29.47(+), 29.37(+), 29.21(+), 28.9(+), 22.7(+), 22.0(+), 14.1(-).

Experiment 7: (*S*)-2,2-Dimethyl-4-((*E/Z*)-(R)-1-methyl-nonadec-3-enyl)-[1,3]dioxolane (160 and 161)



The sulfone (**141**) (13.3 g, 32.2 mmol) was dissolved in dry THF (100 ml) and a solution of the aldehyde (**102**) (4.6 g, 26.8 mmol) in dry THF (70 ml) was added at r.t. This solution was cooled to -10 ^{o}C and lithium bis (trimethylsilyl) amide (48 ml, 50.9 mmol, 1.9 mol eq., 1.06M) was added at between -11 and -4 ^{o}C. The solution was allowed to reach room temperature and stirred for 2 hrs. Petrol / ether (1:1) (100 ml) and sat. aq. NH_4Cl (100 ml) was added. The organic phase was separated and the water layer was extracted with petrol / ether (1:1, 2 x 100 ml). The combined organic layers were dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol / ether (50:1) to give a colourless oil, (*S*)-2,2-dimethyl-4-((*E/Z*)-(*R*)-1-methyl-nonadec-3-enyl)-[1,3]dioxolane (**160** and **161**)[148] (8.2 g, 80 %) as a mixture of two isomers in ratio 2.3:1, {Found M^+: 380.3667, $C_{25}H_{48}O_2$ requires: 380.3654}. This showed ν_{max}/cm^{-1} : 2922, 2853, 1463, 1065, 860; δ_H (major *E* isomer): 5.47–5.40 (1H, m), 5.37–5.31 (1H, m), 4.03–3.99 (1H, m), 3.94–3.87 (1H, m), 3.65–3.60 (1H, m), 2.09–1.97 (3H, m), 1.83–1.77 (1H, m), 1.67–1.59 (1H, m), 1.41 (3H, s), 1.36 (3H, s), 1.33–1.26 (26H, m), 0.97 (3H, d, *J* 6.6 Hz), 0.89 (3H, t, *J* 6.9 Hz); δ_H (minor *Z* isomer): 1.93–1.87 (1H, m), 0.98 (3H, d, *J* 6.6 Hz) (the remaining signals were obscured by the major isomer); δ_C (major isomer): 132.7(-), 127.5(-), 108.5, 80.0(-), 67.8(+), 36.9(-), 36.2(+), 31.9(+), 29.7(+), 29.67(+), 29.57(+), 29.55(+), 29.52(+), 29.4(+), 29.2(+), 26.6(-), 25.6(-), 22.7(+), 15.7(-), 14.2(-); δ_C (minor isomer): 131.7(-), 127.1(-), 79.9(-), 67.7(+), 37.0(-), 30.6(+), 27.3(+), 25.5(-), 15.6(-).

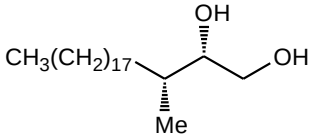
Experiment 8: (*S*)-2,2-Dimethyl-4-((*R*)-1-methyl-nonadecyl)-[1,3]dioxolane (162)



Palladium 10% on carbon (1.4 g) was added to a stirred solution of the alkenes (**160** and **161**) (7.6 g, 19.9 mmol) in ethanol (150 ml) and methanol (50 ml). Hydrogenation

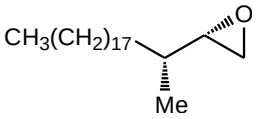
was carried out for 2 hrs. The solution was filtered on a bed of celite and the solvent was evaporated to give a colourless oil, (*S*)-2,2-dimethyl-4-((*R*)-1-methyl-nonadecyl)-[1,3]dioxolane (**162**)[148] (7.2 g, 95 %), $[\alpha]_D^{23}$ = + 23.0 (*c* 0.62, $CHCl_3$), {Found: C, 78.36; H, 12.68. $C_{25}H_{50}O_2$ requires: C, 78.47; H, 13.17}. This showed ν_{max}/cm^{-1}: 2919, 2851, 1467, 1076, 856; δ_H: 4.00 (1H, dd, *J* 6.3, 7.9), 3.87 (1H, br.q, *J* 7.3 Hz), 3.60 (1H, br.t, *J* 7.6 Hz), 1.57–1.54 (1H, m), 1.41 (3H, s), 1.36 (3H, s), 1.26 (34H, m), 0.97 (3H, d, *J* 6.6 Hz), 0.89 (3H, t, *J* 6.6 Hz); δ_C: 108.5, 80.4(-), 67.8(+), 35.6(-), 32.7(+), 31.9(+), 29.87(+), 29.70(+), 29.65(+), 29.62(+), 29.4(+), 27.0(+), 26.6(-), 25.5(-), 22.7(+), 15.6(-), 14.1(-).

Experiment 9: (2*S*,3*R*)-3-Methyl-henicosane-1,2-diol (163)



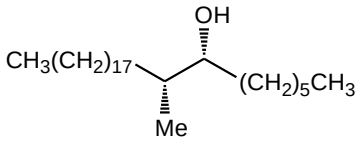
The acetal protected compound (**162**) (7 g 18.3 mmol) was dissolved in THF (35 ml) and methanol (50 ml) and water (5 ml) were added. Then PTSA (1 g) was added and the mixture was refluxed for 2 hrs. TLC showed that no starting material was left. Ether (60 ml), petrol (60 ml) and then Na_2CO_3 (50 ml) were added. The mixture was separated and the aqueous layer re-extracted with petrol / ether (1:1, 50 ml). The combined organic layers were washed with brine (2 x 50 ml) and dried. The solvent was evaporated and the crude product was purified by column chromatography eluting with petrol / ether (1:1) to give a white solid, (2*S*,3*R*)-3-methyl-henicosane-1,2-diol (**163**)[148] (5.3 g, 85 %), m.p.: 68 ^oC, $[\alpha]_D^{23}$ = + 12.7 (*c* 0.79, $CHCl_3$), {Found: C, 76.93; H, 13.17. $C_{22}H_{46}O_2$ requires: C, 77.13; H, 13.53}. This showed ν_{max}/cm^{-1}: 3283, 2919, 2850, 1472, 1025, 884; δ_H: 3.69–3.65 (1H, m), 3.61–3.53 (2H, m), 2.02 (1H, d, *J* 4.1 Hz), 1.93 (1H, dd, *J* 4.8, 7.0 Hz), 1.63–1.52 (1H, m), 1.43–1.26 (34H, m), 0.93 (3H, d, *J* 7.0 Hz), 0.89 (3H, t, *J* 7.0 Hz); δ_C: 75.8(-), 65.2(+), 35.7(-), 33.0(+), 31.9(+), 29.9(+), 29.7(+), 29.4(+), 27.2(+), 22.7(+) 14.6(-), 14.1(-).

Experiment 10: (*S*)-2-((*R*)-1-Methyl-nonadecyl)-oxirane (127)



The diol (**163**) (5.02 g, 14.68 mmol) was dissolved in CH_2Cl_2 (230 ml) by heating and then cetrimide (0.5 g) was added. NaOH solution (19 ml, 50 % in water) and p-toluenesulfonyl chloride (3.49 g, 18.3 mmol) in CH_2Cl_2 (25 ml) were added to the well stirred mixture and stirred for 45 min at r.t. TLC indicated that the reaction was complete. Water (250 ml) and dichloromethane (100 ml) were added. The mixture was separated and the aqueous layers re-extracted with CH_2Cl_2 (2 x 50 ml). The combined organic layers were dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol / ether (9.5:0.5) to give a white solid, (*S*)-2-((*R*)-1-methyl-nonadecyl)-oxirane (**127**)[148] (4.4 g, 92 %), m.p.: 45–46 ^{o}C, $[\alpha]_D^{23}$ = + 0.6 (*c* 0.84, $CHCl_3$), {Found: C, 81.75; H, 13.41. $C_{22}H_{44}O$ requires: C, 81.41; H, 13.66}. This showed ν_{max}/cm^{-1}: 2919 (v. br.), 1473, 1261, 892, 729; δ_H: 2.77 (1H, dd, *J* 4.1, 5.0 Hz), 2.71–2.67 (1H, m), 2.54 (1H, dd, *J* 2.6, 4.8 Hz), 1.38–1.14 (35H, m), 1.03 (3H, d, *J* 6.3 Hz), 0.89 (3H, t, *J* 7.0 Hz); δ_C: 57.2(-), 47.1(+), 36.3(-), 33.6(+), 31.9(+), 29.9(+), 29.7(+), 29.68(+), 29.65(+), 29.61(+), 29.4(+), 27.1(+), 22.7(+), 17.2(-), 14.1(-).

Experiment 11: (7*R*,8*R*)-8-Methyl-hexacosan-7-ol (164)



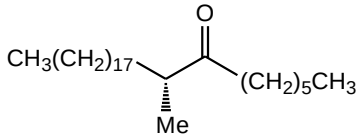
I) Mg (0.27 g, 11.3 mmol) was weighed in a dry three neck flask under argon and then dry THF (5 ml) added. 1-Bromopentane (0.839 g, 5.55 mmol) was dissolved in dry THF (5 ml) under argon. A few drops of this solution were added by syringe to the Mg suspension and the reaction was started by heating gently. The addition was continued and then the solution was refluxed for 45 min.

II) CuI (0.12 g, 0.63 mmol) was dissolved in dry THF (10 ml) at r.t. under argon and cooled to -30 ^{o}C. The above Grignard reagant was added dropwise via a syringe at -30 ^{o}C and stirred for 30 min. at -30 ^{o}C.

III) The epoxide (**127**) (0.6 g, 1.9 mmol) dissolved in dry THF (8 ml) under argon, was added to above solution dropwise at -30 ^{o}C and the reaction was kept at -30 ^{o}C for

2.5 hrs then warmed to r.t. and stirred 18 hrs. Sat. aq. of NH_4Cl (20 ml) was added and extracted with ether (20 ml). The water layers were re-extracted with ether (20 ml), the combined organic layers were washed with water (30 ml) and dried. The solvent was evaporated and the crude product was purified by column chromatography eluting with petrol / ether (9:1) to give a white solid, *(7R,8R)-8-methyl-hexacosan-7-ol* (**164**) (0.65 g, 89 %), m.p.: 38–39 ^oC, $[\alpha]_D^{24}$ = + 12.9 (*c* 0.84, $CHCl_3$), {Found $(M + Na)^+$: 419.4204, $C_{27}H_{56}NaO$ requires: 419.4223}. This showed ν_{max}/cm^{-1}: 3360, 2920, 2853, 1466, 1378, 721; δ_H: 3.51–3.49 (1H, m), 1.47–1.26 (44H, m), 1.17–1.14 (1H, m), 0.90–0.85 (9H, m); δ_C: 75.2(-), 38.2(-), 34.5(+), 33.4(+), 31.94(+), 31.88(+), 30.0(+), 29.7(+), 29,67(+), 29.4(+), 29.3(+), 27.4(+), 26.3(+), 22.7(+), 22.6(+), 14.1(-), 14.07(-), 13.6(-).

Experiment 12: (*R*)-8-Methyl-hexacosan-7-one (165)



A solution of the secondary alcohol (**164**) (0.38 g, 0.96 mmol) in CH_2Cl_2 (10 ml) was added to a stirred solution of PCC (0.44 g, 2.06 mmol) in CH_2Cl_2 (50 ml). Addition was done portion and portion, and during the addition a black colour appeared. The reaction was stirred for 30 min, refluxed for 30 min, and then cooled, diluted first with ether (50 ml) and then petrol (50 ml). The solution was filtered through a bed of silica and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol / ether (9.5:0.5) to give a white solid, *(R)-8-methyl-hexacosan-7-one* (**165**) (0.35 g, 93 %), m.p.: 34–35 ^oC, $[\alpha]_D^{22}$ = – 12.1 (*c* 1.24, $CHCl_3$), {Found $(M + Na)^+$: 417.4056, $C_{27}H_{54}NaO$ requires: 417.4067}. This showed ν_{max}/cm^{-1}: 2919, 2851, 1704, 1464, 1376, 1124, 1075; δ_H: 2.51 (1H, sext, *J* 6.9 Hz), 2.44 (1H, dt, *J* 17.0, 7.6 Hz), 2.40 (1H, dt, *J* 17.0, 7.3 Hz), 1.67–1.60 (1H, m), 1.59–1.53 (2H, m), 1.37–1.26 (39H, m), 1.05 (3H, d, *J* 6.9 Hz), 0.89 (6H, t, *J* 6.9 Hz); δ_C: 215.2, 46.3(-), 41.1(+), 33.1(+), 31.9(+), 31.6(+), 29.71(+), 29.68(+), 29.61(+), 29.5(+), 29.4(+), 29.0(+), 27.3(+), 23.7(+), 22.7(+), 22.5(+), 16.4(-), 14.1(-), 14.0(-).

Experiment 13: 6-Bromo-hexan-1-ol (167)

$Br(CH_2)_6OH$

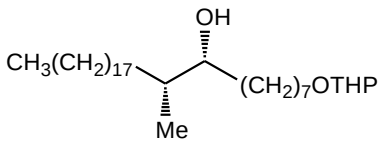
1,6-Hexanediol (47.27 g, 0.4 mol) was dissolved in toluene (200 ml) and aqueous HBr (56.6 ml, 0.5 mol, 48 % w.w.) was added then the mixture was refluxed for 18 hrs. The mixture was washed with water (200 ml) and then extracted with sat. aq. $NaHCO_3$ (3 x 150 ml). The toluene was removed by simple distillation at atm. pressure and the crude product was purified by column chromatography eluting with first petrol / ether (3:1) and then petrol / ether (1:1) to give a colourless oil, 6-bromo-hexan-1-ol (**167**)[180] (40.2 g, 55 %). This showed ν_{max}/cm^{-1}: 3357, 2937, 1638, 1054; δ_H: 3.67 (2H, t, *J* 6.6 Hz), 3.43 (2H, q, *J* 6.6 Hz), 1.90–1.89 (2H, m), 1.62–1.59 (2H, m), 1.49–1.45 (2H, m), 1.42–1.39 (2H, m); δ_C: 70.7(+), 62.8(+), 33.8(+), 32.4(+), 27.9(+), 24.9(+).

Experiment 14: 2-(6-Bromo-hexyloxy)-tetrahydropyran (168)

$Br(CH_2)_6OTHP$

3,4-Dihydro-2*H*-pyran (31.2 g, 0.37 mol. 2.1 mol eq.) and pyridinium-*p*-toluene-sulfonate (3 g) were added to a stirred solution of 6-bromo-hexan-1-ol (**167**) (32 g, 0.13 mol) in dry CH_2Cl_2 (250 ml) under argon at r.t. The reaction was stirred at r.t for 3 hrs and filtered through a bed of silica and washed with CH_2Cl_2 (200 ml) The solvent was evaporated and the crude product purified by column chromatography eluting with petrol / ether (9:1) to give a colourless oil, 2-(6-bromo-hexyloxy)-tetrahydropyran (**168**)[228] (39.8 g, 85 %), {Found $(M + Na)^+$: 287.0630, $C_{11}H_{21}BrNaO_2$ requires: 287.0617}. This showed ν_{max}/cm^{-1}: 2939, 2865, 1440, 1120; δ_H: 4.59–4.57 (1H, m), 3.89–3.85 (1H, m), 3.75 (1H, dt, *J* 6.6, 9.5 Hz), 3.53–3.48 (1H, m), 3.43 (2H, t, *J* 6.6 Hz), 3.39 (1H, dt, *J* 6.6, 9.5 Hz), 1.90–1.82 (3H, m), 1.75–1.71 (1H, m), 1.65–1.41 (10H, m); δ_C: 98.9(-), 67.4(+), 62.4(+), 33.9(+), 32.6(+), 30.8(+), 29.6(+), 28.0(+), 25.5(+), 25.4(+), 19.7(+).

Experiment 15: (8*R*,9*R*)-9-Methyl-1-(tetrahydropyran-2-yloxy)-heptacosan-8-ol (170)



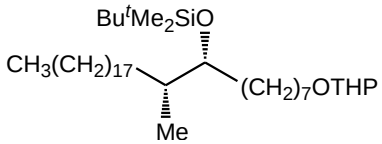
I) Mg (3.29 g, 137 mmo) was weighed in a dry three neck flask under argon and then dry THF (20 ml) added. 2-(6-Bromo-hexyloxy)-tetrahydropyran (**168**) (18.16 g, 68.5

mmol) was dissolved in dry THF (25 ml) under argon. A few drops of this solution were added by syringe to the Mg suspension and the reaction was started by heating gently. The addition was continued and then the solution was refluxed for 1 h.

II) CuI (1g, 5.25 mmol) was dissolved in dry THF (60 ml) at r.t. under argon and cooled to -30 ^{o}C. The above Grignard reagant was added dropwise via a syringe at -30 ^{o}C and stirred for 30 min at -30 ^{o}C.

III) The epoxide (**127**) (7.4 g, 22.83 mmol) dissolved in dry THF (60 ml) under argon, was added to above solution dropwise at -30 ^{o}C and the reaction was kept at -30 ^{o}C for 2.5 hrs then warmed to r.t. and stirred 18 hrs. Sat. aq. of NH_4Cl (50 ml) was added and extracted with ether (50 ml). The water layers were re-extracted with ether (50 ml), the combined organic layers were washed with water (50 ml) and dried. The solvent was evaporated and the crude product was purified by column chromatography eluting with first petrol / ether (9:1) and then petrol / ether (2:1) to give a colourless oil, (8*R*,9*R*)-9-methyl-1-(tetrahydropyran-2-yloxy)-heptacosan-8-ol (**170**)[148] (9.9 g, 85 %), $[\alpha]_D^{24}$ = + 10.9 (*c* 1.42, $CHCl_3$), {Found $(M + Na)^+$: 533.4900, $C_{33}H_{66}NaO_3$ requires: 533.4904}. This showed ν_{max}/cm^{-1}: 3450 (b), 2921, 2851, 1465, 1033; δ_H: 4.58 (1H, dd, *J* 2.9, 4.1 Hz), 3.90–3.85 (1H, m), 3.74 (1H, dt, *J* 7.0, 9.8 Hz), 3.51–3.49 (2H, m), 3.39 (1H, dt, *J* 6.6, 9.8 Hz), 1.86–1.81 (1H, m), 1.74–1.70 (1H, m), 1.61–1.54 (6H, m), 1.44–1.35 (10H, m), 1.34–1.26 (34H, m), 1.17–1.13 (1H, m), 0.89 (3H, t, *J* 6.7 Hz), 0.87 (3H, d, *J* 7.0 Hz); δ_C: 98.9(-), 75.2(-), 67.7(+), 62.4(+), 38.2(-), 34.5(+), 33.4(+), 31.9(+), 30.8(+), 29.97(+), 29.75(+), 29.71(+), 29.67(+), 29.5(+), 29.4(+), 27.4(+), 26.3(+), 26.2(+), 25.5(+), 22.7(+), 19.7(+), 14.1(-), 13.6(-).

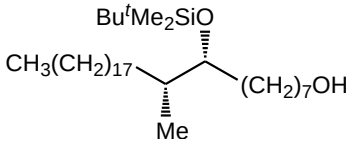
Experiment 16: *tert*-Butyl-dimethyl-{(1*R*,2*R*)-2-methyl-1-[7-(tetrahydropyran-2-yloxy)-heptyl]-eicosyloxy}-silane (173)



Imidazole (1 g, 14.7 mmol) was added to a stirred solution of the secondary alcohol (**170**) (3 g, 5.9 mmol) in dry DMF (35 ml) at r.t. followed by the addition of *tert*-butyl-dimethylsilylchloride (1.15 g, 7.65 mmol). The mixture was stirred at r.t. for 18 hrs and TLC showed there was still some alcohol remaining, so the reaction was warmed to 50 ^{o}C for 5 h when TLC showed the reaction was almost complete. The mixture was

quenched with water (200 ml) and extracted with CH_2Cl_2 (3 x 75 ml). The combined organic layers were washed with water (100 ml), dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol / ether (5:1) to give a colourless oil, *tert-butyl-dimethyl-{(1R,2R)-2-methyl-1-[7-(tetrahydropyran-2-yloxy)-heptyl]-eicosyloxy}-silane* (**173**) (3.1 g, 84 %), $[\alpha]_D^{24}$ = + 6.2 (*c* 1.42, $CHCl_3$), {Found $(M + Na)^+$: 647.5761, $C_{39}H_{80}NaO_3Si$ requires: 647.5769}. This showed ν_{max}/cm^{-1}: 2825, 2853, 1464, 1253, 1079, 1035, 836, 773; δ_H: 4.58 (1H, br.t, J = 2.9 Hz), 3.90–3.85 (1H, m), 3.74 (1H, dt, *J* 7.0, 9.8 Hz), 3.51–3.49 (2H, m), 3.38 (1H, dt, *J* 6.6, 9.5 Hz), 1.87–1.81 (1H, m), 1.75–1.70 (1H, m), 1.62–1.26 (52H, m, v.br.), 1.09–1.01 (1H, m), 0.89 (9H, s), 0.88 (3H, t, *J* 7.0 Hz), 0.80 (3H, d, *J* 6.6 Hz), 0.03 (3H, s), 0.02 (3H, s); δ_C: 98.9(-), 75.9(-), 67.7(+), 62.3(+), 37 8(-), 33.5(+), 32.5(+), 31.9(+), 30.8(+), 30.0(+), 29.9(+), 29.8(+), 29.77(+), 29.71(+, v.br.), 29.7(+), 29.5(+), 29.4(+), 27.7(+), 26.2(+), 26.0(+), 22.7(+), 19.7(+), 18.2, 14.4(-), 14.1(-), - 4.2(-), - 4.4(-).

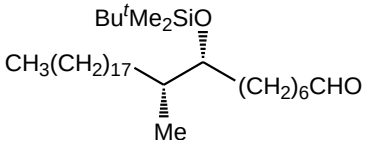
Experiment 17: (8*R*,9*R*)-8-(*tert*-Butyldimethylsilanyloxy)-9-methylheptacosan-1-ol (174)



A solution of pyridinium-*p*-toluenesulfonate (0.73 g, 0.5 mol eq.) in methanol (40 ml) was added to a stirred solution of the THP protected compound (**173**) (3.63 g, 5.8 mmol) in THF (30 ml) and stirred at 40 ^oC for 2.5 hrs. TLC showed that the reaction was almost complete. Sat. aq. $NaHCO_3$ (10 ml) and water (20 ml) were added and extracted with ether (3 x 25 ml). The combined organic layers were dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ether (first 5:1 then 3:1 and then 1:1) to give a colourless oil, *(8R,9R)-8-(tert-butyldimethylsilanyloxy)-9-methylheptacosan-1-ol* (**174**) (2.7 g, 86 %), $[\alpha]_D^{23}$ = + 12.9 (*c* 1.39, $CHCl_3$), {Found $(M + Na)^+$: 563.5213, $C_{34}H_{72}NaO_2Si$ requires: 563.5194}. This showed ν_{max}/cm^{-1}: 3321, 2925, 2854, 1464, 1379, 1253, 1058; δ_H: 3.65 (2H, q, *J* 6.5 Hz), 3.50 (1H, dt, *J* 3.5, 6.0 Hz), 1.58 (2H, quintet, *J* 7.3 Hz), 1.49–1.14 (44H, m, v.br.), 1.08–1.01 (1H, m), 0.90–0.86 (12H, s and t, *J* 6.9 Hz), 0.80 (3H, d, *J* 6.6 Hz), 0.03 (3H, s), 0.02 (3H, s); δ_C: 75.9(-), 63.1(+), 37.8(-), 33.5(+), 32.8(+),

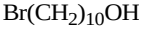
32.4(+), 31.9(+), 30.0(+), 29.9(+), 29.7(+, v.br.), 29.68(+), 29.5(+), 29.4(+), 27.7(+), 26.0(-), 25.9(+), 25.7(+), 22.7(+), 18.2, 14.5(-), 14.1(-), - 4.2(-), - 4.4(-).

Experiment 18: (8*R*,9*R*)-8-(*tert*-Butyldimethylsilanyloxy)-9-methylheptacosanal (176)



A solution of the alcohol (**174**) (3.8 g, 7.04 mmol) in CH_2Cl_2 (70 ml) was added to a stirred solution of PCC (3.8 g, 17.6 mmol) in CH_2Cl_2 (160 ml). Addition was done portion and portion, and during the addition a black colour appeared. The reaction was stirred for 2 hrs at r.t. and TLC showed that the reaction was complete. The reaction was diluted with ether (250 ml), filtered through a bed of silica and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol / ether (3:1) to give a colourless oil, *(8R,9R)-8-(tert-butyldimethysilanyloxy)-9-methylheptacosanal* (**176**) (3.7 g, 97 %), $[\alpha]_D^{25}$ = + 7.2 (*c* 1.10, $CHCl_3$), {Found M^+: 538.5131, $C_{34}H_{70}O_2Si$ requires: 538.5145}. This showed ν_{max}/cm^{-1}: 2925, 2854, 1731, 1464, 1379, 1253, 1073; δ_H: 9.77 (1H, t, *J* 1.6 Hz), 3.51–3.48 (1H, m), 2.43 (2H, dt, *J* 1.6, 7.3 Hz), 1.64 (2H, quintet, *J* 7.3 Hz), 1.48–1.20 (42H, m, v.br.), 1.09–1.01 (1H, m), 0.90–0.88 (12H, s and t, *J* 6.9 Hz), 0.80 (3H, d, *J* 6.6 Hz), 0.03 (3H, s), 0.02 (3H, s); δ_C: 202.9(-), 75.9(-), 37.8(+), 33.4(+), 32.4(+), 31.9(+), 30.0(+), 29.72(+, v.br.), 29.68(+), 29.39(+), 29.21(+), 27.7(+), 25.9(-), 25.8(+), 22.7(+), 21.1(+), 18.2, 14.5(-), 14.2(-), - 4.2(-), - 4.4(-).

Experiment 19: 10-Bromo-decan-1-ol (178)



1,10-Decanediol (25 g, 0.14 mol) was dissolved in toluene (300 ml) and aqueous HBr (30 ml, 0.27 mol, 48 % w.w.) was added then the mixture was refluxed for 18 h. The mixture was washed with water (200 ml) and then extracted with sat. aq. $NaHCO_3$ (3 x 150 ml). The toluene was removed by simple distillation at atm. pressure and the crude product was purified by column chromatography eluting with petrol / ether (4:1 then 1:1) to give a colourless oil, 10-bromo-decan-1-ol (**178**)[180] (24.52 g, 72 %). This showed ν_{max}/cm^{-1}: 3331, 2928, 2854, 1464, 1256, 1057; δ_H: 3.64 (2H, t, *J* 6.6 Hz), 3.41

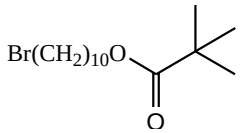
(2H, t, *J* 7.0 Hz), 1.85 (2H, quintet, *J* 7.2 Hz), 1.56 (2H, quintet, *J* 6.9 Hz), 1.47–1.42 (2H, m), 1.36–1.30 (11H, m); δ_C: 63.0(+), 34.0(+), 32.8(+), 32.7(+), 29.4(+), 29.33(+), 29.31(+), 28.7(+), 28.1(+), 25.7(+).

Experiment 20: 9-Bromo-nonan-1-ol (236)

$Br(CH_2)_9OH$

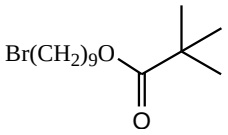
The procedure used in **Experiment 19** was repeated in order to convert 1,9-nonanediol (26 g, 0.16 mol) into a white solid, 9-bromo-nonan-1-ol (**236**)[180] (29.4 g, 81 %). This showed δ_H: 3.65 (2H, t, *J* 6.6 Hz), 3.42 (2H, t, *J* 6.6 Hz), 1.89–1.83 (2H, m), 1.60–1.55 (2H, m), 1.48–1.41 (2H, m), 1.37–1.32 (8H, m); δ_C: 63.04(+), 34.0(+), 32.8(+), 32.7(+), 29.4(+), 29.3(+), 28.7(+), 28.1(+), 25.7(+).

Experiment 21: 2,2-Dimethyl-propionic acid 10-bromodecyl ester (179)



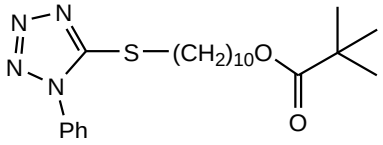
A solution of trimethylacetyl chloride (7.3 g, 60.8 mmol, 1.2 mol eq.) in CH_2Cl_2 (25 ml) was added to a stirred solution of 10-bromo-decan-1-ol (**178**) (12 g, 50.6 mmol), CH_2Cl_2 (80 ml), pyridine (8.2 ml, 101 mmol, 2 mol eq.) and 4-dimethylaminopyridine (0.25 g, 2 mmol) over a period of 15 min at 5 ^oC and stirred at r.t. 18 hrs. Dilute HCl (150 ml) was added and the organic phase separated. This was then washed with dilute HCl (100 ml) and brine (2 x 200 ml). The organic phase was dried ant the solvent was evaporated. The crude product was dissolved in petrol (200 ml) and filtered through a pad of silica washed with petrol (50 ml). The silica pad was finally washed with petrol / ether (1:1, 150 ml) and the solvent was evaporated to give a colourless oil, *2,2-dimethyl-propionic acid 10-bromodecyl ester* (**179**) (13.8 g, 85 %), {Found: C, 56.15; H, 9.23; $C_{15}H_{29}BrO_2$ requires: C, 56.07; H, 9.10.}. This showed ν_{max}/cm^{-1}: 2930, 2856, 1729, 1480, 1398, 1285, 1158; δ_H: 4.05 (2H, t, *J* 6.6 Hz), 3.41 (2H, t, *J* 7.0 Hz), 1.86 (2H, quintet, *J* 7.2 Hz), 1.62 (2H, quintet, *J* 6.9 Hz), 1.44–1.40 (2H, m), 1.36–1.30 (10H, m), 1.20 (9H, s); δ_C: 178.6, 64.4(-), 38.7, 34.0(-), 32.8(-), 29.4(-), 29.3(-), 29.1(-), 28.7(-), 28.6(-), 28.1(-), 27.2(+), 25.9(-) [+ = CH, CH_3, - = CH_2].

Experiment 22: 2,2-Dimethyl-propionic acid 9-bromo-nonyl ester (237)



The procedure used in **Experiment 21** was repeated using 9-bromo-nonan-1-ol (**236**) (28.5 g, 127.8 mmol), trimethylacetyl chloride (18.5 g, 153.4 mmol), triethylamine (38.7 ml, 383.4 mmol) and 4-dimethylaminopyridine (0.5 g, 4 mmol) in CH_2Cl_2 (200 ml). The crude product was purified by column chromatography eluting with petrol / ether (4:1) to give a colourless oil, *2,2-dimethyl-propionic acid 9-bromo-nonyl ester* (**237**) (37.58 g, 96 %), {Found: C, 54.23; H, 8.75; $C_{14}H_{27}BrO_2$ requires: C, 54.72; H, 8.86}. This showed ν_{max}/cm^{-1}: 2932, 2857, 1729, 1285, 1158; δ_H: 4.05 (2H, t, *J* 6.6 Hz), 3.41 (2H, t, *J* 6.6 Hz), 1.88–1.83 (2H, m), 1.65–1.59 (2H, m), 1.46–1.40 (2H, m), 1.36–1.31 (8H, m), 1.20 (9H, s); δ_C: 178.6, 64.4(-), 38.7, 33.9(-), 32.8(-), 29.3(-), 29.1(-), 28.6(-), 28.5(-), 28.1(-), 27.2(+), 25.8(-).

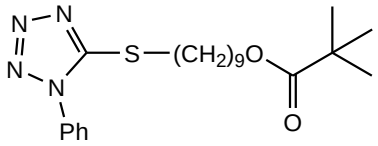
Experiment 23: 2,2-Dimethyl-propionic acid 10-(1-phenyl-1*H*-tetrazol-5-ylsulfanyl)-decyl ester (180)



1-Phenyl-1*H*-tetrazole-5-thiol (5.75 g, 32.3 mmol), 2,2-dimethyl-propionic acid 10-bromodecyl ester (**179**) (10 g, 31.2 mmol) and anhydrous potassium carbonate (9.5 g, 65.4 mmol) in acetone (160 ml) were mixed. The mixture was vigorously stirred for 18 hrs at r.t. TLC indicated the reaction to be complete. Water (1 L) was added to mixture and this was extracted with CH_2Cl_2 (1 x 150 ml, 2 x 25 ml). The combined organic phases were washed with brine (2 x 200 ml), dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol / ether (7:2.5 and then 1:1) to give a colourless oil, *2,2-dimethyl-propionic acid 10-(1-phenyl-1H-tetrazol-5-ylsulfanyl)-decyl ester* (**180**) (12.1 g, 93 %), {Found $(M + Na)^+$: 441.2292, $C_{22}H_{34}N_4NaO_2S$ requires: 441.2295}. This showed ν_{max}/cm^{-1}: 2929, 2856, 1726, 1500, 1461, 1388, 1285, 1159; δ_H: 7.61–7.54 (5H, m), 4.04 (2H, t, *J* 6.6 Hz), 3.40 (2H, t, *J* 7.4 Hz), 1.82 (2H, quintet, *J* 7.4 Hz), 1.62 (2H, quintet, *J* 6.9 Hz), 1.48–1.42 (2H, m), 1.33–1.28 (10H, m), 1.20 (9H, s); δ_C: 178.6, 154.5, 133.8, 130.0(+),

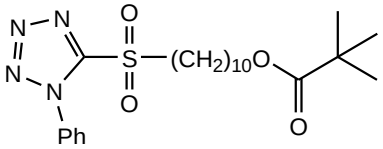
129.7(+), 123.8(+), 64.4(-), 38.7, 33.3(-), 29.4(-), 29.3(-), 29.2(-), 29.1(-), 29.0(-), 28.6(-), 28.5(-), 27.2(+), 25.9(-) [+ = CH, CH_3, - = CH_2].

Experiment 24: 2,2-Dimethyl-propionic acid 9-(2-phenyl-2*H*-pentazol-1-ylsulfanyl)-nonyl ester (238)



The procedure used in **Experiment 23** was repeated using 2,2-dimethyl-propionic acid 9-bromo-nonyl ester (**237**) (20 g, 65.1 mmol), 1-phenyl-1*H*-tetrazole-5-thiol (11.03 g, 61.9 mmol) and anhydrous potassium carbonate (18.9 g, 136.8 mmol) in acetone (320 ml) to give a colourless oil, *2,2-dimethyl-propionic acid 9-(2-phenyl-2H-pentazol-1-ylsulfanyl)-nonyl ester* (**238**) (23.85 g, 91 %), {Found: C, 62.74; H, 8.28; N, 14.10; $C_{21}H_{32}N_4O_2S$ requires: C, 62.34; H, 7.97; N, 13.85; Found $(M + Na)^+$: 427.2139, $C_{21}H_{32}N_4NaO_2S$ requires: 427.2138}. This showed ν_{max}/cm^{-1}: 2930, 2856, 1726, 1500, 1159; δ_H: 7.58–7.54 (5H, m), 4.05 (2H, t, *J* 6.6 Hz), 3.40 (2H, t, *J* 7.4 Hz), 1.82 (2H, quintet, *J* 7.5 Hz), 1.62 (2H, quintet, *J* 6.8 Hz), 1.48–1.42 (2H, m), 1.35–1.30 (8H, m), 1.20 (9H, s); δ_C: 178.6, 154.5, 133.7, 130.0 (+), 129.7 (+), 123.8 (+), 64.4 (-), 38.7, 33.3 (-), 29.3 (-), 29.1 (-), 29.05 (-), 28.9 (+), 28.57 (-), 28.55 (-), 27.2 (+), 25.8(-) [- = CH_2, + = CH, CH_3].

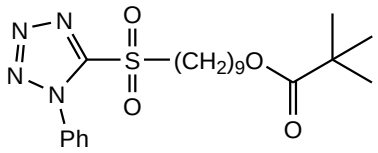
Experiment 25: 2,2-Dimethylpropionic acid 10-(1-phenyl-1*H*-tetrazole-5-sulfonyl)-decyl ester (181)



A solution of ammonium molybdate (VI) tetrahydrate (13.9 g, 11.25 mmol) in 35 % H_2O_2 (37 ml), prepared and cooled in an ice bath, was added to a stirred solution of the sulfide (**180**) (10 g, 23.9 mmol) in THF (140 ml) and IMS (280 ml) at 10 ^oC and stirred at r.t. for 2 hrs. A further solution of ammonium molybdate (VI) tetrahydrate (7.5 g, 6.1 mmol) in 35% H_2O_2 (20 ml) was added and the mixture was stirred at r.t. for 18 hrs. The mixture was poured into water (1.2 L) and extracted with CH_2Cl_2 (1 x 200 ml, 3 x 30 ml). The combined organic phases were washed with water (500 ml), dried and the solvent was evaporated. The crude product was purified by column chromatography

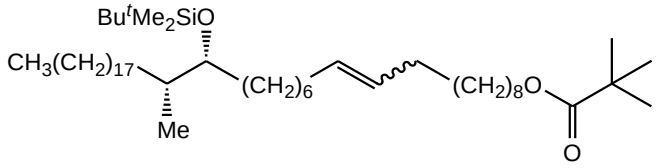
eluting with petrol / ether (3:1 and then 1:1) to give a yellow oil, *2,2-dimethylpropionic acid 10-(1-phenyl-1H-tetrazole-5-sulfonyl)-decyl ester* (**181**) (10.5 g, 97 %), {Found $(M + Na)^+$: 473.2186, $C_{22}H_{34}N_4NaO_4S$ requires: 473.2199}. This showed ν_{max}/cm^{-1}: 2930, 2857, 1725, 1498, 1480, 1342, 1286, 1154; δ_H: 7.1–7.69 (2H, m), 7.63–7.60 (3H, m), 4.04 (2H, t, *J* 6.6 Hz), 3.75–3.72 (2H, m), 2.01–1.94 (2H, m), 1.62 (2H, quintet, *J* 6.9 Hz), 1.50 (2H, quintet, *J* 7.4 Hz), 1.34–1.29 (10H, m), 1.20 (9H, s); δ_C: 178.6, 153.4, 133.0, 131.4(+), 129.7(+), 125.0(+), 64.3(-), 55.9(-), 38.7, 29.3(-), 29.1(-), 29.0(-), 28.8(-), 28.5(-), 28.1(-), 27.2(+), 25.8(-), 21.9(-) [+ = CH, CH_3, - = CH_2].

Experiment 26: 2,2-Dimethyl-propionic acid 9-(2-phenyl-2*H*-pentazole-1-sulfonyl)-nonyl ester (239)



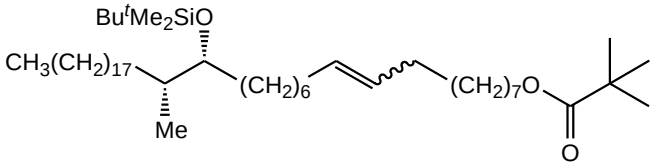
The procedure used in **Experiment 25** was repeated using the sulfane (**238**) (20.8 g, 51.49 mmol), ammonium molybdate (VI) tetrahydrate (35.6 g, 28.8 mmol) in 35 % H_2O_2 (80 ml), in THF (200 ml) and IMS (400 ml) and further ammonium molybdate (VI) tetrahydrate (15.9 g, 12.87 mmol) in 35% H_2O_2 (40.6 ml) to give a colourless oil, *2,2-dimethyl-propionic acid 9-(2-phenyl-2H-pentazole-1-sulfonyl)-nonyl ester* (**239**) (21.8 g, 97 %), {Found: C, 57.55; H, 7.62; N, 12.70; $C_{21}H_{32}N_4O_4S$ requires: C, 57.77; H, 7.39; N, 12.83; Found $(M + Na)^+$: 459.2015, $C_{21}H_{32}N_4NaO_4S$ requires: 459.2036}. This showed ν_{max}/cm^{-1}: 2932, 2858, 1725, 1499, 1342, 1154; δ_H: 7.70–7.69 (2H, m), 7.65–7.59 (3H, m), 4.05 (2H, t, *J* 6.6 Hz), 3.75–3.72 (2H, m), 1.99–1.93 (2H, m), 1.64–1.59 (2H, m), 1.53–1.47 (2H, m), 1.38–1.30 (8H, m), 1.20 (9H, s); δ_C: 178.6, 153.5, 133.0, 131.4(+), 129.7(+), 125.0(+), 64.3(-), 56.0(-), 38.7, 29.03(-), 29.01(-), 28.8(-), 28.5(-), 28.1(-), 27.2(+), 25.8(-), 21.9(-).

Experiment 27: 2,2-Dimethylpropionic acid (*E*/*Z*)-(18*R*,19*R*)-18-(*tert*-butyldimethylsilanyloxy)-19-methylheptatriacont-10-enyl ester (182)



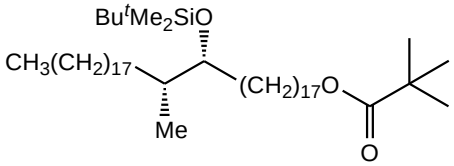
The sulfone (**181**) (3.9 g, 8.7 mmol) was dissolved in dry THF (60 ml) and a solution of the aldehyde (**176**) (3.8 g, 7.1 mmol) in dry THF (60 ml) was added at r.t. This solution was cooled to -12 ^{o}C and lithium bis(trimethylsilyl) amide (13 ml, 13.49 mmol, 1.9 mol eq., 1.06M) was added at between -12 and -4 ^{o}C. The solution was allowed to reach room temperature and stirred for 2 hrs. TLC analysis indicated that the reaction was complete. Ether (75 ml) and sat. aq. NH_4Cl (25 ml) were added. The organic phase was separated and water layer was extracted with ether (2 x 75 ml). The combined organic layers were dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol / ether (10:0.6) to give a colourless oil, *2,2-dimethylpropionic acid (E/Z)-(18R,19R)-18-(tert-butyldimethyl-silanyloxy)-19-methylheptatriacont-10-enyl ester* (**182**) (4.94 g, 92 %) as a mixture of two isomers in ratio 2.3:1, {Found $(M + Na)^+$: 785.7205, $C_{49}H_{98}NaO_3Si$ requires: 785.7177}. This showed ν_{max}/cm^{-1}: 2925, 2854, 1732, 1463, 1362, 1284, 1253, 1155; δ_H (major *E* isomer): 5.41–5.39 (2H, m), 4.05 (2H, t *J* 6.6 Hz), 3.51–3.48 (1H, m), 2.0–1.96 (4H, m), 1.64–1.61 (2H, m), 1.48–1.27 (58H, m, v.br.), 1.20 (9H, s), 1.08–1.00 (1H, m), 0.90–0.88 (12H, m, including a s), 0.80 (3H, d, *J* 6.6 Hz), 0.04 (3H, s), 0.03 (3H, s); δ_H (minor *Z* isomer): 5.37–5.34 (2H, m), 2.05–2.01 (4H, m), the remaining signals were obscured by the major isomer; δ_C (two isomers): 178.6, 130.3(-), 129.9(-), 75.9(-), 64.5(+), 38.7, 37.7(-), 33.5(+), 32.6(+), 32.58(+), 32.5(+), 31.9(+), 30.0(+), 29.98(+), 29.75(+), 29.7(+,v.br.), 29.67(+), 29.63(+), 29.5(+), 29.4(+), 29.36(+), 29.33(+), 29.30(+), 29.24(+), 29.17(+), 29.15(+), 28.6(+), 27.7(+), 27.2(-), 26.0(-), 25.92(+), 25.89(+), 22.7(+), 18.2, 14.4(-), 14.1(-), - 4.2(-), - 4.4(-).

Experiment 28: 2,2-Dimethyl-propionic acid (*E*/*Z*)-(17*R*,18*R*)-17-(*tert*-butyl-dimethyl-silanyloxy)-18-methyl-hexatriacont-9-enyl ester (240)



The procedure used in **Experiment 27** was repeated in order to couple the sulfone (**239**) (10.21 g, 23.4 mmol) with the aldehyde (**176**) (10.5 g, 19.5 mmol) using lithium bis(trimethylsilyl) amide (30 ml, 31.2 mmol, 1.06M) in dry THF to give a colourless oil, *2,2-dimethyl-propionic acid (E/Z)-(17R,18R)-17-(tert-butyl-dimethyl-silanyloxy)-18-methyl-hexatriacont-9-enyl ester* (**240**) (12.1 g, 83 %) as a mixture of two isomers in ratio 2.1:1, {Found $(M + Na)^+$: 771.7002, $C_{48}H_{96}NaO_3Si$ requires: 771.7021}. This showed ν_{max}/cm^{-1}: 2926, 2854, 1732, 1463, 1154; δ_H (major *E* isomer): 5.40–5.38 (2H, m), 4.05 (2H, t, *J* 6.6 Hz), 3.52–3.49 (1H, m), 1.97–1.95 (4H, m), 1.65–1.60 (2H, m), 1.47–1.26 (54H, m, v.br.), 1.20 (9H, s), 1.07–1.01 (1H, m), 0,89 (12H, m, including a s), 0.80 (3H, d, J = 6.9 Hz), 0.04 (3H, s), 0.03 (3H, s); δ_H (minor *Z* isomer): 5.37–5.35 (2H, m), 2.03–2.00 (4H, m) (the remaining signals were obscured by the major isomer); δ_C (two isomers): 178.6, 130.4(-), 130.3(-), 129.9(-), 129.8(-), 75.9(-), 64.5(+), 38.7, 37.7(-), 33.5(+), 32.6(+), 32.5(+), 31.9(+), 30.01(+), 29.79(+), 29.74(+), 29.70(+, v.br.), 29.65(+), 29.61(+), 29.42(+), 29.36(+), 29.3(+), 29.2(+), 29.19(+), 29.16(+), 29.06(+), 28.6(+), 27.7(+), 27.2(-), 26.0(-), 25.9(+), 22.7(+), 18.2, 14.4(-), 14.1(-), - 4.2(-), - 4.4(-).

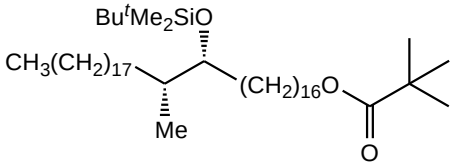
Experiment 29: 2,2-Dimethylpropionic acid (18*R*,19*R*)-18-(*tert*-butyldimethyl-silanyloxy)-19-methylheptatriacontyl ester (183)



Palladium 10% on carbon (1.5 g) was added to a stirred solution of the alkene (**182**) (4.94 g, 6.5 mmol) in ethanol (150 ml) and ethyl acetate (70 ml). Hydrogenation was carried out 1 h. The solution was filtered over a bed of celite and the solvent was evaporated to give pure colourless oil, *2,2-dimethylpropionic acid (18R,19R)-18-(tert-butyldimethylsilanyloxy)-19-methyl-heptatriacontyl ester* (**183**) (4.9 g, 99 %), $[\alpha]_D^{25} = +4.8$ (*c* 1.10, $CHCl_3$), {Found $(M + Na)^+$: 787.7355, $C_{49}H_{100}NaO_3Si$ requires: 787.7334}.

