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Novel bio-active fatty acids

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Abstract

New methods were developed to produce thiolated analogues of Mycobacteria components. Thiolated tuberculostearic acid, (*S*)-18-mercapto-10-methyloctadecanoic acid, was firstly produced in seven steps in an overall yield of 7.6 %. This was followed by the first synthesis of a thiolated simple mycolic acid, the disulfide, ((2*R*,2'*R*,3*R*,3'*R*)-2,2'-(disulfanediyl*bis*(tetradecane-14,1-diyl))*bis*(3-hydroxyhenicosanoic acid, in 11 steps in an overall yield of 2.6 %. The first synthesis of a thiolated α-methyl-*trans*-cyclopropane methoxy mycolic acid was also achieved using the newly developed methods with the thiol introduced at two different positions within the molecule. (*S*,*S*,*S*,*R*,*S*,*R*,2*R*,2*R*')-26-26'-Disulfanediyl*bis*(2-((*R*)-1-hydroxy-19-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonadecyl)hexacosanoic acid, which includes the thiolated disulfide at the end of the α-alkyl chain, was synthesised in 18 steps from synthetically prepared starting materials in an overall yield of 2.6 %. (*S*)-2-((*S*)-1-Hydroxy-19-((1*R*,2*S*)-2-((2*R*,19*R*,20*R*)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonadecyl)-*N*-(2-((2-((*R*)-2-((*R*)-1-hydroxy-19-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatria-contan-2-yl)cyclopropyl)nonadecyl)hexacosanamido)ethyl)disulfanyl)ethyl)hexacosan-amide, which contais a thiolated linker on the carboxylic acid, was synthesised in two steps from the free synthetic mycolic acid in an overall yield of 8.7 %. The different methods attempted for the formation of the thiolated analogues are discussed.

To attempt to maximise the inhibitory effect of sterculic acid against *Plasmodium falciparum* Δ^9 desaturase, which is essential for parasite growth, analogues of sterculic acid were designed and synthesised. 7-(2-Octyl-cycloprop-1-enyl)-heptanoic acid methyl ester and 9-(2-octyl-cycloprop-1-enyl)-nonanoic acid methyl ester which contain one more and one less carbon atoms than sterculic acid in their chain lengths respectively were both synthesised in five steps in overall yields of 8 % and 4.6 % respectively. (±)-8-Methoxy-8-(2-octyl-cycloprop-1-enyl)-octanoic acid methyl ester was subsequently synthesised in three steps in an overall yield of 36 % whilst (±)-8-hydroxy-8-(2-octyl-cycloprop-1-enyl)-octanoic acid methyl ester was also synthesised in three steps in an overall yield of 25 %. In four steps both (±)-8-(*tert*-butyldimethylsilyloxy)-8-(2-octyl-cycloprop-1-enyl)-octanoic

acid methyl ester and (±)-8-acetoxy-8-(2-octyl-cycloprop-1-enyl)-octanoic acid methyl ester were synthesised in an overall yield of 22.7 % and 34.9 % respectively. The inhibitory effects of these analogues were investigated.

Abbreviations

°C – degree Celsius

Ac – acetyl

ACP – acyl carrier protein

AIBN – azobisisobutyronitrile

ATP – adenosine triphosphate

b – broad

BACTEC - bactenecin

BCG – Bacillus Calmette-Guérin

Bn – benzyl

BuLi – butyllithium

cetrimide – hexadecyltrimethylammonium bromide

CoA – coenzyme A

d – doublet

DCC – *N*,*N*'-dicyclohexylcarbodiimide

DEAD – diethyl azodicarboxylate

DEZn – diethylzinc

DIBAL – diisobutylaluminium hydride

DL – racemic

DMAP – 4-dimethylaminopyridine

DMF – dimethylformamide

DMSO – dimethyl sulfoxide

DNA – deoxyribonucleic acid

DOTS – Directly Observed Treatment Short course

EDC – 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide

ELISA – enzyme linked immunosorbent assay

Ether – diethyl ether

GC-MS – gas chromatography-mass spectroscopy

HIV – human immunodeficiency virus

HMPA – hexamethylphosphoramide

Hz – hertz

IMS – industrial methylated spirits

IR – infra-red

J – coupling constant

LAH – lithium aluminium hydride

LiHMDS – lithium *bis*(trimethylsilyl)amide

m – *meta*-

m – multiplet

m.p. – melting point

MALDI – Matrix-assisted laser desorption/ionization

MDR-TB – multidrug-resistant tuberculosis

2-MEA – 2-mercaptoethylamine hydrochloride

mmol – millimols

MS – mass spectrometry

NADPH – nicotinamide adenine dinucleotide phosphate

NBS – *N*-bromosuccinimide

NCS – *N*-chlorosuccinimide

NHS – *N*-hydroxysuccinimide

NMR – nuclear magnetic resonance

PCC – pyridinium chlorochromate

PCR – polymerase chain reaction

Petrol – petroleum spirit (boiling point 40 – 60 °C)

PPTS – pyridinium *p*-toluenesulfonate

PTSA – *p*-toluenesulfonic acid monohydrate

PVCl – pivaloyl chloride

Py – pyridine

q – quartet

RNA – ribonucleic acid

s – singlet

t – triplet

TBAH – tetrabutylammonium hydroxide

TBAF – tetra-n-butylammonium fluoride

TBSA – tuberculostearic acid

THF – tetrahydrofuran

THP – tetrahydropyranyl

TLC – thin layer chromatography

TsCl – p-toluenesulfonyl chloride

UNICEF – United Nations Children's Fund (Previously United Nations International Children's Emergency Fund)

WHO – World Health Organisation

XDR-TB – extensively drug-resistance tuberculosis

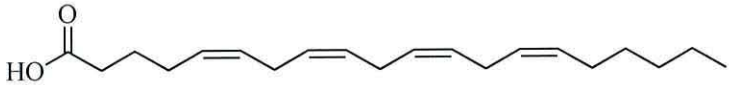
1. Introduction

1.1 - Fatty Acids

Fatty acids are a widely occurring group of compounds that are of significant biological importance. They generally consist of a carboxylic acid and a straight long alkyl chain and predominantly contain an even number of carbon atoms, typically between ten and twenty. Common fatty acids are saturated, unsaturated or polyunsaturated with the configuration of the alkene units generally *cis* interrupted by a methylene group in a divinyl methane or skip conjugated pattern.

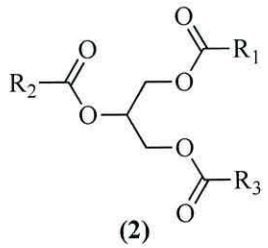
1.1.1 - Roles of Fatty Acids

Fatty acids have three major roles within a biological system. Firstly, they act as chemical transmitters and hormones, which control a variety of processes within biological systems. Some examples of these are prostaglandins and thromboxanes, which are derived from the polyunsaturated fatty acid arachidonic acid **(1)**.[1] Prostaglandins control a wide range of physiological and pharmacological effects, whilst thromboxanes have a major role in clot formation.



(1)

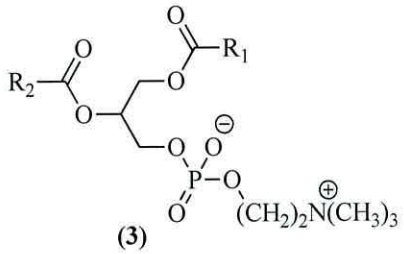
Secondly, and most commonly, they take the form of triglycerides **(2)**.[2] Triglycerides **(2)** consist of a glycerol backbone and three, often different, fatty acid chains and are highly concentrated, stable sources of metabolic energy. This is due to their highly reduced and non polar nature. From the complete oxidation of triglycerides **(2)** the energy yield is around 39 kJg^{-1} in comparison with 13 kJg^{-1} for carbohydrates and proteins.



(2)

Finally, fatty acids are present as membrane constituents in the form of phospholipids and glycolipids, which surround all living cells. Complex eukaryotic cells are composed of up

to 80 % lipid, the majority being phospholipid. Phospholipids that are derived from glycerols are called phosphoglycerides **(3)**. These contain a glycerol backbone bound by an ester link to two fatty acid chains and a phosphorylated alcohol. The phosphate group is linked to an amino acid such as choline.



(3)

Phospholipid molecules are amphipathic, possessing a polar head group and a hydrophobic fatty acid tail. In aqueous media the phospholipids form a bilayer structure with the polar head group being external to the membrane and facing the aqueous media. These are held together by electrostatic interactions. The non polar tails remain internal and are held together by Van der Waals interactions. The viscosity across the cell membrane is affected by two factors concerning these fatty acids. The membrane becomes less permeable as the chain length becomes longer as there are more Van der Waals forces present. However, introducing a double bond to the system causes a kink of around 30 °, which leads to a more open arrangement that results in less efficient packing and weaker Van der Waals interactions, making the membrane more permeable (Figure 1).[3]

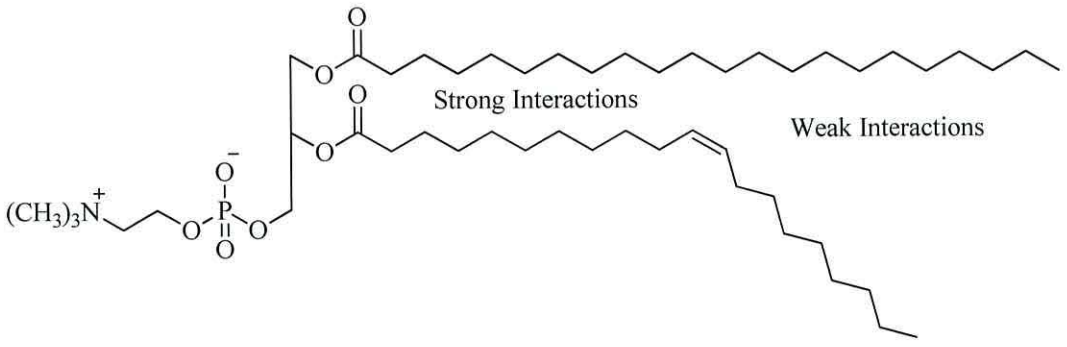
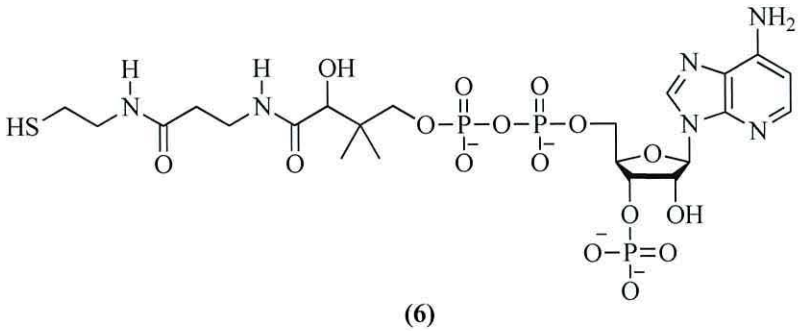


Figure 1: A model for the effects of straight chains and unsaturation on the Van der Waals interactions between two fatty acid chains.

1.1.2 - Saturated Fatty Acid Biosynthesis

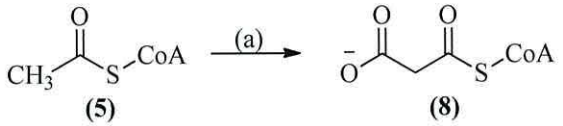
Fatty acids predominantly contain an even number of carbon atoms due to the mechanism of their biosynthesis. In 1907 it was firstly suggested that the fatty acid hydrocarbon chains

were composed of linked two carbon units[4] but it wasn't until the development of ^{13}C NMR that the two carbon unit was found to be acetic acid **(4)**.[5] It was later discovered that acetyl-CoA **(5)**, the thioester of Coenzyme A **(6)** was the source of the two carbon unit.[6] For unusual fatty acids that contain an odd number of carbon atoms, propionyl CoA **(7)** is the starting unit.[7]



(6)

It has been demonstrated that fatty acid biosynthesis requires ATP as an energy source, NADPH as an electron donor and bicarbonate as a catalyst.[8] The *de novo* biosynthesis also requires the action of two multifunctional enzymes, acetyl-CoA carboxylase and fatty acid synthetase.[9] Acetyl-CoA carboxylase is responsible for the irreversible carboxylation of acetyl-CoA **(5)** to malonyl-CoA **(8)** and is the initial step of the biosynthesis (Scheme 1).

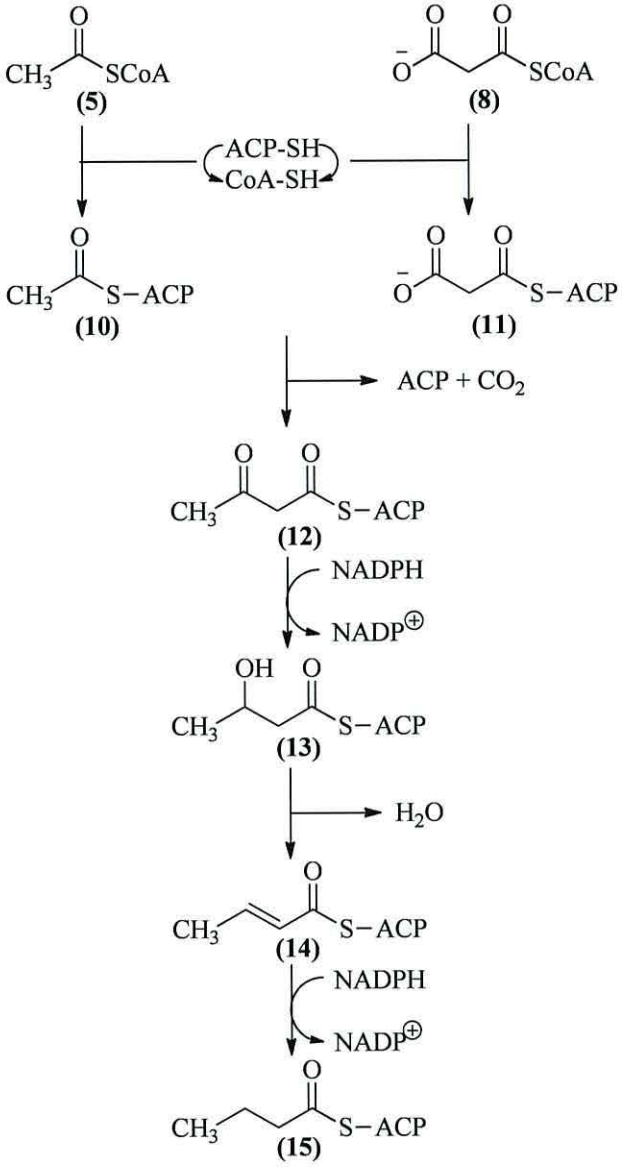


(5) **(8)**

Scheme 1: Biosynthesis of malonyl-CoA **(8)**. (a) Acetyl-CoA carboxylase, ATP, HCO_3^-.

Following carboxylation, the remaining reactions in the synthesis of palmitate **(9)** are catalysed by the fatty acid synthetase enzymes. The mechanism of biosynthesis of saturated fatty acids in this stage in *E. coli* has been elucidated (Scheme 2).[10] The first stage involves the formation of the acyl-ACP intermediates **(10)**, **(11)** from their respective acyl-CoA's **(5)**, **(8)**. The β-ketoester, acetoacetyl-ACP **(12)** can then be formed by a condensation reaction between the activated acyl-ACP **(10)** and malonyl-ACP **(11)**. D-β-Hydroxybutyryl-ACP **(13)** is then formed by the reduction of the β-ketoester **(12)** followed by dehydration to form α,β-*trans*-Δ^2-butenoyl-ACP **(14)**. Finally α,β-*trans*-Δ^2-butenoyl-ACP **(14)** is reduced to butyryl-ACP **(15)**. The process may then be repeated by condensing butyryl-ACP **(15)** with another molecule of malonyl-ACP **(11)** in order to

extend the chain further. This process can be repeated until the 16 carbon atom palmitoyl-ACP is generated, from which free palmitate **(9)** can be released.



Scheme 2: Mechanism of biosynthesis in *Escherichia coli.*

In order to synthesise fatty acids with chain lengths of longer than 16 carbon atoms, a chain elongation system is required. This process can happen in the endoplasmic reticulum and in the mitochondria[11] and is believed to occur in a very similar way to the *de novo* synthesis. The main differences are believed to be that in the endoplasmic reticulum the two carbon unit is derived from malonyl-CoA **(8)** and that different enzymes also catalyse this process.

1.1.3 - Unsaturated Fatty Acid Biosynthesis

Unsaturated fatty acids are formed by the oxidative desaturation of saturated fatty acids by the action of an oxygen-dependent multi-enzyme complex. This multi-enzyme complex is known as the fatty acid desaturation system. Each desaturase enzyme is different depending on where along the chain they introduce the double bond and each enzyme is named according to this position. Although there are several different desaturase systems, the Δ^9 desaturase enzyme is the most widely studied.

It was firstly determined that unsaturated fatty acids were formed from their saturated precursors in 1936 following deuterium labelling experiments.[12] This study was then extended to show that palmitic acid **(16)** is directly converted into palmitoleic acid **(17)**.[13] Following studies using cell free extracts of yeast it was demonstrated that it is the Coenzyme A of the saturated fatty acid that is converted into the Coenzyme A of the monounsaturated fatty acid using molecular oxygen and NADPH as co factors.[14] The desaturase system consists of a membrane bound three protein enzyme. These are NADPH-cytochrome b_5 reductase, cytochrome b_5 and a terminal desaturase component.[15] It is believed that each desaturase enzyme contains NADPH-cytochrome b_5 reductase and cytochrome b_5, which are involved in the electron transfer but that they differ in the terminal desaturase unit, which is responsible for the actual conversion. This belief was strengthened when it was discovered that cytochrome b_5 was a component of Δ^6 desaturase as well as Δ^9 desaturase.[16] The mechanism of desaturation is believed to proceed *via* a syn-elimination of a vicinal pair of pro-*R* protons to generate a *cis* double bond (Scheme 3).[7]

(18) **(19)**

Scheme 3: Biosynthesis of oleoyl-CoA **(19)** from stearoyl-CoA **(18)**.

It was shown during the investigation of the desaturation of stearic acid **(20)** to oleic acid **(21)** using isotopic labelling experiments that only the hydrogen atoms on C_9 and C_{10} were removed during desaturation.[17] It was also suggested that the hydrogen removal was a stepwise process as an isotope effect was seen for the loss of a hydrogen atom only at the C_9 position. This led to the suggestion that the first step may be a hydroxylation at this carbon followed by the elimination of water forming a double bond. However, the Δ^9

desaturase enzyme could not desaturate 9- or 10-hydroxystearyl-CoA.[18] There are alternative theories for the mechanism of unsaturation. Again, following isotopic labelling experiments a substantial isotope effect was seen at both C_9 and C_{10} positions, which led to the suggestion that the mechanism of desaturation was concerted.[19] Generally the first double bond is introduced to a saturated fatty acid precursor at the Δ^9 position. The monounsaturated fatty acid produced may then undergo further desaturation and chain elongation (Figure 2). Higher plants and fungi may then desaturate at certain positions above Δ^9 in order to form polyunsaturated fatty acids, whilst animals may only desaturate further at specific positions below Δ^9. This shows the importance of consuming essential fatty acid through diet, as the human body is not capable of synthesising these fatty acids. As expected, there are a few exceptions to this rule as a house cricket was found to have the ability to desaturate at the Δ^{12} position.[20]

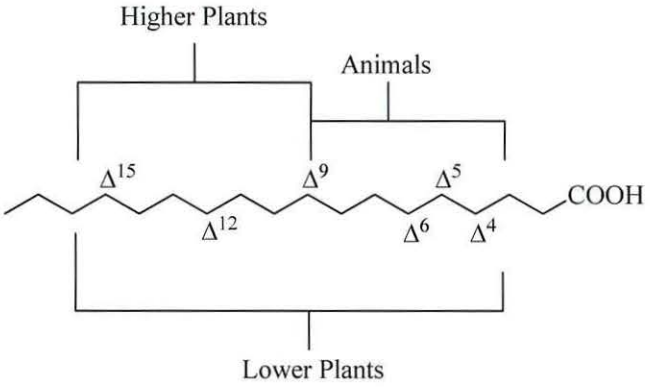
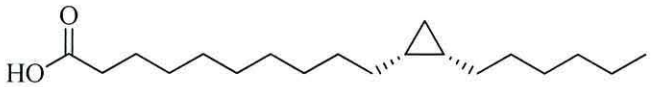


Figure 2: Positions where various organisms are able to generate a double bond.

1.2 - Cyclopropane Fatty Acids

Several naturally occurring cyclopropane-containing fatty acids have been isolated, with the most common being lactobacillic acid **(22)**. Lactobacillic acid **(22)** was first discovered in 1950 during the analysis of the fatty acid content of *Lactobacillus arabinosus* and was named accordingly.[21] It was believed to be a branched fatty acid with the chemical composition being $C_{19}H_{36}O_2$. Subsequently, on the basis of its melting point, infrared absorption, X-ray diffraction data and its chemical transformation it was believed to contain a cyclopropane ring, although its exact position within the chain remained unknown. The presence of the cyclopropane ring was confirmed in 1954 as a close correlation of its chemical behaviour to the synthetically prepared *trans*-DL-11,12-methyleneoctadecanoic acid was observed.[22] This work also suggested that the ring was

present between the C_{11} and C_{12} positions but this was not confirmed until later X-ray diffraction studies.[23] The absolute structure of lactobacillic acid **(22)** however remained unknown until 1972.[24, 25] Lactobacillic acid **(22)** was oxidised to an α-cyclopropyl ketone that was similar to ketones with a known configuration and compared by circular dichroism identifying the structure as 11*R*,12*S*-methyleneoctadecanoic acid. Following the synthesis of 11*R*,12*S*-methyleneoctadecanoic acid and its enantiomer the absolute stereochemistry was further confirmed.[26]

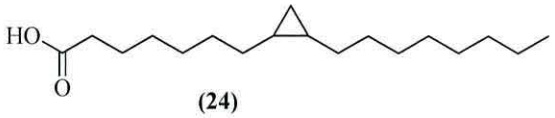


(22)

1.2.1 - Occurrence of Cyclopropane Fatty Acids

With the development of gas chromatography, cyclopropane fatty acids were discovered in many bacteria with C_{19} and C_{17} cyclopropane fatty acids being the most abundant. Dihydrosterculic acid **(23)**,[27] another C_{19} cyclopropane fatty acid that is a positional isomer of lactobacillic acid **(22)**, was found to be very abundant in the seed oil of *Euphoria longana*. Analysis of the seed oil of *Litchi chinensis* was also seen to contain a staggering 41 % of dihydrosterculic acid **(23)**.[28] A closely related species, dihydromalvalic acid **(24)** has also been isolated from a variety of plant species as well as other, shorter chains such as *cis*-7*R*,8*S*-methylenehexadecanoic acid **(25)**, *cis*-5,6-methylene-tetradecanoic acid **(26)** and *cis*-3,4-methylenedodecanoic acid **(27)**.[29] Although much less common, some *trans* cyclopropane fatty acids have also been isolated like cascarillic acid **(28)**.[30]

(23)



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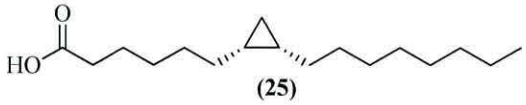

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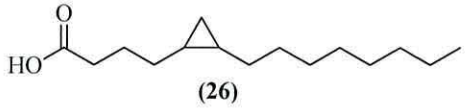

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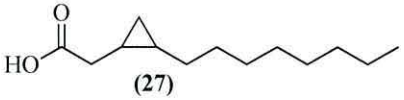

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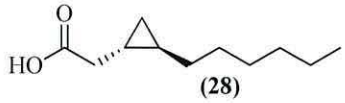
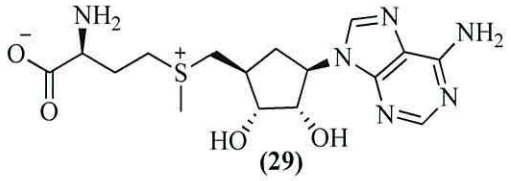


(28)

1.2.2 - Biosynthesis

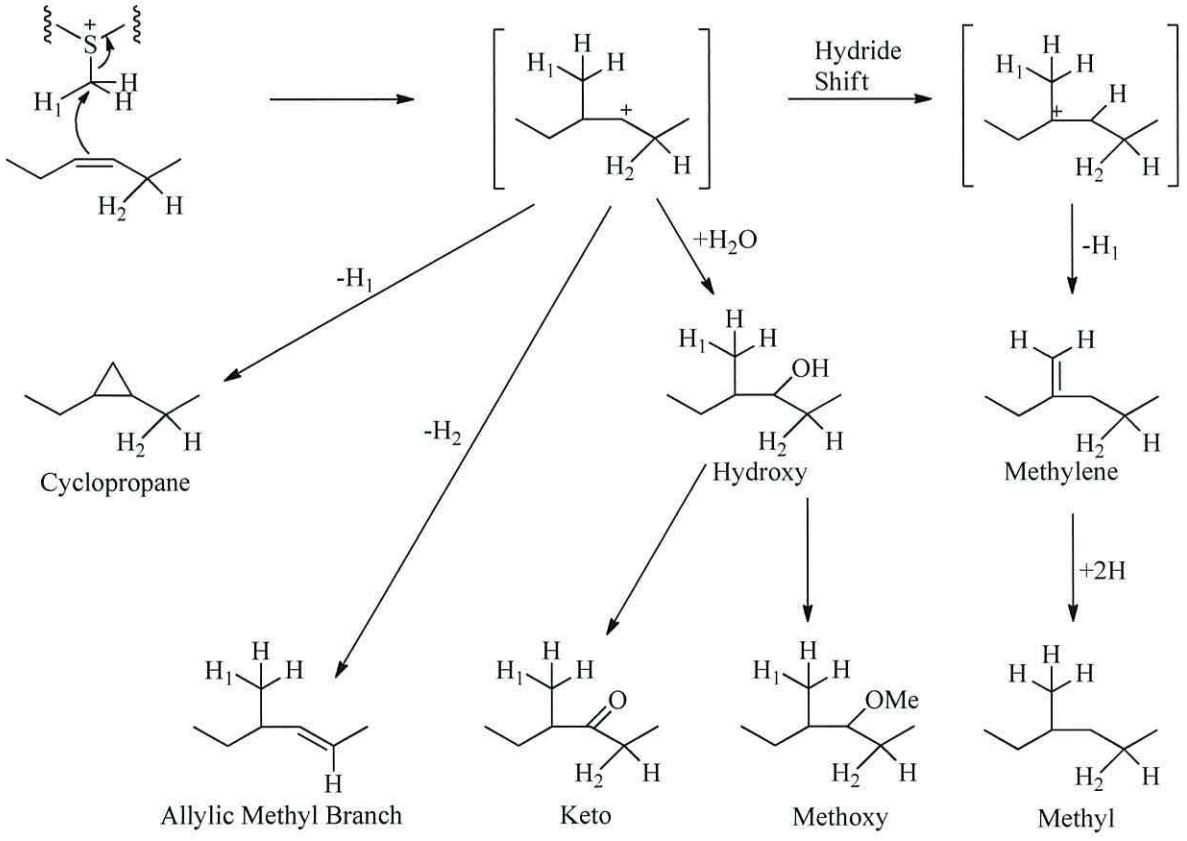
The biosynthesis of cyclopropane fatty acids has been widely studied and the mechanism is well established. Very soon after the discovery of lactobacillic acid **(22)** it was believed that the cyclopropane fatty acids may be synthesised from their unsaturated precursor with the addition of a one carbon unit.[31] It was soon confirmed that the single carbon unit was

derived from *S*-adenosyl methionine **(29)** with a variety of isotopic labelling experiments.[32] It was also demonstrated that the biosynthesis of the ring occurred from its unsaturated precursor whilst it was part of a phospholipid molecule.[33] Cyclopropane biosynthesis, however, shows no preference as to the position of unsaturation within the phospholipid.



(29)

In 1969 a mechanism was proposed for the biosynthesis of cyclopropane fatty acids[34] and the currently accepted mechanism is based on these findings (Scheme 4).[35] The mechanism proposed also accounts for the biosynthesis of methoxy, methylene, keto, methyl and allylic methyl branched fatty acids. For the biosynthesis of a cyclopropane fatty acid the double bond is firstly methylated with *S*-adenosyl methionine **(29)** and leads to the first carbocation. Abstraction of the methyl proton from the carbocation then generates the cyclopropane ring. This carbocation can be quenched in a variety of different ways to result in the different types of substituents stated earlier on the fatty acid.



Scheme 4: Mechanism of the addition of a one carbon unit to a double bond.

1.2.3 - Mycolic Acids

From the high lipid content of the cell wall of tuberculosis bacilli, a hydroxy acid with a high molecular weight was isolated.[36] This hydroxy acid was named mycolic acid and believed to have an empirical formulae of either $C_{88}H_{172}O_4$ or $C_{88}H_{176}O_4$.[37] Mycolic acid was believed to also contain a methoxy group and have low dextro-rotation. Work by Asselineau and Lederer confirmed that the hydroxy group was present at the β position with respect to the acid and a long alkyl chain was present at the α-position. It was also confirmed that mycolic acid did indeed have a high molecular weight.[38] With the advancement in spectroscopic techniques in the 1960s came more knowledge about the structural features of mycolic acids. It was then known that what was initially believed to be a single compound was actually a mixture of numerous different compounds. Therefore, before attempting to identify the structural features, the natural mixtures of mycolic acids were firstly separated into three separate fractions by chromatography.[39] Using a variety of analytical techniques, as well as chemical analysis, the specific features within the fractions were then analysed. The first fraction, named α-mycolic acids appeared to contain only cyclopropane rings in a *cis* configuration and it was on the analysis of this fraction that the first proposed complete structure of a mycolic acid was based.[40] The second fraction, named β-mycolic acids contained a methoxy group and a cyclopropane ring in both the *cis* and *trans* configuration and the third fraction, named γ-mycolic acids contained a keto group as well as a cyclopropane ring again in both the *cis* and *trans* configurations.[41] It was observed that the *trans* cyclopropane group, the keto group and the methoxy groups were also associated with a methyl group on the adjacent carbon. Based on the early separations of mycolic acids into the three sub classes by chromatography, major mycolic acid classes are still considered as either α, methoxy or keto.[42] There are some differences however, as mycolic acids have now been discovered to contain different functional groups such as *cis* and *trans* olefins, hydroxyl groups and epoxy groups to name only a few.[43-45] Mycolic acids do not only differ in the functional groups they include but they also differ in their chain length. This has led to the belief that over 500 mycolic acids may exist[46] and has also made the identification of different mycolic acids extremely difficult due the problems in separating the mycolic acids of different chain lengths. Watanabe *et al.*[47] have, however, successfully catalogued over 50 different mycolic acids with accurate chain lengths. The structure of a mycolic acid is now considered as two parts, the mycolic motif and the meromycolate moiety (Figure 3). The mycolic motif consists of the terminal acid group, the β-hydroxyl and the α-branch. This unit is relatively

constant in all mycolic acids, the difference only being in the length of the alkyl chain. The remaining functionality is found in the meromycolate chain. Two functional groups are found in the meromycolate moiety at the distal [X] and proximal [Y] positions. These groups are separated by alkyl chains of various lengths.

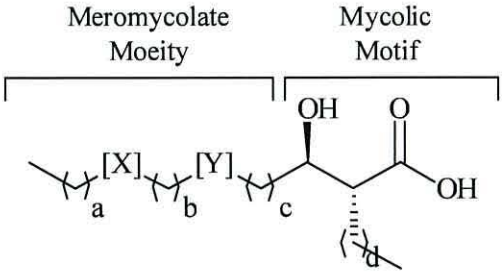


Figure 3: A generic mycolic acid.

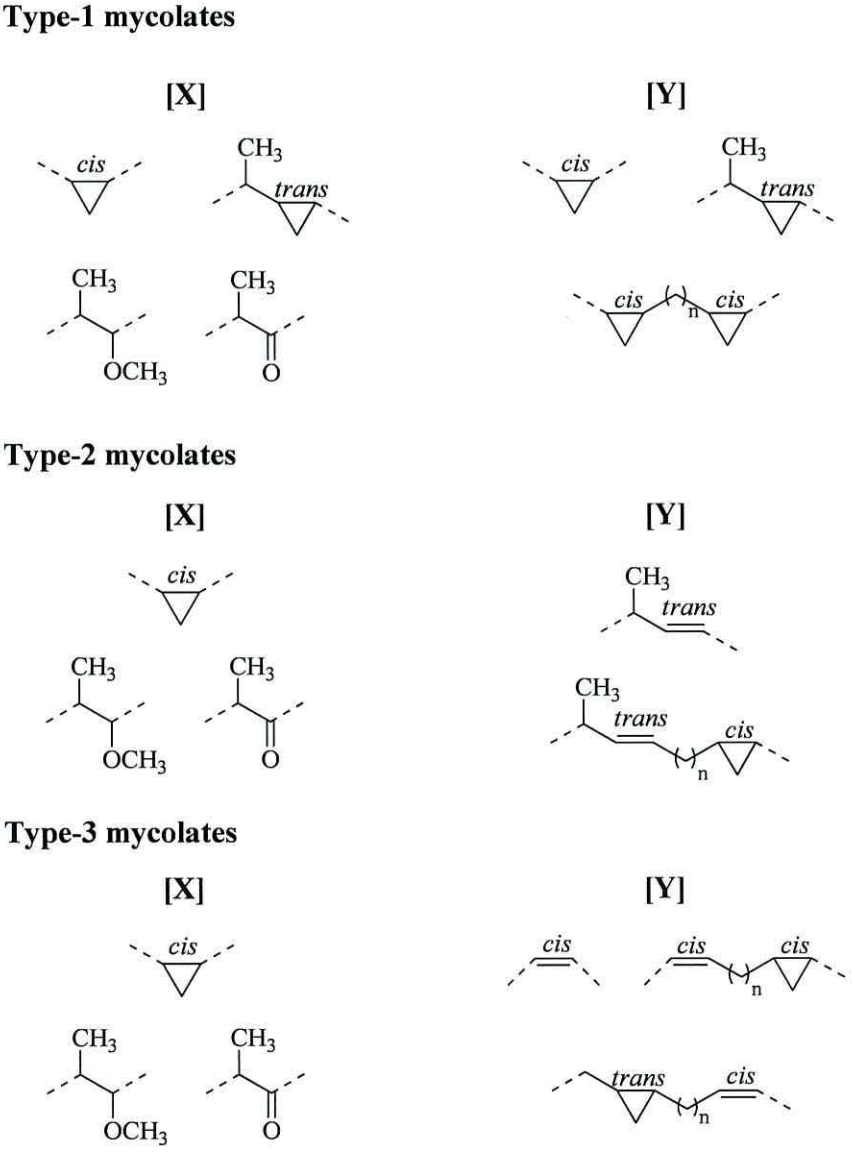


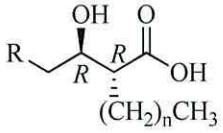
Figure 4: The generalised structures of type-1, type-2 and type-3 mycolic acids.

As there are so many different mycolic acids in existence, a broad classification system was generated to describe generalised structures of major mycolic acids (Figure 4).[45] This system shows the likely combinations of functional groups that may be present in mycolic acids. The major mycolic acids were separated into three types, type 1, type 2 and type 3. Type 1 mycolic acids contain no double bonds, type 2 mycolic acids contain *trans* double bonds and type 3 mycolic acids contain *cis* double bonds.

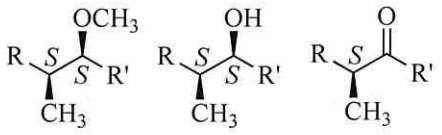
Mycolic acids are believed to exist in a triple folded, four chain segment conformation[48] and it is the functional groups within the mycolic acid structure that allow the chain to fold up. It is believed that the β-hydroxyl and the distal functional group are associated with the hydrophilic surface whilst the two terminal methyl groups as well as the proximal functional group are associated with the hydrophobic unit. Changes do occur in the mycolic acid conformation depending on the environmental conditions and these changes are dependent on the functional groups present.[49] A keto mycolic acid is believed to be able to withstand most external factors and remain in its constrained W configuration whilst α and methoxy mycolic acids are more likely to extend with higher temperature and surface pressure. Differences in the stereochemistry of the cyclopropane ring however are not believed to have any effect on the folding of the mycolic acid[50] but some differences may be observed due to differences in chain lengths.[51]

1.2.3.1 - Stereochemistry

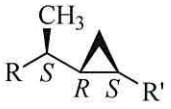
Much work has been carried out in order to determine the absolute stereochemistries of various mycolic acids. For the α-alkyl, β-hydroxy fragment it has been determined that in all cases these groups are in an *R*,*R* configuration.[52] It is the configurations of these two groups that are necessary for T cell recognition and subsequently for generating an immune response.[53]



For the oxygenated mycolic acids there is some evidence that the oxygenated functional groups as well as the methyl branch are in an *S*, *S* configuration.[54] This provides a biosynthetic link between the three oxygenated groups.



Not much is known about the configuration of the *cis* cyclopropane unit but the α-methyl-*trans*-cyclopropane unit was discovered to be in an *S*, *R*, *S* configuration.[55]

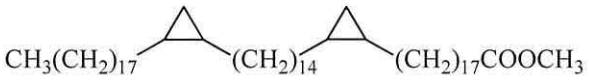


1.2.3.2 - Biosynthesis

The biosynthesis of the various functional groups that are part of the mycolic acids occur from the same intermediate as for the biosynthesis of a cyclopropane ring (see Scheme 4, page 8) where the carbocation intermediate is quenched in a variety of different ways to produce various substituents.[35] This mechanism accounts for the biosynthesis of all of the major functional groups present in mycolic acids apart from the α-methyl-*trans*-cyclopropane unit. This may be formed by converting the double bond of the allylic methyl branch into a *trans*-cyclopropane by the action of an unknown methylene transferase enzyme. The mycolic motif and the meromycolate fragment are believed to be biosynthesised separately and a full mycolic acid may be formed following a Claisen type condensation reaction between the two fatty acid moieties.[56] Not much more is currently known about the biosynthesis of mycolic acids although further understanding of the biosynthetic pathway might provide alternative tools in order to combat disease.

1.2.3.3 - Mycolic Acid Synthesis

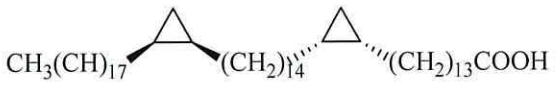
In 1977, one of the first syntheses of a major fragment of a mycolic acid was reported by Gensler *et al.*[57] A meromycolate fragment **(30)** was synthesised that contained two *cis*-cyclopropane rings of non-specific absolute stereochemistry, resulting in a mixture of four different isomers.



(30)

The first enantiomerically pure meromycolic fragment **(31)** was not synthesised until 2000 by Al Dulayymi *et al.*[58] This structure was the same as Gensler's structure in that it

contained two *cis*-cyclopropane rings in the distal and proximal positions but differed in the number of carbon atoms in the chain length.



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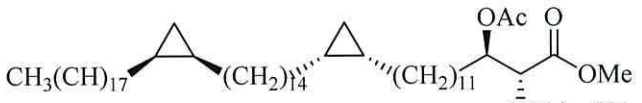
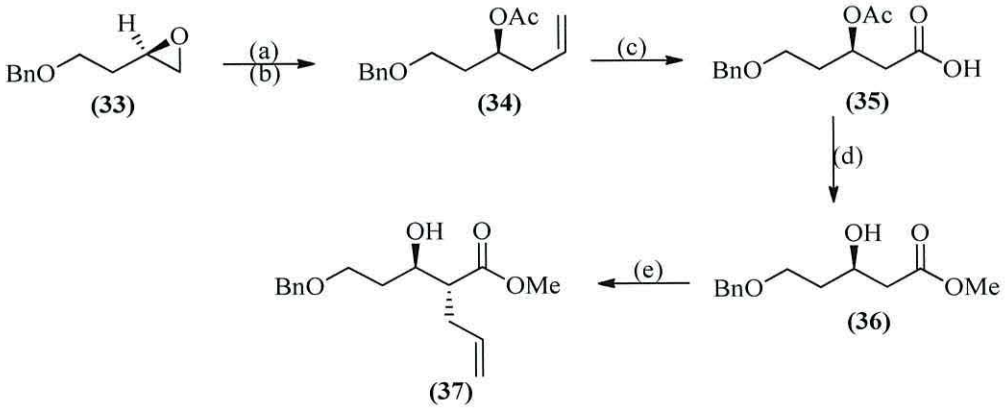
Quickly following the synthesis of the first enantiomerically pure meromycolic acid came the first synthesis of a complete enantiomerically pure mycolic acid **(32)**.[59]



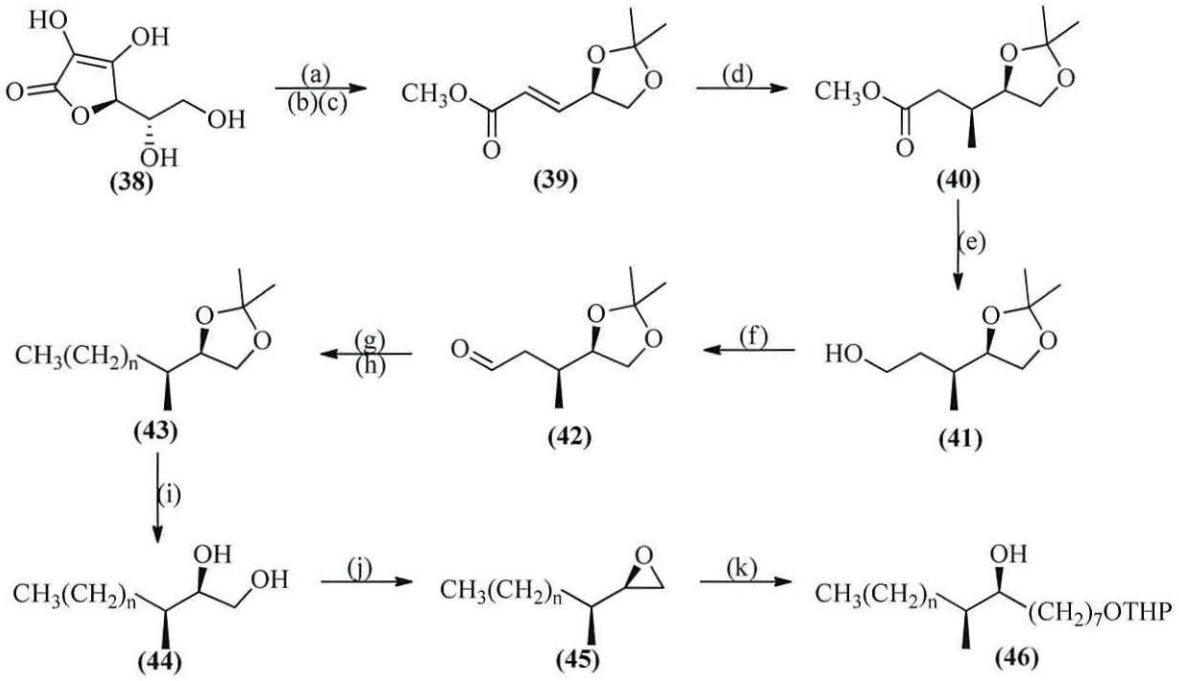
(32)

Various other mycolic acids have since been synthesised with single absolute stereochemistries.[55, 60] In order to synthesise more mycolic acids, various fragments have been synthesised on a large scale in order to utilise them in the synthesis of numerous different mycolic acids. As the mycolic motif is constant in all mycolic acids apart from the length of the α-alkyl chain, unit **(37)** has been synthesised that contains all the required features but allows for variation in the length of the α-alkyl chain (Sheme 5). This unit **(37)** may be coupled to any meromycolate fragment in order to generate a full mycolic acid.[61]



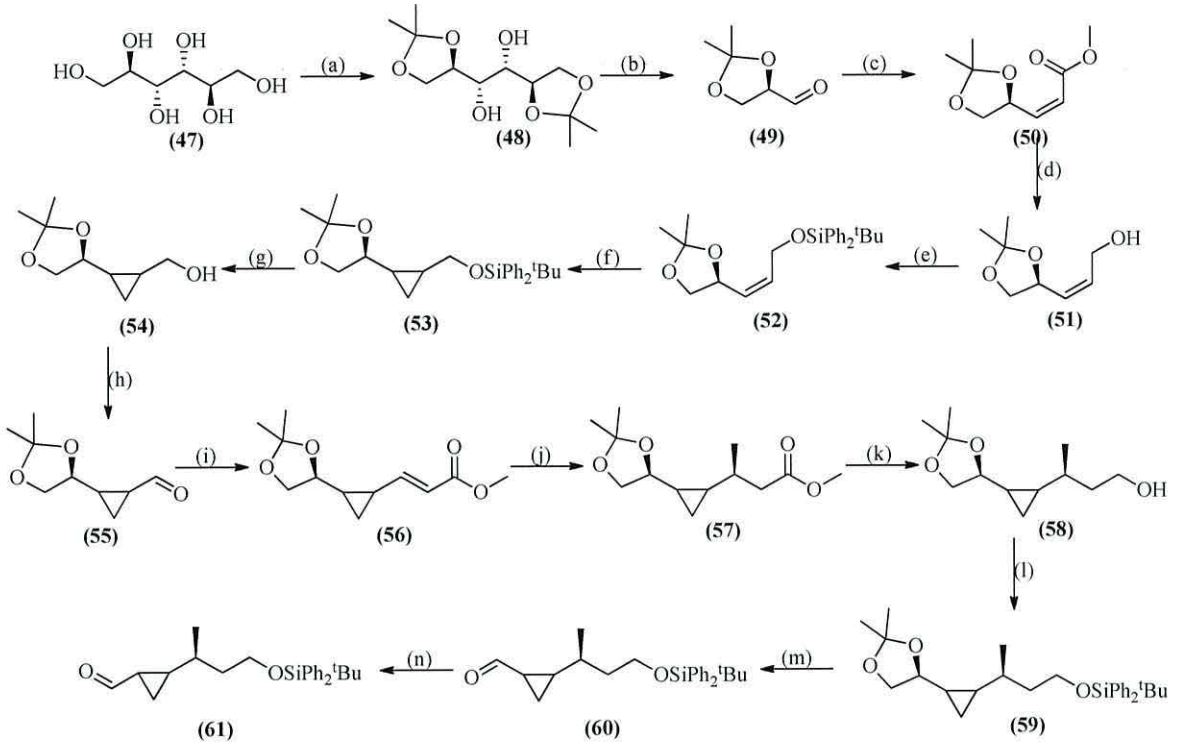
Scheme 5: Synthesis of (*R*)-methyl 2-((*R*)-3-(benzyloxy)-1-hydroxypropyl)pent-4-enoate **(37)**. (a) CuI, THF, vinylmagnesium bromide; (b) Ac_2O, pyridine, toluene; (c) DMF, oxone, OsO_4 (2.5% in 2-methyl-2-propanol); (d) conc. H_2SO_4, MeOH; (e) Diisopropylamine, dry THF, MeLi, allyl iodide.

Another important intermediate is the alcohol **(46)**, which may be used for the synthesis of any mycolic acid that is oxygenated at the distal position and contains an *S*, *S* configuration (Scheme 6).[60] The synthesis of this fragment is a long process but again it may be generated on a large scale for the synthesis of numerous different mycolic acids.



Scheme 6: Synthesis of a distal oxygenated intermediate **(46)**. (a) pyridinium *p*-toluene sulfonate, 2-methoxypropene, DMF; (b) $NaIO_4$; (c) methyl diisopropoxyphosphinyl acetate, K_2CO_3; (d) MeLi, Et_2O; (e) $LiAlH_4$, THF; (f) PCC, CH_2Cl_2; (g) LiHMDS, sulfone, dry THF; (h) H_2, Pd/C, EtOH, MeOH; (i) PTSA, THF, MeOH, H_2O; (j) NaOH, cetrimide, pTsCl, CH_2Cl_2 (k) $BrMg(CH_2)_6OTHP$, CuI, THF.

Another unit that may be synthesised is the cyclopropane unit (Scheme 7).[55,62] The syntheses of both the *cis*-cyclopropane unit **(60)** and the α-methyl-*trans*-cyclopropane unit **(61)** follow the same initial method, with the *cis*-cyclopropane intermediate **(60)** then being converted into the α-methyl-*trans*-cyclopropane unit **(61)**.



Scheme 7: Synthesis of a *cis*-cyclopropane unit **(60)** and an α-methyl-*trans*-cyclopropane unit **(61)**. (a) $ZnCl_2$, AcO, K_2CO_3; (b) $NaIO_4$, CH_2Cl_2; (c) methyl (triphenylphosphoranylide)acetate, MeOH; (d) DIBAL, THF, NH_4Cl, H^+; (e) $^tBuPh_2SiCl$, CH_2Cl_2; (f) EDC, CH_2I_2, DEZn; (g) TBAF, THF; (h) PCC, CH_2Cl_2; (i) methyl (triphenylphospharanylide)acetate, toluene; (j) MeMgBr, CuBr, THF; (k) $LiAlH_4$, THF; (l) DMF, imidazole, $^tBuPh_2SiCl$; (m) periodic acid, Et_2O; (n) NaOMe, MeOH.