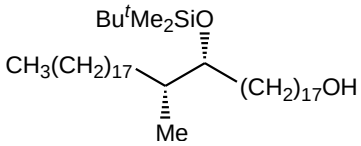
This showed ν_{max}/cm^{-1}: 2923, 2854, 1733, 1464, 1155, 836; δ_H: 4.05 (2H, t, *J* 6.6 Hz), 3.51–3.48 (1H, m), 1.62 (2H, quintet, *J* 6.6 Hz), 1.49–1.26 (64H, m, v.br.), 1.20 (9H, s), 1.08–1.01 (1H, m), 0.90–0.87 (12H, m), 0.80 (3H, d, *J* 6.6 Hz), 0.03 (3H, s), 0.02 (3H, s); δ_C: 178.7, 75.9(-), 64.5(+), 38.7, 37.7(-), 33.5(+), 32.5(+), 31.9(+), 30.0(+), 29.9(+), 29.72(+, v.br.), 29.68(+), 29.59(+), 29.55(+), 29.4(+), 29.3(+), 28.6(+), 27.7(+), 27.2(-), 26.0(-), 25.9(+), 22.7(+), 18.2, 14.4(-), 14.2(-), - 4.2(-), - 4.4(-).

Experiment 30: 2,2-Dimethyl-propionic acid (17*R*,18*R*)-17-(*tert*-butyl-dimethyl-silanyloxy)-18-methyl-hexatriacontyl ester (241)



The procedure used in **Experiment 29** was repeated using the alkene (**240**) (11.2 g, 14.97 mmol), palladium 10% on carbon (2.5 g) in IMS (200 ml) and THF (100 ml) to give a colourless oil, *2,2-dimethyl-propionic acid (17R,18R)-17-(tert-butyl-dimethyl-silanyloxy)-18-methyl-hexatriacontyl ester* (**241**) (4.9 g, 99 %), $[\alpha]_D^{23}$ = + 5.3 (*c* 1.07, $CHCl_3$), {Found $(M + Na)^+$: 773.7171, $C_{48}H_{98}NaO_3Si$ requires: 773.7177}. This showed ν_{max}/cm^{-1}: 2926, 2854, 1733, 1464, 1155, 836; δ_H: 4.05 (2H, t, *J* 6.6 Hz), 3.52–3.49 (1H, m), 1.65–1.60 (2H, m), 1.51–1.26 (62H, m, v.br.), 1.20 (9H, s), 1.08–1.01 (1H, m), 0.9–0.87 (12H, m, including a s) 0.80 (3H, d, *J* 7.0 Hz), 0.04 (3H, s), 0.03 (3H, s); δ_C: 178.6, 75.8(-), 64.5(+), 38.7, 37.7(-), 33.5(+), 32.5(+), 31.9(+), 30.0(+), 29.9(+), 29.7(+, v.br.), 29.67(+), 29.59(+), 29.54(+), 29.4(+), 29.3(+), 28.6(+), 27.7(+), 27.2(-), 26.0(-), 25.9(+), 22.7(+), 18.2, 14.4(-), 14.1(-), - 4.2(-), - 4.4(-).

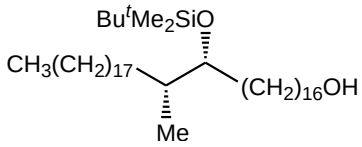
Experiment 31: (18*R*,19*R*)-18-(*tert*-Butyldimethylsilanyloxy)-19-methyl-heptatriacontan-1-ol (184)



$LiAlH_4$ (0.37 g, 9.6 mmol) was added to stirred dry THF (60 ml) at – 20 ^oC under argon. A solution of the *tert*-butyl ester (**183**) (4.9 g, 6.4 mmol) in dry THF (50 ml) was added slowly, allowed to warm and refluxed for 1 h. Sat. aq. sodium sulphate was added to the mixture at – 20 ^oC until a white precipitate had formed and THF (60 ml)

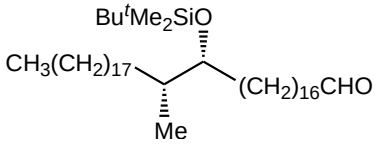
was added. The mixture was stirred at r.t. for 30 min, filtered through a bed of silica and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol / ether (3:1) and then (4:3) to give a colourless oil, *(18R,19R)-18-(tert-butyldimethylsilanyloxy)-19-methyl-heptatriacontan-1-ol* (**184**) (4.3 g, 98 %), $[\alpha]_D^{24}$ = + 5.1 (*c* 0.95, $CHCl_3$), {Found $(M + H)^+$: 681.6921, $C_{44}H_{93}O_2Si$ requires: 681.6939}. This showed ν_{max}/cm^{-1}: 3330, 2927, 2848, 1465, 1378, 1253, 1058; δ_H: 3.65 (2H, q, *J* 6.7 Hz), 3.52–3.48 (1H, m), 1.58 (2H, quintet, *J* 6.9 Hz), 1.48–1.12 (64, m, v.br.), 1.08–1.01 (1H, m), 0.90–0.88 (2H, m, including a s), 0.80 (3H, d *J* 6.7 Hz), 0.03 (3H, s), 0.02 (3H, s); δ_C: 75.9(-), 63.1(+), 37.7(-), 33.5(+), 32.8(+), 32.4(+), 31.9(+), 30.0(+), 29.9(+), 29.7(+), 29.6(+, v.br.), 29.5(+), 29.4(+), 27.7(+), 26.0(-), 25.9(+), 25.8(+), 22.7(+), 18.2, 14.4(-), 14.2(-), - 4.2(-), - 4.4(-).

Experiment 32: (17*R*,18*R*)-17-(*tert*-Butyl-dimethyl-silanyloxy)-18-methyl-hexatriacontan-1-ol (242)



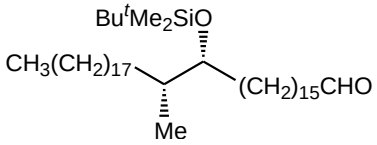
The procedure used in **Experiment 31** was repeated in order to reduce the *tert*-butyl ester (**241**) (10.9 g, 14.54 mmol) using $LiAlH_4$ (0.83 g, 21.8 mmol) to a colourless oil, *(17R,18R)-17-(tert-butyl-dimethyl-silanyloxy)-18-methyl-hexatriacontan-1-ol* (**242**) (9.6 g, 99 %), $[\alpha]_D^{25}$ = + 5.8 (*c* 1.22, $CHCl_3$), {Found $(M + Na)^+$: 689.6581, $C_{43}H_{90}NaO_2Si$ requires: 689.6602}. This showed ν_{max}/cm^{-1}: 3332, 2924, 2854, 1465, 1253, 1058; δ_H: 3.65 (2H, t, *J* 6.6 Hz), 3.53–3.50 (1H, m), 1.61–1.55 (2H, m), 1.51–1.43 (2H, m), 1.40–1.26 (60H, m, v.br), 1.10–1.02 (1H, m), 0.89 (12H, m, including a s), 0.81 (3H, d, *J* 6.6 Hz), 0.04 (3H, s), 0.03 (3H, s); δ_C: 75.9(-), 63.1(+), 37.8(-), 33.6(+), 32.9(+), 32.6(+), 31.9(+), 30.0(+), 29.9(+), 29.7(+, v.br.), 29.68(+), 29.65(+), 29.62(+), 29.6(+), 29.5(+), 29.4(+), 27.7(+), 26.0(-), 25.9(+), 25.8(+), 22.7(+), 18.2, 14.4(-), 14.1(-), - 4.2(-), - 4.4(-).

Experiment 33: (18*R*,19*R*)-18-(*tert*-Butyldimethylsilanyloxy)-19-methyl-heptatriacontanal (126)



The procedure used in **Experiment 18** was repeated in order to oxidise the alcohol (**184**) (2.0 g, 2.94 mmol) using PCC (1.6 g, 7.35 mmol) in CH_2Cl_2 (120 ml). The crude product was purified by column chromatography eluting with petrol / ether (4:1) to give a colourless oil, *(18R,19R)-18-(tert-butyldimethylsilanyloxy)-19-methylheptatri-acontanal* (**126**) (1.95 g, 98 %), $[\alpha]_D^{23}$ = + 5.8 (c 0.92, $CHCl_3$), {Found M^+: 678.6723, $C_{44}H_{90}O_2Si$ requires: 678.6710}. This showed ν_{max}/cm^{-1}: 2925, 2854, 1731, 1465, 1379, 1253, 1072; δ_H: 9.77 (1H, t, *J* 1.6 Hz), 3.51–3.48 (1H, m), 2.42 (2H, dt, *J* 1.6, 7.3 Hz), 1.64 (2H, quintet, *J* 7.3 Hz), 1.50–1.13 (62H, m, v.br.), 1.08–1.01 (1H, m), 0.90–0.88 (12 H, m, including a s), 0.80 (3H, d, *J* 6.7 Hz), 0.03 (3H, s), 0.02 (3H, s); δ_C: 202.9(-), 75.9(-), 43.9(+), 37.7(-), 33.5(+), 32.5(+), 31.9(+), 30.0(+), 29.9(+, v.br.), 29.65(+), 29.6(+), 29.5(+), 29.4(+), 29.2(+), 27.7(+), 26.0(-), 25.9(+), 22.7(+), 22.1(+), 18.2, 14.4(-), 14.1(-), - 4.2(-), - 4.4(-).

Experiment 34: (17*R*,18*R*)-17-(*tert*-Butyl-dimethyl-silanyloxy)-18-methyl-hexatriacontan-1-ol (215)



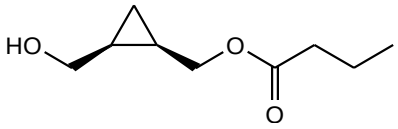
The procedure used in **Experiment 33** was repeated using the alcohol (**242**) (2.0 g, 3.0 mmol), and PCC (1.62 g, 7.5 mmol) in CH_2Cl_2 (120 ml) to give a colourless oil, *(17R,18R)-17-(tert-butyl-dimethyl-silanyloxy)-18-methyl-hexatriacon-tan-1-ol* (**215**) (1.94 g, 97 %), $[\alpha]_D^{25}$ = + 5.8 (*c* 1.3, $CHCl_3$), {Found $(M + Na)^+$: 687.6451, $C_{43}H_{88}NaO_2Si$ requires: 687.6446}. This showed ν_{max}/cm^{-1}: 2925, 2854, 1731, 1465, 1253, 1075, 836; δ_H: 9.77 (1H, t, *J* 1.9 Hz), 3.51–3.49 (1H, m), 2.43 (2H, dt, *J* 1.9, 7.6 Hz), 1.67–1.61 (2H, m), 1.50–1.45 (2H, m), 1.42–1.26 (58H, m, v.br.), 1.08–1.01 (1H, m), 0.89 (12H, m, including a s), 0.80 (3H, d, *J* 6.6 Hz), 0.04 (3H, s), 0.03 (3H, s); δ_C: 202.9(-), 75.9(-), 43.9(+), 37.7(-), 33.5(+), 32.5(+), 31.9(+), 30.0(+), 29.9(+), 29.7(+,

v.br.), 29.68(+), 29.67(+), 29.65(+), 29.59(+), 29.5(+), 29.4(+), 29.2(+), 27.7(+), 26.0(-), 25.9(+), 22.7(+), 22.1(+), 18.2, 14.4(-), 14.1(-), - 4.2(-), - 4.4(-).

Experiment 35: Butyric acid *cis*-2-butyryloxymethylcyclopropylmethyl ester (186)

Butyric anhydride (27.3 g, 172.6 mmol, 2.2 mol eq.) was added to the (*cis*-2-hydroxymethyl-cyclopropyl)-methanol (**125**)[184] (8 g, 78.4 mmol) and the mixture was refluxed at 120 ^{o}C for 1 h. then cooled to r.t. CH_2Cl_2 (100 ml) and sodium NaOH solution (7 g in 100 ml water) were added, then extracted. The aqueous layer was re-extracted with CH_2Cl_2 (2 x 25 ml) and the combined organic layers were washed with aq. $NaHCO_3$ (50 ml). The solution was dried, the solvent was evaporated and excess of butyric anhydride was distilled at high vacuum. The crude product was purified by column chromatography eluting with petrol / ether (5:1 then 1:1) to give a colourless oil, butyric acid *cis*-2-butyryloxymethylcyclopropylmethyl ester (**186**)[184] (13.4 g, 71 %).

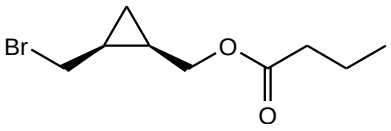
Experiment 36: Butyric acid *cis*-2-hydroxymethylcyclopropylmethyl ester (124)



Lipase (1 g, PPL, type II, crude, Sigma cat. no. L 3126) was added to a flask fitted with a combined glass electrode which had been accurately calibrated, containing a gently stirred solution of ethylene glycol (41 ml) and distilled water (161 ml) at 3 ^{o}C under nitrogen. The pH was 6.8 and butyric acid *cis*-2-butyryloxymethyl-cyclopropylmethyl ester (**186**) (10.5 g, 43.4 mmol) was then added. When hydrolysis began, pH was lowered due to the formation of butyric acid. The pH was brought back to 6.5 by the careful addition via the syringe of NaOH solution (1M) whilst maintaining the temperature at 3 ^{o}C throughout. Further lipase (0.75 g) was then added to the reaction mixture after 1 h and NaOH solution was added dropwise during the reaction to pH 6.5. Total added NaOH solution was 44 ml and it took 5 h. The mixture was filtered through a bed of celite and the celite bed was first washed with water (25 ml) and then ether (50 ml). Sat. aq. $NaHCO_3$ (60 ml) was added, pH 8.3, and then

neutralised by NH_4Cl solution to pH 7.2. The mixture was extracted with ether (2 x 300 ml) and the combined organic layers were dried. The solvent was evaporated and the crude product was purified by column chromatography eluting with petrol / ether (1:1 then 1:3) to give a colourless oil, butyric acid *cis*-2-hydroxymethyl-cyclopropylmethyl ester (**124**)[184] (5.54 g, 74 %), $[\alpha]_D^{23}$ = + 18.9. (*c* 0.715, $CHCl_3$). This showed ν_{max}/cm^{-1}: 3417, 2966, 1732, 1461, 1367, 1255, 1186, 1093; δ_H: 4.48 (1H, dd, *J* 5.7, 12.0 Hz), 3.85 (1H, dd, *J* 5.4, 12.0 Hz), 3.81 (1H, dd, *J* 9.8, 12.0 Hz), 3.40 (1H, dd, *J* 9.2, 12.0 Hz), 2.31 (2H, t, *J* 7.6 Hz), 2.16–2.11 (1H, m), 1.65 (2H, sext, *J* 7.4 Hz), 1.37–1.24 (2H, m), 0.95 (3H, t, *J* 7.4 Hz), 0.87–0.83 (1H, dt, *J* 5.1, 8.5 Hz), 0.22 (1H, br.q, *J* 5.4 Hz); δ_C: 173.6, 64.4(+), 62.5(+) 36.2(+), 18.6(-), 18.4(+), 14.4(-), 13.6(-), 7.7(+).

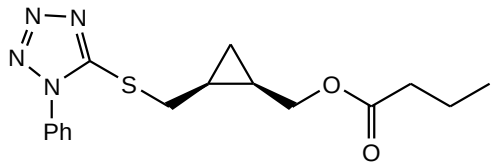
Experiment 37: Butyric acid *cis*-2-bromomethylcyclopropylmethyl ester (188)



PPh_3 (1.75 g, 6.7 mmol) was added to a stirred solution of the primary alcohol (**124**) (1 g, 5.8 mmol) in CH_2Cl_2 (35 ml) and then $NaHCO_3$ (0.15 g) was added. The mixture was cooled to 0 ^oC and NBS (1.31 g, 7.38 mmol) was added portion wise over 10 min. at 0–4 ^oC. Stirring was continued at 0–3 ^oC for further 1 h, when TLC examination indicated that the reaction was completed. A saturated solution of sodium bisulphate (40 ml) was added and the mixture was extracted. The aqueous layer was re-extracted with CH_2Cl_2 (2 x 20 ml) and the combined organic layers were washed with water (50 ml). The solution was dried, the solvent was evaporated and ether (40 ml) and petrol (30 ml) were added. The mixture was stirred for 30 min., the triphenylphosphonium oxide was filtered and washed well with a mixture of petrol / ether (1:1) (40 ml). The solvent was evaporated and the crude product was purified by column chromatography eluting with petrol / ether (4:1 then 2:1) to give a colourless oil, butyric acid *cis*-2-bromomethyl-cyclopropylmethyl ester (**188**)[148] (1.1 g, 80 %), $[\alpha]_D^{23}$ = – 10.6 (*c* 0.805, $CHCl_3$). This showed ν_{max}/cm^{-1}: 2966, 2876, 1734, 1459, 1364, 1181, 1091; δ_H: 4.24 (1H, dd, *J* 6.7, 12.0 Hz), 4.04 (1H, dd, *J* 8.2, 12.0 Hz), 3.53 (1H, dd, *J* 7.6, 10.4 Hz), 3.40 (1H, dd, *J* 8.2, 10.7 Hz), 2.31 (2H, t, *J* 7.4 Hz), 1.67 (2H, sext, *J* 7.4 Hz), 1.56–

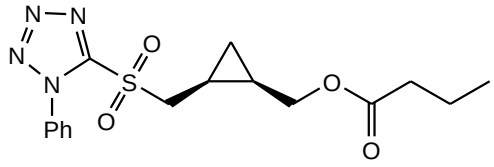
1.45 (2H, m), 1.03 (1H, dt, *J* 5.4, 8.5 Hz), 0.96 (3H, t, *J* 7.4 Hz), 0.38 (1H, br.q, *J* 5.4 Hz); δ_C: 173.6, 63.4(+), 36.2(+), 34.0(+), 19.3(-), 18.4(+), 18.0(-), 13.7(-), 12.5(+).

Experiment 38: Butyric acid (1*R*,2*S*)-2-(1-phenyl-1*H*-tetrazol-5-ylsulfanylmethyl)-cyclopropylmethyl ester (189)



The procedure used in **Experiment 23** was repeated in order to convert the bromo compound (**188**) (4.9 g, 20.85 mmol) using 1-phenyl-1*H*-tetrazole-5-thiol (3.7 g, 20.85 mmol), anhydrous potassium carbonate (6.06 g, 43.79 mmol) and acetone (150 ml) into a crude product. The crude product was purified by column chromatography eluting with petrol / ether (2:1 then 1:1) to give a yellow oil, butyric acid (1*R*,2*S*)-2-(1-phenyl-1*H*-tetrazol-5-ylsulfanylmethyl)cyclopropylmethyl ester (**189**)[148] (6.15 g, 89 %), $[\alpha]_D^{23} = -1.2$ (*c* 1.06, $CHCl_3$). This showed ν_{max}/cm^{-1}: 3071, 2965, 2875, 1732, 1599, 1500, 1461, 1386, 1174, 1090; δ_H: 7.58–7.55 (5H, m), 4.33 (1H, dd, *J* 6.6, 12.0 Hz), 3.95 (1H, dd, *J* 9.2, 12.0 Hz), 3.58 (1H, dd, *J* 7.7, 13.4 Hz), 3.42 (1H, dd, *J* 8.0, 13.4 Hz), 2.26 (2H, t, *J* 7.6 Hz), 1.63 (2H, sext, *J* 7.4 Hz), 1.54–1.47 (1H, m), 1.43–1.36 (1H, m), 0.97 (1H, dt, *J* 5.4, 8.2 Hz), 0.92 (3H, t, *J* 7.4 Hz), 0.39 (1H, br.q, *J* 5.7 Hz); δ_C: 173.5, 154.2, 133.7, 130.1(+), 129.7(+), 123.8(+), 63.7(-), 36.1(-), 34.1(-), 18.3(-), 16.3(+), 15.4(+), 13.6(+), 10.9(-) [+ = CH, CH_3, - = CH_2].

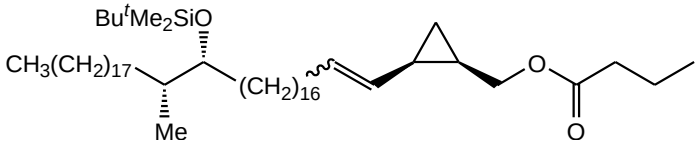
Experiment 39: Butyric acid (1*R*,2*S*)-2-(1-phenyl-1*H*-tetrazole-5-sulfonylmethyl)-cyclopropylmethyl ester (123)



The procedure used in **Experiment 25** was repeated in order to oxidise the sulfane (**189**) (5.6 g, 16.7 mmol) using ammonium molybdate (VI) tetrahydrate (10.42 g, 8.4 mmol) in 35 % H_2O_2 (28 ml) in THF (75 ml) and IMS (150 ml) and further ammonium molybdate (VI) tetrahydrate (5.6 g, 4.5 mmol) in 35% H_2O_2 (15 ml) to give a crude product. The crude product was purified by column chromatography eluting with petrol / ether (2:1 and then 1:1) to give a thick colourless oil, butyric acid (1*R*,2*S*)-2-(1-

phenyl-1*H*-tetrazole-5-sulfonylmethyl)cyclopropylmethyl ester (**123**)[148] (5.82 g, 95 %), $[\alpha]_D^{23}$ = + 52.7 (*c* 1.45, $CHCl_3$). This showed ν_{max}/cm^{-1}: 2967, 2877, 1732, 1498, 1461, 1343, 1258, 1101; δ_H: 7.71–7.69 (2H, m), 7.66–7.60 (3H, m), 4.38 (1H, dd, *J* 5.4, 12.3 Hz), 4.05 (1H, dd, *J* 5.1, 14.8 Hz), 3.91 (1H, dd, *J* 8.2, 12.3 Hz), 3.67 (1H, dd, *J* 8.5, 14.8 Hz), 2.31 (2H, t, *J* 7.4 Hz), 1.66 (2H, sext, *J* 7.4 Hz), 1.52–1.45 (2H, m), 1.03 (1H, dt, *J* 6.0, 8.5 Hz), 0.96 (3H, t, *J* 7.4 Hz), 0.60 (1H, br.q, *J* 6.0 Hz); δ_C: 173.4, 153.6, 133.0, 131.5(-), 129.7(-), 125.1(-), 63.4(+), 56.6(+), 36.1(+), 18.4(+), 14.6(-), 13.6(-), 9.7(+), 8.6(-).

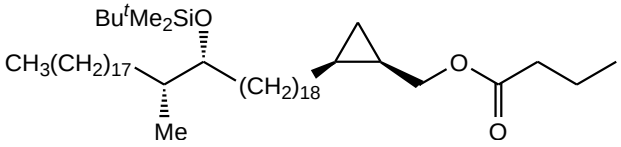
Experiment 40: Butyric acid (1*R*,2*S*)-2-[(*E*/*Z*)-(19*R*,20*R*)-19-(tert-butyldimethyl-silanyloxy)-20-methyl-octatriacont-1-enyl]-cyclopropylmethyl ester (190)



The procedure used in **Experiment 27** was repeated in order to couple the aldehyde (**126**) (1.87 g, 2.76 mmol) with the sulfone (1.2 g, 3.31 mmol) (**123**) using lithium bis(trimethylsilyl) amide (4.9 ml, 5.2 mmol, 1.06M) in dry THF. The crude product was purified by column chromatography eluting with petrol / ether (20:1) to give a colourless oil, *butyric acid (1R,2S)-2-[(E/Z)-(19R,20R)-19-(tert-butyldimethyl-silanyloxy)-20-methyl-octatriacont-1-enyl]-cyclopropylmethyl ester* (**190**) (2.05 g, 91 %) as a mixture of two isomers in ratio 1.6:1, {Found $(M + Na)^+$: 839.7642, $C_{53}H_{104}NaO_3Si$ requires: 839.7647}. This showed ν_{max}/cm^{-1}: 2958, 2854, 1739, 1464, 1252, 1180, 1080; δ_H (major *E* isomer): 5.57 (1H, dt, *J* 15.2, 7.0 Hz), 5.22 (1H, dd, *J* 15.2, 7.6 Hz), 4.16–4.12 (1H, m), 4.01–3.93 (1H, m), 3.51–3.48 (1H, m), 2.30 (2H, t, *J* 7.6 Hz), 2.00 (2H, br.q, *J* 7.0 Hz), 1.66 (2H, sext, *J* 7.4 Hz), 1.62–1.57 (1H, m), 1.51–1.13 (66H, m, v.br.), 1.08–1.01 (1H, m), 0.96 (3H, t, *J* 7.6 Hz), 0.90–0.88 (12H, m, including a s), 0.80 (3H, d, *J* 6.7 Hz), 0.43 (1H, br.q, *J* 5.4 Hz), 0.04 (3H, s), 0.03 (3H, s); δ_H (minor *Z* isomer): 5.47 (1H, dt, *J* 11.0, 7.3 Hz), 5.04 (1H, br.t, *J* 10.4 Hz), 2.18–2.13 (2H, m), 1.80–1.73 (1H, m), 0.39 (1H, br.q, *J* 5.4 Hz) (the remaining signals were obscured by the major isomer); δ_C (two isomers): 173.8, 132.3(-), 132.2(-), 127.4(-), 127.3(-), 75.9(-), 65.1(+), 64.9(+), 37.7(-), 36.3(+), 33.6(+), 32.6(+), 32.5(+), 31.9(+), 30.0(+), 29.9(+), 29.7(+, v.br.), 29.66(+), 29.63(+), 29.60(+), 29.5(+), 29.4(+), 29.2(+),

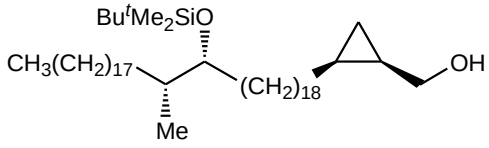
27.72(+), 27.67(+), 26.0(-), 25.9(+), 22.7(+), 18.53(+), 18.51(+), 18.4(-), 18.2, 16.6(-), 16.3(-), 14.4(-), 14.2(-), 14.1(-), 13.7(-), 12.3(+), 10.4(+), - 4.2(+), - 4.4(-).

Experiment 41: Butyric acid (1*R*,2*S*)-2-[(19R,20R)-19-(*tert*-butyl-dimethyl-silanyloxy)-20-methyl-octatriacontyl]-cyclopropylmethyl ester (122)



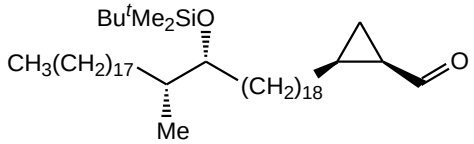
The alkene (**190**) (2.5 g, 3.06 mmol) and TPBSH (3.2 g, 10.7 mmol) were dissolved in dry THF (50 ml) and stirred at 40 ^{o}C for 3 hrs. Further TPBSH (1 g, 3.34 mmol) was added and stirred at 40 ^{o}C for 24 hrs. The mixture was diluted with petrol / ether (1:1, 200 ml) and aq. NaOH (80 ml, 2 %) was added and extracted. The aqueous layer was re-extracted with petrol / ether (1:1, 2 x 60 ml) and the combined organic layers were washed with water (80 ml), dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol / ether (25:1) to give a colourless oil which contained a small amount of olefin. To remove the olefin, the mixture was dissolved in CH_2Cl_2 (35 ml) and water (35 ml) then acetic acid (1 ml), cetrimide (0.15 g) and $KMnO_4$ (0.7 g) were added respectively and stirred at r.t. for 1 h. Sodium metabisulfite was added until the dark colour disappeared and the products were extracted with CH_2Cl_2 (3 x 30 ml). The combined organic layers were dried, filtered and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol / ether (25:1) to give a colourless oil, *butyric acid (1R,2S)-2-[(19R,20R)-19-(tert-butyl-dimethyl-silanyloxy)-20-methyl-octatriacontyl]-cyclopropylmethyl ester* (**122**) (1.75 g, 70 %), $[\alpha]_D^{24}$ = + 9.8 (*c* 1.26, $CHCl_3$), {Found $(M + Na)^+$: 841.7820, $C_{53}H_{106}NaO_3Si$ requires: 841.7803}. This showed ν_{max}/cm^{-1}: 2924, 2854, 1739, 1665, 1252, 1181; δ_H: 4.20 (1H, dd, *J* 6.9, 11.7 Hz), 3.95 (1H, dd, *J* 8.5, 11.7 Hz), 3.51 (1H, dt, *J* 3.5, 6.3 Hz), 2.31 (2H, t, *J* 7.4 Hz), 1.68 (2H, sext, *J* 7.4 Hz), 1.50–1.13 (71H, m, v. br.), 1.16–1.12 (1H, m), 1.08–1.01 (1H, m), 0.97 (3H, t, *J* 7.4 Hz), 0.91–0.88 (12H, s and t, *J* 6.9 Hz), 0.81 (3H, d, *J* 6.9 Hz), 0.75 (1H, dt, *J* 5.1, 8.5 Hz), 0.04 (3H, s), 0.03 (3H, s), 0.01 (1H, br.q, *J* 5.1 Hz); δ_C: 173.8, 75.9(-), 65.1(+), 37.8(-), 36.4(+), 33.6(+), 32.5(+), 31.9(+), 30.0(+), 29.9(+), 29.7(+, v.br.), 29.6(+), 29.4(+), 28.6(+), 27.7(+), 26.0(-), 25.9(+), 22.7(+), 18.8(+), 18.2, 16.3(-), 14.4(-), 14.2(-), 14.1(-), 13.7(-), 9.8(+), - 4.2(-), - 4.4(-).

Experiment 42: {(1*R*,2*S*)-2-[(19*R*,20*R*)-19-(*tert*-Butyl-dimethyl-silanyloxy)-20-methyl-octatriacontyl]-cyclopropyl}-methanol (194)



The procedure used in **Experiment 31** was repeated in order to reduce the ester (**122**) (1.48 g, 1.81 mmol) using $LiAlH_4$ (0.17 g, 4.35 mmol). The crude product was purified by column chromatography eluting with petrol / ether (4:3) to give a colourless oil, *{(1R,2S)-2-[(19R,20R)-19-(tert-butyl-dimethyl-silanyloxy)-20-methyl-octatriacontyl]-cyclopropyl}-methanol* (**194**) (1.31 g, 97 %), $[\alpha]_D^{22}$ = + 10.5 (*c* 0.89, $CHCl_3$), {Found $(M + Na)^+$: 771.7363, $C_{49}H_{100}NaO_2Si$ requires: 771.7385}. This showed ν_{max}/cm^{-1}: 3346, 2924, 2853, 1465, 1253, 1033; δ_H: 3.67–3.64 (1H, m), 3.61–3.57 (1H, m), 3.52–3.48 (1H, m), 1.51–1.21 (71H, m, v.br), 1.15–1.09 (1H), 1.07–1.01 (1H, m), 0.90–0.88 (12H, s and t, *J* 6.7 Hz), 0.80 (3H, d, *J* 6.6 Hz), 0.71 (1H, dt, *J* 4.4, 8.2 Hz), 0.04 (3H, s), 0.03 (3H, s), - 0.03 (1H, br.q, *J* 5.4 Hz); δ_C: 75.9(-), 63.4(+), 37.7(-), 33.5(+), 32.5(+), 31.9(+), 30.2(+), 30.0(+), 29.9(+), 29.7(+, v.br.), 29.67(+), 29.65(+), 29.6(+), 29.4(+), 28.6(+), 27.7(+), 26.0(-), 25.9(+), 22.7(+), 18.2(-), 16.2(-), 14.4(-), 14.1(-), 9.5(+), - 4.2(-), - 4.4(-).

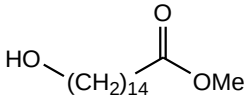
Experiment 43: (1*R*,2*S*)-2-[(19*R*,20*R*)-19-(*tert*-Butyldimethylsilanyloxy)-20-methyl-octatriacontyl]-cyclopropanecarbaldehyde (121)



The procedure used in **Experiment 18** was repeated in order to oxidise the alcohol (**194**) (0.9 g, 1.2 mmol) using PCC (0.65 g, 3.0 mmol) in CH_2Cl_2 (120 ml). The crude product was purified by column chromatography eluting with petrol / ether (6:1) to give a colourless oil, *(1R,2S)-2-[(19R,20R)-19-(tert-butyldimethylsilanyloxy)-20-methyl-octatriacontyl]-cyclopropane-carbaldehyde* (**121**) (0.9 g, 100 %), $[\alpha]_D^{25}$ = + 7.6 (*c* 1.19, $CHCl_3$), {Found $(M + H)^+$: 747.7397, $C_{49}H_{99}O_2Si$ requires: 747.7409}. This showed ν_{max}/cm^{-1}: 2925, 2853, 1706, 1465, 1253, 1057; δ_H: 9.36 (1H, d, *J* 5.7 Hz), 3.52–3.49 (1H, m), 1.87 (1H, ddt, *J* 8.2, 5.7, 5.4 Hz), 1.62–1.56 (1H, m), 1.50–1.18 (72H, m, v.br.), 1.07–1.01 (1H, m), 0.90–0.87 (12H, m, including a s), 0.80 (3H, d, *J*

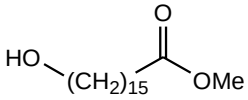
6.7 Hz), 0.04 (3H, s), 0.03 (3H, s); δ_C: 201.7(-), 75.9(-), 37.7(-), 33.6(+), 32.5(+), 31.9(+), 30.0(+), 29.99(+), 29.9(+), 29.7(+, v.br.), 29.69(+), 29.67(+), 29.62(+), 29.56(+), 29.4(+), 29.3(+), 28.2(+), 27.8(-), 27.7(+), 26.0(-), 25.9(+), 24.8(-), 22.7(+), 18.2, 14.7(+), 14.4(-), 14.1(-), - 4.2(-), - 4.4(-).

Experiment 44: 15-Hydroxypentadecanoic acid methyl ester (196)



Sodium (0.95 g, 41.5 mmol) was added to methanol (50 ml) at 0 ^oC with stirring. The mixture was warmed to r.t. and stirred until all of the sodium was consumed. ω-Pentadecalactone (**195**) (2 g, 8.3 mmol) was added with stirring, and the solution was stirred at 80 ^oC for 3 hrs. The reaction was quenched with aq. HCl (70 ml, 1N) and diluted with water (100 ml). The mixture was extracted with ether (3 x 70 ml), the combined organic layers were washed with water (250 ml) and then brine (100 ml) and dried. The solvent was evaporated to give a white solid and the product was a mixture of the ester and acid, so the white solid was dissolved with methanol (60 ml) and 8 drops of H_2SO_4 in methanol (1 ml) was added. The solution was refluxed for 90 min, cooled to r.t. and methanol was evaporated. The product was dissolved with ether (80 ml), washed with sat. aq. $NaHCO_3$ (50 ml) and then brine (60 ml) and dried. The ether was evaporated and the crude product was purified by column chromatography eluting with petrol / ethyl acetate (2:1) to give a white solid, 15-hydroxy-pentadecanoic acid methyl ester (**196**)[190] (2.05 g, 91 %), m.p.: 44–46 ^oC. This showed ν_{max}/cm^{-1}: 3298, 2919, 2850, 1742, 1464, 1178; δ_H: 3.67 (3H, s), 3.64 (2H, t, *J* 6.6 Hz), 2.31 (2H, t, *J* 7.6 Hz), 1.65–1.54 (4H, m), 1.36–1.26 (20H, m); δ_C: 174.3, 63.1(+), 51.4(-), 34.1(+), 32.8(+), 29.6(+), 29.5(+), 29.4(+), 29.3(+), 29.2(+), 25.7(+), 25.0(+).

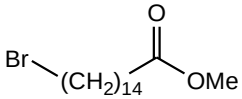
Experiment 45: 16-Hydroxy-hexadecanoic acid methyl ester (227)



The procedure used in **Experiment 44** was repeated in order to ring open 16-hexadecanolide (**226**) (10 g, 39.3 mmol) using sodium (4.52 g, 196.5 mmol) in methanol (220 ml) to give a white solid, 16-hydroxy-hexadecanoic acid methyl ester (**227**)[229] (11.12 g, 90 %), m.p.: 57–58 ^oC, {Found $(M + Na)^+$: 309.2375, $C_{17}H_{34}NaO_3$

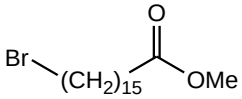
requires: 309.2400}. This showed ν_{max}/cm^{-1}: 3404, 2921, 2850, 1744; δ_H: 3.67 (3H, s), 3.65 (2H, t, *J* 6.6 Hz), 2.31 (2H, t, *J* 7.6 Hz), 1.66–1.60 (2H, m), 1.59–1.55 (2H, m), 1.36–1.27 (22H, m); δ_C: 174.3, 63.1(+), 51.4(-), 34.1(+), 32.8(+), 29.62(+), 29.60(+), 29.58(+), 29.56(+), 29.4(+), 29.2(+), 29.1(+), 25.7(+), 25 0(+).

Experiment 46: 15-Bromopentadecanoic acid methyl ester (197)



PPh_3 (1.44 g, 5.5 mmol) was added to a stirred solution of 15-hydroxypentadecanoic acid methyl ester (**196**) (1.3 g, 4.8 mmol) in CH_2Cl_2 (50 ml) and then sodium bicarbonate (0.15 g) was added. The mixture was cooled to 0 ^oC and NBS (1.08 g, 6.1 mmol) was added portion wise over 10 min at 0–4 ^oC. Stirring was continued at 0–3 ^oC for a further 1 h, when TLC indicated that the reaction was complete. A saturated solution of sodium bisulphate (40 ml) was added and the mixture was extracted. The aqueous layer was re-extracted with CH_2Cl_2 (2 x 20 ml) and the combined organic layers were washed with water (50 ml). The solution was dried, the solvent was evaporated and ether (40 ml) and petrol (30 ml) were added. The mixture was stirred for 30 min, the triphenylphosphonium oxide was filtered and washed well with a mixture of petrol / ether (1:1, 40 ml). The solvent was evaporated and the crude product was purified by column chromatography eluting with petrol / ether (20:1) to give a white solid, 15-bromopentadecanoic acid methyl ester (**197**)[190] (1.35 g, 84 %), m.p.: 38–39 ^oC. This showed ν_{max}/cm^{-1}: 2918, 2848, 1737, 1435, 1251, 1173; δ_H: 3.67 (3H, s), 3.41 (2H, t, *J* 6.6 Hz), 2.31 (2H, t, *J* 7.6 Hz), 1.89–1.83 (2H, quintet, *J* 7.0 Hz), 1.65–1.60 (2H, m), 1.45–1.40 (2H, m), 1.30–1.26 (18H, m); δ_C: 174.3, 51.4(-), 34.1(+), 34.0(+), 32.9(+), 29.59(+), 29.57(+), 29.52(+), 29.4(+), 29.3(+), 29.2(+), 28.8(+), 28.2(+), 25.0(+).

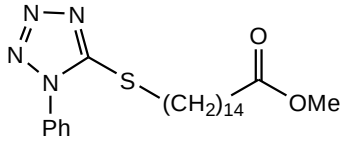
Experiment 47: 16-Bromo-hexadecanoic acid methyl ester (228)



The procedure used in **Experiment 46** was repeated in order to convert the alcohol (**227**) (9.85 g, 34.4 mmol) using NBS (7.79 g, 43.7 mmol) and PPh_3 (10.4 g, 39.6 mmol) in CH_2Cl_2 (250 ml) into a white solid, *16-bromo-hexadecanoic acid methyl*

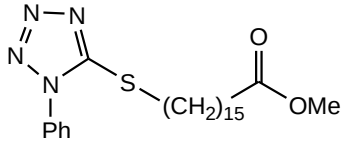
ester (**228**) (11.4 g, 95 %), m.p.: 34–35 ^{o}C, {Found (M + Na)$^{+}$: 371.1529, $C_{17}H_{33}BrNaO_2$ requires: 371.1556}. This showed ν_{max}/cm^{-1}: 2916, 2851, 1746, 1473, 1167; δ_H: 3.67 (3H, s), 3.41 (2H, t, *J* 7.0 Hz), 2.31 (2H, t, *J* 7.6 Hz), 1.86 (2H, quintet, *J* 7.0 Hz), 1.65–1.60 (2H, m), 1.45–1.42 (2H, m), 1.30–1.27 (20H, m); δ_C: 174.3, 51.4(-), 34.1(+), 34.0(+), 32.9(+), 29.6(+), 29.57(+), 29.52(+), 29.4(+), 29.2(+), 29.1(+), 28.8(+), 28.2(+), 25.0(+).

Experiment 48: 15-(1-Phenyl-1*H*-tetrazol-5-ylsulfanyl)-pentadecanoic acid methyl ester (198)



The procedure used in **Experiment 23** was repeated using 15-bromopentadecanoic acid methyl ester (**197**) (1.1 g, 3.28 mmol), 1-phenyl-1*H*-tetrazole-5-thiol (0.58 g, 3.25 mmol), anhydrous potassium carbonate (0.95 g, 6.89 mmol) and acetone (50 ml). The crude product was purified by column chromatography eluting with petrol / ether (1:1) to give a white solid, *15-(1-phenyl-1H-tetrazol-5-ylsulfanyl)-pentadecanoic acid methyl ester* (**198**) (1.2 g, 86 %), m.p.: 62–64 ^{o}C, {Found: C, 63.80; H, 8.61; N, 12.90. $C_{23}H_{36}N_4O_2S$ requires: C, 63.85; H, 8.39; N, 12.95; Found (M + Na)$^{+}$: 455.2448, $C_{23}H_{36}N_4NaO_2S$ requires: 455.2451}. This showed ν_{max}/cm^{-1}: 2916, 2850, 1742, 1499, 1472, 1250, 1171; δ_H: 7.60–7.7.56 (5H, m), 3.67 (3H, s), 3.39 (2H, t, *J* 7.3 Hz), 2.30 (2H, t, *J* 7.6 Hz), 1.82 (2H, quintet, *J* 7.4 Hz), 1.62 (2H, quintet, *J* 7.4 Hz), 1.47–1.41 (2H, m), 1.32–1.25 (18H, m); δ_C: 174.3, 154.5, 133.8, 130.1(-), 129.8(-), 123.9(-), 51.4(-), 34.1(+), 33.4(+), 29.59(+), 29.56(+), 29.52(+), 29.4(+), 29.3(+), 29.2(+), 29.1(+), 29.0(+), 28.6(+), 25.0(+).

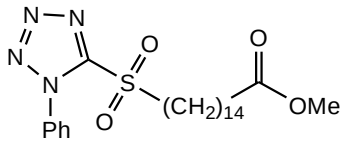
Experiment 49: 16-(1-Phenyl-1*H*-tetrazol-5-ylsulfanyl)-hexadecanoic acid methyl ester (229)



The procedure used in **Experiment 23** was repeated using 16-bromo-hexadecanoic acid methyl ester (**228**) (11 g, 31.5 mmol), 1-phenyl-1*H*-tetrazole-5-thiol (5.61 g, 31.5 mmol), anhydrous potassium carbonate (9.15 g, 66.2 mmol) and acetone (200 ml). The

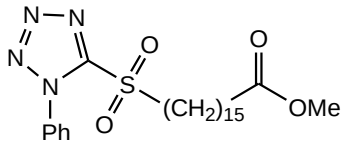
solid crude product was re-crystallised with petrol / ether (2:1, 700 ml) to give a white solid, *16-(1-phenyl-1H-tetrazol-5-ylsulfanyl)-hexadecanoic acid methyl ester* (**229**) (12.67 g, 90 %), m.p.: 71–72 ^{o}C, {Found (M + Na)$^{+}$: 469.2614, $C_{24}H_{38}N_4NaO_2S$ requires: 469.2608}. This showed ν_{max}/cm^{-1}: 2916, 2851, 1743, 1503, 1474; δ_H: 7.60–7.54 (5H, m), 3.67 (3H, s), 3.40 (2H, t, *J* 7.6 Hz), 2.31 (2H, t, *J* 7.6 Hz), 1.86–1.80 (2H, m), 1.65–1.60 (2H, m), 1.48–1.42 (2H, m), 1.30–1.26 (20H, m); δ_C: 174.3, 154.5, 133.9, 130.0(-), 129.7(-), 123.9(-), 51.4(-), 34.1(+), 33.4(+), 29.6(+), 29.58(+), 29.57(+), 29.52(+), 29.4(+), 29.2(+), 29.15(+), 29.11(+), 29.0(+), 28.6(+), 25.0(+).

Experiment 50: 15-(1-Phenyl-1*H*-tetrazole-5-sulfonyl)-pentadecanoic acid methyl ester (199)



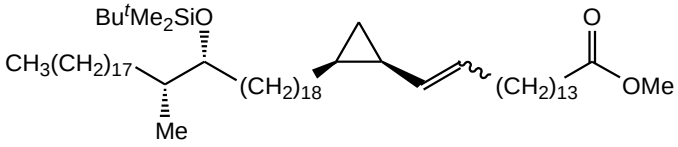
The procedure used in **Experiment 25** was repeated using the sulfane (**198**) (1.1 g, 2.55 mmol), ammonium molybdate (VI) tetrahydrate (1.57 g, 1.27 mmol) in 35 % H_2O_2 (4.2 ml) in THF (40 ml) and IMS (40 ml), and further ammonium molybdate (VI) tetrahydrate (0.8 g, 0.65 mmol) in 35% H_2O_2 (2.2 ml). The crude product was purified by column chromatography eluting with petrol / ether (1:1) to give a white solid, *15-(1-phenyl-1H-tetrazole-5-sulfonyl)-pentadecanoic acid methyl ester* (**199**) (0.95 g, 81 %), m.p.: 72–73 ^{o}C, {Found: C, 60.10; H, 7.60; N, 12.22. $C_{23}H_{36}N_4O_4S$ requires: C, 59.46; H, 7.81; N, 12.06; Found (M + Na)$^{+}$: 487.2343, $C_{23}H_{36}N_4NaO_4S$ requires: 487.2349}. This showed ν_{max}/cm^{-1}: 2918, 2948, 1729, 1497, 1464, 1343, 1255, 1199, 1157; δ_H: 7.70–7.69 (2H, m), 7.64–7.58 (3H, m), 3.75–3.72 (2H, m), 3.67 (3H, s), 2.30 (2H, t, *J* 7.6 Hz), 1.98–1.92 (2H, m), 1.64–1.58 (2H, m), 1.52–1.46 (2H, m), 1.35–1.26 (18H, m); δ_C: 174.3, 153.5, 133.1, 131.4(+), 129.7(+), 125.1(+), 56.0(-), 51.4(+), 34.1(-), 29.57(-), 29.55(-), 29.53(-), 29.44(-), 29.3(-), 29.2(-), 29.1(-), 28.9(-), 28.1(-), 25.0(-), 21.9(-) [+ = CH, CH_3, - = CH_2].

Experiment 51: 16-(1-Phenyl-1*H*-tetrazole-5-sulfonyl)-hexadecanoic acid methyl ester (230)



The procedure used in **Experiment 25** was repeated using the sulfane (**229**) (12.5 g, 28.03 mmol), ammonium molybdate (VI) tetrahydrate (16.3 g, 13.2 mmol) in 35 % H_2O_2 (43.4 ml) in THF (160 ml) and IMS (320 ml), and further ammonium molybdate (VI) tetrahydrate (8.8 g, 7.1 mmol) in 35% H_2O_2 (23.5 ml). The solid crude product was re-crystallised with petrol / ether (2:1, 500 ml) and methanol (100 ml) to give a white solid, *16-(1-phenyl-1H-tetrazole-5-sulfonyl)-hexadecanoic acid methyl ester* (**230**) (11.92 g, 89 %), m.p.: 78–79 ^{o}C, {Found $(M + Na)^+$: 501.2519, $C_{24}H_{38}N_4NaO_4S$ requires: 501.2506}. This showed ν_{max}/cm^{-1} : 2918, 2852, 1737, 1343, 1155; δ_H: 7.71–7.69 (2H, m), 7.65–7.59 (3H, m), 3.75–3.72 (2H, m), 3.67 (3H, s), 2.31 (2H, t, *J* 7.6 Hz), 1.99–1.93 (2H, m), 1.65–1.60 (2H, m), 1.53–1.49 (2H, m), 1.30–1.27 (20H, m); δ_C: 174.3, 153.6, 133.1, 131.4 (-), 129.7 (-), 125.1 (-), 56.1 (+), 51.4 (-), 34.1 (+), 29.6 (+), 29.55 (+), 29.52 (+), 29.4 (+), 29.2 (+), 29.17 (+), 29.1 (+), 28.9 (+), 28.1 (+), 25.0 (+), 22.0 (+).

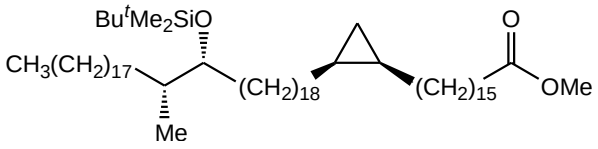
Experiment 52: (*E*/*Z*)-16-{(1*R*,2*S*)-2-[(19*R*,20*R*)-19-(*tert*-Butyldimethylsilanyloxy)-20-methyl-octatriacontyl]-cyclopropyl}-hexadec-15-enoic acid methyl ester (200)



The procedure used in **Experiment 27** was repeated in order to couple the aldehyde (**121**) (0.8 g, 1.07 mmol) with the sulfone (**199**) (0.6 g, 1.29 mmol) using lithium bis (trimethylsilyl) amide (1.5 ml, 1.6 mmol, 1.06M) in dry THF. The crude product was purified by column chromatography eluting with petrol / ether (20:1) to give a colourless oil, *(E/Z)-16-{(1R,2S)-2-[(19R,20R)-19-(tert-butyldimethyl-silanyloxy)-20-methyl-octatriacontyl]-cyclopropyl}-hexadec-15-enoic acid methyl ester* (**200**) (0.86 g, 82 %) as a mixture of two isomers in ratio 5:1, {Found $(M + Na)^+$: 1007.9551, $C_{65}H_{128}NaO_3Si$ requires: 1007.9525}. This showed ν_{max}/cm^{-1}: 2924, 2853, 1743, 1465, 1253, 1171, 1074; δ_H (major *E* isomer): 5.52 (1H, dt, *J* 15.2, 6.9 Hz), 5.18 (1H, dd, *J* 15.2, 8.5 Hz), 3.67 (3H, s), 3.52–3.48 (1H, m), 2.31 (2H, t, *J* 7.6 Hz), 2.00 (2H, br.q, *J*

7.0 Hz), 1.66–1.60 (2H, m), 1.51–1.12 (90H, m, v.br.), 1.09–1.01 (1H, m), 0.89–0.87 (14H, m, including a s), 0.80 (4H, m, including a doublet, *J* 7.0 Hz), 0.11 (1H, br.q, *J* 5.4 Hz), 0.04 (3H, s), 0.03 (3H, s); δ_H (minor *Z* isomer): 5.41 (1H, dt, *J* 10.4, 7.5 Hz), 5.07–5.02 (1H, m), 2.16–2.14 (2H, m) (the remaining signals were obscured by the major isomer); δ_C (two isomers): 174.3, 130.5(-), 129.5(-), 75.9(-), 41.4(-), 37.7(-), 34.1(+), 33.6(+), 32.8(+), 32.5(+), 31.9(+), 30.0(+), 29.9(+), 29.8(+), 29.7(+, v.br.), 29.62(+), 29.58(+), 29.5(+), 29.4(+), 29.3(+), 29.2(+), 27.7(+), 26.0(-), 25.9(+), 25.0(+), 2.7(+), 22.6(+), 18.4(-), 18.3(-), 18.2, 14.4(-), 14.2(-), 12.3(+), - 4.2(-), - 4.4(-).

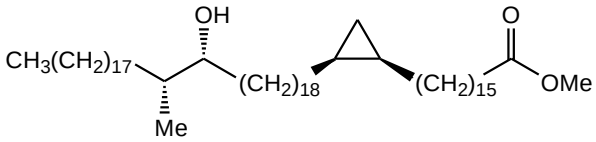
Experiment 53: 16-{(1*R*,2*S*)-2-[(19*R*,20*R*)-19-(*tert*-Butyldimethylsilanyloxy)-20-methyl-octatriacontyl]-cyclopropyl}-hexadecanoic acid methyl ester (120)



TPBSH (1.6 g, 5.4 mmol) was added to a stirred solution of the alkene (**200**) (0.76 g, 0.77 mmol) in THF (25 ml) and triethylamine (0.5 ml) and stirred at 65 ^oC for 18 hrs. NMR showed the reaction was not yet complete and further TPBSH (1 g, 3.35 mmol) was added and stirred at 40 ^oC for 18 hrs. The reaction was allowed to reach r.t., water (25 ml) and ether (40 ml) were added and extracted. The aqueous layer was re-extracted with ether (2 x 30 ml) and the combined organic layers were dried. The solvent was evaporated and the crude product was purified by column chromatography eluting with petrol / ether (70:2) to give a very viscous oil, *16-{(1R,2S)-2-[(19R,20R)-19-(tert-butyldimethylsilanyloxy)-20-methyl-octatriacontyl]-cyclopropyl}-hexadecanoic acid methyl ester* (**120**) (0.68 g, 90 %). The NMR showed that there was small amount of unsaturated compound, so this was separated on a silver nitrate column eluting with petrol / ether (60:1) to give product (0.60 g, 79 %). [**To prepare silver nitrate column:** Silica (30 g) was added to a solution of silver nitrate (1 g) in distilled water (20 ml) and IMS (50 ml) and the solvent was evaporated then this mixture was dried at 110 ^oC for 1h. This was cooled to r.t. in a dark place and dissolved with petrol / ether (60:1) then added to a column which was covered with a dark paper], $[\alpha]_D^{25}$ = + 4.0 (*c* 1.23, $CHCl_3$), {Found: C, 79.45; H, 12.89, $C_{65}H_{130}O_3Si$ requires: C, 79.03; H, 13.26}. This showed ν_{max}/cm^{-1}: 2922, 2852, 1746, 1464, 1252, 836; δ_H: 3.67 (3H, s), 3.52–3.48 (1H, m), 2.31 (2H, t, *J* 7.3 Hz), 1.66–1.60 (2H, m), 1.48–1.14 (96H, m,

v.br.), 1.09–1.02 (1H, m), 0.90–0.87 (12H, m, including a s), 0.80 (3H, d, *J* 6.6 Hz), 0.67–0.64 (2H, m), 0.56 (1H, br.dt, *J* 4.1, 8.2 Hz), 0.04 (3H, s), 0.03 (3H, s), - 0.32 (1H, br.q, *J* 5.4 Hz); δ_C: 174.3, 75.9(-), 51.4(-), 37.7(-), 34.1(+), 33.5(+), 32.5(+), 31.9(+), 30.2(+), 30.0(+), 29.9(+), 29.8(+), 29.7(+, v.br.), 29.67(+), 29.61(+), 29.5(+), 29.4(+), 29.3(+), 29.2 (+), 28.7(+), 27.7(+), 26.0(-), 25.9(+), 25.0(+), 22.7(+), 18.2, 15.8(-), 14.4(-), 14.2(-), 10.9(+), - 4.2(-), - 4.4(-).

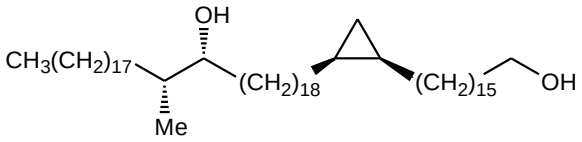
Experiment 54: 16-[(1*R*,2*S*)-2-((19*R*,20*R*)-19-Hydroxy-20-methyl-octatriacontyl)-cyclopropyl]-hexadecanoic acid methyl ester (201)



Tetra-n-butylammonium fluoride (1.05 ml, 1.03 mmol) was added to a stirred solution of the silyl protected compound (**120**) (0.6 g, 0.61 mmol) in dry THF (30 ml) at r.t. and the mixture was stirred at r.t. for 3 h. TLC showed that there was no product and it was heated to 50 ^oC for 6 hrs. TLC also showed no product; n-TBAF (0.3 ml) was added and stirred at 70 ^oC for 18 hrs. There was very small amount product and further n-TBAF (1.2 ml) was added and refluxed for 18 hrs. TLC showed half starting material and half product; further n-TBAF (1 ml) was added and refluxed for 18 hrs. TLC showed that the starting material had almost gone. Sat. aq. NH_4Cl (20 ml) and petrol / ether (1:1, 40 ml) were added and extracted. The aqueous layer was re-extracted with petrol / ether (1:1, 30 ml) and the combined organic layers dried. The solvent was evaporated and the crude product was purified by column chromatography eluting with petrol / ether (5:1 and then 1:1) and just ether to give a white solid, *16-[(1R,2S)-2-((19R,20R)-19-hydroxy-20-methyl-octatriacontyl)-cyclopropyl]-hexadecanoic acid methyl ester* (**201**) (260 mg, 49 %), m.p.: 70–72 ^oC, $[\alpha]_D^{26}$ = + 5.9 (*c* 1.38, $CHCl_3$), {Found: C, 80.90; H, 13.52, $C_{59}H_{116}O_3$ requires: C, 81.12; H, 13.38}. This showed ν_{max}/cm^{-1}: 3511, 2918, 2849, 1726, 1465, 1256, 1178; δ_H: 3.67 (3H, s), 3.52–3.48 (1H, m), 2.31 (2H, t, *J* 7.6 Hz), 1.65–1.60 (2H, m), 1.48–1.12 (97H, m, v.br.), 0.90 (3H, t, *J* 6.6 Hz), 0.87 (3H, d, *J* 6.9 Hz), 0.68–0.63 (2H, m), 0.56 (1H, br.dt, *J* 4.1, 8.2 Hz), -0.33 (1H, br.q, *J* 5.4 Hz); δ_C: 174.3, 75.2(-), 51.4(-), 38.2(-), 34.5(+), 34.1(+), 33.4(+), 31.9(+), 30.2(+), 30.0(+), 29.7(+, v.br.), 29.6 (+), 29.5(+), 29.3(+), 29.2(+), 28.7(+), 27.4(+), 26.3(+), 25.0(+), 22.7(+), 15.8(-), 14.1(-), 13.6(-), 10.9(+) and a white solid,

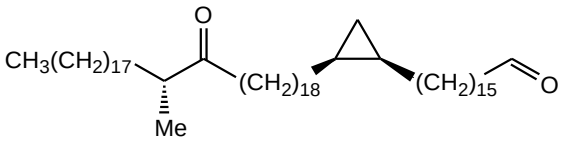
16-[(1R,2S)-2-((19R,20R)-19-hydroxy-20-methyl-octatriacontyl)-cyclopropyl]-hexa-decanoic acid (150 mg, 29 %), m.p.: 58–60 ^{o}C, $[\alpha]_{D}^{26}$ = + 5.66 (c 1.06, $CHCl_3$), {Found $(M + Na)^+$: 881.8677, $C_{58}H_{114}NaO_3$ requires: 881.8660}. This showed ν_{max}/cm^{-1}: 3449, 2918, 2849, 1710, 1465, 1024, 720; δ_H : 3.52–3.48 (1H, m), 2.33 (2H, t, *J* 7.6 Hz), 1.64 (2H, sext, *J* 7.6 Hz), 1.49–1.12 (97H, m, v.br.), 0.86 (3H, t, *J* 6.6 Hz), 0.83 (3H, d, *J* 7.0 Hz), 0.68–0.66 (2H, m), 0.57 (1H, br.dt, *J* 4.1, 8.2 Hz), - 0.33 (1H, br.q, *J* 5.4 Hz); δ_C: 178.7, 75.3(-), 51.2(+), 38.2(-), 34.5(+), 33.4(+), 31.9(+), 30.2(+), 30.0(+), 29.7(+, v.br.), 29.6(+), 29.5(+), 29.4(+), 29.3(+), 29.2(+), 28.7(+), 27.4(+), 26.2(+), 25.0(+), 22.7(+), 20.3(+), 15.7(-), 14.1(-), 13.7(-), 13.6 (-), 10.9(+).

Experiment 55: (19*R*,20*R*)-1-[(1*R*,2*S*)-2-(16-Hydroxy-hexadecyl)-cyclopropyl]-20-methyl-octatriacontan-19-ol (202)



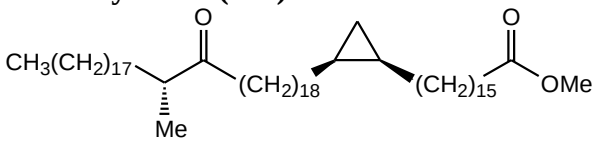
$LiAlH_4$ (30 mg, 0.79 mmol) was added to a stirred dry THF (20 ml) at – 20 ^{o}C under argon and a solution of the methyl ester (**201**) (180 mg, 0.21 mmol) in dry THF (15 ml) was added. The mixture was allowed to reach r.t. and refluxed for 1 h. A saturated solution of sodium sulphate in water was added to the mixture at – 20 ^{o}C until a white precipitate had formed and THF (20 ml) was added. The mixture was stirred at r.t. for 30 min, filtered through a bed of silica, dried and the solvent was evaporated. There was no product so the silica bed was washed with CH_2Cl_2; no product was eluted. The silica bed was washed with hot chloroform and the chloroform was evaporated to give a white solid, *(19R,20R)-1-[(1R,2S)-2-(16-hydroxy-hexadecyl)-cyclopropyl]-20-methyl-octatriacontan-19-ol* (**202**) (150 mg, 88 %), m.p.: 82–83 ^{o}C, $[\alpha]_{D}^{35}$ = + 5.2 (*c* 0.575, $CHCl_3$), {Found: C, 82.56; H, 13.81, $C_{58}H_{116}O_2$ requires: C, 82.39; H, 13.83}. This showed ν_{max}/cm^{-1}: 3345, 2918, 2850, 1464, 1080; δ_H: 3.69–3.64 (2H, m), 3.52–3.48 (1H, m), 1.60–1.55 (2H, m), 1.45–1.16 (99H, m, v.br.), 0.90 (3H, t, *J* 6.6 Hz), 0.87 (3H, d, *J* 6.6 Hz), 0.68–0.64 (2H, m), 0.58 (1H, br.dt, *J* 4.1, 8.2 Hz), - 0.31 (1H, br.q, *J* 5.4 Hz); δ_C: 75.3(-), 63.1(+), 38.3(-), 34.7(+), 33.5(+), 32.9(+), 31.9(+), 30.2(+), 30.0(+), 29.8(+), 29.74(+), 29.7(+, v.br.), 29.63(+), 29.6(+), 29.5(+), 29.4(+), 28.7(+), 27.4(+), 26.3(+), 25.8(+), 22.7(+), 15.9(-), 14.0(-), 13.6(-), 10.98(+).

Experiment 56: 16-[(1*R*,2*S*)-2-((*R*)-20-Methyl-19-oxo-octatriacontyl)-cyclopropyl]-hexadecanal (114)



A solution of the diol (**202**) (100 mg, 0.12 mmol) in CH_2Cl_2 (10 ml) was added to a stirred solution of PCC (128 mg, 0.59 mmol) in CH_2Cl_2 (15 ml) at 40 ^oC. Addition was done portion and portion and during the addition a black colour appeared. The reaction was stirred at 40 ^oC for 1.5 hrs. and TLC showed that the reaction completed. Ether (40 ml) was added and filtered through a bed of silica. The solvent was evaporated and the crude product was purified by column chromatography eluting with petrol / ether (6:1) to give a white solid, *16-[(1R,2S)-2-((R)-20-methyl-19-oxo-octatriacontyl)-cyclopropyl]-hexadecanal* (**114**) (90 mg, 90 %), m.p.: 61–62 ^oC, $[\alpha]_D^{24} = -4.8$ (*c* 0.56, $CHCl_3$), {Found $(M + H)^+$: 841.8702, $C_{58}H_{113}O_2$ requires: 841.8735; Found: C, 82.45; H, 13.29, $C_{58}H_{112}O_2$ requires: C, 82.78; H, 13.42}. This showed ν_{max}/cm^{-1}: 2918, 2849, 1716, 1699, 1472, 1407, 1374, 1070; δ_H: 9.77 (1H, t, *J* 1.9 Hz), 2.51 (1H, sext, *J* 6.9 Hz), 2.46–2.36 (2H, m), 2.42 (2H, dt, *J* 1.9, 7.3 Hz), 1.66–1.54 (4H, m), 1.40–1.12 (92H, m, v.br.), 1.05 (3H, d, *J* 6.9 Hz), 0.89 (3H, t, *J* 7.0 Hz), 0.67–0.63 (2H, m), 0.56 (1H, br.dt, *J* 4.1, 8.2 Hz), -0.33 (1H, br.q, *J* 5.4 Hz); δ_C: 215.2, 202.9(-), 46.3(-), 43.9(+), 41.1(+), 33.0(+), 31.9(+), 30.2(+), 29.7(+, v. br.), 29.60(+), 29.59(+), 29.51(+), 29.49(+), 29.46(+), 29.44(+), 29.4(+), 29.3(+), 29.2(+), 28.7(+), 27.3(+), 23.7(+), 22.7(+), 22.1(+), 16.4(-), 15.8(-), 14.1(-), 10.9(+).

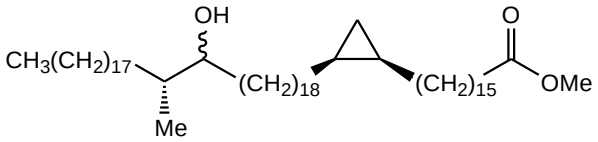
Experiment 57: 16-[(1*R*,2*S*)-2-((*R*)-20-Methyl-19-oxo-octatriacontyl)-cyclopropyl]-hexadecanoic acid methyl ester (203)



The procedure used in **Experiment 56** was repeated in order to oxidise the alcohol (**201**) (80 mg, 0.092 mmol) using PCC (79 mg, 0.37 mmol). The crude product was purified by column chromatography eluting with petrol / ether (6:1) to give a white solid, *16-[(1R,2S)-2-((R)-20-methyl-19-oxo-octatriacontyl)-cyclopropyl]-hexadecanoic acid methyl ester* (**203**) (70 mg, 88 %), m.p.: 67–69 ^oC, $[\alpha]_D^{22} = -4.4$ (*c* 0.59, $CHCl_3$), {Found $(M + Na)^+$: 893.8647, $C_{59}H_{114}NaO_3$ requires: 893.8660; Found: C,

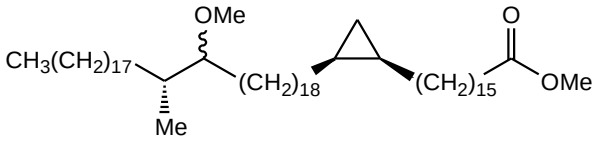
80.85; H, 13.19, $C_{59}H_{114}O_3$ requires: C, 81.31; H, 13.18}. This showed ν_{max}/cm^{-1}: 2919, 2850, 1725, 1699, 1463, 1251; δ_H: 3.67 (3H, s), 2.51 (1H, sext, *J* 6.9 Hz), 2.43 (1H, dt, *J* 16.7, 7.3 Hz), 2.40 (1H, dt, *J* 16.7, 7.3 Hz), 2.31 (2H, t, *J* 7.6 Hz), 1.66–1.60 (2H, m), 1.57–1.52 (2H, m), 1.38–1.12 (92H, m, v.br.), 1.05 (3H, d, *J* 7.0 Hz), 0.89 (3H, t, *J* 6.7 Hz), 0.67–0.63 (2H, m), 0.56 (1H, br.dt, *J* 4.1, 8.2 Hz), - 0.33 (1H, br.q, *J* 5.4 Hz); δ_C: 215.2, 174.3, 51.4(-), 46.3(-), 41.1(+), 34.1(+), 33.0(+), 31.9(+), 30.2(+), 29.7(+, v.br.), 29.65(+), 29.60(+), 29.49(+), 29.46(+), 29.4(+), 29.3(+), 29.2(+), 29.1(+), 28.7(+), 27.3(+), 25.0(+), 23.7(+), 22.7(+), 16.4(-), 15.8(-), 14.1(-), 10.9(+).

Experiment 58: 16-[(1*R*,2*S*)-2-((19*R*/*S*,20*R*)-19-Hydroxy-20-methyl-octatriacontyl)-cyclopropyl]-hexadecanoic acid methyl ester (201 and 204)



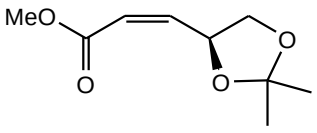
The ketone (**203**) (60 mg, 0.07 mmol) was dissolved in THF (10 ml) and methanol (4 ml) and treated with sodium borohydride (1 mg, 0.03 mmol). The mixture was stirred for 18 hrs. and then quenched by addition of water (10 ml). The mixture was extracted with ether (3 x 15 ml), the combined organic layers were dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol / ether (5:1) to give a white solid, *16-[(1R,2S)-2-((19R/S,20R)-19-hydroxy-20-methyl-octatriacontyl)-cyclopropyl]-hexadecanoic acid methyl ester* (**201** and **204**) (55 mg, 92%), m.p.: 69–71 ^oC, {Found: 895.8786, $C_{59}H_{116}NaO_3$ requires: 895.8817; Found: C, 80.97; H, 13.59, $C_{59}H_{116}O_3$ requires: C, 81.12; H, 13.38}. This showed ν_{max}/cm^{-1}: 3508, 2919, 2850, 1723, 1465, 1256, 1178; δ_H: 3.67 (3H, s), 3.50 (1H, m), 3.43 (1H, m), 2.31 (2H, t, *J* 7.6 Hz), 1.62–1.10 (99H, m, v. br.), 0.90–0.86 (3H, m), 0.68–0.63 (2H, m), 0.56 (1H, br.dt, *J* 4.1, 8.2 Hz), - 0.33 (1H, br.q, *J* 5.4 Hz); δ_C: 174.3, 76.09(-), 75.2(-), 51.4(-), 38.8(-), 38.2(-), 34.5(+), 34.1(+), 33.4(+), 31.9(+), 31.8(+), 30.2(+), 30.0(+), 29.7(+, v.br.), 29.6 (+), 29.5(+), 29.3(+), 29.2(+), 28.7(+), 27.4(+), 27.3 (+), 26.3(+), 26.1(+), 25.0(+), 22.7(+), 15.8(-), 15.3 (-), 14.1(-), 13.6(-), 10.9(+).

Experiment 59: 16-[(1*R*,2*S*)-2-((19*R*/*S*,20*R*)-19-Methoxy-20-methyl-octatriacont-yl)-cyclopropyl]-hexadecanoic acid methyl ester (205 and 206)



Sodium hydride (10 mg, 0.42 mmol) washed with petrol and dissolved with dry THF (10 ml) under argon. The alcohols (**201** and **204**) (40 mg 0.046 mmol) was dissolved with dry THF (8 ml) and added to sodium hydride solution. Iodomethane (1.5 ml) was added and the mixture was stirred at r.t. for 18 hrs. The reaction was cooled to – 20 ^{o}C and water (5 ml) was added dropwise and extracted with ether (3 x 15 ml). The combined organic layers were dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol / ether (35:2) to give a white solid, *16-[(1R,2S)-2-((19R/S,20R)-19-methoxy-20-methyl-octatriacontyl)-cyclopro-pyl]-hexadecanoic acid methyl ester* (**205** and **206**) (32 mg, 80%), m.p.: 55–57 ^{o}C, {Found $(M + Na)^{+}$: 909.8945, $C_{60}H_{118}NaO_3$ requires: 909.8974}. This showed ν_{max}/cm^{-1}: 2923, 2852, 1720, 1462; δ_H: 3.67 (3H, s), 3.35 (3H, s), 3.33 (3H, s), 2.95 (1H, m), 2.31 (2H, t, *J* 7.6 Hz), 1.73–1.05 (99H, m, v. br.), 0.90–0.83 (6H, m), 0.65 (2H, m), 0.57 (1H, br.dt, *J* 4.1, 8.5 Hz), - 0.33 (1H, br.q, *J* 5.1 Hz); δ_C: 174.3, 85.5, 85.4, 57.7, 57.3, 51.4, 35.4, 34.9, 34.1, 32.8, 32.4, 31.9, 31.6, 31.4, 30.5, 30.2, 30.0, 29.9, 29.7 (v. br.), 29.66, 29.61, 29.52, 29.47, 29.37, 29.27, 29.17, 28.7, 28.2, 27.62, 27.59, 26.2, 26.1, 25.0, 22.7, 15.8, 14.9, 14.6, 14.1, 10.9.

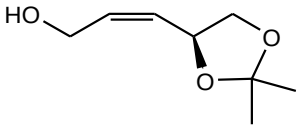
Experiment 60: (*Z*)-3-((*S*)-2,2-Dimethyl-[1,3]dioxolan-4-yl)-acrylic acid methyl ester (217)



2,3-O-Isopropylidene-D-glyceraldehyde (34 g, 0.26 mol) was dissolved in methanol (300 ml) and cooled to 5 ^{o}C. Methyl (triphenylphosphoranylidene)acetate (94.5 g, 0.28 mol) was added to the solution portionwise between 2 and 5 ^{o}C and the mixture was stirred for 1 hr. at 0 ^{o}C. The solvent was removed on a rotary evaporator and the residue was refluxed for 30 min. with a mixture of petrol / ether (7:3, 300 ml). The mixture was filtered, the solvent was evaporated and the process was repeated three times. The crude product was purified by column chromatography eluting with petrol /

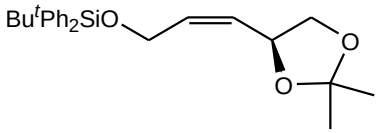
ethyl acetate (9:1) to give a colourless oil, (*Z*)-3-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-acrylic acid methyl ester (**217**)[192,193] (38.62 g, 80%).

Experiment 61: (*Z*)-3-((*S*)-2,2-Dimethyl-[1,3]dioxolan-4-yl)-prop-2-en-1-ol (218)



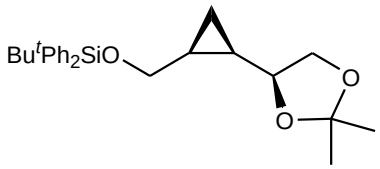
The methyl ester (**217**) (25 g, 0.134 mol) was dissolved in dry CH_2Cl_2 (200 ml) and cooled to -70 ^oC under argon. Diisobutylaluminium hydride (DIBAL-H, 268 ml, 0.268 mol, 2 mol eq., 1M) was added dropwise between -70 ^oC to -55 ^oC. The reaction was stirred for 2 hr. at r.t. Methanol (100 ml) was added at -35 ^oC dropwise to produce a white solid then a saturated aq. sol. of NH_4Cl (20 ml) was added. HCl (8M) was added dropwise to the stirred mixture to dissolve some white solid. The reaction mixture was filtered and on a bed of celite and the precipitate washed with CH_2Cl_2. The solvent was evaporated and the crude product was purified by column chromatography eluting with petrol / ethyl acetate (5:2 then 1:1) to give a colourless oil, (*Z*)-3-((*S*)-2,2-diethyl-[1,3]dioxolan-4-yl)-prop-2-en-1-ol (**218**)[192,193] (18.35 g, 86%).

Experiment 62: *tert*-Butyl-[(*Z*)-3-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-allyloxy]-diphenyl-silane (207)



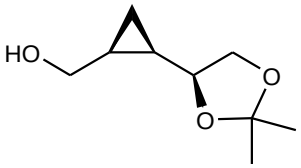
Imidazole (17.36 g, 0.26 mol) was added to a stirred solution of the alcohol (**218**) (18.31 g, 0.116 mol) in dry DMF (150 ml) at 5 ^oC followed by the addition of *tert*-butyldiphenylchlorosilane (35.04 g, 0.127 mol). The mixture was stirred at that temperature for further 15 min and then at r.t. for 18 hrs. The mixture was quenched with water (500 ml) and the product was extracted with CH_2Cl_2 (3 x 250 ml), dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol / ether (7:1) to give a colourless oil, *tert*-butyl-[(*Z*)-3-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-allyloxy]-diphenyl-silane (**207**)[192,193] (43.65 g, 95 %).

Experiment 63: *tert*-Butyl-[(1*S*,2*R*)-2-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-cyclopropylmethoxy]-diphenyl-silane (219)



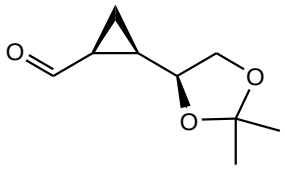
Diiodomethane (175.7 g, 0.66 mol) was added to a stirred solution of the alkene (**207**) (43.3 g, 0.11 mol) in 1,2-dichloroethane (300 ml) at r.t. under argon and the solution was cooled to – 28 ^{o}C. Diethyl zinc (328 ml, 0.33 mol, 1M) was added by dropwise between – 28 and – 14 ^{o}C, then the solution warmed to 0 ^{o}C and stirred for 4 hrs. The reaction was quenched with sat. aq. NH_4Cl (250 ml), extracted with chloroform (3 x 200 ml), the combined organic phases washed with water (500 ml) and dried. The solvent was evaporated and the crude product was purified by column chromatography eluting with petrol / ether (8:1) to give a colourless oil, *tert*-butyl-[(1*S*,2*R*)-2-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-cyclopropylmethoxy]-diphenyl-silane (**219**)[192,193] (36.81 g, 82 %).

Experiment 64: [(1*S*,2*R*)-2-((*S*)-2,2-Dimethyl-[1,3]dioxolan-4-yl)-cyclopropyl]-methanol (220)



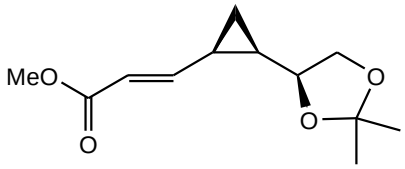
n-TBAF (118 ml, 117.5 mmol) was added to a stirred solution of the protected compound (**219**) (34.4 g, 83.9 mmol) in dry THF (150 ml) at 0 ^{o}C under argon. The mixture was allowed to reach room temperature and stirred for 18 hrs, when T.L.C. showed no starting material. The mixture was cooled to 5 ^{o}C and quenched with sat. aq. NH_4Cl (75 ml) and the product was extracted with ethyl acetate (3 x 200 ml). The combined organic layers were washed with brine (150 ml), dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol / ethyl acetate (1:1) to give a colourless oil, [(1*S*,2*R*)-2-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-cyclopropyl]-methanol (**220**)[138] (12.25 g, 85 %).

Experiment 65: (1*S*,2*R*)-2-((*S*)-2,2-Dimethyl-[1,3]dioxolan-4-yl)-cyclopropane-carbaldehyde (221)



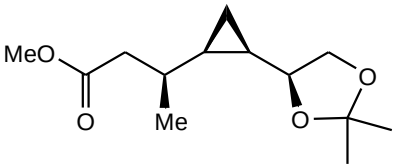
The procedure used in **Experiment 18** was repeated in order to oxidise the alcohol (**220**) (11.1 g, 64.5 mmol) using PCC (31.9 g, 148.4) in CH_2Cl_2 (450 ml). The crude product was purified by column chromatography eluting with petrol / ethyl acetate (1:1) to give a colourless oil, (1*S*,2*R*)-2-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-cyclopropanecarbaldehyde (**221**)[138] (9.85 g, 90 %).

Experiment 66: (*E*/*Z*)- 3-[(1*R*,2*R*)-2-((*S*)-2,2-Dimethyl-[1,3]dioxolan-4-yl)-cyclopropyl]-acrylic acid methyl ester (208)



Methyl (triphenylphosphoranylidene)acetate (23.1 g, 69.1 mmol) was added to a stirred solution of the aldehyde (**221**) (9.8 g, 57.6 mmol) in toluene (250 ml) at r.t. and stirred for 18 hrs. at r.t. The toluene was evaporated, the residue was diluted with petrol / ether 300 ml, 1:1) and refluxed for 15 min. The suspension was filtered and the precipitate was washed with petrol / ether (1:1, 3 x 100 ml). The solvent was evaporated and the crude product was purified by column chromatography on silica eluting with petrol / ether (5:2) to give a colourless oil, (*E*/*Z*)- 3-[(1*R*,2*R*)-2-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-cyclopropyl]-acrylic acid methyl ester (**208**)[138] (11.61 g, 89 %) in a ratio 2:1.

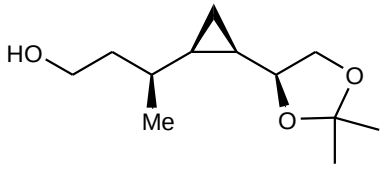
Experiment 67: (*S*)-3-[(1*R*,2*R*)-2-((S)-2,2-Dimethyl-[1,3]dioxolan-4-yl)-cyclopropyl]-butyric acid methyl ester (209)



Methyl magnesium bromide (51.3 ml, 153.9 mmol, 3M in ether) was added dropwise to a stirred suspension of copper bromide (11.04 g, 76.9 mmol) in dry THF (250 ml) at

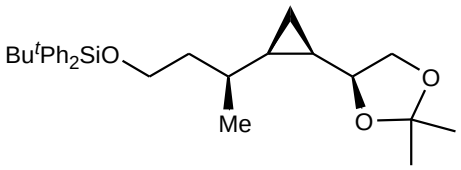
– 40 °C under argon and the mixture was stirred at – 20 °C for 30 min. The alkene (**208**) (11.6 g, 51.3 mmol) in dry THF (40 ml) was added drowise at – 30 °C and the mixture was allowed to reach – 8 °C in cooling bath. The mixture was cooled to – 40 °C and sat. aq. NH_4Cl (500 ml) was added dropwise and allowed to reach r.t. The mixture was extracted with ethyl acetate (2 x 200 ml) and the combined organic phases were dried. The solvent was evaporated and the crude product was purified by column chromatography eluting with petrol / ethyl acetate (5:1) to give a bright yellow oil, (*S*)-3-[(1*R*,2*R*)-2-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-cyclopropyl]-butyric acid methyl ester (**209**)[138] (8.96 g, 72 %), $[\alpha]_D^{25}$ = + 13.3 (*c* 0.93, $CHCl_3$).

Experiment 68: (*S*)-3-[(1*R*,2*R*)-2-((*S*)-2,2-Dimethyl-[1,3]dioxolan-4-yl)-cycloprop-yl]-butan-1-ol (222)



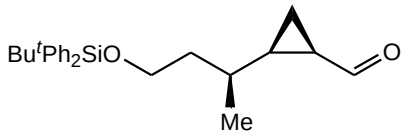
The procedure used in **Experiment 31** was repeated in order to reduce the ester (**209**) (8.94 g, 36.8 mmol) using $LiAlH_4$. The crude product was purified by column chromatography eluting with petrol / ethyl acetate (1:1) to give a colourless oil, (*S*)-3-[(1*R*,2*R*)-2-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-cyclopropyl]-butan-1-ol (**222**)[138] (7.15 g, 91 %), $[\alpha]_D^{23}$ = – 9.8 (*c* 1.07, $CHCl_3$).

Experiment 69: *tert*-Butyl-{(*S*)-3-[(1R,2R)-2-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-cyclopropyl]-butoxy}-diphenyl-silane (210)



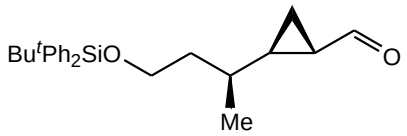
The procedure used in **Experiment 62** was repeated in order to protect the alcohol (**222**) (6.7 g, 31.3 mmol) using imidazole (4.69 g, 68.88 mol) and *tert*-butyldiphenylchlorosilane (9.47 g, 34.4 mol) in dry DMF (100 ml) to give a colourless oil, *tert*-butyl-{(*S*)-3-[(1*R*,2*R*)-2-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-cyclopropyl]-butoxy}-diphenyl-silane (**210**)[89] (13.5 g, 95 %).

Experiment 70: *cis*-(1*R*,2*R*)-2-[(*S*)-3-(*tert*-Butyl-diphenylsilanyloxy)-1-methyl-propyl]-cyclopropanecarbaldehyde (211)



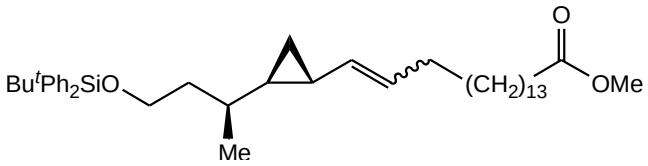
Periodic acid (7.82 g, 34.3 mmol) was added to a stirred solution of the acetal compound (**210**) (6.2 g, 13.7 mmol) in dry ether (150 ml) at r.t. under argon and stirred 17 hrs. The mixture was filtered through a bed of celite and washed with ether. The solvent was evaporated and the crude product was purified by column chromatography eluting with petrol / ethyl acetate (10:1) to give a colourless oil, *cis*-(1*R*,2*R*)-2-[(*S*)-3-(*tert*-butyl-diphenylsilanyloxy)-1-methyl-propyl]-cyclopropanecarbaldehyde (**211**)[89] (5.1 g, 98 %).

Experiment 71: *trans*-(1*S*,2*R*)-2-[(*S*)-3-(*tert*-Butyl-diphenylsilanyloxy)-1-methyl-propyl]-cyclopropanecarbaldehyde (212)



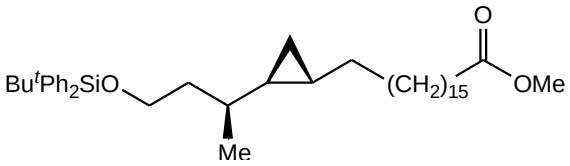
Sodium methoxide (0.8 g, 14.8 mmol) was added to stirred methanol (180 ml) and followed by addition of the *cis*-aldehyde (**211**) (5.1 g, 13.4 mmol) in methanol (27 ml) then refluxed for 69 hrs. Ether (250 ml) and sat.aq. NH_4Cl (100 ml) were added and then extracted with ether (2 x 250 ml). The combined organic layers were dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol / ethyl acetate (10:1, then 1:1) to give a colourless oil, *trans*-(1*S*,2*R*)-2-[(*S*)-3-(*tert*-butyl-diphenylsilanyloxy)-1-methylpropyl]-cyclopropane-carbaldehyde (**212**) (1.3 g 25 %) and *trans*-(1S,2R)-2-((S)-3-hydroxy-1-methyl-propyl)-cyclopropanecarbaldehyde (**223**)[89] (0.99 g, 52 %) which then this diprotected alcohol was protected with *tert*-butyldiphenylchlorosilane with the same procedure as experiment 63.

Experiment 72: (*E*/*Z*)-17-{(1*R*,2*R*)-2-[(*S*)-3-(*tert*-Butyl-diphenylsilanyloxy)-1-methylpropyl]-cyclopropyl}-heptadec-16-enoic acid methyl ester (231)



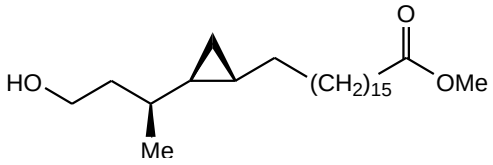
The sulfone (**230**) (4.53 g, 9.47 mmol) was dissolved in dry THF (70 ml) and a solution of the aldehyde (**212**) (3 g, 7.89 mmol) in dry THF (60 ml) was added at r.t. This solution was cooled to -12 ^{o}C and lithium bis(trimethylsilyl) amide (13.4 ml, 14.2 mmol, 1.06M) was added at between -12 and -4 ^{o}C. The solution was allowed to reach room temperature and stirred for 3 hrs. TLC analysis indicated that the reaction was complete. Ether (200 ml) and sat. aq. NH_4Cl (200 ml) were added. The organic phase was separated and water layer was extracted with ether (2 x 50 ml). The combined organic layers were dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol / ether (25:1) to give a colourless oil, *(E/Z)-17-{(1R,2R)-2-[(S)-3-(tert-butyldiphenylsilanyloxy)-1-methyl-propyl]-cyclopropyl}-heptadec-16-enoic acid methyl ester* (**231**) (3.75 g, 75 %) as a mixture of two isomers in ratio 3.7:1, {Found $(M + Na)^+$: 655.4542, $C_{41}H_{64}NaO_3Si$ requires: 655.4517}. This showed ν_{max}/cm^{-1}: 2926, 2855, 1743, 1111, 738; δ_H (major *E* isomer): 7.68–7.66 (4H, m), 7.44–7.35 (6H, m), 5.35 (1H, dt, *J* 15.2, 6.6 Hz), 4.94 (1H, dd, *J* 15.2, 8.5 Hz), 3.79–3.76 (2H, m), 3.67 (3H, s), 2.31 (2H, t, *J* 7.6 Hz), 1.94–1.90 (2H, m), 1.71–1.57 (4H, m), 1.31–1.27 (22H, m), 1.02–0.99 (1H, m), 0.95 (9H, s), 0.90–0.85 (1H, m), 0.80 (3H, d, *J* 6.6 Hz), 0.33–0.28 (3H, m); δ_H (minor *Z* isomer): 5.24 (1H, dt, *J* 10.7, 7.3 Hz), 4.72 (1H, br.t, *J* 10.7 Hz) (the remaining signals were obscured by the major isomer); δ_C (two isomers): 174.3, 135.59(+), 135.57(+), 134.4, 133.3(+), 133.1(+), 129.5(+), 127.9(+), 127.8(+), 127.5(+), 62.3(-), 62.2(-), 51.4(+), 40.3(-), 40.1(-), 34.7(+), 34.6(+), 34.1(-), 32.5(-), 29.9(-), 29.7(-), 29.68(-), 29.66(-), 29.64(-), 29.59(-), 29.5(-), 29.4(-), 29.3(-), 29.2(-), 29.1(-), 27.9(+), 27.6(-), 27.4(+), 26.9(+), 25.0(-), 21.3(+), 19.9(+), 19.6(+), 19.2, 17.4(+), 12.9(-), 12.01(-).

Experiment 73: 17-{(1*S*,2*R*)-2-[(*S*)-3-(*tert*-Butyldiphenylsilanyloxy)-1-methyl-propyl]-cyclopropyl}-heptadecanoic acid methyl ester (213)



The alkene (**231**) (3.5 g, 5.54 mmol) and TPBSH (6.6 g, 22.15 mmol) were dissolved in dry THF (60 ml) and stirred at 40 ^{o}C for 18 hrs. Further TPBSH (2.6 g, 8.7 mmol) was added and stirred at 50 ^{o}C for 23 hrs. ^{1}H NMR showed that hydrogenation was completed. The mixture was diluted with petrol / ether (1:1, 200 ml,) and aq. NaOH (80 ml, 2 %) was added and extracted. The aqueous layer was re-extracted with petrol / ether (1:1, 2 x 60 ml) and the combined organic layers were washed with water (50 ml), dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol / ether (15:1) to give a colourless oil, *17-{(1S,2R)-2-[(S)-3-(tert-butyldiphenylsilanyloxy)-1-methyl-propyl]-cyclopropyl}-heptadecanoic acid methyl ester* (**213**) (3.27 g, 93 %), $[\alpha]_D^{25}$ = + 6.9 (*c* 1.01, $CHCl_3$), {Found (M + Na)$^+$: 657.4697, $C_{41}H_{66}NaO_3Si$ requires: 657.4673}. This showed ν_{max}/cm^{-1}: 3071, 2925, 2854, 1743, 1463, 1428, 1171, 703; δ_H: 7.69–7.67 (4H, m), 7.44–7.37 (6H, m), 3.79–3.70 (2H, m), 3.67 (3H, s), 2.31 (2H, t, *J* 7.6 Hz), 1.74–1.69 (1H, m), 1.66–1.60 (2H, m), 1.56–1.50 (1H, m), 1.34–1.26 (28H, m), 1.17–1.12 (1H, m), 1.05 (9H, s), 0.89 (3H, br.s), 0.47–0.40 (1H, m), 0.18–0.08 (3H, m); δ_C: 174.3, 135.6(+), 134.2, 129.5(+), 127.5(+), 62.3(-), 51.4(+), 40.2(-), 34.8(+), 34.3(-), 34.1(-), 29.7(-), 29.68(-), 29.65(-), 29.64(-), 29.59(-), 29.5(-), 29.3(-), 29.2(-), 26.9(+), 25.9(+), 25.0(-), 19.8(+), 19.2, 18.6(+), 10.5(-) [- = CH_2, + = CH, CH_3].

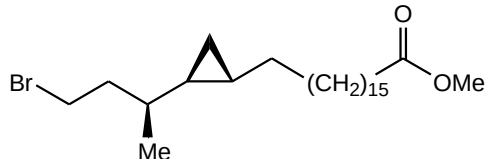
Experiment 74: 17-[(1*S*,2*R*)-2-((*S*)-3-Hydroxy-1-methyl-propyl)-cyclopropyl]-heptadecanoic acid methyl ester (232)



n-TBAF (6.5 ml, 6.5 mmol, 1M sol. in THF) was added to a stirred solution of the silyl ether (**213**) (3.15 g, 4.97 mmol) in dry THF (90 ml) at 0 ^{o}C under argon. The mixture was allowed to reach room temperature and stirred for 18 hrs. When T.L.C. showed no starting material, the mixture was diluted with petrol / ethyl acetate (1:1, 150 ml),

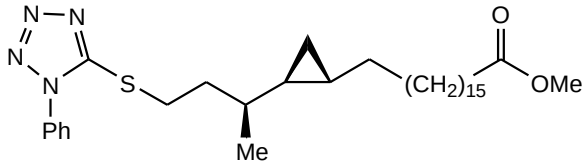
cooled to 5 ^{o}C and quenched with sat. aq. NH_4Cl (100 ml) then extracted. The water layer was re-extracted with petrol / ethyl acetate (1:1, 2 x 50 ml), the combined organic layers were washed with brine (75 ml), dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol / ethyl acetate (5:1 and then 4:1) to give a white solid, *17-[(1S,2R)-2-((S)-3-hydroxy-1-methyl-propyl)-cyclopropyl]-heptadecanoic acid methyl ester* (**232**) (1.92 g, 98 %), m.p.: 29–30 ^{o}C, $[\alpha]_D^{21}$ = + 13.5 (*c* 0.99, $CHCl_3$), {Found $(M + Na)^+$: 419.3492, $C_{25}H_{48}NaO_3$ requires: 419.3496}. This showed ν_{max}/cm^{-1}: 3386, 2924, 2852, 1743, 1467, 1172, 792; δ_H: 3.79–3.69 (2H, m), 3.67 (3H, s), 2.31 (2H, t, *J* 7.6 Hz), 1.76–1.70 (1H, m), 1.66–1.60 (2H, m), 1.59–1.52 (1H, m), 1.37–1.20 (27H, m), 1.18–1.11 (1H, m), 0.96 (3H, d, *J* 6.6 Hz), 0.90–0.81 (1H, m), 0.51–0.46 (1H, m), 0.26–0.14 (3H, m); δ_C: 174.3, 61.4(+), 51.4(-), 40.4(+), 35.0(-), 34.3(+), 34.1(+), 29.68(+), 29.62(+), 29.61(+), 29.60(+), 29.58(+), 29.4(+), 29.2(+), 29.1(+), 25.9(-), 24.9(+), 19.8(-), 18.7(-), 10.6(+).