Synthesising mycolic acids provides valuable information in determining the stereochemistry of naturally occurring mycolic acids, which subsequently may provide further understanding on the biosynthetic pathway. Synthetic mycolic acids may also be significant in tuberculosis therapy and diagnosis.

1.2.3.4 - Tuberculosis

Tuberculosis is a deadly, infectious disease which led to over nine million cases and nearly two million deaths in 2009.[63] It is caused by *Mycobacterium tuberculosis*. Although estimated to have originated over a million years ago, the earliest detection of tuberculosis was found by DNA analysis of the remains of an extinct bison that was dated at around 17,000 years ago.[64] The earliest human detection discovered was in the eastern

Mediterranean 9,000 years ago.[65] Over the following years, tuberculosis remained prevalent in history until the Middle Ages where there are not many written accounts of tuberculosis but much archaeological evidence.

Tuberculosis was much more widely recognised in the 18th and 19th centuries when the disease was even romanticised by society. People suffering from tuberculosis were thought to be extremely attractive due to their pale complexion. This belief translated to novels written during that period by Charles Dickens and Emily Bronte. During this time much more research was done on tuberculosis, which led in 1720 to the belief that the disease was infectious.[66] The next discovery came in 1821 when the pathogenesis of tuberculosis was first elucidated.[67] This work has formed the basis of the modern understanding of the disease. The next significant period for the knowledge of tuberculosis came in 1882 when Hermann Heinrich Robert Koch gave his now famous presentation on the identification of the tubercle bacillus.[68] He also postulated a link between microbe and disease. In 1890 he made another presentation on the isolation of a substance from tubercle bacillus, which was named tuberculin. This was believed to 'render harmless the pathogenic bacteria' and was subsequently used as a form of treatment of tuberculosis. However, this was quickly discredited. Koch then injected himself with tuberculin and developed a rise in body temperature but he never developed tuberculosis. He then concluded that tuberculin may be used diagnostically. Following Koch's findings, the existence of latent tuberculosis was firstly suggested in 1907. It was discovered that a positive reaction to tuberculin indicated that a person had latent tuberculosis. With all these discoveries came a decline in the mortality rate in the mid 19th century.[69] It is not known for certain why this decline occurred but it is believed to be due to better living conditions and in particular the segregation of patients infected with the disease. However, the number of people infected with tuberculosis did again begin to increase towards the end of the 20th century. This is believed to be due to the decline in living conditions and due to the spreading of HIV.[70] Patients with HIV have an increased susceptibility to *M. tb* and both diseases work together to form a lethal combination as each disease increases the rate of the other disease.

1.2.3.4.1 - Prevention

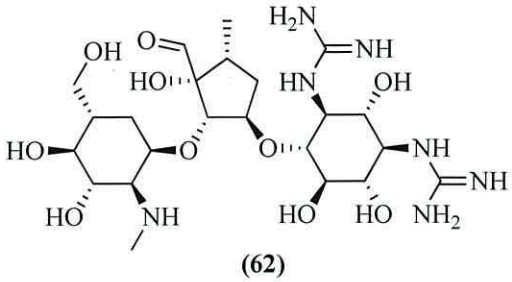
A vaccine for tuberculosis was developed in 1921 from *Mycobacterium bovis* and named Bacillus Calmette-Guérin (BCG) after the two French scientists responsible for its discovery. Although it is one of the most wildly used vaccines worldwide, its use has been

plagued with controversy and complication. From the beginning, its use was not accepted as it was not believed to be safe as it was based on live tuberculosis bacteria. This belief was not aided by the Lübeck disaster in 1930 where, following vaccination, nearly all of the 249 infants vaccinated developed tuberculosis, which resulted in 75 deaths.[71] Although it was subsequently discovered that the BCG sample in question had been contaminated, the incident had damaged people's perception of the vaccine.

Following this incident the use of BCG did become widespread, in a large part due to the support for the vaccine by the WHO and UNICEF.[72] However, the results of trials continued to show inconsistency towards the effectiveness of the BCG vaccination, with some trials showing an effectiveness of up to 80 % whilst others indicated that BCG showed no benefit in preventing tuberculosis. These varying results are believed to be accounted for by the fact that the vaccine is based on live bacteria and that therefore different BCG vaccine strains are present, which may have different potency.[73] Due to the continued controversy surrounding BCG, the WHO supported a large scale trial in the Chingleput district of India in 1968 in order to discover the protective value of BCG.[72] This trial was conducted on 360,000 people over nine years and indicated that BCG showed no protective effects against tuberculosis. The results of this trial were surprising but the WHO continued its support for BCG and suggested various reasons for the lack of benefit of the vaccine during the trial. One of these was that the trial was conducted on adults whereas it was believed that BCG administered to infants remained effective to protect against severe forms of tuberculosis. The use of BCG to protect infants from severe forms of childhood tuberculosis has since been proven on numerous occasions but has showed variable results against adult pulmonary tuberculosis.[74]

1.2.3.4.2 - Treatment

In 1946, streptomycin **(62)** was first used as an antibiotic against tuberculosis but this form of treatment was soon adapted and, by 1955, the treatment involved combining streptomycin **(62)**, *p*-aminosalicylic acid **(63)** and isoniazid **(64)**, each of which has a different mechanism of action and helps prevent the development of drug resistance to tuberculosis.[75]



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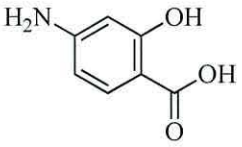

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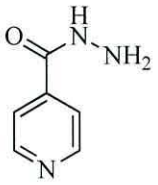
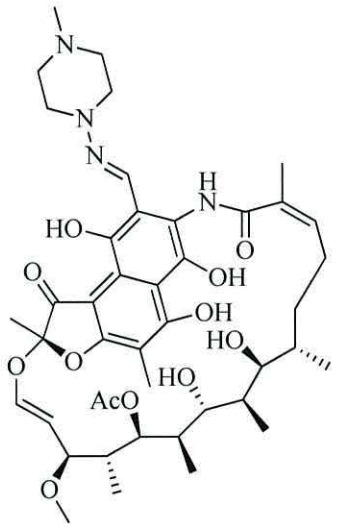


(64)

Currently a Directly Observed Therapy, Short-course (DOTS) treatment is recommended for the treatment of tuberculosis. This therapy involves a two month course of isoniazid **(64)**, rifampcin **(65)**, pyrazinamide **(66)** and ethambutol **(67)** followed by a four month course of isoniazid **(64)** and rifampcin **(65)**. This therapy is considered a first line treatment and again involves a multidrug combination in order to attempt to combat resistance.[76] However, the effectiveness of the therapy is compromised by some adverse side effects and the long duration of the treatment.



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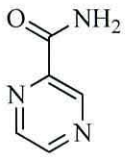

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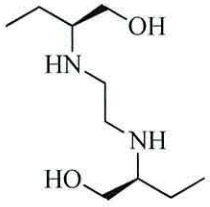


(67)

Although multi-drug therapies are used to try and avoid the development of drug resistance, multidrug-resistant tuberculosis (MDR-TB) does occur where the patient becomes resistant to at least isoniazid **(64)** or rifampcin **(65)**.[77] This is extremely prevalent in China and India and an estimated nearly half a million cases were believed to occur in 2008 with only 7 % of these cases being treated correctly.[78] Currently treatment for MDR-TB involves the use of different, effective drugs and lasts between 18 and 24 months. However, treatment for MDR-TB is more costly and complex and has much more severe side effects. Patients with MDR-TB may also become resistant to these second-line drugs and are then known to have developed extensively drug-resistance tuberculosis (XDR-TB).[79] XDR-TB involves individualised treatment to avoid the amplification of resistance

and involves drugs that it has been proven to be susceptible to. Treatment in this case is again more costly and complex and results in a higher mortality rate. Some cases have also been seen that show resistance to all available drugs against tuberculosis, which severely threatens tuberculosis control.

1.2.3.4.3 - Detection

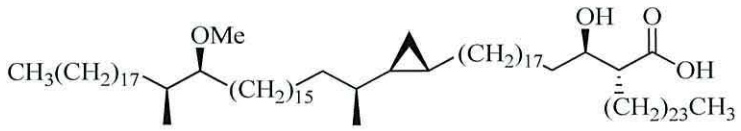
There are numerous methods available for diagnosing tuberculosis but only some of these are regularly used. The most wildly used way of detecting pulmonary tuberculosis is by sputum smear microscopy. Sputum smear microscopy generally involves staining smears of direct sputum with a dye such as the Ziehl-Neelsen stain or by auramine-rhodamine staining. These dyes stain acid fast bacilli that can then be identified by microscopy.

According to the WHO's DOTS strategy, every case of tuberculosis should be detected by smear microscopy.[80] This method is favoured in low to middle income countries where the number of cases of tuberculosis is higher. In 2007, 83 % of the countries that reported new cases of tuberculosis to the WHO used smear microscopy to detect tuberculosis. There are several advantages to using this method such as that it is inexpensive, rapid and highly specific. The main disadvantage is its low sensitivity, which is the proportion of people who actually have tuberculosis that were also tested positive for the disease,[81] which has been reported to be between 80 % and 20 % with especially low sensitivity seen in cases where individuals are co-infected with HIV.[82] Research is currently being performed in order to improve the general sensitivity[83] of the detection method and to improve sensitivity in cases where individuals are co-infected with HIV.[84]

In highly developed countries, tuberculosis is diagnosed by different methods such as by sputum culture.[85] This form of detection is the only one perceived to give definitive diagnosis of tuberculosis. This process involves growing cultures of *M. tb* from patient sputum using a Lowenstein Jensen media or a BACTEC media.[86] The BACTEC medium is favoured as it results in earlier detection times. Although this method can give a definite answer if a person is infected with tuberculosis it is a very slow process as *M. tb* is very slow growing and may result in the cultures being incubated for periods of between two to eight weeks. This form of detection is also limited in high burden countries due to its high cost. Newly developed diagnostic techniques are always compared to sputum culture as it still is considered the best method for detecting tuberculosis.[87]

1.2.3.4.3.1 – Immunodiagnostic Methods

New, alternative methods are continuously being developed in order to try and improve the detection methods that are currently available, which include a number of immunodiagnostic methods. These methods may involve modifications of an enzyme-linked immunosorbent assay, which can detect actual tuberculosis disease from various immunologically active components of *M. tb*. ELISA tests have shown that one possible method to diagnose tuberculosis involves detecting the anti cord factor antibody IgG.[88] Further work in this area suggested that IgG recognises the actual mycolic acid structures in the cord factor as the sera of patients with tuberculosis was highly reactive against *M. tb* cord factor but less reactive against *Mycobacterium avium* cord factor and the other way around. This discovery led to research into the effect of the different sub classes of mycolic acids on the detection of IgG, which showed that the sera of patients with tuberculosis was most reactive with methoxy mycolic acids compared to the α and the keto forms.[89] The antigenic effect of some synthetic mycolic acids have also now been discovered, which agreed with previous work showing that the methoxy mycolic acids were the strongest antigens but also showed that the antigenicity was dependent on both functional groups in the distal and proximal positions as well as on the stereochemistry of the various functional groups.[90] From the synthetic molecules tested, (*R*)-2-((*R*)-1-hydroxy-19-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl)cyclo-propyl)nonadecyl)hexacosanoic acid **(68)** was found to be the most antigenic.



(68)

Although much information has been gained from the ELISA results there are disadvantages to this method, such as its low sensitivity and specificity. This prevents ELISA from being considered as a potential commercial diagnostic method.

An alternative potential method for diagnosing tuberculosis is by biosensor analysis. Biosensor analysis involves converting a biological response into an observable response.[91] A substrate or an analyte firstly responds to a biological substance that gives rise to a signal that is transformed by a tranducer into another signal, such as an electrical signal, which can be measured.

Thanyani *et al.*[92] developed an IAsys (interaction assay system) affinity biosensor that detects anti-mycolic acid antibodies. On the IAsys cuvette surface, mycolic acids containing liposomes are immobilised and a serum sample is added. The biosensor then monitors and quantifies the binding between the antigen and antibody in real time by looking at the changes in the refractive index values. The change in the refractive index is directly related to the change in the accumulated mass, which is a major advantage of this method. However, as a number of false positive and false negative samples were obtained and the biosensor was only tested on 61 samples, this shows that there are some disadvantages to this method.

Another biosensor developed is an optoelectronic biosensor developed by Silva *et al.*[93] This method involves the molecular recognition of DNA that has been amplified by PCR to a nanoprobe of gold nanoparticles mixed with oligonucleotides that have been modified to include a thiol group. By then looking at an aggregation profile a positive or negative analysis of tuberculosis can be made. There are several advantages to this method such as it is portable and is of low cost. It can also distinguish between different types of tuberculosis such as *M. tb* and *M. bovis*. Although only a small number of samples have been analysed it has shown great promise in the accuracy of the results. This form of detection is also very rapid and only takes 3 hours to give an accurate result but 2½ hours are due to the amplification of the DNA by PCR, which is the main disadvantage of this method.

A potential sensor for detecting tuberculosis had also been proposed by integrating natural mycolic acids into a self assembled monolayer of *N*-(2-mercaptoethyl)octadecanamide **(69)**.[94] This involves firstly creating the gold-thiolate **(69)** monolayer and then incubating the electrode in a solution of mycolic acids where these may integrate on to the electrode by strong Van der Waals interactions. The electrode is then modified to block non-specific binding sites. By incubating the electrode in the patients' sera diluted in empty liposomes the difference in the electrode polarisation resistances can distinguish between positive and negative tuberculosis.

1.2.3.4.4 - Mycobacteria

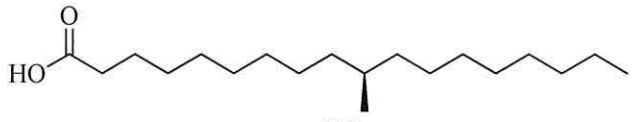
M. tb is a rod shaped bacillus, 1-4 x 0.3-0.6 μm in size, classified as an acid fast Gram positive bacterium. It is classed as acid fast because during the staining process the bacilli are not decolourised by acids. However, a Ziehl-Neelsen stain may be used to identify acid

fast bacilli, as they appear red after staining.[95] An alternative to this would be to use fluorescence. Although mycobacteria are generally classed as Gram positive on the basis of their ribosomal RNA, there is some controversy surrounding this, as *M. tb* has also been also found to contain some aspects of what would be expected for a Gram negative bacterium.[96] *M. tb* is responsible for causing tuberculosis in humans but there are several other mycobacteria in existence. A number of these mycobacteria again cause tuberculosis but in different animals. *M. bovis* is responsible for causing tuberculosis in cattle,[97] *M. microti* for causing tuberculosis in small mice, shrews and voles,[98] *M. avium* for causing tuberculosis in birds[99] and *M. marinum* for causing tuberculosis in fish.[100] There are also non-tuberculosis mycobacteria such as *M. ulcerans*, which causes skin lesions known as Buruli ulcer,[101] *M. leprae*, which causes leprosy,[102] and *M. canettii*, which causes lymphadenitis in children.[103]

M. tb has a complex cell envelope with low permeability to hydrophilic solutes.[104] This results in the mycobacterium being resistant to common antibiotics and chemotherapeutic agents.[105] Knowledge of the cell envelope could therefore be important for drug discovery. Much more is now known about the cell envelope of *M. tb* since the determination of the complete genome in 1998.[106] The cell envelope can be broken down into three separate units; the plasma membrane, the cell wall and the capsule.[107] The plasma membrane is consistent with the plasma membrane of other organisms but the cell wall of the mycobacterium is unique. The thick cell wall core consists of peptidoglycan linked to arabinogalactan, which is esterified to mycolic acids forming the mycolyl arabinogalactan-peptidoglycan complex, of which 40 % are mycolic acids. The cell capsule consists of polysaccharides, proteins and a large amount of lipid. This unique cell envelope results in an asymmetric lipid bi-layer where the fluidity changes across the cell membrane. This kind of feature is characteristic of a Gram negative bacterium, which again questions whether *M. tb* is Gram positive or Gram negative. Although the majority of mycolic acids present in the cell envelope are covalently bound to arabinogalactan they are also present in the cell envelope as cord factors. Cord factors consist of a trehalose backbone esterified at the 6 and 6' positions to two mycolic acids.[108] They are widely distributed in the cell envelope but they are not covalently bound and are therefore referred to as readily extractable free lipids.

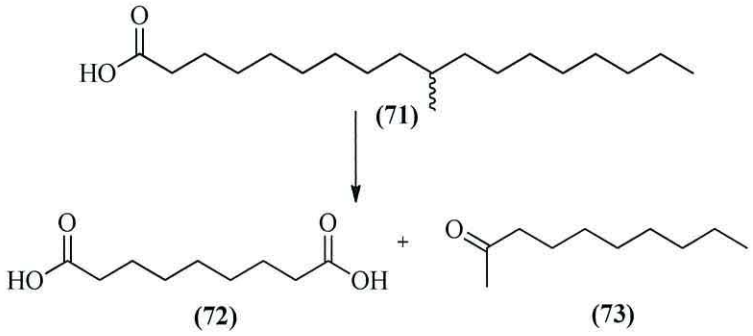
1.2.3.4.5 - Tuberculostearic acid (70)

Another unusual fatty acid isolated from *M. tb* is tuberculostearic acid **(70)**.[109] TBSA **(70)** is a branched C_{19} fatty acid that was firstly reported as a liquid saturated fatty acid found in the phosphatide fraction of the *M. tb*. It is found as 10 % of all the lipid content in the phospholipid membrane.



(70)

Following isolation of the lipid in 1929 from the acetone soluble fat in *M. tb*,[110] the peculiar fatty acid was named tuberculostearic acid **(70)**, as analysis of its methyl ester showed the liquid fatty acid to be isomeric with stearic acid **(20)** and to have the formula $C_{18}H_{36}O_2$.[111] TBSA **(70)** was also believed at this stage to be optically inactive. Various bacillus species were then investigated to see if they also contained the unusual fatty acid. It was not found in *M. avium*[112] but was present in *M. bovis*,[113] *M. phlei*[114] and in *M. leprae*.[115] It was during the analysis of the liquid saturated fatty acids of *M. leprae* by titration and silver salt experiments that the formula $C_{19}H_{38}O_2$ was firstly suggested - although reluctantly so due to the rarity of fatty acids containing an odd number of carbon atoms.[115] In 1934, the formula was confirmed and the structure proposed to be 10-methylstearic acid **(71)**.[116] This structure was suggested following analysis of its degradation products from vigorous oxidation (Scheme 8).



(71) **(72)** **(73)**

Scheme 8: Oxidation products of 10-methylstearic acid **(71)**.

Following the synthesis of racemic 10-methylstearic acid **(71)** most of its properties were found to be very similar to natural tuberculostearic acid **(70)**. However, some discrepancy was seen between the synthetic and the natural sample. A difference of 10 °C was seen between the melting points of the two compounds and their crystalline form was also

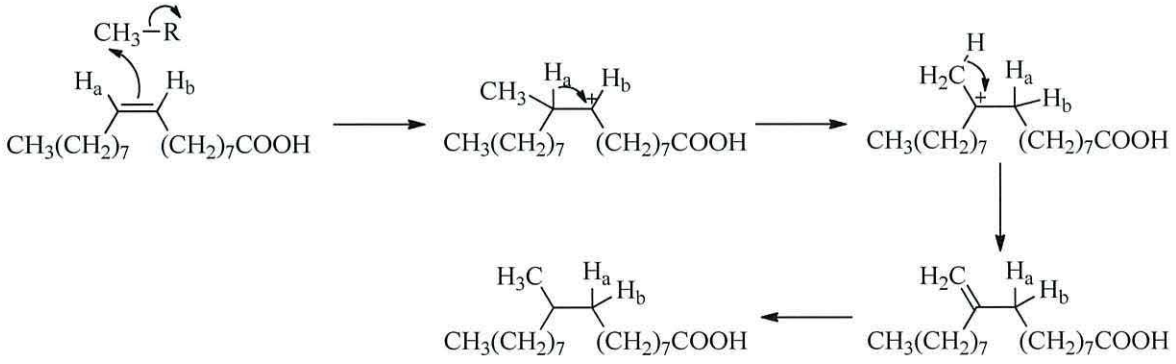
significantly different. It was at this stage that the suggestion of the existence of a chiral carbon was proposed but that the optical rotation was too small to be measured.

A racemic mixture of TBSA **(71)** as well as the two single enantiomers were synthesised in 1948.[117,118] This synthesis did involve a long-winded method but it was subsequently discovered that the sample with a negative optical rotation was identical in every way to natural TBSA **(70)**. TBSA **(70)** was therefore assigned to the L-series.

Following the original synthesis of TBSA **(70)**, the racemic mixture of the compound has been synthesised on numerous occasions.[119,120] However, the synthesis of the enantiomerically pure compound **(70)** is less common. The two enantiomers have been successfully synthesised by electrochemical methods in 1951[121] and by a novel short synthesis in 2006.[122] From the results of these experiments it could be confirmed that the actual structure of TBSA **(70)** is 10*R*-methyloctadecanoic acid by comparison of the natural and synthetic optical rotations.

1.2.3.4.5.1 - Biosynthesis of tuberculostearic acid (70)

The biosynthesis of TBSA **(70)** has been successfully deduced (Scheme 9). Using isotopic labelling experiments, it was firstly discovered that the precursors to TBSA **(70)** are oleic acid **(21)** and a single carbon unit, with the likely source being methionine.[123] Again by isotopic labelling, it was shown that the transfer of the methionine methyl group occurred with the loss of a proton.[124] This discovery, combined with the finding that during addition of the methyl group there was a shift in the olefinic proton from C_{10} of oleic acid to C_9, led to the proposed mechanism for the biosynthesis.[125]



Scheme 9: Mechanism of the biosynthesis of TBSA **(70)**.

This proposed mechanism was confirmed in 1966, again using labelling experiments.[126] In 1970, the mechanism was further confirmed but in this case various one carbon donors were tested for the biosynthesis of TBSA **(70)**. These results showed that the only effective substrate was *S*-adenosyl methionine **(29)** and that the final hydrogenation step proceeded using NADPH.[127] This biosynthetic procedure is consistent with that proposed earlier for the biosynthesis of a methyl substituent.[35]

1.2.3.4.5.2 - Tuberculostearic acid (70) in the detection of tuberculosis

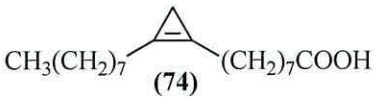
As TBSA **(70)** is detected in some Mycobacteria, one of its proposed uses is for the detection of pulmonary tuberculosis. Detecting TBSA **(70)** by GC-MS could also be developed to be a much more rapid way of diagnosing pulmonary tuberculosis than existing methods. Some initial experiments showed that it could be detected by GC-MS in five out of six samples where cultures were visually observed.[128] More importantly, it could be detected by GC-MS before cultures were visually observed.

Although these results were promising, much work has since been done to try and optimise the conditions required for the detection of TBSA **(70)** and ultimately the detection of tuberculosis. These investigations found more accurate results using frequency pulsed electron capture gas-liquid chromatography.[129] This procedure was further optimised using quantitative chemical ionisation mass spectrometry capillary gas chromatography to satisfy the sensitivity and specificity of the above test.[130] This test gives quantitative results, which in itself is a great advance.

During GC analysis, it is the methyl ester and not the free acid that is monitored. This is done by saponifying body fluid or by adding methanolic hydrochloric acid. However, this method does have one great obstacle, in that 4 ml of celebrospinal fluid is needed in order to be able to perform the test. More recent advances in this area include an alternative method for detection using stir bar sorptive extraction – thermal desorption – gas chromatography – mass spectrometry.[131] One of the main advantages of this method is that the analysis can be performed directly on sputum samples, making detection a much quicker process. The results from these analyses show the same trends as for direct microscopy and mycobacterial culture test. Being able to quantify the amount of TBSA **(70)** present allows a correlation to be made between the extent of disease and the concentration of TBSA **(70)**; however, the main disadvantage of these methods is the high cost involved in purchasing and maintaining the necessary equipment.

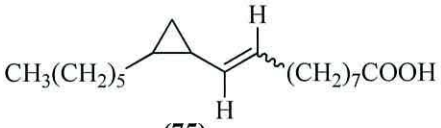
1.3 - Cyclopropene Fatty Acids

The first naturally occurring cyclopropene fatty acid was isolated in 1952 by Nunn and co-workers from the seed oil of the slow growing tree *Sterculia foetida*.[132] Due to the chemical transformations of the yellow oil obtained, the structure was proposed to be 8-(2-octyl-cycloprop-1-enyl)-octanoic acid and was named sterculic acid **(74)**.



(74)

This structure was rejected by Verma *et al.*[133] who proposed an alternative one due to incorrect interpretation of infra-red data. The structure was instead suggested to be 10-(2-hexylcycloprop-1-yl)dec-9-enoic acid **(75)**.



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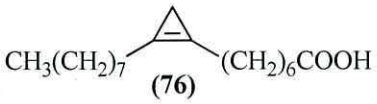
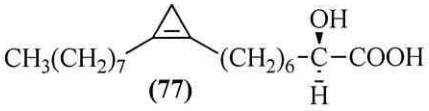
This structure was discounted when it was discovered that the absorption signal in the infra-red spectrum, which indicated the presence of a di-substitited double bond, was found to be due to a polymeric impurity.[134] Numerous other investigations also disregarded Verma's structure in favour of Nunn's original structure.[135] Nunn's structure was further confirmed by X-ray crystallography.[136] X-ray diffraction data showed that the synthetically prepared *cis*-9,10-methylene octadecanoic acid was identical to dihydrosterculic acid **(23)**, which had been prepared by hydrogenating natural sterculic acid **(74)**. The structure was again confirmed by analysis of the compound by ^{1}H NMR spectroscopy.[137] In 1969, the methyl ester of sterculic acid **(74)** was successfully synthesised by Gensler *et al.*,[138] which finally confirmed the structure.

A second cyclopropene fatty acid was isolated in 1956 from the seed oil of various plants belonging to the *Malvaceae* family.[139] It was named malvalic acid **(76)** and analysis of its infra-red spectrum suggested that it was a homologue of sterculic acid **(74)**.[140] Crystal structure analysis of dihydromalvalic acid **(24)** indicated that malvalic acid **(76)** was indeed a homologue of sterculic acid **(74)** and had the structure 7-(2-octylcycloprop-1-enyl)heptanoic acid.[141] This structure was again further confirmed in 1970 following the synthesis of its methyl ester.[142]



(76)

Only two other cyclopropene containing fatty acids have been isolated naturally, D-2-hydroxysterculic acid **(77)**,[143] which is believed to be an intermediate in the malvalic acid **(76)** biosynthesis and sterculynic acid **(78)**,[144] which was isolated from *Sterculia alata*.



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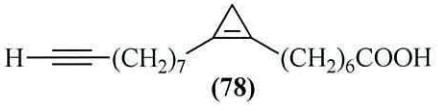


(78)

1.3.1 - Occurrence and Composition of cyclopropene fatty acids

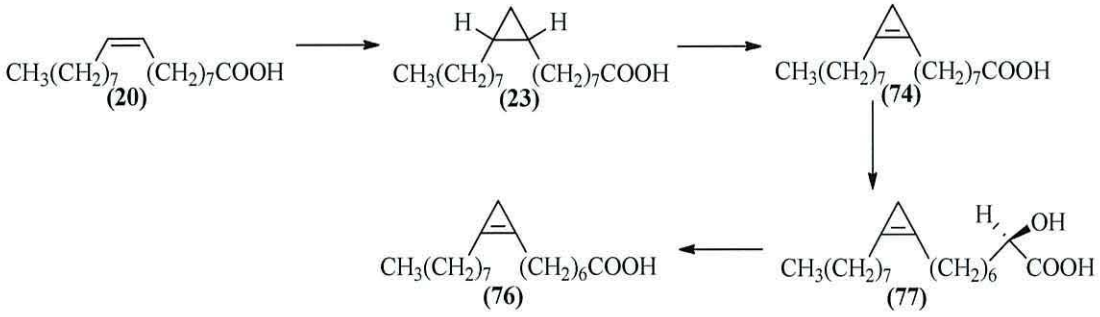
Although firstly isolated from a limited number of plant families in the *Malvales* order including *Malvaceae* and *Sterculiaceae,* currently cyclopropene fatty acids have been isolated from a variety of different families within the *Malvales* order as well as within other plant orders (Figure 5). However, it is clear to see why the first cyclopropene fatty acid was isolated from *Sterculia foetida*, as it contains the largest percentage by far of cyclopropene fatty acid in comparison to other plant families. Cyclopropene fatty acid content in plants also varies with season,[145] location within the plant[146] and plant maturity[147].

			% Fatty acid in seed oil	
Order	Family	Species	Malvalic Acid **(76)**	Sterculic Acid **(74)**
Malvaceae	*Sterculiaceae*	*Sterculia foetida*[148]	6.7	54.5
		Sterculia oblonga[149]	-	19.0
	Malvaceae	*Gossypium hirsutum*[149]	1.1	0.4
		Triumfetta pilosa[150]	2.4	6.6
	Sarcolaenaceae	*Schizolaena rosea*[151]	0.8	0.4
Urticales	*Moraceae*	*Ficus benghalensis*[152]	3.7	1.6
Fabales	*Leguminosae*	*Cassia corymbosa*[153]	3.2	2.8
		Telia babul[154]	2.5	2.1
Not known	*Boraginaceae*	*Trichodesma zeylanicum*[155]	8.2	7.8

Figure 5: Table showing the percentage of malvalic acid **(76)** and sterculic acid **(74)** in various plant species.

1.3.2 - Biosynthesis of cyclopropene fatty acids

Not much research has been done on the biosynthesis of cyclopropene fatty acids. However, it is believed that sterculic acid **(74)** is formed from its cyclopropane precursor, dihydrosterculic acid **(23)**. Malvalic acid **(76)** may then be formed from sterculic acid **(74)** by an α-oxidation reaction. This theory is supported by the fact that D-2-hydroxysterculic acid **(77)** does occur naturally, however, the exact mechanism of biosynthesis remains unknown.[156]



Scheme 10: Biosynthesis of sterculic acid **(74)** and malvalic acid **(76)**.

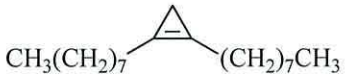
1.3.3 - Biological Effects of Cyclopropene Fatty Acids

It has been known for a long time that cyclopropene fatty acids have significant effects on biological systems but the reasons for these effects were not known. Over a century ago, pigs fed on a diet that contained cotton seed oils were seen to produce harder fats. Lactating cows were also seen to produce milk that resulted in butter with a higher melting point than average. The common cotton plant *Gossypium hirsutus* is known to contain cyclopropene fatty acids and now these effects can be accounted for by the cyclopropene fatty acids in the cotton seed oil that was part of the animals' diets.

Unusual biological effects were also seen with hens also fed on a diet containing cotton seed oil, which produced eggs with pink-coloured whites and a pasty yolk.[157] The pasty yolk is believed to be due to the increase in proportion of saturated to unsaturated fatty acids in the yolk and the pink white disorder is believed to be due to an increase in the permeability of the vitellin membrane surrounding the yolk.[158] This increase in permeability allows iron to diffuse out of the yolk into the white where it chelates to conalbumin, resulting in the pink colouration. These effects were later discovered to be due to the cyclopropene fatty acids in the cotton seed oil.[159] They led to the realisation that cyclopropene fatty acids could potentially be very important as desaturase inhibitors.

1.3.4 - Cyclopropene Fatty Acids as Desaturase Inhibitors

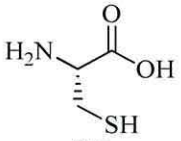
When rats were given dietary sterculic acid **(74)** it caused a dramatic difference in the ratios of stearic acid **(20)** to oleic acid **(21)**, which confirmed that cyclopropene fatty acids inhibited this conversion.[160] A later study on hen livers also showed that both malvalic acid **(76)** and sterculic acid **(74)** inhibited the Δ^9 desaturation of stearic acid **(20)**.[161] In this case, the degree of inhibition was found to be concentration dependent and the methyl esters of the cyclopropene compounds gave greater inhibition. The use of cyclopropene fatty acids as inhibitors of Δ^9 desaturase has since been confirmed on numerous occasions.[162, 163] However, it has been shown that the actual inhibitor of Δ^9 desaturase is sterculyl-CoA rather than the free acid.[160] This suggests that the reason why sterculine **(79)** and sterculyl alcohol **(80)** could not inhibit Δ^9 desaturase to the same degree is because of their inability to form the acyl-CoA derivative.



(79)

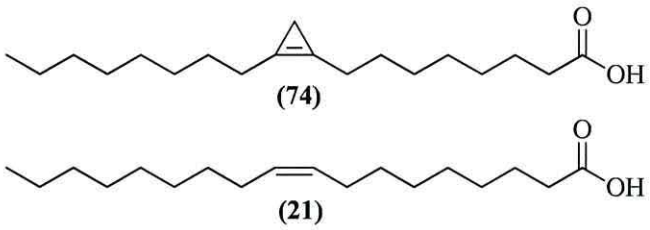
(80)

The exact mechanism of the inhibition of Δ^9 desaturase by cyclopropene fatty acids is still unknown. However, it is known that for the compound to be able to inhibit Δ^9 desaturase, the cyclopropene ring must be present on the C_9 and/or C_{10} positions. It is also suggested that the inhibitory effect of sterculic acid **(74)** is due to the cyclopropene ring itself as 1,2-dihydroxy sterculene showed very little or no effect as an inhibitor.[164] It is believed by some that the process is also irreversible, as adding additional stearic acid **(20)** did not cause any reversibility. Methyl sterculate **(81)** reacts rapidly with thiols, which suggests that sterculic acid **(74)** may be inhibiting Δ^9 desaturase by reacting with a thiol group within the enzyme. This suggestion is supported by the fact that the enzyme is inhibited by compounds such as *N*-ethylmaleimide, which is a thiol specific reagent, therefore showing that the thiol groups are important binding sites within the active site of Δ^9 desaturase.[160] When different amino acid mimics were tested, 1-propanethiol was the only one that reacted with the cyclopropene containing compounds, which implies that the inhibition is specific and that the cyclopropene fatty acids can only react with the cysteine **(82)** unit within the active site of the enzyme.[165]



(82)

It is also believed that sterculic acid **(74)** is a good inhibitor of Δ^9 desaturase as it has the same basic shape as oleic acid **(21)**. It can therefore mimic the structure of oleic acid **(21)**, react with the enzyme and block the active site.



(74) **(21)**

1.3.5 - Uses of Cyclopropene Fatty Acids

As sterculic acid **(74)** has been shown to be a potent inhibitor of Δ^9 desaturase there are potentially numerous industrial applications for the cyclopropene-containing fatty acid.

One application is in the food industry. Cocoa butter is industrially very important but also very expensive and it was believed that a substitute oil could be produced utilising the fact that sterculic acid **(74)** inhibits Δ^9 desaturase. Fermenting yeast from inexpensive carbohydrate feedstocks gives large quantities of lipids including 5 % stearic acid **(20)** and around 50 % oleic acid **(21)**. As cocoa butter has higher levels of stearic acid **(20)** and lower levels of oleic acid **(21)**, the addition of sterculic acid **(74)** to the yeast culture should produce a fat of similar composition to cocoa butter. This did occur, but the levels of linoleic acid in the fat also increased; therefore a combination of sterculic acid **(74)** and a substrate to inhibit Δ^{12} desaturase should give the correct composition of fatty acids in the oil.

One of the main drivers for the use of sterculic acid **(74)** is in the field of medicine. Sterculic acid **(74)** has become an important target in body weight regulation. Studies on mice with a targeted disruption in Δ^9 desaturase have shown that they have increased energy expenditure, reduced body adiposity and are resistant to diet-induced obesity.[166] Therefore, this mutation in the Δ^9 desaturase gene has led to global changes in the gene expression and altered the metabolic activity, which accounts for the loss of body fat. The disruption in Δ^9 desaturase is, therefore, a promising therapeutic target in the treatment of the metabolic syndrome.[167] As sterculic acid **(74)** is an inhibitor of Δ^9 desaturase, cells were treated with sterculic acid **(74)** and this was found to reduce their adiposity.[168] As obesity has now reached epidemic proportions with high levels of obesity-related diseases, this research is very significant.

Increased adiposity is also associated with insulin resistance and increased risk of type 2 diabetes. As rats that were made to be diabetic were found to have increased activity of Δ^9 desaturase, these findings have led researchers to consider sterculic acid **(74)** as a potential therapeutic target for the treatment of diabetes. However, alternative compounds that also inhibit Δ^9 desaturase are also being investigated as potential new drugs.[169]

Human tumour cells are known to contain higher levels of oleic acid **(21)** due to the higher levels and activity of Δ^9 desaturase. This leads to a more permeable membrane, which leads to an increase in the metabolic rate and cell division, which causes the development of cancers. However, until recently a causal relationship between Δ^9 desaturase and cancer remained unknown. It has now been shown that, in lung cancer cells when the Δ^9 desaturase expression was reduced, the biosynthesis of fatty acids was also significantly impaired, which shows a causal relationship between Δ^9 desaturase and abnormal lipid metabolism in cancer cells.[170] Numerous different tumour cells exhibit higher levels and higher activity of Δ^9 desaturase such as in human larynx tumour cells[171] and in simian transformed human lung fibroblasts.[172] Research has also showed that males with prostatic malignancy also had higher levels of Δ^9 desaturase and a threefold increase in some monounsaturated fatty acids. Δ^9 Desaturase inhibitors could therefore be promising anti-cancer agents.[173] Interferon[174] is a commercially available drug used to treat certain types of cancers. Studies have shown that interferon increases the amount of saturated fatty acids *in vitro* by inhibiting the action of Δ^9 desaturase. This inhibition decreases membrane fluidity, making the membrane denser.[175] This increase in the membrane rigidity may explain the capacity to inhibit virus release and possibly cell division. Interferon's inhibitory properties may therefore be responsible for its anti-cancer properties. Conjugated linoleic acid has also been found to possess some inhibitory effects on some forms of mammary carcinogenesis by decreasing the levels and activity of Δ^9 desaturase.[176] As sterculic acid **(74)** is a more potent inhibitor of Δ^9 desaturase it is believed that it would have a greater effect on the fluidity of the membrane and would therefore be a better anti-cancer agent. Sterculic acid **(74)** has already displayed some anti-tumour effects in vitro and in a tumour model in rats.[177]

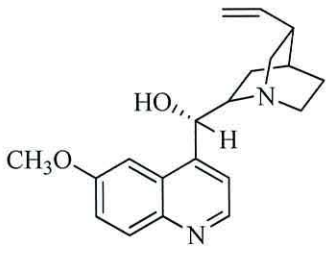
Sterculic acid **(74)** may also be effective in the treatment of tuberculosis. The thiourea drug isoxyl is used for the treatment of tuberculosis, and has been found to be active against multi drug resistant tuberculosis.[178] Isoxyl decreases the levels of oleic acid **(21)** and therefore TBSA **(70)** and has some effect on the synthesis of mycolic acids. This suggests that isoxyl works by inhibiting Δ^9 desaturase. By comparing the effect of isoxyl with that

of sterculic acid **(74)** on *M. bovis* BCG it was shown that both have the same effect on the levels of oleic acid **(21)**. However, sterculic acid **(74)** had no effect on the synthesis of mycolic acids, which suggests that isoxyl has different effects on different fatty acids. However, the primary effect of isoxyl is to inhibit oleic acid **(21)** biosynthesis, which is a vital part of the mycobacterial membrane. This suggests that alternative Δ^9 desaturase inhibitors could also be a potential drug target for the treatment of tuberculosis.

1.3.6 - Malaria

The most severe form of human malaria is caused by the parasite *Plasmodium falciparum*. It accounts for nearly a million deaths annually from around 250 million cases. The majority of the deaths caused by the *P. falciparum* parasite are among children living in Africa. Every 45 seconds a child in Africa dies from malaria and this accounts for 20 % of all childhood deaths.[179] Malaria is transmitted through the bites of infected Anopheles mosquitoes. Transmission is more intense in places where the mosquito is relatively long lived and where it prefers to bite humans rather than other animals. In areas of high transmission human immunity is also an important factor for the development of malaria. Human immunity is developed over years of exposure and this is why most malarial deaths in Africa occur in young children who have not yet developed this immunity.

1.3.6.1 - Treatment



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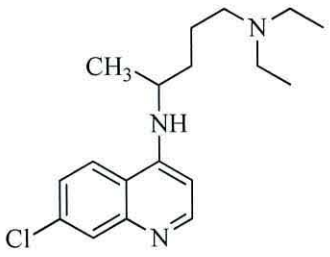
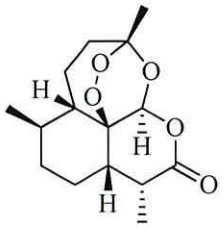


(84)

Malaria is preventable and treatable. The first effective drug for the treatment of *P. falciparum* malaria was quinine **(83)**.[180] Quinine **(83)** was firstly isolated from the bark of the Cinchona tree in 1820 and used for the treatment of malaria until the 1940s when it was replaced by chloroquine **(84)** and other synthetic quinine derivatives.[181] Chloroquine **(84)** was firstly discovered in 1934 but it was not used to treat malaria until 1947. Since then it has been extensively used for the treatment of malaria but due to the widespread resistance

of *P. falciparum,*[181,182] other drugs have since been investigated and chloroquine **(84)** is now not recommended to treat *P. falciparum* malaria.



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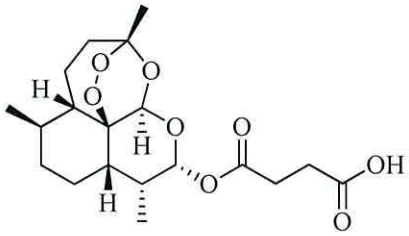

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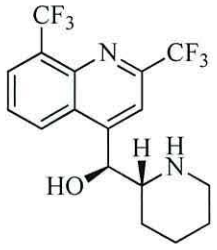


(87)

An artemisinin **(85)** based combination therapy is currently recommended for the treatment of *P. falciparum* malaria. Artemisinin **(85)** was firstly discovered in 1972 in the leaves of the common herb plant *Artemisia annua.* Since then derivatives of artemisinin **(85)**, such as artesunate **(86)** have been synthesised to increase the effectiveness of artemisinin **(85)** as an anti-malarial drug. Due to the short half-life of artemisinin **(85)** and its derivatives, it should be taken in combination with another partner drug such as mefloquine **(87)** that has a longer half-life.[183] However, as new cases of artemisinin resistance[184] are seen, this again prompts the investigation into new anti-malarial drugs.

1.3.6.2 - Fatty Acids in *Plasmodium falciparum*

A large increase in the lipid content of infected red blood cells is seen during *P. falciparum* infection.[185] However, it has been reported that *P. falciparum* only has a limited capacity to elongate fatty acids itself during infection. These fatty acids are therefore scavenged from human serum and experiments have shown that the parasite growth is not sustained in a culture without them. It has been shown that oleic acid **(21)** in particular is essential for parasite growth.[186] It is believed that these fatty acids may be incorporated by passive diffusion through the membranes of *P. falciparum*'s infected red blood cells.[187] Research has shown the parasite contains members of the type II fatty acid biosynthetic pathway, which contradicts earlier reports and suggests that *P. falciparum* can indeed synthesise fatty acids *de novo.*[188] However, it has been reported that this pathway is only active in the liver stages and that it is not essential for the blood-stage parasite. It can therefore be concluded that blood-stage *P. falciparum* relies on host fatty acids, but not much research has been done to see if these fatty acids can be modified by the parasite. Δ^9 Desaturase was subsequently discovered to be present in the parasite, which suggests that the fatty acids can be modified. Further investigations were made by members of the University of

Montpellier into the importance of Δ^9 desaturase to the *P. falciparum* parasite.[189] This research showed that *P. falciparum* can desaturate scavenged stearic acid **(20)** and that the activity of Δ^9 desaturase is essential for parasite growth in the blood stages.

1.4 - Project Aims

1.4.1 – Detection of tuberculosis

Tuberculosis is currently a major problem and, with the existence of tuberculosis/HIV co-infection, the accurate and rapid detection of tuberculosis is of growing importance. It is believed that eventually a novel biosensor may be formed that would involve antibodies from serum samples being recognised by mycolic acids. In a number of proposed sensors to do this the mycolic acids would have to be firstly bound to gold nanoparticles by a gold-sulfur bond, e.g. to form an electrode. Although this has been achieved using natural mycolic acids and a thiolated linker group,[94] there would be more control if thiolated mycolic acids were synthesised in order for the mycolic acids to be directly bound to the gold surface. It has already been shown by ELISA methods that some synthetic mycolic acids have higher reactivity towards the antibodies than others, therefore, using specific synthetic mycolic acids should result in better recognition of antibody-antigen. In this case, the position of the thiol group within the mycolic acid could also be very important, as the antibodies may only recognise certain units within the mycolic acids. Mycolic acid analogues could then be formed that contained thiol groups at different positions in order to determine where the best antibody response is observed. This would be a novel approach, as thiolated mycolic acids have not been previously synthesised. Following the synthesis of thiolated mycolic acids they may be bound to the gold nanoparticles to form an electrode. The electrode may then be subjected to serum samples and a transducer could transform the signal for the interaction between the electrode and the serum sample to an electrochemical signal that could be measured and quantified, allowing for the detection of active tuberculosis. Eventually it is believed that this may be extended to be able to distinguish between the different types of mycobacterial disease, as it is known that some mycolic acids may be unique to certain Mycobacterial diseases.[104] Therefore, one ultimate goal of this project is to synthesise thiolated analoges of components of Mycobacteria, including thiolated tuberculostearic acid and thiolated mycolic acids, which could then be potentially used for the detection of tuberculosis.

As stated previously another form of detection of tuberculosis is by the detection of TBSA **(70)** by a variety of GC methods. In order to be able to quantify the amount of TBSA **(70)** more accurately, it could be detected against a standard of labelled TBSA **(70)**. By comparing the known amount of labelled TBSA present to the unknown amount of TBSA present in the patients sample by a method such as mass spectrometry, this would allow for the amount of TBSA **(70)** to be quantified much more precisely. This would allow a correlation to be made between the amount of TBSA **(70)** present and the extent of disease. The synthesis of labelled tuberculostearic acid is therefore also a target of this project.

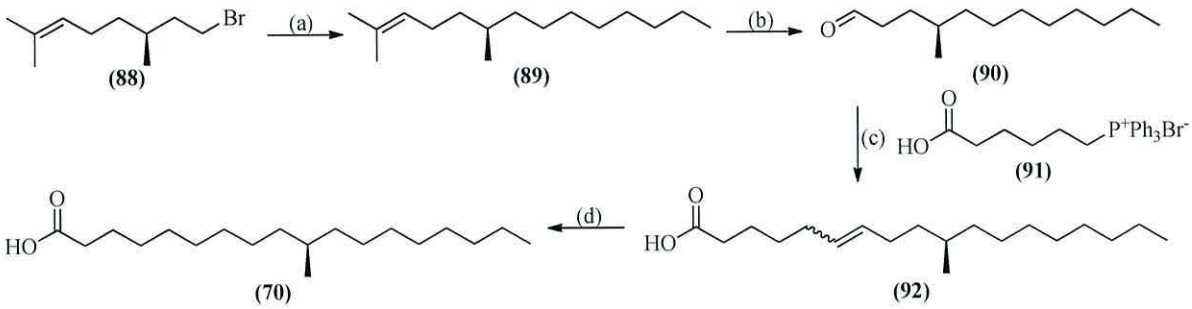
1.4.2 – Δ^9 Desaturase inhibitors

As it has been discovered that unsaturated fatty acids are essential for *P. falciparum* growth, this strengthens the impetus to investigate Δ^9 desaturase inhibitors as potential anti-malarial drugs. As sterculic acid **(74)** is a potent inhibitor of Δ^9 desaturase, analogues of sterculic acid **(74)** will be synthesised in order to try and maximise the inhibition against *P. falciparum* Δ^9 desaturase. This could then guide us towards a better inhibitor and may also convey more information about the active site of the enzyme and could be an important target in anti malarial treatment. The effectiveness of the Δ^9 desaturase inhibitors against *P. falciparum* would then be tested at the University of Montpellier.

2. Results and Discussion

2.1 - The Synthesis of Tuberculostearic Acid (70)

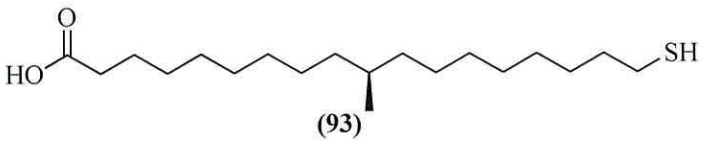
Before attempting to synthesise any thiolated components of Mycobacteria, TBSA **(70)** was synthesised following an existing procedure (Scheme 11).[122] TBSA **(70)** was synthesised in four steps from (*S*)-citronellyl bromide **(88)**, derived from the naturally occurring monoterpenoid (*S*)-citronellol. To date, this is the shortest route to enantiomerically pure TBSA **(70)**.