Experiment 75: 17-[(1*S*,2*R*)-2-((*S*)-3-Bromo-1-methylpropyl)-cyclopropyl]-heptadecanoic acid methyl ester (233)



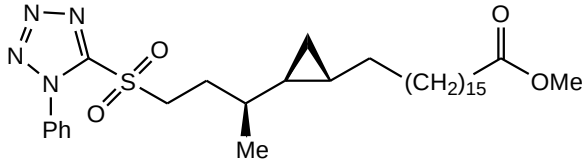
The procedure used in **Experiment 47** was repeated using the alcohol (**232**) (1.77 g, 4.47 mmol), NBS (1.01 g, 5.68 mmol) and PPh_3 (1.35 g, 5.14 mmol) in CH_2Cl_2 (100 ml). The crude product was purified by column chromatography eluting with petrol / ether (15:1) to give a white solid, *17-[(1S,2R)-2-((S)-3-bromo-1-methylpropyl)-cyclopropyl]-heptadecanoic acid methyl ester* (**233**) (1.79 g, 84 %), m.p.: 33–34 ^{o}C, $[\alpha]_D^{21}$ = + 15.2 (*c* 1.3, $CHCl_3$), {Found $(M + Na)^+$: 481.2653, $C_{25}H_{47}BrNaO_2$ requires: 481.2652}. This showed ν_{max}/cm^{-1}: 2920, 2851, 1732, 1473, 1167, 792; δ_H: 3.67 (3H, s), 3.56–3.46 (2H, m), 2.31 (2H, t, *J* 7.6 Hz), 2.02–1.95 (1H, m), 1.88–1.81 (1H, m), 1.65–1.59 (2H, m), 1.37–1.20 (28H, m), 1.18–1.11 (1H, m), 0.95 (3H, br.s), 0.61–0.52 (1H, m), 0.23–0.14 (3H, m); δ_C: 174.3, 51.4(-), 40.7(+), 36.8(-), 34.3(+), 34.1(+), 32.5(+), 29.7(+), 29.6(+), 29.58(+), 29.53(+), 29.4(+), 29.2(+), 29.1(+), 25.2(-), 25.0(+), 19.3(-), 18.6(-), 10.4(+).

Experiment 76: 17-{(1*S*,2*R*)-2-[(*S*)-1-Methyl-3-(1-phenyl-1*H*-tetrazol-5-ylsulfanyl)-propyl]-cyclopropyl}-heptadecanoic acid methyl ester (234)



The procedure used in **Experiment 23** was repeated using compound (**233**) (1.67 g, 3.6 mmol), 1-phenyl-1*H*-tetrazole-5-thiol (0.65 g, 3.6 mmol), anhydrous potassium carbonate (1.06 g, 7.64 mmol) and acetone (60 ml). The crude product was purified by column chromatography eluting with petrol / ether (4:1) to give a white solid, *17-{(1S,2R)-2-[(S)-1-methyl-3-(1-phenyl-1H-tetrazol-5-ylsulfanyl)-propyl]-cyclopropyl}-heptadecanoic acid methyl ester* (**234**) (1.91 g, 95 %), m.p.: 48–49 ^{o}C, $[\alpha]_{D}^{25}$ = + 10.3 (*c* 1.18, $CHCl_3$), {Found $(M + Na)^+$: 579.3697, $C_{32}H_{52}N_4NaO_2S$ requires: 579.3703}. This showed ν_{max}/cm^{-1}: 2920, 2851, 1739, 1500, 1468, 1389, 1166, 766; δ_H: 7.60–7.54 (5H, m), 3.67 (3H, s), 3.53–3.44 (2H, m), 2.31 (2H, t, *J* 7.6 Hz), 1.94–1.87 (1H, m), 1.85–1.78 (1H, m), 1.65–1.60 (2H, m), 1.35–1.25 (26H, m), 1.17–1.09 (1H, m), 0.99 (3H, d, *J* 7.0 Hz), 0.94–0.84 (2H, m), 0.53–0.48 (1H, m), 0.29–0.23 (1H, m), 0.21–0.16 (2H, m); δ_C: 174.4, 154.5, 133.8, 130.0(-), 129.7(-), 123.8(-), 51.4(-), 37.4(-), 37.6(+), 34.2(+), 34.1(+), 31.6(+), 29.7(+), 29.64(+), 29.58(+), 29.55(+), 29.4(+), 29.2(+), 29.1(+), 25.3(-), 24.9(+), 19.5(-), 18.8(-), 10.4(+).

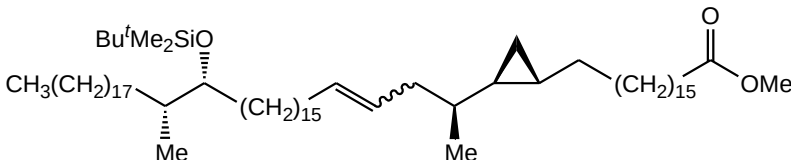
Experiment 77: 17-{(1*S*,2*R*)-2-[(*S*)-1-Methyl-3-(1-phenyl-1*H*-tetrazole-5-sulfonyl)-propyl]-cyclopropyl}-heptadecanoic acid methyl ester (214)



The procedure used in **Experiment 25** was repeated using the sulfane (**234**) (1.8 g, 3.2 mmol), ammonium molybdate (VI) tetrahydrate (1.8 g, 1.46 mmol) in 35 % H_2O_2 (4 ml) and THF (25 ml) and IMS (50 ml), and then further ammonium molybdate (VI) tetrahydrate (0.7 g, 0.57 mmol) in 35% H_2O_2 (1.8 ml). The crude product was purified by column chromatography eluting with petrol / ether (2:1) to give a white solid, *17-{(1S,2R)-2-[(S)-1-methyl-3-(1-phenyl-1H-tetrazole-5-sulfonyl)-propyl]-cyclopropyl}-heptadecanoic acid methyl ester* (**214**) (1.67 g, 88 %), m.p.: 61–62 ^{o}C, $[\alpha]_{D}^{26}$ = + 3.6 (*c* 1.04, $CHCl_3$), {Found $(M + Na)^+$: 611.3597, $C_{32}H_{52}N_4NaO_4S$ requires: 611.3601}.

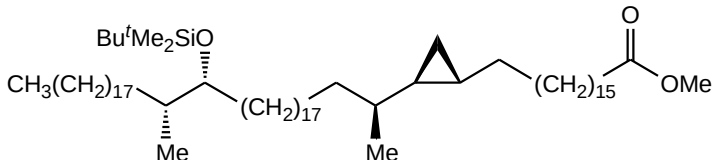
This showed ν_{max}/cm^{-1}: 2921, 2851, 1730, 1344, 1155; δ_H: 7.72–7.70 (2H, m), 7.65–7.59 (3H, m), 3.89–3.76 (2H, m), 3.67 (3H, s), 2.31 (2H, t, *J* 7.6 Hz), 2.06–1.92 (2H, m), 1.65–1.59 (2H, m), 1.36–1.25 (26H, m), 1.14–1.08 (1H, m), 1.01 (3H, d, *J* 6.4 Hz), 0.99–0.84 (2H, m), 0.56–0.50 (1H, m), 0.29–0.22 (3H, m); δ_C: 174.4, 153.5, 133.1, 131.4(-), 129.7(-), 125.1(-), 54.5(+), 51.4(-), 37.2(-), 34.1(+), 29.7(+), 29.6(+), 29.59(+), 29.51(+), 29.4(+), 29.2(+), 29.14(+), 29.06(+), 25.0(+), 24.8(-), 19.5(-), 19.0(-), 10.5(+).

Experiment 78: 17-{(1*S*,2*R*)-2-[(*E*/*Z*)-(1*S*,20*R*,21*R*)-20-(*tert*-Butyl-dimethyl-silanyloxy)-1,21-dimethyl-nonatriacont-3-enyl]-cyclopropyl}-heptadecanoic acid methyl ester (242)



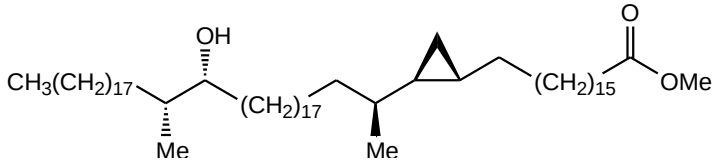
The procedure used in **Experiment 72** was repeated in order to couple the aldehyde (**215**) (1.7 g, 2.56 mmol) and the sulfone (**214**) (1.58 g, 2.69 mmol) using lithium bis(trimethylsilyl) amide (4.6 ml, 4.8 mmol, 1.06M) in dry THF. The crude product was purified by column chromatography eluting with petrol / ether (32:1) to give a colourless oil, *17-{(1S,2R)-2-[(E/Z)-(1S,20R,21R)-20-(tert-butyl-dimethyl-silanyloxy)-1,21-dimethyl-nonatriacont-3-enyl]-cyclopropyl}-heptadecanoic acid methyl ester* (**242**) (1.85 g, 71 %) as a mixture of two isomers in ratio 2:1, {Found $(M + Na)^+$: 1050.0003, $C_{68}H_{134}NaO_3Si$ requires: 1049.9994}. This showed ν_{max}/cm^{-1}: 2924, 2853, 1746, 1465, 1253, 836; δ_H (two isomers): 5.45–5.37 (2H, m), 3.67 (3H, s), 3.52–3.49 (1H, m), 2.31 (2H, t, *J* 7.6 Hz), 2.19–2.11 (1H, m), 2.05–1.91 (3H, m), 1.65–1.60 (2H, m), 1.50–1.12 (90H, m, v.br.), 1.08–1.01 (1H, m), 0.92–0.87 (15H, m, including a s), 0.80 (3H, d, *J* 7.0 Hz), 0.76–0.71 (1H, m), 0.50–0.43 (1H, m), 0.26–0.10 (3H, m), 0.04 (3H, s), 0.03 (3H, s); δ_C (two isomers): 174.3, 131.4(-), 130.4(-), 128.9(-), 128.4(-), 75.9(-), 51.4(-), 40.3(+), 38.9(-), 37.7(-), 34.7(+), 34.4(+), 34.1(+), 33.5(+), 32.7(+), 32.5(+), 31.9(+), 30.0(+), 29.9(+), 29.7(+, v.br.), 29.66(+), 29.61(+), 29.56(+), 29.46(+), 29.36(+), 29.26(+), 29.22(+), 29.1(+), 27.7(+), 27.3(+), 26.0(-), 25.9(+), 25.7(-), 25.0(+), 22.7(+), 19.3(+), 19.2(-), 18.6(-), 18.2, 14.4(-), 14.1(-), 10.8(+), 10.7(+), - 4.2(-), - 4.4(-).

Experiment 79: 17-{(1*S*,2*R*)-2-[(1*S*,20*R*,21*R*)-20-(*tert*-Butyl-dimethyl-silanyloxy)-1,21-dimethyl-nonatriacontyl]-cyclopropyl}-heptadecanoic acid methyl ester (216)



The alkene (**242**) (1.5 g, 1.46 mmol) and TPBSH (1.75 g, 5.85 mmol) were dissolved in dry THF (50 ml) and stirred at 40 ^{o}C for 18 hrs. Further TPBSH (0.69 g, 2.34 mmol) was added and stirred at 50 ^{o}C for 23 hrs. ^{1}H NMR showed the reaction was not yet complete and further TPBSH (0.69 g, 2.34 mmol) was added and stirred at 60 ^{o}C for 23 hrs. The mixture was diluted with petrol / ether (1:1, 250 ml,) and aq. NaOH (15 ml, 2 %) was added and extracted. The aqueous layer was re-extracted with petrol / ether (1:1, 2 x 30 ml) and the combined organic layers were washed with water (40 ml), dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol / ether (32:1) to give a colourless oil, *17-{(1S,2R)-2-[(1S,20R,21R)-20-(tert-butyl-dimethyl-silanyloxy)-1,21-dimethyl-nonatriacontyl]-cyclopropyl}-heptadecanoic acid methyl ester* (**216**) (0.95 g, 63 %), $[\alpha]_{D}^{24}$= + 5.9 (*c* 1.05, $CHCl_3$), {Found $(M + Na)^+$: 1052.0190, $C_{68}H_{136}NaO_3Si$ requires: 1052.0151}. This showed ν_{max}/cm^{-1}: 2924, 2854, 1746, 1464, 1253, 836; δ_H: 3.67 (3H, s), 3.52–3.49 (1H, m), 2.31 (2H, t, *J* 7.6 Hz), 1.66–1.60 (2H, m), 1.49–1.16 (98H, m, v.br.), 1.08–1.01 (1H, m), 0.91–0.88 (15H, m, including a s), 0.80 (3H, d, *J* 7.0 Hz), 0.71–0.64 (1H, m), 0.49–0.43 (1H, m), 0.22–0.09 (3H, m), 0.04 (3H, s), 0.03 (3H, s); δ_C: 174.3, 75.9(-), 51.4(-), 38.1(-), 37.7(-), 37.4(+), 34.5(+), 34.1(+), 33.6(+), 32.5(+), 31.9(+), 30.1(+), 30.0(+), 29.9(+), 29.73(+), 29.71(+, v.br.), 29.67(+), 29.62(+), 29.5(+), 29.4(+), 29.3(+), 29.2(+), 27.7(+), 27.3(+), 26.2(-), 26.0(-), 25.9(+), 25.0(+), 22.7(+), 19.7(-), 18.6(-), 18.2, 14.4(-), 14.1(-), 10.5(+), - 4.2(-), - 4.4(-).

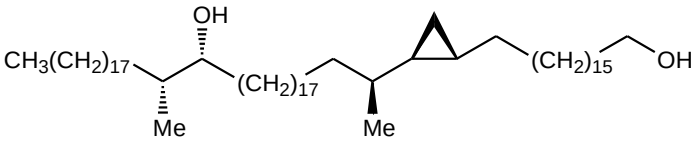
Experiment 80: 17-[(1*S*,2*R*)-2-((1*S*,20*R*,21*R*)-20-Hydroxy-1,21-dimethyl-nonatriacontyl)-cyclopropyl]-heptadecanoic acid methyl ester (243)



n-TBAF (3.2 ml, 3.2 mmol) was added to a stirred solution of the silyl ether (**216**) (0.82 g, 0.8 mmol) in dry THF (50 ml) at r.t under argon. The mixture was refluxed for

18 hrs., and further n-TBAF (2 ml, 2 mmol) was added and refluxed for 23 hrs. T.L.C. showed almost no starting material, the mixture was cooled to r.t. and quenched with sat. aq. NH_4Cl (25 ml) and diluted with petrol / ether (1:1, 50 ml) then extracted. The water layer was re-extracted with petrol / ether (1:1, 2 x 30 ml), the combined organic layers were washed with brine (40 ml), dried and the solvent was evaporated. The crude product was treated by diazomethane in ether for 10 min. and purified by column chromatography eluting with petrol / ether (4:1) to give a white solid, *17-[(1S,2R)-2-((1S,20R,21R)-20-hydroxy-1,21-dimethyl-nonatriacontyl)-cyclo-propyl]-heptadecanoic acid methyl ester* (**243**) (0.51 g, 70 %), m.p.: 58–60 ^oC, $[\alpha]_D^{23}$ = + 8.7 (*c* 0.85, $CHCl_3$), {Found $(M + Na)^+$: 937.9272, $C_{62}H_{122}NaO_3$ requires: 937.9286}. This showed ν_{max}/cm^{-1}: 3521, 2918, 2850, 1736, 1471, 1168, 720; δ_H: 3.67 (3H, s), 3.51–3.49 (1H, m), 2.31 (2H, t, *J* 7.6 Hz), 1.64–1.60 (2H, m), 1.43–1.16 (99H, m, v.br.), 0.90 (3H, d, *J* 6.6 Hz), 0.89 (3H, t, *J* 6.7 Hz), 0.87 (3H, d, *J* 7.0 Hz), 0.71–0.65 (1H, m), 0.49–0.42 (1H, m), 0.22–0.09 (3H, m); δ_C: 174.3, 75.2(-), 51.4(-), 38.2(-), 38.1(-), 37.4(+), 34.53(+), 34.49(+), 34.1(+), 33.4(+), 31.9(+), 30.1(+), 29.9(+), 29.8(+), 29.71(+), 29.70(+, v.br.), 29.65(+), 29.6(+), 29.5(+), 29.4(+), 29.3(+), 29.2(+), 27.4(+), 27.3(+), 26.3(+), 26.2(-), 25.0(+), 22.7(+), 19.7(-), 18.6(-), 14.1(-), 13.6(-), 10.5(+).

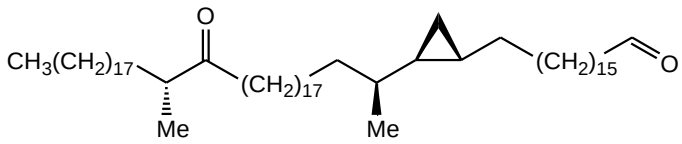
Experiment 81: (19*R*,20*R*,39*S*)-39-[(1*R*,2*S*)-2-(17-Hydroxy-heptadecyl)-cyclopropyl]-19-methyl-tetracontan-20-ol (244)



The procedure used in **Experiment 55** was repeated in order to reduce the ester (**243**) (270 mg, 0.3 mmol) using $LiAlH_4$ (50 mg, 1.32 mmol) to give a white solid, *(19R,20R,39S)-39-[(1R,2S)-2-(17-hydroxy-heptadecyl)-cyclopropyl]-19-methyl-tetracontan-20-ol* (**244**) (220 mg, 85 %), m.p.: 61–63 ^oC, $[\alpha]_D^{35}$ = + 3.7 (*c* 0.98, $CHCl_3$), {Found: C, 82.35; H, 13.95, $C_{61}H_{122}O_2$ requires: C, 82.54; H, 13.85}. This showed ν_{max}/cm^{-1}: 3408, 2916, 2850, 1471, 1026; δ_H: 3.64 (2H, t, *J* 6.6 Hz), 3.51–1.48 (1H, m), 1.59–1.54 (2H, m), 1.43–1.15 (101H, m, v.br.), 0.90–0.86 (9H, m), 0.70–0.64 (1H, m), 0.48–0.42 (1H, m), 0.22–0.09 (3H, m); δ_C: 75.2(-), 63.0(+), 38.2(-), 38.1(-), 37.4(+), 34.5(+), 34.4(+), 33.3(+), 32.8(+), 31.9(+), 30.0(+), 29.9(+), 29.7(+), 29.68(+),

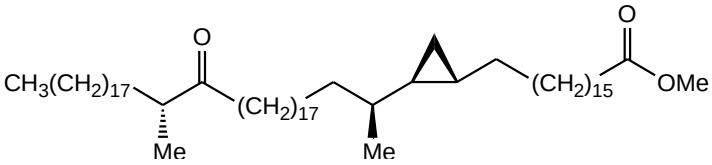
29.65(+, v.br.), 29.57(+), 29.4(+), 29.3(+), 27.4(+), 27.2(+), 26.3(+), 26.1(-), 25.7(+), 22.6(+), 19.6(-), 18.6(-), 14.1(-), 13.6(-), 10.5(+).

Experiment 82: 17-[(1*S*,2*R*)-2-((1*S*,21*R*)-1,21-Dimethyl-20-oxo-nonatriacontyl)-cyclopropyl]-heptadecanal (115)



The procedure used in **Experiment 56** was repeated in order to oxidise the diol (**244**) (180 mg, 0.2 mmol) using PCC (220 mg, 1.02 mmol) in CH_2Cl_2. The crude product was purified by column chromatography eluting with petrol / ether (8:1) to give a white solid, *17-[(1S,2R)-2-((1S,21R)-1,21-dimethyl-20-oxo-nonatriacontyl)-cyclopropyl]-heptadecanal* (**115**) (165 mg, 92 %), m.p.: 69–71 ^{o}C, $[\alpha]_D^{24} = -1.8$ (*c* 1.05, $CHCl_3$), {Found $(M + H)^+$: 883.9197, $C_{61}H_{119}O_2$ requires: 883.9205; Found: C, 82.78; H, 13.62, $C_{61}H_{118}O_2$ requires: C, 82.92; H, 13.46}. This showed ν_{max}/cm^{-1}: 2918, 2850, 1716, 1699, 1471; δ_H: 9.77 (1H, t, *J* 1.9 Hz), 2.51 (1H, sext, *J* 6.9 Hz), 2.47–2.37 (4H, m), 1.69–1.61 (4H, m), 1.58–1.53 (2H, m), 1.43–1.16 (92H, m, v.br.), 1.05 (3H, d, *J* 7.0 Hz), 0.91 (3H, d, *J* 6.6 Hz), 0.89 (3H, t, *J* 7.0 Hz), 0.72–0.65 (1H, m), 0.49–0.43 (1H, m), 0.23–0.09 (3H, m); δ_C: 215.1, 202.8(-), 46.3(-), 43.9(+), 41.1(+), 38.1(-), 37.4(+), 34.5(+), 33.1(+), 31.9(+), 30.1(+), 29.72(+), 29.69(+, v.br.), 29.64(+), 29.6(+), 29.5(+), 29.49(+), 29.47(+), 29.4(+), 29.3(+), 29.2(+), 27.33(+), 27.26(+), 26.2(-), 23.7(+), 22.7(+), 19.7(-), 18.6(-), 16.4(-), 14.1(-), 10.5(+).

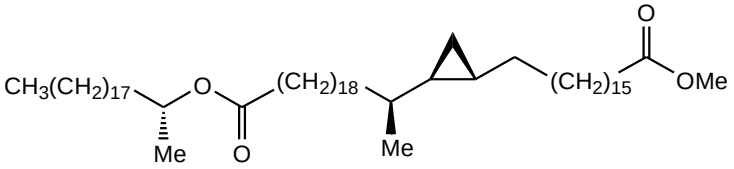
Experiment 83: 17-[(1*S*,2*R*)-2-((1*S*,21*R*)-1,21-Dimethyl-20-oxo-nonatriacontyl)-cyclopropyl]-heptadecanoic acid methyl ester (245)



The procedure used in **Experiment 57** was repeated in order to oxidise the alcohol (**243**) (180 mg, 0.20 mmol) using PCC (130 mg, 0.59 mmol) in CH_2Cl_2. The crude product was purified by column chromatography eluting with petrol / ether (12:1) to give a white solid, *17-[(1S,2R)-2-((1S,21R)-1,21-dimethyl-20-oxo-nonatriacontyl)-cyclopropyl]-heptadecanoic acid methyl ester* (**245**) (160 mg, 89 %), m.p.: 60–62 ^{o}C,

$[\alpha]_D^{24} = -4.1$ (*c* 0.61, $CHCl_3$), {Found $(M + Na)^+$: 935.9143, $C_{62}H_{120}NaO_3$ requires: 930.9130}. This showed ν_{max}/cm^{-1}: 2918, 2849, 1736, 1699, 1471, 1250, 1212, 1169; δ_H: 3.67 (3H, s), 2.51 (1H, sext, *J* 6.9 Hz), 2.43 (1H, dt, *J* 16.7, 7.3 Hz), 2.38 (1H, dt, *J* 16.7, 7.3 Hz), 2.31 (2H, t, *J* 7.6 Hz), 1.67–1.60 (2H, m), 1.57–1.52 (2H, m), 1.42–1.16 (94H, m, v.br.), 1.05 (3H, d, *J* 6.9 Hz), 0.91 (3H, d, *J* 6.6 Hz), 0.89 (3H, t, *J* 6.7 Hz), 0.71–0.64 (1H, m), 0.48–0.42 (1H, m), 0.22–0.09 (3H, m); δ_C: 215.1, 174.3, 51.4(-), 46.3(-), 41.1(+), 38.1(-), 37.4(+), 34.5(+), 34.1(+), 33.0(+), 31.9(+), 30.1(+), 29.72(+), 29.7(+, v.br.), 29.65(+), 29.6(+), 29.51(+), 29.49(+), 29.46(+), 29.4(+), 29.3(+), 29.2(+), 29.1(+), 27.3(+), 27.2(+), 26.2(-), 25.0(+), 23.7(+), 22.7(+), 19.7(-), 18.6(-), 16.4(-), 14.1(-), 10.5(+).

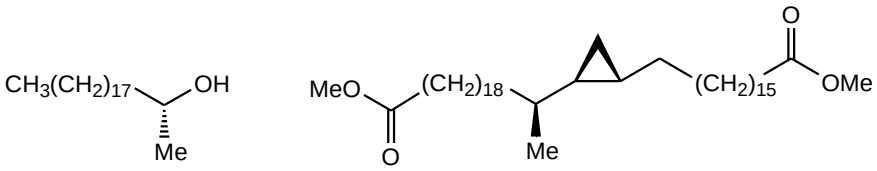
Experiment 84: (*S*)-20-[(1*R*,2*S*)-2-(16-Methoxycarbonyl-hexadecyl)-cyclopropyl]-henicosanoic acid (*R*)-1-methyl-nonadecyl ester (246)



A solution of the ketone (**245**) (140 mg, 0.15 mmol) in dry CH_2Cl_2 (15 ml) and then sodium hydrogen carbonate (61 mg, 0.72 mmol) were added to a stirred solution of *m*-chloroperbenzoic acid (80 mg, 0.46 mmol) in dry CH_2Cl_2 (15 ml). The mixture was stirred at r.t. for 4 days and refluxed for 24 hrs. at 45 ^oC. The mixture was diluted with CH_2Cl_2 (20 ml) and washed with 10 % aq. Na_2SO_3 (10 ml), water (10 ml) and brine (10 ml). The organic layer was dried, filtered and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol / ether (12:1) to give a white solid, *(S)-20-[(1R,2S)-2-(16-methoxycarbonyl-hexadecyl)-cyclopropyl]-henicosanoic acid (R)-1-methyl-nonadecyl ester* (**246**) (90 mg, 63 %). NMR showed that the product contain 20 % starting ketone, m.p.: 54–56 ^oC, {Found $(M + Na)^+$: 951.9093, $C_{62}H_{120}NaO_4$ requires: 951.9079}. This showed ν_{max}/cm^{-1}: 2918, 2850, 1735, 1698, 1470, 1407, 1376, 1251, 1097; δ_H : 4.91 (1H, sext, *J* 6.0 Hz), 3.67 (3H, s), 2.31 (2H, t, *J* 7.6 Hz), 2.27 (2H, t, *J* 7.6 Hz), 1.64–1.60 (4H, m), 1.49–1.15 (94H, m, v.br.), 1.20 (3H, d, *J* 6.3 Hz), 0.90 (3H, d, *J* 7.0 Hz), 0.89 (3H, t, *J* 7.0 Hz), 0.71–0.64 (1H, m), 0.48–0.42 (1H, m), 0.22–0.09 (3H, m); δ_C: 174.3, 173.6, 70.7(-), 51.4(-), 38.1(-), 37.4(+), 36.0(+), 34.8(+), 34.5(+), 34.1(+), 31.9(+), 30.1(+), 29.73(+), 29.7(+, v.br.), 29.65(+), 29.61(+), 29.58(+), 29.55(+), 29.50(+), 29.45(+), 29.4(+),

29.3(+), 29.2(+), 29.1(+), 27.3(+), 26.1(-), 25.4(+), 25.1(+), 25.0(+), 22.7(+), 20.0(-), 19.7(-), 18.6(-), 14.1(-), 10.5(+).

Experiment 85: (*S*)-20-[(1*R*,2*S*)-2-(16-Methoxycarbonyl-hexadecyl)-cyclopropyl]-henicosanoic acid methyl ester (248), and (*R*)-icosan-2-ol (249)

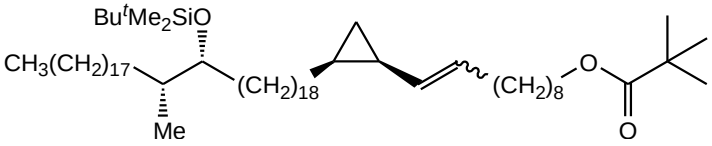


The α-methyl ester (**246**) (80 mg, 0.086 mmol) in THF (5 ml) was added to a stirred solution of KOH (73 mg, 1.3 mmol), in methanol (7.5 ml), THF (7.5 ml) and water (1 ml) at r.t. A white bulky solution formed, so more THF (5 ml) was added to dissolve and refluxed at 75 ^{o}C for 4 hrs, then TLC showed no starting material was left. The mixture was cooled to 4 ^{o}C then a white precipitate formed and filtered on a sinter funnel, then washed with ether (2 x 15 ml). The liquid layers were washed with water (2 x 30 ml) and the organic layers dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol / ether (5:2 then 2:1) to give a white solid, (*R*)-icosan-2-ol (**249**)[200] (12 mg, 67 %), m.p.: 57–59 ^{o}C, $[\alpha]_D^{23} = -3.8$ (*c* 0.47, $CHCl_3$), it was reported as m.p.: 61.7–61.8 ^{o}C and as $[\alpha]_D^{23} = -3.8$ (*c* 10, $CHCl_3$).[200] This showed δ_H: 3.80 (1H, sext, *J* 6.3 Hz), 1.49–1.25 (34H, m, v.br.), 1.20 (3H, d, *J* 6.0 Hz), 0.89 (3H, t, *J* 6.6 Hz); δ_C: 68.2(-), 38.4(+), 31.9(+), 29.7(+, v.br.), 29.67(+), 29.63(+), 29.6(+), 29.3(+), 25.8(+), 23.5(-), 22.7(+), 14.1(-).

The above white precipitate was dissolved in hot water, cooled and mixed with petrol / ethyl acetate (1:1, 25 ml) and then acidified with H_2SO_4 (20 %). The product was extracted with hot petrol / ethyl acetate (1:1, 2 x 30 ml), the organic layers dried and the solvent was evaporated. The product was treated with excess diazomethane[‡] solution in ether and left overnight. The ether was evaporated and the crude product was purified by column chromatography eluting with petrol / ether (9:1) to give a white solid, *(S)-20-[(1R,2S)-2-(16-methoxycarbonyl-hexadecyl)-cyclopropyl]-henicosanoic acid methyl ester* (**248**) (18 mg, 45 %), m.p.: 61–63 ^{o}C, $[\alpha]_D^{23} = +3.3$ (*c* 0.6, $CHCl_3$), {Found $(M + H)^+$: 663.6280, $C_{42}H_{83}O_4$ requires: 663.6286}. This showed ν_{max}/cm^{-1}: 2924, 2854, 1738, 1461, 1377, 1169; δ_H: 3.67 (6H, s), 2.31 (4H, t, *J* 7.6 Hz), 1.65–1.60 (4H, m), 1.43–1.16 (60H, m, v.br.), 0.90 (3H, d, *J* 6.6 Hz), 0.71–0.64 (1H,

m), 0.48–0.42 (1H, m), 0.22–0.09 (3H, m); δ_C: 174.3, 51.4(-), 38.1(-), 37.4(+), 34.5(+), 34.1(+), 30.1(+), 29.7(+, v.br.), 29.65(+), 29.60(+), 29.5(+), 29.3(+), 29.2(+), 27.3(+), 26.2(-), 25.0(+), 19.6(-), 18.6(-), 10.5(+).

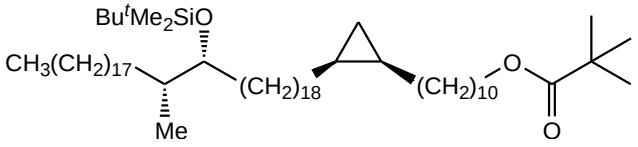
Experiment 86: 2,2-Dimethyl-propionic acid (*E*/*Z*)-10-{(1*R*,2*S*)-2-[(19*R*,20*R*)-19-(*tert*-butyl-dimethyl-silanyloxy)-20-methyl-octatriacontyl]-cyclopropyl}-dec-9-enyl ester (253)



The procedure used in **Experiment 27** was repeated in order to couple the aldehyde (**121**) (1.3 g, 1.74 mmol) with the sulfone (**239**) (0.95 g, 2.18 mmol) using lithium bis(trimethylsilyl) amide (2.96 ml, 3.14 mmol, 1.06M) in dry THF. The crude product was purified by column chromatography eluting with petrol / ether (33:1) to give a colourless oil, *2,2-dimethyl-propionic acid (E/Z)-10-{(1R,2S)-2-[(19R,20R)-19-(tert-butyl-dimethyl-silanyloxy)-20-methyl-octatriacontyl]-cyclopropyl-}-dec-9-enyl ester* (**253**) (1.45 g, 87 %) as a mixture of two isomers in ratio 5.9:1, {Found $(M + Na)^+$: 979.9184, $C_{63}H_{124}NaO_3Si$ requires: 979.9212}. This showed ν_{max}/cm^{-1}: 2925, 2854, 1732, 1464, 1285, 1253, 1157; δ_H (major *E* isomer): 5.52 (1H, dt, *J* 6.6, 15.4 Hz), 5.18 (1H, dd, *J* 8.5, 15.2 Hz), 4.05 (2H, t, *J* 6.6 Hz), 3.52–3.49 (1H, m), 2.03–1.98 (2H, m), 1.66–1.60 (2H, m), 1.49–1.22 (80H, m, v.br.), 1.20 (9H, s), 1.09–1.02 (1H, m), 0.91–0.88 (12H, s and t, *J* 6.9 Hz), 0.89–0.84 (2H, m), 0.83–0.77 (1H, m), 0.80 (3H, d, *J* 7.0 Hz), 0.12 (1H, br.q, *J* 5.4 Hz), 0.04 (3H, s), 0.03 (3H, s); δ_H (minor *Z* isomer): 5.40 (1H, dt, *J* 7.3, 11.1 Hz), 5.06–5.02 (1H, m) (the remaining signals were obscured by the major isomer); δ_C (two isomers): 178.6, 130.4(-), 130.1(-), 129.6(-), 129.5(-), 75.9(-), 64.5(+), 38.7, 37.8(-), 33.6(+), 32.7(+), 32.5(+), 31.9(+), 30.0(+), 29.9(+), 29.8(+), 29.72(+), 29.7(+, v.br.), 29.6(+), 29.5(+), 29.4(+), 29.3(+), 29.2(+), 29.1(+), 29.0(+), 28.6(+), 27.7(+), 27.2(-), 26.0(-), 25.9(+), 22.7(+), 18.4(-), 18.3(-), 18.2, 14.4(-), 14.1(-), 12.3(+), - 4.2(-), - 4.4(-).

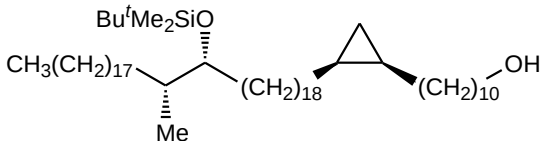
‡ I thank to J. R. Al Dulayymi for providing the diazomethane

Experiment 87: 2,2-Dimethyl-propionic acid 10-{(1*R*,2*S*)-2-[(19*R*,20*R*)-19-(*tert*-butyl-dimethyl-silanyloxy)-20-methyl-octatriacontyl]-cyclopropyl}-decyl ester (254)



The alkene (**253**) (1.4 g, 1.46 mmol) and TPBSH (1.53 g, 5.13 mmol) were dissolved in dry THF (40 ml) and stirred at 45 ^{o}C for 24 hrs. Further TPBSH (0.52 g, 1.75 mmol) was added and stirred at 45 ^{o}C for another 24 hrs. The mixture was diluted with petrol / ether (1:2, 100 ml) and aq. NaOH (30 ml, 2 %) was added and extracted. The aqueous layer was re-extracted with petrol / ether (1:2, 2 x 25 ml) and the combined organic layers were washed with water (100 ml), dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol / ether (33:1) to give a colourless oil, *2,2-dimethyl-propionic acid 10-{(1R,2S)-2-[(19R,20R)-19-(tert-butyl-dimethyl-silanyloxy)-20-methyl-octatriacontyl]-cyclopropyl}-decyl ester* (**254**) (1.3 g, 93 %), $[\alpha]_D^{19}$ = + 5.6 (*c* 1.3, $CHCl_3$), {Found $(M + Na)^+$: 981.9332, $C_{63}H_{126}NaO_3Si$ requires: 981.9368}. This showed ν_{max}/cm^{-1}: 2925, 2854, 1733, 1464, 1284, 1253, 1155; δ_H: 4.05 (2H, t, *J* 6.6 Hz), 3.51 (1H, dt, *J* 3.5, 6.3 Hz), 1.66–1.60 (2H, m), 1.50–1.12 (86H, m, v.br.), 1.21 (9H, s), 1.10–1.02 (1H, m), 0.91–0.88 (12H, s and t, *J* 6.7 Hz), 0.81 (3H, d, *J* 6.6 Hz), 0.67–0.63 (2H, m), 0.58 (1H, br.dt, *J* 4.1, 8.2 Hz), 0.04 (3H, s), 0.03 (3H, s), - 0.32 (1H, br.q, *J* 5.1 Hz); δ_C: 178.6, 75.9(-), 64.5(+), 38.7, 37.7(-), 33.6(+), 32.5(+), 31.9(+), 30.2(+), 30.0(+), 29.9(+), 29.75(+), 29.7(+, v.br.), 29.57(+), 29.52(+), 29.4(+), 29.3(+), 28.7(+), 28.6(+), 27.7(+), 27.2(-), 26.0(-), 25.9(+), 22.7(+), 18.2, 15.8(-), 14.4(-), 14.1(-), 10.9(+), - 4.2(-), - 4.4(-).

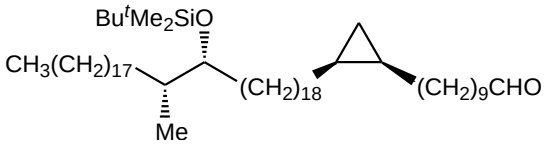
Experiment 88: 10-{(1*R*,2*S*)-2-[(19*R*,20*R*)-19-(*tert*-Butyl-dimethyl-silanyloxy)-20-methyl-octatriacontyl]-cyclopropyl}-decan-1-ol (255)



The procedure used in **Experiment 31** was repeated in order to reduce ester (**254**) (1.2 g, 1.25 mmol) using $LiAlH_4$ (71 mg, 1.88 mmol). The crude product was purified by column chromatography eluting with petrol / ether (2:1) to give a colourless oil, *10-{(1R,2S)-2-[(19R,20R)-19-(tert-butyl-dimethyl-silanyloxy)-20-methyl-octatriacontyl]-*

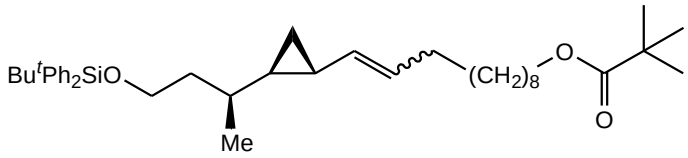
cyclopropyl}-decan-1-ol (**255**) (0.75 g, 69 %), $[\alpha]_D^{25}$ = + 5.9 (*c* 1.16, $CHCl_3$), {Found $(M - H)^+$: 873.8788, $C_{58}H_{117}O_2Si$ requires: 873.8817}. This showed ν_{max}/cm^{-1}: 3332, 2924, 2854, 1465, 1253, 1058; δ_H: 3.65 (2H, t, *J* 6.6 Hz), 3.51 (1H, dt, *J* 3.5, 6.3 Hz), 1.60–1.55 (2H, m), 1.51–1.14 (86H, m, v.br.), 1.09–1.02 (1H, m), 0.91–0.88 (12H, s and t, *J* 6.9 Hz), 0.80 (3H, d, *J* 6.7 Hz), 0.68–0.63 (2H, m), 0.57 (1H, br.dt, *J* 4.1, 8.2 Hz), 0.04 (3H, s), 0.03 (3H, s), - 0.32 (1H, br.q, *J* 5.4 Hz); δ_C: 75.9(-), 63.1(+), 37.7(-), 33.6(+), 32.8(+), 32.5(+), 31.9(+), 30.2(+), 30.0(+), 29.9(+), 29.7(+, v.br.), 29.62(+), 29.6(+), 29.5(+), 29.4(+), 28.7(+), 27.7(+), 26.0(-), 25.9(+), 25.8(+), 22.7(+), 18.2, 15.8(-), 14.4(-), 14.1(-), 10.9(+), - 4.2(-), - 4.4(-).

Experiment 89: 10-{(1*R*,2*S*)-2-[(19*R*,20*R*)-19-(*tert*-Butyl-dimethyl-silanyloxy)-20-methyl-octatriacontyl]-cyclopropyl}-decanal (250)



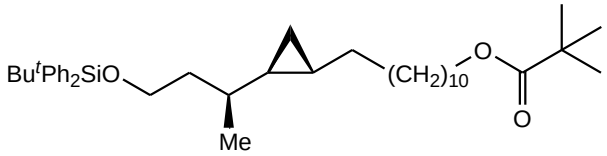
The procedure used in **Experiment 18** was repeated in order to oxidise the alcohol (**255**) (0.62 g, 0.73 mmol) using PCC (0.38 g, 1.77 mmol) in CH_2Cl_2 (60 ml). The crude product was purified by column chromatography eluting with petrol / ether (15:1) to give a colourless oil, *10-{(1R,2S)-2-[(19R,20R)-19-(tert-butyl-dimethyl-silanyloxy)-20-methyl-octatriacontyl]-cyclopropyl}-decanal* (**250**) (0.59 g, 95 %), $[\alpha]_D^{24}$ = + 5.1 (*c* 0.9, $CHCl_3$), {Found $(M + Na)^+$: 895.8638, $C_{58}H_{116}NaO_2Si$ requires: 895.8637}. This showed ν_{max}/cm^{-1}: 2932, 2854, 1731, 1465, 1361, 1253, 1074; δ_H: 9.77 (1H, t, *J* 1.9 Hz), 3.52–3.48 (1H, m), 2.43 (2H, dt, *J* 1.9, 7.4 Hz), 1.64 (2H, quintet, *J* 7.3 Hz), 1.51–1.14 (84H, m, v.br.), 1.09–1.01 (1H, m), 0.90–0.87 (12H, m, including a s), 0.80 (3H, d, *J* 6.7 Hz), 0.67–0.64 (2H, m), 0.57 (1H, br.dt, *J* 4.1, 8.2 Hz), 0.04 (3H, s), 0.03 (3H, s), - 0.32 (1H, br.q, *J* 5.4 Hz); δ_C: 202.9(-), 75.9(-), 43.9(+), 37.7(-), 33.6(+), 32.5(+), 31.9(+), 30.2(+), 30.2(+), 30.0(+), 29.9(+), 29.7(+, v.br.), 29.64(+), 29.62(+), 29.44(+), 29.36(+), 29.2(+), 28.7(+), 28.7(+), 27.7(+), 26.0(-), 25.9(+), 22.7(+), 22.1(+), 18.2, 15.8(-), 15.8(-), 14.4(-), 14.1(-), 10.9(+), - 4.2(-), - 4.4(-).

Experiment 90: 2,2-Dimethyl-propionic acid (*E*/*Z*)-11-{(1*R*,2*R*)-2-[(*S*)-3-(*tert*-butyl-dimethyl-silanyloxy)-1-methyl-propyl]-cyclopropyl}-undec-10-enyl ester (256)



The procedure used in **Experiment 27** was repeated in order to couple the *trans*-aldehyde (**212**) (4.1 g, 10.8 mmol) with the sulfone (**181**) (6.06 g, 13.5 mmol) using lithium bis(trimethylsilyl) amide (18.32 ml, 19.4 mmol, 1.06M) in dry THF. The crude product was purified by column chromatography eluting with petrol / ether (30:1) to give a colourless oil, *2,2-dimethyl-propionic acid (E/Z)-11-{(1R,2R)-2-[(S)-3-(tert-butyl-dimethyl-silanyloxy)-1-methyl-propyl]-cyclopropyl}-undec-10-enyl ester* (**256**) (5.55 g, 85 %) as a mixture of two isomers in ratio 3.6:1, {Found $(M + Na)^+$: 627.4190 $C_{39}H_{60}NaO_3Si$ requires: 627.4204}. This showed ν_{max}/cm^{-1}: 2929, 2856, 1730, 1462, 1284, 1156, 1111; δ_H (major *E* isomer): 7.69–7.66 (4H, m), 7.44–7.36 (6H, m), 5.35 (1H, dt, *J* 15.4, 6.7 Hz), 4.94 (1H, dd, *J* 8.5, 15.2 Hz), 4.05 (2H, t, *J* 6.6 Hz), 3.81–3.73 (2H, m), 1.93–1.90 (2H, m), 1.73–1.50 (4H, m), 1.35–1.25 (12H, m), 1.21 (9H, s), 1.15–1.11 (1H, m), 1.05 (9H, s), 1.01–0.96 (1H, m), 0.91 (3H, d, *J* 6.7 Hz), 0.53–0.40 (3H, m); δ_H (minor *Z* isomer): 5.24 (1H, dt, *J* 10.8, 6.7 Hz), 4.73 (1H, br. t, *J* 10.7 Hz), 2.14–2.10 (1H, m), 2.07–2.02 (1H, m) (the remaining signals were obscured by the major isomer); δ_C (two isomers): 178.6, 135.57(+), 135.56(+), 134.2, 133.3(+), 133.1(+), 129.5(+), 127.8(+), 127.7(+), 127.5(+), 64.5(-), 62.3(-), 30.3(-), 40.1(-), 34.67(+), 34.65(+), 32.5(-), 29.9(-), 29.7(-), 29.5(-), 29.47(-), 29.41(-), 29.3(-), 29.2(-), 29.1(-), 28.6(-), 27.9(+), 27.6(-), 27.3(+), 27.2(+), 26.9(+), 25.9(-), 21.3(+), 19.9(+), 19.6(+), 19.2, 17.4(+), 12.9(-), 12.0(-) [- = CH_2, + = CH, CH_3].