Scheme 11: Synthesis of TBSA **(70)**. (a) $CH_3(CH_2)_5MgBr$, $LiCuCl_4$, dry THF, 75 %; (b) O_3 (g), PPh_3, CH_2Cl_2, -78 °C, 62 %; (c) LiHMBS (1.06 M in THF), toluene/DMSO, 55 %; (d) Pd/C (10 %), H_2 (g), MeOH, 85 %.

TBSA **(70)** was successfully synthesised in an overall yield of 22 % and all data were comparable with those in the literature. As no problems were encountered synthesising standard TBSA **(70)**, synthesis of thiolated TBSA was then attempted.

2.2 - The synthesis of (*S*)-18-mercapto-10-methyloctadecanoic acid (93)

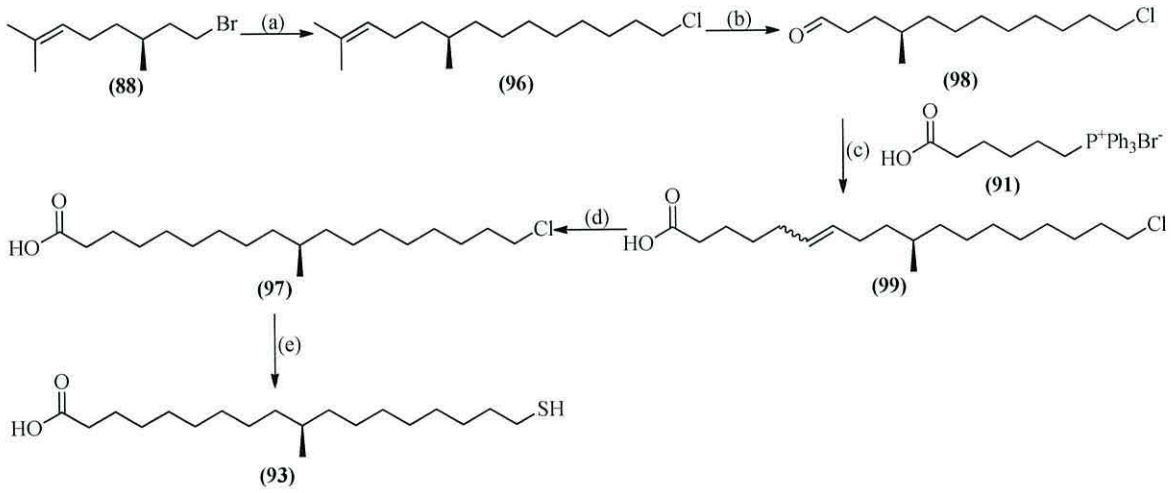


Before synthesising thiolated TBSA, the position where the thiol group would be introduced had to be selected. It was important to choose a position that ensured that the thiol would not interfere with any functionality within the molecule and that it would have the least effect on any kind of antibody recognition ability that the molecule might possess.

Therefore, it was decided that initially the thiol group would be included at the end of the alkyl chain to form (*S*)-18-mercapto-10-methyloctadecanoic acid **(93)**.

2.2.1 - The Original Methodology

The initial method that was to be attempted for the synthesis of enantiomerically pure (*S*)-18-mercapto-10-methyloctadecanoic acid **(93)** was adapted from the existing method for the synthesis of TBSA **(70)** (Scheme 12). The initial Grignard reaction in this case would be performed between (*S*)-citronellyl bromide **(88)** and the Grignard reagent of 1-bromo-6-chlorohexane **(94)** rather than 1-bromohexane **(95)**. This would allow for the formation of (*R*)-14-chloro-2,6-dimethyltetradec-2-ene **(96)** which includes a chloride at the end of the alkyl chain which could eventually be converted into a thiol. It was expected that the following three reactions would proceed as per the previous work, finally forming (*S*)-18-chloro-10-methyloctadecanoic acid **(97)**. The chloride could be converted to the thiol by reacting (*S*)-18-chloro-10-methyloctadecanoic acid **(97)** with potassium hydrogen sulfide[190] to finally form thiol **(93)**.

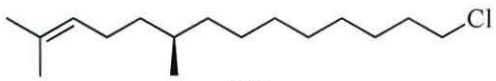


Scheme 12: Attempted synthesis of (*S*)-18-mercapto-10-methyloctadecanoic acid **(93)**. (a) $Cl(CH_2)_6MgBr$, dry THF, -78 °C, $LiCuCl_4$; (b) O_3 (g), CH_2Cl_2, -78 °C, PPh_3; (c) LiHMBS (1.06 M in THF), toluene/DMSO, 0 °C; (d) H_2 (g), Pd/C (10 %), MeOH; (e) KHS, EtOH.

Existing work has shown that for the preparation of a mono-Grignard reagent from a dihalide, the two halogens must be at least three atoms apart and must also be different.[191] This was true in the conversion of bromide **(88)** to the coupled product **(96)** and the

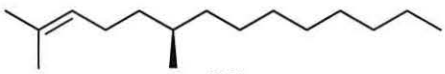
Grignard reagent was prepared by slowly adding a solution of 1-bromo-6-chlorohexane **(94)** to a solution of magnesium turnings and the mixture was heated gently with a heat gun where the activation of the magnesium could be clearly seen by bubbles being emitted from the turnings. The resulting solution of chlorohexylmagnesium bromide was then added to a cooled solution of (*S*)-citronellyl bromide **(88)**, followed by lithium tetrachlorocuprate to synthesise (*R*)-14-chloro-2,6-dimethyltetradec-2-ene **(96)**.

The residue of the reaction was purified by column chromatography and gave two different fractions, (*R*)-14-chloro-2,6-dimethyltetradec-2-ene **(96)** and (*R*)-(2,6)-dimethyl-tetradec-2-ene **(89)**, but the chloride **(96)** was only formed in a 1.7 % yield.



(96)

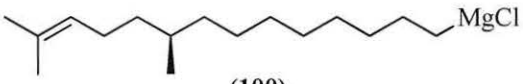
The formation of the chloride **(96)** was confirmed by the ^{1}H NMR spectrum. A two-hydrogen triplet at 3.54 ppm was seen, representing the methylene group adjacent to the chlorine whilst all the remaining peaks were nearly identical to the peaks for (*R*)-(2,6)-dimethyl-tetradec-2-ene **(89)**, which represented the remainder of the structure. Although its formation was confirmed, it is not a viable method for the formation of chloride **(96)** due to the extremely low yield.



(89)

(*R*)-(2,6)-Dimethyl-tetradec-2-ene **(89)** accounted for 77 % of the reaction yield and its formation in such high quantities was not expected or desired. The synthesis of this compound was confirmed as all the data obtained was consistent with (*R*)-(2,6)-dimethyl-tetradec-2-ene **(89)**, which was synthesised previously.[122]

There are several possible reasons why (*R*)-(2,6)-dimethyl-tetradec-2-ene **(89)** was formed. The reaction may have worked exactly as expected, but, after coupling with (*S*)-citronellyl bromide **(88)** the magnesium may have exchanged and formed (*R*)-(9,13-dimethyltetradec-12-en-1-yl)magnesium chloride **(100)**. When the reaction was then quenched with a saturated aqueous solution of ammonium chloride, Grignard **(100)** may have also been hydrolysed, resulting in the formation of (*R*)-(2,6)-dimethyl-tetradec-2-ene **(89)**.[192]



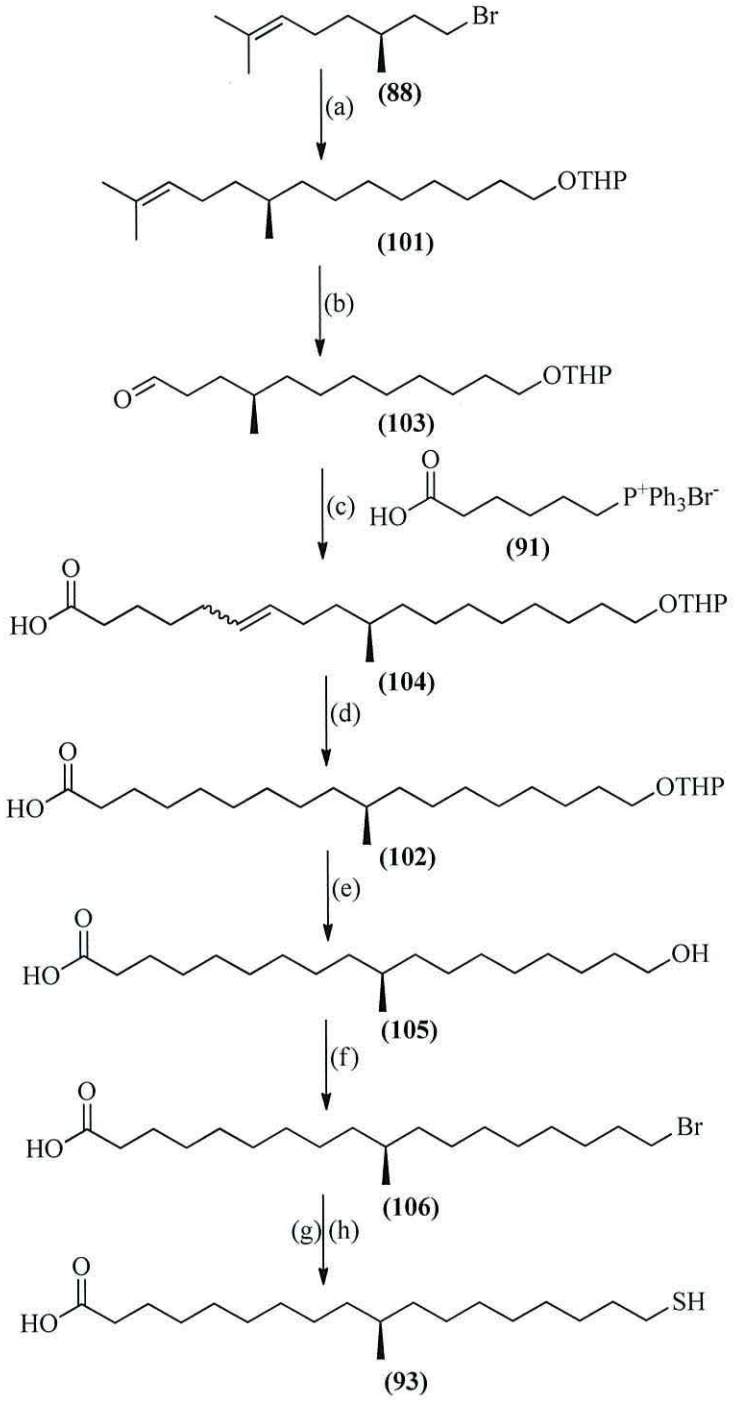
(100)

Another possible reason for the formation of (*R*)-(2,6)-dimethyl-tetradec-2-ene **(89)** may be that when forming the Grignard reagent a di-Grignard reagent was prepared. This would again result in the terminal end being hydrolysed and forming (*R*)-(2,6)-dimethyl-tetradec-2-ene **(89)**. However, this is unlikely as only 1.3 equivalents of magnesium were added, which would not be sufficient to fully generate the di-Grignard. It was also evident that not all of the magnesium was used in the reaction as some residual magnesium was still present at the end of the reaction.

The reaction was then repeated under exactly the same conditions and gave similar results. The same two products were produced in similar ratios, (*R*)-(2,6)-dimethyl-tetradec-2-ene **(89)** in a 62.1 % yield and (*R*)-14-chloro-2,6-dimethyltetradec-2-ene **(96)** in a 5.5 % yield. The yield of the desired product, chloride **(96)** may have increased but the increase was so insignificant that an alternative route for the formation of (*S*)-18-mercapto-10-methyloctadecanoic acid **(93)** was sought rather than attempting to optimise this reaction.

2.2.2 - The Final Method

The new route for the formation of (*S*)-18-mercapto-10-methyloctadecanoic acid **(93)** would follow the same basic principle as that for the synthesis of TBSA **(70)**. However, in this case the initial Grignard reaction would be performed between (*S*)-citronellyl bromide **(88)** and (6-(tetrahydro-2*H*-pyran-2-yloxy)hexyl)magnesium bromide. This avoids the complications caused by the Grignard reagent being a di-halide and should not result in the loss of a functional group from the product. From the Grignard coupling product, 2-((*R*)-9,13-dimethyltetradec-12-enyloxy)tetrahydro-2*H*-pyran **(101)**, the synthesis would proceed as the original synthesis of TBSA **(70)**, finally forming (10*S*)-10-methyl-18-(tetrahydro-2*H*-pyran-2-yloxy)octadecanoic acid **(102)**. From acid **(102)**, the tetrahydropyranyl group would be deprotected to the alcohol which would then be converted into the bromide, before being converted again into the thiol. The thiol would be formed by reacting bromide **(106)** with thiourea as this is a more efficient method for generating thiols from bromides.[193] This new route (Scheme 13) does constitute seven steps from the Grignard reaction rather than the original five, but the advantage of preventing the complications of the di-halide far outweighs the need for more steps.



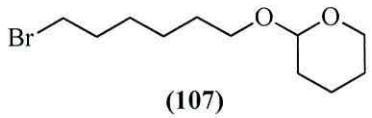
Scheme 13: Synthesis of (*S*)-18-mercapto-10-methyloctadecanoic acid **(93)**. (a) $THPO(CH_2)_6MgBr$ **(107)**, dry THF, -78°C, $LiCuCl_4$; (b) O_3 (g), CH_2Cl_2, -78 °C, PPh_3; (c) LiHMBS (1.06 M in THF), toluene/DMSO, 0 °C; (d) H_2 (g), Pd/C (10 %), MeOH; (e) PTSA, THF, MeOH, H_2O, reflux; (f) NBS, PPh_3, CH_2Cl_2, 0°C; (g) thiourea, EtOH, reflux; (h) 5 M NaOH, HCl.

As the Grignard stage of the previous synthesis resulted in so many problems, to ensure the maximum possible yield, 2.5 equivalents of magnesium turnings were used in this synthesis. A large excess of Grignard reagent would then be formed which should have a

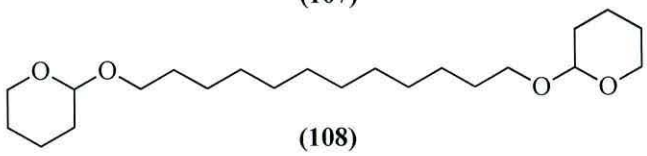
profound effect on the yield of the reaction. The reaction proceeded as expected with the purification of the product being a little problematic with a distillation and column chromatography necessary to isolate the coupled product **(101)** pure due to the excess of 2-(6-bromohexyloxy)tetrahydro-2*H*-pyran **(107)** used. However, 2-((*R*)-9,13-dimethyltetradec-12-enyloxy)tetrahydro-2*H*-pyran **(101)** was successfully isolated in a 70 % yield.

The ^{1}H NMR spectrum confirmed the formation of ether **(101)** as it showed two, three-proton singlets at 1.60 and 1.68 ppm for the two CH_3 groups next to the double bond and a single proton doublet of triplets at 5.10 ppm representing the olefinic proton. The tetrahydropyranyl group was also clearly evident with a characteristic pattern between 3.37 and 4.57 ppm. The acetal proton was seen as a single-proton triplet at 4.57 ppm and the four-protons next to the oxygen atoms were seen as four separate peaks. Two clear single-hydrogen doublets of triplets were seen at 3.37 and 3.73 ppm as well as two, single-hydrogen multiplets at 3.48 and 3.87 ppm.

The two major impurities of the reaction and the cause of the purification problems are believed to be some unreacted 2-(6-bromohexyloxy)tetrahydro-2*H*-pyran **(107)** as well as 1,12-*bis*((tetrahydro-2H-pyran-2-yl)oxy)dodecane **(108)**. As a large excess of bromide **(107)** was used, some of it, which had formed the Grignard reagent, may have coupled to unreacted **(107)** to form the dimer **(108)**.

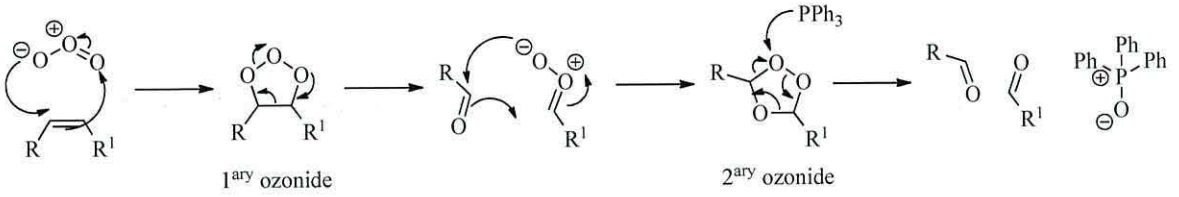


(107)



(108)

An ozonolysis reaction was then performed on 2-((*R*)-9,13-dimethyltetradec-12-enyloxy)tetrahydro-2*H*-pyran **(101)** using commercial ozonolysis apparatus, and triphenylphosphine was added for a reductive work up (Scheme 14). Ozone reacts with the olefin through a 1,3-dipolar cycloaddition reaction to form an unstable primary ozonide that immediately decomposes by a reverse 1,3-dipolar cycloaddition to give an aldehyde and a carbonyl oxide. The carbonyl oxide is also a 1,3-dipole and therefore reacts with the aldehyde in another 1,3-dipolar cycloaddition reaction to give the secondary ozonide. This can be decomposed by reacting with triphenylphosphine to give two carbonyl-containing compounds and triphenylphosphine oxide.



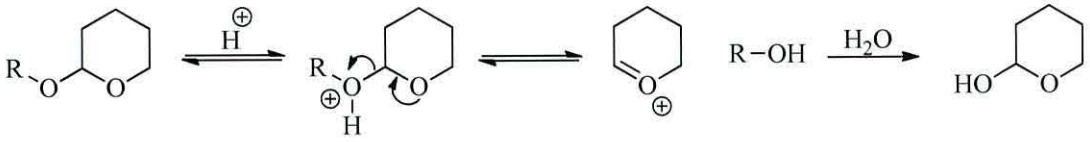
Scheme 14: Mechanism of ozonolysis.

This ozonolysis proceeded as expected with the only small problem being the separation of (4*R*)-4-methyl-12-(tetrahydro-2*H*-pyran-2-yloxy)dodecanal **(103)** from the triphenylphosphine oxide produced. Acetone was the second carbonyl compound produced in this reaction, which could be easily separated from the desired product **(103)** by rotary evaporation. Both ^{1}H and ^{13}C NMR spectra for aldehyde **(103)** showed the disappearance of the peaks representing the double bond and the two terminal methyl groups and clear signals for the aldehyde were present at 9.77 ppm and 203.1 ppm in the ^{1}H and ^{13}C NMR spectra respectively. All the peaks for the tetrahydropyranyl group and the remainder of the molecule were as before.

Aldehyde **(103)** was immediately used in a Wittig reaction with (6-carboxyhexyl)triphenylphosphonium bromide **(91)** using lithium *bis*(trimethylsilyl)amide as a base. This resulted in the coupled product **(104)** in a 51 % yield. Peaks could be seen representing the olefin at 5.35 ppm in the ^{1}H NMR spectrum and at 128.8 and 130.7 ppm in the ^{13}C NMR spectrum. A peak could also be seen in the ^{13}C NMR spectrum representing the carbonyl of the acid at 178.6 ppm. No peak was observed for the acidic proton in the ^{1}H NMR spectrum as the peak may be very broad due to its rapid exchange with the traces of acid in the deuterated solvent. The IR spectrum exhibited some unusual properties as it showed two peaks in the 1700 cm^{-1} region suggesting that two carbonyl groups were present. As the initial Grignard reaction involved reacting 2-(6-bromohexyloxy)tetrahydro-2*H*-pyran **(107)** with (*S*)-citronellyl bromide **(88)**, which is a chiral molecule, this caused the formation of a mixture of diastereomers. Two different peaks may then be seen in the IR spectrum as a mixture of compounds was present, though it is unclear why they should be different enough to see. An alternative theory is that the two acid groups dimerised together. This would give rise to two different carbonyl groups in the IR spectrum and the disappearance of the acid peak in the ^{1}H NMR spectrum. This is supported by the presence of a broad peak at 2735 cm^{-1} in the IR spectrum that is a characteristic O-H stretch for dimerised acids. All remaining data for the product **(104)** was as expected.

Olefin **(104)** was then hydrogenated with palladium on carbon (10 %) in the presence of hydrogen gas to give the alkane **(102)**. Much care was taken whilst adding the catalyst as the methanol can ignite if oxygen is present, as it is readily dehydrogenated. This was overcome by blowing a steady stream of nitrogen gas into the flask during the addition of the catalyst. This reaction was completed in a few hours and gave (10*S*)-10-methyl-18-(tetrahydro-2*H*-pyran-2-yloxy)octadecanoic acid **(102)** in an 81 % yield. The peaks in the ^{1}H and ^{13}C NMR spectra were very similar to the previous compound. However, the disappearance of the peaks representing the double bond was clearly evident, confirming the formation of the hydrogenated product **(102)**.

The tetrahydropyranyl ether **(102)** was then deprotected by a standard acid-catalysed hydrolysis, using *p*-toluene sulfonic acid monohydrate in a mixture of THF, methanol and water (Scheme 15). The ^{1}H NMR spectrum of the product **(109)** clearly showed the disappearance of the characteristic signals for the tetrahydropyranyl group and the introduction of a two-hydrogen triplet representing the methylene adjacent to the alcohol at 3.64 ppm. However, a three-hydrogen singlet had also been introduced at 3.66 ppm suggesting that the acid had been converted into the methyl ester. This occured as the methanol, which was part of the solvent mixture, was involved in an acid catalysed esterification with the carboxylic acid. Although this was unwanted, it was not a major problem as the methyl ester could be removed during the thiolation stage, not causing extra steps to be included in the synthesis.

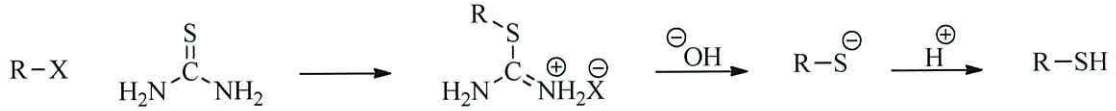


Scheme 15: Deprotection of a tetrahydropyranyl ether to form an alcohol.

The next stage was the trivial reaction of converting alcohol **(109)** to bromide **(110)** using *N*-bromosuccinimide and triphenylphosphine. This reaction occurred with no problems in an 87 % yield, showing the replacement of the two-hydrogen triplet at 3.64 ppm in the ^{1}H NMR spectrum with a two-hydrogen triplet at 3.41 ppm. All other peaks were as before. The formation of the bromide was further confirmed as two peaks of equal height were present in the mass spectrum for the mass ion.

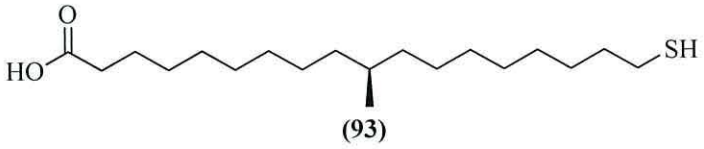
The final stage was then to convert the bromide into the thiol whilst hydrolysing the methyl ester to the acid to form (*S*)-18-mercapto-10-methyloctadecanoic acid **(93)** (Scheme

16). This was done by heating (*S*)-methyl 18-bromo-10-methyloctadecanoate **(110)** with thiourea in ethanol, which firstly forms the isothiouronium salt. The solvent was then evaporated and a solution of sodium hydroxide was added to cleave to the thiolate. The solution was heated again for two hours and the mixture was then acidified and the product extracted.



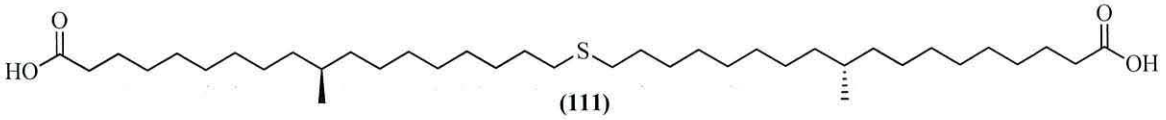
Scheme 16: Preparation of a thiol from an alkyl halide using thiourea.[193]

The thiolation of bromide **(110)** resulted in a mixture of compounds that were separated by column chromatography. The major product was (*S*)-18-mercapto-10-methyloctadecanoic acid **(93)**, the desired compound, in a 51 % yield (Scheme 17). The most significant change seen for the ^{1}H NMR spectrum for this compound was that the two-hydrogen triplet, representing the protons adjacent to the bromide, had been replaced by a two-hydrogen quartet at 2.54 ppm representing the methylene next to the thiol. The three-hydrogen singlet representing the methyl ester at 3.67 ppm had also disappeared; however, there was again no signal in the ^{1}H NMR spectrum representing the acid. Confirmation of the regeneration of the acid could be seen by looking at the IR spectrum where a broad signal was seen at 3027 cm^{-1}. The thiol group itself could not be identified in the NMR spectra; however, a weak signal could be seen in the IR spectrum at 2675 cm^{-1} representing the S-H stretch. Mass spectrometry also confirmed the molecular weight of this compound.



(93)

The minor compound isolated in the reaction was the thioether, (10*S*,10'*S*)-18,18'-thio*bis*(10-methyloctadecanoic acid) **(111)** in a lower yield of 24 %. Both the thioether **(111)** and the thiol **(93)** were practically identical by NMR with the only real difference being in the signal for the methylene group adjacent to the sulfur in the ^{1}H NMR spectrum. For (*S*)-18-mercapto-10-methyloctadecanoic acid **(93)** a two-proton quartet was seen at 2.54 ppm whilst for the disulfide **(111)** a two-hydrogen triplet was seen at 2.51 ppm (Figure 6).



(111)

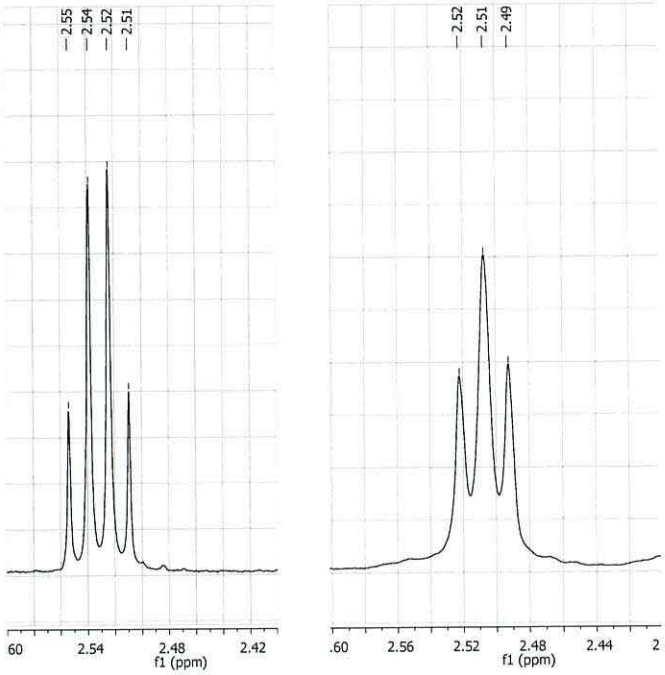
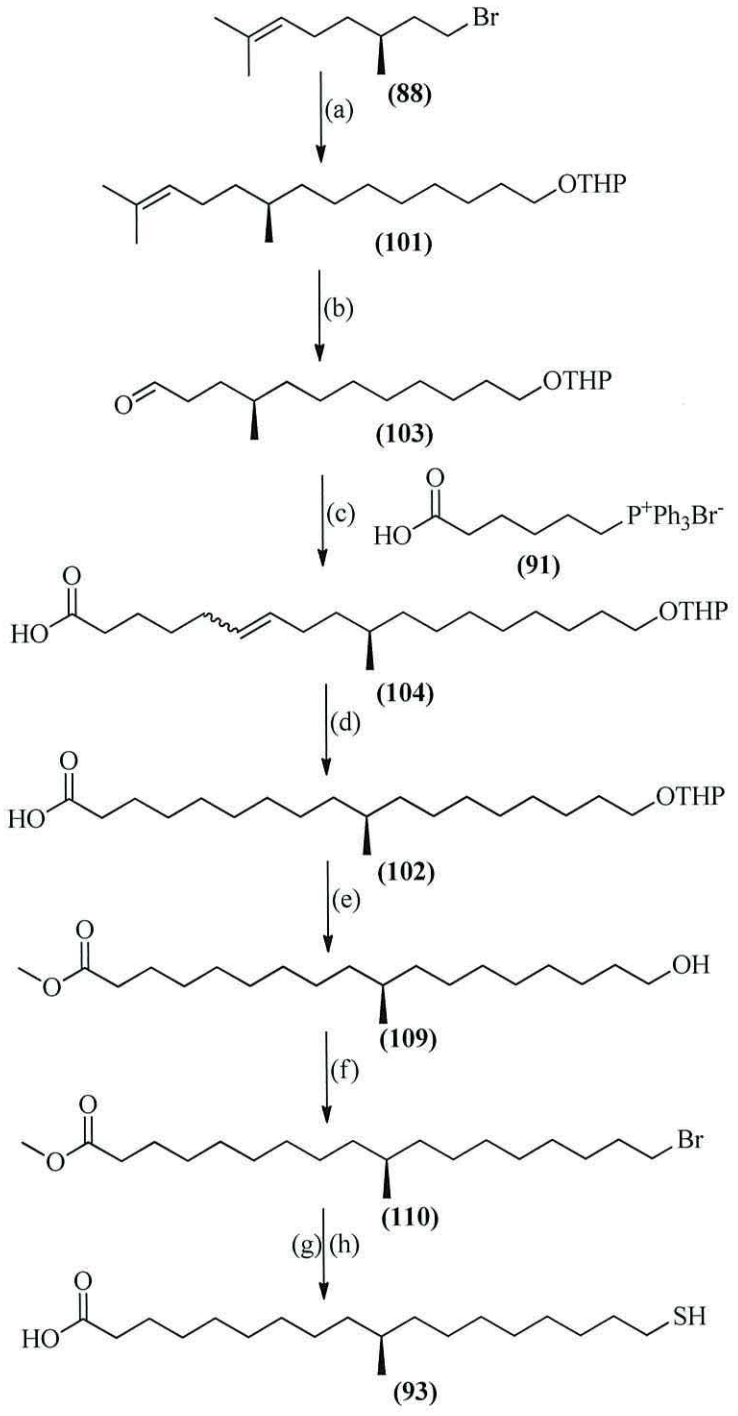


Figure 6: Comparison of a section of the ^{1}H NMR spectra between 2.4 and 2.6 ppm for (*S*)-18-mercapto-10-methyloctadecanoic acid **(93)** and (10*S*,10'*S*)-18,18'-thio*bis*(10-methyloctadecanoic acid) **(111)** showing the signal for the methylene group adjacent to the sulfur in both cases.

The molecular weight of the thioether **(111)** was confirmed by mass spectrometry, which confirmed that only one sulfur atom was present between the two alkyl chains and not two as would have been seen for a dialkyl disulfide. It was further confirmed that a thioether had been synthesised following literature searches that showed that generally a triplet is seen at around 2.50 ppm in the ^{1}H NMR spectrum for the methylene group adjacent to the sulfur in a thioether, which was the case here.[194] For a disulfide a triplet at around 2.68 ppm would be expected.[195]

Thioethers are generally synthesised by the treatment of alkyl halides with thiols in the presence of a base.[196, 197] If some unreacted bromide **(110)** remained that had not formed the isothiouronium salt, during treatment with the base, as the thiouronium salt is cleaved to generate the thiolate, the thiolate may then react with the unreacted bromide **(110)** to form the thioether. This can occur as the thiolates are highly potent nucleophiles.

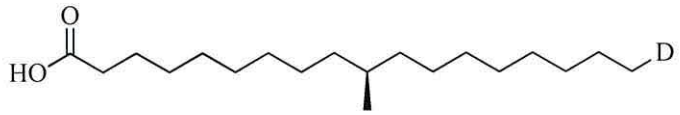


Scheme 17: Synthesis of (*S*)-18-mercapto-10-methyloctadecanoic acid **(93)**. (a) $THPO(CH_2)_6MgBr$, dry THF, -78°C, $LiCuCl_4$, 70 %; (b) O_3 (g), CH_2Cl_2, -78 °C, PPh_3, 80 %; (c) LiHMBS (1.06 M in THF), toluene/DMSO, 0 °C, 51 %; (d) H_2 (g), Pd/C (10 %), MeOH, 81 %; (e) PTSA, THF, MeOH, H_2O, reflux, 74 %; (f) NBS, PPh_3, CH_2Cl_2, 0°C, 87 %; (g) thiourea, EtOH, reflux; (h) 5 M NaOH, HCl, 51 %.

Although some thioether **(111)** had been formed, (*S*)-18-mercapto-10-methyloctadecanoic acid **(93)** had been obtained successfully in an overall yield of 7.6 % (Scheme 17). The

thioether **(111)** could also be used in some antibody recognition experiments as the thioether may also be bound to the gold surface and the difference between the binding of a thiol and a thioether could be investigated.[198]

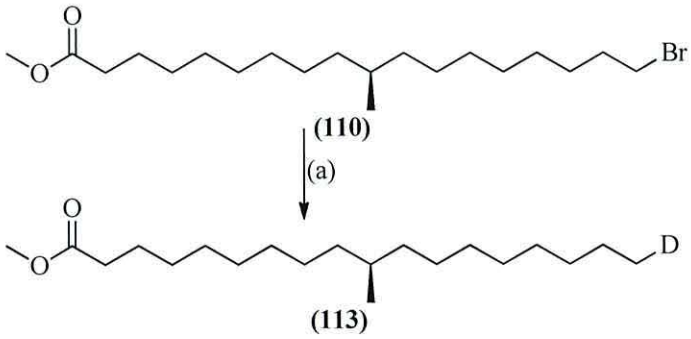
2.3 - The Synthesis of Deuterated Tuberculostearic acid (112)



(112)

Since it is known that TBSA **(70)** is used for the detection of tuberculosis by GC methods, synthesis of labelled TBSA was attempted. Forming labelled TBSA would make it much easier to quantify the amount of TBSA **(70)** present, potentially allowing for the discovery of the extent of the disease.

2.3.1 - The Original Methodology



(113)

Scheme 18: Attempted synthesis of methyl (*R*)-10-methyloctadecanoate deuteride **(113)**. (a) D_2 (g), Pd/C (10 %), MeOH.

During a hydrogenation catalysed by palladium on carbon a bromide can be converted into a proton.[199] Therefore, based on the same principle, exposing a stirred solution of bromide **(110)** and palladium on carbon to deuterium gas should convert the bromide into a deuterium, resulting in the formation of methyl (*R*)-10-methyloctadecanoate deuteride **(113)**. This would give the methyl ester rather than the free acid, which is generally used during testing anyway.[130]

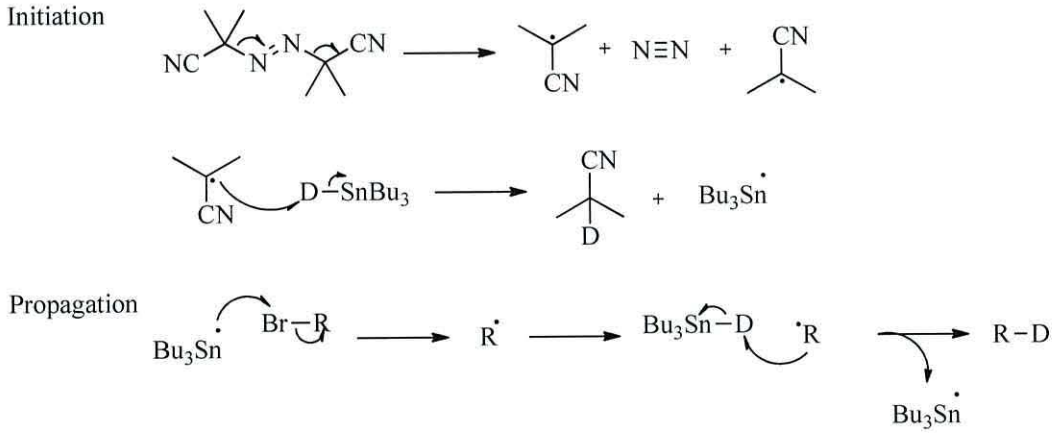
This reaction was performed by stirring a mixture of bromide **(110)** with palladium on carbon in methanol and exposing the reaction mixture to a deuterium filled balloon. The

balloon deflated quite rapidly and was refilled as needed. The reaction mixture was stirred overnight under these conditions but upon work up only the starting material **(110)** was recovered.

The reaction was repeated by stirring for a total of five days whilst the balloon was refilled as required. However, this did not yield **(113)**. This reaction was then repeated for the third time in hexane to see if performing the reaction in a non-polar solvent compared to a protic polar solvent would result in any difference. However, after stirring for five days no product **(113)** was formed. As this method had been unsuccessful, a different route was then devised for the synthesis of methyl (*R*)-10-methyloctadecanoate deuteride **(113)**.

2.3.2 - The Final Method

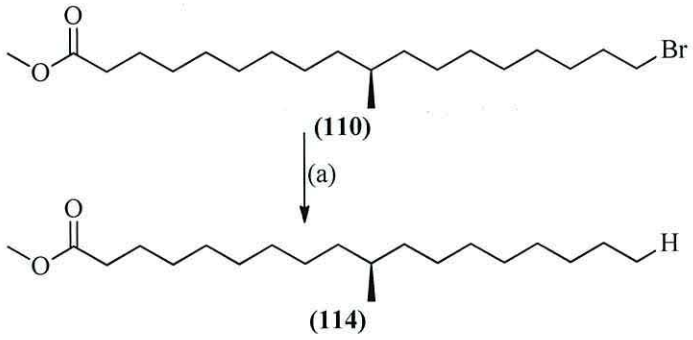
An alternative method was investigated for the formation of deuteride **(113)** that involved the reductive cleavage of the alkyl-halogen bond using tri-n-butyltindeuteride, catalysed by AIBN.



Scheme 19: Radical preparation of a deuterated compound.

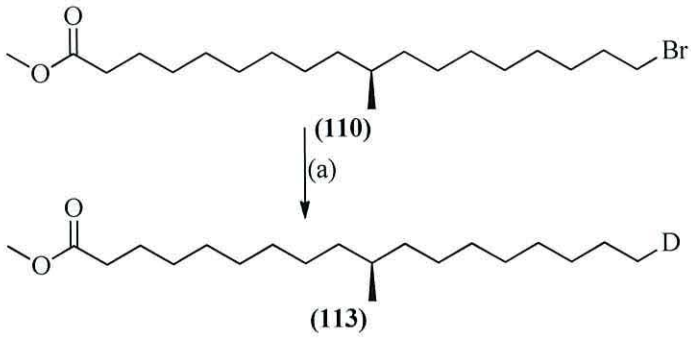
The first stage in this free radical reaction is the initiation stage, which involves the homolysis of tri-n-butyltindeuteride. This is promoted by the initiator AIBN that produces the higher concentration of the free radical $Bu_3Sn^{\cdot}$ needed due to the strength of the C-Br bond. The radical then attacks the bromide, which is substituted for a deuterium atom through a radical process giving rise to the deuterated product (Scheme 19).

Due to the high cost of tri-n-butyltindeuteride and the limited amount of starting material **(110)** available, the reaction was initially executed using tri-n-butyl tin hydride to form methyl (*R*)-10-methyloctadecanoate **(114)** (Scheme 20).



Scheme 20: Synthesis of methyl (*R*)-10-methyloctadecanoate **(114)**. (a) Tri-n-butyltinhydride, dry ether, AIBN, reflux, 87%.

A solution of the bromide **(110)** and AIBN was added to a solution of tri-n-butyltinhydride in ether at reflux and the mixture was heated under reflux for two days. Reflux was required as AIBN undergoes homolysis at 60 °C. Column chromatography of the residue gave the hydrogenated product **(114)** in an 87 % yield. The formation of the product **(114)** could be clearly seen in the ^{1}H NMR spectrum with the replacement of the two-hydrogen triplet at 3.41 ppm by a three-hydrogen triplet at 0.89 ppm, representing the terminal CH_3. The removal of the bromide was further confirmed by mass spectrometry, which showed a single $(M + Na)^+$ peak at 335.2911.



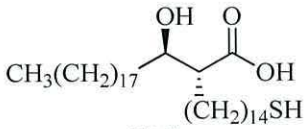
Scheme 21: Synthesis of methyl (*R*)-10-methyloctadecanoate deuteride **(113)**. (a) Tri-n-butyltindeuteride, dry ether, AIBN, reflux, 81 %.

As the reaction using tri-n-butyltinhydride had gone to completion as expected it was repeated using tri-n-butyl tin deuteride under exactly the same conditions (Scheme 21). This resulted in the formation of methyl (*R*)-10-methyloctadecanoate deuteride **(113)** in a good yield of 81 %. Its formation was confirmed again with the disappearance of the two-hydrogen triplet at 3.41 ppm in the ^{1}H NMR spectrum. However, rather than the appearance of a three-hydrogen triplet at 0.89 ppm, a two-hydrogen multiplet could be

seen, due to the coupling to the deuterium. All remaining peaks in the ^{1}H NMR spectrum were as for the starting material **(110)**. The clearest evidence of the inclusion of the deuterium was seen in the proton decoupled ^{13}C NMR spectrum where a triplet was seen at 13.7 ppm, again caused by coupling to the deuterium, spin 1. Comparison of the mass spectra for methyl (*R*)-10-methyloctadecanoate deuteride **(113)** and methyl (*R*)-10-methyloctadecanoate **(114)** also confirmed the formation of the deuteride **(113)** as the mass spectra showed the expected difference of 1 between the two compounds.

2.4 - The Synthesis of a thiolated model of a mycolic acid

In order to see whether a thiolated mycolic acid could be synthesised it was decided to first synthesise a thiolated model of a mycolic acid, as synthesising full mycolic acids is an extremely long and laborious process. As the mycolic motif is a constant in all mycolic acids, a model (**115**) would be synthesised that contained the motif and a straight alkyl chain as the meromycolate fragment forming a simple mycolic acid (Figure 3, page 10). It was decided to include the thiol group at the end of the α-alkyl chain, as it was believed that introducing it at this position would not interfere with any antibody recognition ability that the molecule might possess. The α-alkyl chain, which generally contains 22 or 24 carbon atoms in the full mycolic acid,[46] would also only contain 14 carbon atoms. This was only a model, and it was not believed that making the α-chain shorter would make much difference to the process when later synthesising the complete thiolated mycolic acid. Further studies could also investigate the effects of introducing the thiol group at different positions within the mycolic acid.

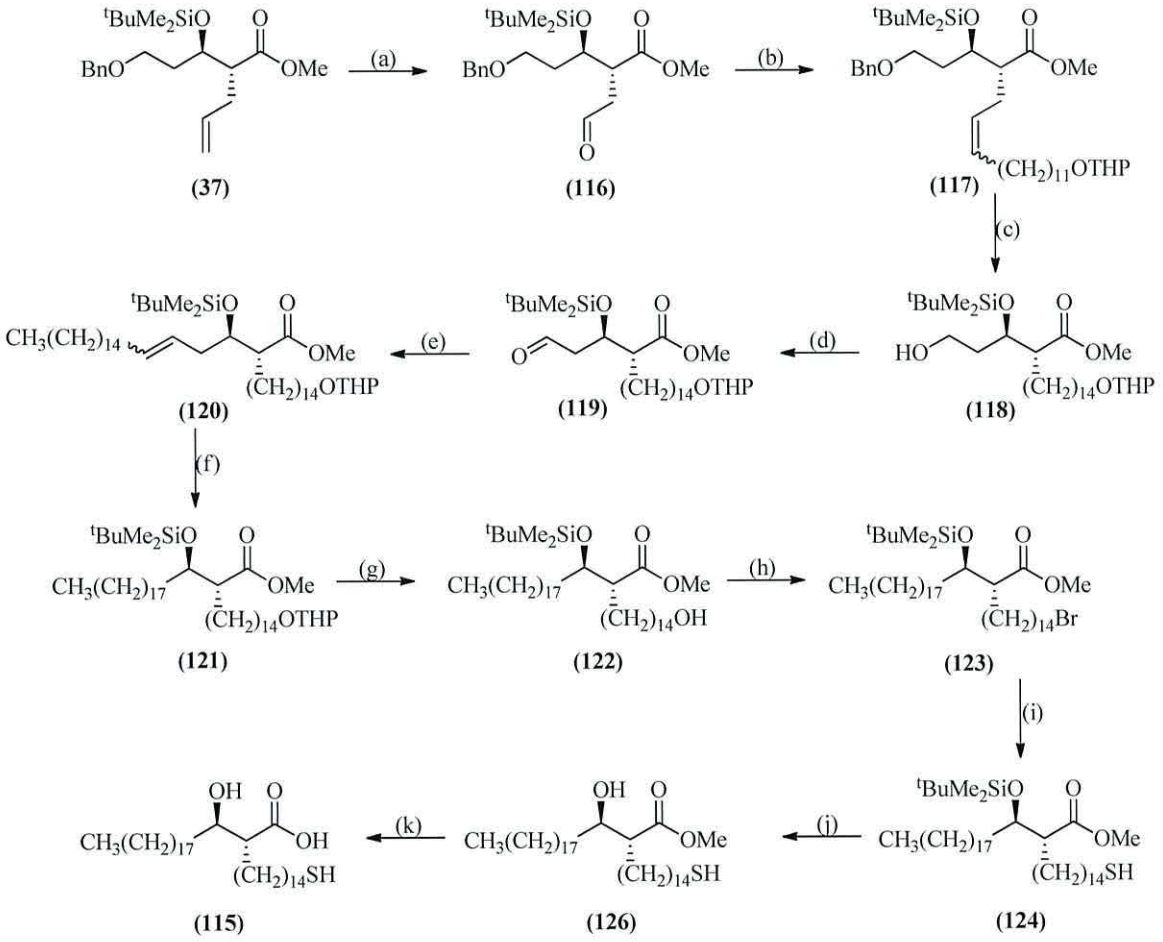


(115)

2.4.1 - The original methodology

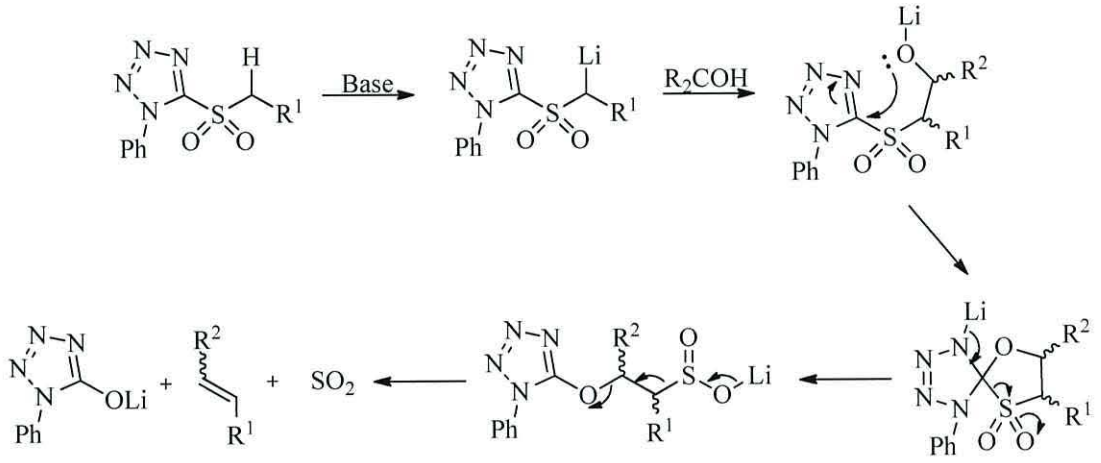
It was decided to combine existing methods for the synthesis of mycolic acids with the method developed for the synthesis of thiolated TBSA **(93)** to synthesise (2*R*,3*R*)-3-hydroxy-2-(14-mercaptotetradecyl)henicosanoic acid **(115)**. This synthesis would begin from the intermediate (*R*)-methyl 2-((*R*)-3-(benzyloxy)-1-(*tert*-butyldimethylsilyloxy)propyl)pent-4-enoate **(37)**,[61] which had been prepared in multigram quantities by members of the Baird group for the synthesis of general mycolic acids. This

fragment is always used, regardless of which mycolic acid is synthesised. After oxidising the double bond of this intermediate, the aldehyde (**116**) could then be coupled to a long chain sulfone that includes a functional group such as a tetrahydropyranyl ether at the non-reacting end. Following hydrogenation to saturate the double bond and remove the benzyl group, the newly formed terminal alcohol (**118**) could be oxidised and coupled again to another sulfone to form the meromycolate alkyl chain. The tetrahydropyranyl ether (**121**) could then be deprotected to the alcohol, converted to the bromide, before being converted again to the thiol. The silyl and ester protecting groups could finally be removed to give the thiolated model of a mycolic acid (**115**) as in Scheme 22.