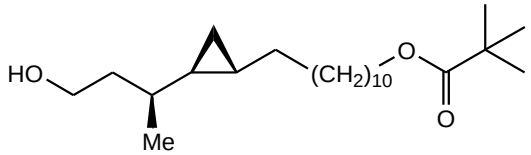
Experiment 91: 2,2-Dimethyl-propionic acid 11-{(1*S*,2*R*)-2-[(*S*)-3-(*tert*-butyl-dimethyl-silanyloxy)-1-methyl-propyl]-cyclopropyl}-undecyl ester (257)



The alkene (**256**) (5.45 g, 9.02 mmol) and TPBSH (7.5 g, 25.13 mmol) were dissolved in dry THF (100 ml) and stirred at 50 ^oC for 22 hrs. Further TPBSH (1.65 g, 5.51

mmol) was added and stirred at 50 ^{o}C for 18 hrs. The mixture was diluted with petrol / ether (1:1, 200 ml) and aq. NaOH (100 ml, 2 %) was added and separated. The aqueous layer was re-extracted with petrol / ether (1:1, 2 x 75 ml) and the combined organic layers were washed with water (60 ml), dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol / ether (30:1) to give a colourless oil which contained small amount of olefin. To remove the olefin, the mixture was dissolved in CH_2Cl_2 (50 ml) and water (50 ml) then acetic acid (1 ml), cetrimide (0.15 g) and potassium permanganate (0.87 g) were added respectively and stirred at r.t. for 1 h. Sodium metabisulfite was added until the dark colour disappeared and the product was extracted with CH_2Cl_2 (3 x 50 ml). The combined organic layers were dried, filtered and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol / ether (30:1) to give a colourless oil, *2,2-dimethyl-propionic acid 11-{(1S,2R)-2-[(S)-3-(tert-butyl-dimethyl-silanyloxy)-1-methyl-propyl]-cyclopropyl}-undecyl ester* (**257**) (4.62 g, 85 %), $[\alpha]_D^{26}$ = + 6.5 (*c* 1.03, $CHCl_3$), {Found $(M + Na)^+$: 629.4344, $C_{39}H_{62}NaO_3Si$ requires: 629.4360}. This showed ν_{max}/cm^{-1} : 2928, 2856, 1731, 1462, 1284, 1155, 1111; δ_H: 7.69–7.67 (4H, m), 7.43–7.37 (6H, m), 4.05 (2H, t, *J* 6.6 Hz), 3.78–3.70 (2H, m), 1.74–1.68 (1H, m), 1.66–1.60 (2H, m), 1.56–1.50 (1H, m), 1.35–1.25 (18H, m), 1.21 (9H, s), 1.18–1.12 (1H, m), 1.05 (9H, s), 0.89 (3H, br. s), 0.47–0.41 (1H, m), 0.19–0.09 (3H, m); δ_C: 178.6, 135.6(+), 134.2, 129.5(+), 127.5(+), 64.5(-), 62.4(-), 40.2(-), 38.7, 34.8(+), 34.3(-), 29.7(-), 29.66(-), 29.63(-), 29.58(-), 29.53(-), 29.2(-), 28.6(-), 27.2(+), 26.9(+), 25.9(-), 19.8(+), 19.2, 18.6(+), 10.5(-) [- = CH_2, + = CH, CH_3].

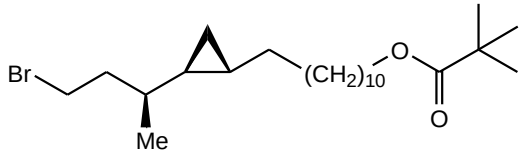
Experiment 92: 2,2-Dimethyl-propionic acid 11-[(1*S*,2*R*)-2-((*S*)-3-hydroxy-1-methyl-propyl)-cyclopropyl]-undecyl ester (258)



The procedure used in **Experiment 74** was repeated using the silyl ether (**257**) (4.5 g, 7.4 mmol), tetra n-butyl ammonium fluoride (9.65 ml, 9.65 mmol, 1M sol. in THF) in THF. This was purified by column chromatography eluting with petrol / ethyl acetate (5:1 and then 4:1) to give a colourless oil, *2,2-dimethyl-propionic acid 11-[(1S,2R)-2-((S)-3-hydroxy-1-methyl-propyl)-cyclopropyl]-undecyl ester* (**258**) (2.65 g, 97 %),

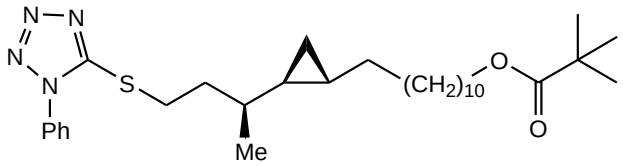
$[\alpha]_D^{25}$ = + 14.7 (*c* 1.12, $CHCl_3$), {Found $(M + Na)^+$: 391.3165, $C_{23}H_{44}NaO_3$ requires: 391.3183}. This showed ν_{max}/cm^{-1}: 3363, 2925, 2854, 1731, 1480, 1286, 1159, 1053; δ_H: 4.05 (2H, t, *J* 6.6 Hz), 3.78–3.69 (2H, m), 1.76–1.69 (1H, m), 1.65–1.60 (2H, m), 1.58–1.52 (1H, m), 1.37–1.23 (17H, m), 1.21 (9H, s), 1.18–1.11 (1H, m), 0.96 (3H, d, *J* 6.6 Hz), 0.89–0.84 (1H, m), 0.51–0.46 (1H, m), 0.27–0.15 (3H, m); δ_C: 178.6, 64.5(-), 61.4(-), 40.4(-), 38.7, 35.0(+), 34.4(-), 29.64(-), 29.61(-), 29.59(-), 29.57(-), 29.53(-), 29.5(-), 29.2(-), 28.6(-), 27.2(+), 25.92(+), 25.90(-), 19.8(+), 18.7(+), 10.6(-) [- = CH_2, + = CH, CH_3].

Experiment 93: 2,2-Dimethyl-propionic acid 11-[(1*S*,2*R*)-2-((*S*)-3-bromo-1-methyl-propyl)-cyclopropyl]-undecyl ester (259)



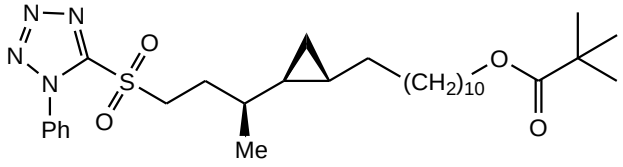
The procedure used in **Experiment 47** was repeated using the alcohol (**258**) (2.6 g, 7.07 mmol), NBS (1.6 g, 8.98 mmol) and PPh_3 (2.13 g, 8.13 mmol) in CH_2Cl_2 (100 ml). The crude product was purified by column chromatography eluting with petrol / ether (15:1) to give a colourless oil, *2,2-dimethyl-propionic acid 11-[(1S,2R)-2-((S)-3-bromo-1-methyl-propyl)-cyclopropyl]-undecyl ester* (**259**) (2.87 g, 94 %), $[\alpha]_D^{25}$ = + 16.2 (*c* 1.3, $CHCl_3$), {Found $(M + Na)^+$: 453.2316, $C_{23}H_{43}BrNaO_2$ requires: 453.2339}. This showed ν_{max}/cm^{-1}: 2925, 2854, 1730, 1480, 1285, 1157; δ_H: 4.05 (2H, t, *J* 6.6 Hz), 3.56–3.45 (2H, m), 2.02–1.97 (1H, m), 1.88–1.83 (1H, m), 1.65–1.60 (2H, m), 1.35–1.23 (18H, m), 1.20 (9H, s), 1.18–1.12 (1H, m), 0.95 (3H, br.s), 0.58–0.54 (1H, m), 0.23–0.15 (3H, m); δ_C: 178.6, 64.5(-), 40.7(-), 38.7, 36.9(+), 34.3(-), 32.4(-), 29.65(-), 29.62(-), 29.57(-), 29.55(-), 29.53(-), 29.5(-), 29.2(-), 28.6(-), 27.2(+), 25.9(-), 25.3(+), 19.3(+), 18.6(+), 10.4(-) [- = CH_2, + = CH, CH_3].

Experiment 94: 2,2-Dimethyl-propionic acid 11-{(1*S*,2*R*)-2-[(*S*)-1-methyl-3-(1-phenyl-1*H*-tetrazol-5-ylsulfanyl)-propyl]-cyclopropyl}-undecyl ester (260)



The procedure used in **Experiment 23** was repeated using compound (**259**) (2.75 g, 6.38 mmol), 1-phenyl-1*H*-tetrazole-5-thiol (1.19 g, 6.7 mmol) and anhydrous potassium carbonate (1.85 g, 13.4 mmol) in acetone (100 ml). This was purified by column chromatography eluting with petrol / ether (7:2) to give a colourless oil, *2,2-dimethyl-propionic acid 11-{(1S,2R)-2-[(S)-1-methyl-3-(1-phenyl-1H-tetrazol-5-ylsulfanyl)-propyl]-cyclopropyl}-undecyl ester* (**260**) (3.2 g, 95 %), $[\alpha]_D^{25}$ = + 11.7 (*c* 1.28, $CHCl_3$), {Found $(M + Na)^+$: 551.3364, $C_{30}H_{48}N_4NaO_2S$ requires: 551.3390}. This showed ν_{max}/cm^{-1}: 2925, 2854, 1727, 1501, 1440, 1284, 1158; δ_H : 7.60–7.54 (5H, m), 4.05 (2H, t, *J* 6.6 Hz), 3.53–3.44 (2H, m), 1.94–1.86 (1H, m), 1.85–1.78 (1H, m), 1.64–1.59 (2H, m), 1.34–1.25 (17H, m), 1.17–1.09 (1H, m), 0.99 (3H, d, *J* 6.6 Hz), 0.94–0.87 (1H, m), 0.53–0.47 (1H, m), 0.29–0.16 (3H, m); δ_C: 178.6, 154.4, 133.8, 130.0(+), 129.7(+), 123.8(+), 64.4(-), 38.7, 37.4(+), 36.5(-), 34.2(-), 31.6(-), 29.62(-), 29.60(-), 29.55(-), 29.52(-), 29.5(-), 29.2(-), 28.6(-), 27.2(+), 25.9(-), 25.3(+), 19.5(+), 18.8(+), 10.4(-) [- = CH_2, + = CH, CH_3].

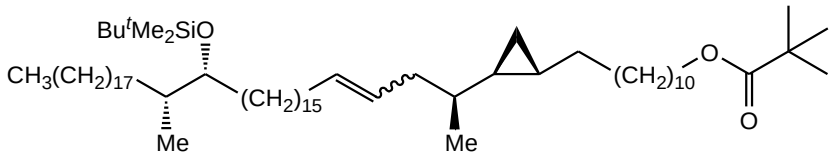
Experiment 95: 2,2-Dimethyl-propionic acid 11-{(1*S*,2*R*)-2-[(*S*)-1-methyl-3-(1-phenyl-1*H*-tetrazole-5-sulfonyl)-propyl]-cyclopropyl}-undecyl ester (261)



The procedure used in **Experiment 25** was repeated using the sulfane (**260**) (3.1 g, 5.87 mmol), ammonium molybdate (VI) tetrahydrate (3.41 g, 2.76 mmol) in 35 % H_2O_2 (7.6 ml) and THF (50 ml) and IMS (100 ml), and a further ammonium molybdate (VI) tetrahydrate (1.25 g, 1.01 mmol) in 35% H_2O_2 (3.2 ml). The crude product was purified by column chromatography eluting with petrol / ether (2:1) to give a colourless oil, *2,2-dimethyl-propionic acid 11-{(1S,2R)-2-[(S)-1-methyl-3-(1-phenyl-1H-tetrazole-5-sulfonyl)-propyl]-cyclopropyl}-undecyl ester* (**261**) (3.2 g, 98 %), $[\alpha]_D^{22}$ = + 2.4 (*c* 1.24, $CHCl_3$), {Found $(M + H)^+$: 561.3490, $C_{30}H_{49}N_4O_4S$

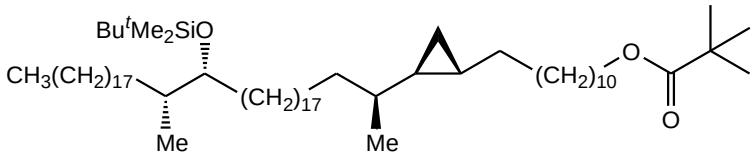
requires: 561.3475}. This showed ν_{max}/cm^{-1}: 2926, 2854, 1727, 1480, 1340, 1285, 1155; δ_H: 7.72–7.70 (2H, m), 7.66–7.59 (3H, m), 4.05 (2H, t, *J* 6.6 Hz), 3.87 (1H, ddd, *J* 5.1, 11.1, 14.2 Hz), 3.79 (1H, ddd, *J* 5.4, 11.1, 14.5 Hz), 2.05–1.92 (2H, m), 1.65–1.60 (2H, m), 1.36–1.25 (17H, m), 1.20 (9H, s), 1.16–1.07 (1H, m), 1.02 (3H, d, *J* 6.6 Hz), 0.99–0.92 (1H, m), 0.56–0.50 (1H, m), 0.30–0.21 (3H, m); δ_C: 178.6, 153.5, 133.1, 131.4(+), 129.7(+), 125.0(+), 64.4(-), 54.5(-), 38.7, 37.2(+), 34.1(-), 29.57(-), 29.53(-), 29.52(-), 29.5(-), 29.2(-), 29.0(-), 28.6(-), 27.2(+), 25.9(-), 24.8(+), 19.5(+), 19.0(+), 10.5(-) [- = CH_2, + = CH, CH_3].

Experiment 96: 2,2-Dimethyl-propionic acid 11-{(1*S*,2*R*)-2-[(*E*/*Z*)-(1*S*,20*R*,21*R*)-20-(*tert*-butyl-dimethyl-silanyloxy)-1,21-dimethyl-nonatriacont-3-enyl]-cyclopropyl}-undecyl ester (262)



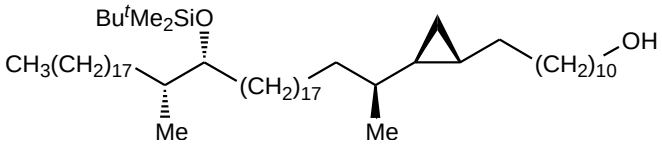
The procedure used in **Experiment 27** was repeated in order to couple the aldehyde (**215**) (3.2 g, 4.82 mmol) with the sulfone (**261**) (3.1 g, 5.54 mmol) using lithium bis(trimethylsilyl) amide (8.2 ml, 8.67 mmol, 1.06M) in dry THF. The crude product was purified by column chromatography eluting with petrol / ether (40:1) to give a colourless oil, *2,2-dimethyl-propionic acid 11-{(1S,2R)-2-[(E/Z)-(1S,20R,21R)-20-(tert-butyl-dimethyl-silanyloxy)-1,21-dimethyl-nonatriacont-3-enyl]-cyclopropyl}-undecyl ester* (**262**) (3.85 g, 80 %) as a mixture of two isomers in ratio 3:1, {Found $(M + Na)^+$: 1021.9724, $C_{66}H_{130}NaO_3Si$ requires: 1021.9681}. This showed ν_{max}/cm^{-1}: 2925, 2854, 1733, 1464, 1284, 1253, 1155, 1077; δ_H (two isomers): 5.45–5.37 (2H, m), 4.05 (2H, t, *J* 6.6 Hz), 3.52–1.49 (1H, m), 2.18–2.12 (1H, m), 2.05–1.90 (3H, m), 1.65–1.60 (2H, m), 1.50–1.12 (80H, m, v.br.), 1.20 (9H, s), 1.08–1.01 (1H, m), 0.92–0.87 (15H, m, including a s), 0.80 (3H, d, *J* 6.6 Hz), 0.77–0.68 (1H, m), 0.51–0.43 (1H, m), 0.29–0.11 (3H, m), 0.04 (3H, s), 0.03 (3H, s); δ_C (two isomers): 178.6, 131.4(-), 130.4(-), 128.9(-), 128.4(-), 75.9(-), 64.5(+), 40.3(+), 38.8(-), 38.7, 37.7(-), 34.7(+), 34.39(+), 34.37, 33.5(+), 32.7(+), 32.5(+), 31.9(+), 30.0(+), 29.9(+), 29.8(+), 29.7(+, v.br.), 29.65(+), 29.61(+), 29.58(+), 29.55(+), 29.5(+), 29.4(+), 29.3(+), 29.2(+), 28.6(+), 27.7(+), 27.3(+), 27.2(-), 26.0(-), 25.9(+), 25.8(-), 25.7(-), 19.3(-), 19.2(-), 18.5(-), 18.2, 14.4(-), 14.1(-), 10.8(+), 10.7(+), - 4.2(-), - 4.4(-).

Experiment 97: 2,2-Dimethyl-propionic acid 11-{(1*S*,2*R*)-2-[(1*S*,20*R*,21*R*)-20-(*tert*-butyl-dimethyl-silanyloxy)-1,21-dimethyl-nonatriacontyl]-cyclopropyl}-undecyl ester (263)



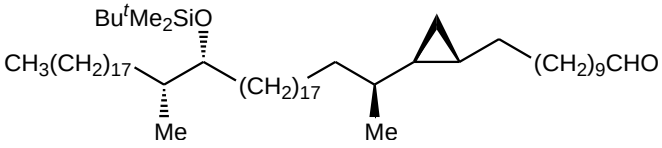
The alkene (**262**) (3.65 g, 3.66 mmol) and TPBSH (3 g, 10.06 mmol) were dissolved in dry THF (75 ml) and stirred at 50 ^{o}C for 19 hrs. Further TPBSH (0.98 g, 3.29 mmol) was added and stirred at 50 ^{o}C for 21 hrs. The mixture was diluted with petrol / ether (1:1, 150 ml) and aq. NaOH (80 ml, 2 %) was added and extracted. The aqueous layer was re-extracted with petrol / ether (1:1, 2 x 50 ml) and the combined organic layers were washed with water (100 ml), dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol / ether (40:1) to give a colourless oil which contained small amount of olefin. To removed the olefin, the mixture was compounds were dissolved in CH_2Cl_2 (45 ml) and water (45 ml) then acetic acid (0.8 ml), cetrimide (0.12 g) and potassium permanganate (0.65 g) were added and stirred at r.t. for 1 hr. Sodium metabisulfite was added until the dark colour disappeared and extracted with CH_2Cl_2 (3 x 50 ml). The combined organic layers were dried, filtered and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol / ether (40:1) to give a colourless oil, *2,2-dimethyl-propionic acid 11-{(1S,2R)-2-[(1S,20R,21R)-20-(tert-butyl-dimethyl-silanyloxy)-1,21-dimethyl-nonatriacontyl]-cyclopropyl}-undecyl ester* (**263**) (3.05 g, 84 %), $[\alpha]_D^{23}$ = + 6.5 (*c* 1.05, $CHCl_3$), {Found $(M + Na)^+$: 1023.9881, $C_{66}H_{132}NaO_3Si$ requires: 1023.9838}. This showed ν_{max}/cm^{-1} : 2924, 2853, 1733, 1464, 1284, 1253, 1154, 1075; δ_H: 4.05 (2H, t, *J* 6.6 Hz), 3.52–3.49 (1H, m), 1.65–1.60 (2H, m), 1.49–1.16 (88H, m, v.br.), 1.21 (9H, s), 1.08–1.01 (1H, m), 0.91–0.88 (15H, m, including a s and a t, *J* 6.6 Hz), 0.80 (3H, d, *J* 7.0 Hz), 0.71–0.64 (1H, m), 0.48–0.42 (1H, m), 0.22–0.09 (3H, m), 0.04 (3H, s), 0.03 (3H, m); δ_C: 178.6, 75.9(-), 64.5(+), 38.7, 38.1(-), 37.7(-), 37.4(+), 34.5(+), 33.5(+), 32.5(+), 31.9(+), 30.7(+), 30.0(+), 29.9(+), 29.72(+), 29.7(+, v.br.), 29.66(+), 29.60(+), 29.59(+), 29.54(+), 29.4(+), 29.2(+), 28.6(+), 27.7(+), 27.3(+), 27.2(-), 26.1(-), 26.0(-), 25.9(+), 22.7(+), 19.7(-), 18.6(-), 18.2, 14.4 (-), 14.1 (-), 10.5 (+), - 4.2 (-), - 4.4 (-).

Experiment 98: 11-{(1*S*,2*R*)-2-[(1*S*,20*R*,21*R*)-20-(*tert*-Butyl-dimethyl-silanyloxy)-1,21-dimethyl-nonatriacontyl]-cyclopropyl}-undecan-1-ol (264)



The procedure used in **Experiment 31** was repeated in order to reduce the ester (**263**) (2.9 g, 2.9 mmol) using $LiAlH_4$ (0.17 g, 4.35 mmol) to a crude alcohol. This was purified by column chromatography eluting with petrol / ether (5:2) to give a colourless oil, *11-{(1S,2R)-2-[(1S,20R,21R)-20-(tert-butyl-dimethyl-silanyloxy)-1,21-dimethyl-nonatriacontyl]-cyclopropyl}-undecan-1-ol* (**264**) (2.58 g, 97 %), $[\alpha]_D^{25}$ = + 6.2 (*c* 1.03, $CHCl_3$), {Found $(M + Na)^+$: 939.9263, $C_{61}H_{124}NaO_2Si$ requires: 939.9263}. This showed ν_{max}/cm^{-1} : 3332, 2924, 2853, 1464, 1253, 1058; δ_H: 3.65 (2H, t, *J* 6.6 Hz), 3.52–3.49 (1H, m), 1.60–1.55 (2H, m), 1.48–1.16 (88H, m, v.br.), 1.08–1.01 (1H, m), 0.91–0.88 (15H, m, including a s), 0.80 (3H, d, *J* 6.6 Hz), 0.71–0.64 (1H, m), 0.48–0.43 (1H, m), 0.21–0.09 (3H, m), 0.04 (3H, s), 0.03 (3H, s); δ_C: 75.9(-), 63.1(+), 38.1(-), 37.7(-), 37.4(+), 34.5(+), 33.6(+), 32.8(+), 32.5(+), 31.9(+), 30.1(+), 30.0(+), 29.9(+), 29.74(+), 29.72(+, v.br.), 29.69(+), 29.67(+), 29.66(+), 29.64(+), 29.5(+), 29.4(+), 27.7(+), 27.3(+), 26.1(-), 26.0(-), 25.9(+), 25.8(+), 22.7(+), 19.7(-), 18.6(-), 18.2, 14.4(-), 14.1(-), 10.5(+), - 4.2(-), - 4.4(-).

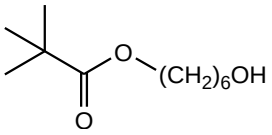
Experiment 99: 11-{(1*S*,2*R*)-2-[(1*S*,20*R*,21*R*)-20-(*tert*-Butyl-dimethyl-silanyloxy)-1,21-dimethyl-nonatriacontyl]-cyclopropyl}-undecanal (251)



The procedure used in **Experiment 18** was repeated in order to oxidise the alcohol (**264**) (0.7 g, 0.76 mmol) using PCC (0.41 g, 1.9 mmol) in CH_2Cl_2 (60 ml). The crude product was purified by column chromatography eluting with petrol / ether (10:1) to give a colourless oil, *11-{(1S,2R)-2-[(1S,20R,21R)-20-(tert-butyl-dimethyl-silanyloxy)-1,21-dimethyl-nonatriacontyl]-cyclopropyl}-undecanal* (**251**) (0.69 g, 98 %), $[\alpha]_D^{24}$ = + 5.9 (*c* 1.14, $CHCl_3$), {Found $(M + Na)^+$: 937.9084, $C_{61}H_{122}NaO_2Si$ requires: 937.9106}. This showed ν_{max}/cm^{-1}: 2925, 2854, 1731, 1465, 1253, 1077; δ_H: 9.77 (1H, t, *J* 1.9 Hz), 3.52–3.49 (1H, m), 2.42 (2H, dt, *J* 1.9, 7.3 Hz), 1.67–1.61 (2H, m), 1.51–1.16 (86H, m,

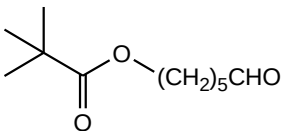
v.br.), 1.08–1.01 (1H, m), 0.91–0.88 (15H, m, including a s and a t, *J* 7.3 Hz), 0.80 (3H, d, *J* 7.0 Hz), 0.71–0.64 (1H, m), 0.48 0.42 (1H, m), 0.22–0.09 (3H, m), 0.04 (3H, s), 0.03 (3H, s); δ_C: 202.9(-), 75.9(-), 43.9(+), 38.1(-), 37.7(-), 37.4(+), 34.5(+), 33.5(+), 32.5(+), 31.9(+), 30.1(+), 30.0(+), 29.9(+), 29.73(+), 29.71(+, v.br.), 29.60(+), 29.58(+), 29.44(+), 29.4(+), 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(-).

Experiment 100: 2,2-Dimethyl-propionic acid 6-hydroxy-hexyl ester (265)



Trimethylacetyl chloride (39.75 ml, 326 mmol) in CH_2Cl_2 (60 ml) was added to a stirred solution of 1,6-hexanediol (35 g, 296 mmol) in CH_2Cl_2 (400 ml), triethylamine (124 ml, 888 mmol) and 4-dimethylaminopyridine (0.75 g, 6.1 mmol) between 20 and 30 ^oC over a period of 40 min. The mixture was stirred at r.t. for 3 hrs, when TLC showed no diol was left. Dilute HCl (250 ml, 10 %) was added and extracted. The organic phase re-washed with dilute HCl (100 ml, 10 %) and brine (2 x 300 ml), then dried and the solvent evaporated. The crude product was purified by column chromatography eluting with petrol / ether (10:1 and then 1:1) to give a pale yellow oil, 2,2-dimethyl-propionic acid 6-hydroxy-hexyl ester (**265**) [230] (35.10 g, 59 %). This showed ν_{max}/cm^{-1}: 3396, 2936, 2865, 1730, 1481, 1287, 1161, 1058; δ_H: 4.06 (2H, t, *J* 6.6 Hz), 3.65 (2H, t, *J* 6.6 Hz), 1.67–1.57 (5H, m), 1.41–1.39 (4H, m), 1.20 (9H, s); δ_C: 178.8, 64.3(-), 62.8(-), 38.7, 32.6(-), 28.6(-), 27.2(+), 25.7(-), 25.3(-) [- = CH_2, + = CH, CH_3].

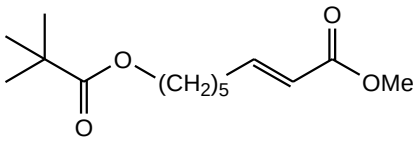
Experiment 101: 2,2-Dimethyl-propionic acid 6-oxo-hexyl ester (266)



The procedure used in **Experiment 18** was repeated in order to oxidise the alcohol (**265**) (35.06 g, 174 mmol) using PCC (75 g, 347 mmol) in CH_2Cl_2 (1.5 L) to a crude product. This was purified by column chromatography eluting with petrol / ether (7:3) to give a pale yellow oil, 2,2-dimethyl-propionic acid 6-oxo-hexyl ester (**266**)[230] (29.45 g, 85 %). This showed ν_{max}/cm^{-1}: 2959, 1728, 1481, 1286, 1159; δ_H: 9.77 (1H, t, *J* 1.6

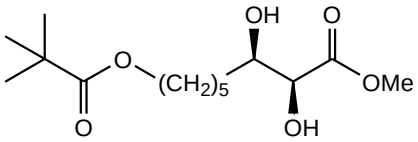
Hz), 4.06 (2H, t, *J* 6.6 Hz), 2.45 (2H, dt, *J* 1.6, 7.3 Hz), 1.71–1.62 (4H, m), 1.44–1.37 (2H, m), 1.19 (9H, s); δ_C: 202.3(+), 178.6, 64.0(-), 43.7(-), 38.7, 28.4(-), 27.2(+), 25.5(-), 21.6(-) [- = CH_2, + = CH, CH_3].

Experiment 102: (*E*)-8-(2,2-Dimethyl-propionyloxy)-oct-2-enoic acid methyl ester (267)



(Methoxycarbonylmethylene) triphenylphosphorane (56.24 g, 168.4 mmol) was added to a stirred solution of the 2,2-dimethyl-propionic acid 6-oxo-hexyl ester (**266**) (29.35 g, 146.8 mmol) in toluene (500 ml) at r.t. and the mixture was stirred at r.t. for 18 hrs. The solvent was evaporated to give a white solid then refluxed with petrol / ether (1:1, 600 ml). The mixture was then filtered and the precipitate washed with petrol / ether (1:1, 300 ml). The solvent was evaporated and the crude product was purified and separated by column chromatography eluting with petrol / ether (9:2) to give a colourless oil, *(E)-8-(2,2-dimethyl-propionyloxy)-oct-2-enoic acid methyl ester* (**267**) (33.25 g, 89 %), {Found $(M + Na)^+$: 279.1552, $C_{14}H_{24}NaO_4$ requires: 279.1561}. This showed ν_{max}/cm^{-1}: 2955, 2866, 1728, 1658, 1481, 1437, 1285, 1158, 1046; δ_H: 6.96 (1H, dt, *J* 7.0, 15.8 Hz), 5.82 (1H, d, *J* 15.8 Hz), 4.05 (2H, t, *J* 6.6 Hz), 3.72 (3H, s), 2.21 (2H, q, *J* 7.3 Hz), 1.64 (2H, quintet, *J* 6.9 Hz), 1.50 (2H, quintet, *J* 7.3 Hz), 1.42–1.36 (2H, m), 1.19 (9H, s); δ_C: 178.6, 167.0, 149.1(+), 121.1(+), 64.1(-), 51.4(+), 38.7, 32.0(-), 28.3(-), 27.6(-), 27.2(+), 25.4(-) [- = CH_2, + = CH, CH_3].

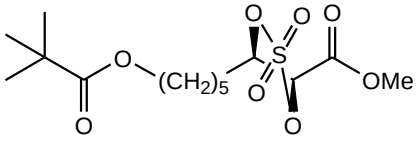
Experiment 103: (2*S*,3*R*)-8-(2,2-Dimethyl-propionyloxy)-2,3-dihydroxy-octanoic acid methyl ester (270)



The $(DHQD)_2PHAL$ ligand (770 mg, 0.99 mmol), $K_3Fe(CN)_6$ (97.6 g, 296.5 mmol), K_2CO_3 (41.0 g, 296.5 mmol) and a solution of 2.5 % 0f OsO_4 in *tert*-butyl alcohol (3.95 ml, 3.95 mmol) were dissolved in a 1:1 mixture of water and tert-butyl alcohol (800 ml) at r.t. Then methanesulfonamide (9.7 g, 98.8 mmol) was added and the mixture was vigorously stirred and cooled to 2 ^oC. (*E*)-8-(2,2-Dimethyl-propionyloxy)-

oct-2-enoic acid methyl ester (**267**) (25.3 g, 98.8 mmol) was added and the mixture was stirred at 2 ^{o}C for 10 hrs. TLC showed the reaction was complete. Sodium metabisulfite (45 g, 237 mmol) was added carefully portionwise then the mixture was warmed to r.t. and stirred for 30 min. The mixture was extracted with CH_2Cl_2 (3 x 600 ml) and the combined organic layers washed with brine, dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol / ethyl acetate (1:1) to give a colourless oil, *(2S,3R)-8-(2,2-dimethyl-propionyloxy)-2,3-dihydroxy-octanoic acid methyl ester* (**270**) (27.5 g, 96 %), $[\alpha]_D^{25}$ = + 13.5 (*c* 1.245, $CHCl_3$), {Found $(M + Na)^+$: 313.1615, $C_{14}H_{26}NaO_6$ requires: 313.1622}. This showed ν_{max}/cm^{-1}: 3482, 2956, 2866, 1729, 1481, 1461, 1366, 1286, 1161, 1088; δ_H: 4.10 (1H, br.s), 4.06 (2H, t, *J* 6.6 Hz), 3.90–3.87 (1H, m), 3.83 (3H, s), 3.15 (1H, br.s), 2.05 (1H, br.s), 1.67–1.58 (4H, m), 1.52–1.38 (4H, m), 1.19 (9H, s); δ_C: 178.7, 174.0, 73.1(+), 72.3(+), 64.2(-), 52.8(+), 38.7, 33.6(-), 28.5(-), 27.2(+), 25.7(-), 25.3(-) [- = CH_2, + = CH, CH_3].

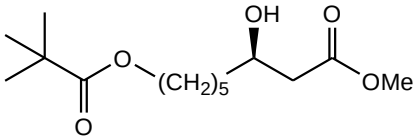
Experiment 104: (4*S*,5*R*)-5-[5-(2,2-Dimethyl-propionyloxy)-pentyl]-2,2-dioxo-2λ^6-[1,3,2]dioxathiolane-4-carboxylic acid methyl ester (272)



In a two neck round bottomed flask with a reflux condenser and topped with a drying tube ($CaCl_2$), the diol (**270**) (27.3 g, 94.1 mmol) was dissolved in carbon tetrachloride (200 ml). Thionyl chloride (15.0 ml, 207.1 mmol) was added and the mixture was vigorously refluxed for 2.5 hrs. After cooling, the solution was diluted with CH_3CN (200 ml) and ruthenium trichloride hydrate (976 mg, 47 mmol, 0.05 mol eq.) and $NaIO_4$ (30.2 g, 141.2 mmol) were added followed by water (300 ml) slowly. The mixture was stirred at r.t. for 3 hrs. then diluted by ether (1 L) and extracted. The aq. layer was re-extracted with ether (2 x 200 ml). The combined organic layers were washed with water (400 ml), sat.aq. $NaHCO_3$ (300 ml) and brine (300 ml) and dried. The solvent was evaporated and the crude product was purified by column chromatography eluting with petrol / ether (1:1) to give a colourless oil, *(4S,5R)-5-[5-(2,2-dimethyl-propionyloxy)-pentyl]-2,2-dioxo-2λ^6-[1,3,2]dioxathiolane-4-carboxylic acid methyl ester* (**272**) (29.80 g, 90 %), $[\alpha]_D^{24}$ = + 39.4 (*c* 1.18, $CHCl_3$), {Found (M +

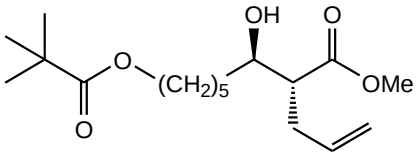
Na)$^+$: 375.1076, $C_{14}H_{24}NaO_8S$ requires: 375.1084}. This showed ν_{max}/cm^{-1}: 2960, 2872, 1775, 1750, 1724, 1482, 1398, 1287, 1212, 1162, 1054; δ_H: 4.97–4.93 (1H, m), 4.88 (1H, br.d, *J* 7.2 Hz), 4.07 (2H, t, *J* 6.6 Hz), 3.91 (3H, s), 2.03–1.99 (2H, m), 1.70–1.42 (6H, m), 1.20 (9H, s); δ_C: 178.6, 165.3, 83.8(+), 79.7(+), 63.9(-), 53.7(+), 38.7, 32.9(-), 28.3(-), 27.2(+), 25.3(-), 24.5(-) [- = CH_2, + = CH, CH_3].

Experiment 105: (*R*)-8-(2,2-Dimethyl-propionyloxy)-3-hydroxy-octanoic acid methyl ester (274)



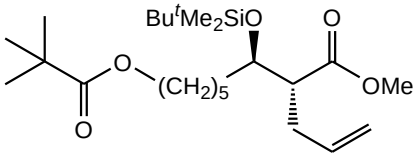
The cyclic compound (**272**) (17.68 g, 50 mmol) was dissolved in DMAC (350 ml) and cooled to – 2 ^oC. $NaBH_4$ (1.9 g, 50 mmol, 1 mol eq.) was added by portionwise between – 2 ^oC and + 2 ^oC. The reaction was stirred at 0 ^oC for 30 min and monitored by TLC. The solvent was carefully removed by distillation under high vacuum. THF (300 ml), water (0.47 ml) and sulphuric acid (1.3 ml) were added and resulting suspension was stirred for 1 h. Then sodium metabisulfite was added. The reaction mixture was stirred for 20 min and filtered on silica gel. The filtrate was concentrated in vacuum. The residue was purified by column chromatography eluting with petrol / ethyl acetate (5:2) to give a colourless oil, *(R)-8-(2,2-dimethyl-propionyloxy)-3-hydroxy-octanoic acid methyl ester* (**274**) (10.75 g, 78 %), $[\alpha]_D^{24}$ = – 12.3 (*c* 1.34, $CHCl_3$), {Found (M + Na)$^+$: 297.1669, $C_{14}H_{26}NaO_5$ requires: 297.1672}. This showed ν_{max}/cm^{-1}: 3510, 2938, 2866, 1729, 1481, 1438, 1287, 1161; δ_H: 4.05 (2H, t, *J* 6.6 Hz), 4.03–3.98 (1H, m), 3.72 (3H, s), 2.52 (1H, dd, *J* 3.2, 16.4 Hz), 2.42 (1H, dd, *J* 9.2, 16.4 Hz), 1.64 (2H, quintet, *J* 6.9 Hz), 1.56–1.37 (6H, m), 1.20 (9H, s); δ_C: 178.6, 173.4, 67.9(+), 64.2(-), 51.7(+), 41.1(-), 38.7, 36.4(-), 28.6(-), 27.2(+), 25.8(-), 25.1(-) [- = CH_2, + = CH, CH_3].

Experiment 106: (2*R*,3*R*)-2-Allyl-8-(2,2-dimethyl-propionyloxy)-3-hydroxy-octanoic acid methyl ester (278)



Diisopropylamine (1.99 g, 19.6 mmol) was dissolved in dry THF (80 ml) and cooled to – 78 ^{o}C. n-BuLi (9.3 ml, 19.6 mmol, 2.15 mol eq.) was added and stirred to + 15.5 ^{o}C for 20 min. then re-cooled to – 65 ^{o}C and (*R*)-8-(2,2-dimethyl-propionyloxy)-3-hydroxy-octanoic acid methyl ester (**274**) (2.5 g, 9.1 mmol) in dry THF (30 ml) was added and the mixture was stirred from – 63 ^{o}C to + 2.5 ^{o}C for 1 h. and 50 min and then at 0 ^{o}C for 10 min. It was re-cooled to – 55 ^{o}C and allyl iodide (1.1 ml, 11.9 mmol) in THF (10 ml) and HMPA (4.7 ml, 27.4 mmol, 3 mol eq.) was added and the mixture was stirred for 2 hrs. from – 50 ^{o}C to – 8 ^{o}C. Sat.aq. NH_4Cl (35 ml) was added and extracted with ether / ethyl acetate (1:1, 3 x 100 ml), dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol / ethyl acetate (3:1) to give a colourless oil, *(2R,3R)-2-allyl-8-(2,2-dimethyl-propionyloxy)-3-hydroxy-octanoic acid methyl ester* (**278**) (1.71 g, 60 %), $[\alpha]_D^{26}$ = + 4.0 (*c* 1.08, $CHCl_3$), {Found $(M + Na)^+$: 337.1985, $C_{17}H_{30}NaO_5$ requires: 337.1981}. This showed ν_{max}/cm^{-1}: 3518, 2939, 2866, 1729, 1643, 1481, 1399, 1286, 1162; δ_H: 5.76 (1H, ddt, *J* 17.0, 10.1, 7.0 Hz), 5.13–5.04 (2H, m), 4.05 (2H, t, *J* 6.6 Hz), 3.71 (3H, s), 3.71–3.68 (1H, m), 2.54 (1H, ddd, *J* 5.1, 6.3, 7.9 Hz), 2.50–2.50 (2H, m), 1.64 (2H, quintet, *J* 7.0 Hz), 1.55–1.35 (6H, m), 1.20 (9H, s); δ_C: 178.6, 175.3, 134.8(+), 117.2(-), 71.6(+), 64.2(-), 51.6(+), 50.5(+), 38.7, 35.4(-), 33.8(-), 28.6(-), 27.2(+), 25.8(-), 25.4(-) [- = CH_2, + = CH, CH_3].

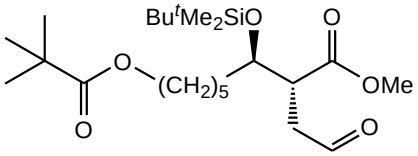
Experiment 107: (2*R*,3*R*)-2-Allyl-3-(*tert*-butyl-dimethyl-silanyloxy)-8-(2,2-dimethyl-propionyloxy)-octanoic acid methyl ester (279)



Imidazole (2.83 g, 41.5 mmol) was added to a stirred solution of the alcohol (**278**) (5.2 g, 16.56 mmol) in dry DMF (58 ml) at r.t., followed by addition of *tert*-butyldimethylchlorosilane (3.26 g, 21.6 mmol). The mixture was stirred at 45 ^{o}C for 18

hrs. TLC showed the reaction was complete. The mixture was quenched with water (350 ml) and extracted with CH_2Cl_2 (3 x 150 ml). The combined organic layers were washed with water (150 ml), dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol / ether (7:1) to give a colourless oil, *(2R,3R)-2-allyl-3-(tert-butyl-dimethyl-silanyloxy)-8-(2,2-dimethyl-propionyloxy)-octanoic acid methyl ester* (**279**) (6.15 g, 87 %), $[\alpha]_D^{24} = -13.7$ (*c* 1.06, $CHCl_3$), {Found $(M + Na)^+$: 451.2831, $C_{23}H_{44}NaO_5Si$ requires: 451.2850}. This showed ν_{max}/cm^{-1}: 2955, 2858, 1732, 1473, 1285, 1255, 1159; δ_H: 5.75 (1H, ddt, *J* 17.0, 10.1, 6.9 Hz), 5.05 (1H, dq, *J* 17.0, 1.6 Hz), 4.99 (1H, br.d, *J* 10.1 Hz), 4.05 (2H, t, *J* 6.6 Hz), 3.96–3.93 (1H, m), 3.66 (3H, s), 2.63 (1H, ddd, *J* 4.3, 6.3, 10.7 Hz), 2.36–2.22 (2H, m), 1.66–1.60 (2H, m), 1.51–1.32 (6H, m), 1.20 (9H, s), 0.87 (9H, s), 0.06 (3H, s), 0.04 (3H, s); δ_C: 178.6, 174.0, 135.9(+), 116.4(-), 72.6(+), 64.3(-), 51.3(+), 51.3(+), 38.7, 33.6(-), 31.5(-), 28.6(-), 27.2(+), 26.1(-), 25.7(+), 23.9(-), 18.0, - 4.4(+), - 4.9(+) [- = CH_2, + = CH, CH_3].

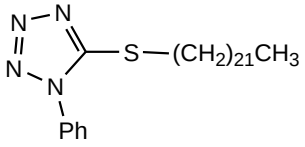
Experiment 108: (2*R*,3*R*)-3-(*tert*-Butyl-dimethyl-silanyloxy)-8-(2,2-dimethyl-propionyloxy)-2-(2-oxo-ethyl)-octanoic acid methyl ester (280)



2,6-Lutidine (1.5 g, 14.02 mmol), OsO_4 2.5 % in 2-methyl-2-propanol (1.4 ml, 0.14 mmol), and then $NaIO_4$ (6.0 g, 28.04 mmol) were added to a stirred solution of the alkene (**279**) (3.0 g, 7.01 mmol) in 1,4-dioxane–water (3:1, 100 ml) at r.t. The reaction was stirred at 25 ^oC for 2 hrs, when TLC showed complete reaction. Water (250 ml) and CH_2Cl_2 (250 ml) were added and extracted. The water layer was re-extracted (2 x 50 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 (7:1 and then 3:1) to give a colourless oil, *(2R,3R)-3-(tert-butyl-dimethyl-silanyloxy)-8-(2,2-dimethyl-propionyloxy)-2-(2-oxo-ethyl)-octanoic acid methyl ester* (**280**) (2.41 g, 80%), $[\alpha]_D^{24} = -12.7$ (*c* 0.6, $CHCl_3$), {Found $(M + Na)^+$: 453.2644, $C_{22}H_{42}NaO_6Si$ requires: 453.2643}. This showed ν_{max}/cm^{-1}: 2956, 2859, 1730, 1463, 1285, 1255, 1159; δ_H: 9.82 (1H, br.s), 4.06–4.03 (1H, m), 4.04 (2H, t, *J* 6.6 Hz), 3.69 (3H, s), 3.21 (1H, dt, *J* 3.5, 10.4 Hz), 2.96 (1H, dd,

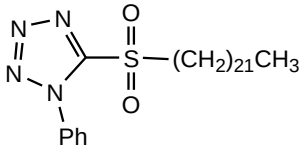
J 10.4, 18.3 Hz), 2.67 (1H, dd, *J* 3.5, 18.3 Hz), 1.63–1.27 (8H, m), 1.20 (9H, s), 0.88 (9H, s), 0.08 (3H, s), 0.07 (3H, s); δ_C: 200.5(+), 178.6, 172.5, 71.7(+), 64.2(-), 51.9(+), 45.2(+), 39.9(-), 38.7, 33.5(-), 28.6(-), 27.2(+), 25.9(-), 25.7(+), 25.4(-), 17.9, - 4.6(+), - 4.7(+) [- = CH_2, + = CH, CH_3].

Experiment 109: 5-Docosylsulfanyl-1-phenyl-1*H*-tetrazole (282)



1-Phenyl-1*H*-tetrazole-5-thiol (6.0 g, 33.7 mmol), 1-bromodocosane (12.5 g, 32.1 mmol), anhydrous potassium carbonate (9.3 g, 67.4 mmol) and acetone (500 ml) were mixed and vigorously stirred and refluxed at 60 ^oC for 15 hrs, 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 CH_2Cl_2 (200 ml). The solution was washed with water (300 ml) and the aqueous layer was re-extracted with CH_2Cl_2 (2 x 50 ml). The combined organic phases were washed with water (300 ml), dried and the solvent was evaporated. The crude product was re-crystallised from acetone (90 ml) and methanol (180 ml) to give a white solid, 5-docosylsulfanyl-1-phenyl-1*H*-tetrazole (**282**)[144] (14.5 g, 73 %), m.p.: 70–72 ^oC (lit.[144] m.p: 70–72 ^oC). This showed ν_{max}/cm^{-1}: 3017, 2925, 2853, 1598, 1500; δ_H: 7.61–7.54 (5H, m), 3.40 (2H, t, *J* 7.3 Hz), 1.82 (2H, quintet, *J* 7.4 Hz), 1.45 (2H, quintet, *J* 7.3 Hz), 1.34–1.26 (36H, m, v.br.), 0.89 (3H, t, *J* 6.9 Hz); δ_C: 154.5, 133.8, 130.0(-), 129.7(-), 123.9(-), 33.9(+), 31.9(+), 29.7(+, v.br.), 29.65(+), 29.60(+), 29.53(+), 29.43(+), 29.35(+), 29.1(+), 29.0(+), 28.6(+), 22.8(+), 14.10(-).