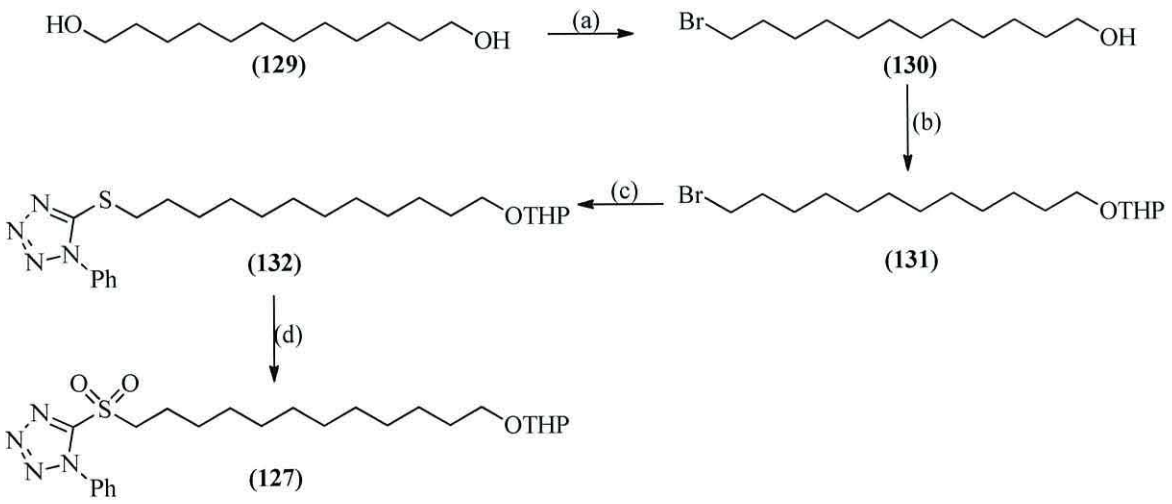


Scheme 22: Attempted synthesis of (2*R*,3*R*)-3-hydroxy-2-(14-mercapto-tetradecyl)henicosanoic acid **(115)**. (a) 2,6-Lutidine, OsO_4, $NaIO_4$, dioxane/H_2O; (b) LiHMDS, 1-phenyl-5-(12-((tetrahydro-2*H*-pyran-2-yloxy)dodecylsulfonyl)-1*H*-tetrazole **(127)**, dry THF; (c) Pd/C (10 %), H_2 (g), IMS/THF; (d) PCC, CH_2Cl_2; (e) LiHMDS, 5-(hexadecylsulfonyl)-1-phenyl-1*H*-tetrazole **(128)**, dry THF; (f) Pd/C (10 %), H_2 (g), IMS/THF; (g) PPTS, THF/MeOH/H_2O; (h) NBS, PPh_3, CH_2Cl_2; (i) Thiourea, EtOH, NaOH, H^+; (j) HF.Pyridine, Py, THF; (k) $LiOH.H_2O$, THF/MeOH/H_2O.

For the general synthesis of mycolic acids, intermediate **(37)** is oxidised to the aldehyde **(116)** and coupled to a long chain sulfone *via* a modified Julia-Kocienski coupling reaction (Scheme 23).[61] This coupling reaction is based on the Julia olefination,[200] which had been adapted by P. J. Kocienski *et al.*[201] to make it a very important reaction in organic synthesis today. It involves reacting an aldehyde with a sulfone in the presence of a base. The addition of a base to a sulfone leads to the metallated sulfone, which on reaction with an aldehyde gives rise to a β-alkoxysulfone. Due to the instability of the β-alkoxysulfone it undergoes the Smiles rearrangement, which results in the sulfinate salt, which undergoes elimination to form sulfur dioxide, lithium 1-phenyl-1*H*-tetrazolone and a mixture of alkenes.

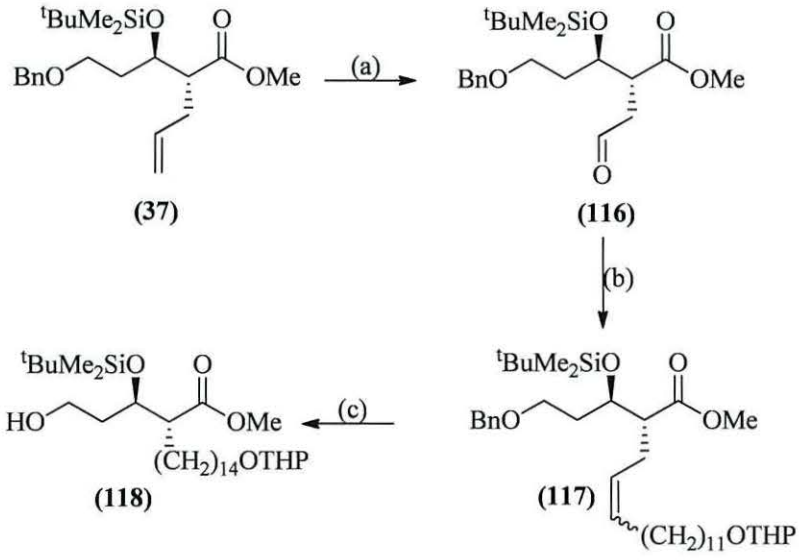


Scheme 23: Mechanism of the modified Julia-Kocienski olefination.



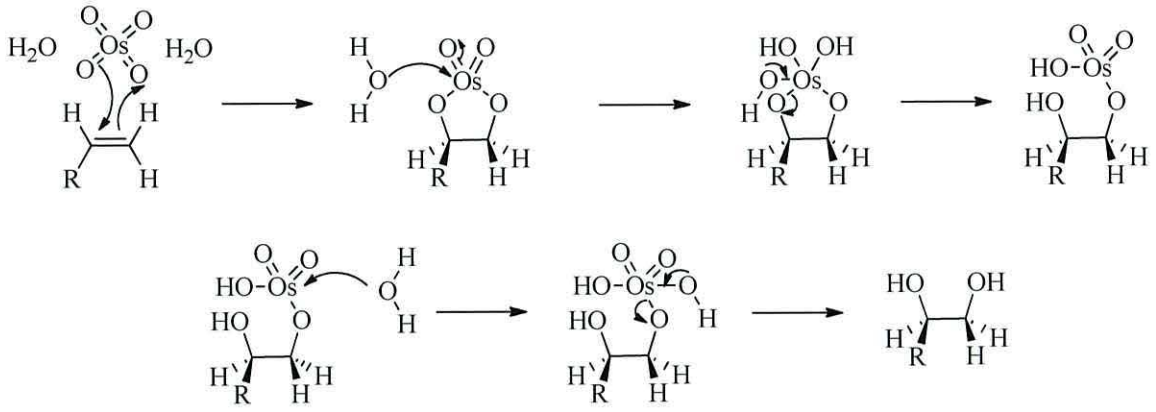
Scheme 24: Synthesis of 1-phenyl-5-(12-(tetrahydro-2*H*-pyran-2-yloxy)dodecylsulfonyl)-1*H*-tetrazole **(127)**. (a) HBr (aq), toluene, reflux, 78 %; (b) 2,3-dihydropyran, PPTS, CH_2Cl_2, 92 %; (c) 1-phenyl-1*H*-tetrazole-5-thiol, K_2CO_3, acetone, reflux, 90 %; (d) ammonium molybdate(VI) tetrahydrate, H_2O_2, THF, IMS, 77 %.

Therefore, before coupling, the sulfone, 1-phenyl-5-(12-(tetrahydro-2*H*-pyran-2-yloxy)dodecylsulfonyl)-1*H*-tetrazole **(127)** was firstly prepared from diol **(129)** (Scheme 24). 1,12-Dodecanediol **(129)** was firstly monobrominated with aqueous hydrobromic acid under reflux. The second alcohol was then protected with a tetrahydropyranyl protecting group. This protecting group is chosen to be different to the others in the intermediate **(37)**, which ensures that it can be removed selectively. The formation of 2-(12-bromododecyloxy)tetrahydro-2*H*-pyran **(131)** was confirmed with the characteristic pattern for the tetrahydropyranyl group clearly seen between 3.39 and 4.57 ppm in the ^{1}H NMR spectrum. The bromide **(131)** was then converted into the sulfide **(132)** with 1-phenyl-1*H*-tetrazole-5-thiol and anhydrous potassium carbonate forming 1-phenyl-5-(12-(tetrahydro-2*H*-pyran-2-yloxy)dodecylthio)-1*H*-terazole **(132)**. Subsequently, the sulfide **(132)** was oxidised to the sulfone **(127)** using ammonium molybdate(VI) tetrahydrate and hydrogen peroxide. Both compounds appear very close on TLC and were very similar by NMR. A slight change in the multiplicity of the aromatic peaks was seen, but the main difference was observed for the methylene group adjacent to the sulfur, as a shift occurred from 3.39 ppm to 3.72 ppm on oxidation. The splitting associated with these peaks could not be seen, as a multiplet was formed, in both cases, by overlap of the signals with those of one of the protons adjacent to the oxygen atoms of the tetrahydropyranyl ether.



Scheme 25: Synthesis of methyl (2*R*)-2-((*R*)-1-(*tert*-butyldimethylsilyloxy)-3-hydroxypropyl)-16-(tetrahydro-2*H*-pyran-yloxy)hexadecanoate **(118)**. (a) 2,6-Lutidine, OsO_4, $NaIO_4$, dioxane/H_2O, 77 %; (b) LiHMDS, 1-phenyl-5-(12-((tetrahydro-2*H*-pyran-2-yloxy)dodecylsulfonyl)-1*H*-tetrazole **(127)**, dry THF, 75 %; (c) Pd/C (10 %), H_2 (g), IMS/THF, 79 %.

Once the sulfone **(127)** was prepared, methyl (2*R*,3*R*)-5-(benzyloxy)-3-(*tert*-butyldimethylsilyloxy)-2-(2-oxoethyl) pentanoate **(37)** (which was kindly contributed by Miss Cornelia Theunissen)[202] was oxidised to the aldehyde **(116)** with 2,6-lutidine, osmium tetroxide and sodium metaperiodate in a mixture of dioxane and water by oxidative cleavage.[203] Osmium tetroxide firstly converts the olefin into a diol (Scheme 26), which is then cleaved by sodium metaperiodate.



Scheme 26: Formation of a diol from an olefin using osmium tetroxide.

Following the oxidation of alkene **(37)**, the aldehyde was clearly present as a broad one-hydrogen singlet at 9.81 ppm could be seen in the ^{1}H NMR spectrum. This aldehyde **(116)** was used immediately in a modified Julia-Kocienski coupling reaction with 1-phenyl-5-(12-(tetrahydro-2*H*-pyran-2-yloxy)dodecylsulfonyl)-1*H*-tetrazole **(127)** in the presence of lithium *bis*(trimethylsilyl)amide as a base.

The coupling product **(117)** could be seen as a black spot high on the TLC plate when this was dipped in a solution of phosphomolybdic acid and charred, in comparison to the starting materials, which appeared quite low on the plate. A mixture of the two alkene products **(117)** was formed in this reaction but this was not important, as the double bond would be hydrogenated in the next step. ^{1}H and ^{13}C NMR spectra of the coupling product **(117)** showed the clear formation of the double bond with two, one-hydrogen multiplets at 5.28 and 5.41 ppm and two peaks at 132.8 and 138.5 ppm observed, respectively. For some of the signals in the ^{1}H NMR spectrum, two peaks were seen close together. This was most evident for the peaks representing the silyl protecting group, as both singlets at 0.04 and 0.86 ppm were associated with smaller singlets. This was due to the existence of the *cis* and *trans* isomers of the olefin **(117)**, which caused slightly different chemical shift values to be observed.

Olefin **(117)** was then hydrogenated using hydrogen gas catalysed by palladium on carbon. A solution of olefin **(117)** was stirred with palladium on carbon (10 %) under a hydrogen atmosphere for a total of three days. This reaction not only required the hydrogenation of the double bond but also the hydrogenolysis of the benzyl ether, which accounted for the long reaction time.

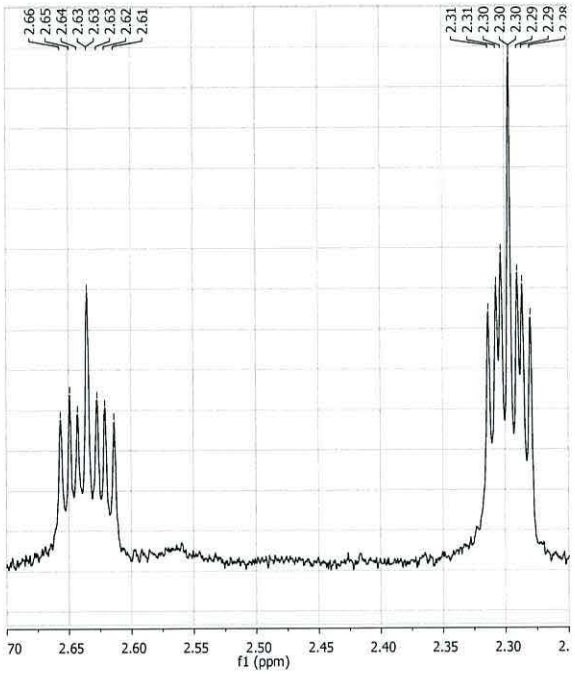
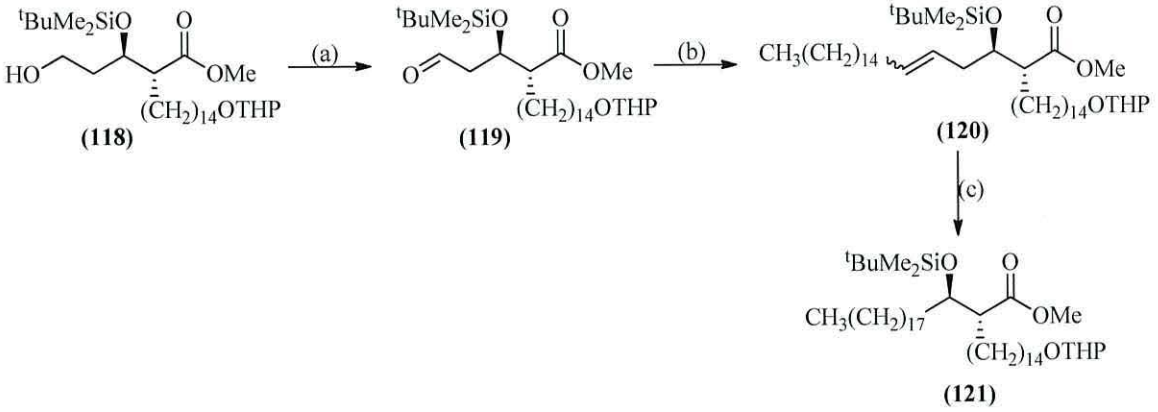


Figure 7: A section of the ^{1}H NMR spectrum for methyl (2*R*)-2-((*R*)-1-(*tert*-butyldimethylsilyloxy)-3-hydroxypropyl)-16-(tetrahydro-2*H*-pyran-yloxy)hexadecanoate **(118)** in $CDCl_3$ between 2.25 and 2.70 ppm showing the multiple peaks for the α-proton.

The formation of methyl (2*R*)-2-((*R*)-1-(*tert*-butyldimethylsilyloxy)-3-hydroxypropyl)-16-(tetrahydro-2*H*-pyran-yloxy)hexadecanoate **(118)** was confirmed with the disappearance of the olefinic and aromatic peaks in both ^{1}H and ^{13}C NMR spectra. However, the remainder of the ^{1}H NMR spectrum for this product was extremely complicated. Two peaks could be seen at different chemical shifts for most of the expected peaks in a ratio of around 1:2 (Figure 7). This was unexpected. One explanation for the occurrence of multiple peaks may be that some of the tetrahydropyranyl ether had migrated to the alcohol of the previous benzyl ether through an acid-catalysed deprotection of the tetrahydropyranyl group and the acid-catalysed protection of the other alcohol. This could have occurred when the compound was in the deuterated chloroform in the NMR tube. It is known that the deprotection of a tetrahydropyranyl ether does occur in deuterated chloroform as a small triplet can often be seen at around 3.64 ppm, between the four peaks for the four-

protons adjacent to the oxygen atoms. This peak was also seen in the ^{1}H NMR spectrum for the present compound. It was therefore believed that the tetrahydropyranyl ether did not migrate in the bulk product synthesised but only in the NMR sample.



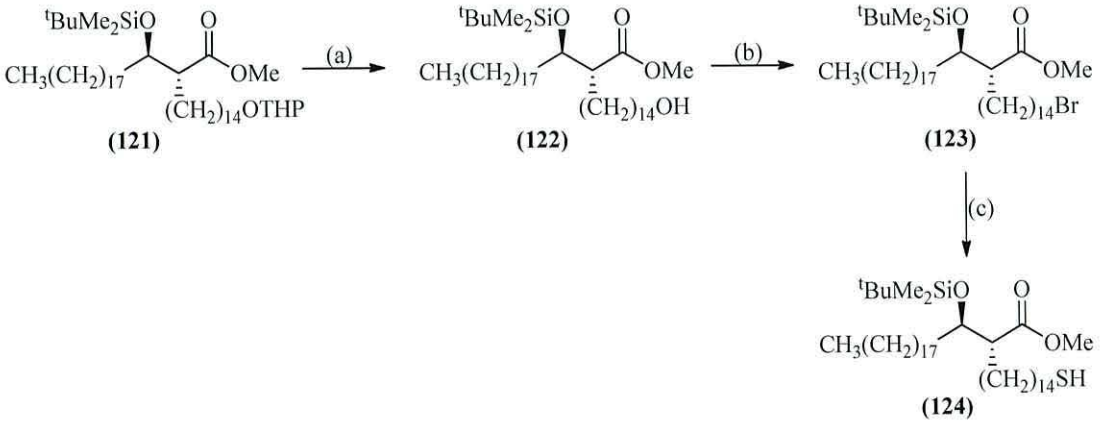
Scheme 27: Synthesis of methyl (2*R*,3*R*)-3-(*tert*-butyldimethylsilyloxy)-2-(14-(tetrahydro-2*H*-pyran-2-yloxy)tetradecyl)henicosanoate **(121)**. (a) PCC, CH_2Cl_2, 82 %; (b) LiHMDS, 5-(hexadecylsulfonyl)-1-phenyl-1*H*-tetrazole **(128)**, dry THF, 75 %; (c) Pd/C (10 %), H_2 (g), IMS/THF, 92 %.

The newly synthesised alcohol in **(118)** was oxidised to the aldehyde **(119)** and coupled to 5-(hexadecylsulfonyl)-1-phenyl-1*H*-tetrazole **(128)**, again *via* a modified Julia-Kocienski coupling reaction using lithium *bis*(trimethylsilyl)amide as a base (Scheme 27). The aldehyde **(119)** was prepared this time by stirring the alcohol **(118)** in a suspension of PCC in dichloromethane and its formation was evident by a one-hydrogen, doublet of doublets in the ^{1}H NMR spectrum at 9.18 ppm. The product **(119)** gave a clean spectrum with only one peak present for each of the signals expected. This concurs with the theory that the change of the alcohol **(118)** occurred only in the NMR tube. This aldehyde **(119)** was again used immediately in a coupling reaction due to its unstable nature.

As for the previous Julia-Kocienski coupling, the reaction proceeded by adding base to a stirred solution of aldehyde **(119)** and sulfone **(128)** (contributed by Dr Juma'a Al-Dulayymi). Peaks could be seen in both ^{1}H and ^{13}C NMR spectra representing the double bond at 5.44 ppm as a two-hydrogen multiplet in the ^{1}H NMR spectrum and at 132.2 and 133.8 ppm in the ^{13}C NMR spectrum. The peaks representing the silyl protecting group were again associated with other smaller peaks. This was again due to the presence of the *cis* and *trans* isomers of the olefin **(120)**.

The coupling product **(120)** was then hydrogenated, catalysed by palladium on carbon. This reaction was completed in a few hours as it only involved the hydrogenation of the double bond. Disappearance of the peaks representing the double bond was evident in both ^{1}H and ^{13}C NMR spectra, and the multiple peaks for the silyl protecting group had also disappeared. This reaction led to a complete model of a mycolic acid **(121)**, which included functionality at the end of the α-alkyl chain that could be converted into a thiol (Scheme 28). The silyl and methyl esters could then also be removed to form an unprotected model of a thiolated mycolic acid **(115)**.

The tetrahydropyranyl ether was firstly deprotected to the alcohol using pyridinium-*p*-toluene sulfonate (Scheme 28). Only a mild acid is needed to deprotect a tetrahydropyranyl ether and pyridinium-*p*-toluene sulfonate is a much better reagent than the *p*-toluene sulfonic acid monohydrate used previously and resulted in a good yield of 82 % for the reaction. The disappearance of the tetrahydropyranyl peaks was evident in the ^{1}H NMR spectrum confirming the deprotection. The alcohol **(122)** was then converted into the bromide **(123)** by the standard method using *N*-bromosuccinimide and triphenylphosphine. This reaction worked as expected with the two-hydrogen triplet at 3.65 ppm in the ^{1}H NMR spectrum shifting to 3.41 ppm to represent the methylene adjacent to the bromide. Other significant peaks observed in the ^{1}H NMR spectrum were a one-hydrogen doublet of triplets at 3.91 ppm representing the β-proton, a three-hydrogen singlet at 3.66 ppm representing the methyl ester, and a one-hydrogen doublet of doublet of doublets at 2.53 ppm representing the α-proton. The peaks for the silyl protecting group were also seen as three separate singlets at 0.02, 0.05 and 0.87 ppm.



Scheme 28: Attempted synthesis of methyl (2*R*,3*R*)-3-(*tert*-butyldimethylsilyloxy)-2-(14-mercaptotetradecyl)henicosanoate **(124)**. (a) PPTS, THF, MeOH, H_2O, 82 %; (b) NBS, PPh_3, CH_2Cl_2, 72 %; (c) Thiourea, EtOH, NaOH, H^+.

It was hypothesised that the bromide **(123)** could then be converted into the thiol **(124)** by the same method as for the synthesis of (*S*)-18-mercapto-10-methyloctadecanoic acid **(93)** (Scheme 16).[204] The bromide **(123)** was firstly heated with thiourea in ethanol, followed by the addition of a solution of sodium hydroxide. The mixture was again heated before being acidified and the product extracted. The crude ^{1}H NMR spectrum of the residue of this reaction was very complicated and contained numerous different peaks. However, it was easily observed that some starting material **(123)** remained. If a thiol had been produced as expected, a quartet would be observed at around 2.50 ppm for the methylene group next to the sulfur as at room temperature sulfhydryl protons exchange at a low rate, resulting in coupling to protons on the adjacent carbon atom.[205] However, no such quartet could be clearly seen as the peak for the α-proton was also present at 2.50 ppm. The multipet at 2.50 ppm may therefore contain signals for the α-proton and the thiol quartet but due to overlapping signals it could not be clearly identified. However, at 2.68 ppm a newly formed triplet could be seen. This may represent a methylene group adjacent to a sulfur in a disulfide.[206] The formation of a disulfide and not a thiol is not considered to be problematic as a disulfide can bond to gold in the same way as a thiol.[207] The triplet at 2.68 ppm was extremely small in comparison to the other peaks in the spectrum, which suggests that the disulfide was formed in a very low yield. It may have been generated in this reaction as they can be easily synthesised from thiols by refluxing in a solution of sodium hydroxide.[208] One clear difference that was seen between the ^{1}H NMR spectrum for this crude residue and that for thiolated TBSA **(93)** was that the methyl ester was still fully present with a singlet integrating to three hydrogens. This procedure hydrolysed the methyl ester in the synthesis of thiolated TBSA **(93)**, but not for this molecule. This may be due to the higher complexity of the molecule in comparison to TBSA **(70)**. As this reaction was only performed on a very small quantity and due to the small amount of product that was believed to have been formed, no attempt was made to purify the residue.

This reaction was then repeated under the same reaction conditions but this time it was initially heated under reflux with thiourea overnight. The crude ^{1}H NMR spectrum of the product for this reaction showed peaks for all the significant protons for the bromide **(123)** apart from the triplet at 3.41 ppm. This indicated that all the starting material **(123)** was used up in the reaction and that increasing the reaction time did have a positive effect on the reaction. Again, it was not possible to clearly state whether any thiol was present due to the overlapping signals at 2.50 ppm, but a stronger triplet was seen at 2.68 ppm suggesting that more of the disulfide had been produced. The residue was attempted to be purified by

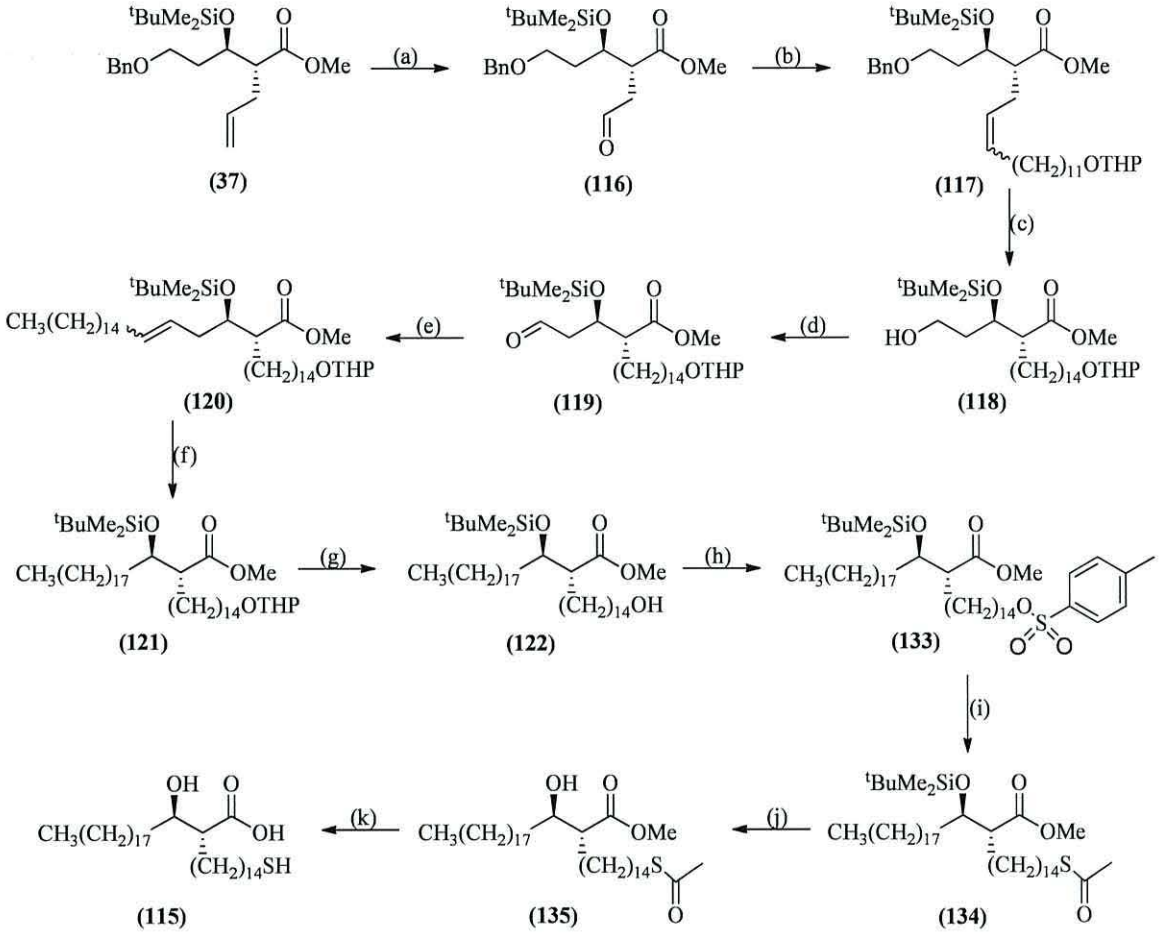
column chromatography but the entire product was lost. This may have occurred due to the small scale of the reaction or due to its decomposition at a high pH.[209]

A slightly different method was then used to try and generate the thiol. The bromide **(123)** was firstly stirred with thiourea in ethanol. The solvent was not evaporated and a solution of sodium hydroxide was added. The mixture was stirred for five minutes at ambient temperature before being acidified and stirred for 20 minutes.[210] The residue of the reaction gave a very busy crude ^{1}H NMR spectrum showing that none of the starting material **(123)** remained but that some disulfide and maybe some thiol was produced. However, after purification by column chromatography the entire residue was lost. This may be due to the scale of the reaction, the decomposition at high pH or that the reaction simply is not suitable for such a complex structure. It is seen from the literature that it is mainly simple molecules that are converted from bromides into thiols using thiourea and that alternative methods are used for more complex structures.[211]

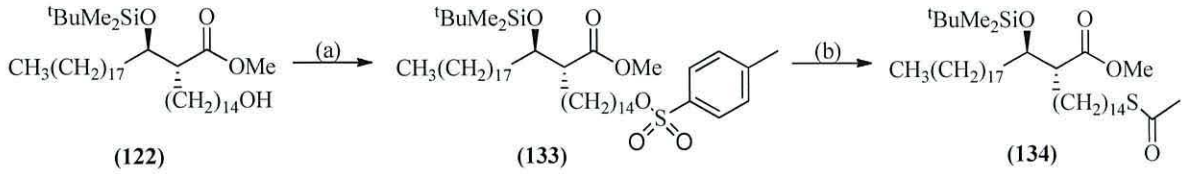
2.4.2 - The second method

As pure methyl (2*R*,3*R*)-3-(*tert*-butyldimethylsilyloxy)-2-(14-mercaptotetradecyl)-henicosanoate **(124)** had not been successfully synthesised and the amount of starting material **(123)** remaining had been substantially reduced, an alternative method was then examined for the synthesis of (2*R*,3*R*)-3-hydroxy-2-(14-mercaptotetradecyl)henicosanoic acid **(115)** (Scheme 29).

This synthesis would use the alcohol **(122)** as described earlier (Scheme 22) which would be converted into a tosylate **(133)**, followed by a thioacetate **(134)** (Scheme 30).[212] The tosylate **(133)** was formed from alcohol **(122)** in this case rather than a halide **(123)** as it is a much better leaving group.[213] The silyl and ester protecting groups would then be removed (Scheme 31). During the hydrolysis of the methyl ester the thioacetate was likely to be converted into the thiol, but this was not essential as a thiol or a thioacetate may be attached to the gold surface, although longer adsorption times are required to have the same surface coverage with thioacetates.[214]



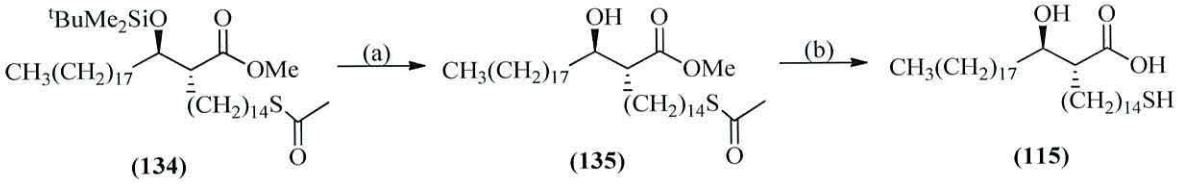
Scheme 29: Attempted synthesis of (2*R*,3*R*)-3-hydroxy-2-(14-mercaptotetradecyl)-henicosanoic acid **(115)**. (a) 2,6-Lutidine, OsO_4, $NaIO_4$, dioxane/H_2O; (b) LiHMDS, 1-phenyl-5-(12-((tetrahydro-2*H*-pyran-2-yloxy)dodecylsulfonyl)-1*H*-tetrazole **(127)**, dry THF; (c) Pd/C (10 %), H_2 (g), IMS/THF; (d) PCC, CH_2Cl_2; (e) LiHMDS, 5-(hexadecylsulfonyl)-1-phenyl-1*H*-tetrazole **(128)**, dry THF; (f) Pd/C (10 %), H_2 (g), IMS/THF; (g) PPTS, THF/MeOH/H_2O; (h) NEt_3, TsCl, CH_2Cl_2; (i) potassium thioacetate, acetone; (j) HF.Pyridine, Py, THF; (k) LiOH.H_2O, THF/MeOH/H_2O, H^+.



Scheme 30: Synthesis of methyl (2*R*,3*R*)-2-(14-(acetylthio)tetradecyl)-3-(*tert*-butyldimethylsilyloxy) henicosanoate **(134)**. (a) NEt_3, TsCl, CH_2Cl_2, 74 %; (b) potassium thioacetate, acetone, 75 %.

To synthesise the tosylate **(133)**, *p*-toluene sulfonyl chloride was added to a stirred solution of ester **(122)** and triethylamine in dichloromethane and the solution was left in the refrigerator overnight. Two doublets could be seen at 7.36 and 7.80 ppm in the ^{1}H NMR spectrum of the product **(133)** showing the formation of the aromatic ring, which was confirmed with peaks at 127.9, 129.8, 133.3 and 144.6 ppm in the ^{13}C NMR spectrum. A three-hydrogen singlet at 2.46 ppm was seen for the methyl on the ring and a two-hydrogen triplet at 4.02 ppm was present for the methylene adjacent to the oxygen.

The tosylate **(133)** was then stirred with potassium thioacetate in acetone for four hours to form methyl (2*R*,3*R*)-2-(14-(acetylthio)tetradecyl)-3-(*tert*-butyldimethylsilyloxy) henicosanoate **(134)** in a yield of 75 %. The ^{1}H and ^{13}C NMR spectra showed the disappearance of the peaks representing the tosylate and these were replaced with peaks representing the thioacetate. The two-hydrogen triplet representing the methylene adjacent to the sulfur shifted upfield to 2.87 ppm and a three-hydrogen singlet could also be seen at 2.32 ppm representing the CH_3 group next to the carbonyl. Two carbonyl peaks could also now be seen in the ^{13}C NMR spectrum, the original peak at 175.1 ppm for the carbonyl of the methyl ester and at 196.0 ppm for the carbonyl of the thioacetate. This reaction was favoured as no sulfide products, such as disulfides, could be formed, which was a problem when using thiourea.[193]



Scheme 31: Attempted synthesis of (2*R*,3*R*)-3-hydroxy-2-(14-mercaptotetradecyl)-henicosanoic acid **(115)**. (a) HF.Pyridine, Py, THF, 84 %; (k) LiOH.H_2O (15 eq), THF, MeOH, H_2O, H^+.

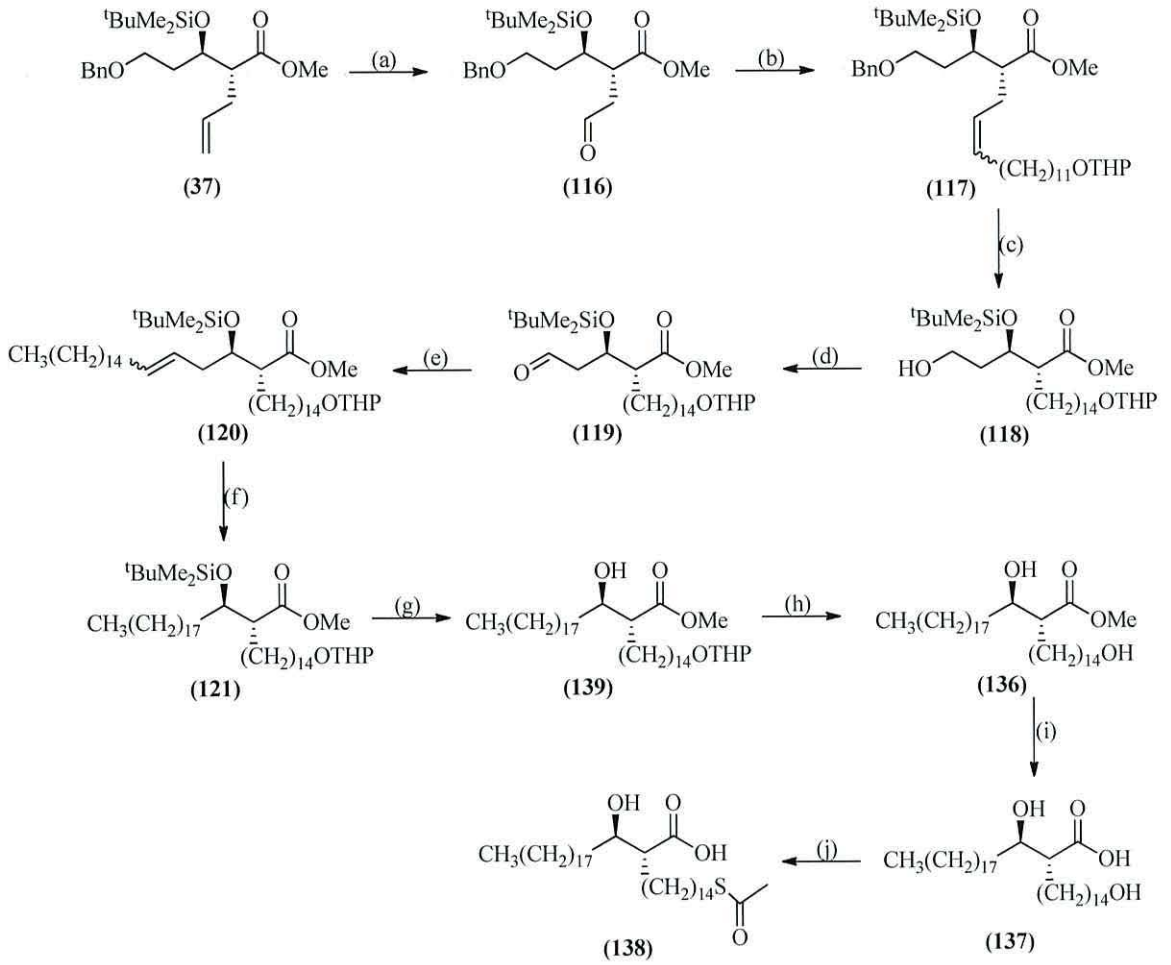
The silyl protecting group on the β-alcohol was then removed by heating thioacetate **(134)** with HF.pyridine and pyridine in dry THF in a dry polyethylene vial. This is a standard method for the deprotection of the silyl group using the additional pyridine to buffer the reaction,[215] however, great care was needed due to the hazards associated with HF.pyridine. This reaction gave the alcohol in an 84 % yield with the removal of the silyl protecting group clear, with the loss of the three characteristic singlets in the ^{1}H NMR spectrum at 0.02, 0.05 and 0.86 ppm. This deprotection also caused a shift in the peak for the β-proton from 3.91 ppm to 3.66 ppm and a slight shift in the α-proton peak from 3.53

to 3.44 ppm. The biggest difference in this peak was that it changed from a doublet of doublet of doublets, which it had consistently been in the silyl ether, to a doublet of triplets. This may be because the alcohol is a smaller group that would ease the rotation of other bonds in close proximity resulting in a similar environment for the two-protons on the C_3 position.

The last stage of the synthesis was then to hydrolyse the methyl ester to the acid. This was done by stirring the methyl ester **(135)** with 15 equivalents of lithium hydroxide in a mixture of THF, methanol and water at 45 °C overnight. The salt was then acidified with dilute hydrochloric acid. This is a standard method for the hydrolysis of a methyl ester for a mycolic acid and is always the last step of the synthesis.[216] This gave an extremely complicated crude ^{1}H NMR spectrum and TLC of the residue also contained numerous spots. Due to these impurities it was not possible to identify if the singlet for the methyl ester at 3.71 ppm remained and therefore if the reaction had gone to completion. However, the hydrolysis of the thioacetate was evident with the removal of the triplet at 2.86 ppm. A quartet at 2.50 ppm representing a methylene group next to an SH could not be identified but a triplet was present at 2.70 ppm. This suggests that the thioacetate was hydrolysed to the disulfide. Purification of the residue was attempted by performing column chromatography numerous times in various solvents but during purification all the desired product **(115)** was lost. As the hydrolysis was not as simple as expected an alternative route was again sought whilst trying to optimise this reaction.

2.4.3 - The third method attempted

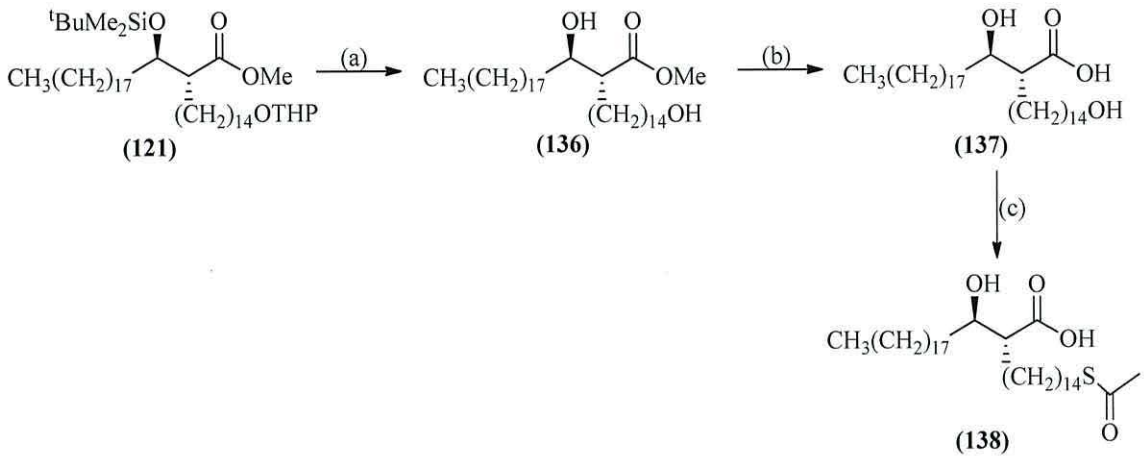
The problems in the previous synthesis were caused during the hydrolysis step, therefore, removing all the protecting groups before synthesising the thiol or the thioacetate should avoid these complications (Scheme 32). This synthesis would use ester **(121)** as described earlier (Scheme 22). The silyl, tetrahydropyranyl and methyl ester protecting groups would then all be removed before converting into the thiol to form (2*R*,3*R*)-2-(14-(acetylthio)tetradecyl)-3-hydroxyhenicosanoic acid **(138)**. It was expected that the thioacetate would be introduced through a Mitsunobu reaction[217] as synthesising the tosylate first would result in the tosylate on both the primary and secondary alcohol groups. The Mitsunobu reaction should result in the formation of the thioacetate on the primary alcohol only.[218] Another advantage of this method is that the thioacetate would be formed from the alcohol in only one step.



Scheme 32: Attempted synthesis of (2*R*,3*R*)-2-(14-(acetylthio)tetradecyl)-3-hydroxyhenicosanoic acid **(138)**. (a) 2,6-lutidine, OsO_4, $NaIO_4$, dioxane/H_2O; (b) LiHMDS, 1-phenyl-5-(12-((tetrahydro-2*H*-pyran-2-yloxy)dodecylsulfonyl)-1*H*-tetrazole **(127)**, dry THF; (c) Pd/C (10 %), H_2 (g), IMS/THF; (d) PCC, Cl_2CH_2; (e) LiHMDS, 5-(hexadecylsulfonyl)-1-phenyl-1*H*-tetrazole **(128)**, dry THF; (f) Pd/C (10 %), H_2 (g), IMS/THF; (g) HF.Pyridine, py, THF; (h) PPTS; (i) LiOH, THF, H_2O, MeOH, HCl; (j) PPh_3, DEAD, thioacetic acid, THF.

Both the silyl and tetrahydropyranyl protecting groups should be removed from ester **(121)** at the same time using HF.pyridine and pyridine. However, when the crude product of this reaction was purified by column chromatography, two fractions were observed. The first consisted of the silyl deprotected molecule **(139)**, whilst the second consisted of the silyl and tetrahydropyranyl deprotected molecule **(136)**. The removal of the different protecting groups was easily identified by the ^1H NMR spectra as both contain very characteristic peaks. Leaving the reaction stirring for a longer period of time may have resulted in the complete deprotection of both protecting groups. For alcohol **(139)** the tetrahydropyranyl

group was deprotected in an additional step using pyridinium-*p*-toluene sulfonate to form the primary alcohol.

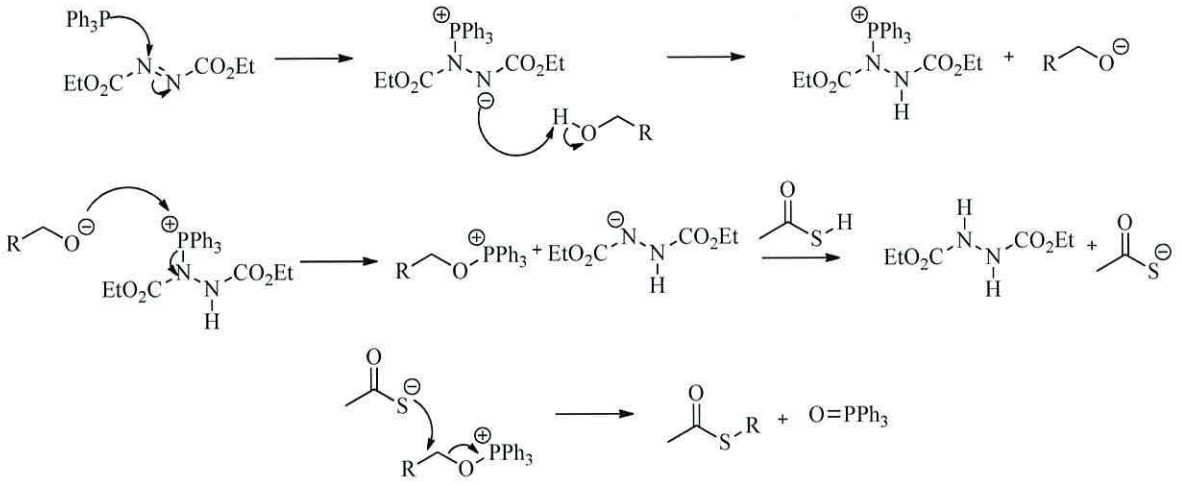


Scheme 33: Attempted synthesis of (2*R*,3*R*)-2-(14-(acetylthio)tetradecyl)-3-hydroxyhenicosanoic acid **(138)**. (a) HF.Pyridine, py, THF; (b) $LiOH.H_2O$, THF, H_2O, MeOH, HCl; (c) PPh_3, DEAD, thioacetic acid, THF.

Methyl ester **(136)** was then hydrolysed to the acid by heating the reaction mixture overnight with 15 equivalents of lithium hydroxide. Following purification, analysis of the product **(137)** was extremely difficult due to its lack of solubility. The deprotected compound **(137)** gave a broad ^1H NMR spectrum, resulting from the numerous alcohol functional groups but it contained all the significant peaks expected. A single-hydrogen multiplet was seen at 3.72 ppm for the β-proton whilst the α-proton was seen as a one-hydrogen doublet of triplets at 2.48 ppm. The other significant peak was seen as a two-hydrogen triplet at 3.67 ppm for the methylene adjacent to the primary alcohol. Mass spectral analyses also confirmed the formation of this compound **(137)** with a mass ion of 577.5107 observed, which was as expected.

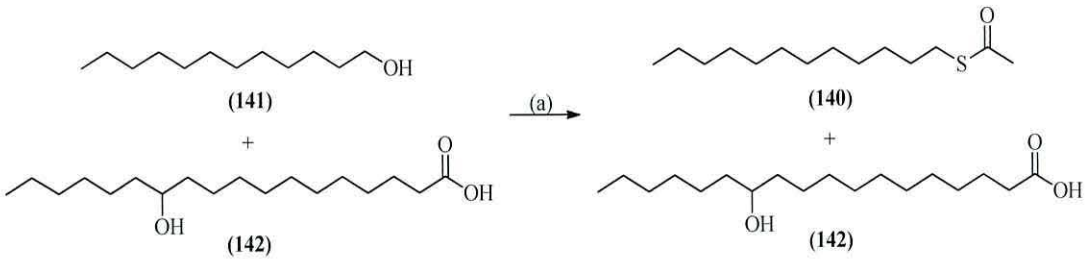
The next stage was to perform a Mitsunobu reaction (Scheme 34). This reaction involves adding DEAD dropwise to a solution of triphenylphosphine. A solution of the acid **(137)** and thioacetic acid would then be added and the mixture stirred. This S_N2 reaction firstly sees triphenylphosphine adding across the weak N=N bond of DEAD to generate an anion. This basic anion then removes a proton from the alcohol to generate an alkoxide ion, which in turn attacks the positively charged phosphorus to generate another anion. This anion now removes a proton from the nucleophile thioacetic acid to produce another anion and diethyl hydrazinedicarboxylate. The anion of thioacetic acid then attacks the phosphorus

derivative of the alcohol in a standard S_N2 reaction, which gives rise to the product and triphenylphosphine oxide.



Scheme 34: Mechanism of the Mitsunobu reaction.

Before performing the Mitsunobu[219, 220] reaction on the alcohol **(137)**, the reaction was first performed on a 1:1 mixture of 1-dodecanol **(141)** and 12-hydroxystearic acid **(142)** using 1.2 and 1.3 equivalents of the reagents to ensure that the reaction would only occur on the primary alcohol (Scheme 35).[217]

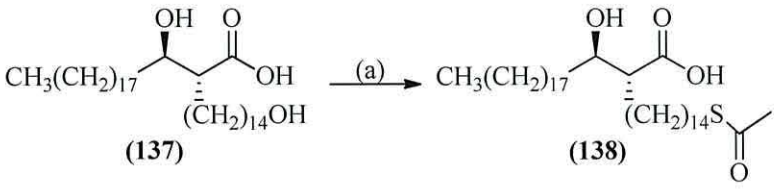


Scheme 35: Synthesis of *S*-Dodecyl ethanethioacetate **(140)**. (a) PPh_3, DEAD, thioacetic acid, THF.

The ^1H NMR spectrum of the residue of this reaction was extremely complicated but confirmed that the starting materials, alcohol **(141)** and acid **(142)** remained present. A triplet at 2.84 ppm and a singlet at 2.30 ppm suggested that the thioacetate had also been synthesised.

The reaction was repeated using two equivalents of each of the reagents. Again, the crude ^1H NMR spectrum of this reaction was extremely complicated but was also much more promising, clearly showing the disappearance of the triplet for 1-dodecanol **(141)** whilst all

the peaks for 12 hydroxystearic acid **(142)** remained. This confirmed that the reaction had only occurred on the primary alcohol. A triplet could again be seen at 2.82 ppm as well as a singlet at 2.26 ppm confirming the formation of a thioacetate. However, a large amount of impurities could again be seen associated with this product. These compounds were not purified at this stage as this was only a model and the crude ^{1}H NMR spectrum had confirmed that the thioacetate would only be formed on the primary alcohol.



Scheme 36: Attempted synthesis of (2*R*,3*R*)-2-(14-(acetylthio)tetradecyl)-3-hydroxyhenicosanoic acid **(138)**. (a) PPh_3, DEAD, thioacetic acid, THF.

The Mitsunobu reaction was then performed on the alcohol **(137)**, using 1.5 equivalents of each reagent to try to minimise the amount of impurities produced (Scheme 36). The ^{1}H NMR spectrum of the product was extremely complicated but a triplet at 2.78 ppm and a singlet at 2.24 ppm suggested that the thioacetate **(138)** had been formed. Numerous purification attempts followed including column chromatography in various solvent mixtures and separation by converting the acid into the salt and back. These were unsuccessful and gave very complicated ^{1}H NMR spectra.

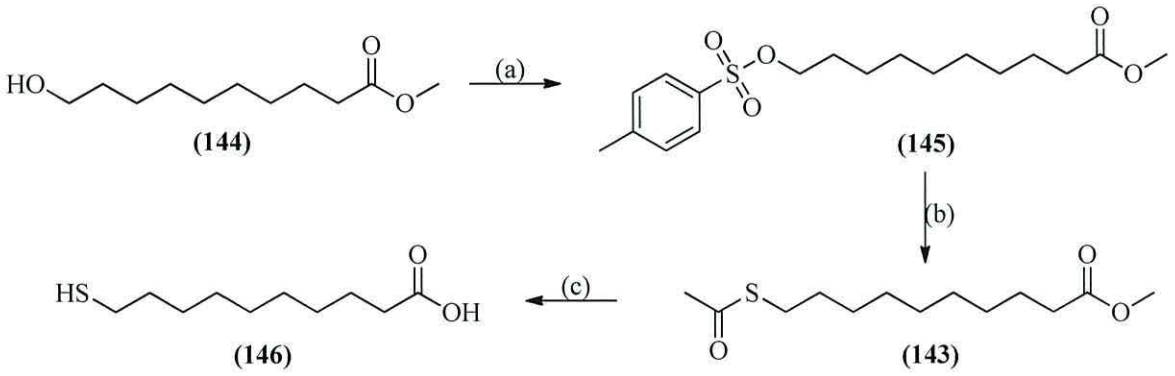
The Mitsunobu reaction was repeated using two equivalents of each of the reagents. This again gave an extremely complicated crude ^{1}H NMR spectrum but appeared to contain the required peaks for the thioacetate. These peaks were however overpowered by the peaks representing the impurities. Purification was again attempted by column chromatography and by converting the acid into the salt and back. Although this did decrease the amount of impurities present, many impurities remained present and the pure product **(138)** could not be isolated.

The Mitsunobu reaction was repeated for the third time, under the original conditions, using 1.5 equivalents of each of the reagents. This should minimise the amount of impurities present and aid purification. The crude ^{1}H NMR spectrum however was again very complicated. Column chromatography in various solvents was attempted several times that did result in a fraction that may have included thioacetate **(138)** without the majority of the impurities; however, the weight of the product had decreased substantially

at this stage and it was not viable to perform another column. These results suggested that (2*R*,3*R*)-2-(14-(acetylthio)tetradecyl)-3-hydroxyhenicosanoic acid **(138)** had been produced but that too many impurities were present to isolate a pure product **(138)** on such a small scale. This is consistent with the literature[218] that states that purification problems do exist with this reaction as the product is associated with the two by-products, diethyl hydrazinedicarboxylate and triphenylphosphine oxide as well as excess/unreacted reagents. It is also seen that yields are sometimes calculated from their NMR ratios and that the products are not actually purified. However, one method that has been successfully used to purify the products of these reactions is preparative TLC. If this procedure was repeated again, the product would be attempted to be purified by preparative TLC but there are several barriers to this purification process such as the difficulty in obtaining a pure product when numerous spots are present, as well as the problem of not being able to chemically develop the TLC plate, which was the method used in these experiments. Alternative and modified reagents are currently being researched in order to minimise the effect of the purification problems for this reaction.[221]

This method was again not a viable way to synthesise (2*R*,3*R*)-2-(14-(acetylthio)tetradecyl)-3-hydroxyhenicosanoic acid **(138)** and therefore more time was concentrated on trying to optimise the reaction conditions for the hydrolysis of the methyl ester of methyl (2*R*,3*R*)-2-(14-(acetylthio)tetradecyl)-3-hydroxyhenicosanoate **(135)**.

2.4.4 - Optimising the hydrolysis step

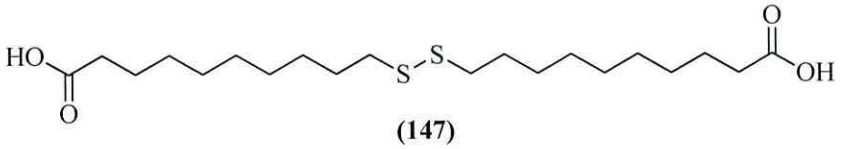


Scheme 37: Attempted synthesis of 10 mercaptodecanoic acid **(146)**. (a) NEt_3, TsCl, CH_2Cl_2, 47 %; (b) potassium thioacetate, acetone, 69 %; (c) varyious conditions.

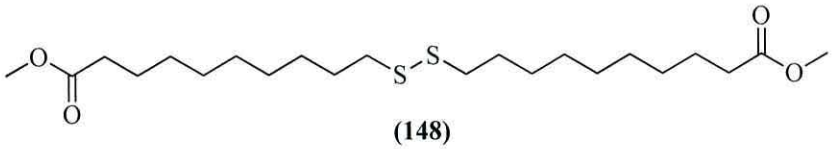
To discover the optimum conditions for the hydrolysis of the methyl ester of methyl (2*R*,3*R*)-2-(14-(acetylthio)tetradecyl)-3-hydroxyhenicosanoate **(135)**, the reaction was

repeated using a model. Methyl 10-(acetylthio)decanoate **(143)** was subsequently synthesised (Scheme 37) for this purpose as it contained a methyl ester and a thioacetate. A better model would be a more complex molecule, more similar in nature to the mycolic acid but no such compound was available.

The first hydrolysis attempt involved heating methyl 10-(acetylthio)decanoate **(143)** with 15 equivalents of lithium hydroxide in a mixture of THF, methanol and water overnight at 45 °C. The crude ^{1}H NMR spectrum of the residue of this reaction was extremely busy but did show the loss of the peaks for the methyl ester and the thioacetate. The thioacetate had also been hydrolysed as heating a thioester under reflux with an alcoholic base and subsequent acidification is a method often used to convert a thioester into a thiol.[193] Replacing the peaks for the thioacetate were two triplets, at 2.68 ppm and 2.50 ppm, which represented a disulfide and a thioether. Purification by column chromatography (petrol/ethyl acetate, 5:2) failed to generate pure disulfide **(147)** as the spectrum was associated with several impurities.



(147)



(148)

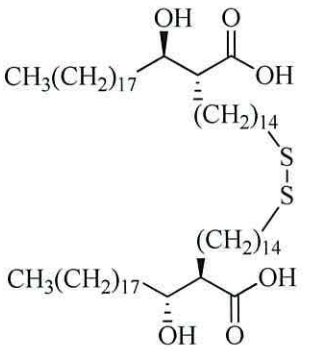
The reaction was then repeated using three equivalents of lithium hydroxide and a slight change in the solvent mixture. Although the crude ^{1}H NMR spectrum for the product of this reaction was not as busy as the previous spectrum, it did contain several unwanted peaks. Closer inspection of the spectrum implied that all the thioacetate had been converted into a mixture of disulfide and thioether but that not all of the methyl ester had been hydrolysed to the carboxylic acid. Purification by column chromatography (petrol/ethyl acetate, 5:2) isolated pure starting material, 10-(acetylthio)decanoate **(143)** and a mixture of 10,10'-disulfanediyl*bis*(decanoic acid) **(147)** and dimethyl(acetylthio)decanoate **(148)** that could not be separated.

The hydrolysis was then repeated using four equivalents of lithium hydroxide in isopropanol and water.[222] This was a literature method for the hydrolysis of a compound that contained a methyl ester and a thioacetate. The mixture was stirred at 23 °C for only

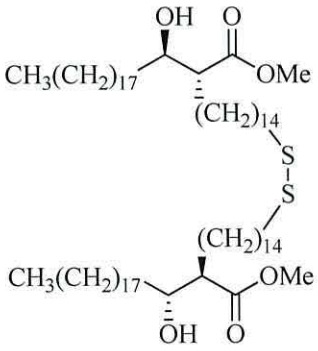
one hour and acidified with dilute hydrochloric acid. The milder conditions may make a difference in the formation of the sulfur by-products. Following work up, the crude ^{1}H NMR spectrum of this reaction was clean and showed the complete removal of the thioacetate and the majority of the methyl ester. However, the majority of the thioacetate had again been converted into the disulfide as a triplet was seen at 2.68 ppm with some thiol also produced as a quartet was observed at 2.53 ppm. The results of this reaction were very promising and due to this, it was repeated on the model of the mycolic acid **(137)**.

When this hydrolysis was attempted, THF was added to a suspension of the methyl ester **(137)** in isopropanol and the mixture was heated to 40 °C as acid **(137)** was not soluble in isopropanol. Although this did improve the solubility of acid **(137)** the reaction was unsuccessful and only the starting material **(137)** was recovered. It was therefore decided not to perform any more hydrolysis reactions on the model, ester **(143)**, as it was obvious at this point that the model did not mimic the structure of the mycolic acid well enough to be worthwhile. Other literature methods for the hydrolysis of a compound that contained a methyl ester and a thioacetate group were all for simple small molecules, therefore, the majority of the time was concentrated on optimising the original hydrolysis method rather than attempting new methods.[211]

The hydrolysis was then repeated by heating acid **(137)** to 45 °C with 15 equivalents of lithium hydroxide in a mixture of THF, methanol and water, the original hydrolysis conditions. This gave an extremely complicated crude ^{1}H NMR spectrum confirming that the complications in this experiment were not due to experimental error.



(149)



(150)

The reaction was repeated using two equivalents of lithium hydroxide. The crude ^{1}H NMR spectrum of this reaction was relatively clean but suggested that a mixture of acid **(149)** and ester **(150)** was formed as a triplet at 2.69 ppm was present for the disulfide and a small singlet for the methyl ester. Purification of the residue was attempted several times

by column chromatography (petrol/ethyl acetate, 5:2) but it did not result in the isolation of the pure product **(149)**.

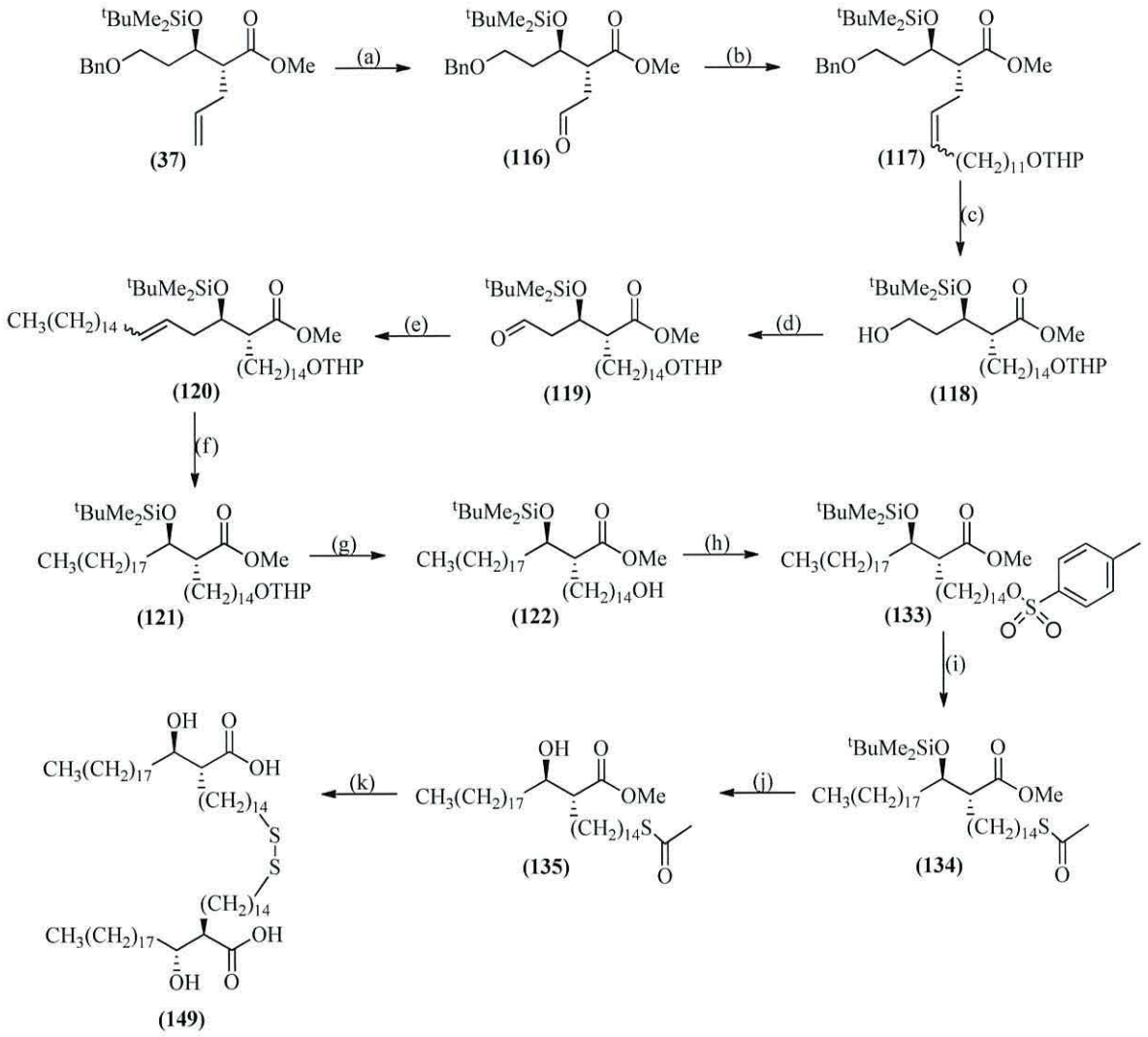
The reaction was then repeated with three equivalents of lithium hydroxide. Again this gave quite a clean crude ^{1}H NMR spectrum containing the triplet for the disulfide at 2.68 ppm present as well as a very small singlet for the methyl ester. This suggested that the majority of the methyl ester had been converted to the acid and a mixture of acid **(149)** and ester **(150)** was again present. Purification of this residue was attempted by column chromatography (petrol/ethyl acetate, 5:2), but, as before, it was not successful.

The reaction was repeated using four equivalents of lithium hydroxide. This gave a very similar crude ^{1}H NMR spectrum to that for the reaction using three equivalents of lithium hydroxide but in this case the singlet for the methyl ester was even smaller. The residue was then purified by column chromatography (petrol/ethyl acetate, 5:2) to give pure (2*R*,2'*R*,3*R*,3'*R*)-2,2'-(disulfanediyl*bis*(tetradecane-14,1-diyl))*bis*(3-hydroxyhenicosanoic acid **(149)**. The ^{1}H NMR spectrum of acid **(149)** contained a multiplet at 3.70 ppm representing the β-proton, a two-hydrogen triplet at 2.70 ppm representing the methylene adjacent to the sulfur and a doublet of triplets at 2.47 ppm representing the α-proton. No peaks could be seen in the ^{1}H NMR spectrum for the methyl ester or the thioacetate. The acid could not be identified in the ^{1}H NMR spectrum but the IR spectrum showed a broad hydroxyl group as well as a carbonyl. Only one carbonyl group could be seen in the ^{13}C NMR spectrum at 180 ppm confirming the removal of the thioacetate. It was again very difficult to obtain a correct mass spectrum for the product but a spectrum was obtained using negative ionisation by direct insertion. This confirmed the synthesis of ((2*R*,2'*R*,3*R*,3'*R*)-2,2'-(disulfanediyl*bis*(tetradecane-14,1-diyl))*bis*(3-hydroxyhenicosanoic acid **(149)** in a yield of 26 %. This may be a low yield but this is the first thiolated mycolic acid that has been synthesised, albeit a simple mycolic acid. The reaction may in the future be further optimised, but obtaining a small amount of pure thiolated mycolic acid was a great achievement.

2.4.5 - The final method

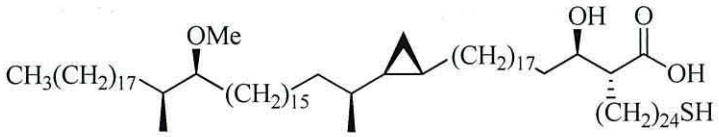
The final method for the synthesis of a thiolated simple mycolic acid involved synthesising a tosylate at the end of the α-alkyl chain, which would in turn be converted into a thioacetate. The silyl protecting group could then be removed using HF.pyridine and pyridine and finally the methyl ester could be removed by reacting the mycolic acid with

four equivalents of lithium hydroxide, which in turn also forms the disulfide **(149)** (Scheme 38).

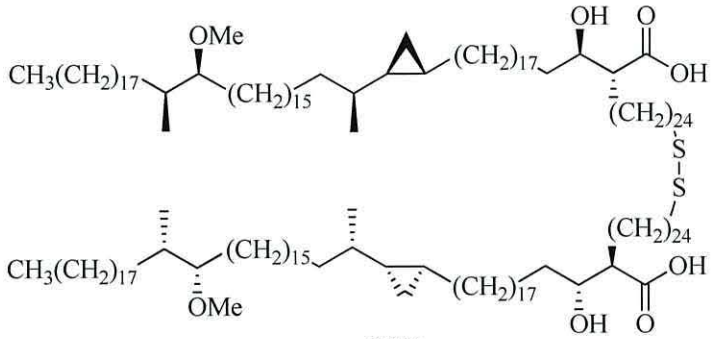


Scheme 38: Synthesis of ((2*R*,2'*R*,3*R*,3'*R*)-2,2'-(disulfanediyl*bis*(tetradecane-14,1-diyl))*bis*(3-hydroxyhenicosanoic acid **(149)**. (a) 2,6-Lutidine, OsO_4, $NaIO_4$, dioxane/H_2O, 77 %; (b) LiHMDS, 1-phenyl-5-(12-((tetrahydro-2*H*-pyran-2-yloxy)dodecylsulfonyl)-1*H*-tetrazole **(127)**, dry THF, 75 %; (c) Pd/C (10 %), H_2 (g), IMS/THF, 79 %; (d) PCC, Cl_2CH_2, 82 %; (e) LiHMDS, 5-(hexadecylsulfonyl)-1-phenyl-1*H*-tetrazole **(128)**, dry THF, 75 %; (f) Pd/C (10 %), H_2 (g), IMS/THF, 92 %; (g) PPTS, THF/MeOH/H_2O, 82 %; (h) NEt_3, TsCl, CH_2Cl_2, 74 %; (i) potassium thioacetate, acetone, 75 %; (j) HF.pyridine, Py, THF, 84 %; (k) $LiOH.H_2O$ (4 eq), THF/MeOH/H_2O, H^+, 26 %.

2.5 - Thiolated Mycolic acids



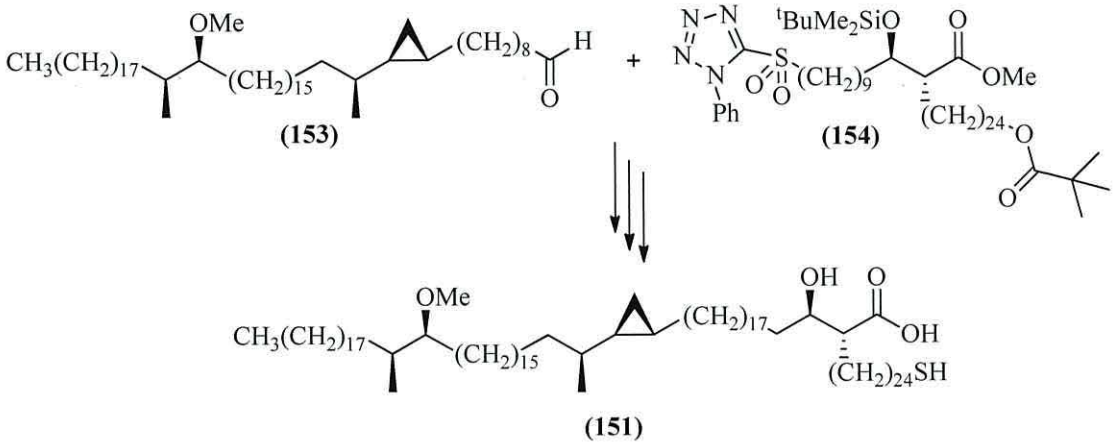
(151)



(152)

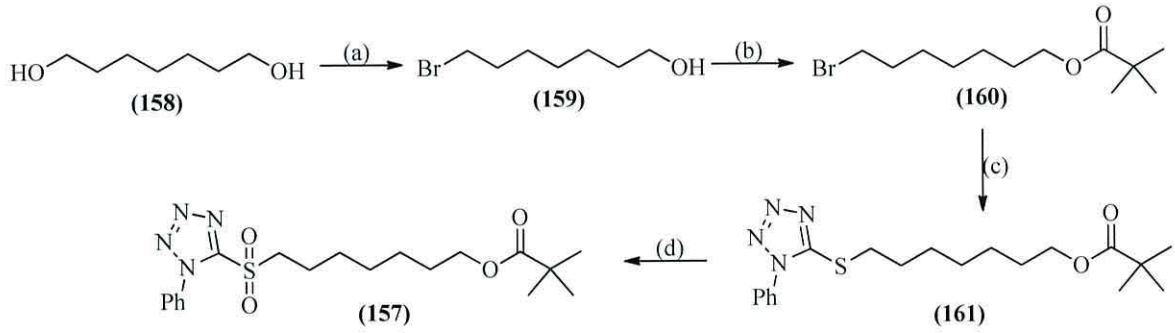
Following the synthesis of a thiolated model of a mycolic acid, the synthesis of a full thiolated mycolic acid was attempted. It was decided to synthesise a thiolated derivative of an existing synthetically-produced mycolic acid, which showed the best reactivity during ELISA tests to distinguish TB positive from TB negative serum.[90] This was (*R*)-2-((*R*)-1-hydroxy-19-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonadecyl)-26-mercaptohexacosanoic acid **(68)**.[223] As for the model, the thiol or disulfide would be introduced at the end of the α-alkyl chain. Therefore, combining and adapting the procedure for the synthesis of mycolic acid **(68)**[223] with the method developed for the synthesis of the thiolated model **(149)** should result in the synthesis of a full thiolated mycolic acid. The synthesis of (*R*)-2-((*R*)-1-hydroxy-19-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonadecyl)-26-mercaptohexacosanoic acid **(151)** was therefore attempted, although it was believed that the disulfide, (*S*,*S*,*S*,*R*,*S*,*R*,2*R*,2*R*')-26-26'-disulfanediyl*bis*(2-((*R*)-1-hydroxy-19-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonadecyl)hexacosanoic acid **(152)** was more likely to be obtained in the final step following the results of the synthesis of the thiolated model **(149)**.

For this synthesis, the meromycolate fragment **(153)** was firstly prepared prior to being coupled to the mycolic motif **(154)**. To prepare the meromycolate fragment, (8*S*,9*S*)-8-methoxy-9-methylheptacosan-1-ol **(155),** which had been prepared according to Al Dulayymi *et al.*[60] and represented the distal position within the fragment was firstly chain-extended before coupling to the α-methyl-*trans*-cyclopropane fragment **(156)** through a modified Julia-Kocienski coupling reaction (Scheme 23).



Scheme 39: Synthesis of (*R*)-2-((*R*)-1-hydroxy-19-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonadecyl)-26-mercaptohexacosanoic acid **(151)** from the meromycolate fragment **(153)** and the mycolic motif **(154)**.

7-(1-Phenyl-1*H*-terazol-5-ylsulfonyl)heptyl pivalate **(157)** was therefore prepared, which contained the appropriate number of carbon atoms and included functionality at the non-reacting end ensuring that the terminal end of the coupled product could be coupled further (Scheme 40).



Scheme 40: Synthesis of 7-(1-phenyl-1*H*-terazol-5-ylsulfonyl)heptyl pivalate **(157)**. (a) HBr (aq), toluene, 79 %; (b) PVCL, Et_3N, DMAP, 86 %; (c) 1-phenyl-1*H*-tetrazole-5-thiol, K_2CO_3, acetone, 78 %; (d) ammonium molybdate(VI) tetrahydrate, H_2O_2, IMS, 86 %.

7-(1-Phenyl-1*H*-tetrazol-5-ylsulfonyl)heptyl pivalate **(157)** was prepared in a similar fashion to **(127)** differing only in the number of carbon atoms in the chain and the type of alcohol protecting group used. 1,7-Heptanediol **(158)** was monobrominated with hydrobromic acid under reflux and the second alcohol protected. A pivalate ester was used for this protection of the alcohol **(159)** as this is much more stable than the tetrahydropyranyl ether and gives a much cleaner ^1H NMR spectrum. The bromide **(160)**

was then converted into the sulfide **(161)** and finally to the sulfone **(157)**. A simple triplet could be seen for the methylene adjacent to the sulfur in the sulfide **(161)** but for the methylene group next to the sulfur in the sulfone **(157)** a distorted triplet could be seen. This signal is characteristic of an AA'BB' system where two pairs of protons are magnetically non-equivalent, therefore H_A would exhibit different splitting to H_B and $H_{B'}$ (Figure 8). The Newman projection below shows H_A splitting *cis* to H_B and *trans* to $H_{B'}$ showing the effect of magnetically non-equivalent protons on the ^1H NMR spectrum.

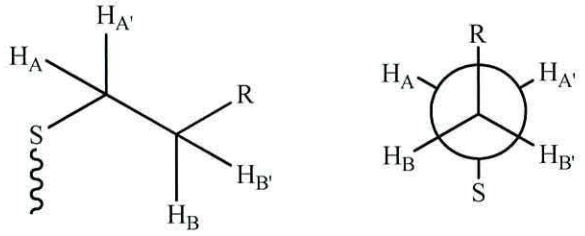
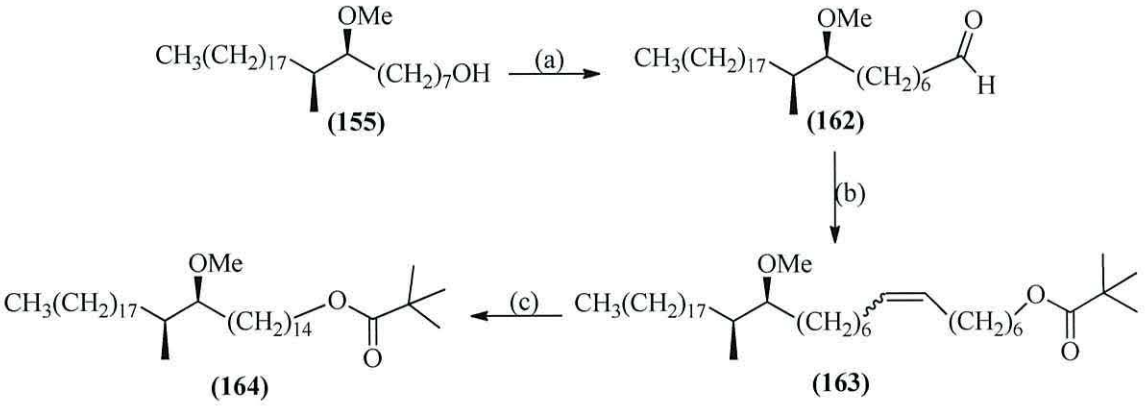


Figure 8: Newman projection of an AA'BB' system.

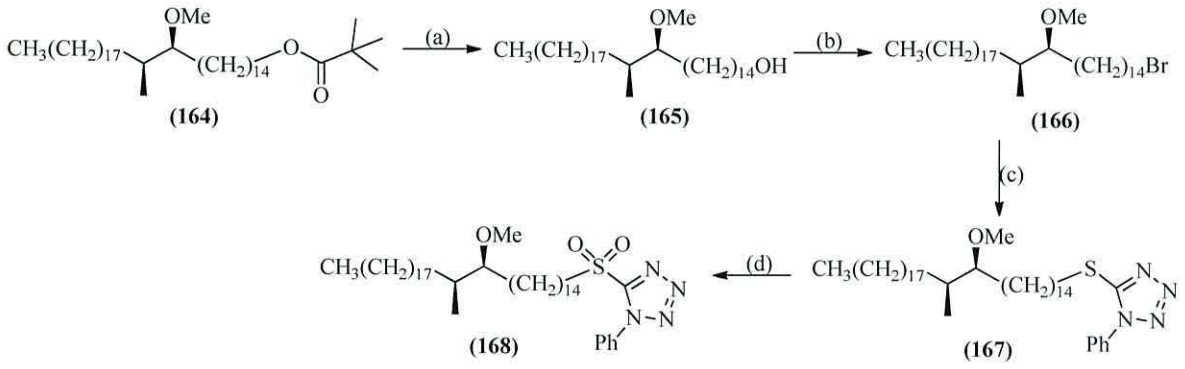


Scheme 41: Synthesis of (15*S*,16*S*)-15-methoxy-16-methyltetratriacontyl pivalate **(164)**. (a) PCC, CH_2Cl_2, 88 %; (b) LiHMDS, 7-(1-phenyl-1*H*-terazol-5-ylsulfonyl)heptyl pivalate **(157)**, dry THF, 80 %; (c) Pd/C (10 %), H_2 (g), IMS, THF, 98 %.

(8*S*,9*S*)-8-Methoxy-9-methylheptacosan-1-ol **(155)** was then oxidised to the aldehyde **(162)** with PCC in dichloromethane and its formation was confirmed by a one-hydrogen triplet at 9.77 ppm. The aldehyde **(162)** was then coupled to sulfone **(157)** *via* a modified Julia-Kocienski coupling reaction (Scheme 41). This coupling reaction was preferred as it gives the coupled product **(163)** in a good yield with no major unwanted side products. However, there are some disadvantages such as having to synthesise the bromide, sulfide and sulfone each time, all of which take considerable time and resources. This reaction again gave a mixture of the *cis* and *trans* isomers with the olefin appearing as a two-

hydrogen multiplet at 5.37 ppm in the ^{1}H NMR spectrum, but, as before, this caused no concern as the next stage was to hydrogenate the double bond.

The coupling product **(163)** was hydrogenated in the presence of hydrogen gas catalysed by palladium on carbon, which was completed in a matter of a few hours. The ^{1}H NMR spectrum showed the disappearance of the two-hydrogen multiplet at 5.37 ppm confirming successful hydrogenation. A nine-hydrogen singlet could be seen for the pivalate ester at 1.20 ppm as well as a two-hydrogen triplet at 4.05 ppm for the methylene adjacent to the oxygen. A three-hydrogen singlet could also be seen at 3.35 ppm for the methoxy group, whilst the proton adjacent to the methoxy group was seen as a one-hydrogen multiplet at 2.96 ppm. The terminal methyl and the branched methyl could be seen as a three-hydrogen triplet and a three-hydrogen doublet respectively at 0.89 and 0.86 ppm.

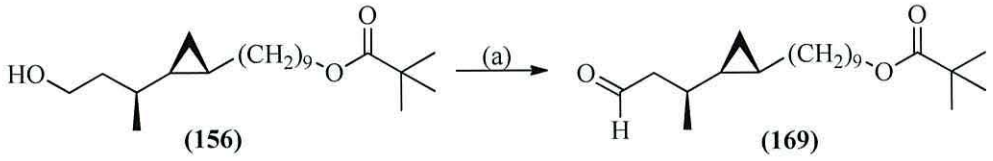


Scheme 42: Synthesis of 5-((15*S*,16*R*)-15-methoxy-16-methyltetratriacontylsulfonyl)-1-phenyl-1*H*-tetrazole **(168)**. (a) LAH, THF, 93 %; (b) NBS, PPh_3, CH_2Cl_2, 92 %; (c) 1–phenyl-1*H*-tetrazole-5-thiol, K_2CO_3, acetone 89 %; (d) ammonium molybdate(VI) tetrahydrate, H_2O_2, IMS, THF, 78 %.

This fragment **(164)** of the meromycolate moiety now contained the appropriate number of carbon atoms to be coupled to the α-methyl-*trans*-cyclopropane fragment (**156**). However, before the coupling the pivalate ester had to be converted into the sulfone (Scheme 42). This process required four additional steps, which highlights some of the disadvantages of coupling *via* the modified Julia-Kocienski coupling method.

(15*S*,16*S*)-15-Methoxy-16-methyltetratriacontyl pivalate **(164)** was firstly deprotected to form (15*S*,16*S*)-15-methoxy-16-methyltetratriacontan-1-ol **(165)** using lithium aluminium hydride in a very high yield of 93 %. This deprotection was easily confirmed by the dissapearance of the characteristic peaks for the pivalate ester. The alcohol **(165)** was then converted into the bromide **(166)** with *N*-bromosuccinimide and triphenylphosphine, which

caused a shift in the triplet for the methylene adjacent to the terminal heteroatom from 3.65 ppm to 3.42 ppm. The bromide **(166)** was then converted into the sulfide **(167)** and subsequently to the sulfone **(168)**. The sulfide **(167)** was synthesised in a good yield of 89 % but some trouble was encountered whilst oxidising the sulfide to the sulfone. Sulfide **(167)** was not soluble in the original solvent mixture, therefore, additional THF was added to increase solubility, which should have resulted in the reaction proceeding as expected. This, however, was not the case. This reaction was monitored by TLC, which in itself was difficult as the R_f value of the sulfide **(167)** and the sulfone **(168)** were extremely close but after stirring the reaction mixture overnight, TLC showed that a large amount of sulfide **(167)** remained. The reaction mixture was stirred for a total of three days. This eventually gave the sulfone **(168)** in a 78 % yield. An alternative method that could have been performed would have been to oxidise the sulfide with *m*-chloroperoxybenzoic acid and sodium hydrogen carbonate in dichloromethane as done for the synthesis of mycolic acid **(68)**. This method gave the sulfone **(168)** in a much higher yield of 89 % and therefore, repeating the same method in this synthesis might have given the sulfone **(168)** in a greater yield. In the ^{1}H NMR spectrum of this sulfone **(168)** a distorted triplet at 3.74 ppm was observed for the methylene group adjacent to the sulfur. Other significant peaks for the compound could be seen as a three-hydrogen singlet at 3.35 ppm for the distal methoxy and a one-hydrogen multiplet at 2.96 ppm representing the methoxy proton. The terminal CH_3 group could be seen as a three-hydrogen triplet at 0.89 ppm whilst the terminal branched CH_3 group was seen as a three-hydrogen doublet at 0.86 ppm.

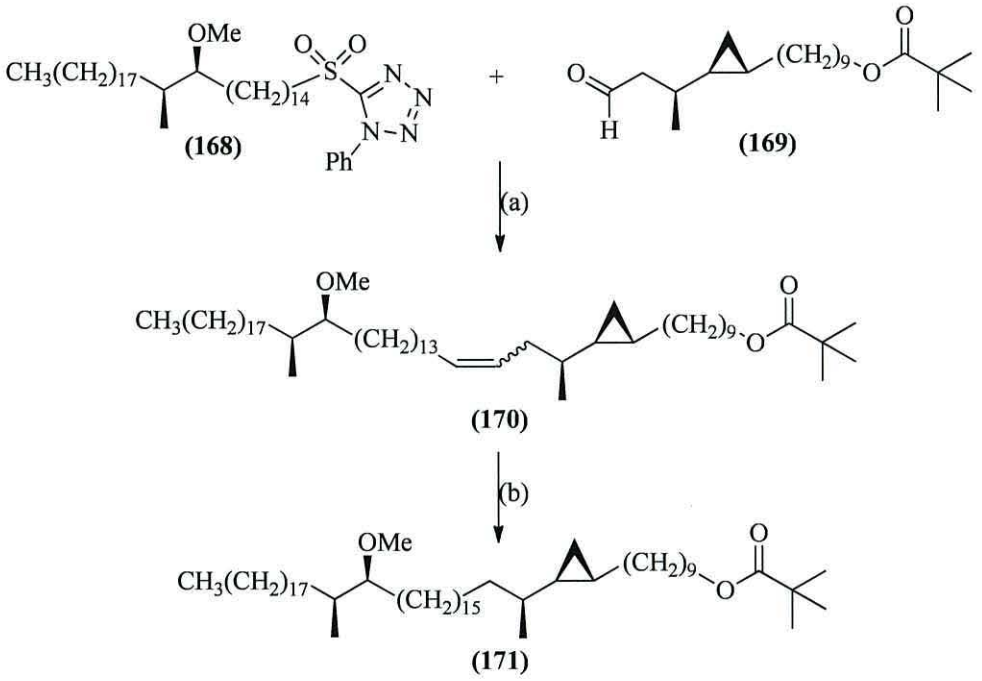


(156) (169)

Scheme 43: Synthesis of 9-((1*S*,2*R*)-2-((*S*)-4-oxobutan-2-yl)cyclopropyl)nonyl pivalate **(169)**. (a) PCC, CH_2Cl_2, 93 %.

The α-methyl-*trans*-cyclopropane unit **(156)**, which was prepared according to Al Dulayymi *et al.*,[55] was then oxidised to the aldehyde **(169)** with PCC (Scheme 43) and was used immediately in the Julia-Kocienski coupling reaction. The aldehyde **(169)** showed several significant peaks in its ^{1}H NMR spectrum. A clear one-hydrogen triplet was seen at 9.79 ppm representing the aldehyde and as before a nine-hydrogen singlet at 1.20 ppm and a two-hydrogen triplet at 4.05 ppm represented the pivalate protecting group. For the *trans*-

cyclopropane ring itself a three-hydrogen multiplet could be seen between 0.19-0.36 ppm as well as a one-hydrogen multiplet between 0.46-0.52 ppm. The branched methyl was seen as a three-hydrogen doublet at 1.04 ppm.

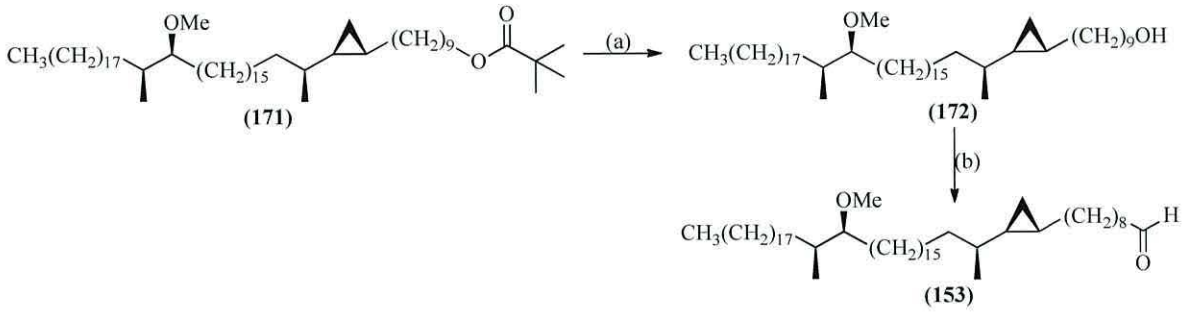


Scheme 44: Synthesis of 9-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonyl pivalate **(171)**. (a) LiHMDS, THF, 72 %; (b) $(NCOO^-K^+)_2$, CH_3COOH, THF, MeOH, 92 %.

Aldehyde **(169)** was then coupled to sulfone **(168)** *via* a modified Julia-Kocienski reaction joining together the distal and proximal fractions of the meromycolate fragment (Scheme 44). This reaction gave the coupling product **(170)** in a reasonable yield of 72 %. A two-hydrogen multiplet could now be seen at 5.39 ppm representing the double bond as well as four peaks in the ^{13}C NMR spectrum at 128.4, 128.8, 130.4 and 131.4 ppm due to the presence of the *cis* and *trans* isomers. Two of the peaks were seen at a greater intensity than the other two confirming that a greater amount of one isomer had been synthesised. Mechanistically the *trans* isomer is favoured in this coupling reaction.

This olefin **(170)** was then hydrogenated using dipotassium azodicarboxylate and acetic acid in a mixture of THF and methanol. In the presence of an acid, azodicarboxylic acid is decoarboxylated to a di-imide, which reacts with the olefin releasing nitrogen gas. The hydrogenation was performed using dipotassium azodicarboxylate rather than hydrogen gas catalysed by palladium on carbon as it is a much milder hydrogenation procedure.[224] Using hydrogen gas and palladium on carbon would have resulted in the hydrogenation of

the cyclopropane ring as well as the double bond.[225] From the ^{1}H NMR spectrum of the hydrogenated product **(171)** it was evident that the peaks for the double bond had been removed whist the signals for the cyclopropane ring between 0.09 and 0.48 ppm were still present. This hydrogenation gave the product **(171)** in a very high yield of 92 % but the major disadvantage of this method was the length of time required for the reaction to go to completion.

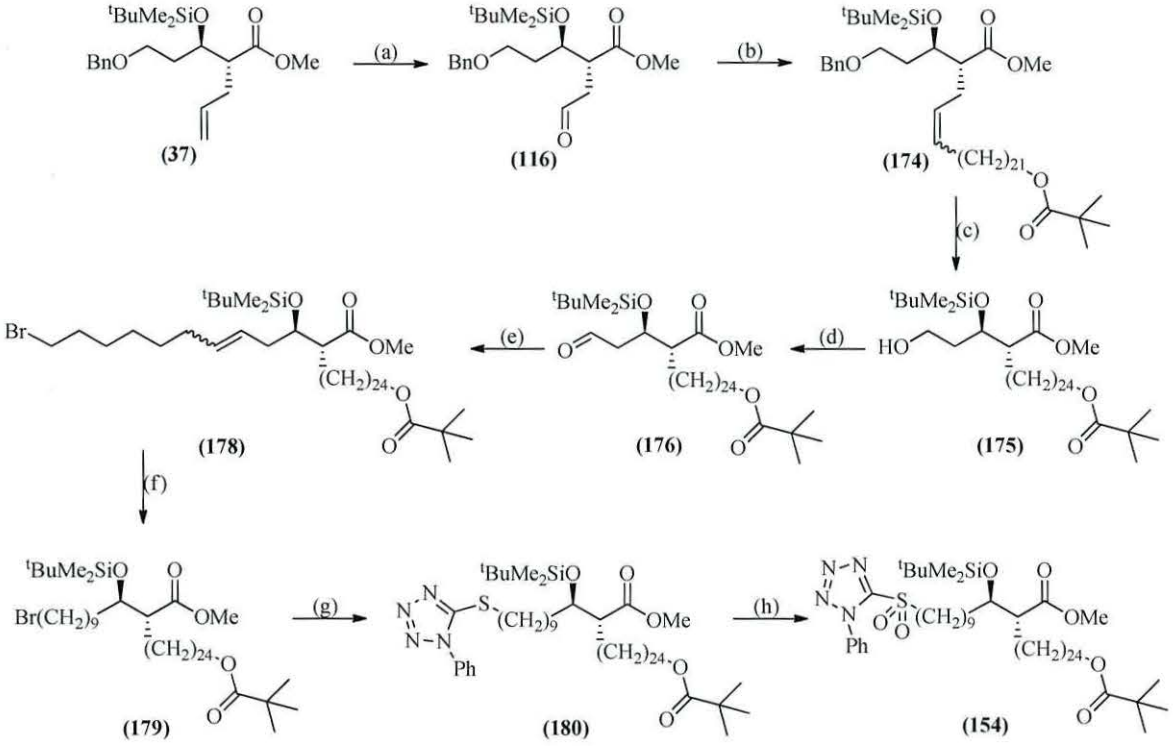


Scheme 45: Synthesis of 9-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonanal **(153)**. (a) LAH, THF, 87 %; (b) PCC, CH_2Cl_2, 85 %.

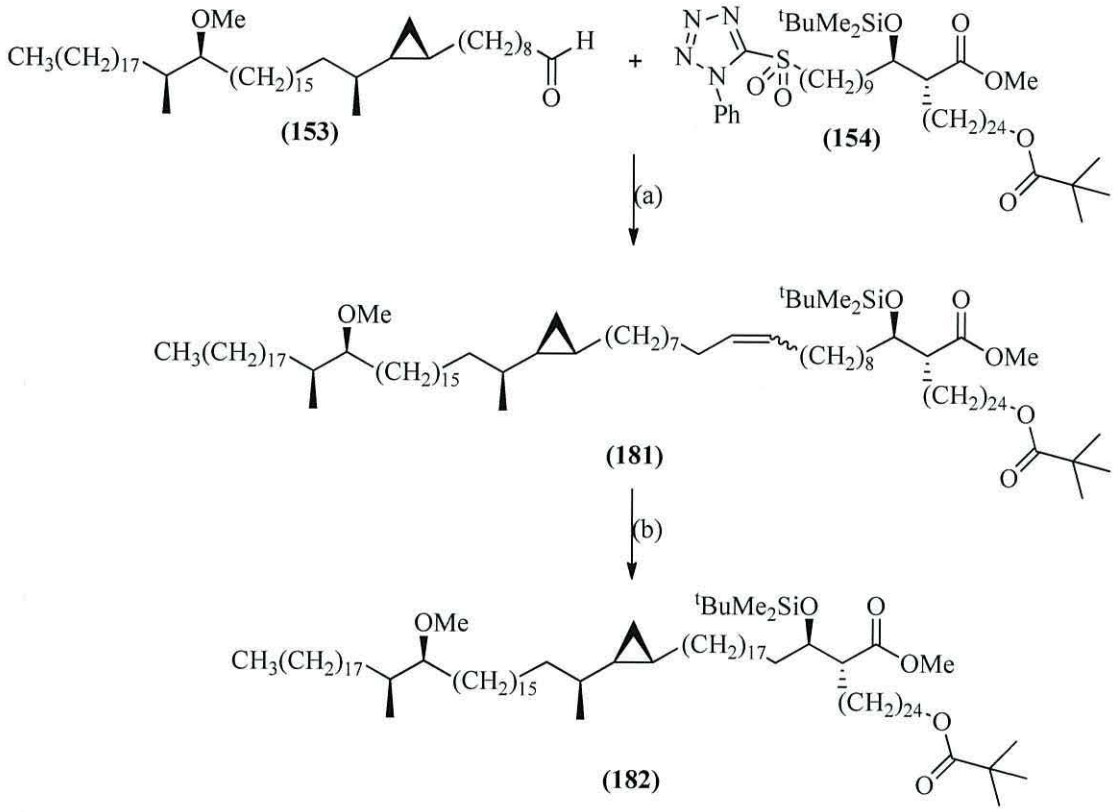
Pivalate **(171)** was now converted into aldehyde **(153)** before being coupled to the mycolic motif **(154)** through another Julia-Kocienski coupling reaction. It was firstly deprotected to give alcohol **(172)** by reacting it with a solution of lithium aluminiumhydride. The removal of the pivalate ester was again clear with the removal of the characteristic nine-hydrogen singlet at 1.20 ppm. The alcohol **(172)** was subsequently oxidised to the aldehyde **(153)** using PCC in dichloromethane, which showed a broad singlet at 9.77 ppm in the ^{1}H NMR spectrum.

Aldehyde **(153)** was then coupled to the mycolic motif, methyl (*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-10-((1-phenyl-1*H*-tetrazol-5-yl)sulfonyl)decyl)-26-(pivaloyloxy)hexacosanoate **(154)**. This sulfone **(154)**, prepared by Mr A. D. Saleh[226] was synthesised in a very similar fashion to the simple mycolic acid **(149)** (Scheme 46). However, in this case the initial coupling reaction to form the α-alkyl chain was performed between the oxidised allyl intermediate **(116)** and 22-((1-phenyl-1*H*-tetrazole-5-yl)sulfonyl)docosyl pivalate **(173)** to give the correct number of carbon atoms in the α-alkyl chain. Again a pivalate ester was used rather than a tetrahydropyranyl ether due to the increased stability and the inclusion of only one clear singlet in the ^{1}H NMR spectrum. The coupling product **(174)** was then hydrogenated, which, as before, resulted in the hydrogenolysis of the benzyl protecting group. The newly regenerated alcohol **(175)** was

oxidised to the aldehyde **(176)** and coupled to 5-((7-bromoheptyl)sulfonyl)-1-phenyl-1*H*-tetrazole **(177)** in order to chain extend at the meromycolate end to give olefin **(178)**, which was then hydrogenated to give ester **(179)**. The terminal bromide in ester **(179)** was converted into the sulfide **(180)** and subsequently to the sulfone **(154)**, ready to couple to the meromycolate fragment **(153)**.