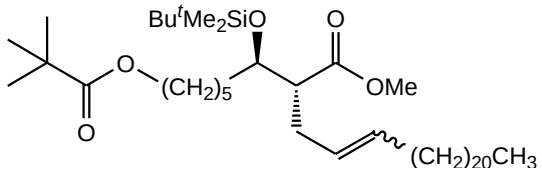
Experiment 110: 5-(Docosane-1-sulfonyl)-1-phenyl-1*H*-tetrazole (97)



A solution of ammonium molybdate (VI) tetrahydrate (16.51 g, 13.36 mmol) in 35 % H_2O_2 (37 ml), prepared and cooled in an ice bath, was added to a stirred solution of 5-docosylsulfanyl-1-phenyl-1*H*-tetrazole (**282**) (14.28 g, 29.4 mmol) in THF (200 ml) and IMS (400 ml) at 12 ^oC and stirred at 15-20 ^oC for 2 hrs. A further solution of ammonium molybdate (VI) tetrahydrate (6.27 g, 5.07 mmol) in 35% H_2O_2 (16 ml) was

added and the mixture was stirred at r.t. for 18 hrs. The mixture was poured into 3 L of water and extracted with CH_2Cl_2 (3 x 200 ml). The combined organic phases were washed with water (2 x 300 ml), dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol / ether (8:1) to give a white solid, 5-(docosane-1-sulfonyl)-1-phenyl-1*H*-tetrazole (**97**)[144] (12.9 g, 85 %), m.p.: 56–58 ^{o}C (lit.[144] m.p.: 56–59 ^{o}C). This showed ν_{max}/cm^{-1}: 3018, 2926, 2854, 1498, 1342, 1152; δ_H: 7.72–7.58 (5H, m), 3.76–3.72 (2H, m), 1.99–1.93 (2H, m), 1.53–1.47 (2H, m), 1.38–1.26 (36H, m, v.br.), 0.89 (3H, t, *J* 6.9 Hz); δ_C: 153.5, 133.1, 131.4(-), 129.7(-), 125.1(-), 56.0(+), 31.9(+), 29.7(+, v.br.), 29.65(+), 29.62(+), 29.55(+), 29.44(+), 29.35(+), 29.2(+), 28.9(+), 28.14(+), 22.7(+), 21.9(+), 14.1(-).

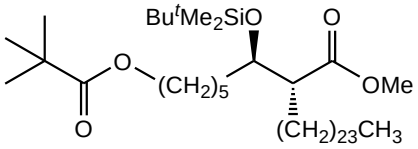
Experiment 111: (*E*/*Z*)-(*R*)-2-[(*R*)-1-(*tert*-Butyl-dimethyl-silanyloxy)-6-(2,2-dimethyl-propionyloxy)-hexyl]-hexacos-4-enoic acid methyl ester (283)



The procedure used in **Experiment 27** was repeated in order to couple the aldehyde (**280**) (2.3 g, 5.35 mmol) with the sulfone (**97**) (3.6 g, 6.95 mmol) using lithium bis(trimethylsilyl) amide (9.6 ml, 10.16 mmol, 1.06M) in dry THF. The crude product was purified by column chromatography eluting with petrol / ether (25:1) to give a colourless oil, *(E/Z)-(R)-2-[(R)-1-(tert-butyl-dimethyl-silanyloxy)-6-(2,2-dimethyl-propionyloxy)-hexyl]-hexacos-4-enoic acid methyl ester* (**283**) (3.21 g, 83 %) as a mixture of two isomers in ratio 2.1:1, {Found $(M + Na)^+$: 745.6399, $C_{44}H_{86}O_5Si$ requires: 745.6413}. This showed ν_{max}/cm^{-1}: 2925, 2854, 1733, 1464, 1363, 1284, 1255, 1158, 1062; δ_H (major *E* isomer): 5.47–5.39 (1H, m), 5.34–5.24 (1H, m), 4.05 (2H, t, *J* 6.6 Hz), 3.97–3.91 (1H, m), 3.64 (3H, s), 2.59–2.55 (1H, m), 2.28–2.14 (2H, m), 1.95 (2H, br.q, *J* 7.0 Hz), 1.66–1.26 (46H, m, v.br.), 1.20 (9H, s), 0.87–0.83 (12H, m, including a s), 0.015 (3H, s), 0.00 (3H, s); δ_H (minor *Z* isomer): 2.03 (2H, br.q, *J* 7.0 Hz), 0.84 (9H, s), 0.022 (3H, s), 0.005 (3H, s) (the remaining signals were obscured by the major isomer); δ_C (two isomers): 179.6, 174.2, 132.9(-), 132.0(-), 126.8(-), 126.2(-), 72.8(-), 72.7(-), 64.3(+), 51.8(-), 51.7(-), 51.3(-), 51.2(-), 38.7, 33.6(+), 33.6(+), 32.5(+), 31.9(+), 30.6(+), 29.7(+, v.br.), 29.65(+), 29.60(+), 29.53(+), 29.49(+),

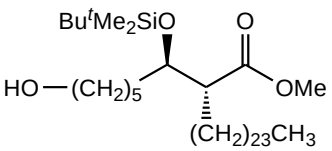
29.36(+), 29.1(+), 28.7(+), 28.7(+), 27.3(+), 27.2(-), 26.2(+), 25.7(-), 25.3(+), 23.8(+), 23.7(+), 22.7(+), 18.0, 14.1(-), - 4.4(-), - 4.9(-).

Experiment 112: (*R*)-2-[(*R*)-1-(*tert*-Butyl-dimethyl-silanyloxy)-6-(2,2-dimethyl-propionyloxy)-hexyl]-hexacosanoic acid methyl ester (284)



The alkene (**283**) (5.8 g, 8.03 mmol) was dissolved in a solution of IMS (150 ml) and ethyl acetate (170 ml) then Pd on C (1 g, 10 %) was added. The mixture was stirred under hydrogen until no more was absorbed. The mixture was filtered on a pad of celite and washed with ethyl acetate (100 ml). The solvent was evaporated and the crude product was purified by column chromatography eluting with petrol / ether (12:1) to give a colourless oil, *(R)-2-[(R)-1-(tert-butyl-dimethyl-silanyloxy)-6-(2,2-dimethyl-propionyloxy)-hexyl]-hexacosanoic acid methyl ester* (**284**) (5.7 g, 98 %), $[\alpha]_D^{26} = -5.0$ (*c* 1.24, $CHCl_3$), {Found $(M + Na)^+$: 747.6273, $C_{44}H_{88}O_5Si$ requires: 747.6293}. This showed ν_{max}/cm^{-1}: 2926, 2854, 1733, 1464, 1284, 1254, 1157; δ_H: 4.05 (2H, t, *J* 6.6 Hz), 3.93–3.89 (1H, m), 3.66 (3H, s), 2.52 (1H, ddd, *J* 3.6, 7.1, 10.9 Hz), 1.64–1.26 (54H, m, v.br.), 1.20 (9H, s), 0.89 (3H, t, *J* 6.9 Hz), 0.87 (9H, s), 0.05 (3H, s), 0.03 (3H, s); δ_C: 178.6, 175.0, 73.1(-), 64.3(+), 51.6(-), 51.2(-), 38.7, 33.6(+), 31.9(+), 29.7(+, v.br.), 29.65(+), 29.60(+), 29.58(+), 29.46(+), 29.35(+), 28.7(+), 27.9(+), 27.4(+), 27.2(-), 26.2(+), 25.7(-), 23.6(+), 22.7(+), 18.0, 14.1(-), - 4.4(-), - 4.9(-).

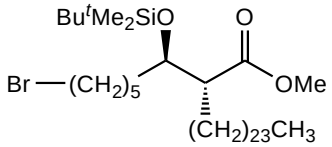
Experiment 113: (*R*)-2-[(*R*)-1-(*tert*-Butyl-dimethyl-silanyloxy)-6-hydroxy-hexyl]-hexacosanoic acid methyl ester (285)



Compound (**284**) (5.6 g, 7.73 mmol) was added to a stirred solution of potassium hydroxide (6.51 g, 116 mmol) dissolved in a mixture of THF : MeOH : H_2O (10:10:1, 315 ml). The mixture was refluxed at 70 ^oC and monitored by TLC. After 3 hrs, the TLC showed no starting material left and the reaction was quenched with water and extracted with ethyl acetate (3 x 300 ml), dried and the solvent was evaporated. The

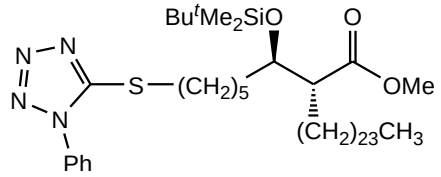
crude product was purified by column chromatography eluting with petrol / ether (2:1 and then 1:1) to give a semi-solid, *(R)-2-[(R)-1-(tert-Butyl-dimethyl-silanyloxy)-6-hydroxy-hexyl]-hexacosanoic acid methyl ester* (**285**) (4.45 g, 90 %), $[\alpha]_D^{26} = -4.9$ (*c* 1.23, $CHCl_3$), {Found $(M + Na)^+$: 663.5638, $C_{39}H_{80}NaO_5Si$ requires: 663.5718}. This showed ν_{max}/cm^{-1}: 3363, 2925, 2854, 1738, 1465, 1254, 1056; δ_H: 3.93–3.89 (1H, m), 3.66 (3H, s), 3.65 (2H, t, *J* 6.7 Hz), 2.53 (1H, ddd, *J* 3.8, 7.0, 10.7 Hz), 1.60–1.26 (54H, m, v.br.), 0.90–0.88 (12H, including a s), 0.05 (3H, s), 0.03 (3H, s); δ_C: 175.0, 73.1(+), 63.0(-), 51.6(+), 51.2(+), 33.7(-), 32.8(-), 31.9(-), 29.7(-, v.br.), 29.65(-), 29.60(-), 29.46(-), 29.35(-), 27.9(-), 27.4(-), 26.0(-), 25.8(+), 23.7(-), 22.7(-), 18.0, 14.1(+), -4.4(+), - 4.9(+) [- = CH_2, + = CH, CH_3].

Experiment 114: (*R*)-2-[(*R*)-6-Bromo-1-(*tert*-butyl-dimethyl-silanyloxy)-hexyl]-hexacosanoic acid methyl ester (286)



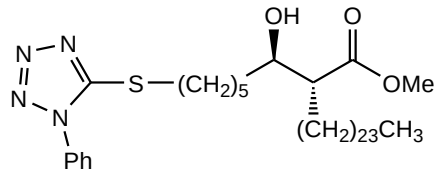
The procedure used in **Experiment 47** was repeated in order to convert the alcohol (**285**) (4.0 g, 6.25 mmol) using NBS (1.41 g, 7.94 mmol) and PPh_3 (1.89 g, 7.19 mmol) in CH_2Cl_2 (110 ml) into a crude product. This was purified by column chromatography eluting with petrol / ether (25:1) to give a colourless oil, *(R)-2-[(R)-6-bromo-1-(tert-butyl-dimethyl-silanyloxy)-hexyl]-hexacosanoic acid methyl ester* (**286**) (3.82 g, 87 %), $[\alpha]_D^{26} = -5.0$ (*c* 1.12, $CHCl_3$), {Found $(M + Na)^+$: 725.4875, $C_{39}H_{79}BrNaO_3Si$ requires: 725.4874}. This showed ν_{max}/cm^{-1}: 2925, 2854, 1741, 1464, 1253, 1167, 1095; δ_H: 3.93–3.91 (1H, m), 3.67 (3H, s), 3.41 (2H, t, *J* 6.9 Hz), 2.53 (1H, ddd, *J* 3.8, 7.0, 10.7 Hz), 1.86 (2H, quintet, *J* 7.0 Hz), 1.58–1.27 (52H, m, v.br.), 0.90 (3H, t, *J* 7.0 Hz), 0.88 (9H, s), 0.06 (3H, s), 0.04 (3H, s); δ_C: 174.9, 73.0(-), 51.5(-), 51.3(-), 33.7(+), 33.5(+), 32.7(+), 31.9(+), 29.7(+, v.br.), 29.65(+), 29.58(+), 29.45(+), 29.36(+), 28.4(+), 27.9(+), 27.4(+), 25.8(-), 23.1(+), 22.7(+), 18.0, 14.1(-), - 4.4(-), - 4.9(-).

Experiment 115: (*R*)-2-[(*R*)-1-(*tert*-Butyl-dimethyl-silanyloxy)-6-(1-phenyl-1*H*-tetrazol-5-ylsulfanyl)-hexyl]-hexacosanoic acid methyl ester (287)



The procedure used in **Experiment 23** was repeated using compound (**286**) (3.65 g, 5.20 mmol), 1-phenyl-1*H*-tetrazole-5-thiol (0.97 g, 5.46 mmol), anhydrous potassium carbonate (1.51 g, 10.92 mmol) and acetone (110 ml). The crude product was purified by column chromatography eluting with petrol / ether (4:1) to give a colourless oil, *(R)-2-[(R)-1-(tert-butyl-dimethyl-silanyloxy)-6-(1-phenyl-1H-tetrazol-5-ylsulfanyl)-hexyl]-hexacosanoic acid methyl ester* (**287**) (4.03 g, 97 %), $[\alpha]_D^{23} = -4.5$ (*c* 0.84, $CHCl_3$), {Found $(M + H)^+$: 801.6092, $C_{46}H_{85}N_4O_3SSi$ requires: 801.6106}. This showed ν_{max}/cm^{-1}: 2925, 2854, 1739, 1599, 1501, 1464, 1251, 1168, 1090; δ_H: 7.55–7.53 (5H, m), 3.92–3.89 (1H, m), 3.66 (3H, s), 3.40 (2H, t, *J* 7.3 Hz), 2.52 (1H, ddd, *J* 3.8, 6.9, 10.7 Hz), 1.84 (2H, quintet, *J* 7.3 Hz), 1.60–1.24 (52H, m, v.br.), 0.90 (3H, t, *J* 6.6 Hz), 0.86 (9H, s), 0.04 (3H, s), 0.03 (3H, s); δ_C: 174.9, 154.4, 133.8, 130.0(-), 129.8(-), 123.9(-), 73.0(-), 51.6(-), 51.3(-), 33.5(+), 33.3(+), 31.9(+), 29.7(+, v.br.), 29.65(+), 29.61(+), 29.57(+), 29.46(+), 29.35(+), 29.1(+), 28.9(+), 27.9(+), 27.3(+), 25.8(-), 23.5(+), 22.7(+), 18.0, 14.1(-), - 4.4(-), - 4.9(-).

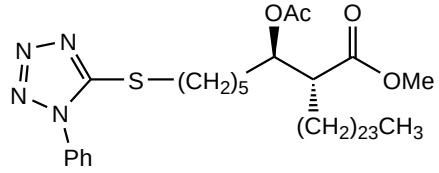
Experiment 116: (*R*)-2-[(*R*)-1-Hydroxy-6-(1-phenyl-1*H*-tetrazol-5-ylsulfanyl)-hexyl]-hexacosanoic acid methyl ester (288)



Compound (**287**) (1 g, 1.25 mmol) was dissolved with THF (75 ml) and a mixture of acetic acid (25 ml), water (13 ml), THF (20 ml) and 2 N HCl (17 ml) was added at r.t. and the mixture was stirred for 18 hrs. The mixture was cooled to 8 ^oC and ethyl acetate (20 ml) and then sat.aq. $NaHCO_3$ were added slowly until the acid was neutralised. Brine (20 ml) and ethyl acetate (30 ml) was added and extracted. The aqueous layer re-extracted with ethyl acetate (2 x 25 ml) and the combined organic layers were dried and the solvent was evaporated. The crude product was purified by

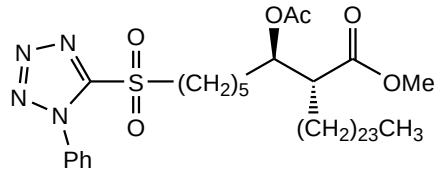
column chromatography eluting with petrol / ether (2:1, then 1:2) to give a white solid, *(R)-2-[(R)-1-hydroxy-6-(1-phenyl-1H-tetrazol-5-ylsulfanyl)-hexyl]-hexacosanoic acid methyl ester* (**288**) (0.645 g, 75 %), m.p.: 69–70 ^{o}C, $[\alpha]_{D}^{26}$ = + 6.4 (*c* 0.78, $CHCl_3$), {Found $(M + H)^+$: 687.5211, $C_{40}H_{71}N_4O_3S$ requires: 687.5241}. This showed ν_{max}/cm^{-1}: 3505, 2918, 2849, 1713, 1597, 1500, 1463, 1390, 1241, 1170, 1142, 1071; δ_H: 7.61–7.54 (5H, m), 3.72 (3H, s), 3.65 (1H, m), 3.40 (2H, t, *J* 7.4 Hz), 2.43 (1H, dt, *J* 5.4, 8.9 Hz), 1.85 (2H, quintet, *J* 7.3 Hz), 1.75–1.62 (52H, m, v.br.), 0.89 (3H, t, *J* 6.9 Hz); δ_C: 176.2, 154.4, 133.8, 130.1(-), 129.8(-), 123.9(-), 72.1(-), 51.6(-), 51.0(-), 35.5(+), 33.2(+), 31.9(+), 29.7(+, v.br.), 29.67(+), 29.65(+), 29.63(+), 29.57(+), 29.51(+), 29.43(+), 29.35(+), 29.1(+), 28.5(+), 27.4(+), 25.2(+), 22.7(+), 14.1(-).

Experiment 117: (*R*)-2-[(*R*)-1-Acetoxy-6-(1-phenyl-1*H*-tetrazol-5-ylsulfanyl)-hexyl]-hexacosanoic acid methyl ester (289)



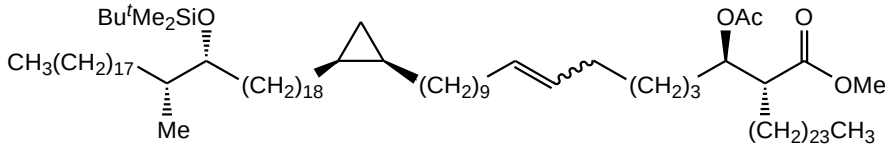
A mixture of acetic anhydride (30 ml) and anhydrous pyridine (30 ml) was added to stirred solution of the alcohol (**288**) (1.95 g, 2.84 mmol) in dry toluene (40 ml) at r.t. and the mixture was stirred for 18 hrs, then diluted with toluene (30 ml) and the solvent evaporated under reduced to give a solid. This was purified by column chromatography eluting with petrol / ether (3:1, then 2:1) to give a white solid, *(R)-2-[(R)-1-acetoxy-6-(1-phenyl-1H-tetrazol-5-ylsulfanyl)-hexyl]-hexacosanoic acid methyl ester* (**289**) (2.03 g, 98 %), m.p.: 51–52 ^{o}C, $[\alpha]_{D}^{26}$ = + 7.3 (*c* 0.78, $CHCl_3$), {Found $(M + H)^+$: 729.5380, $C_{42}H_{73}N_4O_4S$ requires: 729.5347}. This showed ν_{max}/cm^{-1}: 2918, 2849, 1742, 1597, 1500, 1464, 1384, 1244, 1169; δ_H: 7.60–7.54 (5H, m), 5.08 (1H, ddd, *J* 3.8, 6.8, 8.4 Hz), 3.68 (3H, s), 3.39 (2H, t, *J* 7.4 Hz), 2.61 (1H, ddd, *J* 4.4, 6.8, 10.7 Hz), 2.03 (3H, s), 1.86–1.80 (2H, m), 1.66–1.21 (52H, m, v.br.), 0.89 (3H, t, *J* 6.9 Hz); δ_C: 173.5, 170.3, 154.4, 133.7, 130.1(-), 129.8(-), 123.8(-), 73.8(-), 51.6(-), 49.6(-), 33.1(+), 31.9(+), 31.5(+), 29.7(+, v.br.), 29.64(+), 29.62(+), 29.54(+), 29.46(+), 29.4(+), 29.3(+), 29.0(+), 28.4(+), 28.1(+), 27.5(+), 24.6(+), 22.7(+), 21.0(-), 14.1(-).

Experiment 118: (*R*)-2-[(*R*)-1-Acetoxy-6-(1-phenyl-1*H*-tetrazole-5-sulfonyl)-hexyl]-hexacosanoic acid methyl ester (252)



The procedure used in **Experiment 25** was repeated using the sulfane (**289**) (2.0 g, 2.75 mmol), ammonium molybdate (VI) tetrahydrate (1.54 g, 1.25 mmol) in 35 % H_2O_2 (3.5 ml) and THF (40 ml) and IMS (80 ml), and further ammonium molybdate (VI) tetrahydrate (0.6 g, 0.49 mmol) in 35% H_2O_2 (1.5 ml). The crude product was purified by column chromatography eluting with petrol / ether (1:1) to give a white solid, *(R)-2-[(R)-1-acetoxy-6-(1-phenyl-1H-tetrazole-5-sulfonyl)-hexyl]-hexacosanoic acid methyl ester* (**252**) (2.02 g, 97 %), m.p.: 69–70 ^{o}C, $[\alpha]_D^{26}$ = + 9.0 (*c* 0.9, $CHCl_3$), {Found $(M + Na)^+$: 783.5039, $C_{42}H_{72}N_4NaO_6S$ requires: 783.5065}. This showed ν_{max}/cm^{-1}: 2917, 2849, 1742, 1595, 1463, 1331, 1244, 1159, 1059; δ_H : 7.71–7.58 (5H, m), 5.08 (1H, ddd, *J* 3.8, 6.8, 8.4 Hz), 3.75–3.72 (2H, m), 3.69 (3H, s), 2.61 (1H, ddd, *J* 4.3, 6.8, 10.7 Hz), 2.04 (3H, s), 1.98–1.95 (2H, m), 1.66–1.26 (52H, m, v.br.), 0.89 (3H, t, *J* 7.1 Hz); δ_C: 173.4, 170.3, 153.4, 133.0, 131.5 (-), 129.7 (-), 125.1 (-), 73.6 (-), 55.8 (+), 51.6 (-), 49.6 (-), 31.9 (+), 31.3 (+), 29.7 (+, v.br.), 29.65 (+), 29.62 (+), 29.56 (+), 29.48 (+), 29.41 (+), 29.35 (+), 28.1 (+), 27.9 (+), 27.5 (+), 24.5 (+), 22.7 (+), 22.0 (+), 21.0 (-), 14.1 (-).

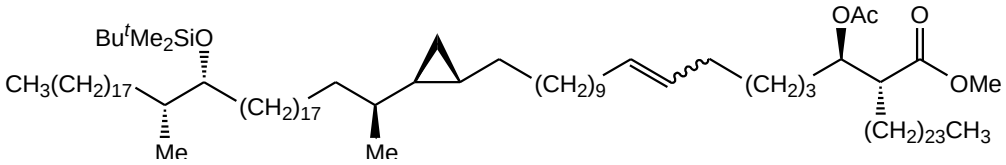
Experiment 119: (*R*)-2-((*E*/Z)-(*R*)-1-Acetoxy-16-{(1*R*,2*S*)-2-[(19*R*,20*R*)-19-(*tert*-butyl-dimethyl-silanyloxy)-20-methyl-octatriacontyl]-cyclopropyl}-hexadec-6-enyl)-hexacosanoic acid methyl ester (290)



The procedure used in **Experiment 27** was repeated in order to couple the aldehyde (**250**) (0.5 g, 0.57 mmol) with the sulfone (**252**) (0.54 g, 0.72 mmol) using lithium bis(trimethylsilyl) amide (1.05 ml, 1.09 mmol, 1.06M) in dry THF. The crude product was purified by column chromatography eluting with petrol / ether (18:1) to give a colourless oil, *(R)-2-((E/Z)-(R)-1-acetoxy-16-{(1R,2S)-2-[(19R,20R)-19-(tert-butyl-dimethyl-silanyloxy)-20-methyl-octatriacontyl]-cyclopropyl}-hexadec-6-enyl)-*

hexacosanoic acid methyl ester (**290**) (0.45 g, 56 %) as a mixture of two isomers in ratio 2:1, {Found $(M + Na)^+$: 1430.3699, $C_{93}H_{182}NaO_5Si$ requires: 1430.3649}. This showed ν_{max}/cm^{-1}: 2924, 2853, 1747, 1465, 1372, 1236, 1022; δ_H (two isomers): 5.41–5.33 (2H, m), 5.11–5.07 (1H, m), 3.69 (3H, s), 3.52–3.48 (1H, m), 2.62 (1H, ddd, *J* 4.2, 6.8, 10.7 Hz), 2.03 (3H, s), 2.04–1.95 (4H, m), 1.64–1.11 (138H, m, v.br.), 1.08–1.01 (1H, m), 0.90–0.88 (15H, m, including a s), 0.80 (3H, d, *J* 7.0 Hz), 0.68–0.63 (2H, m), 0.57 (1H, br.dt, *J* 4.1, 8.2 Hz), 0.04 (3H, s), 0.03 (3H, s), - 0.32 (1H, br.q, *J* 5.2 Hz); δ_C (two isomers): 173.6, 173.6, 170.3, 130.8(-), 130.3(-), 129.7(-), 129.3(-), 75.9(-), 74.1(-), 51.5(-), 49.6(-), 37.7(-), 33.5(+), 32.6(+), 32.5(+), 32.3(+), 31.9(+), 31.6(+), 31.5(+), 30.2(+), 30.0(+), 29.9(+), 29.7(+), 29.7(+, v.br.), 29.64(+), 29.61(+), 29.57(+), 29.48(+), 29.41(+), 29.36(+), 29.2(+), 28.7(+), 28.1(+), 27.7(+), 27.5(+), 27.3(+), 27.0(+), 26.0(-), 25.9(+), 24.6(+), 24.4(+), 22.7(+), 21.0(-), 18.2, 15.8(-), 14.4(-), 14.1(-), 10.9(+), - 4.2(-), - 4.4(-).

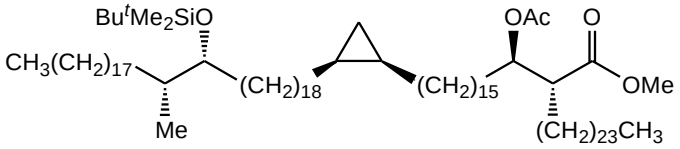
Experiment 120: (*R*)-2-((*E*/*Z*)-(*R*)-1-Acetoxy-17-{(1*S*,2*R*)-2-[(1*S*,20*R*,21*R*)-20-(*tert*-butyl-dimethyl-silanyloxy)-1,21-dimethyl-nonatriacontyl]-cyclopropyl}-heptadec-6-enyl)-hexacosanoic acid methyl ester (294)



The procedure used in **Experiment 27** was repeated in order to couple the aldehyde (**251**) (1.6 g, 1.75 mmol) with the sulfone (**252**) (1.5 g, 1.96 mmol) using lithium bis(trimethylsilyl) amide (3.2 ml, 3.33 mmol, 1.06M) in dry THF. The crude product was purified by column chromatography eluting with petrol / ether (15:1) to give a colourless oil, *(R)-2-((E/Z)-(R)-1-acetoxy-17-{(1S,2R)-2-[(1S,20R,21R)-20-(tert-butyl-dimethyl-silanyloxy)-1,21-dimethyl-nonatriacontyl]-cyclopropyl}-heptadec-6-enyl)-hexacosanoic acid methyl ester* (**294**) (1.32 g, 52 %) as a mixture of two isomers in ratio 2:1, {Found $(M + Na)^+$:1472.23, $C_{96}H_{188}NaO_5Si$ requires: 1472.41}. This showed ν_{max}/cm^{-1}: 2923, 2853, 1746, 1465, 1372, 1236, 1022; δ_H (two isomers): 5.42–5.29 (2H, m), 5.11–5.07 (1H, m), 3.68 (3H, s), 3.52–3.48 (1H, m), 2.62 (1H, ddd, *J* 4.2, 6.9, 10.7 Hz), 2.03 (3H, s), 2.04–1.94 (4H, m), 1.64–1.14 (140H, m, v.br.), 1.08–1.01 (1H, m), 0.91–0.86 (18H, m, including a s), 0.80 (3H, d, *J* 7.0 Hz), 0.71–0.64 (1H, m), 0.48–0.42 (1H, m), 0.22–0.09 (3H, m), 0.04 (3H, s), 0.03 (3H, s); δ_C (two isomers): 173.6,

170.3, 130.8(-), 130.3(-), 129.7(-), 129.3(-), 75.9(-), 74.0(-), 51.5(-), 49.6(-), 38.1(-), 37.7(-), 37.4(+), 34.5(+), 33.5(+), 32.6(+), 32.5(+), 32.3(+), 31.9(+), 31.6(+), 31.5(+), 30.1(+), 30.0(+), 29.9(+), 29.73(+), 29.70(+, v.br.), 29.66(+), 29.57(+), 29.48(+), 29.41(+), 29.36(+), 29.3(+), 28.1(+), 27.7(+), 27.5(+), 27.3(+), 27.0(+), 26.1(+), 26.0(-), 25.9(+), 24.4(+), 22.7(+), 21.0(-), 19.7(-), 18.6(-), 18.2, 14.4(-), 14.1(-), 10.5(+), -4.2(-), - 4.4(-).

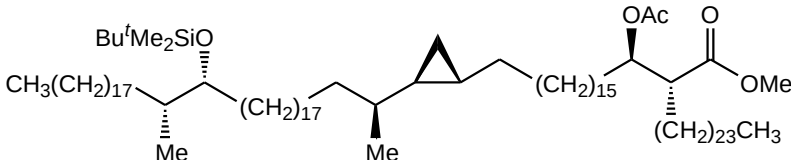
Experiment 121: (*R*)-2-((*R*)-1-Acetoxy-16-{(1*R*,2*S*)-2-[(19*R*,20*R*)-19-(*tert*-butyl-dimethyl-silanyloxy)-20-methyl-octatriacontyl]-cyclopropyl}-hexadecyl)-hexacosanoic acid methyl ester (291)



Dipotassium azodicarboxylate (2 g, 10.3 mmol) was added to a stirred solution of the alkene (**290**) (400 mg, 0.28 mmol) in THF (20 ml) and methanol (4 ml) at 5 ^{o}C. A solution of glacial acetic acid (2.5 ml) an THF (2.5 ml) was prepared and half of this solution was added at 5 ^{o}C by dropwise and the mixture was stirred at r.t. for 2 hrs. The other half of the glacial acetic acid solution was added at r.t. and the mixture was stirred at r.t for overnight. Dipotassium azodicarboxylate (1.5 g) and glacial acetic acid (2 ml) were added and stirred overnight. This mixture was added to sat. aq. NH_4Cl slowly and extracted with petrol / ether (1:1, 3 x 80 ml,) and the combined organic layers were washed with water (50 ml) and the solvent was evaporated. The procedure was repeated. The crude product was purified by column chromatography eluting with petrol / ether (15:1) to give a white solid, *(R)-2-((R)-1-acetoxy-16-{(1R,2S)-2-[(19R,20R)-19-(tert-butyl-dimethyl-silanyloxy)-20-methyl-octatriacontyl]-cyclopropyl}-hexadecyl)-hexacosanoic acid methyl ester* (**291**) (350 mg, 88 %), m.p.: 29–30 ^{o}C, $[\alpha]_D^{24}$ = + 7.9 (*c* 0.79, $CHCl_3$), {Found $(M + Na)^+$: 1432.3752, $C_{93}H_{184}NaO_5Si$ requires: 1432.3805}. This showed ν_{max}/cm^{-1}: 2924, 2853, 1747, 1465, 1372, 1236, 1164, 1022; δ_H: 5.10 (1H, dt, *J* 3.8, 7.9 Hz), 3.69 (3H, s), 3.52–3.49 (1H, m), 2.63 (1H, ddd, *J* 4.2, 6.8, 10.7 Hz), 2.04 (3H, s), 1.68–1.14 (146H, m, v.br.), 1.07–1.02 (1H, m), 0.91–0.88 (15H, m, including a s), 0.80 (3H, d, *J* 6.6 Hz), 0.69–0.65 (2H, m), 0.57 (1H, br.dt, *J* 4.1, 8.2 Hz), 0.04 (3H, s), 0.03 (3H, s), - 0.32 (1H, br.q, *J* 5.4 Hz); δ_C: 173.6, 170.3, 75.9(-), 74.1(-), 51.5(-), 49.6(-), 37.7(-), 33.6(+), 32.5(+), 31.9(+),

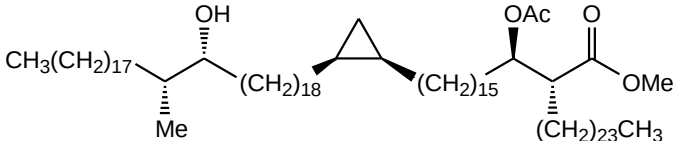
31.7(+), 30.2(+), 30.0(+), 29.9(+), 29.7(+, v.br.), 29.7(+), 29.6(+), 29.5(+), 29.4(+), 29.4(+), 29.4(+), 28.7(+), 28.1(+), 27.7(+), 27.5(+), 26.0(-), 25.9(+), 25.0(+), 22.7(+), 21.0(-), 18.2, 15.8(-), 14.4(-), 14.1(-), 10.9(+), - 4.2(-), - 4.4(-).

Experiment 122: (*R*)-2-((*R*)-1-Acetoxy-17-{(1*S*,2*R*)-2-[(1*S*,20*R*,21*R*)-20-(*tert*-butyl-dimethyl-silanyloxy)-1,21-dimethyl-nonatriacontyl]-cyclopropyl}-heptadecyl)-hexacosanoic acid methyl ester (295)



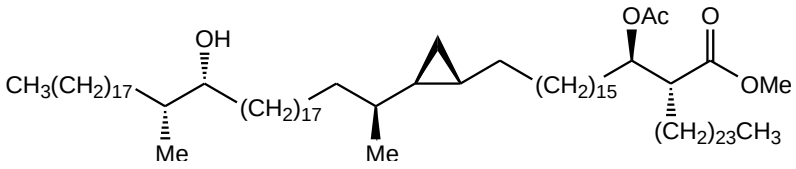
The procedure used in **Experiment 121** was repeated in order to hydrogenate the alkene (**294**) (1.1 mg, 0.76 mmol) using dipotassium azodicarboxylate (4.45 g, 22.94 mmol) in THF (25 ml), methanol (5 ml) a solution of glacial acetic acid (6 ml) to a white solid, *(R)-2-((R)-1-Acetoxy-17-{(1S,2R)-2-[(1S,20R,21R)-20-(tert-butyl-dimethyl-silanyloxy)-1,21-dimethyl-nonatriacontyl]-cyclopropyl}-heptadecyl)-hexacosanoic acid methyl ester* (**295**) (1.0 mg, 91 %), m.p.: 30–31 ^{o}C, $[\alpha]_{D}^{24}$ = + 8.7 (*c* 0.91, $CHCl_3$), {Found $(M + Na)^+$: 1474.29, $C_{96}H_{190}NaO_5Si$ requires: 1474.43}. This showed ν_{max}/cm^{-1}: 2925, 2853, 1746, 1465, 1372, 1248, 1023; δ_H: 5.10 (1H, ddd, *J* 4.2, 6.9, 8.1 Hz), 3.69 (3H, s), 3.52–3.49 (1H, m), 2.62 (1H, ddd, *J* 4.2, 7.0, 10.7 Hz), 2.04 (3H, s), 1.68–1.16 (148H, m, v.br.), 1.09–1.02 (1H, m), 0.91–0.88 (18H, m, including a s), 0.80 (3H, d, *J* 6.6 Hz), 0.70–0.65 (1H, m), 0.49–0.43 (1H, m), 0.23–0.09 (3H, m), 0.04 (3H, s), 0.03 (3H, s); δ_C: 173.6, 170.3, 75.9(-), 74.1(-), 51.5(-), 49.6(-), 38.1(-), 37.8(-), 37.4(+), 34.5(+), 33.6(+), 32.5(+), 31.9(+), 31.7(+), 30.1(+), 30.0(+), 29.9(+), 29.74(+), 29.72(+, v.br.), 29.67(+), 29.64(+), 29.59(+), 29.57(+), 29.49(+), 29.47(+), 29.45(+), 29.41(+), 29.37(+), 28.1(+), 27.7(+), 27.5(+), 27.3(+), 26.2(-), 26.0(-), 25.9(+), 25.0(+), 22.7(+), 21.0(-), 19.7(-), 18.6(-), 18.2, 14.4(-), 14.1(-), 10.5(+), - 4.2(-), - 4.4(-).

Experiment 123: (*R*)-2-{(*R*)-1-Acetoxy-16-[(1*R*,2*S*)-2-((19*R*,20*R*)-19-hydroxy-20-methyl-octatriacontyl)-cyclopropyl]-hexadecyl}-hexacosanoic acid methyl ester (292)



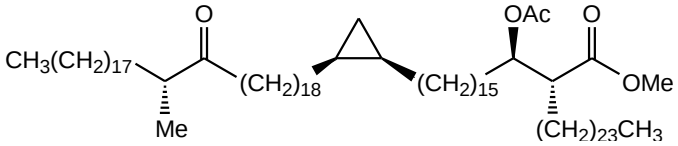
The silyl ether (**291**) (300 mg, 0.21 mmol) was dissolved in dry THF (15 ml) in a dry polyethylene vial under argon at r.t. and stirred. Pyridine (0.3 ml) and HF.pyridine (1.2 ml) were added and the mixture was stirred for 17 hrs. at 40 ^{o}C TLC showed the reaction was complete. The reaction was diluted with petrol / ether (1:1, 70 ml,) and neutralized with sat.aq. $NaHCO_3$ until no more carbon dioxide was liberated. The mixture was extracted and the aqueous layer was re-extracted with petrol / ether (1:1, 2 x 50 ml,). The combined organic layers were washed with brine and dried. The solvent was evaporated and the crude product was purified by column chromatography eluting with petrol / ether (4:1) to give a white solid, *(R)-2-{(R)-1-acetoxy-16-[(1R,2S)-2-((19R,20R)-19-hydroxy-20-methyl-octatriacontyl)-cyclopropyl]-hexadecyl}-hexacosanoic acid methyl ester* (**292**) (260 mg, 94 %), m.p.: 47–48 ^{o}C, $[\alpha]_D^{21}$ = + 9.3 (*c* 0.95, $CHCl_3$), {Found $(M + Na)^+$: 1318.3003, $C_{87}H_{170}NaO_5$ requires: 1318.2940}. This showed ν_{max}/cm^{-1}: 3449, 2918, 2850, 1743, 1470, 1374, 1238, 1022; δ_H: 5.08 (1H, br.dt, *J* 3.8, 7.9 Hz), 3.69 (3H, s), 3.51–3.48 (1H, m), 2.62 (1H, ddd, *J* 4.3, 6.8, 10.7 Hz), 2.04 (3H, s), 1.66–1.12 (147H, m, v.br.), 0.90 (3H, t, *J* 6.9 Hz), 0.86 (3H, d, *J* 7.0 Hz), 0.67–0.64 (2H, m), 0.57 (1H, br.dt, *J* 4.1, 8.2 Hz), - 0.32 (1H, br.q, *J* 5.4 Hz); δ_C: 173.7, 170.3, 75.2(-), 74.1(-), 51.5(-), 49.6(-), 38.2(-), 34.5(+), 33.4(+), 31.9(+), 31.7(+), 30.2(+), 27.0(+), 29.8(+), 29.7(+, v.br.), 29.66(+), 29.57(+), 29.47(+), 29.44(+), 29.40(+), 29.36(+), 28.7(+), 28.1(+), 27.5(+), 27.4(+), 26.3(+), 25.0(+), 22.7(+), 21.0(-), 15.8(-), 14.1(-), 13.6(-), 10.9(+).

Experiment 124: (*R*)-2-{(*R*)-1-Acetoxy-17-[(1*S*,2*R*)-2-((1*S*,20*R*,21*R*)-20-hydroxy-1,21-dimethyl-nonatriacontyl)-cyclopropyl]-heptadecyl}-hexacosanoic acid methyl ester (296)



The procedure used in **Experiment 123** was repeated in order to desilylate the silyl ether (**295**) (800 mg, 0.55 mmol) using HF.pyridine (2 ml) and pyridine (0.5 ml) in THF (20 ml) to give a white solid, *(R)-2-{(R)-1-acetoxy-17-[(1S,2R)-2-((1S,20R,21R)-20-hydroxy-1,21-dimethyl-nonatriacontyl)-cyclopropyl]-heptadecyl}-hexacosanoic acid methyl ester* (**296**) (680 mg, 92 %), m.p.: 47–48 ^{o}C, $[\alpha]_{D}^{21}$ = + 10.0 (*c* 0.83, $CHCl_3$), {Found $(M + Na)^+$: 1360.22, $C_{90}H_{176}O_5$ requires: 1360.34}. This showed ν_{max}/cm^{-1}: 3489, 2850, 1739, 1470, 1375, 1240, 1176, 1021; δ_H: 5.09 (1H, ddd, *J* 4.4, 6.9, 8.2 Hz), 3.68 (3H, s), 3.52–3.49 (1H, m), 2.62 (1H, ddd, *J* 4.4, 6.9, 10.7 Hz), 2.04 (3H, s), 1.67–1.14 (150H, m, v.br.), 0.90 (3H, d, *J* 6.6 Hz), 0.89 (3H, t, *J* 6.9 Hz), 0.86 (3H, d, *J* 6.9 Hz), 0.71–0.64 (1H, m), 0.48–0.42 (1H, m), 0.22–0.09 (3H, m); δ_C: 173.6, 170.3, 75.2(-), 74.1(-), 51.5(-), 49.6(-), 38.2(-), 38.1(-), 37.4(+), 34.51(+), 34.49(+), 33.4(+), 31.9(+), 31.7(+), 30.1(+), 30.0(+), 29.72(+), 29.70(+, v.br.), 29.65(+), 29.56(+), 29 46(+), 29 44(+), 29.39(+), 29.36(+), 28.1(+), 27.5(+), 27.4(+), 27.3(+), 26.3(+), 16.1(-), 25.0(+), 22.7(+), 21.0(-), 19.7(-), 18.6(-), 14.1(-), 13.6(-), 10.5(+).

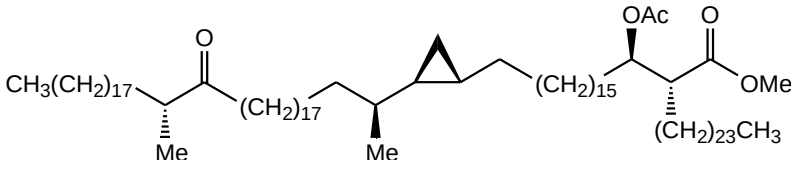
Experiment 125: (*R*)-2-{(*R*)-1-Acetoxy-16-[(1*R*,2*S*)-2-((*R*)-20-methyl-19-oxo-octatriacontyl)-cyclopropyl]-hexadecyl}-hexacosanoic acid methyl ester (293)



The alcohol (**292**) (150 mg, 0.12 mmol) was dissolved in CH_2Cl_2 (5 ml) and added to a stirred solution of PCC (62.5 mg, 0.29 mmol) in CH_2Cl_2 (25 ml) at r.t. Addition was done portionwise and during the addition a black colour appeared. The mixture was stirred for 3 hrs at r.t. TLC showed complete reaction. The mixture was diluted with ether (40 ml) and filtered through a bed of silica. The solvent was evaporated and the crude product was purified by column chromatography eluting with petrol /ether (6:1) to give a white solid, *(R)-2-{(R)-1-acetoxy-16-[(1R,2S)-2-((R)-20-methyl-19-oxo-*

octatriacontyl)-cyclopropyl]-hexadecyl}-hexacosanoic acid methyl ester (**293**) (150 mg, 100 %), m.p.: 49–50 ^{o}C, $[\alpha]_{D}^{20}$ = + 3.0 (*c* 0.7, $CHCl_3$), {Found $(M + Na)^+$: 1316.2779, $C_{87}H_{168}NaO_5$ requires: 1316.2784}. This showed ν_{max}/cm^{-1}: 2919, 2850, 1740, 1708, 1471, 1376, 1241, 1166, 1020; δ_H: 5.09 (1H, dt, *J* 3.8, 7.9 Hz), 3.69 (3H, s), 2.62 (1H, ddd, *J* 4.4, 6.9, 10.7 Hz), 2.50 (1H, sext, *J* 6.8 Hz), 2.43 (1H, dt, *J* 14.7, 7.3 Hz), 2.40 (1H, dt, *J* 14.7, 7.3 Hz), 2.03 (3H, s), 1.68–1.14 (144H, m, v.br.), 1.05 (3H, d, *J* 7.0 Hz), 0.89 (6H, t, *J* 7.0 Hz), 0.68–0.64 (2H, m), 0.57 (1H, br.dt, *J* 4.1, 8.2 Hz), - 0.32 (1H, br.q, *J* 5.0 Hz); δ_C: 215.1, 173.6, 170.3, 74.1(-), 51.5(-), 49.6(-), 46.3(-), 41.1(+), 33.1(+), 31.9(+), 31.7(+), 30.2(+), 29.8(+), 29.7(+, v.br.), 29.65(+), 29.63(+), 29.60(+), 29.56(+), 29.51(+), 29.49(+), 29.46(+), 29.44(+), 29.40(+), 29.36(+), 28 7(+), 28 1(+), 27.5(+), 27.3(+), 25.0(+), 23.7(+), 22.7(+), 21.0(-), 16.4(-), 15.8(-), 14.1(-), 10.9(+).

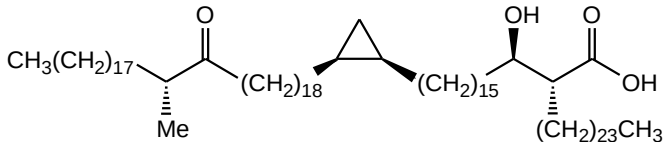
Experiment 126: (*R*)-2-{(*R*)-1-Acetoxy-17-[(1*S*,2*R*)-2-((1*S*,21*R*)-1,21-dimethyl-20-oxo-nonatriacontyl)-cyclopropyl]-heptadecyl}-hexacosanoic acid methyl ester (297)



The procedure used in **Experiment 125** was repeated in order to oxidise the alcohol (**296**) (450 mg, 0.34 mmol) using PCC (0.18 g, 0.84 mmol) in CH_2Cl_2 (40 ml) to a white solid, *(R)-2-{(R)-1-acetoxy-17-[(1S,2R)-2-((1S,21R)-1,21-dimethyl-20-oxo-nonatriacontyl)-cyclopropyl]-heptadecyl}-hexacosanoic acid methyl ester* (**297**) (450 mg, 100 %), m.p.: 48–49 ^{o}C, $[\alpha]_{D}^{20}$ = + 3.1 (*c* 0.96, $CHCl_3$), {Found $(M + Na)^+$: 1358.20, $C_{90}H_{174}NaO_5$ requires: 1358.33}. This showed ν_{max}/cm^{-1}: 2919, 2850, 1737, 1703, 1471, 1375, 1241, 1162; δ_H: 5.10 (1H, br.dt, *J* 4.0, 8.1 Hz), 3.69 (3H, s), 2.62 (1H, ddd, *J* 4.4, 7.0, 10.7 Hz), 2.51 (1H, sext, *J* 6.7 Hz), 2.43 (1H, dt, *J* 14.6, 7.6 Hz), 2.40 (1H, dt, *J* 14.6, 7.6 Hz), 2.03 (3H, s), 1.65–1.17 (146H, m, v.br.), 1.05 (3H, d, *J* 7.0 Hz), 0.90 (3H, d, *J* 7.0 Hz), 0.89 (6H, t, *J* 6.8 Hz), 0.70–0.65 (1H, m), 0.48–0.42 (1H, m), 0.22–0.09 (3H, m); δ_C: 215.1, 173.6, 170.3, 74.1(-), 51.5(-), 49.6(-), 46.3(-), 41.1(+), 38.1(-), 37.4(+), 34.5(+), 33.0(+), 31.9(+), 31.7(+), 30.1(+), 29.72(+), 29.70(+, v.br.), 29.67(+), 29.65(+), 29.60(+), 29.57(+), 29.55(+), 29.51(+), 29.48(+), 29.46(+),

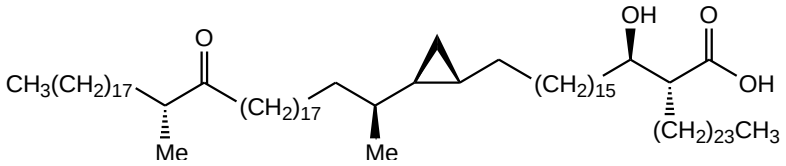
29.44(+), 29.39(+), 29.35(+), 28.1(+), 27.5(+), 27.4(+), 27.3(+), 26.1(-), 25.0(+), 23.7(+), 22.7(+), 21.0(-), 19.7(-), 18.6(-), 16.4(-), 14.1(-), 10.5(+).