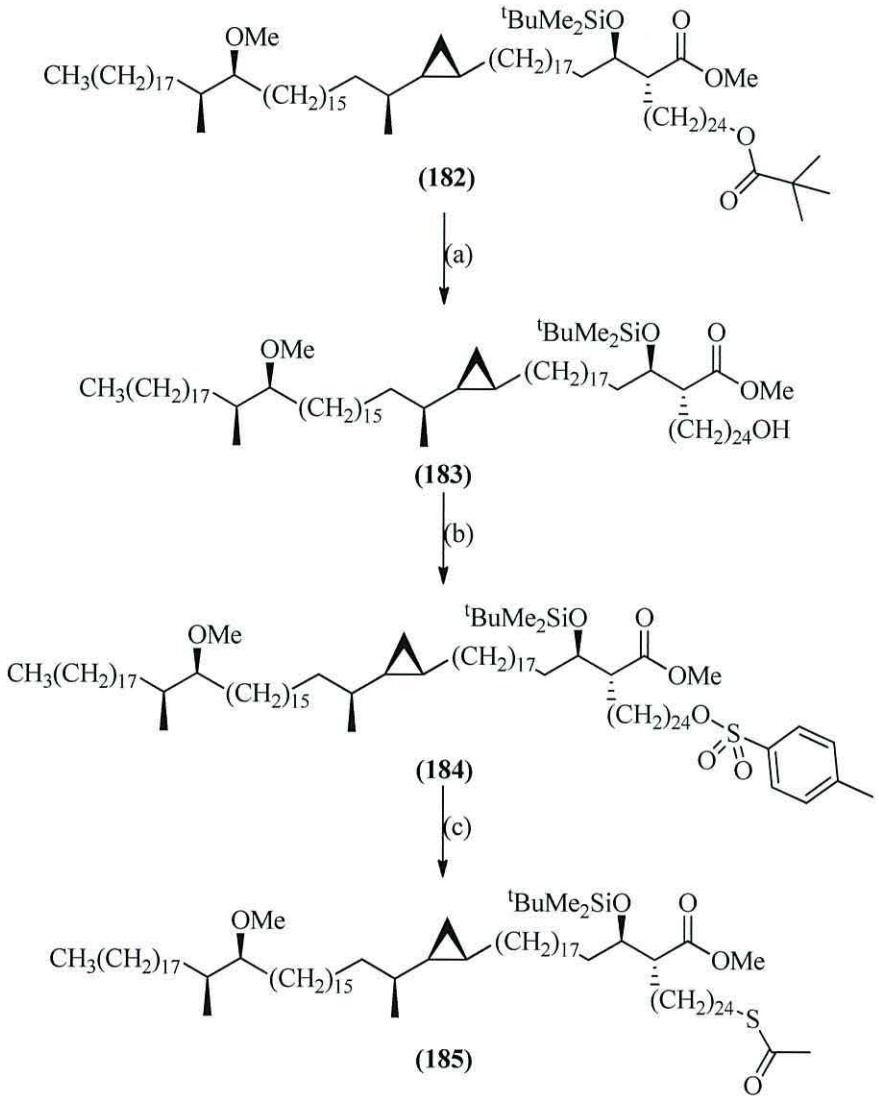
Scheme 46: Synthesis of methyl (*R*)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-10-((1-phenyl-1*H*-tetrazol-5-yl)sulfonyl)decyl)-26-(pivaloyloxy)hexacosanoate **(154)**. (a) 2,6-lutidine, OsO_4, $NaIO_4$, dioxane/H_2O; (b) LiHMDS, 22-((1-phenyl-1*H*-tetrazole-5-yl)sulfonyl)docosyl pivalate **(173)**, dry THF; (c) Pd/C (10 %), H_2; (d) PCC, Cl_2CH_2; (e) LiHMDS, 5-((7-bromoheptyl)sulfonyl)-1-phenyl-1*H*-tetrazole **(176)**, dry THF; (f) Pd/C (10 %), H_2; (g) 1-phenyl-1*H*-tetrazole-5-thiol, K_2CO_3, acetone, reflux; (h) ammonium molybdate(VI) tetrahydrate, H_2O_2, THF, IMS.



Scheme 47: Synthesis of methyl (*R*)-2-((*R*)-1-(*tert*-butyldimethylsilyloxy)-19-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonadecyl)-26-(pivaloyloxy)hexacosanoate **(182)**. (a) LiHMDS, THF, 86 %; (b) $(NCOO^{-}K^{+})_2$, CH_3COOH, THF, 86 %.

The meromycolate chain **(153)** and the mycolic motif **(154)** were coupled through a modified Julia-Kocienski coupling reaction using lithium(*bis*)trimethylsilyl amide as the base, to form a mixture of alkenes **(181)**, which were isolated in a high yield of 86 % (Scheme 47). This is much higher than the yield of the coupling reaction for the same mycolic acid without the pivalate ester, which was 28 % under exactly the same conditions.[223] It was believed that during this reaction in the synthesis of a standard mycolic acid **(68)**, the sulfone-lithium complex had been sterically hindered, which accounts for the low yield, but as the present reaction gave such a significantly different yield this cannot be the case. For this coupling product **(181)**, all the peaks for the distal methoxy and proximal α-methyl-*trans*-cyclopropane were seen as for aldehyde **(153)**, together with several new peaks. A two-hydrogen multiplet was seen at 5.37 ppm in the ^1H NMR spectrum as well as the three characteristic singlets for the silyl protecting group at 0.02, 0.05 and 0.87. The β-proton was seen as a single proton multiplet at 3.91 ppm whilst the α-proton was seen as a single proton doublet of doublets of doublets at 2.53 ppm. A

three-hydrogen singlet was also seen for the methyl ester at 3.66 ppm as well as the characteristic singlet for the pivalate protecting group at 1.20 ppm. This alkene **(181)** was hydrogenated using dipotassium azodicarboxylate, again due to the presence of the easily modified cyclopropane ring. This reaction proceeded as expected giving ester **(182)** in an 86 % yield. The success of the hydrogenation was clear with the loss of the two-hydrogen alkene multiplet at 5.37 ppm in the ^{1}H NMR spectrum.



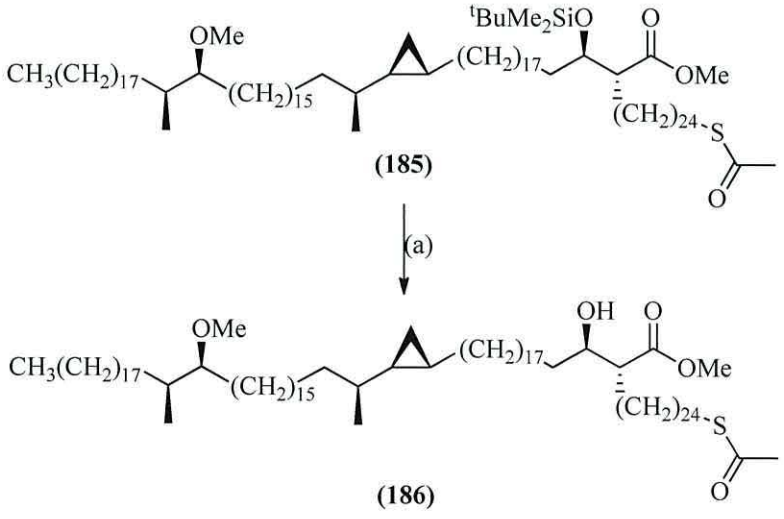
Scheme 48: Synthesis of methyl (*R*)-26-(acetylthio)-2-((*R*)-1-(*tert*-butyldimethylsilyloxy)-19-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonadecyl)hexanoate **(185)**. (a) KOH, THF, MeOH, H_2O, 86 %; (b) TsCl, Et_3N, CH_2Cl_2, 65 %; (c) potassium thioacetate, acetone, 61%.

The pivalate ester of the hydrogenated compound **(182)** was then deprotected to give the alcohol **(183)** using potassium hydroxide in a mixture of THF, methanol and water

(Scheme 48). Removal of the pivalate ester was confirmed with the loss of the characteristic singlet in the ^{1}H NMR spectrum and of one of the carbonyl peaks in the ^{13}C NMR spectrum. A broad hydroxyl peak could also be seen in the IR spectrum.

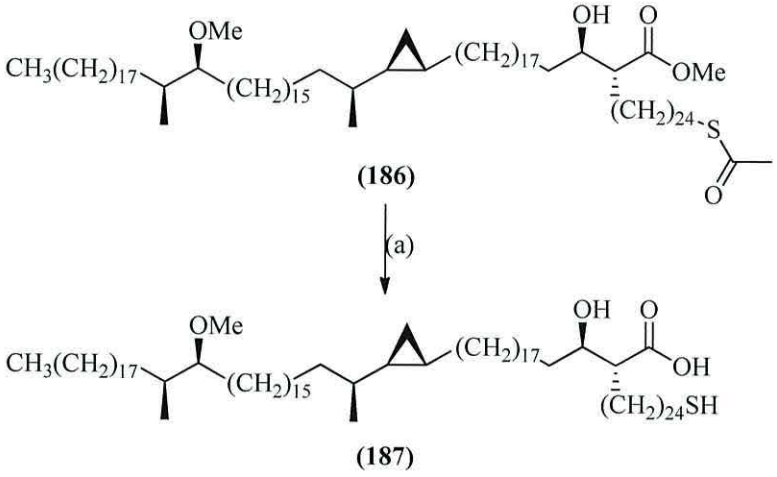
A tosylate **(184)** was formed by reacting alcohol **(183)** with *p*-toluene sulfonyl chloride and triethylamine in dichloromethane. This gave methyl (*R*)-2-((*R*)-1-(*tert*-butyldimethyl-silyl)oxy)-19-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatria-contan-2-yl)cyclo-propyl)nonadecyl)-26-(tosyloxy) hexacosanoate **(184)** in a lower yield than expected of 65 %. The formation of the tosylate could be seen in the ^{1}H NMR spectrum as two, two-hydrogen doublets at 7.36 and 7.80 ppm as well as a three-hydrogen singlet at 2.46 ppm. This was also confirmed by the ^{13}C NMR spectrum, where four peaks were present in the aromatic region at 127.9, 129.8, 133.3 and 144.5 ppm.

The tosylate **(184)** was then converted into the thioacetate **(185)** by reacting tosylate **(184)** with potassium thioacetate in acetone. This did give the thioacetate **(185)** but again in a much lower yield than expected of 61 %. The high complexity of these molecules may be causing the decrease in the yields of these reactions. The ^{1}H NMR spectrum showed the disappearance of all the peaks representing the tosylate and a new carbonyl peak could be seen in the ^{13}C NMR spectrum at 195.9 ppm as well as new peaks in the ^{1}H NMR spectrum as a three-hydrogen singlet at 2.31 ppm for the CH_3 group next to the newly formed carbonyl and a two-hydrogen triplet for the methylene group adjacent to the sulfur at 2.87 ppm.



Scheme 49: Synthesis of methyl (*R*)-26-(acetylthio)-2-((*R*)-1-hydroxy-19-((1*S*,2*R*)-2-((2*S*,19*S*,20S)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonadecyl)hexa-cosanoate **(186)**. (a) HF.Pyridine, Py, THF, 90 %.

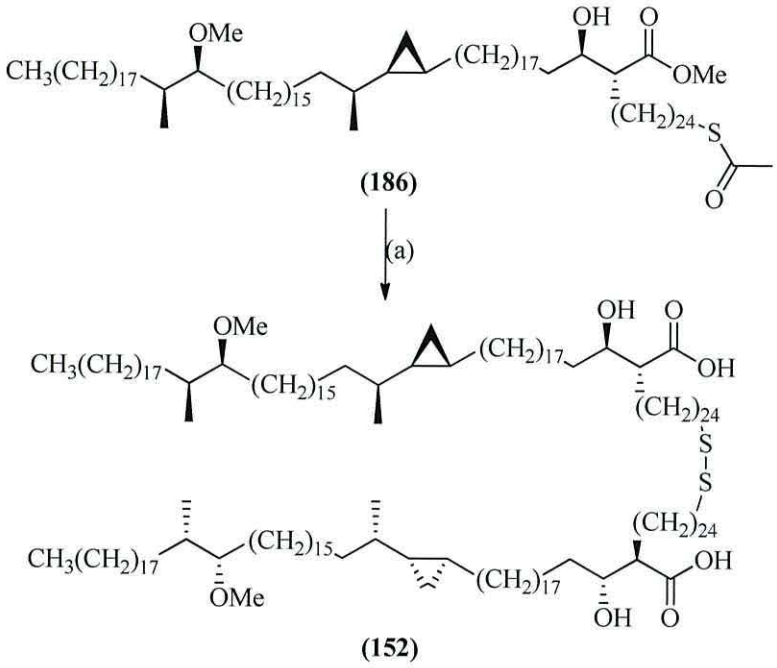
A fully-protected thiolated mycolic acid **(185)** was now synthesised, the last stage being to remove both protecting groups that remained in the molecule. The silyl was firstly deprotected by heating the compound overnight with HF.pyridine buffered by additional pyridine (Scheme 49). This reaction was completed as expected in a high yield of 90 %. This was again much higher than the yield for this reaction for the mycolic acid without the thiol (which was 54 %), but it is not believed that the thioacetate group aids this reaction in any additional way due to the distance between the two groups. The formation of thioacetate **(186)** was confirmed with the loss of the characteristic singlets representing the silyl group at 0.02, 0.04 and 0.86 ppm in the ^{1}H NMR spectrum. MALDI-MS also confirmed the molecular weight of this molecule.



Scheme 50: Attempted synthesis of (*R*)-2-((*R*)-1-hydroxy-19-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonadecyl)-26-mercaptohexacosanoic acid **(187)**. (a) LiOH.H_2O, THF, H_2O, MeOH.

The last stage was then to hydrolyse the methyl ester (Scheme 50). Due to the extensive problems observed for this reaction when synthesising the thiolated model of the mycolic acid **(149)** more problems were anticipated. This reaction was firstly attempted by heating thioacetate **(186)** with 15 equivalents of lithium hydroxide and not the four equivalents that had been found to be successful for the thiolated model **(149)** as the simple model may perform differently to the full mycolic acid. However, the crude ^{1}H NMR spectrum of the residue was again very complicated and also contained numerous spots on TLC. The triplet at 2.87 ppm for the methylene next to the sulfur in the thioacetate was not present suggesting that the thioacetate had been hydrolysed but the singlet for the methyl ester at 3.72 ppm remained although the intensity of the peak had decreased. This implied that even under these harsh conditions not all of the methyl ester had been hydrolysed.

Purification of the crude product of this reaction was not attempted as the crude ^{1}H NMR spectrum was so complicated and not very encouraging. The hydrolysis was not repeated using four equivalents of lithium hydroxide, which was found to be successful for the synthesis of the thiolated model of a mycolic acid **(149)**, as the peak representing the methyl ester was still present after using 15 equivalents of lithium hydroxide. Therefore, the probability of hydrolysing the methyl ester with four equivalents of lithium hydroxide was extremely low.



Scheme 51: Synthesis of (*S*,*S*,*S*,*R*,*S*,*R*,2*R*,2*R*')-26-26'-disulfanediylbis(2-((*R*)-1-hydroxy-19-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)-nonadecyl)hexacosanoic acid **(152)**. (a) (i) TBAH (aq), (ii) H^+, 58 %.

An alternative hydrolysis procedure was therefore used (Scheme 51). This method involved heating the methyl ester to 100 °C in a 5 % aqueous solution of tetrabutylammonium hydroxide. The solution was then acidified and extracted. This method was more likely to succeed as it had been previously used to extract natural mycolic acids that were covalently bound to the cell wall, without breaking their overall structure.[227] The crude ^{1}H NMR spectrum for this reaction was very promising. It was extremely clean showing the removal of the thioacetate singlet at 2.33 ppm and the triplet at 2.87 ppm and a newly formed triplet was observed at 2.69 ppm, implying that the disulfide **(152)** had been synthesised. It was also observed that the majority of the methyl ester had been removed. This compound was subsequently purified by column

chromatography and gave pure (*S*,*S*,*S*,*R*,*S*,*R*,2*R*,2*R*')-26-26'-disulfanediyl*bis*(2-((*R*)-1-hydroxy-19-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonadecyl)hexacosanoic acid **(152)** in a 58 % yield. This is again a significant step forward, as it was the first thiolated mycolic acid ever synthesised and the desired product **(152)** was synthesised in a much cleaner fashion and in a higher yield compared to the yield of the hydrolysis reaction of the model **(149)**.

The formation of the disulfide **(152)** was confirmed by a variety of analytical techniques including ^{1}H NMR, ^{13}C NMR, IR and optical rotation. However, an accurate mass spectrum could not be obtained for this product using either positive or negative ionisation modes by direct insertion mass spectroscopy or by MALDI mass spectroscopy. The ^{1}H NMR spectrum gave the majority of information about the structure of the disulfide and expansions of the various regions are shown in Figures 9a and 9b.

Figure 9a mainly represents the α-methyl-*trans*-cyclopropane fragment that had been analysed previously.[223,228] Three of the cyclopropane protons were seen as a large multiplet between 0.09 and 0.19 ppm and extensive splitting was seen in this case as none of the protons are equivalent, each giving rise to its own splitting pattern. For the cyclopropane proton nearest the the methyl branch, a multiplet was seen due to the extensive splitting, but this signal was further downfield due to its close proximity to the methyl branch. The proton on the methyl branch was also seen, again in this region, but further downfield at 0.66 ppm. In this region peaks could also be seen for the three terminal methyl groups between 0.85 and 0.91 ppm. Two doublets were seen at 0.86 and 0.90 for the two methyl branches whilst the terminal methyl was seen as a triplet at 0.89 ppm. The other significant peaks in the spectrum came above 2 ppm but a large multiplet was present at 1.26 ppm representing the alkyl chains within the molecule. The α-proton was seen as a single proton doublet of triplets at 2.47 ppm whilst the methylene group next to the sulfur was seen as a two-hydrogen triplet at 2.69 confirming the formation of a disulfide. The proton adjacent to the methoxy was seen as a single proton multiplet at 2.97 ppm and the methoxy itself was seen as a three-hydrogen singlet at 3.35 ppm. The only other significant peak in the spectrum was a one-hydrogen multiplet at 3.91 ppm representing the β-proton.

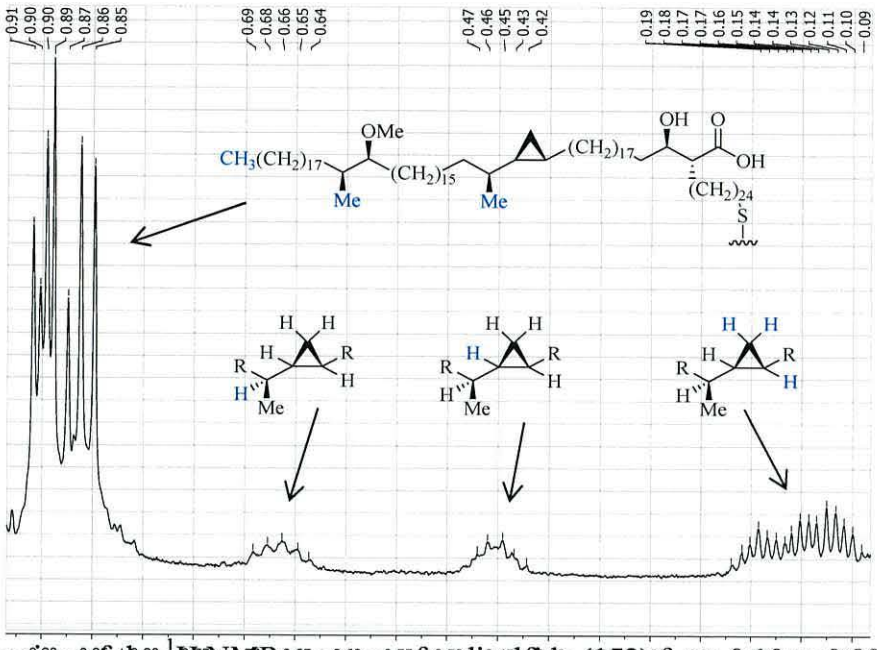


Figure 9a: Expansion of the ^{1}H NMR spectrum for disulfide **(152)** from 0.10 to 0.93 ppm.

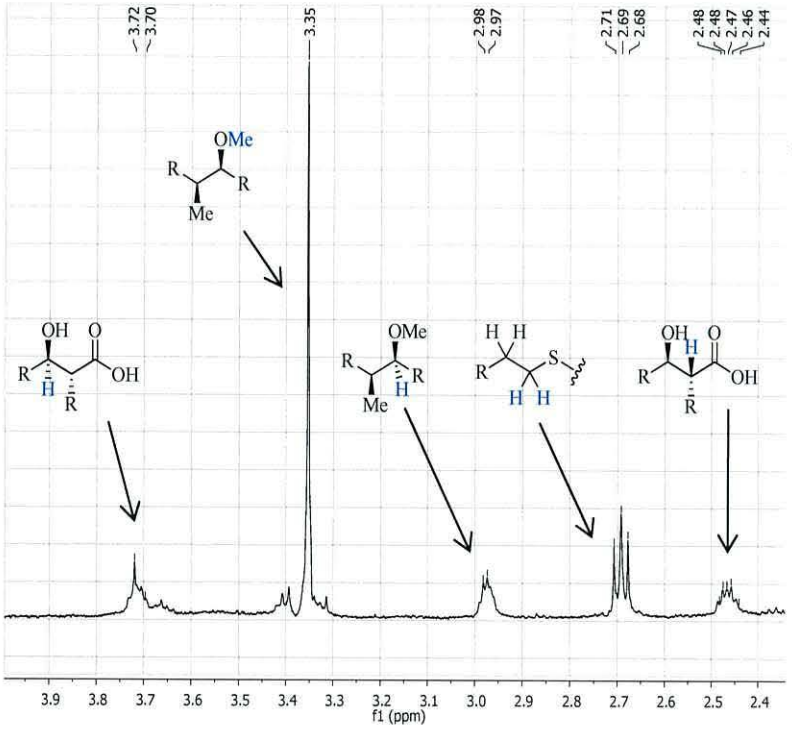
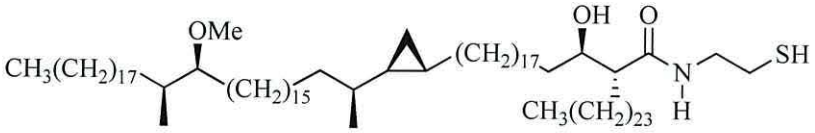


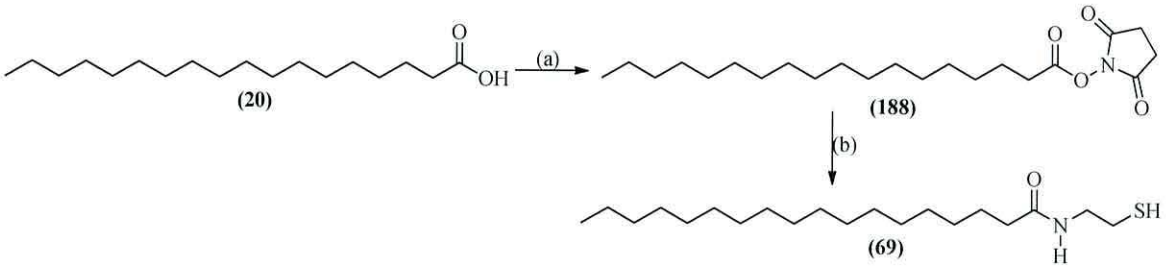
Figure 9b: Expansion of the ^{1}H NMR spectrum for disulfide **(152)** from 2.35 to 4.0 ppm.

2.6 – A Thiolated linker on a carboxylic acid



(187)

The position of the thiol within the mycolic acid structure could dramatically affect the antibody recognition results, therefore, the synthesis of a thiolated mycolic acid was attempted with the thiol group introduced as a thiolated linker on the carboxylic acid. A comparison could then be made between the antibody recognition effects of the mycolic acids with a thiol group at both positions. In order to have a direct comparison, this thiolated linker would be included into mycolic acid **(68)** to form (*R*)-2-((*R*)-1-hydroxy-19-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonadecyl)-*N*-(2-mercaptoethyl)hexacosanamide **(187)**.



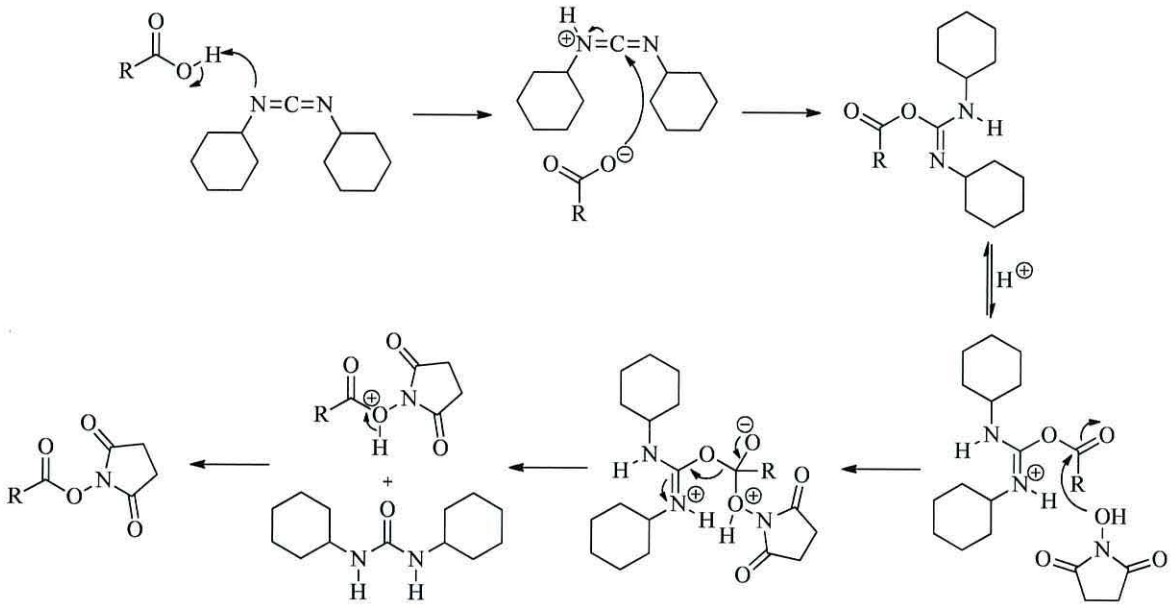
Scheme 52: Synthesis of *N*-(2-mercaptoethyl)stearamide **(69)**. (a) DCC, NHS, EtOAc, 74 %; (b) 2-MEA, CH_2Cl_2, NEt_3, 56 %.

The formation of the thiolated linker on a carboxylic acid was firstly attempted using stearic acid **(20)** (Scheme 52). This would also allow for determination of the antibody recognition effect of stearic acid **(20)**. To synthesise *N*-(2-mercaptoethyl)stearamide **(69)** the *N*-hydroxy succinimide ester of stearic acid **(20)** was firstly generated as an activating reagent for the fatty acid that generally consists of an ester and a good leaving group.

Formation of an activated reagent facilitates amide synthesis, as nucleophilic attack on the carbonyl by the amine in the next stage is aided due to the high reactivity of the succinimide ester because of the inductive effect of the nitrogen. The inductive effect of the nitrogen is reinforced by the adjacent carbonyl group.[229] This process has been widely used in peptide synthesis.

The *N*-hydroxy succinimide ester was synthesised by stirring stearic acid **(20)** in a solution of *N*-hydroxysuccinimide at room temperature followed by the addition of a solution of DCC (Scheme 53).[230] The carboxylic acid firstly reacts with DCC to form a very reactive

O-acylisourea intermediate. This activated intermediate then reacts with *N*-hydroxy succinimide to form DCU and an *N*-hydroxy succinimide ester.



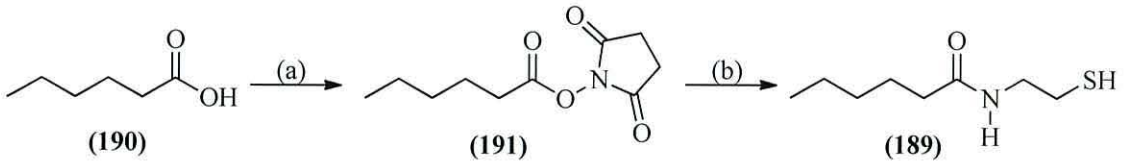
Scheme 53: Mechanism of formation of an *N*-hydroxy succinimide ester.

This resulted in the synthesis of 2,5-dioxopyrrolidin-1-yl stearate **(188)** in a 72 % yield. The main difference in the ^{1}H NMR spectrum for this product **(188)** in comparison to the starting material **(20)** was that a broad four-hydrogen singlet could be seen at 2.84 ppm representing the two equivalent CH_2 groups in the ring. In the ^{13}C NMR spectrum only two peaks could be seen representing carbonyl groups at 168.7 and 169.1 ppm due to the two equivalent carbonyl groups in the ring. Three carbonyl peaks however could be seen in the IR spectrum, which became characteristic for these compounds.

2,5-Dioxopyrrolidin-1-yl stearate **(188)** was then stirred in a solution of 2-mercaptoethylamine hydrochloride followed by the addition of triethylamine to form *N*-(2-mercaptoethyl)stearamide **(69)** in a low yield of 56 %.[231] The product, *N*-(2-mercaptoethyl)stearamide **(69)** was purified by recrystallisation rather than by column chromatography, which may have contributed towards the low yield. This method would eventually be performed using full mycolic acids on a very small scale and for these molecules a recrystallisation would not be viable and column chromatography would be a preferable purification method. The formation of *N*-(2-mercaptoethyl)stearamide **(69)** was confirmed by analysis of its ^{1}H NMR spectrum. The methylene adjacent to the sulfur and the methylene adjacent to the nitrogen were seen as a two-hydrogen doublet of triplets at 2.68 ppm and a two-hydrogen quartet at 3.45 ppm, respectively, in the ^{1}H NMR spectrum.

A signal could also be seen representing the NH as a broad one-hydrogen singlet at 5.83 ppm. In the ^{13}C NMR spectrum only one carbonyl peak was now observed, further confirming the formation of the product **(69)**. This synthesis produced *N*-(2-mercaptoethyl)stearamide **(69)** in an overall yield of 41 % from the corresponding acid **(20)**.

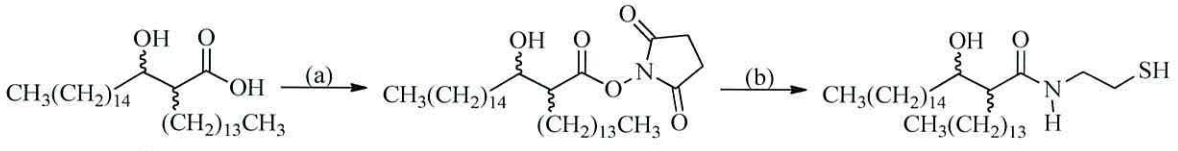
The synthesis of an *N*-(2-mercaptoethyl)amide was then attempted with a much shorter chain to discover if antibody recognition is dependent on chain length. *N*-(2-Mercaptoethyl)hexanamide **(189)** was therefore synthesised from hexanoic acid **(190)** (Scheme 54).



(190) (191) (189)

Scheme 54: Synthesis of *N*-(2-mercaptoethyl)hexanamide **(189)**. (a) DCC, NHS, EtOAc, 72 %; (b) 2-MEA, CH_2Cl_2, NEt_3, 65 %.

2,5-Dioxopyrrolidin-1-yl hexanoate **(191)** was firstly formed in a similar yield to *N*-hydroxy succinimide ester **(188)**; it showed the same significant peaks in the NMR spectra. This was followed by the synthesis of *N*-(2-mercaptoethyl)hexanamide **(189).** This was purified by column chromatography that resulted in a slightly higher yield, and the overall synthesis yield also increased from 41 to 47 %.



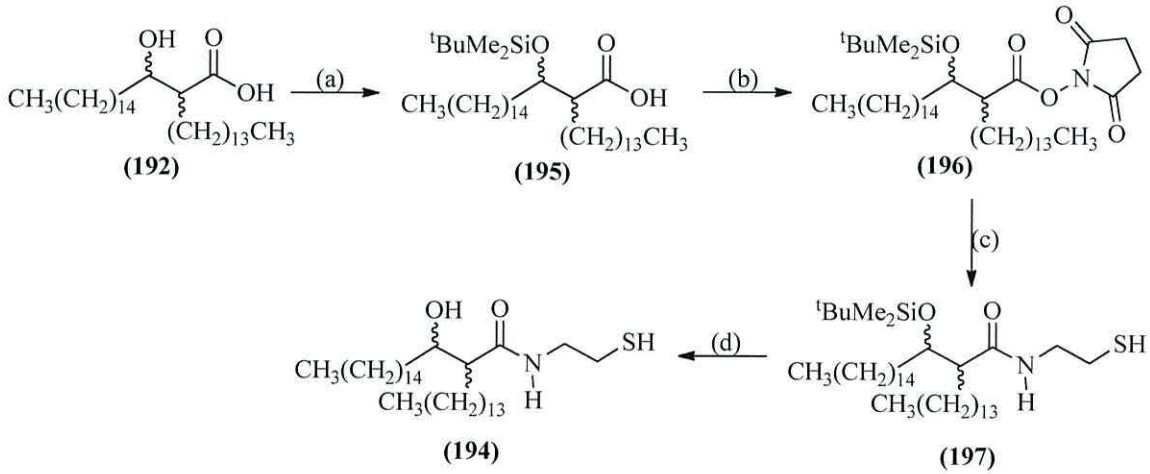
(192) (193) (194)

Scheme 55: Attempted synthesis of 3-hydroxy-*N*-(2-merccaptoethyl)-2-tetradecyloctadecanamide **(194)**. a) DCC, NHS, EtOAc, 68 %; (b) 2-MEA, CH_2Cl_2, NEt_3.

The synthesis of an *N*-(2-mercaptoethyl)amide was successful on simple molecules, but before attempting the synthesis of the thiolated linker on a full mycolic acid it was tried on a model of a mycolic acid **(192)** (Scheme 55). This model was again a simple mycolic acid **(192)** but in this case it did not have a single stereochemistry and was a mixture of four enantiomers. However, as it contained the full motif it was an excellent model. The *N*-

hydroxysuccinimide ester **(193)** was firstly synthesised from acid **(192)**, the reaction proceeding as expected in a yield of 68 %. In this case a four-hydrogen doublet was seen for the two methylene groups on the ring at 2.85 ppm in the ^{1}H NMR spectrum whilst the α and β-protons were seen as a doublet of triplets at 2.73 ppm and a multiplet at 3.79 ppm respectively. There was also a slight difference from **(188)** and **(191)** in the ^{13}C NMR spectrum as two signals could be seen at 169.3 and 169.4 ppm but the signal at 169.3 ppm was split into two accounting for the two different carbonyl groups.

The aminolysis was then performed to attempt to synthesise 3-hydroxy-*N*-(2-merccaptoethyl)-2-tetradecyloctadecanamide **(194)**. This reaction was unsuccessful and the crude ^{1}H NMR spectrum of the residue contained mainly starting material **(192)**. Therefore, no attempt was made to purify the residue. The reaction was then repeated by stirring the reaction mixture for a longer period of time, which again gave mainly starting material **(192)**.



Scheme 56: Attempted synthesis of 3-hydroxy-*N*-(2-merccaptoethyl)-2-tetradecyclocta-decanamide **(194)**. (a) Imidazole, DMF, toluene, TBDMSCl, DMAP, K_2CO_3, H^+, 83 %; (b) DCC, NHS, EtOAc, 70 %; (c) 2-MEA, CH_2Cl_2, NEt_3; (d). HF.Pyridine, Py, THF.

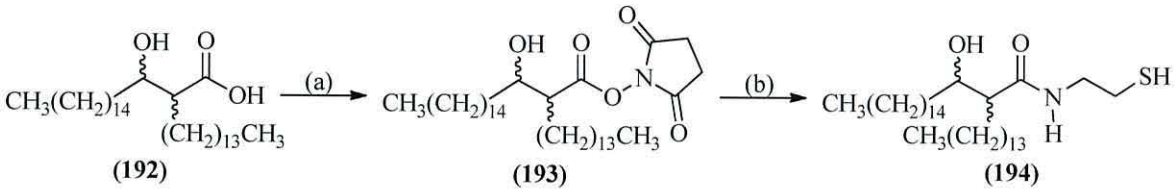
An alternative method was then attempted for the synthesis of 3-hydroxy-*N*-(2-mercaptoethyl)-2-tetradecyloctadecanamide **(194)** that involved firstly protecting the alcohol with a silyl protecting group (Scheme 56). This was done in case the close proximity of the hydroxyl group somehow interfered with the reaction.

The acid **(192)** was firstly protected with *tert*-butyldimethylsilyl by stirring a solution of the acid **(192)** with imidazole, *tert*-butyldimethylsilylchloride and DMAP. The formation of this protected compound **(195)** was extremely evident with three characteristic singlets

at 0.12, 0.13 and 0.92 ppm present in the ^{1}H NMR spectrum representing the silyl protecting group.

The *N*-hydroxy succinimide ester **(196)** was then synthesised from silyl **(195)** in a good yield of 70 %. Here, a five-hydrogen multiplet was seen at 2.81 ppm in the ^{1}H NMR spectrum representing the two methylene groups in the ring and the α-proton and the β-proton was seen as a one-hydrogen quartet at 4.03 ppm. Only two peaks representing carbonyl groups could be seen in the ^{13}C NMR spectrum of this compound but the characteristic three peaks representing the three carbonyl groups was seen in the IR spectrum.

It was then attempted to prepare 3-((*tert*-butyldimethylsilyl)oxy)-*N*-(2-mercaptoethyl)-2-tetradecyloctadecanamide **(197)** by stirring *N*-hydroxy succinimide ester **(196)** in a suspension of 2-mercaptoethylamine hydrochloride and triethylamine overnight. However, from the crude ^{1}H NMR spectrum of the residue it was seen that only the starting material **(196)** was recovered. The reaction was then repeated and stirred for a full week. In this case, the crude ^{1}H NMR spectrum again contained peaks representing the starting material **(196)** but also a small amount of the desired product **(197)**. This implied that this synthesis was not a viable method for the formation of thiol **(194)** and it was decided to try and optimise the original method.



Scheme 57: Synthesis of 3-hydroxy-*N*-(2-merccaptoethyl)-2-tetradecyloctadecanamide **(194)**. (a) DCC, NHS, EtOAc, 68 %; (b) 2-MEA, CH_2Cl_2, NEt_3, reflux, 79 %.

In order to optimise the aminolysis of *N*-hydroxy succinimide ester **(193)**, different reaction parameters were investigated. The reaction between *N*-hydroxy succinimide ester **(193)** and 2-mercaptoethylamine hydrochloride with triethylamine was therefore heated under reflux. After 24 hours, the TLC was not very clear and a large streak could be seen across the plate regardless of the solvent mixture and the visualisation method used. Due to this, there was no certain way to determine if the reaction had proceeded as expected. To try and ensure a complete reaction, the mixture was heated under reflux for a total of 130

hours with additional 2-mercaptoethylamine hydrochloride added halfway though this period.

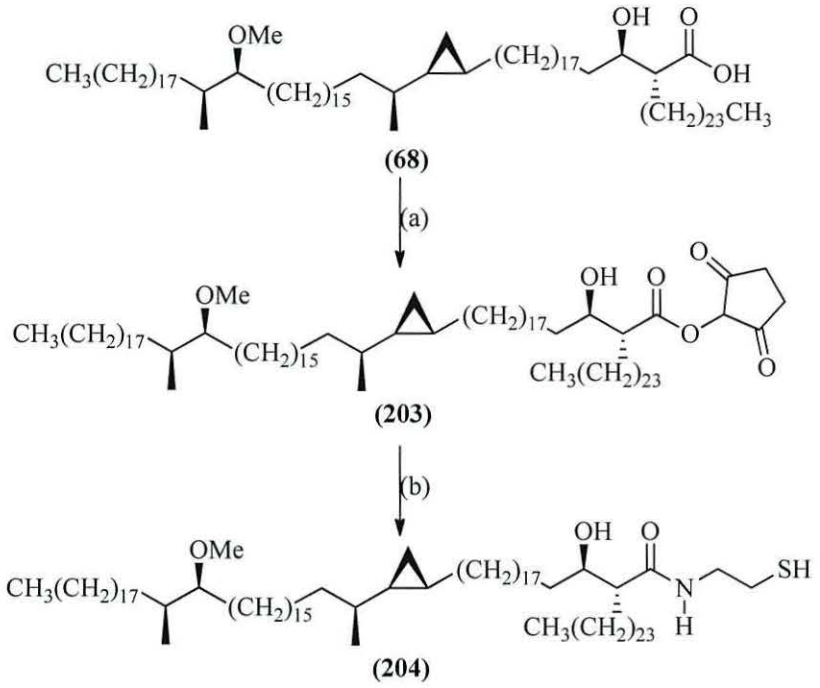
Following work up, the crude ^{1}H NMR spectrum for this reaction was very encouraging. No peaks were present representing the starting material **(193)** and all the peaks expected for the product **(194)** were observed. A MALDI-MS confirmed the formation of the product. The crude product was then purified by column chromatography obtaining pure 3-hydroxy-*N*-(2-merccaptoethyl)-2-tetradecyloctadecanamide **(194)** in a good yield of 79 %. The ^{1}H NMR spectrum for the product **(194)** showed all the expected peaks for the thiolated linker including a triplet for the NH at 6.18 ppm, a quartet for the methylene adjacent to the NH at 3.47 ppm and a multiplet for the methylene group next to the thiol at 2.70 ppm. It is understandable that this peak appears as a multiplet as the methylene group behaves as an AA'BB' system, as for a sulfone, accounting for the multiple peaks. The correct signals were also seen for the remainder of the molecule, which included a one-hydrogen multiplet for the β-proton at 3.66 ppm and a one-hydrogen doublet of triplets at 2.10 ppm for the α-proton.

Before synthesising the *N*-(2-mercaptoethyl)amide of the synthetic mycolic acid **(68)** the synthesis was attempted on a natural mixture of mycolic acids **(198).** The synthesis of the *N*-hydroxysuccinimide ester of the mycolic acid **(199)** was performed using only 18 mg of the natural material **(198)** and, as numerous mycolic acids were present, analysis of the NMR spectra for the product **(199)** was very difficult due to their complexity. The ^{1}H NMR spectrum of the product **(199)** contained various signals corresponding to the different mycolic acids in the mixture including singlets at 3.34 and 3.71 ppm representing methoxy groups and multiplets in the -0.4 to 0.6 ppm region representing the *cis* and *trans* cyclopropane. Due to the existence of all these peaks, the formation of the *N*-hydroxy succinimide ester was confirmed by comparing two specific peaks. The signal for the β-proton should be the same for all the mycolic acids in the mixture, therefore, the integration of this peak was compared to that of the doublet expected for the two methylene groups of the succinimide ester. A single proton multiplet could be seen at 3.80 ppm for the β-proton in comparison to a four-hydrogen doublet seen for the two succinimide methylene groups at 2.86 ppm. For 2,5-diocopyrrolidin-1-yl 3-hydroxy-2-tetradecyloctadecanoate **(193),** the α-proton was seen as a pentet at 2.73 ppm and a similar peak was seen for this mixture of mycolic acids **(199)** with a single proton pentuplet present at 2.74 ppm. The IR spectrum further confirmed the formation of the *N*-hydroxy

succinimide ester as the characteristic three signals at 1738, 1780 and 1810 cm^{-1} could also be seen. However, a MALDI mass spectrum could not be obtained for these molecules.

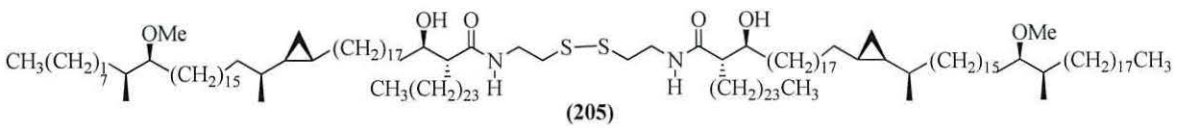
The *N*-hydroxy succinimide ester of the natural mycolic acid **(199)** mixture was then reacted with 2-mercaptoethylamine hydrochloride and triethylamine under the same conditions as for the synthesis of **(194)**. The formation of the *N*-(2-mercaptoethyl)amide of the natural mycolic acid mixture **(200)** was confirmed by comparing the signals for the α and β-protons to the peaks for the thiol linker group in the ^{1}H NMR spectrum, as previously. The α-proton could be seen as a one-hydrogen multiplet at 2.18 ppm but the peak for the β-proton was associated with the peaks expected for the methylene group adjacent to the nitrogen between 3.56 and 3.65 ppm. The new peaks expected for the thiolated linker could not, therefore, be compared to the peak for the β-proton. However, as this multiplet was integrated to three it was believed to account correctly for the β-proton and the methylene group adjacent to the nitrogen. Another significant peak expected was a multiplet for the methylene next to the thiol, which was expected to be seen at around 2.70 ppm. This, however, was not the case and a two-hydrogen triplet was seen at 2.86 ppm. The presence of the triplet in itself suggests that this compound might have formed a dialkyldisulfide. With disulfides in the literature, this was also seen to give rise to a triplet.[232] However, again no MALDI-MS could be obtained for the sample, which would further confirm this dimerisation. The data available therefore confirms not the formation of the *N*-(2-mercaptoethyl)amide of the natural mixture of mycolic acids **(201)** but the formation of the disulfide **(202)**.

The synthesis was then repeated on a pure synthetic sample of a mycolic acid **(68)** (Scheme 58). Mycolic acid **(68)** was reacted with *N*-hydroxysuccinimide and DCC to form *N*-hydroxysuccinimide ester **(203)** in a low yield of 38 %. This yield was much lower than that previously obtained for such reactions and this may be due to the starting material **(68)** not being completely soluble in the solvent at room temperature or to the complexity of the molecule. This could be attempted if the reaction was repeated. The formation of the succinimide ester was confirmed with a four-hydrogen doublet at 2.86 ppm in the ^{1}H NMR spectrum as well as three peaks representing the three different carbonyl groups in the ^{13}C NMR spectrum at 167.1, 167.3 and 167.9 ppm. The IR spectrum also showed the characteristic three peaks for the carbonyl groups. This reaction may be encouraged by catalysis with DMAP or by heating the reaction mixture under reflux.[233]



Scheme 58: Attempted synthesis of (*R*)-2-((*R*)-1-hydroxy-19-((1*S*,2*R*)-2-((2S,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonadecyl)-*N*-(2-mercaptoethyl)hexacosanamide **(204)**. (a) DCC, NHS, EtOAc, 38 %; (b) 2-MEA, CH_2Cl_2, NEt_3, reflux.

The synthesis of (*R*)-2-((*R*)-1-hydroxy-19-((1*S*,2*R*)-2-((2S,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonadecyl)-*N*-(2-mercaptoethyl)hexacosanamide **(204)** was then attempted by the same method as for **(194)**. However, from the ^{1}H NMR spectrum of the purified product, a two-hydrogen triplet could again be seen for the methylene adjacent to the sulfur, implying that the disulfide **(205)** was synthesised. Again, no MALDI-MS could be obtained for this compound to confirm this theory, but as for the natural mixture, comparison with the literature suggests that the disulfide had been generated due to the formation of a triplet. The remainder of the data were as expected with the majority of the information about the structure again being gained from the ^{1}H NMR spectrum. Expansions of specific regions in this spectrum can be seen in Figures 10a and 10b.



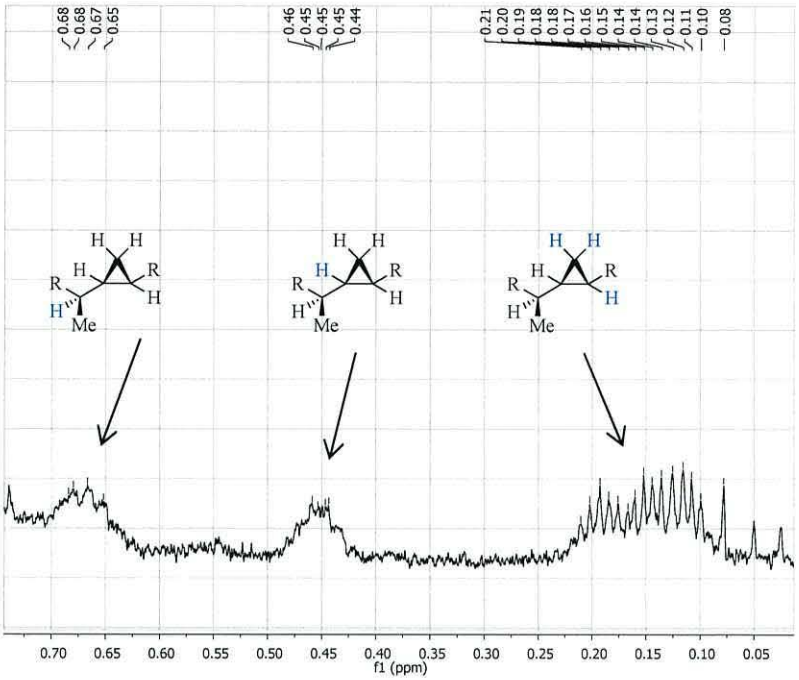


Figure 10a: Expansion of the ^{1}H NMR spectrum for thiol **(205)** from 0.0 to 0.75 ppm.