Experiment 127: (*R*)-2-{(*R*)-1-Hydroxy-16-[(1*R*,2*S*)-2-((*R*)-20-methyl-19-oxo-octatriacontyl)-cyclopropyl]-hexadecyl}-hexacosanoic acid (116)



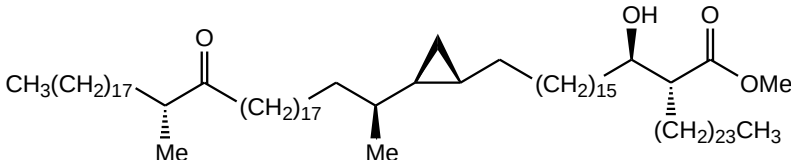
Lithium hydroxide monohydrate (68 mg, 1.62 mmol) was added to a stirred solution of the acetyl protected methyl ester (**293**) (70 mg, 0.054 mmol) in THF (12 ml), methanol (1.2 ml) and water (1 ml) at r.t. The mixture was stirred at 45 ^{o}C for 18 hrs, when TLC showed a small amount of starting material was left. It was cooled to r.t. and a mixture of petrol / ether (1:1, 10 ml) and then sat aq. NH_4Cl (10 ml) was added and the mixture was acidified to pH = 1 with 5 % HCl by dropwise. Further petrol / ether (1:1, 20 ml) was added and extracted. The aq. layer was re-extracted with petrol / ether (1:1, 2 x 20 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 (7:2) to give a white solid, *(R)-2-{(R)-1-hydroxy-16-[(1R,2S)-2-((R)-20-methyl-19-oxo-octatriacontyl)-cyclopropyl]-hexadecyl}-hexacosanoic acid* (**116**) (54 mg, 81 %). m.p.: 70–72 ^{o}C; $[\alpha]_D^{26}$ = + 4.4 (c 1.02, $CHCl_3$); {Found $(M + Na)^+$: 1260.12, $C_{84}H_{164}NaO_4$ requires: 1260.25; Found: C, 81.94; H, 13.24, $C_{84}H_{164}O_4$ requires: C, 81.48; H, 13.35}. This showed ν_{max}/cm^{-1}: 3285, 2919, 2850, 1707, 1470, 1377, 1204, 1019; δ_H: 3.74–3.70 (1H, m), 2.51 (1H, sext, *J* 6.9 Hz), 2.46 (1H, dt, *J* 8.8, 5.4 Hz), 2.43 (1H, dt, *J* 14.8, 7.3 Hz), 2.40 (1H, dt, *J* 14.8, 7.3 Hz), 1.75–1.12 (144H, m, v.br.), 1.05 (3H, d, *J* 7.0 Hz), 0.89 (6H, t, *J* 7.0 Hz), 0.69–0.64 (2H, m), 0.57 (1H, br.dt, *J* 4.1, 8.2 Hz), - 0.32 (1H, br.q, *J* 5.4 Hz); δ_C: 215.4, 179.6, 72.1(-), 50.8(-), 46.3(-), 41.1(+), 35.5(+), 33.0(+), 31.9(+), 30.2(+), 29.7(+, v.br.), 29.66(+), 29.60(+), 29.52(+), 29.50(+), 29.46(+), 29.43(+), 29.36(+), 29.33(+), 28.7(+), 27.3(+), 25.7(+), 23.7(+), 22.7(+), 16.4(-), 15.8(-), 14.1(-), 10.9(+).

Experiment 128: (*R*)-2-{(*R*)-17-[(1*S*,2*R*)-2-((1*S*,21*R*)-1,21-Dimethyl-20-oxo-nonatriacontyl)-cyclopropyl]-1-hydroxy-heptadecyl}-hexacosanoic acid (117)



The procedure used in **Experiment 127** was repeated in order to hydrolyse the keto-acetyl protected methyl ester (**297**) (200 mg, 0.15 mmol) using lithium hydroxide monohydrate (190 mg, 4.49 mmol) in THF (20 ml), methanol (2 ml) and water (1.7 ml) to a white solid, *(R)-2-{(R)-17-[(1S,2R)-2-((1S,21R)-1,21-dimethyl-20-oxo-nonatriacontyl)-cyclopropyl]-1-hydroxy-heptadecyl}-hexacosanoic acid* (**117**) (158 mg, 83 %), m.p.: 70–71 ^{o}C, $[\alpha]_{D}^{26}$ = + 5.3 (*c* 0.96, $CHCl_3$), {Found $(M + Na)^+$: 1302.18, $C_{87}H_{170}NaO_4$ requires: 1302.30; Found: C, 81.97; H, 13.31, $C_{87}H_{170}O_4$ requires: C, 81.62; H, 13.38}. This showed ν_{max}/cm^{-1}: 3348, 2919, 2850, 1697, 1471, 1376, 1185, 1023; δ_H: 3.74–3.70 (1H, m), 2.51 (1H, sext, *J* 6.9 Hz), 2.46 (1H, dt, *J* 8.8, 5.4 Hz), 2.43 (1H, dt, *J* 14.8, 7.3 Hz), 2.40 (1H, dt, *J* 14.8, 7.3 Hz), 1.78–1.14 (146H, m, v.br.), 1.05 (3H, d, *J* 6.9 Hz), 0.90 (3H, d, *J* 6.9 Hz), 0.89 (6H, t, *J* 6.9 Hz), 0.71–0.64 (1H, m), 0.48–0.42 (1H, m), 0.22–0.08 (3H, m); δ_C: 215.4, 180.0, 72.1(-), 50.9(-), 46.3(-), 41.1(+), 38.1(-), 37.4(+), 35.5(+), 34.5(+), 33.0(+), 31.9(+), 30.1(+), 29.7(+, v.br.), 29.66(+), 29.61(+), 29.52(+), 29.50(+), 29.47(+), 29.44(+), 29.37(+), 29.34(+), 27.3(+), 27.2(+), 26.1(-), 25.7(+), 23.7(+), 22.7(+), 19.7(-), 18.6(-), 16.3(-), 14.1(-), 10.5(+).

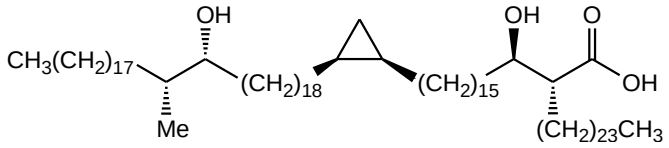
Experiment 129: (*R*)-2-{(*R*)-17-[(1*S*,2*R*)-2-((1*S*,21*R*)-1,21-Dimethyl-20-oxo-nonatriacontyl)-cyclopropyl]-1-hydroxy-heptadecyl}-hexacosanoic acid methyl ester (298)



Potassium carbonate (140 mg, 1.0 mmol) was added to a stirred solution of the ester (**297**) (80 mg, 0.06 mmol) in THF (15 ml), methanol (5 ml) and water (1 ml). The mixture was stirred at 60 ^{o}C for 18 hrs, when TLC showed complete reaction, and extracted with petrol / ether (1:1, 3 x 25 ml). The combined organic layers were dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol / ether (4:1) to give a white solid, *(R)-2-{(R)-17-*

[(1S,2R)-2-((1S,21R)-1,21-dimethyl-20-oxo-nonatriacontyl)-cyclopropyl]-1-hydroxy-heptadecyl}-hexacosanoic acid methyl ester (**298**) (65 mg, 81 %), m.p.: 59–60 ^{o}C, $[\alpha]_{D}^{24}$ = + 3.0 (*c* 1.0, $CHCl_3$), {Found $(M + Na)^+$: 1316.20, $C_{88}H_{172}NaO_4$ requires: 1316.31; Found: C, 81.87; H, 13.35, $C_{88}H_{172}O_4$ requires: C, 81.66; H, 13.39}. This showed ν_{max}/cm^{-1}: 3450, 2920, 2851, 1711, 1463, 1377, 1164; δ_H: 3.72 (3H, s), 3.69–3.64 (1H, m), 2.51 (1H, sext, *J* 6.9 Hz), 2.46–2.37 (3H, m), 1.74–1.52 (6H, m), 1.48–1.14 (140H, m, v.br.), 1.05 (3H, d, *J* 7.0 Hz), 0.90 (3H, d, *J* 6.6 Hz), 0.89 (6H, t, *J* 6.9 Hz), 0.69–0.63 (1H, m), 0.48–0.42 (1H, m), 0.22–0.09 (3H, m); δ_C: 215.2, 176.2, 72.3(-), 51.5(-), 50.9(-), 46.3(-), 41.1(+), 38.1(-), 37.4(+), 35.7(+), 34.5(+), 33.0(+), 31.9(+), 30.1(+), 29.73(+), 29.71(+, v.br.), 29.68(+), 29.66(+), 29.62(+), 29.59(+), 29.57(+), 29.55(+), 29.52(+), 29.50(+), 29.47(+), 29.43(+), 29.36(+), 29.33(+), 27.4(+), 27.3(+), 27.2(+), 26.1(-), 25.7(+), 23.7(+), 22.7(+), 19.7(-), 18.6(-), 16.4(-), 14.1(-), 10.5(+).

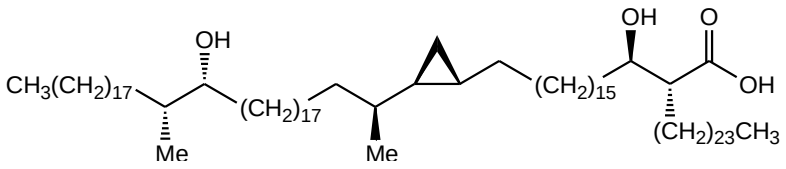
Experiment 130: (*R*)-2-{(*R*)-1-Hydroxy-16-[(1*R*,2*S*)-2-((19*R*,20*R*)-19-hydroxy-20-methyl-octatriacontyl)-cyclopropyl]-hexadecyl}-hexacosanoic acid (299)



The procedure used in **Experiment 127** was repeated in order to hydrolyse the ester (**292**) (24 mg, 0.019 mmol) using lithium hydroxide monohydrate (23 mg, 0.55 mmol) in THF (5 ml), methanol (0.5 ml) and water (0.4 ml) to a crude product. This was purified by column chromatography eluting with petrol / ethyl acetate (3:1) to give a white solid, *(R)-2-{(R)-1-hydroxy-16-[(1R,2S)-2-((19R,20R)-19-hydroxy-20-methyl-octatriacontyl)-cyclopropyl]-hexadecyl}-hexacosanoic acid* (**299**) (14 mg, 61 %), m.p.: 64–66 ^{o}C, $[\alpha]_{D}^{25}$ = + 9.1 (*c* 0.6, $CHCl_3$); {Found $(M + Na)^+$: 1262.16, $C_{84}H_{166}NaO_4$ requires: 1262.27; Found: C, 81.38; H, 13.25, $C_{84}H_{166}O_4$ requires: C, 81.35; H, 13.49}. This showed ν_{max}/cm^{-1}: 3331, 2921, 2852, 1688, 1463, 1377, 1201; δ_H: 3.74–3.70 (1H, m), 3.53–3.50 (1H, m), 2.46 (1H, dt, *J* 8.8, 5.4 Hz), 1.77–1.60 (1H, m), 1.67–1.12 (146H, m), 0.89 (6H, t, *J* 7.0 Hz), 0.87 (3H, d, *J* 7.0 Hz), 0.68–0.64 (2H, m), 0.57 (1H, br.dt, *J* 4.1, 8.2 Hz), - 0.32 (1H, br.q, *J* 5.4 Hz); δ_C: 178.4, 75.4(-), 72.1(-), 50.7(-), 38.1(-), 35.6(+), 34.4(+), 33.3(+), 31.9(+), 30.2(+), 29.9(+), 29.7(+, v.br.), 29.65(+),

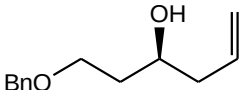
29.61(+), 29.59(+), 29.55(+), 29.51(+), 29.43(+), 29.36(+), 28.7(+), 27.4(+), 27.3(+), 26.3(+), 25.7(+), 22.7(+), 15.8(-), 14.1(-), 13.6(-), 10.9(+).

Experiment 131: (*R*)-2-{(*R*)-1-Hydroxy-17-[(1*S*,2*R*)-2-((1*S*,20*R*,21*R*)-20-hydroxy-1,21-dimethyl-nonatriacontyl)-cyclopropyl]-heptadecyl}-hexacosanoic acid (300)



The procedure used in **Experiment 127** was repeated in order to hydrolyse the ester (**296**) (50 mg, 0.037 mmol) using lithium hydroxide monohydrate (47 mg, 1.12 mmol) in THF (10 ml), methanol (1 ml) and water (0.8 ml) to a white solid, *(R)-2-{(R)-1-hydroxy-17-[(1S,2R)-2-((1S,20R,21R)-20-hydroxy-1,21-dimethyl-nonatriacontyl)-cyclopropyl]-heptadecyl}-hexacosanoic acid* (**300**) (32 mg, 67 %), m.p.: 69–70 ^{o}C, $[\alpha]_{D}^{26}$ = + 11.4 (*c* 0.60, $CHCl_3$), {Found $(M + Na)^+$: 1304.19, $C_{87}H_{172}NaO_4$ requires: 1304.31}. This showed ν_{max}/cm^{-1}: 3331, 2920, 2851, 1686, 1460, 1377, 1201; δ_H: 3.73–3.69 (1H, m), 3.53–3.50 (1H, m), 2.46 (1H, dt, *J* 8.8, 5.4 Hz), 1.75–1.59 (1H, m), 1.55–1.12 (148H, m, v.br.), 0.91–0.86 (12H, m), 0.69–0.63 (1H, m), 0.48–0.42 (1H, m), 0.22–0.08 (3H, m); δ_C: 178.8, 75.4(-), 72.1(-), 50.7(-), 38.2(-), 38.1(-), 37.4(+), 35.6(+), 34.5(+), 34.4(+), 33.3(+), 31.9(+), 30.1(+), 30.0(+), 29.73(+), 29.71(+, v.br.), 29.66(+), 29.60(+), 29.54(+), 29.43(+), 29.36(+), 27.4(+), 27.3(+), 27.2(+), 26.3(+), 26.1(-), 25.7(+), 22.7(+), 19.7(-), 18.6(-), 14.1(-), 13.6(-), 10.5(+).

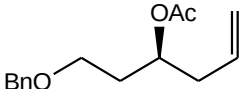
Experiment 132: (*S*)-1-Benzyloxy-hex-5-en-3-ol (314)



Copper iodide (4.6 g, 24.2 mmol) was dissolved in dry THF (300 ml) at r.t. under argon and cooled to – 75 ^{o}C. Vinylmagnesium bromide (155 ml, 155 mmol, 1M in THF) was added between – 75 ^{o}C to – 50 ^{o}C and the mixture was stirred at – 50 ^{o}C to – 40 ^{o}C for 30 min. It was re-cooled to – 75 ^{o}C and a solution of the (*R*)-(2-benzyloxyethyl)oxirane (**302**) (14.3 g, 80.3 mmol) in dry THF (100 ml) was added between – 75 ^{o}C to – 40 ^{o}C and the reaction was stirred at – 40 ^{o}C to – 30 ^{o}C for 1 h then at – 20 ^{o}C for 15 min. Sat. aq. NH_4Cl (400 ml) was added and extracted with ethyl acetate (3 x 300 ml) and the combined organic layers were washed with water, dried

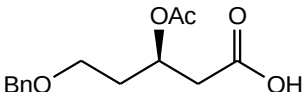
and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol / ethyl acetate (2:1) to give a colourless oil, (*S*)-1-benzyloxy-hex-5-en-3-ol (**314**) [231,232] (15.7 g, 95 %), $[\alpha]_D^{24} = -5.3$ (*c* 1.2, $CHCl_3$), {Found $(M + Na)^+$: 229.1194, $C_{13}H_{18}NaO_2$ requires: 229.1199}. This showed ν_{max}/cm^{-1}: 3425, 3069, 3031, 2919, 1863, 1641, 1496, 1454, 1363, 1206, 1098; δ_H: 7.38–7.28 (5H, m), 5.85 (1H, ddt, *J* 17.4, 10.1, 7.3 Hz), 5.14–5.10 (2H, m), 4.54 (2H, br.t, *J* 12.0 Hz), 3.91–3.87 (1H, m), 3.73 (1H, dt, *J* 9.5, 5.4 Hz), 3.66 (1H, ddd, *J* 9.5, 7.3, 5.4 Hz), 2.85 (1H, d, *J* 2.2 Hz), 2.28–2.25 (2H, m), 1.82–1.73 (2H, m); δ_C: 138.0, 134.9(-), 128.4(-), 127.7(-), 127.6(-), 117.6(+), 73.3(+), 70.3(-), 68.9(+), 41.9(+), 35.9(+).

Experiment 133: Acetic acid (*S*)-1-(2-benzyloxy-ethyl)-but-3-enyl ester (315)



The procedure used in **Experiment 119** was repeated in order to acetylate the alcohol (**314**) (23.5 g, 114.1 mmol) using acetic anhydride (80 ml) and then anhydrous pyridine (80 ml) in dry toluene (180 ml) to a crude product. 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* (**315**) (28.05 g, 99 %), $[\alpha]_D^{23} = +49.0$ (*c* 1.13, $CHCl_3$), {Found $(M + H)^+$: 249.1485, $C_{15}H_{21}O_3$ requires: 249.1485}. This showed ν_{max}/cm^{-1}: 3066, 3031, 2923, 2861, 1737, 1643, 1496, 1454, 1372, 1241, 1100, 1026; δ_H: 7.37–7.27 (5H, m), 5.76 (1H, ddt, *J* 17.0, 10.4, 7.0 Hz), 5.13–5.06 (3H, m), 4.50 (1H, d, *J* 12.0 Hz), 4.47 (1H, d, *J* 12.0 Hz), 3.54–3.46 (2H, m), 2.40–2.30 (2H, m), 2.00 (3H, m), 1.94–1.82 (2H, m); δ_C: 170.6, 138.3, 133.5(-), 128.3(-), 127.7(-), 127.6(-), 117.8(+), 73.0(+), 70.8(-), 66.6(+), 38.8(+), 33.7(+), 21.1(-)

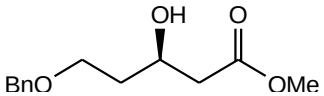
Experiment 134: (*R*)-3-Acetoxy-5-benzyloxy-pentanoic acid (316)



Acetic acid (*S*)-1-(2-benzyloxy-ethyl)-but-3-enyl ester (**315**) (17.8 g, 71.77 mmol) was dissolved in dry DMF (450 ml) and oxone (176.5 g, 287.1 mmol) then OsO_4 2.5 % in 2-methyl-2-propanol (9 ml, 0.72 mmol) were added at 10 ^oC. The mixture was allowed to reach 32 ^oC and stirred 3 hrs. The mixture was dissolved with water (3 L) and extracted with ethyl acetate (1 x 500 ml, 2 x 250 ml). The combined organic layers

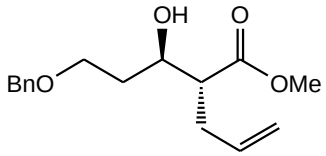
were washed with water (700 ml), dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol / ethyl acetate (1:1 then 1:2) to give a colourless oil, *(R)-3-acetoxy-5-benzyloxy-pentanoic acid* (**316**) (14.85 g, 78 %), $[\alpha]_D^{22}$ = + 15.2 (*c* 0.89, $CHCl_3$), {Found $(M + H)^+$: 267.1216, $C_{14}H_{19}O_5$ requires: 267.1227}. This showed ν_{max}/cm^{-1}: 3457, 3064, 3032, 2930, 2864, 1740, 1680, 1454, 1374, 1242, 1176, 1100; δ_H: 7.37–7.27 (5H, m), 5.36 (1H, quintet, *J* 6.3 Hz), 4.49 (2H, br.t, *J* 12.5 Hz), 3.56 (1H, dt, *J* 15.8, 6.0 Hz), 3.53 (1H, dt, *J* 16.1, 6.3 Hz), 2.71 (1H, dd, *J* 5.7, 15.8 Hz), 2.69 (1H, dd, *J* 6.9, 16.1 Hz), 2.01 (3H, s), 1.97 (2H, br.q, *J* 6.0 Hz); δ_C: 175.4, 170.4, 138.0, 128.4(-), 127.73(-), 127.66(-), 73.1(+), 68.2(-), 66.2(+), 38.9(+), 33.8(+), 21.0(-).

Experiment 135: (*R*)-5-Benzyloxy-3-hydroxy-pentanoic acid methyl ester (303)



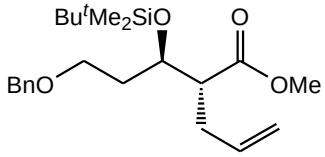
Conc. H_2SO_4 (70 drops) was added to a stirred solution of (*R*)-3-acetoxy-5-benzyloxy-pentanoic acid (**316**) (14.75 g, 55.45 mmol) in methanol (300 ml) and refluxed for 3.5 hrs. TLC showed complete reaction, methanol was evaporated and ethyl acetate (250 ml) and sat. aq. $NaHCO_3$ (200 ml) were added. The mixture was extracted and the aq. layer re-extracted with ethyl acetate (2 x 150 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-hydroxy-pentanoic acid methyl ester (**303**)[141] (10.23 g, 78 %), $[\alpha]_D^{26}$ = – 12.2 (*c* 1.23, $CHCl_3$), {Found $(M + Na)^+$: 261.1085, $C_{13}H_{18}NaO_4$ requires: 261.1097}. This showed ν_{max}/cm^{-1}: 3467, 3031, 2951, 2864, 1737, 1496, 1438, 1168, 1100; δ_H: 7.37–7.28 (5H, m), 4.53 (2H, s), 4.26 (1H, tdd, *J* 6.3, 4.1, 7.9 Hz), 3.72 (1H, ddd, *J* 5.1, 6.3, 9.5 Hz), 3.71 (3H, s), 3.66 (1H, ddd, *J* 5.1, 6.9, 9.5 Hz), 3.38 (1H, d, *J* 3.2 Hz), 2.52 (2H, d, *J* 6.3 Hz), 1.87–1.77 (2H, m); δ_C: 172.8, 138.0, 128.4(-), 127.7(-), 127.6(-), 73.3(+), 68.0(+), 67.0(-), 51.7(-), 41.4(+), 36.0(+).

Experiment 136: (*R*)-2-((*R*)-3-Benzyloxy-1-hydroxy-propyl)-pent-4-enoic acid methyl ester (320)



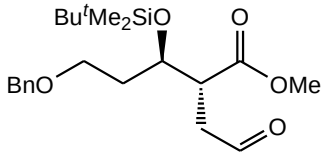
Diisopropylamine (7.86 g, 77.7 mmol) was dissolved in dry THF (100 ml) and cooled to – 78 ^{o}C. MeLi (54.4 ml, 81.6 mmol, 1.5M) was added and stirred to + 16 ^{o}C for 30 min., then re-cooled to – 61 ^{o}C and (*R*)-5-benzyloxy-3-hydroxy-pentanoic acid methyl ester (**303**) (8.6 g, 36.1 mmol) in dry THF (50 ml) was added and the mixture was stirred at – 45 ^{o}C for 1 h, - 20 ^{o}C for 40 min. and then at - 20 ^{o}C to – 10 ^{o}C for 20 min. It was re-cooled to – 62 ^{o}C and allyl iodide (5.0 ml, 54.2 mmol) in dry THF (20 ml) and HMPA (12.6 ml, 72.3 mmol) were added and the mixture was stirred at – 45 ^{o}C for 1 h., - 45 ^{o}C to – 20 ^{o}C for 30 min. and then – 20 ^{o}C for 30 min. Further allyl iodide (0.9 ml) was added and stirred at – 20 ^{o}C to – 10 ^{o}C for 30 min. and then – 10 ^{o}C for 30 min. Sat. aq. NH_4Cl (70 ml) was added and extracted with ether / ethyl acetate (1:1, 3 x 100 ml), dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol / ethyl acetate (2:1) to give a colourless oil, *(R)-2-((R)-3-benzyloxy-1-hydroxy-propyl)-pent-4-enoic acid methyl ester* (**320**) (7.64 g, 76 %), $[\alpha]_D^{21} = -6.9$ (*c* 1.09, $CHCl_3$), {Found $(M + H)^+$: 279.1582, $C_{16}H_{23}O_4$ requires: 279.1591}. This showed ν_{max}/cm^{-1} : 3494, 3066, 3030, 2951, 2863, 1735, 1643, 1438, 1367, 1170, 1100; δ_H: 7.38–7.28 (5H, m), 5.75 (1H, ddt, *J* 17.0, 10.1, 6.9 Hz), 5.12–5.03 (2H, m), 4.52 (2H, s), 3.97 (1H, dtd, *J* 8.8, 5.7, 2.9 Hz), 3.72 (1H, ddd, *J* 4.8, 6.0, 9.2 Hz), 3.70 (3H, s), 3.66 (1H, ddd, *J* 5.1, 7.4, 9.5 Hz), 3.20 (1H, d, *J* 5.7 Hz), 2.58 (1H, td, *J* 5.7, 8.8 Hz), 2.47–2.35 (2H, m), 1.88–1.74 (2H, m); δ_C: 174.8, 137.9, 134.9(-), 128.4(-), 127.7(-), 127.6(-), 117.1(+), 73.3(+), 70.9(-), 68.3(+), 51.6(-), 51.0(-), 34.6(+), 33.3(+).

Experiment 137: (*R*)-2-[(*R*)-3-Benzyloxy-1-(*tert*-butyl-dimethyl-silanyloxy)-propyl]-pent-4-enoic acid methyl ester (304)



The procedure used in **Experiment 107** was repeated in order to protect the alcohol (**320**) (6.0 g, 21.83 mmol) using imidazole (3.67 g, 53.96 mmol) and *tert*-butyldimethylchlorosilane (4.23 g, 28.06 mmol) in dry DMF (100 ml). 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* (**304**) (7.4 g, 87 %), $[\alpha]_D^{26} = -17.2$ (*c* 0.93, $CHCl_3$), {Found $(M + Na)^+$: 415.2256, $C_{22}H_{36}NaO_4Si$ requires: 415.2275}. This showed ν_{max}/cm^{-1}: 3032, 2954, 2930, 2857, 1739, 1643, 1437, 1361, 1254, 1171, 1100; δ_H: 7.37–7.27 (5H, m), 5.73 (1H, ddt, *J* 17.0, 10.1, 6.9 Hz), 5.06–4.97 (2H, m), 4.49 (2H, br.t, *J* 12.3 Hz), 4.13 (1H, br.q, *J* 5.7 Hz), 3.65 (3H, s), 3.59 (1H, td, *J* 6.3, 9.2 Hz), 3.55 (1H, td, *J* 6.6, 9.5 Hz), 2.69–2.65 (1H, m), 2.37–2.33 (2H, m), 1.84–1.80 (2H, m), 0.87 (9H, s), 0.05 (6H, s); δ_C: 173.7, 138.5, 135.9(-), 128.3(-), 127.6(-), 127.5(-), 116.3(+), 72.9(+), 70.2(-), 66.3(+), 51.7(-), 51.3(-), 33.7(+), 31.3(+), 25.7(-), 17.9, -4.4(-), - 4.9(-).

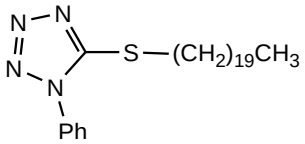
Experiment 138: (2*R*,3*R*)-5-Benzyloxy-3-(*tert*-butyl-dimethyl-silanyloxy)-2-(2-oxo-ethyl)-pentanoic acid methyl ester (324)



The procedure used in **Experiment 108** was repeated in order to oxidise the alkene (**304**) (4.0 g, 10.99 mmol) using 2,6-lutidine (2.36 g, 21.98 mmol), OsO_4 2.5 % in 2-methyl-2-propanol (2 ml, 0.2 mmol), and $NaIO_4$ (9.4 g, 43.96 mmol) in 1,4-dioxane–water (160 ml, 3:1). The crude product was purified by column chromatography eluting with petrol / ether (2:1) to give a colourless oil, *(2R,3R)-5-benzyloxy-3-(tert-butyl-dimethyl-silanyloxy)-2-(2-oxo-ethyl)-pentanoic acid methyl ester* (**324**) (3.52 g, 88 %), $[\alpha]_D^{26} = -18.4$ (*c* 0.97, $CHCl_3$), {Found $(M + H)^+$: 395.2244, $C_{21}H_{35}O_5Si$ requires: 395.2248}. This showed ν_{max}/cm^{-1}: 3031, 2954, 2930, 2857, 1736, 1496,

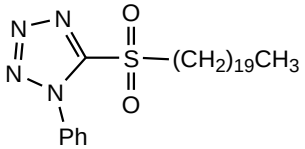
1437, 1315, 1254, 1100; δ_H: 9.81 (1H, s), 7.37–7.28 (5H, m), 4.50 (1H, d, *J* 12.0 Hz), 4.45 (1H, d, *J* 12.0 Hz), 4.27 (1H, td, *J* 4.4, 7.9 Hz), 3.68 (3H, s), 3.54–3.50 (2H, m), 3.23 (1H, ddd, *J* 3.2, 7.6, 10.4 Hz), 2.97 (1H, ddd, *J* 1.0, 10.4, 18.3 Hz), 2.70 (1H, dd, *J* 3.2, 18.3 Hz), 1.71–1.63 (2H, m), 0.87 (9H, m), 0.08 (3H, s), 0.07 (3H, s); δ_C: 200.4(-), 172.4, 138.3, 128.3(-), 127.6(-), 127.5(-), 72.8(+), 68.8(-), 66.5(+), 52.0(-), 45.3(-), 40.0(+), 33.7(+), 25.7(-), 17.9, - 4.7(-), - 4.9(-).

Experiment 139: 5-Icosylsulfanyl-1-phenyl-1*H*-tetrazole (322)



The procedure used in **Experiment 109** was repeated using 1-bromoeicosane (15 g, 41.5 mmol), 1-phenyl-1*H*-tetrazole-5-thiol (7.77 g, 43.57 mmol), anhydrous potassium carbonate (12.04 g, 87.15 mmol) and acetone (500 ml). The crude product was recrystallised from acetone (80 ml) and methanol (170 ml) to give a white solid, *5-icosylsulfanyl-1-phenyl-1H-tetrazole* (**322**) (18.85 g, 99 %), m.p.: 62–64 ^oC, {Found $(M + H)^+$: 459.3500, $C_{27}H_{46}N_4S$ requires: 459.3516}. This showed ν_{max}/cm^{-1}: 2916, 2850, 1597, 1502, 1473, 1420, 1390, 1247, 1091; δ_H: 7.61–7.54 (5H, m), 3.40 (2H, t, *J* 7.3 Hz), 1.83 (2H, quintet, *J* 7.3 Hz), 1.48–1.42 (2H, m), 1.32–1.26 (32H, m, v.br.), 0.89 (3H, t, *J* 6.9 Hz); δ_C: 154.5, 133.8, 130.0(-), 127.7(-), 123.9(-), 33.4(+), 31.9(+), 29.7(+), 29.65(+), 29.6(+), 29.5(+), 29.4(+), 29.3(+), 29.1(+), 29.0(+), 28.6(+), 22.7(+), 14.1(-).

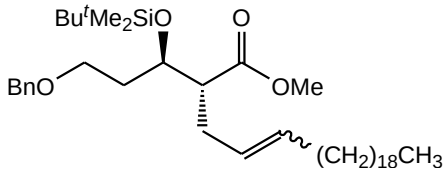
Experiment 140: 5-(Icosane-1-sulfonyl)-1-phenyl-1*H*-tetrazole (323)



The procedure used in **Experiment 110** was repeated in order to oxidise the sulfane (**322**) (3.1 g, 5.87 mmol) using ammonium molybdate (VI) tetrahydrate (22.35 g, 18.08 mmol) in 35 % H_2O_2 (50 ml) and THF (300 ml) and IMS (400 ml), and further ammonium molybdate (VI) tetrahydrate (8.5 g, 6.88 mmol) in 35% H_2O_2 (21.5 ml). The crude product was purified by column chromatography eluting with petrol / ether (8:1) to give a white solid, *5-(icosane-1-sulfonyl)-1-phenyl-1H-tetrazole* (**323**) (16.25 g, 84 %), m.p.: 69–70 ^oC, {Found $(M + H)^+$: 491.3403, $C_{27}H_{47}O_2S$ requires: 491.3414}.

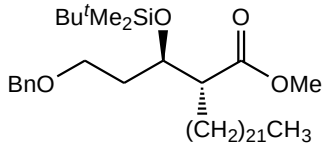
This showed ν_{max}/cm^{-1}: 2920, 2849, 1595, 1499, 1470, 1338, 1153; δ_H: 7.71–7.70 (2H, m), 7.64–7.59 (3H, m), 3.76–3.72 (2H, m), 1.99–1.93 (2H, m), 1.53–1.47 (2H, m), 1.35–1.26 (32H, m, v.br.), 0.89 (3H, t, *J* 7.0 Hz); δ_C: 153.5, 133.1, 131.4(-), 129.7(-), 125.1(-), 56.0(+), 31.9(+), 29.7(+), 29.65(+), 29.6(+), 29.5(+), 29.4(+), 29.3(+), 29.2(+), 28.9(+), 28.1(+), 22.7(+), 21.9(+), 14.1 (-).

Experiment 141: (*E*/*Z*)-(*R*)-2-[(*R*)-3-Benzyloxy-1-(*tert*-butyl-dimethyl-silanyloxy)-propyl]-tetracos-4-enoic acid methyl ester (325)



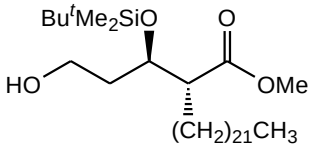
The procedure used in **Experiment 72** was repeated in order to couple the aldehyde (**324**) (3.4 g, 8.63 mmol) and the sulfone (**323**) (5.07 g, 10.36 mmol) using lithium bis(trimethylsilyl) amide (15.47 ml, 16.40 mmol) in dry THF. The crude product was purified by column chromatography eluting with petrol / ether (20:1) to give a colourless oil, *(E/Z)-(R)-2-[(R)-3-benzyloxy-1-(tert-butyl-dimethyl-silanyloxy)-propyl]-tetracos-4-enoic acid methyl ester* (**325**) (3.21 g, 83 %) as a mixture of two isomers in ratio 2:1, {Found $(M + H)^+$: 659.5409, $C_{41}H_{75}O_4Si$ requires: 659.5426}. This showed ν_{max}/cm^{-1}: 2923, 2852, 1739, 1467, 1362, 1256, 1169, 1100; δ_H (two isomers): 7.36–7.26 (5H, m), 5.46–5.38 (1H, m), 5.33–5.23 (1H, m), 4.49 (2H, s), 4.15–4.08 (1H, m), 3.64 (3H, s), 3.59–3.54 (2H, m), 2.63–2.58 (1H, m), 2.41–2.25 (2H, m), 2.04–1.92 (2H, m), 1.86–1.81 (2H, m), 1.31–1.26 (34H, m, v. br.), 0.89 (3H, t, *J* 6.9 Hz), 0.86 (9H, s), 0.06 (3H, s), 0.03 (3H, s); δ_C (major isomer): 173.98, 138.49, 132.8(-), 128.3(-), 127.6(-), 127.5(-), 126.8(-), 72.9(+), 70.3(-), 66.3(+), 52.3(-), 51.2(-), 33.7(+), 32.5(+), 31.9(+), 30.4(+), 29.7(+, v.br.), 29.63(+), 29.53(+), 29.49(+), 29.4(+), 29.1(+), 25.7(-), 22.7(+), 18.0, 14.1(-), - 4.6(-), - 4.9(-); δ_C (minor isomer): 174.0, 138.47, 131.9(-), 126.2(-), 70.4(-), 52.2(-), 51.3(-). The remaining signals were obscured by the major isomer.

Experiment 142: (*R*)-2-[(*R*)-3-Benzyloxy-1-(*tert*-butyl-dimethyl-silanyloxy)-propyl]-tetracosanoic acid methyl ester (326)



Palladium 10% on carbon (1.0 g) was added to a stirred solution of the alkene (**325**) (4.9 g, 7.45 mmol) in ethyl acetate (100 ml). Hydrogenation was carried out for 1 h. 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 colourless oil, *(R)-2-[(R)-3-benzyloxy-1-(tert-butyl-dimethyl-silanyloxy)-propyl]-tetracosanoic acid methyl ester* (**326**) (4.6 g, 94 %), $[\alpha]_D^{23} = -5.4$ (*c* 1.13, $CHCl_3$), {Found $(M + H)^+$: 661.5570, $C_{41}H_{77}O_4Si$ requires: 661.5586}. This showed ν_{max}/cm^{-1}: 2925, 2854, 1740, 1664, 1361, 1254, 1195, 1168, 1102; δ_H: 7.37–7.27 (5H, m), 4.50 (2H, s), 4.09 (1H, br.q, *J* 5.1 Hz), 3.67 (3H, s), 3.59 (1H, ddd, *J* 6.6, 9.2, 11.4 Hz), 3.55 (1H, ddd, *J* 6.6, 9.2, 11.7 Hz), 2.57 (1H, ddd, *J* 3.8, 6.6, 10.4 Hz), 1.82 (2H, br.q, *J* 6.6 Hz), 1.66–1.16 (42H, m, v.br.), 0.89 (3H, t, *J* 7.0 Hz), 0.87 (9H, s), 0.06 (3H, s), 0.05 (3H, s); δ_C: 174.7, 138.5, 128.3(-), 127.5(-), 127.4(-), 72.9(+), 70.7(-), 66.2(+), 52.0(-), 51.2(-), 33.6(+), 31.9(+), 29.7(+, v.br.), 29.65(+), 29.57(+), 29.44(+), 29.35(+), 27.9(+), 27.2(+), 25.7(+), 22.7(+), 17.9, 14.1(-), - 4.6(-), - 4.9(-).

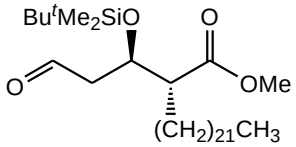
Experiment 143: (*R*)-2-[(*R*)-1-(*tert*-Butyl-dimethyl-silanyloxy)-3-hydroxy-propyl]-tetracosanoic acid methyl ester (327)



Palladium 10% on carbon (1.0 g) was added to a stirred solution of the benzyl compound (**326**) (4.5 g, 6.82 mmol) in ethyl acetate (100 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, *(R)-2-[(R)-1-(tert-butyl-dimethyl-silanyloxy)-3-hydroxy-propyl]-tetracosanoic acid methyl ester* (**327**) (3.21 g, 83 %), m.p.: 35–37 ^oC, $[\alpha]_D^{22} = -8.3$ (*c* 0.4, C_6H_6); {Found $(M + H)^+$: 571.5101, $C_{34}H_{72}O_4Si$ requires: 571.5116}. This showed ν_{max}/cm^{-1}: 3449, 2924, 2854, 1741, 1465, 1361,

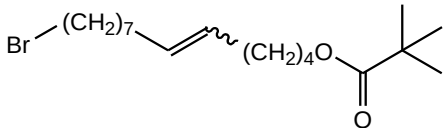
1255, 1196, 1167, 1094; δ_H (C_6D_6): 4.24 (1H, dt, *J* 4.1, 6.6 Hz), 3.63–3.52 (2H, m), 3.41 (3H, s), 2.79 (1H, ddd, *J* 3.8, 6.3, 10.1 Hz), 1.79–1.31 (44H, m, v.br.), 0.98 (9H, s), 0.92 (3H, t, *J* 6.9 Hz), 0.14 (3H, s), 0.11 (3H, s); δ_C (C_6D_6): 174.3, 71.7(-), 59.0(+), 52.3(-), 51.0(-), 36.2(+), 32.4(+), 30.2(+, v.br.), 30.16(+), 30.15(+), 30.13(+), 30.1(+), 29.9(+), 29.8(+), 28.5(+), 27.6(+), 26.0(-), 23.1(+), 18.2, 14.4(-), - 4.4(-), - 4.7(-).

Experiment 144: (*R*)-2-[(*R*)-1-(*tert*-Butyl-dimethyl-silanyloxy)-3-oxo-propyl]-tetracosanoic acid methyl ester (305)



The procedure used in **Experiment 18** was repeated in order to oxidise the alcohol (**327**) (3.15 g, 5.52 mmol) using PCC (3.0 g, 13.82 mmol) in CH_2Cl_2 (400 ml) to give a crude product. The crude product was purified by column chromatography eluting with petrol / ether (4:1) to give a colourless oil, *(R)-2-[(R)-1-(tert-butyl-dimethyl-silanyloxy)-3-oxo-propyl]-tetracosanoic acid methyl ester* (**305**) (2.6 g, 83 %), $[\alpha]_D^{26}$ = – 5.0 (*c* 1.23, $CHCl_3$), {Found $(M + Na)^+$: 591.4774, $C_{34}H_{68}NaO_4Si$ requires: 591.4779}. This showed ν_{max}/cm^{-1}: 2925, 2854, 1736, 1465, 1362, 1255, 1196, 1168, 1098; δ_H: 9.81 (1H, dd, *J* 1.6, 2.7 Hz), 4.43 (1H, dt, *J* 4.7, 6.0 Hz), 3.69 (3H, s), 2.66 (1H, ddd, *J* 1.6, 4.7, 6.3 Hz), 2.61 (1H, ddd, *J* 2.7, 6.3, 8.8 Hz), 2.59 (1H, ddd, *J* 4.1, 6.3, 10.4 Hz), 1.61–1.26 (42H, m, v.br.), 0.90 (3H, t, *J* 6.6 Hz), 0.86 (9H, s), 0.08 (3H, s), 0.07 (3H, s); δ_C: 201.3(-), 174.0, 68.8(-), 52.3(-), 51.5(-), 48.1(+), 31.9(+), 29.7(+, v.br.), 29.66(+), 29.62(+), 29.54(+), 29.5(+), 29.4(+), 29.3(+), 27.8(+), 27.0(+), 25.6(-), 22.7(+), 17.9, 14.1(-), - 4.6(-), - 4.9(-).

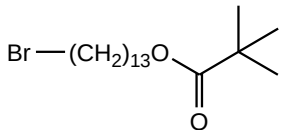
Experiment 145: 2,2-Dimethyl-propionic acid (*E*/*Z*)-13-bromo-tridec-5-enyl ester (332)



The procedure used in **Experiment 27** was repeated in order to couple 8-bromo-octanal (7.9 g, 38.16 mmol) with the sulfone (**331**) (17.4 g, 45.8 mmol) using lithium bis(trimethylsilyl) amide (68.4 ml, 72.5 mmol, 1.06M) in dry THF. The crude product was purified by column chromatography eluting with petrol / ether (15:1) to give a

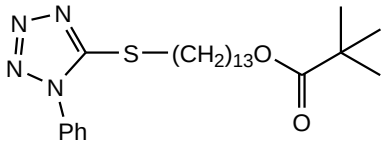
colourless oil, *2,2-dimethyl-propionic acid (E/Z)-13-bromo-tridec-5-enyl ester* (**332**) (9.63 g, 70 %) as a mixture of two isomers in ratio 2.4:1, {Found $(M + H)^+$: 371.1737, $C_{18}H_{34}BrO_2$ requires: 361.1737}. This showed ν_{max}/cm^{-1}: 2930, 2856, 1729, 1480, 1460, 1285, 1157; δ_H (two isomers): 5.44–5.32 (2H, m), 4.06 (2H, t, *J* 6.6 Hz), 3.41 (2H, t, *J* 6.9 Hz), 2.09–1.96 (4H, m), 1.89–1.83 (2H, m), 1.68–1.60 (2H, m), 1.46–1.30 (10H, m), 1.20 (9H, s); δ_C (major isomer): 178.6, 130.8(-), 129.7(-), 64.3(+), 38.7, 34.0(+), 32.8(+), 32.5(+), 32.1(+), 29.4(+), 28.9(+), 28.6(+), 28.2(+), 28.1(+), 27.2(-), 25.9(+); δ_C (minor isomer): 130.3(-), 129.2(-), 64.2(+), 29.6(+), 29.0(+), 28.7(+), 28.3(+), 27.1(-), 26.7(+), 26.0(+) (The remaining signals were obscured by the major isomer).

Experiment 146: 2,2-Dimethyl-propionic acid 13-bromo-tridecyl ester (333)



Palladium 10% on carbon (1.8 g) was added to a stirred solution of the alkene (**332**) (9.3 g, 25.76 mmol) in ethyl acetate (150 ml) and methanol (50 ml). Hydrogenation was carried out 1 h. 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 (15:1) to give a colourless oil, *2,2-dimethyl-propionic acid 13-bromo-tridecyl ester* (**333**) (8.2 g, 88 %), {Found $(M + Na)^+$: 385.1695, $C_{18}H_{35}BrNaO_2$ requires: 385.1713}. This showed ν_{max}/cm^{-1}: 2927, 2855, 1730, 1480, 1462, 1285, 1157; δ_H: 4.05 (2H, t, *J* 6.6 Hz), 3.42 (2H, t, *J* 6.9 Hz), 1.86 (2H, quintet, *J* 6.9 Hz), 1.62 (2H, quintet, *J* 6.6 Hz), 1.46–1.27 (18H, m), 1.20 (9H, s); δ_C: 178.7, 64.5(+), 38.7, 34.0(+), 32.8(+), 29.6(+), 29.5(+), 29.49(+), 29.4(+), 29.2(+), 28.8(+), 28.6(+), 28.2(+), 27.2(-), 25.9(+).