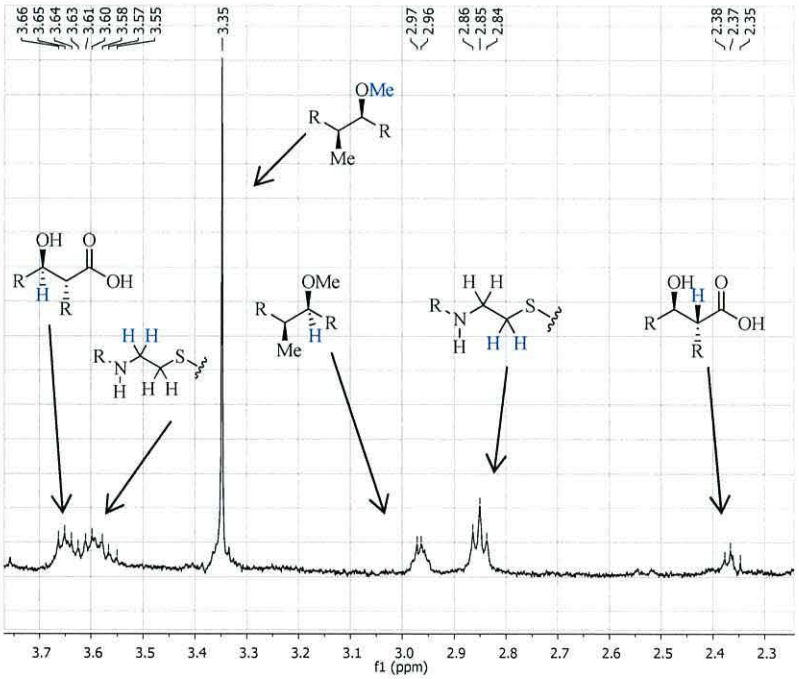


Figure 10b: Expansion of the ^{1}H NMR spectrum for thiol **(205)** from 2.25 to 3.75 ppm.

From the ^{1}H NMR spectrum of the product **(205)** the same pattern was observed between 0.05 and 0.68 ppm representing the *trans*-cyclopropane ring and the methyl proton. A multiplet was now seen between 0.85 and 0.91 ppm, which included signals for four different terminal methyl groups, which made the identification of the separate signals impossible. A large multiplet was seen at around 1.26 ppm, which represented the

numerous alkyl chains within the molecule. The next significant peak was at 2.37 ppm where a multiplet was seen for the α-proton. A triplet was seen at 2.85 ppm representing the methylene adjacent to the sulphur, which suggests that the disulfide has been formed. As for the synthesis of the mycolic acid with the thiol group at the end of the alkyl chain, the formation of the disulfide is not a problem as it can be bound to gold in the same way as a thiol would. A one-hydrogen singlet was seen for the methoxy proton at 2.97 ppm, while the methoxy was seen as a three-hydrogen singlet at 3.35 ppm. The peaks for the β-proton and the methylene next to the nitrogen were seen as a large three-hydrogen multiplet at 3.61 ppm. The only other significant peak in the molecule came at 6.76 ppm as a one-hydrogen triplet representing the NH. This data confirms the formation of the thiolated linker but also confirms the formation of disulfide **(205)**.

2.7 - The Synthesis of Cyclopropene Fatty Acids

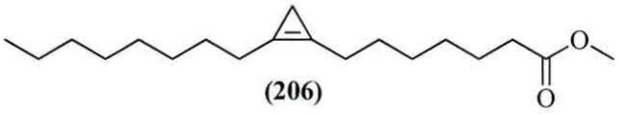
In order to attempt to maximise the inhibition against *P. falciparum* Δ^9 desaturase, small modifications were made to the sterculic acid **(74)** structure to try and produce a better inhibitor. These alterations to the sterculic acid **(74)** may cause a large difference in the inhibitory effects of the molecule and may also result in a better understanding of the active site of the enzyme. To achieve this, analogues of sterculic acid **(74)** were designed and synthesised. These analogues included structures with varying chain lengths and with additional functional groups.

2.7.1 - Changing the chain length

The first analogues of sterculic acid **(74)** prepared were synthesised with varying chain lengths. From previous literature it is known that the cyclopropene fatty acids only show inhibitory properties when the cyclopropene ring is on the C_9 and/or C_{10} positions,[234] therefore, it seemed reasonable to form analogues that still contained these functional features. The first two analogues synthesised were, therefore, 7-(2-octyl-cycloprop-1-enyl)-heptanoic acid methyl ester **(206)** and 9-(2-octyl-cycloprop-1-enyl)-nonanoic acid methyl ester **(207)**, both of which contain the ring on one of the stated positions. For the analogues, the methyl esters rather than the free acids were synthesised due to their increased stability. This does not interfere with the ability of the molecules to inhibit as it is the acyl CoA of the molecules that are the actual inhibitors,[162] which can be readily

generated from the methyl esters. In addition, it does not change the number of steps that are required for the synthesis to be completed.

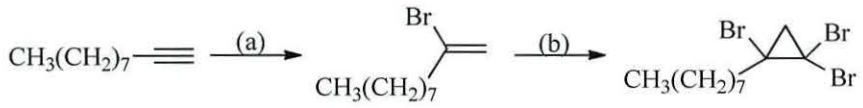
2.7.1.1 - The Synthesis of 7-(2-octylcycloprop-1-enyl)heptanoic acid methyl ester (206)



(206)

7-(2-Octyl-cycloprop-1-enyl)heptanoic acid methyl ester **(206)** or to give its trivial name, methyl malvalate, was the first analogue synthesised. This is also one of the few naturally occurring cyclopropene fatty acids and contains one less carbon atom in its acid side chain in comparison to sterculic acid **(74)** but it still has the second cyclopropene carbon at the C_9 position.

In order to synthesise ester **(206)**, 1,1,2-tribromo-2-octyl cyclopropane **(208)** was firstly prepared as the basic building block for all the analogues synthesised. This compound forms the alkyl chain as well as allowing for the formation of the cyclopropene ring from the cyclopropane *via* a coupling reaction with an electrophile.



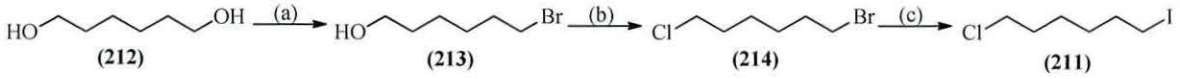
(209) **(210)** **(208)**

Scheme 59: Synthesis of 1,1,2-tribromo-2-octyl cyclopropane **(208)**. (a) HBr (g), $Et_4N^+Br^-$, CH_2Cl_2, 68 %; (b) $CHBr_3$, NaOH, Cetrimide, 89 %.

Following a procedure from the literature[235] gaseous HBr was firstly bubbled into a solution of tetraethylammonium bromide in dichloromethane. This was followed by the addition of 1-decyne **(209)** and the solution was heated under reflux for two hours. This reaction proceeded as a Markovnikov type addition with the HBr adding regioselectively across the triple bond forming 2-bromodec-1-ene **(210)**. The ^{13}C NMR spectrum of this clearly showed two peaks at 116.2 ppm and 134.9 ppm showing the presence of the new alkene carbons but it was the ^1H NMR spectrum that confirmed the regiochemistry of the structure. The ^1H NMR spectrum showed two, single proton doublets at 5.38 ppm and 5.55 ppm, each with small germinal coupling constants that confirmed that the bromine did add by a Markovnikov type addition across the double bond.

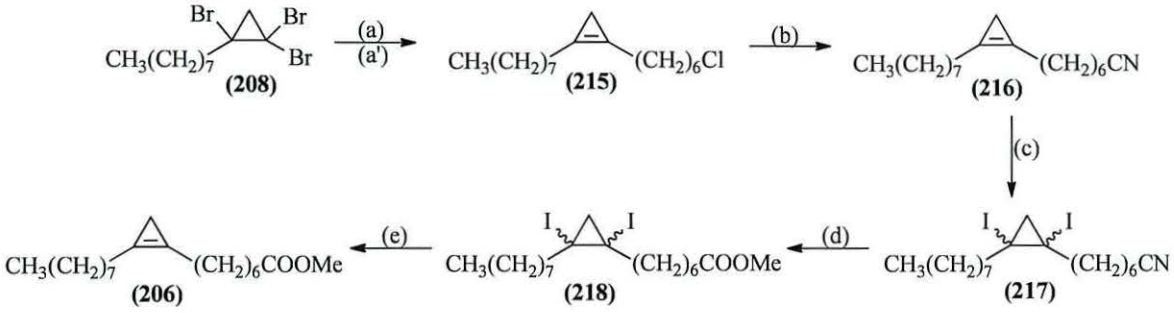
2-Bromodec-1-ene **(210)** was then stirred overnight with sodium hydroxide, bromoform and cetrimide in dichloromethane. This reaction proceeded as a phase transfer catalysis[236] reaction to form 1,1,2-tribromo-2-octylcyclopropane **(208)**. Both ^{1}H and ^{13}C NMR spectra clearly showed the disappearance of the alkene and a series of new peaks could be seen between 1.84 ppm and 2.05 ppm in the ^{1}H NMR spectrum representing the four protons adjacent to the bromines. All of the data confirmed the formation of the tribromo **(208)**.

Before coupling 1,1,2-tribromo-2-octyl cyclopropane **(208)** to an electrophile to form the cyclopropene ring and the acid side chain, 1-chloro-6-iodo-hexane **(211)** was synthesised (Scheme 60). Iodine is a much better leaving group than a chlorine or bromine, therefore, synthesising 1-chloro-6-iodo-hexane **(211)** should make the coupling reaction more favourable and result in an increase in the yield of the reaction.



Scheme 60: Synthesis of 1 chloro-6-iodo-hexane **(211)**. (a) HBr (aq), toluene, 78 %; (b) NCS, PPh_3, CH_2Cl_2, 68 %; (c) NaI, $NaHCO_3$, acetone, 74 %.

1-Chloro-6-iodo-hexane **(211)** was synthesised from 1,6-hexanediol **(212)** in three steps and in an overall yield of 39 % (Scheme 60).



Scheme 61: Synthesis of 7-(2-octyl-cycloprop-1-enyl)-heptanoic acid methyl ester **(206)**. (a) *n*-BuLi; (a') HMPA, $Cl(CH_2)_6I$ **(211)**, 39 %; (b) NaCN, DMSO, 78 %; (c) I_2, ether, 78 %; (d) HCl (g), MeOH, ether, 45 %; (e) *n*-BuLi, ether, 72 %.

1,1,2-Tribromo-2-octyl cyclopropane **(208)** was firstly reacted with two equivalents of *n*-butyllithium to give a lithiated cyclopropene (Scheme 62). In situ, HMPA and 1-chloro-

6-iodo-hexane **(211)** were added, which trapped the lithiated cyclopropene[237] and formed 1-(6-chloro-hexyl)-2-octyl-cyclopropene **(215)**, in a moderate yield of 39 %. It is seen in the literature that this reaction is reported to give a yield of around 50 - 60 %.[238] The presence of the cyclopropene ring in this product **(215)** could be easily confirmed as the ^{1}H NMR showed a characteristic two-hydrogen singlet at 0.77 ppm for the cyclopropene protons. The double bond of the ring could also be easily identified in the ^{13}C NMR spectrum with two peaks present at 109.1 ppm and 109.6 ppm. It was also detected by TLC. When the silica backed TLC plate was stained with iodine, normally the different compounds appeared as dark spots. When the compound contained a cyclopropene ring, a white spot could be seen on the back of the TLC plate as the cyclopropene ring reacts with the iodine absorbed on the plate and removes the colour.[238] All remaining data were as expected for chloride **(215)** with the only other significant peak in the ^{1}H NMR occurring as a two-hydrogen triplet at 3.54 ppm for the methylene group adjacent to the chloride.

Scheme 62: Formation of a lithiated cyclopropene.

Chloride **(215)** was heated to 60 °C with sodium cyanide in DMSO.[239] This reaction converted the chloride into the nitrile to form 7-(2-octyl-cycloprop-1-enyl)-heptanenitrile **(216)**. The formation of the nitrile **(216)** could again be easily confirmed with characteristic peaks in the IR spectrum and ^{13}C NMR spectrum at 2246 cm^{-1} and 119.8 ppm respectively. The data for the cyclopropene ring were as before with one clear difference evident in the IR spectrum. The IR spectrum exhibited a weak band at 1871 cm^{-1}. This band, not seen for chloride **(215)** represents the carbon-carbon double bond of the cyclopropene ring. This is at a much higher frequency than what would be expected for a normal double bond due to the high strain exhibited by the ring.

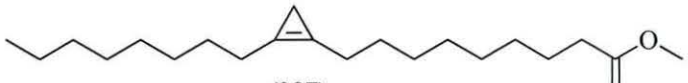
The next stage should then be to convert the nitrile to the methyl ester. However, due to the harsh conditions of the acid hydrolysis and the high reactivity of the cyclopropene ring, the ring was firstly protected with iodine.[240] This ensured that the ring remained intact during the hydrolysis but allows for the facile regeneration of the cyclopropene when needed. Nitrile **(216)** was therefore reacted with iodine in dry ether to form 7-(1,2-diiodo-2-octyl-3cyclopropyl)-heptanenitrile **(217)**. The protection was confirmed with the disappearance of the characteristic peaks for the cyclopropene.

Gaseous HCl was then bubbled through a solution of ether and methanol, and the nitrile **(217)** was added. This acid hydrolysis gave 7-(1,2-diiodo-2-octyl-cyclopropyl)-heptanoic acid methyl ester **(218)** in a yield of 45 %. The formation of the product **(218)** was confirmed with the loss of the signals representing the nitrile and the emergence of a peak in the ^{13}C NMR spectrum at 174.3 ppm confirming a carbonyl was present and a three-hydrogen singlet at 3.68 ppm in the ^{1}H NMR spectrum representing the methoxy. All remaining peaks were identical to that of the starting material **(217)**.

The iodine protection was then removed to regenerate the cyclopropene ring by stirring methyl ester **(218)** in a solution of *n*-butyllithium. This formed 7-(2-octyl-cycloprop-1-enyl)-heptanoic acid methyl ester **(206)** as a yellow oil. Both ^{1}H and ^{13}C NMR spectra confirmed the return of the cyclopropene with a two-hydrogen singlet at 0.78 ppm in the ^{1}H NMR spectrum and two peaks at 109.2 ppm and 109.4 ppm in the ^{13}C NMR spectrum. All other peaks were as for 7-(1,2-diiodo-2-octyl-cyclopropyl)-heptanoic acid methyl ester **(218)**.

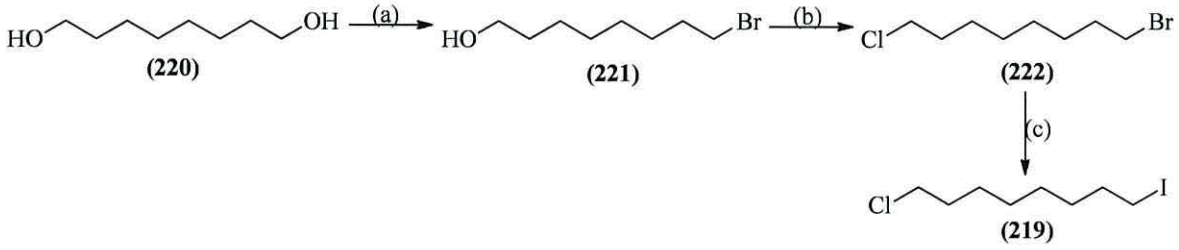
The synthesis from 1,1,2-tribromo-2-octyl cyclopropane **(208)** to 7-(2-octyl-cycloprop-1-enyl)-heptanoic acid methyl ester **(206)** proceeded in an overall yield of 8 %. This method is therefore not as effective as existing methods for the production of methyl malvalate **(206)** including first synthesis of **(206)**, which was reported in an overall yield of 15 % by Gensler *et al.*[142]

2.7.1.2 - The Synthesis of 9-(2-octyl-cycloprop-1-enyl)-nonanoic acid methyl ester (207)



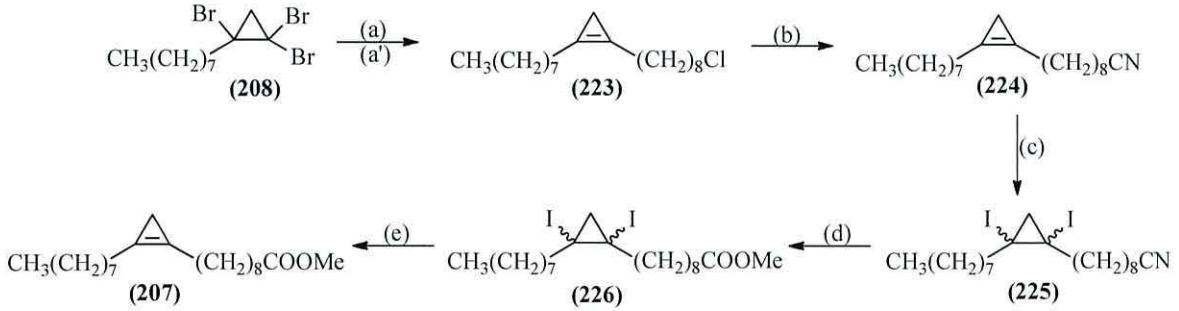
(207)

9-(2-Octyl-cycloprop-1-enyl)-nonanoic acid methyl ester **(207)** was then prepared to see if increasing the number of carbon atoms would cause a difference in the inhibitory capacity of the analogue (Scheme 64). Compound **(207)** also contains the cyclopropene on the C_{10} position and therefore should exhibit inhibitory qualities.



Scheme 63: Synthesis of 1-chloro-8-iodo-octane **(219)**. (a) HBr (aq), toluene, 82 %; (b) NCS, PPh_3, CH_2Cl_2, 73 %; (c) NaI, $NaHCO_3$, acetone, 90 %.

The synthesis of **(207)** proceeded as for **(206),** the only difference occurring in the initial coupling reaction. In this case tribromide **(208)** was coupled with 1-chloro-8-iodo-octane **(219)** rather than 1-chloro-6-iodo-hexane **(211)**. 1-Chloro-8-iodo-octane **(219)** was prepared by the same method as for 1-chloro-6-iodo-hexane **(211)** from its corresponding diol, 1,8-octanediol **(220)** (Scheme 63). This reaction resulted in an overall yield of 54 %, which is slightly higher than in the previous synthesis.



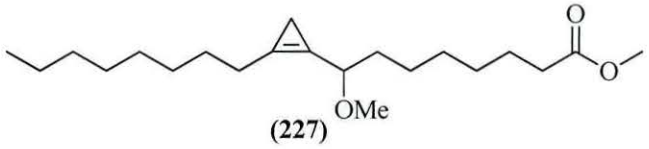
Scheme 64: Synthesis of 7-(2-octyl-cycloprop-1-enyl)-heptanoic acid methyl ester **(207)**. (a) *n*-BuLi; (a') HMPA, $Cl(CH_2)_8I$ **(219)**, 43 %; (b) NaCN, DMSO, 73 %; (c) I_2, ether, 83 %; (d) HCl (g), MeOH, ether, 61 %; (e) *n*-BuLi, ether, 29 %.

The synthesis of ester **(207)** proceeded as expected in an overall yield of 5 % (Scheme 64) compared to 8 % for the previous synthesis. This overall yield was lower with the main difference in the individual reactions occurring in the iodine deprotection step (e). For the synthesis of ester **(207)** this proceeded in a yield of 29 % compared to 72 % for the synthesis of ester **(206)**. This low yield may be accounted for by some moisture being in the system or it may be due to the *n*-butyllithium not being fresh. Starting material **(226)** was recovered during purification, which could be deprotected again to generate more product **(207)** and increase the reaction yield.

2.7.2 - Including New Functional Groups

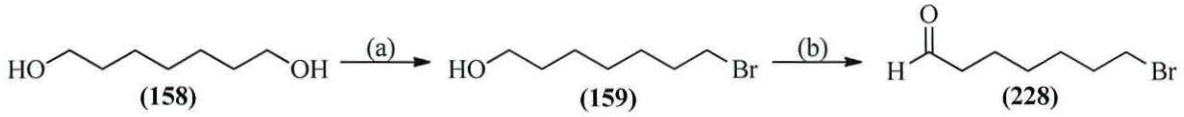
Various functional groups were then included into the sterculic acid **(74)** structure to investigate their effects on the level of inhibition. The functional groups would be included on the C_8 position, which is adjacent the cyclopropene ring on the carboxyl side chain. It is known that the cyclopropene reacts with the enzymes active site,[164] therefore, the C_8 position should also be either close to, or part of the active site. Adding functional groups at this position should therefore have a profound effect in one way or another on the compounds ability to inhibit Δ^9 desaturase. The chain length in this case would be identical to that of sterculic acid (**74**) as changing more than one aspect of the molecule would make it difficult to know the effect of each individual aspect on the inhibitory qualities of the molecule.

2.7.2.1 - The Synthesis of (±)-8-methoxy-8-(2-octyl-cycloprop-1-enyl)-octanoic acid methyl ester (227)



(227)

(±)-8-Methoxy-8-(2-octyl-cycloprop-1-enyl)-octanoic acid methyl ester **(227)** was first synthesised. To synthesise it, 7-bromo heptanal **(228)** was prepared (Scheme 65). This aldehyde **(228)** was then used immediately as the electrophile in the coupling reaction with the lithiocyclopropene derived from 1,1,2-tribromo-2-octylcyclopropane **(208)**.[241]



(158) **(159)** **(228)**

Scheme 65: Synthesis of 7-bromo heptanal **(228)**. (a) HBr (aq), toluene, reflux, 79 %; (b) PCC, CH_2Cl_2, 78 %.

1,1,2-Tribromo-2-octylcyclopropane **(208)** was firstly reacted with two equivalents of *n*-butyllithium, followed by the addition of HMPA and 7-bromoheptanal **(228)**. The solution was stirred for three hours and iodomethane was added, which results in the formation of the methoxy group. Following column chromatography, **(229)** was isolated as expected. The presence of a methoxy group was confirmed as a three-hydrogen singlet at

3.31 ppm in the ^{1}H NMR spectrum and the proton on the carbon atom next to the oxygen was seen as a one-hydrogen triplet at 3.18 ppm, as expected. Two peaks were observed in the ^{13}C NMR spectrum at 108.6 ppm and 114.1 ppm representing the two carbon atoms of the double bond that is part of the cyclopropene ring. However, the characteristic singlet at around 0.78 ppm in the ^{1}H NMR spectrum was not apparent. In this case two, single proton doublets were seen at 0.91 ppm and 0.95 ppm, each with a coupling constant of 8.5 Hz (Figure 11). The inclusion of a functional group at the C_8 position, therefore, caused the two cyclopropene protons to become non-equivalent. This reaction also resulted in a higher yield of 53 % compared to around 40 % for the first two syntheses as aldehydes are more reactive than halides towards organolithium compounds.

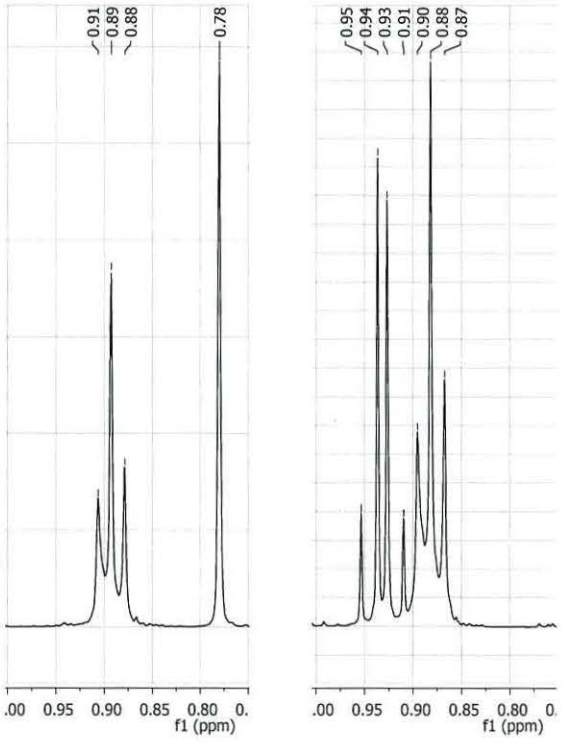


Figure 11: Comparison of a section of the ^{1}H NMR spectra between 0.75 ppm and 1.0 ppm for 1-(6-chloro-hexyl)-2-octyl-cyclopropene **(215)** and (±)-1-(7-bromo-1-methoxy-heptyl)-2-octyl-cyclopropene **(229)** showing the terminal methyl triplet and the singlet/two doublets representing the cyclopropene ring.

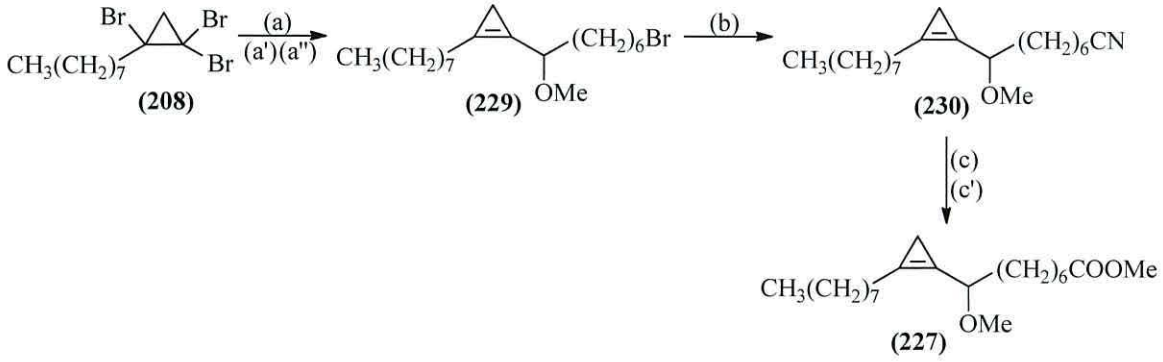
(±)-1-(7-Bromo-1-methoxy-heptyl)-2-octyl-cyclopropene **(229)** was then heated at 60 °C with sodium cyanide in DMSO as before. This formed nitrile **(230)**, which again showed the distinctive peaks at 2246 cm^{-1} in the IR spectrum and at 119.8 ppm in the ^{13}C NMR spectrum, whilst all other peaks were as for the starting material **(229)** with the two

cyclopropene protons again observed as two single-hydrogen doublets and the methoxy as a three-hydrogen singlet.

For the synthesis of ester **(206)** and ester **(207)**, the cyclopropene had been protected at this stage with iodine before acid hydrolysing the nitrile to the methyl ester. This required two extra steps that did not always proceed in a good yield. A base hydrolysis eliminates the need for the iodine protection due to the milder reaction conditions. This allows for the formation of the final product in fewer steps and in a better overall yield.

(±)-8-Methoxy-8-(2-octyl-cycloprop-1-enyl)-octanenitrile **(230)** was therefore reacted with a solution of sodium hydroxide and heated under reflux to give the carboxylate. TBAH and iodomethane were then added and the mixture was stirred for 12 hours, converting the sodium salt directly into the methyl ester using TBAH as a phase transfer catalyst. This method is preferable to the alternative method reported in the literature for the conversion of the nitrile to the methyl ester for cyclopropene containing compounds, which uses the highly toxic and potentially explosive compound, diazomethane.[242]

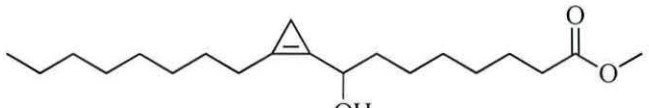
The disappearance of the peaks representing the nitrile was evident in both the IR and ^{13}C NMR spectra. In their place, a peak representing the carbonyl was seen at 174.2 ppm in the ^{13}C NMR spectrum and two three-hydrogen singlets could be seen in the ^{1}H NMR spectrum at 3.30 ppm and 3.66 ppm, representing the two methoxy groups. The presence of the cyclopropene could also be confirmed with a characteristic weak peak in the IR spectrum at 1863 cm^{-1}, two signals in the ^{13}C NMR spectrum at 108.6 ppm and 114.0 ppm and two, single proton doublets at 0.90 ppm and 0.92 ppm in the ^{1}H NMR spectrum, confirming that the ring did not decompose during this reaction.



Scheme 66: Synthesis of (±)-8-methoxy-8-(2-octyl-cycloprop-1-enyl)-octanoic acid methyl ester **(227)**. (a) *n*-BuLi; (a') HMPA, $Br(CH_2)_6COH$ **(228)**; (a'') MeI, 53 %; (b) NaCN, DMSO, 73 %; (c) NaOH, EtOH, H_2O; (c') TBAH, MeI, CH_2Cl_2, 93 %.

This synthesis resulted in an overall reaction yield of 36 % from tribromide **(208)** (Scheme 66). This is a much higher yield than that for the two previous syntheses, showing that substituting the acid hydrolysis for the base hydrolysis was a worthwhile change. This method is therefore much more cost and time effective as it requires less steps and proceeds in a better yield.

2.7.2.2 - The Synthesis of (±)-8-hydroxy-8-(2-octylcycloprop-1-enyl)-octanoic acid methyl ester (231)



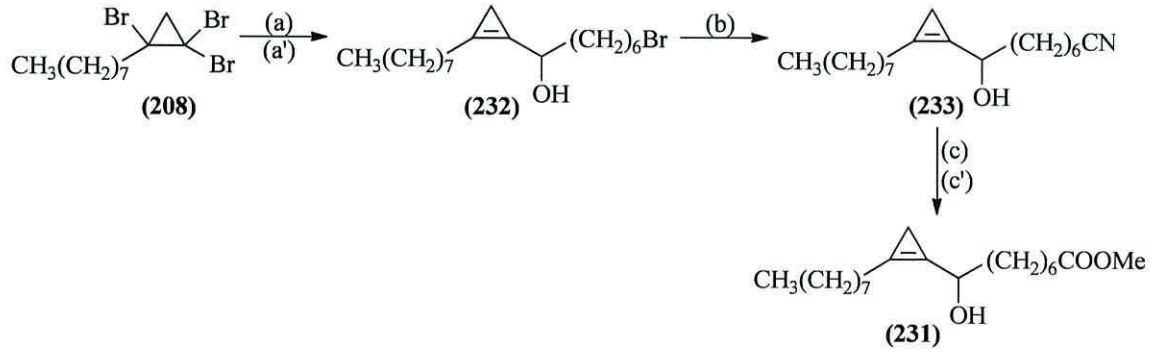
(231)

The synthesis of the alcohol analogue **(231)** followed the same principles as that of the methoxy compound **(227),** with some minor variations. For the initial coupling reaction between tribromide **(208)** and 7-bromoheptanal **(228),** no iodomethane was included. This led to the formation of an alcohol on the C_8 position. The synthesis of bromide **(232)** could be confirmed as a broad hydroxyl peak could be seen in the IR spectrum at 3356 cm^{-1}. The IR spectrum also showed the characteristic peak at 1868 cm^{-1} for the cyclopropene double bond. For this product **(232)** a two-hydrogen singlet could be seen for the cyclopropene protons at 0.95 ppm in the ^1H NMR spectrum and not two, one-hydrogen doublets as seen for the methoxy **(227)**. These protons are further downfield than what would be expected for a characteristic cyclopropene singlet due to the effect of the close proximity of the oxygen atom. A singlet may be seen rather than two doublets as the alcohol group is a rather smaller group than the methoxy.

The two remaining reactions were performed precisely as for the methoxy analogue **(227)**, the bromide **(232)** being converted into nitrile **(233)** before being converted again into methyl ester **(231)**, *via* base hydrolysis conditions. The IR spectrum confirmed the presence of the alcohol in the product **(231)** as a clear broad peak could be observed at 3430 cm^{-1}. The presence of the cyclopropene ring could also be confirmed by the IR spectrum as the weak characteristic signal could be seen at 1869 cm^{-1}. The existence of the ring was further confirmed by the ^1H NMR spectrum, which continued to show the cyclopropene protons as a two-hydrogen singlet. The formation of the methyl ester was also evident with a three-hydrogen singlet at 3.66 ppm in the ^1H NMR spectrum

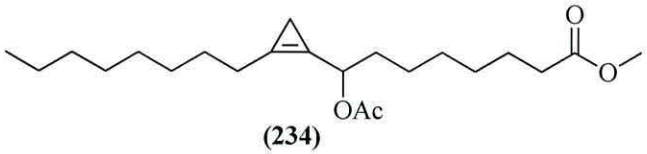
representing the methoxy and a signal at 174.3 ppm in the ^{13}C NMR spectrum represented the carbonyl.

This synthesis gave (±)-8-hydroxy-8-(2-octylcycloprop-1-enyl)-octanoic acid methyl ester **(231)** in an overall reaction yield of 24.9 % (Scheme 67). Again this is much higher than the overall reaction yield for the synthesis of ester **(206)** and ester **(207)**, which again confirms the effectiveness of this method. However, the yield for this synthesis is lower than that for methoxy **(227)**. The main difference in the yields between the two syntheses came for the base hydrolysis reaction where a yield of 66 % was seen for the alcohol **(231)** compared to 93 % for the methoxy **(227)**. Although the first of these yields is low, an even lower yield of 31 % has been reported in the literature.[241] Leaving the reaction to stir for a longer period of time might result in an increased yield.



Scheme 67: Synthesis of (±)-8-hydroxy-8-(2-octylcycloprop-1-enyl)-octanoic acid methyl ester **(231)**. (a) *n*-BuLi; (a') HMPA, $Br(CH_2)_6COH$ **(228)**, 54 %; (b) NaCN, DMSO, 70 %; (c) NaOH, EtOH, H_2O; (c') TBAH, MeI, CH_2Cl_2, 66 %.

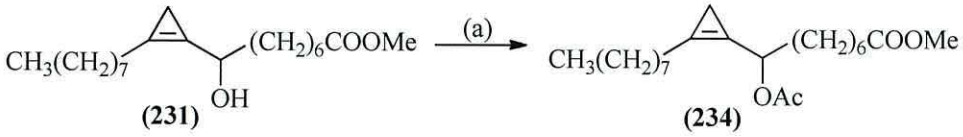
2.7.2.3 - The Synthesis of (±)-8-acetoxy-8-(2-octylcycloprop-1-enyl)octanoic acid methyl ester (234)



(234)

From alcohol **(231)**, other analogues could be synthesised. Acetate **(234)** was obtained in a very high yield by stirring alcohol **(231)** with pyridine and acetic anhydride (Scheme 68). The formation of the acetate was confirmed with a three-hydrogen singlet at 2.06 ppm in the ^1H NMR spectrum. Two carbonyl peaks could also be seen in the ^{13}C NMR spectrum, one for the acetate and the other for the methyl ester. The inclusion of the acetate also

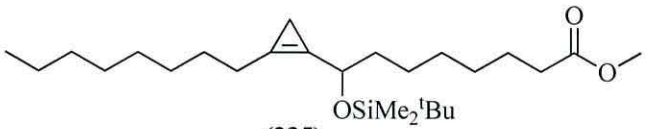
caused a shift in the position of the one-hydrogen triplets for the C_8 proton downfield to 5.65 ppm. Two, one-hydrogen doublets could also be seen for the cyclopropene protons. The remainder of the spectra were as for alcohol **(231)**.



(231) (234)

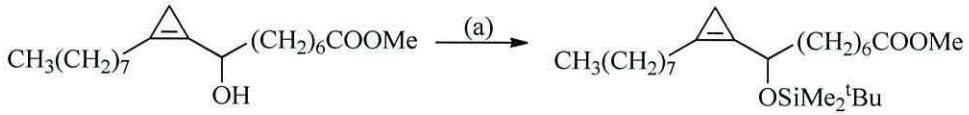
Scheme 68: Synthesis of (±)-8-acetoxy-8-(2-octylcycloprop-1-enyl)octanoic acid methyl ester **(234)**. (a) Py, Ac_2O, toluene, 97 %.

2.7.2.4 - The synthesis of (±)-8-(*tert*-butyldimethylsilyloxy)-8-(2-octylcycloprop-1-enyl)octanoic acid methyl ester (235)



(235)

(±)-8-(*tert*-Butyldimethylsilyloxy)-8-(2-octylcycloprop-1-enyl)octanoic acid methyl ester **(235)** was also synthesised from alcohol **(231)** (Scheme 69). Heating a stirred solution of imidazole and *tert* butyldimethylsilyl chloride with alcohol **(231)** gave methyl ester **(235)** in a 63 % yield.



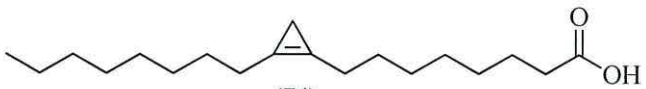
(231) **(235)**

Scheme 69: Synthesis of (±)-8-(*tert*-butyldimethylsilyloxy)-8-(2-octylcycloprop-1-enyl)octanoic acid methyl ester **(235)**. (a) imidazole, DMF, TBDMSCl, DMAP, 63 %.

The formation of this analogue was confirmed by the characteristic peaks for the silyl group in the ^1H NMR spectrum. The two silyl methyl groups were seen as two, three-hydrogen singlets at 0.03 and 0.05 ppm whilst, although a nine-hydrogen singlet was expected for the *tert*-butyl group, this signal was present as a multiplet at 0.89 ppm incorporated with several overlapping signals for various protons. Included in this multiplet were the peaks for the cyclopropene protons. In this case it could not be determined whether the signals for the cyclopropene protons appeared as a two-hydrogen

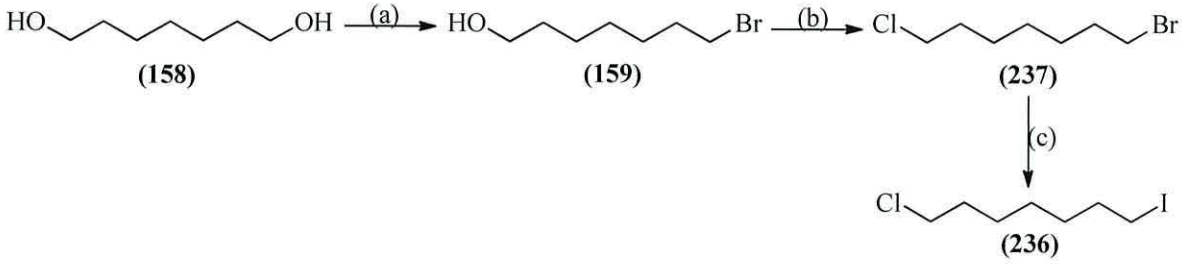
singlet or as two, one-hydrogen doublets but due the number of peaks in the multiplet it can be predicted that the cyclopropene protons do exhibit as two, one-hydrogen doublets for this compound. The existence of the cyclopropene double bond, however, could be confirmed with two peaks in the ^{13}C NMR spectrum at 111.1 and 111.3 ppm and a signal at 1870 cm^{-1} in the IR spectrum.

2.7.3 - The Synthesis of Free Sterculic Acid (74)



(74)

Free sterculic acid **(74)** was also synthesised to ensure that there was no change in the inhibitory effect of the free acid in comparison to the methyl ester. The initial coupling reaction was performed between tribromide **(208)** with two equivalents of *n*-butyllithium, followed by the addition of HMPA and 1-chloro-7-iodo-heptane **(236)**. Compound **(236)** was synthesised from 1,7-heptanediol **(156)** as for the synthesis of **(211)** and **(219)** (Scheme 70).

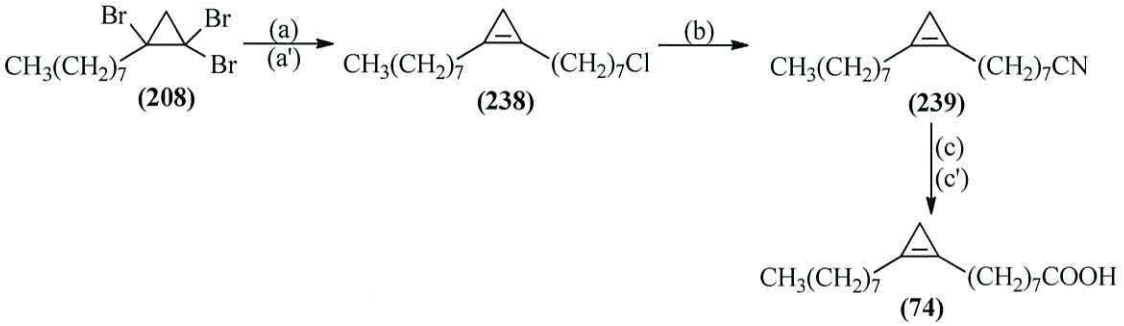


(158) **(159)** **(237)** **(236)**

Scheme 70: Synthesis of 1-chloro-7-iodo-hexane **(236)**. (a) HBr (aq), toluene, 79 %; (b) NCS, PPh_3, CH_2Cl_2, 68 %; (c) NaI, $NaHCO_3$, acetone, 76 %.

This coupling reaction gave 1-(7-chloroheptyl)-2-octylcycloprop-1-ene **(238)** in a 42 % yield. This is comparable to the yields for the coupling reaction with an alkyl iodide but lower than that for the coupling reaction with an aldehyde, again confirming that halides are less reactive than the aldehydes towards organolithium compounds. All peaks in the NMR spectra of chloride **(238)** were as expected with the significant peaks in the ^1H NMR spectrum occurring at 0.77 ppm for the cyclopropene protons and at 3.54 ppm for the methylene group adjacent to the chloride.

1-(7-Chloroheptyl)-2-octylcycloprop-1-ene **(238)** was then reacted with sodium cyanide in DMSO to form nitrile **(239)**. This conversion was confirmed with the presence of the characteristic peaks in the IR and ^{13}C NMR spectra for the nitrile. A base hydrolysis reaction was then performed to form the free acid **(74)**. This reaction was different to the base hydrolysis for the methoxy **(227)** and alcohol **(231)**, as the iodomethane was omitted and the solution was acidified with potassium hydrogen sulphate. Free sterculic acid **(74)** was successfully synthesised in a yield of 63 %. The IR spectrum confirmed the presence of an acid group with a broad peak at 3027 cm^{-1} representing the alcohol group and a peak representing the carbonyl group at 1707 cm^{-1}. The presence of the carbonyl group was again confirmed by the ^{13}C NMR spectrum with a peak at 179.8 ppm. The characteristic two-hydrogen singlet at 0.77 ppm in the ^{1}H NMR spectrum also confirmed that the cyclopropene ring remained intact.



Scheme 71: Synthesis of free sterculic acid **(74)**. (a) *n*-BuLi; (a') HMPA, $Cl(CH_2)_7I$ **(236)**, 42 %; (b) NaCN, DMSO, 81 %; (c) NaOH, MeOH, H_2O; (c') TBAH, CH_2Cl_2, $KHSO_4$, 63 %.

2.7.4 - Biological Activity

All of the analogues of sterculic acid **(74)** synthesised were sent to Dr Laurent Kremer's group at the Laboratoire de Dynamique des Interactions Membranaires Normales et Pathologiques in the University of Montpellier. Here, the inhibitory activities of these compounds were tested against *P. falciparum* Δ^9 desaturase.[189]

The inhibitory effects of these analogues were tested specifically against *P. falciparum* blood stage parasite using a modified Desjardins test.[243] Serial dilutions of the analogues were prepared on microtitration plates and the parasites were subcultured on these plates. Wells also contained [^3H]-hypoxanthine and the antimalarial activity of the analogues were determined by measuring the [^3H]-hypoxanthine incorporation.

As seen from the results in Figure 12, decreasing the number of carbon atoms in the acid side chain had a large effect on the IC_{50} value. By comparison to methyl sterculate **(81),** the IC_{50} value had nearly doubled for **(206)**. This shows that, although methyl malvalate **(206)** does inhibit Δ^9 desaturase, it does it to a lesser degree than methyl sterculate **(81)**. Increasing the number of carbon atoms had less of an effect, with the IC_{50} value being very similar to that of methyl sterculate **(81)**. The IC_{50} value for **(207)** was slightly lower than for methyl sterculate **(81)** but this difference was within error.

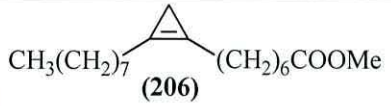
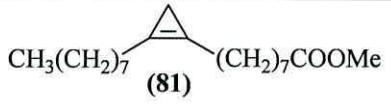
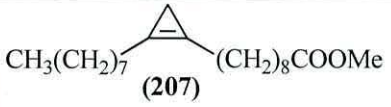
Compound	IC_{50} (μM)
 **(206)**	167 (± 48)
 **(81)**	87 (± 13)
 **(207)**	80 (± 15)

Figure 12: IC_{50} values for methyl sterculate **(81)** and the sterculic acid analogues with varying chain length.

Including functional groups into the structure caused a more profound effect on the IC_{50} values (Figure 13). (±)-8-Methoxy-8-(2-octyl-cycloprop-1-enyl)-octanoic acid methyl ester **(227)** reproducibly showed an IC_{50} value of 41 μM, which is less than half that for methyl sterculate **(81)**. This compound is therefore a much better inhibitor of Δ^9 desaturase. None of the other analogues synthesised showed any inhibitory effect towards Δ^9 desaturase. For acetate **(234)** and silyl **(235)** this may be due to the steric effects of a larger bulkier group. This however cannot be said about alcohol **(231)** as it has a very similar structure to the methoxy analogue **(227)**. As yet, there is no definite explanation as to why the alcohol analogue **(231)** would not inhibit Δ^9 desaturase. Although a better inhibitor had been synthesised the IC_{50} values are still extremely high, compared to other *P. falciparum* inhibitors such as artesunate **(86)** that has an IC_{50} value of 0.05 μM.[244]

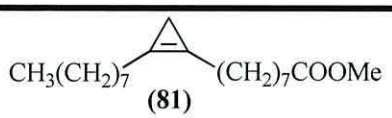
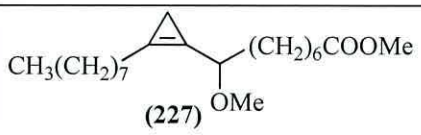
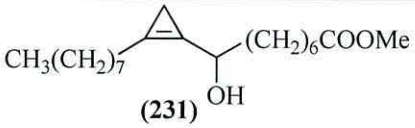
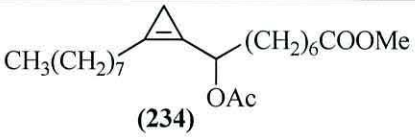
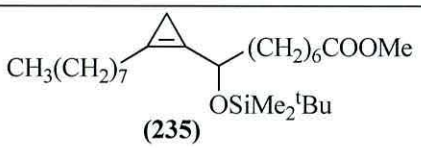
Compound	IC_{50} (μM)
 **(81)**	87 (± 13)
 **(227)**	41 (± 3)
 **(231)**	No Effect
 **(234)**	No Effect
 **(235)**	No Effect

Figure 13: The IC_{50} values of methyl sterculate **(81)** and various analogues against *P. falciparum* Δ^9 desaturase.

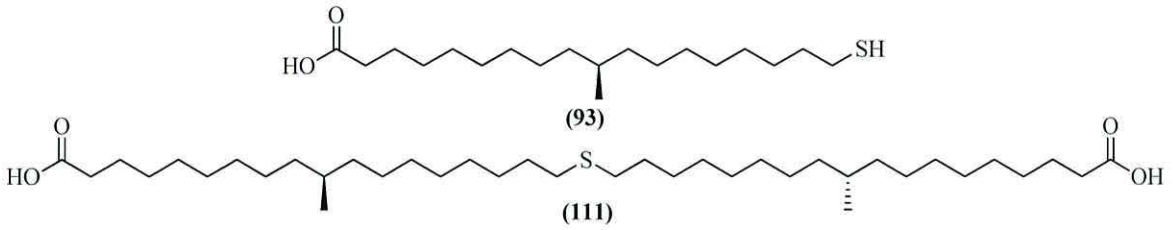
In order to try to further maximise the inhibitory effect against *P. falciparum* Δ^9 desaturase, a single enantiomer of the methoxy analogue **(227)** could be synthesised. It is possible that only one of the enantiomers of **(227)** would show inhibitory properties, which could increase the inhibitory capabilities but also provide evidence for the stereochemistry of the active site.

Another factor that may be investigated to further maximise the inhibitory properties against Δ^9 desaturase would be to include functional groups at the C_{11} position. As nothing is known about the active site it may be that having the functional group on the other side of the ring or on both sides would have more of an effect than having the functional group on only the C_8 position.

3. Conclusions

Using newly developed methods, thiolated components of mycobacteria were synthesised that could be eventually bound to a gold surface and their activity against tuberculosis antibodies tested.