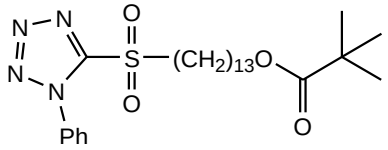
Experiment 147: 2,2-Dimethyl-propionic acid 13-(1-phenyl-1*H*-tetrazol-5-ylsulfanyl)-tridecyl ester (334)



The procedure used in **Experiment 23** was repeated using compound (**333**) (7.25 g, 19.97 mmol), 1-phenyl-1*H*-tetrazole-5-thiol (3.74 g, 20.97 mmol) and anhydrous

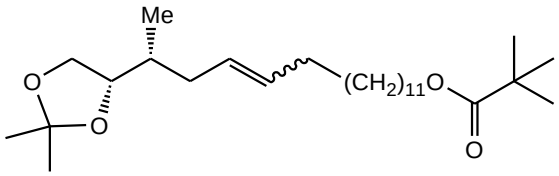
potassium carbonate (5.8 g, 41.94 mmol) in acetone (250 ml). The crude product was purified by column chromatography eluting with petrol / ether (3:2) to give a colourless oil, *2,2-dimethyl-propionic acid 13-(1-phenyl-1H-tetrazol-5-ylsulfanyl)-tridecyl ester* (**334**) (7.92 g, 86 %), {Found $(M + H)^+$: 461.2962, $C_{25}H_{41}N_4O_2S$ requires: 461.2945}. This showed ν_{max}/cm^{-1}: 2927, 2854, 1727, 1598, 1501, 1480, 1462, 1388, 1285, 1159; δ_H: 7.6–7.52 (5H, m), 4.04 (2H, t, *J* 6.6 Hz), 3.39 (2H, t, *J* 7.4 Hz), 1.82 (2H, quintet, *J* 7.4 Hz), 1.62 (2H, quintet, *J* 6.6 Hz), 1.49–1.26 (18H, m), 1.19 (9H, s); δ_C: 178.6, 154.5, 133.7, 130.0(-), 129.7(-), 123.8(-), 64.4(+), 38.7, 33.3(+), 29.5(+), 29.48(+), 29.46(+), 29.38(+), 29.2(+), 29.1(+), 29.0(+), 28.6(+), 28.5(+), 27.2(-), 25.9(+).

Experiment 148: 2,2-Dimethyl-propionic acid 13-(1-phenyl-1*H*-tetrazole-5-sulfonyl)-tridecyl ester (335)



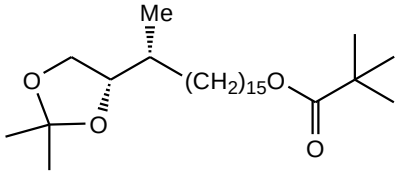
The procedure used in **Experiment 25** was repeated using the sulfane (**334**) (7.7 g, 16.74 mmol), ammonium molybdate (VI) tetrahydrate (10.0 g, 8.09 mmol) in 35 % H_2O_2 (27 ml) and THF (190 ml) and IMS (200 ml), and further ammonium molybdate (VI) tetrahydrate (5.0 g, 4.0 mmol) in 35% H_2O_2 (13 ml). The crude product was purified by column chromatography eluting with petrol / ether (1:1) to give a white solid, *2,2-dimethyl-propionic acid 13-(1-phenyl-1H-tetrazole-5-sulfonyl)-tridecyl ester* (**335**) (7.81 g, 95 %), m.p.: 46–47 ^oC, {Found $(M + H)^+$: 493.2843, $C_{25}H_{41}N_4O_4S$ requires: 493.2843}. This showed ν_{max}/cm^{-1}: 3072, 2917, 2853, 1727, 1593, 1476, 1420, 1358, 1285, 1150; δ_H: 7.71–7.68 (2H, m), 7.64–7.27 (3H, m), 4.04 (2H, t, *J* 6.6 Hz), 3.74–3.71 (2H, m), 1.98–1.92 (2H, m), 1.62 (2H, quintet, *J* 6.6 Hz), 1.52–1.26 (18H, m), 1.19 (9H, m); δ_C: 178.6, 153.5, 133.0, 131.4(-), 129.7(-), 125.0(-), 64.4(+), 56.0(+), 38.7, 29.44(+), 29.37(+), 29.2(+), 29.1(+), 28.8(+), 28.6(+), 28.1(+), 27.2(-), 25.8(+), 21.9(+).

Experiment 149: 2,2-Dimethyl-propionic acid (*E*/*Z*)-(*R*)-16-((S)-2,2-dimethyl-[1,3]dioxolan-4-yl)-heptadec-13-enyl ester (336)



The procedure used in **Experiment 27** was repeated using the aldehyde (**102**) (2.5 g, 14.53 mmol), sulfone (**335**) (7.67 g, 15.55 mmol) and lithium bis (trimethylsilyl) amide (26.05 ml, 26.72 mmol, 1.06M) in dry THF. The crude product was purified by column chromatography eluting with petrol / ether (10:1) to give a colourless oil, *2,2-dimethyl-propionic acid (E/Z)-(R)-16-((S)-2,2-dimethyl-[1,3]dioxolan-4-yl)-heptadec-13-enyl ester* (**336**) (5.47 g, 86 %) as a mixture of two isomers in ratio 2.5:1, {Found $(M + Na)^+$: 439.3768, $C_{27}H_{51}O_4$ requires: 439.3782}. This showed ν_{max}/cm^{-1}: 2927, 2855, 1731, 1480, 1460, 1368, 1284, 1213, 1158; δ_H (major isomer): 5.46–5.31 (2H, m), 4.04 (2H, t, *J* 6.6 Hz), 4.00 (1H, dd, *J* 6.3, 7.9 Hz), 3.87 (1H, br.q, *J* 7.0 Hz), 3.60 (1H, br.t, *J* 7.9 Hz), 2.08–1.77 (4H, m), 1.65–1.59 (3H, m), 1.40 (3H, s), 1.35 (3H, s), 1.34–1.26 (18H, m), 0.96 (3H, d, *J* 6.6 Hz); δ_H (minor isomer): 4.02 (1H, dd, *J* 6.3, 7.9 Hz), 3.92 (1H, br.q, *J* 6.6 Hz), 3.63 (1H, br.t, *J* 7.6 Hz), 0.97 (3H, d, *J* 6.6 Hz) (the remaining signals were obscured by the major isomer); δ_C (major isomer): 178.6, 132.6(-), 127.5(-), 108.4, 79.9(-), 67.8(+), 64.4(+), 38.7, 36.8(-), 36.2(+), 32.64(+), 29.60(+), 29.53(+), 29.51(+), 29.49(+), 29.47(+), 29.3(+), 29.2(+), 29.1(+), 28.6(+), 27.2(-), 26.6(-), 25.9(+), 25.6(-), 15.6(-); δ_C (minor isomer): 131.6(-), 127.1(-), 79.8(-), 36.9(-), 30.6(+), 26.59(-), 25.5(-), 15.57(-) (the remaining signals were obscured by the major isomer).

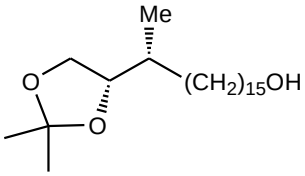
Experiment 150: 2,2-Dimethyl-propionic acid (*R*)-16-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-heptadecyl ester (337)



Palladium 10% on carbon (1.5 g) was added to a stirred solution of the alkene (**336**) (5.37 g, 12.26 mmol) in ethyl acetate (150 ml) and ethanol (100 ml). Hydrogenation was carried out 1.5 hrs. The solution was filtered over a bed of celite and the solvent was evaporated. The crude product was purified by column chromatography eluting

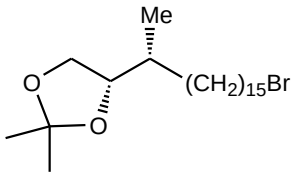
with petrol / ether (8:1) to give a colourless oil, *2,2-dimethyl-propionic acid (R)-16-((S)-2,2-dimethyl-[1,3]dioxolan-4-yl)-heptadecyl ester* (**337**) (5.15 g, 96 %), $[\alpha]_D^{26}$ = + 16.1 (*c* 1.14, $CHCl_3$), {Found $(M + H)^+$: 441.3920, $C_{27}H_{53}O_4$ requires: 441.3938}. This showed ν_{max}/cm^{-1}: 2926, 2855, 1731, 1463, 1368, 1284, 1158; δ_H: 4.05 (2H, t, *J* 6.6 Hz), 4.00 (1H, dd, *J* 6.3, 7.9 Hz), 3.87 (1H, br.q, *J* 6.9 Hz), 3.60 (1H, br.t, *J* 7.9 Hz), 1.62 (2H, quintet, *J* 6.6 Hz), 1.40 (3H, s), 1.35 (3H, s), 1.32–1.26 (26H, m), 1.11–1.05 (1H, m), 0.96 (3H, d, *J* 6.6 Hz); δ_C: 178.6, 108.5, 80.4(-), 67.8(+), 64.5(+), 38.7, 36.5(-), 32.7(+), 29.9(+), 29.7(+), 29.6(+), 29.59(+), 29.54(+), 29.50(+), 29.2(+), 28.6(+), 27.2(-), 27.0(+), 26.6(-), 25.9(+), 25.5(-), 15.6(-).

Experiment 151: (*R*)-16-((*S*)-2,2-Dimethyl-[1,3]dioxolan-4-yl)-heptadecan-1-ol (338)



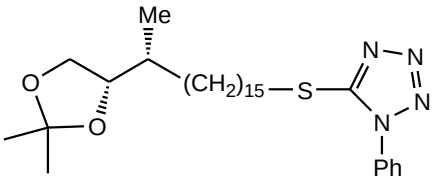
The procedure used in **Experiment 113** was repeated in order to remove the pivalate group (**337**) (5.0 g, 11.36 mmol) using KOH (9.56 g, 170.5 mmol) in a mixture of THF : MeOH : H_2O (10:10:1, 315 ml). The crude product was purified by column chromatography eluting with petrol / ethyl acetate (2:1) to give a white solid, *(R)-16-((S)-2,2-dimethyl-[1,3]dioxolan-4-yl)-heptadecan-1-ol* (**338**) (3.95 g, 97 %), $[\alpha]_D^{23}$ = + 19.3 (*c* 0.95, $CHCl_3$), {Found $(M + H)^+$: 357.3350, $C_{22}H_{45}O_3$ requires: 357.3363}. This showed ν_{max}/cm^{-1}: 3395, 2926, 2853, 1466, 1377, 1266, 1205, 1155, 1058; δ_H (C_6D_6): 3.84 (1H, dd, *J* 6.2, 7.7 Hz), 3.77 (1H, br.q, *J* 7.0 Hz), 3.50 (1H, br.t, *J* 7.7 Hz), 3.41 (2H, t, *J* 6.6 Hz), 1.52–1.47 (1H, m), 1.45 (3H, s), 1.43–1.40 (1H, m), 1.36 (3H, s), 1.34–1.15 (17H, m), 1.01 (3H, d, *J* 6.9 Hz); δ_C (C_6H_6): 108.7, 80.6(-), 68.2(+), 62.7(+), 37.0(-), 33.3(+), 33.2(+), 30.4(+), 30.2(+), 30.18(+), 30.15(+), 30.14(+), 30.12(+), 30.0(+), 27.4(+), 27.0(-), 26.3(+), 25.9(-), 15.9(-).

Experiment 152: (*S*)-4-((*R*)-16-Bromo-1-methyl-hexadecyl)-2,2-dimethyl-[1,3]dioxolane (339)



The procedure used in **Experiment 46** was repeated using the alcohol (**338**) (3.2 g, 8.99 mmol), NBS (2.03 g, 11.42 mmol), PPh_3 (2.71 g, 10.34 mmol), $NaHCO_3$ (0.25 g) in CH_2Cl_2 (130 ml). The crude product was purified by column chromatography eluting with petrol / ether (12:1) to give a colourless oil, *(S)-4-((R)-16-bromo-1-methyl-hexadecyl)-2,2-dimethyl-[1,3]dioxolane* (**339**) (3.45 g, 92 %), $[\alpha]_D^{26}$ = + 15.5 (*c* 1.05, $CHCl_3$), {Found $(M + H)^+$: 419.2503, $C_{22}H_{44}BrO_3$ requires: 419.2519}. This showed ν_{max}/cm^{-1}: 2926, 2854, 1465, 1369, 1250, 1214, 1162, 1066; δ_H: 4.00 (1H, dd, *J* 6.3, 7.9 Hz), 3.87 (1H, br.q, *J* 7.0 Hz), 3.60 (1H, br.t, *J* 7.9 Hz), 3.41 (2H, t, *J* 7.0 Hz), 1.86 (2H, quintet, *J* 7.9 Hz), 1.60–1.53 (1H, m), 1.44–1.40 (2H, m), 1.41 (3H, s), 1.36 (3H, s), 1.31–1.26 (23H, m), 1.11–1.06 (1H, m), 0.96 (3H, d, *J* 6.6 Hz); δ_C: 108.5, 80.4(-), 67.8(+), 36.5(-), 34.0(+), 32.8(+), 32.7(+), 29.9(+), 29.6(+), 29.59(+), 29.5(+), 29.4(+), 28.8(+), 28.2(+), 27.0(+), 26.6(-), 25.5(-), 15.6(-).

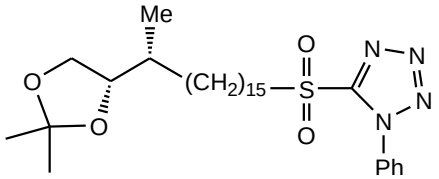
Experiment 153: 5-[(*R*)-16-((*S*)-2,2-Dimethyl-[1,3]dioxolan-4-yl)-heptadecylsulfanyl]-1-phenyl-1*H*-tetrazole (340)



The procedure used in **Experiment 23** was repeated using compound (**339**) (3.1 g, 7.40 mmol), 1-phenyl-1*H*-tetrazole-5-thiol (1.45 g, 8.14 mmol) and anhydrous potassium carbonate (2.15 g, 15.54 mmol) in acetone (120 ml). The crude product was purified by column chromatography eluting with petrol / ether (5:2) to give a colourless oil, *5-[(R)-16-((S)-2,2-dimethyl-[1,3]dioxolan-4-yl)-heptadecylsulfanyl]-1-phenyl-1H-tetrazole* (**340**) (3.63 g, 95 %), $[\alpha]_D^{23}$ = + 13.6 (*c* 0.88, $CHCl_3$), {Found $(M + Na)^+$: 539.3383, $C_{29}H_{48}N_4NaO_2S$ requires: 539.3390}. This showed ν_{max}/cm^{-1}: 2925, 2854, 1598, 1501, 1464, 1380, 1245, 1161, 1065; δ_H: 7.60–7.52 (5H, m), 4.00 (1H, dd, *J* 6.3, 7.9 Hz), 3.87 (1H, br.q, *J* 7.1 Hz), 3.60 (1H, br.t, *J* 7.6 Hz), 3.39 (2H, t, *J* 7.4 Hz),

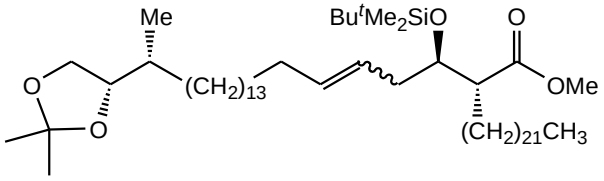
1.82 (2H, quintet, *J* 7.3 Hz), 1.58–1.53 (1H, m), 1.46–1.41 (2H, m), 1.40 (3H, s), 1.35 (3H, s), 1.33–1.25 (23H, m), 1.11–1.04 (1H, m), 0.96 (3H, d, *J* 6.6 Hz); δ_C: 154.5, 133.8, 130.0(-), 129.7(-), 123.8(-), 108.4, 80.4(-), 67.8(+), 36.5(-), 33.3(+), 32.7(+), 29.8(+), 29.6(+), 29.59(+), 29.51(+), 29.4(+), 29.1(+), 29.0(+), 28.6(+), 27.0(+), 26.6(-), 25.5(-), 15.6(-).

Experiment 154: 5-[(*R*)-16-((*S*)-2,2-Dimethyl-[1,3]dioxolan-4-yl)-heptadecane-1-sulfonyl]-1-phenyl-1*H*-tetrazole (306)



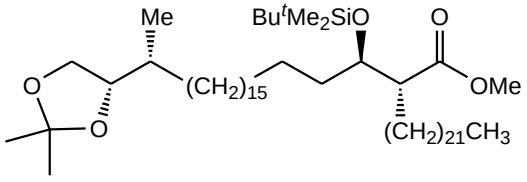
The procedure used in **Experiment 25** was repeated using the sulfane (**340**) (3.38 g, 6.55 mmol), ammonium molybdate (VI) tetrahydrate (3.81 g, 3.01 mmol) in 35 % H_2O_2 (8.5 ml) and THF (55 ml) and IMS (110 ml), and further ammonium molybdate (VI) tetrahydrate (2.3 g, 1.82 mmol) in 35% H_2O_2 (5.4 ml) to a crude product. The crude product was purified by column chromatography eluting with petrol / ether (3:2) to give a white solid, *5-[(R)-16-((S)-2,2-dimethyl-[1,3]dioxolan-4-yl)-heptadecane-1-sulfonyl]-1-phenyl-1H-tetrazole* (**306**) (2.90 g, 81 %), m.p.: 51–52 ^oC, $[\alpha]_D^{25}$ = + 12.6 (*c* 1.01, $CHCl_3$), {Found $(M + Na)^+$: 571.3268, $C_{29}H_{48}N_4NaO_4S$ requires: 571.3289; Found: C, 63.16; H, 8.71; N, 10.43, $C_{29}H_{48}N_4O_4S$ requires: C, 63.47; H, 8.82; N, 10.21}. This showed ν_{max}/cm^{-1}: 3069, 2917, 2854, 1594, 1498, 1473, 1356, 1259, 1209, 1150, 1064; δ_H: 7.71–7.69 (2H, m), 7.65–7.59 (3H, m), 4.00 (1H, dd, *J* 6.3, 7.9 Hz), 3.87 (1H, br.q, *J* 7.0 Hz), 3.75–3.72 (2H, m), 3.60 (1H, br.t, *J* 7.9 Hz), 1.99–1.92 (2H, m), 1.53–1.47 (2H, m), 1.41 (3H, s), 1.35 (3H, s), 1.32–1.26 (24H, m), 1.11–1.04 (1H, m), 0.96 (3H, d, *J* 6.6 Hz); δ_C: 153.5, 133.0, 131.4(-), 129.7(-), 125.0(-), 108.5, 80.4(-), 67.8(+), 56.0(+), 36.5(-), 33.7(+), 29.8(+), 29.6(+), 29.58(+), 29.5(+), 29.4(+), 29.2(+), 28.9(+), 28.1(+), 27.0(+), 26.6(-), 25.5(-), 21.9(+), 15.6(-).

Experiment 155: (*R*)-2-[(*E*/*Z*)-(1*R*,19*R*)-1-(*tert*-Butyl-dimethyl-silanyloxy)-19-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-icos-3-enyl]-tetracosanoic acid methyl ester (341)



The procedure used in **Experiment 72** was repeated using the aldehyde (**305**) (2.62 g, 4.61 mmol), sulfone (**306**) (2.78 g, 5.07 mmol) and lithium bis(trimethylsilyl) amide (8.5 ml, 8.76 mmol, 1.06M) in dry THF. The crude product was purified by column chromatography eluting with petrol / ether (15:1) to give a colourless oil, *(R)-2-[(E/Z)-(1R,19R)-1-(tert-butyl-dimethyl-silanyloxy)-19-((S)-2,2-dimethyl-[1,3]dioxolan-4-yl)-icos-3-enyl]-tetracosanoic acid methyl ester* (**341**) (3.55 g, 86 %) as a mixture of two isomers in ratio 2.2:1, {Found $(M + H)^+$: 891.8155, $C_{56}H_{111}O_5Si$ requires: 891.8195}. This showed ν_{max}/cm^{-1}: 2925, 2854, 1740, 1465, 1368, 1253, 1165, 1071; δ_H (two isomers): 5.50–5.39 (2H, m), 4.00 (1H, dd, *J* 6.3, 7.9 Hz), 3.98–3.89 (1H, m), 3.89 (1H, br.q, *J* 6.9 Hz), 3.66 (3H, s), 3.61 (1H, br.t, *J* 7.9 Hz), 2.56–2.50 (1H, m), 2.35–2.16 (2H, m), 2.01–1.98 (2H, m), 1.59–1.51 (3H, m), 1.41 (3H, s), 1.36 (3H, s), 1.39–1.26 (65H, m, v.br.), 1.12–1.07 (1H, m), 0.97 (3H, d, *J* 6.6 Hz), 0.89 (3H, t, *J* 6.9 Hz), 0.87 (9H, s), 0.06 (3H, s), 0.02 (3H, s); δ_C (major isomer): 175.1, 133.8(-), 124.8(-), 108.5, 80.4(-), 73.2(-), 67.8(+), 51.4(-), 51.16(-), 36.5(-), 32.8(+), 32.7(+), 31.9(+), 29.9(+), 29.7(+, v.br.), 29.66(+), 29.64(+), 29.62(+), 29.60(+), 29.57(+), 29.53(+), 29.49(+), 29.43(+), 29.36(+), 29.25(+), 27.7(+), 27.6(+), 27.0(+), 26.6(-), 25.7(-), 25.5(-), 22.7(+), 18.0, 15.6(-), 14.1(-), - 4.3 (-), - 5.0 (-); δ_C (minor isomer): 175.0, 132.1(-), 124.3(-), 73.24(-), 51.5(-), 51.18(-) (the remaining signals were obscured by the major isomer).

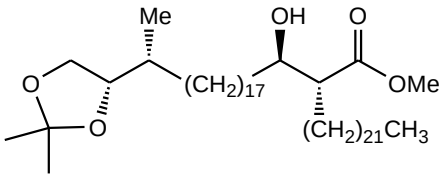
Experiment 156: (*R*)-2-[(1*R*,19*R*)-1-(*tert*-Butyl-dimethyl-silanyloxy)-19-((S)-2,2-dimethyl-[1,3]dioxolan-4-yl)-icosyl]-tetracosanoic acid methyl ester (307)



Palladium 10% on carbon (1.0 g) was added to a stirred solution of the alkene (**341**) (3.45 g, 3.88 mmol) in ethyl acetate (150 ml). Hydrogenation was carried out 1.5 hrs.

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 (15:1) to give a colourless oil, *(R)-2-[(1R,19R)-1-(tert-butyl-dimethyl-silanyloxy)-19-((S)-2,2-dimethyl-[1,3]dioxolan-4-yl)-icosyl]-tetracosanoic acid methyl ester* (**307**) (3.20 g, 96 %), $[\alpha]_D^{22}$ = + 3.6 (*c* 0.94, $CHCl_3$), {Found $(M + H)^+$: 893.8321, $C_{56}H_{113}O_5Si$ requires: 893.8352}. This showed ν_{max}/cm^{-1}: 2925, 2854, 1741, 1465, 1368, 1253, 1165, 1068; δ_H: 4.00 (1H, dd, *J* 6.3, 7.9 Hz), 3.93–3.90 (1H, m), 3.87 (1H, br.q, *J* 7.0 Hz), 3.66 (3H, s), 3.61 (1H, br.t, *J* 7.9 Hz), 2.53 (1H, ddd, *J* 3.8, 7.3, 11.1 Hz), 1.59–1.52 (4H, m), 1.41 (3H, s), 1.36 (3H, s), 1.35–1.26 (72H, m, v.br.), 1.10–1.07 (1H, m), 0.97 (3H, d, *J* 6.6 Hz), 0.89 (3H, t, *J* 6.9 Hz), 0.87 (9H, s), 0.05 (3H, s), 0.03 (3H, s); δ_C: 175.1, 108.5, 80.4(-), 73.2(-), 76.8(+), 51.6(-), 51.2(-), 36.5(-), 33.7(+), 32.7(+), 31.9(+), 29.9(+), 29.8(+), 29.7(+, v.br.), 29.66(+), 29.62(+), 29.60(+), 29.58(+), 29.5(+), 29.4(+), 29.3(+), 27.8(+), 27.5(+), 27.0(+), 26.6(-), 25.7(-), 25.5(-), 23.7(+), 22.7(+), 18.0, 15.6(-), 14.1(-), - 4.4(-), - 4.9(-).

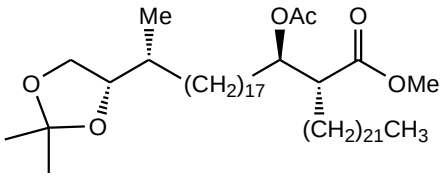
Experiment 157: (*R*)-2-[(1*R*,19*R*)-19-((*S*)-2,2-Dimethyl-[1,3]dioxolan-4-yl)-1-hydroxy-icosyl]-tetracosanoic acid methyl ester (342)



The procedure used in **Experiment 123** was repeated using the silyl ether (**307**) (3.22 g, 3.61 mmol), HF.pyridine (5.0 ml) and pyridine (1.2 ml) in THF (40 ml). The crude product was purified by column chromatography eluting with petrol / ether (5:2) to give a white solid, *(R)-2-[(1R,19R)-19-((S)-2,2-dimethyl-[1,3]dioxolan-4-yl)-1-hydroxy-icosyl]-tetracosanoic acid methyl ester* (**342**) (2.34 g, 83 %), m.p.: 67–68 ^oC, $[\alpha]_D^{25}$ = + 14.8 (*c* 0.97, $CHCl_3$), {Found $(M + H)^+$: 779.7450, $C_{50}H_{99}O_5$ requires: 779.7487}. This showed ν_{max}/cm^{-1}: 3520, 2924, 2854, 1710, 1461, 1377, 1264, 1189, 1164; δ_H: 4.00 (1H, dd, *J* 6.3, 7.9 Hz), 3.87 (1H, br.q, *J* 6.9 Hz), 3.71 (3H, s), 3.68–3.64 (1H, m), 3.60 (1H, br.t, *J* 7.7 Hz), 2.44 (1H, dt, *J* 5.4, 10.4 Hz), 1.74–1.26 (76H, m, v.br.), 1.41 (3H, s), 1.36 (3H, s), 1.11–1.07 (1H, m), 0.96 (3H, d, *J* 7.0 Hz), 0.88 (3H, t, *J* 7.0 Hz); δ_C: 176.2, 108.5, 80.4(-), 72.3(-), 67.8(+), 51.5(-), 50.9(-), 36.5(-), 35.7(+), 32.7(+), 31.9(+), 29.9(+), 29.7(+, v.br.), 29.65(+), 29.62(+), 29.59(+),

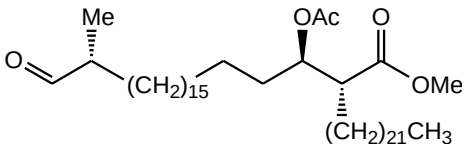
29.57(+), 29.55(+), 29.53(+), 29.48(+), 29.4(+), 29.3(+), 27.4(+), 27.0(+), 26.6(-), 25.7(+), 25.5(-), 22.7(+), 15.6(-), 14.1(-).

Experiment 158: (*R*)-2-[(1*R*,19*R*)-1-Acetoxy-19-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-icosyl]-tetracosanoic acid methyl ester (343)



The procedure used in **Experiment 117** was repeated using the alcohol (**342**) (2.24 g, 2.88 mmol), acetic anhydride (30 ml) and anhydrous pyridine (30 ml) in dry toluene (70 ml). The crude product was purified by column chromatography eluting with petrol / ether (5:1) to give a white solid, *(R)-2-[(1R,19R)-1-acetoxy-19-((S)-2,2-dimethyl-[1,3]dioxolan-4-yl)-icosyl]-tetracosanoic acid methyl ester* (**343**) (2.21 g, 94 %), m.p.: 37–38 ^{o}C, $[\alpha]_{D}^{25}$ = + 15.5 (*c* 1.03, $CHCl_3$), {Found $(M + Na)^{+}$: 843.7431, $C_{52}H_{110}NaO_6$ requires: 843.7412}. This showed ν_{max}/cm^{-1}: 2924, 2854, 1747, 1465, 1369, 1236, 1163, 1066; δ_H: 5.11–5.07 (1H, m), 4.01 (1H, dd, *J* 6.0, 7.9 Hz), 3.88 (1H, br.q, *J* 6.9 Hz), 3.69 (3H, s), 3.61 (1H, br.t, *J* 7.7 Hz), 2.62 (1H, ddd, *J* 4.4, 6.9, 10.9 Hz), 2.04 (3H, s), 1.62–1.26 (76H, m, v.br.), 1.41 (3H, s), 1.36 (3H, s), 1.13–1.06 (1H, m), 0.96 (3H, d, *J* 7.0 Hz), 0.89 (3H, t, *J* 7.0 Hz); δ_C: 173.6, 170.3, 108.5, 80.4(-), 74.1(-), 67.8(+), 51.5(-), 49.6(-), 36.5(-), 32.7(+), 31.9(+), 31.7(+), 29.9(+), 29.7(+, v.br.), 29.64(+), 29.61(+), 29.54(+), 29.46(+), 29.42(+), 29.4(+), 29.3(+), 28.1(+), 27.5(+), 27.0(+), 26.6(-), 25.5(-), 25.0(+), 22.7(+), 21.0(-), 15.6(-), 14.1(-).

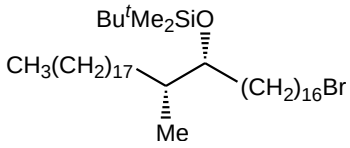
Experiment 159: (*R*)-2-((1*R*,19*R*)-1-Acetoxy-19-methyl-20-oxo-icosyl)-tetracosanoic acid methyl ester (308)



Periodic acid (1.0 g, 4.39 mmol) was added to a stirred solution of the acetal (**343**) (1.2 g, 1.46 mmol) in dry ether (60 ml) at r.t. under argon and stirred 18 hrs. The mixture was filtered through a bed of celite and washed with ether. The solvent was evaporated and the crude product was purified by column chromatography eluting with petrol / ethyl acetate (4:1) to give a white solid; *(R)-2-((1R,19R)-1-acetoxy-19-methyl-20-oxo-*

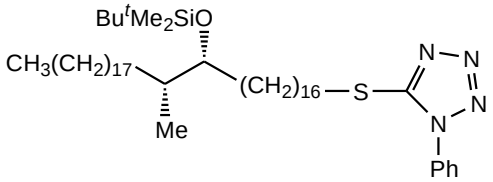
icosyl)-tetracosanoic acid methyl ester (**308**) (0.87 g, 80 %), m.p.: 31–31 ^{o}C, $[\alpha]_D^{23}$ = + 3.2 (*c* 0.69, $CHCl_3$), {Found $(M + Na)^+$: 771.6834, $C_{48}H_{92}NaO_5$ requires: 771.6837}. This showed ν_{max}/cm^{-1}: 2922, 2852, 1744, 1467, 1372, 1237, 1167, 1022; δ_H: 9.62 (1H, d, *J* 1.9 Hz), 5.11–5.07 (1H, m), 3.68 (3H, s), 2.62 (1H, ddd, *J* 4.1, 7.0, 10.4 Hz), 2.33 (1H, d sext, *J* 1.9, 7.0 Hz), 2.03 (3H, s), 1.73–1.26 (76H, m, v.br.), 1.09 (3H, d, *J* 7.0 Hz), 0.88 (3H, t, *J* 7.0 Hz); δ_C: 205.4(-), 173.7, 170.3, 74.1(-), 51.5(-), 49.6(-), 46.3(-), 31.9(+), 31.7(+), 30.5(+), 29.7(+, v.br.), 29.62(+), 29.57(+), 29.54(+), 29.45(+), 29.42(+), 29.4(+), 29.3(+), 28.1(+), 27.5(+), 26.9(+), 25.0(+), 22.7(+), 21.0(-), 14.1(-), 13.3(-).

Experiment 160: [(1*R*,2*R*)-1-(16-Bromo-hexadecyl)-2-methyl-icosyloxy]-*tert*-butyl-dimethyl-silane (344)



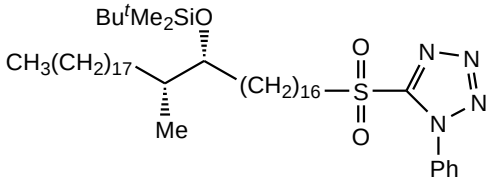
The procedure used in **Experiment 47** was repeated using the alcohol (**242**) (1.5 g, 2.25 mmol), NBS (0.51 g, 2.86 mmol) and PPh_3 (0.68 g, 2.59 mmol) in CH_2Cl_2 (40 ml). The crude product was purified by column chromatography eluting with petrol / ether (30:1) to give a colourless oil, *[(1R,2R)-1-(16-bromo-hexadecyl)-2-methyl-icosyloxy]-tert-butyl-dimethyl-silane* (**344**) (1.27 g, 77 %), $[\alpha]_D^{23}$ = + 4.6 (*c* 1.06, $CHCl_3$). This showed ν_{max}/cm^{-1}: 2925, 2854, 1464, 1361, 1253, 1076; δ_H: 3.52–3.48 (1H, m), 3.42 (2H, t, *J* 6.7 Hz), 1.86 (2H, quintet, *J* 7.0 Hz), 1.51–1.15 (62H, m, v.br.), 1.09–1.02 (1H, m), 0.91–0.88 (12H, m, including a s), 0.81 (3H, d, *J* 7.0 Hz), 0.04 (3H, s), 0.03 (3H, s); δ_C: 75.9(-), 37.7(-), 34.0(+), 33.5(+), 32.9(+), 32.5(+), 31.9(+), 30.0(+), 29.9(+), 29.7(+, v.br.), 29.68(+), 29.64(+), 29.6(+), 29.5(+), 29.4(+), 28.8(+), 28.2(+), 27.7(+), 26.0(-), 25.9(+), 22.7(+), 18.2, 14.4(-), 14.1(-), - 4.2(-), - 4.4(-).

Experiment 161: 5-[(17*R*,18*R*)-17-(*tert*-Butyl-dimethyl-silanyloxy)-18-methyl-hexatriacontylsulfanyl]-1-phenyl-1*H*-tetrazole (345)



The procedure used in **Experiment 23** was repeated using compound (**344**) (1.12 g, 1.54 mmol), 1-phenyl-1*H*-tetrazole-5-thiol (0.29 g, 1.61 mmol) and anhydrous potassium carbonate (0.45 g, 3.22 mmol) in acetone (50 ml) at 45 ^{o}C. The crude product was purified by column chromatography eluting with petrol / ether (5:1) to give a colourless oil, *5-[(17R,18R)-17-(tert-butyl-dimethyl-silanyloxy)-18-methyl-hexatriacontylsulfanyl]-1-phenyl-1H-tetrazole* (**345**) (1.13 g, 89 %), $[\alpha]_D^{23}$ = + 3.3 (*c* 0.92, $CHCl_3$), {Found $(M + H)^+$: 827.7021, $C_{50}H_{95}N_4OSSi$ requires: 827.6990}. This showed ν_{max}/cm^{-1}: 2924, 2854, 1599, 1501, 1464, 1385, 1250, 1075; δ_H: 7.61–7.52 (5H, m), 3.52–3.48 (1H, m), 3.40 (2H, t, *J* 7.4 Hz), 1.83 (2H, quintet, *J* 7.4 Hz), 1.50–1.15 (62H, m, v.br.), 1.08–1.01 (1H, m), 0.90–0.87 (12H, m, including a s), 0.81 (3H, d, *J* 6.7 Hz), 0.04 (3H, s), 0.03 (3H, s); δ_C: 154.5, 133.8, 130.0(-), 129.7(-), 123.9(-), 75.9(-), 37.7(-), 33.5(+), 33.4(+), 32.5(+), 31.9(+), 30.0(+), 29.9(+), 29.7(+, v.br.), 29.65(+), 29.62(+), 29.6(+), 29.4(+), 29.3(+), 29.1(+), 29.0(+), 28.6(+), 27.7(+), 26.0(-), 25.9(+), 22.7(+), 18.2, 14.4(-), 14.1(-), - 4.2(-).

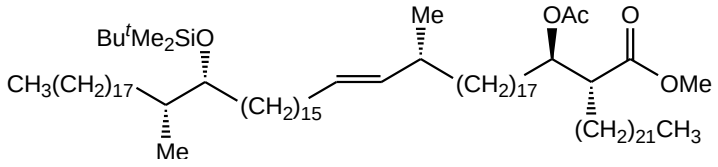
Experiment 162: 5-[(17*R*,18*R*)-17-(*tert*-Butyl-dimethyl-silanyloxy)-18-methyl-hexatriacontylsulfanyl]-1-phenyl-1*H*-tetrazole (309)



The procedure used in **Experiment 25** was repeated using the sulfane (**344**) (1.0 g, 1.21 mmol), ammonium molybdate (VI) tetrahydrate (1.0 g, 0.81 mmol) in 35 % H_2O_2 (2.3 ml) and THF (20 ml) and IMS (40 ml), and further ammonium molybdate (VI) tetrahydrate (0.4 g, 0.32 mmol) in 35% H_2O_2 (1.0 ml). The crude product was purified by column chromatography eluting with petrol / ether (9:2) to give a colourless oil, *5-[(17R,18R)-17-(tert-butyl-dimethyl-silanyloxy)-18-methyl-hexatriacontylsulfanyl]-1-phenyl-1H-tetrazole* (**309**) (0.90 g, 87 %), $[\alpha]_D^{23}$ = + 4.1 (*c* 1.28, $CHCl_3$), {Found (M +

Na)$^+$: 881.6693, $C_{50}H_{94}N_4NaO_3SSi$ requires: 881.6708}. This showed ν_{max}/cm^{-1}: 2925, 2854, 1596, 1499, 1464, 1343, 1253, 1153, 1076; δ_H: 7.72–7.70 (2H, m), 7.66–7.59 (3H, m), 3.76–3.72 (2H, m), 3.52–3.48 (1H, m), 1.99–1.93 (2H, m), 1.57–1.16 (62H, m, v.br.), 1.09–1.02 (1H, m), 0.90–0.88 (12H, m), 0.81 (3H, d, *J* 7.0 Hz), 0.04 (3H, s), 0.03 (3H, s); δ_C: 153.5, 133.1, 131.4(-), 129.7(-), 125.1(-), 75.9(-), 56.0(+), 37.7(-), 33.5(+), 32.5(+), 31.9(+), 30.0(+), 29.9(+), 29.7 (+, v.br.), 29.65(+), 29.57(+), 29.5(+), 29.4(+), 29.2(+), 28.9(+), 28.1(+), 27.7(+), 26.0(-), 25.9(+), 22.7(+), 21.9(+), 18.2, 14.4(-), 14.1(-), - 4.2(-), - 4.4(-).

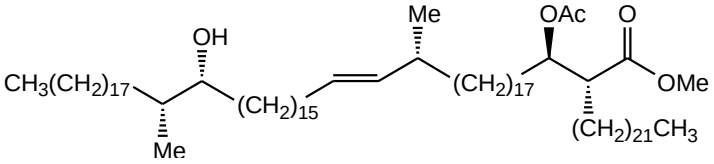
Experiment 163: *trans*-Alkene (310)



The aldehyde (**308**) (0.76 g, 1.02 mmol) in dry 1,2-dimethoxyethane (20 ml) was added to a stirred solution of the sulfone (**309**) (1.1 g, 1.27 mmol) in dry 1,2-dimethoxy ethane (40 ml) under nitrogen at r.t. The mixture was cooled to – 20 ^oC and potassium bis (trimethylsilyl) amide (4.06 ml, 2.03 mmol, 0.5 M in toluene) was added and the mixture was allowed to reach r.t. and stirred at r.t. for 3 hrs. Sat. aq. NH_4Cl (50 ml) and a mixture of petrol / ether (1:1, 100 ml) were added and extracted. The aq. layer re-extracted with petrol / ether (1:1, 2 x 80 ml), the combined organic layers were dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol / ether (18:1) to give a white solid, *compound* (**310**) (0.62 g, 44 %), m.p.: 25–26 ^oC, $[\alpha]_D^{22}$ = + 4.4 (*c* 0.77, $CHCl_3$), {Found (M + Na)$^+$: 1404.26, $C_{91}H_{180}NaO_5Si$ requires: 1404.35}. This showed ν_{max}/cm^{-1}: 2924, 2853, 1747, 1465, 1371, 1236, 1164, 1024; δ_H: 5.33 (1H, td, *J* 6.6, 15.1 Hz), 5.24 (1H, dd, *J* 7.6, 15.1 Hz), 5.09 (1H, ddd, *J* 4.1, 7.3, 8.2 Hz), 3.69 (3H, s), 3.50 (1H, dt, *J* 3.5, 6.3 Hz), 2.62 (1H, ddd, *J* 4.4, 7.0, 10.8 Hz), 2.04 (3H, s), 1.97 (2H, q, *J* 6.9 Hz), 1.68–1.26 (139H, m, v.br.), 1.08–1.01 (1H, m), 0.94 (3H, d, *J* 7.0 Hz), 0.90 (6H, t, *J* 6.7 Hz), 0.89 (9H, s), 0.80 (3H, d, *J* 7.0 Hz), 0.04 (3H, s), 0.03 (3H, s); δ_C: 173.6, 170.3, 136.5(-), 128.4(-), 75.9(-), 74.1(-), 51.5(-), 49.6(-), 37.7(-), 37.2(+), 36.7(-), 33.5(+), 32.6(+), 32.5(+), 31.9(+), 31.7(+), 30.0(+), 29.9(+), 29.8(+), 29.7(+, v.br.), 29.66(+), 29.63(+), 29.56(+), 29.54(+), 29.46(+), 29.44(+), 29.40(+), 29.36(+), 29.1(+), 28.1(+), 27.7(+),

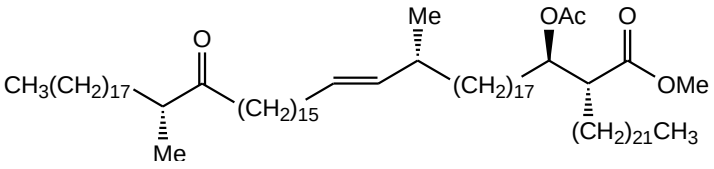
27.5(+), 27.4(+), 26.0(-), 25.9(+), 25.0(+), 22.7(+), 21.0(-), 20.9(-), 18.2, 14.4(-), 14.1(-), - 4.2(-), - 4.4(-).

Experiment 164: Hydroxy mycolic acid (346)



The silylated compound (**310**) (550 mg, 0.40 mmol) was dissolved in dry THF (20 ml) in a dry polyethylene vial under argon at r.t. and stirred. Pyridine (0.45 ml) and HF.Pyridine (1.8 ml) were added and the mixture was stirred for 18 hrs at 45 ^{o}C, when TLC showed complete reaction. The mixture was diluted with petrol / ether (1:1, 50 ml) and neutralized with sat. aq. $NaHCO_3$ until no more carbon dioxide was liberated, then extracted. The aqueous layer was re-extracted with petrol / ether (1:1, 2 x 50 ml). The combined organic layers were washed with brine and dried. The solvent was evaporated and the crude product was purified by column chromatography eluting with petrol / ether (4:1) to give a white solid, *hydroxy mycolic acid* (**346**) (460 mg, 91 %), m.p.: 38–39 ^{o}C, $[\alpha]_D^{24}$ = + 4.9 (*c* 0.85, $CHCl_3$), {Found $(M + Na)^+$: 1290.2653, $C_{85}H_{166}NaO_5$ requires: 1290.2627}. This showed ν_{max}/cm^{-1}: 3583, 2923, 2854, 1747, 1465, 1374, 1236, 1166; δ_H: 5.33 (1H, td, *J* 6.6, 15.5 Hz), 5.24 (1H, dd, *J* 7.6, 15.5 Hz), 5.09 (1H, dt, *J* 3.8, 7.9 Hz), 3.69 (3H, s), 3.52–3.49 (1H, m), 2.62 (1H, ddd, *J* 4.4, 7.0, 10.8 Hz), 2.04 (3H, s), 1.97 (2H, q, *J* 6.9 Hz), 1.63–1.26 (139H, m, v.br.), 1.18–1.14 (1H, m), 0.94 (3H, d, *J* 6.6 Hz), 0.89 (6H, t, *J* 6.7 Hz), 0.86 (3H, d, *J* 7.0 Hz); δ_C: 173.6, 170.3, 136.5(-), 128.4(-), 75.2(-), 74.1(-), 51.5(-), 49.6(-), 38.2(-), 37.2(+), 36.7(-), 34.5(+), 33.4(+), 32.6(+), 31.9(+), 31.7(+), 30.0(+), 29.8(+), 29.75(+), 29.7(+, v.br.), 29.65(+), 29.57(+), 29.54(+), 29.47(+), 29.44(+), 29.4(+), 29.3(+), 29.1(+), 28.1(+), 27.5(+), 27.4(+), 27.3(+), 26.3(+), 25.0(+), 22.7(+), 21.0(-), 20.9(-), 14.1(-), 13.6(-).

Experiment 165: Keto mycolic acid (311)



The hydroxy mycolic acid (**346**) (180 mg, 0.14 mmol) was dissolved in CH_2Cl_2 (10 ml) and added to a stirred solution of PCC (0.10 g, 0.43 mmol) in dichloromethane (30 ml) at r.t. Addition was done portionwise and during the addition a black colour appeared. The mixture was stirred for 3 hrs at r.t. when TLC showed complete reaction. It was diluted with ether (40 ml) and filtered through a bed of silica. The solvent was evaporated and the crude product was purified by column chromatography eluting with petrol / ether (6:1) to give a white solid, *keto mycolic acid* (**311**) (165 mg, 92 %), m.p.: 48–50 ^{o}C, $[\alpha]_D^{24} = -2.4$ (*c* 0.55, $CHCl_3$), {Found $(M + Na)^+$: 1288.2427, $C_{85}H_{164}O_5$ requires: 1288.2471}. This showed ν_{max}/cm^{-1}: 2922, 2852, 1746, 1715, 1467, 1372, 1236, 1165; δ_H: 5.33 (1H, td, *J* 6.6, 15.5 Hz), 5.24 (1H, dd, *J* 7.6, 15.5 Hz), 5.09 (1H, dt, *J* 3.8, 7.9 Hz), 3.69 (3H, s), 2.62 (1H, ddd, *J* 4.4, 7.0, 10.7 Hz), 2.50 (1H, sext, *J* 6.9 Hz), 2.43 (1H, dt, *J* 16.7, 7.3 Hz), 2.40 (1H, dt, *J* 16.7, 7.3 Hz), 2.04 (3H, s), 1.97 (2H, q, *J* 6.9 Hz), 1.63–1.14 (137H, m, v.br.), 1.05 (3H, d, *J* 6.9 Hz), 0.94 (3H, d, *J* 6.9 Hz), 0.89 (6H, t, *J* 7.0 Hz); δ_C: 215.1, 173.6, 170.3, 136.4(-), 128.4(-), 74.1(-), 51.5(-), 49.6(-), 46.3(-), 41.1(+), 37.2(+), 36.7(-), 33.0(+), 32.6(+), 31.9(+), 31.7(+), 29.8(+), 29.7(+, v.br.), 29.65(+), 29.63(+), 29.60(+), 29.55(+), 29.53(+), 29.50(+), 29.49(+), 29.46(+), 29.43(+), 29.38(+), 29.35(+), 29.3(+), 29.1(+), 28.1(+), 27.5(+), 27.4(+), 27.3(+), 25.0(+), 23.7(+), 22.7(+), 21.0(-), 20.9(-), 16.3(-), 14.1(-).

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