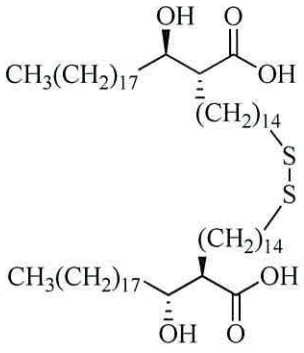
Adapting an existing method for the synthesis of TBSA **(70)**, one of the unusual fatty acid components in Mycobacteria, gave (*S*)-18-mercapto-10-methyloctadecanoic acid **(93)** as well as the thioether, (10*R*,10'*R*)-18,18'-thio*bis*(10-methyloctadecanoic acid) **(111)** in a reasonable yield. Both of which will separately be bound to a gold surface and their activity against tuberculosis antibodies analysed.



(93)

(111)

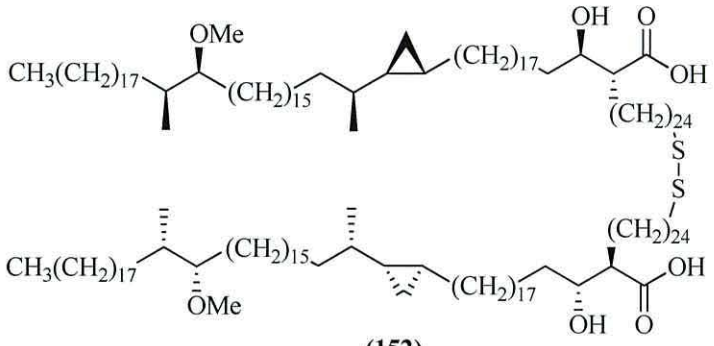
Following numerous explorations, a method was successfully developed to also give a thiolated model of a mycolic acid with the thiol group introduced at the end of the α alkyl chain. This gave the disulfide, (2*R*,2'*R*,3*R*,3'*R*)-2,2'-disulfanediyl*bis*(tetradecane-14,1-diyl))*bis*(3-hydroxyhenicosanoic acid **(149)** as a final product. Although this method did not produce a simple thiol, the formation of the disulfide is as important as it may bind to the gold surface in precisely the same way as a thiol, allowing for the same antibody recognition experiments. This is currently being examined by others.



(149)

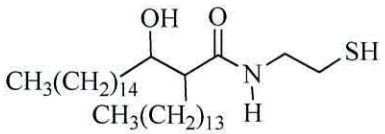
Following the successful synthesis of a simple thiolated mycolic acid, a full thiolated mycolic acid was synthesised by combining and adapting the method developed for the

synthesis of disulfide **(149)** with existing methods for the synthesis of full mycolic acids.[223] The α-methyl-*trans*-cyclopropane containing methoxy mycolic acid was chosen as it had exhibited the best reactivity results during initial ELISA tests.[90] The thiol was again introduced at the end of the α alkyl chain. Rather than producing the free thiol, this method again gave the disulfide, (*S*,*S*,*S*,*R*,*S*,*R*,2*R*,2*R*')-26-26'-disulfanediyl*bis*(2-((*R*)-1-hydroxy-19-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonadecyl)hexacosanoic acid **(152)**. As before, this was not a problem as the disulfide could be attached to the gold surface in the same way as a thiol would be.



(152)

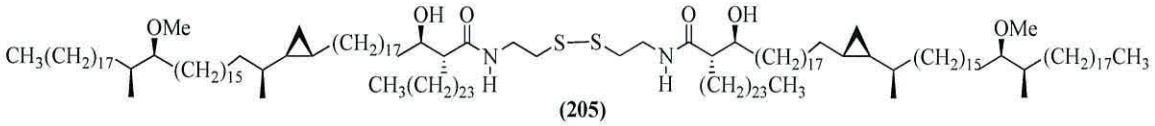
The position of the thiol within the mycobacterial component could make a large difference on the effect of the interaction of the molecule with the antibodies and therefore the thiol was introduced to the Mycobacterial components at an alternative position. This was firstly done with stearic acid **(20)** and hexanoic acid **(190)** where the thiol group was introduced through a thiolated linker on the carboxylic acid. This would allow for the antibody recognition of a straight alkyl chain to be tested as well as allowing for the discovery of the effect of the chain length on reactivity. Following the synthesis of the thiolated linker on the straight alkyl chains, the synthesis was performed on a model of a mycolic acid. Due to the complexity of these molecules the method was slightly adapted to include harsher conditions to form **(194)**.



(194)

Following the successful synthesis of 3-hydroxy-*N*-(2-merccaptoethyl)-2-tetradecyloctadecanamide **(194)**, the thiolated linker was successfully formed on a natural mixture of mycolic acids. It was then included at the acid group of an enaniomericaly pure mycolic acid, (*R*)-2-((*R*)-1-hydroxy-19-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyl-

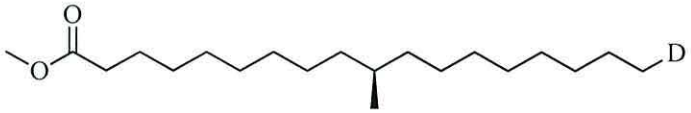
octatriacontan-2-yl)cyclopropyl)nonadecyl)-26-mercaptohexacosanoic acid **(68)** to synthesise the disulfide **(205)**. This structure is the same as the previous thiolated mycolic acid synthesised, which allows a true comparison to be made regarding the effect of the position of the thiol within the structure towards antibody reactivity.



(205)

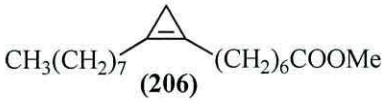
Further work in this area would be to also see the effect of synthesising a thiol at the terminal methyl end of the meromycolate. After comparing the three positions, various other thiolated mycolic acids could then be synthesised and their activities tested. Furthermore, a thiol group could also be attached to other Mycobacterial componenets such as cord factors. Work is currently being carried out to establish if these compounds can be used in the detection of tuberculosis. Initial results are positive but these results are being kept confidential.

Some current methods for the detection of tuberculosis use the identification of TBSA **(70)** by a variety of GC-MS methods. Labelled TBSA **(113)** was subsequently synthesised. This could be used in order to be able to quantify the amount of TBSA **(70)** present, which would lead to the discovery of the extent of disease. Addition of a known amount of **(113)** and measurement of the H/D ratio in the mass ion would then provide direct quantification of the acid.

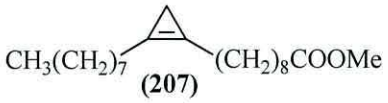


(113)

In order to attempt to maximise the inhibitory effect of sterculic acid on Δ^9-desaturase, analogues of sterculic acid **(74)** were synthesised. Two analogues were produced, 7-(2-octyl-cycloprop-1-enyl)-heptanoic acid methyl ester **(206)** and 9-(2-octyl-cycloprop-1-enyl)-nonanoic acid methyl ester **(207)**, which differed in the length of the carbon chain between the methyl ester and the cyclopropene ring and the inhibitory effect of these compounds against *Plasmodium falciparum* Δ^9-desaturase was tested. It was seen that although both compounds inhibited Δ^9-desaturase, **(206)** was a weaker inhibitor whilst **(207)** showed very similar inhibitory properties to sterculic acid **(74)**.

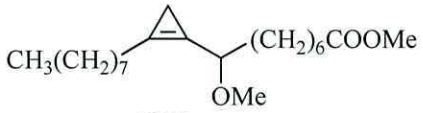


(206)

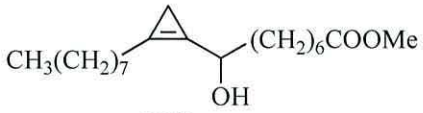


(207)

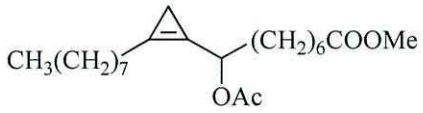
Four analogues were then synthesised that contained the same number of carbon atoms as sterculic acid **(74)** but included various functional groups at the C_8 position. The analogues synthesised were (±)-8-methoxy-8-(2-octyl-cycloprop-1-enyl)-octanoic acid methyl ester **(227)**, (±)-8-hydroxy-8-(2-octylcycloprop-1-enyl)-octanoic acid methyl ester **(231)**, (±)-8-acetoxy-8-(2-octylcycloprop-1-enyl)octanoic acid methyl ester **(234)** and (±)-8-(*tert*-butyldimethylsilyloxy)-8-(2-octylcycloprop-1-enyl)octanoic acid methyl ester **(235).** The inhibitory effects of these compounds against Δ^9-desaturase were also tested. Including the functional groups had a much more profound effect on the inhibitory effect than changing the chain length, as the alcohol **(231)**, the acetate **(234)** and the silyl **(235)** showed no inhibitory effect at all. The acetyl **(234)** and the silyl **(235)** groups are bulky groups that may account for the lack of inhibition but the same cannot be said of the alcohol **(231)** and as of yet there are no firm reasons why the alcohol **(231)** could not inhibit Δ^9 desaturase. On the other hand, the methoxy derivative **(227)** did inhibit Δ^9 desaturase and was twice as effective at doing so than sterculic acid **(74)**. The methoxy must then interact with the enzyme in an additional way to further block the enzyme's active site. As the methoxy derivative **(227)** was a racemic mixture, further work in this area could be to synthesise enantiomerically pure analogues as it may be that one of the enantiomers inhibits Δ^9 desaturase better than the other. Removing the weaker inhibitor should then result in a stronger overall inhibitor. Another area that could be investigated would be to include the functional groups at different positions along the chain, such as the C_{11} or to include different functional groups.



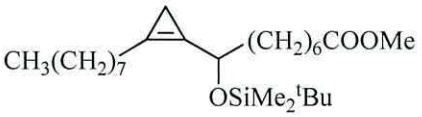
(227)



(231)



(234)



(235)

4. Experimental

4.1 - General Techniques

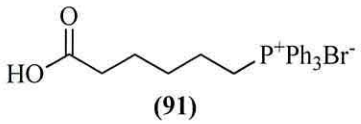
All chemicals and reagents used were purchased from Alfa Aesar, Acros or Sigma-Aldrich. Diethyl ether and THF were dried over sodium wire whilst dichloromethane was dried over calcium hydride or were obtained from an Innovative Technology Pure Solv MD-3 Solvent Purification System. Acetonitrile was dried over phosphorus pentoxide. Other dry solvents were purchased from the commercial suppliers Alfa Aesar, Acros or Sigma-Aldrich. Anhydrous magnesium sulfate was used to dry the organic solutions. For low temperature reactions a cooling bath of IMS and liquid nitrogen was used. Reactions under inert conditions were carried out using nitrogen balloons and all glassware were pre dried in an oven.

Fluorochem silica gel 60 (35-70 μm) from DBH was used for column chromatography and thin layer chromatography was performed on Merck Silica gel 60 F_{254} plates. The TLC components were visualised using UV light, iodine, phosphomolybdic acid solution followed by charring or potassium permanganate solution followed by charring.

A Bruker Advance λ500 NMR spectrometer was used to record both ^{1}H and ^{13}C NMR spectra in $CDCl_3$ unless otherwise stated. The chemical shifts were recorded in parts per million with respect to TMS and J values were reported in Hertz. The IR spectra were recorded on a Bruker Tensor 27 FTIR spectrometer as thin films, nujol mulls or as KBr disks. Melting points were obtained using a Griffin Melting Point Apparatus. Optical rotation values were obtained from a POLAAR 2001 Optical Activity polarimeter or by a Bellingham & Stanley ADP440 Polarimeter. Mass spectra samples were run on a Bruker Microtof by direct infusion and matrix-assisted laser desorption/ionisation mass spectra were run on a Bruker Reflex IV.

4.2 - Experimentals

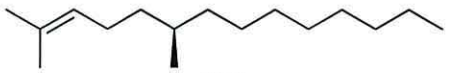
(6-Carboxyhexyl)triphenylphosphonium bromide (91)[149]



(91)

6-Bromohexanoic acid (25.0 g, 0.128 mol) and triphenylphosphine (35.6 g, 0.135 mol) were dissolved in dry acetonitrile (250 ml) under N_2 (g) and the solution was heated under reflux for 72 hours. The solution was then allowed to cool and approximately 80 % of the solvent was removed by rotary evaporation. The resultant oil was stirred briskly and dry ether (250 ml) was added in one portion. The mixture was heated under reflux for 1 hour. Upon cooling, the white suspension was filtered off and washed with dry ether (2 x 50 ml) forming (6-carboxyhexyl)triphenylphosphonium bromide **(91)**[149] (58.1 g, 0.127 mol, 99 %) as a white solid, m.p. 198-200 °C (lit m.p. 201-204 °C). This showed ν_{max}(KBr)/cm^{-1}: 3390 (broad O-H), 2949 (C-H saturated) and 1705 (C=O); δ_H: 1.72 (6H, m, 3 x C*H*$_2$), 2.43 (2H, t, *J* 6.6, CH$_2$C*H*$_2$CO), 3.62 (2H, m, C*H*$_2$) and 7.74 (15H, m, 15 x aromatic C*H*); δ_C: 21.7, 22.8, 23.9, 29.5, 34.3, 117.8 (split into 2 – 118.5), 130.6 (split into 2 – 130.5), 133.7 (split into 2 – 133.6), 135.2 (split into 2 -135.1) and 175.6.

(*R*)-(2,6)-Dimethyl-tetradec-2-ene (89)[122]



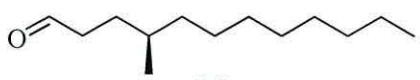
(89)

Magnesium turnings (7.2 g, 0.30 mol) were stirred in dry THF (200 ml) under N_2 (g). A solution of 1-bromohexane **(95)** (37.6 g, 0.23 mol) in dry THF (200 ml) was added slowly whilst heating gently with a heat gun. After all the solution was added the reaction mixture was heated under reflux for 30 minutes.

A stirred solution of (*S*)-citronellyl bromide **(88)** (10.00 g, 45.6 mmol) in dry THF (100 ml) was cooled to -78 °C under N_2 (g). The THF solution of hexyl magnesium bromide was transferred into the same vessel and the resultant mixture was cooled to -78 °C. $LiCuCl_4$ (0.1 M in THF, 8 ml, 0.08 mmol) was added slowly in one portion and the temperature was seen to rise. The solution was left in the cooling bath for 2 days during which it slowly warmed to ambient temperature. The reaction was quenched with a saturated aqueous solution of NH_4Cl (100 ml) and the resultant bright blue solution was extracted with ether (3 x 100 ml). The combined organic phases were washed with brine (100 ml), dried, filtered and evaporated. Flash distillation gave (*R*)-(2,6)-dimethyl-tetradec-2-ene **(89)**[122] (7.64 g, 34.1 mmol, 75 %) as a colourless oil. This showed ν_{max}(film)/cm^{-1}: 2957 (C-H saturated), 2925 (C-H saturated), 2855 (C-H saturated) and 1464; δ_H: 0.89 (3H, d, *J* 6.6, C*H*$_3$CH), 0.91 (3H, t, *J* 7.0, C*H*$_3$CH$_2$), 1.15 (2H, m), 1.29 (14H, m), 1.41 (1H, m, C*H*), 1.63 (3H, s, C*H*$_3$C), 1.71 (3H, s, C*H*$_3$C), 1.98 (2H, m, C*H*$_2$) and 5.13 (1H, br t, *J* 7.2,

1.3, C=C*H*CH_2); δ_C: 14.1, 17.6, 19.6, 22.7, 25.6, 25.7, 27.0, 29.4, 29.7, 30.0, 31.9, 32.4, 37.0, 37.2, 125.1 and 130.9.

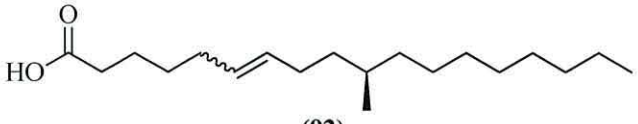
(*R*)-4-Methyldodecanal (90)[122]



(90)

Following a known procedure,[245] a stirred solution of (*R*)-(2,6)-dimethyl-tetradec-2-ene **(89)** (7.46 g, 33.3 mmol) in dichloromethane (200 ml) was cooled to -78 °C prior to treatment with O_3 until a blue colour (liquid O_3) was seen to persist in the cooled solution. N_2 (g) was subsequently bubbled through the solution to remove excess O_3 and avoid danger on warming. Triphenylphosphine (8.74 g, 33.3 mmol) was added to the cold solution, which was warmed to ambient temperature and stirred overnight. The solvent was removed by rotary evaporation. Column chromatography (petrol/ether, 4:1) gave (*R*)-4-methyldodecanal **(90)**[122] (4.09 g, 20.6 mmol, 62 %) as a colourless oil. This showed ν_{max}(film)/cm^{-1}: 2925 (C-H saturated), 2855 (C-H saturated), 1711 (C=O) and 1464; δ_H: 0.90 (6H, m, C*H*$_3$CH and C*H*$_3$CH$_2$), 1.28 (14H, m), 1.45 (2H, m), 1.67 (1H, m, C*H*), 2.42 (2H, m, C*H*$_2$) and 9.79 (1H, t, *J* 1.9, CH$_2$CO*H*); δ_C: 14.1, 19.4, 22.7, 26.9, 28.9, 29.3, 29.6, 29.9, 31.9, 32.4, 36.7, 41.7 and 203.0.

(*R*)-10-Methyloctadec-6-enoic acid (92)[122]

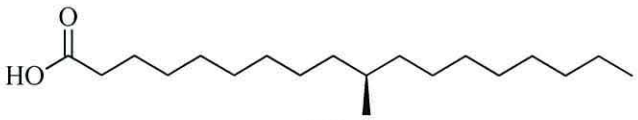


(92)

(6-Carboxyhexyl)triphenylphosphonium bromide **(91)** (13.85 g, 30.3 mmol) was dissolved in dry toluene/dry DMSO (3:1, 150 ml). The solution was cooled to 0 °C and lithium *bis*(trimethylsilyl)amide (1.06 M in THF, 58.4 ml, 61.9 mmol) was slowly added maintaining a temperature of less than 0.3 °C. The resultant bright red orange solution was warmed to ambient temperature over 3 hours and stirred at ambient temperature for a further 1 hour. The solution was cooled to -15 °C and (*R*)-4-methyldodecanal **(90)** (2.84 g, 14.3 mmol) was added as a solution in dry toluene (25 ml); the temperature was seen to rise on this addition. The solution was allowed to slowly return to ambient temperature and stirred overnight. A saturated aqueous solution of NH_4Cl (200 ml) was added and the

mixture was extracted with ethyl acetate (4 x 100 ml). The combined organic phases were washed with brine (100 ml), dried, filtered and evaporated. Column chromatography (petrol/ethyl acetate, 5:2) gave (*R*)-10-methyloctadec-6-enoic acid **(92)**[122] (2.32 g, 7.83 mmol, 55 %) as a colourless oil. This showed ν_{max}(film)/cm^{-1}: 2924 (C-H saturated), 2854 (C-H saturated), 1712 (C=O) and 1462; $\delta_{H:}$ 0.89 (6H, m, C*H*$_3$CH and C*H*$_3$CH$_2$), 1.13 (2H, m), 1.29 (14H, m), 1.43 (3H, m), 1.68 (2H, m, C*H*$_2$), 2.03 (4H, m, 2 x C*H*$_2$), 2.38 (2H, t, *J* 7.6, CH$_2$C*H*$_2$CO) and 5.37 (2H, m, 2 x C*H*); δ_C: 14.1, 19.6, 22.7, 24.3, 24.8, 26.8, 27.0, 29.1, 29.4, 29.7, 30.0, 31.9, 32.4, 34.0, 37.0, 37.1, 128.8, 130.8 and 180.2.

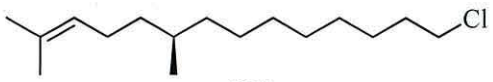
(*R*)-10-Methyloctadecanoic acid (70)[122]



(70)

Palladium on carbon (10 %, 0.5 g) was slowly added under a stream of N_2 (g) to a stirred solution of (*R*)-10-methyloctadec-6-enoic acid **(92)** (2.21 g, 7.47 mmol) in methanol (70 ml). The flask was connected to a hydrogenation apparatus which was purged of any air by repeated application of vacuum followed by refilling the system with H_2 (g). The reaction was monitored by observing the amount of H_2 (g) absorbed by a burette that is part of the apparatus. When the burette reading was steady the reaction was complete. The reaction mixture was then filtered through a pad of Celite®, which was washed with copious methanol. The solvent was removed by rotary evaporation. The crude product was suspended in dichloromethane (50 ml), washed with brine (20 ml), dried, filtered and evaporated. Column chromatography (petrol/ether, 7:3) gave (*R*)-10-methyloctadec-6-enoic acid **(70)**[122] (1.89 g, 6.34 mmol, 85 %) as a colourless oil, $[\alpha]_D^{19.4}$ -0.57 (*c* 1.28, $CHCl_3$) {Found (M + Na)$^+$: 321.2744, $C_{19}H_{38}O_2Na$ requires: 321.2769}. This showed ν_{max}(film)/cm^{-1}: 2955 (C-H saturated), 2925 (C-H saturated), 2854 (C-H saturated), 1711 (C=O) and 1465; δ_H: 0.87 (3H, d, *J* 6.6, C*H*$_3$CH), 0.91 (3H, t, *J* 6.9, C*H*$_3$CH$_2$), 1.29 (25H, m), 1.65 (2H, m) and 2.37 (2H, t, *J* 7.6 CH$_2$C*H*$_2$CO); δ_C: 14.1, 19.7, 22.7, 24.7, 27.0, 27.1, 29.1, 29.2, 29.3, 29.5, 29.7, 29.9, 30.0, 31.9, 32.7, 34.1, 37.0, 37.1 and 180.1.

(*R*)-14-Chloro-2,6-dimethyltetradec-2-ene (96)



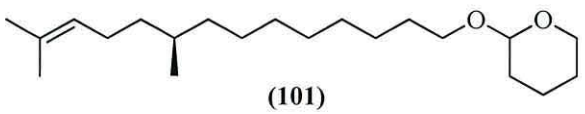
(96)

(i) Magnesium turnings (0.72 g, 0.030 mol) were stirred in dry THF (25 ml) under N_2 (g). 1-Bromo-6-chlorohexane **(94)** (4.58 g, 0.023 mol) in dry THF (25 ml) was added slowly whilst heating gently with a heat gun. After all the solution had been added the reaction mixture was heated under reflux for 30 minutes.

A stirred solution of (*S*)-citronellyl bromide **(88)** (1.00 g, 4.6 mmol) in dry THF (20 ml) was cooled to -78 °C under N_2 (g). The THF solution of chlorohexylmagnesium bromide was transferred into the same vessel and the resultant mixture was cooled to -78 °C. $LiCuCl_4$ (0.1 M in THF, 0.8 ml, 0.08 mmol) was added slowly in one portion and the temperature was seen to rise. The solution was left in the cooling bath for 2 days during which it slowly warmed to ambient temperature. The reaction was quenched with a saturated aqueous solution of NH_4Cl (25 ml) and the resultant bright blue solution was extracted with ether (3 x 25 ml). The combined organic phases were washed with brine (25 ml), dried, filtered and evaporated. Column chromatography (petrol) gave (*R*)-(2,6)-dimethyl-tetradec-2-ene **(89)** (0.80 g, 3.57 mmol, 78 %) as a colourless oil. This showed ν_{max}(film)/cm^{-1}: 2959 (C-H saturated), 2925 (C-H saturated), 2853 (C-H saturated) and 1464; δ_H: 0.88 (3H, d, *J* 6.4, C*H*$_3$CH), 0.90 (3H, t, *J* 7.0, C*H*$_3$CH$_2$), 1.13 (2H, m), 1.27 (14H, m), 1.40 (1H, m, C*H*), 1.62 (3H, s, C*H*$_3$C), 1.70 (3H, s, C*H*$_3$C), 1.96 (2H, m, C*H*$_2$) and 5.13 (1H, br t, *J* 7.1, C=C*H*CH$_2$); δ_C: 14.1, 17.6, 19.6, 22.7, 25.6, 25.7, 27.0, 29.4, 29.7, 30.0, 31.9, 32.4, 37.0, 37.2, 125.1 and 130.9. The second fraction was (*R*)-14-chloro-2,6-dimethyltetradec-2-ene **(96)** (0.02 g, 0.077 mmol, 1.7 %) as a colourless oil. This showed ν_{max}(film)/cm^{-1}: 2927 (C-H saturated), 2851 (C-H saturated) and 1463; δ_H: 0.87 (3H, d, *J* 6.3, C*H*$_3$CH), 1.12 (2H, m), 1.30 (10H, m), 1.43 (3H, m), 1.61 (3H, s, C*H*$_3$C), 1.69 (3H, s, C*H*$_3$C), 1.78 (2H, p, *J* 7.1, CH$_2$C*H*$_2$CH$_2$Cl), 1.98 (2H, m, C*H*$_2$), 3.54 (2H, t, *J* 6.8, CH$_2$C*H*$_2$Cl) and 5.11 (1H, br t, *J* 6.3, C=C*H*CH$_2$); δ_C: 17.6, 19.6, 25.6, 25.7, 26.9, 27.0, 28.9, 29.5, 29.9, 32.4, 32.7, 37.0, 37.2, 45.2, 125.1 and 131.0.

(ii) The reaction was repeated as above to give (*R*)-(2,6)-dimethyl-tetradec-2-ene **(89)** (0.64 g, 2.86 mmol, 62 %) and (*R*)-14-chloro-2,6-dimethyltetradec-2-ene **(96)** (0.06 g, 0.253 mmol, 5.5 %).

2-((*R*)-9,13-Dimethyltetradec-12-enyloxy)tetrahydro-2*H*-pyran (101)

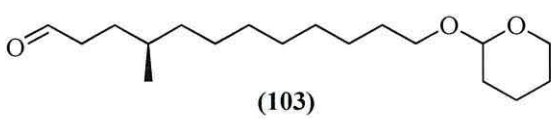


(101)

Magnesium turnings (13.50 g, 0.56 mol) were stirred in dry THF (100 ml) under N_2 (g). 2-(6-Bromohexyloxy)tetrahydro-2*H*-pyran **(107)** (60.95 g, 0.23 mol) in dry THF (100 ml) was added slowly whilst heating gently with a heat gun. After all the solution had been added the reaction mixture was heated under reflux for 30 minutes.

A stirred solution of (*S*)-citronellyl bromide **(88)** (10.00 g, 45.6 mmol) in dry THF (100 ml) was cooled to -78 °C under N_2 (g). The THF solution of (6-(tetrahydro-2*H*-pyran-2-yloxy)hexyl)magnesium bromide was transferred into the same vessel and the resultant mixture was cooled to -78 °C. $LiCuCl_4$ (0.1 M in THF, 8 ml, 0.8 mmol) was added slowly in one portion and the temperature was seen to rise. The solution was left in the cooling bath for 2 days during which it slowly warmed to ambient temperature. The reaction was quenched with a saturated aqueous solution of NH_4Cl (100 ml) and the resultant bright blue solution was extracted with ether (3 x 100 ml). The combined organic phases were washed with brine (100 ml), dried, filtered and evaporated. Flash distillation gave 2-((*R*)-9,13-dimethyltetradec-12-enyloxy)tetrahydro-2*H*-pyran **(101)** (10.28 g, 31.7 mmol, 70 %) as a colourless oil, $[\alpha]_D^{20}$ +1.95 (*c* 1.05, $CHCl_3$) {Found $(M + Na)^+$: 347.2930, $C_{21}H_{40}O_2Na$ requires: 347.2921}. This showed ν_{max}(film)/cm^{-1}: 2925 (C-H saturated), 2854 (C-H saturated) and 1454; δ_H: 0.86 (3H, d, *J* 6.3, CH_3CH), 1.09 (2H, m), 1.29 (13H, m), 1.53 (6H, m), 1.60 (3H s, CH_3C), 1.68 (3H, s, CH_3C), 1.70 (1H, m, C*H*), 1.82 (1H, m, C*H*), 1.96 (2H, m, CH_2), 3.37 (1H, dt, *J* 6.7, 9.3, OC*H*(H)CH_2), 3.48 (1H, m, OC*H*(H)CH_2), 3.73 (1H, dt, *J* 6.9, 9.5, OC*H*(H)CH_2), 3.87 (1H, m, OC*H*(H)CH_2), 4.57 (1H, t, *J* 3.5, O_2CHCH_2) and 5.10 (1H, dt, *J* 1.3, 7.1, C=C*H*CH_2); δ_C: 17.6, 19.6, 19.7, 25.5, 25.6, 25.7, 26.3, 27.0, 29.5, 29.6, 29.8, 30.0, 30.8, 32.4, 37.0, 37.1, 62.3, 67.7, 98.8, 125.1 and 130.9.

(4*R*)-4-Methyl-12-(tetrahydro-2*H*-pyran-2-yloxy)dodecanal (103)

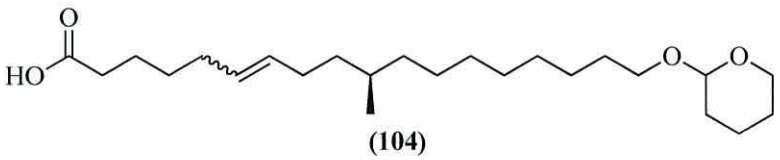


(103)

Following a known procedure,[245] a stirred solution of 2-((*R*)-9,13-dimethyltetradec-12-enyloxy)tetrahydro-2*H*-pyran **(101)** (8.00 g, 24.7 mmol) in dichloromethane (200 ml) was

cooled to -78 °C prior to treatment with O_3 until a blue colour (liquid O_3) was seen to persist in the cooled solution. N_2 (g) was subsequently bubbled through the solution to remove excess O_3 and avoid danger on warming. Triphenylphosphine (6.47 g, 24.7 mmol) was added to the cold solution, which was warmed to ambient temperature and stirred overnight. The solvent was removed by rotary evaporation. Column chromatography (petrol/ether, 2:1) gave (4*R*)-4-methyl-12-(tetrahydro-2*H*-pyran-2-yloxy)dodecanal **(103)** (5.81 g, 19.5 mmol, 80 %) as a colourless oil, $[\alpha]_D^{21}$ +2.17 (*c* 1.02, $CHCl_3$) {Found (M + Na)$^+$: 321.2511, $C_{18}H_{34}O_3Na$ requires: 321.2405}. This showed ν_{max}/cm^{-1}: 2927 (C-H saturated), 2854 (C-H saturated), 1726 (C=O) and 1465; δ_H: 0.87 (3H, d, *J* 6.0, C*H*$_3$CH), 1.13 (1H, m, C*H*), 1.29 (12H, m), 1.42 (2H, m), 1.63 (9H, m), 1.82 (2H, m, C*H*$_2$), 2.41 (2H, m, C*H*$_2$), 3.38 (1H, dt, *J* 6.7, 9.6, OC*H*(H)CH$_2$), 3.49 (1H, m, OC*H*(H)CH$_2$), 3.73 (1H, dt, *J* 7.0, 9.4 OC*H*(H)CH$_2$), 3.87 (1H, m, OC*H*(H)CH$_2$), 4.57 (1H, t, *J* 3.6, O$_2$C*H*CH$_2$) and 9.77 (1H, t, *J* 1.9, CH$_2$CO*H*); δ_C: 19.4, 19.7, 25.5, 26.2, 26.9, 28.9, 29.5, 29.6, 29.7, 29.8, 30.8, 32.4, 36.7, 41.7, 62.4, 67.7, 98.9 and 203.1.

(10*R*)-10-Methyl-18-(tetrahydro-2*H*-pyran-2-yloxy)octadec-6-enoic acid (104)

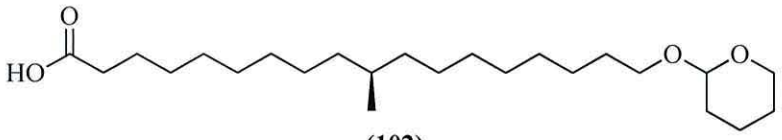


(104)

(5-Carboxyhexyl)triphenylphosphonium bromide **(91)** (18.3 g, 40.2 mmol) was dissolved in dry toluene/dry DMSO (3:1, 200 ml). The solution was cooled to 0 °C and lithium *bis*(trimethylsilyl)amide (1.06 M in THF, 79.4 ml, 84.2 mmol) was slowly added maintaining a temperature of less than 0.3 °C. The resultant bright red orange solution was warmed to ambient temperature over 3 hours and stirred at ambient temperature for a further 1 hour. The solution was cooled to -15 °C and (4*R*)-4-methyl-12-(tetrahydro-2*H*-pyran-2-yloxy)dodecanal **(103)** (5.7 g, 19.1 mmol) was added as a solution in dry toluene (15 ml); the temperature was seen to rise on this addition. The solution was allowed to slowly return to ambient temperature and stirred overnight. A saturated aqueous solution of NH_4Cl (200 ml) was added and the mixture extracted with ethyl acetate (4 x 100 ml). The combined organic phases were washed with brine (100 ml), dried, filtered and evaporated. Column chromatography (petrol/ethyl acetate, 5:2) gave (10*R*)-10-methyl-18-(tetrahydro-2*H*-pyran-2-yloxy)octadec-6-enoic acid **(104)** (3.85 g, 9.72 mmol, 51 %) as a colourless oil, $[\alpha]_D^{25.6}$ +1.70 (*c* 0.57, $CHCl_3$) {Found (M + Na)$^+$; 419.3135, $C_{24}H_{44}O_4Na$ requires:

419.3132}. This showed ν_{max}(film)/cm^{-1} 2926 (C-H saturated), 2854 (C-H saturated), 2735 (broad OH), 1737 (C=O), 1710 (C=O) and 1456; δ_H: 0.86 (3H, d, *J* 6.3 C*H*$_3$CH), 1.14 (2H, m), 1.36 (15H, m), 1.58 (6H, m), 1.66 (2H, m, C*H*$_2$), 1.72 (1H, m, C*H*), 1.83 (1H, m, C*H*), 2.04 (4H, m, 2 x C*H*$_2$), 2.36 (2H, t, *J* 7.4, CH$_2$C*H*$_2$CO), 3.40 (1H, dt, *J* 9.7, 6.7, OC*H*(H)CH$_2$), 3.51 (1H, m, OC*H*(H)CH$_2$), 3.74 (1H, dt, *J* 9.7, 7.0, OC*H*(H)CH$_2$), 3.88 (1H, m, OC*H*(H)CH$_2$), 4.59 (1H, dd, *J* 3.0, 4.3, O$_2$C*H*CH$_2$), 5.35 (2H, m, CH$_2$C*H*=C*H*CH$_2$); δ_C: 19.6, 19.7, 24.4, 24.8, 25.5, 26.2, 26.8, 27.0, 29.1, 29.5, 29.6, 29.7, 29.9, 30.8, 32.3, 33.8, 36.9, 37.0, 62.4, 67.7, 98.9, 128.8, 130.7 and 178.6.

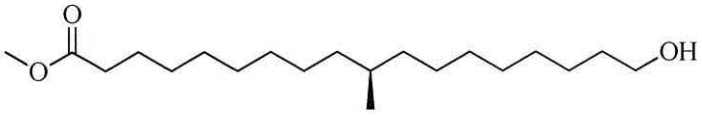
(10*S*)-10-Methyl-18-(tetrahydro-2*H*-pyran-2-yloxy)octadecanoic acid (102)



(102)

Palladium on carbon (10 %, 2.0 g) was slowly added under a stream of N_2 (g) to a stirred solution of (10*R*)-10-methyl-18-(tetrahydro-2*H*-pyran-2-yloxy)octadec-6-enoic acid **(104)** (3.84 g, 9.69 mmol) in methanol (50 ml). The flask was connected to a hydrogenation apparatus which was purged of any air by repeated application of vacuum followed by refilling the system with H_2 (g). The reaction was monitored by observing the amount of H_2 (g) absorbed by a burette that is part of the apparatus. When the burette reading was steady the reaction was complete. The reaction mixture was then filtered through a pad of Celite®, which was washed with copious methanol. The solvent was removed by rotary evaporation. The residue was suspended in dichloromethane (50 ml), washed with brine (20 ml), dried, filtered and evaporated. Column chromatography (petrol/ether, 1:1) gave (10*S*)-10-methyl-18-(tetrahydro-2*H*-pyran-2-yloxy)octadecanoic acid **(102)** (3.14 g, 7.88 mmol, 81 %) as a colourless oil, $[\alpha]_D^{25.9}$ -1.98 (c 0.73, $CHCl_3$) {Found (M + Na)$^+$; 421.3294, $C_{24}H_{46}O_4Na$ requires: 421.3288}. This showed ν_{max}(film)/cm^{-1}: 3159 (broad OH), 2927 (C-H saturated), 2854 (C-H saturated), 1742 (C=O), 1710 (C=O) and 1464; δ_H: 0.84 (3H, d, *J* 6.3, C*H*$_3$CH), 1.07 (2H, m), 1.22 (23H, m), 1.58 (8H, m), 1.71 (1H, m, C*H*), 1.84 (1H, m, C*H*), 2.34 (2H, t, *J* 7.6, CH$_2$C*H*$_2$CO), 3.39 (1H, dt, *J* 6.8, 9.6, OC*H*(H)CH$_2$), 3.51 (1H, m, OC*H*(H)CH$_2$), 3.73 (1H, dt, *J* 6.9, 9.5, OC*H*(H)CH$_2$), 3.88 (1H, m, OC*H*(H)CH$_2$), and 4.59 (1H, t, *J* 3.6, O$_2$C*H*CH$_2$); δ_C: 19.6, 19.7, 24.7, 25.5, 26.2, 27.0, 27.1, 29.1, 29.3, 29.4, 29.5, 29.6, 29.7, 29.9, 30.0, 30.8, 32.7, 34.0, 37.0, 37.1, 62.3, 67.7, 98.8 and 179.2.

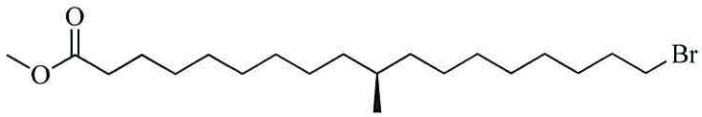
Methyl (*S*)-18-hydroxy-10-methyloctadecanoate (109)



(109)

p-Toluene sulfonic acid monohydrate (372 mg, 1.95 mmol) was added to a stirred solution of (10*S*)-10-methyl-18-(tetrahydro-2*H*-pyran-2-yloxy)octadecanoic acid **(102)** (3.05 g, 7.82 mmol) in THF (25 ml), methanol (100 ml) and water (5 ml) at room temperature. The mixture was heated under reflux for 30 minutes. When TLC showed no starting material remaining a saturated aqueous solution of $NaHCO_3$ (100 ml) and petrol/ethyl acetate (1:1, 100 ml) were added. The layers were separated and the aqueous layer was extracted with petrol/ethyl acetate (1:1, 3 x 100 ml). The combined organic phases were washed with brine (100 ml), dried, filtered and evaporated. Column chromatography (petrol/ether, 2:1) gave methyl (*S*)-18-hydroxy-10-methyloctadecanoate **(109)** (1.87 g, 5.70 mmol, 74 %) as a colourless oil, $[\alpha]_D^{25.8}$ -2.00 (c 0.73, $CHCl_3$) {Found $(M + Na)^+$; 351.2865, $C_{20}H_{40}O_3Na$ requires: 351.2870}. This showed ν_{max}(film)/cm^{-1}: 3356 (broad OH), 2927 (C-H saturated), 2854 (C-H saturated), 1742 (C=O) and 1463; δ_H: 0.83 (3H, d, *J* 6.6, CH_3CH), 1.06 (2H, m), 1.28 (23H, m), 1.59 (4H, m), 2.30 (2H, t, *J* 7.6, CH_2CH_2CO), 3.64 (2H, t, *J* 6.6, CH_2CH_2OH) and 3.66 (3H, s, OCH_3); δ_C: 19.7, 25.0, 25.8, 27.0, 29.2, 29.3, 29.4, 29.5, 29.7, 30.0, 32.7, 32.8, 34.1, 37.1, 51.4, 63.1 and 174.4.

Methyl (*S*)-18-bromo-10-methyloctadecanoate (110)

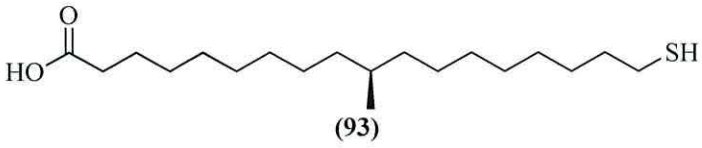


(110)

N-Bromosuccinimide (1.22 g, 6.86 mmol) was added in portions to a stirred solution of methyl (*S*)-18-hydroxy-10-methyloctadecanoate **(109)** (1.50 g, 4.57 mmol) and triphenylphosphine (1.80 g, 6.86 mmol) in dichloromethane (50 ml) at 0 °C. The reaction was stirred at room temperature for 1 hour when there was no starting material remaining. The reaction was quenched with a saturated aqueous solution of $Na_2S_2O_5$ (50 ml). The reaction mixture was separated and the aqueous layer extracted with dichloromethane (2 x 50 ml). The combined organic layers were washed with water, dried, filtered and evaporated to give a residue. This was treated with a mixture of petrol/ethyl acetate (1:1,

50 ml) and heated under reflux for 30 minutes. The solution was filtered, washed with petrol/ethyl acetate (1:1) and the solvent evaporated. Column chromatography (petrol/ethyl acetate, 5:1) gave methyl (*S*)-18-bromo-10-methyloctadecanoate **(110)** (1.56 g, 3.98 mmol, 87 %) as a colourless oil, $[\alpha]_D^{25.8}$ -1.23 (*c* 0.92, $CHCl_3$) {Found $(M + Na)^+$: 413.2005 and 415.1985, $C_{20}H_{39}O_2BrNa$ requires: 413.2031 and 415.2010}. This showed ν_{max}(film)/cm^{-1}: 2926 (C-H saturated), 2854 (C-H saturated), 1742 (C=O) and 1463; δ_H: 0.84 (3H, d, *J* 6.6, CH_3CH), 1.07 (2H, m), 1.29 (21H, m), 1.43 (2H, m), 1.62 (2H, m), 1.86 (2H, p, *J* 7.2, $CH_2CH_2CH_2$), 2.30 (2H, t, *J* 7.6, CH_2CH_2CO), 3.41 (2H, t, *J* 6.8, CH_2CH_2Br) and 3.67 (3H, s, OCH_3); δ_C: 19.7, 25.0, 27.0, 27.1, 28.2, 28.8, 29.2, 29.3, 29.5, 29.5, 29.9, 30.0, 32.7, 32.9, 34.0, 34.1, 37.0, 37.1, 51.4 and 174.5.

(*S*)-18-Mercapto-10-methyloctadecanoic acid (93)

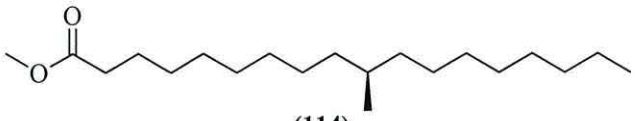


(93)

A solution of methyl (*S*)-18-bromo-10-methyloctadecanoate **(110)** (500 mg, 1.28 mmol) and thiourea (148 mg, 1.94 mmol) in ethanol (10 ml) was heated under reflux for 2.5 hours. The solvent was evaporated and 5 M NaOH (4 ml) was added slowly with stirring and the solution was heated under reflux for another 2 hours. The aqueous solution was cooled in an ice bath and acidified with dilute HCl. The solution was extracted with ether (3 x 15 ml), dried, filtered and evaporated. Column chromatography (petrol/ether, 1:1) gave (*S*)-18-mercapto-10-methyloctadecanoic acid **(93)** (217 mg, 0.66 mmol, 51 %) as a colourless oil, $[\alpha]_D^{19.4}$ -3.27 (*c* 0.53, $CHCl_3$) {Found $(M + Na)^+$: 353.2471, $C_{19}H_{38}O_2SNa$ requires: 353.2485}. This showed ν_{max}(film)/cm^{-1}: 3027 (broad OH), 2924 (C-H saturated), 2853 (C-H saturated), 2674 (S-H), 1708 (C=O) and 1463; δ_H: 0.85 (3H, d, *J* 6.3, CH_3CH), 1.08 (2H, m), 1.29 (23H, m), 1.63 (4H, m), 2.36 (2H, t, *J* 7.4, CH_2CH_2CO) and 2.54 (2H, q, *J* 7.4, CH_2CH_2SH); δ_C: 19.7, 24.6, 24.7, 27.0, 28.4, 29.0, 29.1, 29.3, 29.5, 29.6, 29.9, 32.7, 34.0, 34.1, 37.1 and 180.3. The second fraction was (10*R*,10'*R*)-18,18'-thio*bis*(10-methyloctadecanoic acid) **(111)** (193 mg, 0.31 mmol, 24 %) as a brownish solid, m.p. 42-44 °C, $[\alpha]_D^{20.5}$ -3.19 (*c* 0.94, $CHCl_3$) {Found $(M + Na)^+$: 649.5190, $C_{38}H_{74}O_4SNa$ requires: 649.5205}. This showed ν_{max}(nujol)/cm^{-1}: 3450 (broad OH), 2923 (C-H saturated), 2853 (C-H saturated), 2685 (S-H), 1711 (C=O) and 1462; δ_H: 0.85 (3H, d, *J* 6.7, CH_3CH), 1.08 (2H, m), 1.28 (23H, m), 1.61 (4H, m), 2.36 (2H, t, *J* 7.4, CH_2CH_2CO) and

2.51 (2H, t, *J* 7.4, CH_2CH_2S); δ_C: 19.8, 24.7, 26.9, 27.0, 28.9, 29.0, 29.2, 29.3, 29.4, 29.6, 29.7, 29.8, 29.9, 32.2, 32.7, 34.0, 37.0 and 179.8.

Methyl (*R*)-10-methyloctadecanoate (114)



(114)

A solution of tri-n-butyl tin hydride (0.31 g, 0.29 ml, 1.08 mmol) in dry ether (5 ml) was heated under reflux under N_2 (g). A solution of AIBN (4 mg, 0.018 mmol) and methyl (*S*)-18-bromo-10-methyloctadecanoate **(110)** (50 mg, 0.13 mmol) in dry ether (1 ml) was added and the reaction mixture was heated under reflux for two days. The solvent was then removed by rotary evaporation. Column chromatography (petrol/ethyl acetate, 10:1) gave methyl (*R*)-10-methyloctadecanoate **(114)** (35 mg, 0.10 mmol, 87 %) as a colourless oil {Found $(M + Na)^+$: 335.2911, $C_{20}H_{40}O_2Na$ requires: 335.2926}. This showed ν_{max}(film)/cm^{-1}: 2924 (C-H saturated), 2853 (C-H saturated), 1743 (C=O) and 1457; δ_H: 0.84 (3H, d, *J* 6.6, CH_3CH), 0.89 (3H, t, *J* 6.5, CH_3CH_2), 1.09 (2H, m), 1.27 (24H, m), 1.50 (1H, m, C*H*), 1.63 (2H, m, CH_2), 2.31 (2H, t, *J* 7.6, CH_2CH_2CO) and 3.67 (3H, s, OCH_3); δ_C: 14.1, 19.7, 22.7, 25.0, 17.0, 27.1, 29.2, 29.3, 29.4, 29.5, 29.7, 29.9, 30.0, 31.9, 32.7, 34.1, 37.1, 37.1, 51.4 and 174.3.

Methyl (*R*)-10-methyloctadecanoate deuteride (113)

(113)

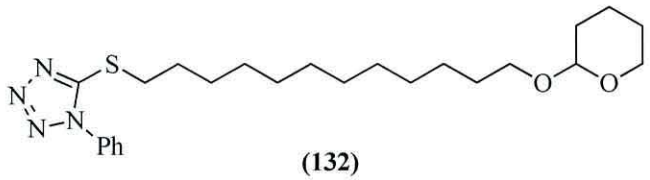
(i) Palladium on carbon (10%, 100 mg) was slowly added under a stream of N_2 (g) to a stirred solution of methyl (*S*)-18-bromo-10-methyloctadecanoate **(110)** (100 mg, 0.256 mmol) in methanol (5 ml). The reaction mixture was stirred overnight whilst being exposed to a balloon filled with D_2 (g). The following day the reaction mixture was filtered through a pad of Celite® which was washed with copious methanol. The solvent was then removed by rotary evaporation to give the starting material, methyl (*S*)-18-bromo-10-methyloctadecanoate **(110)**.

(ii) The reaction was repeated using palladium on carbon (10 %, 100 mg) and methyl (*S*)-18-bromo-10-methyloctadecanoate **(110)** (100 mg, 0.256 mmol) in methanol (5 ml) which were stirred for 5 days whilst attached to a balloon filled with D_2 (g) to give the starting material, methyl (*S*)-18-bromo-10-methyloctadecanoate **(110)**.

(iii) The reaction was repeated using palladium on carbon (10 %, 50 mg) and (*S*)-methyl 18-bromo-10-methyloctadecanoate **(110)** (50 mg, 0.128 mmol) in hexane (5 ml) which were stirred for 5 days whilst attached to a balloon filled with D_2 (g) to give the starting material, methyl (*S*)-18-bromo-10-methyloctadecanoate **(110)**.

(iv) A solution of tri-n-butyl tin deuteride (0.32 g, 0.29 ml, 1.08 mmol) in dry ether (5 ml) was heated under reflux under N_2 (g). A solution of AIBN (4 mg, 0.018 mmol) and methyl (*S*)-18-bromo-10-methyloctadecanoate **(110)** (50 mg, 0.13 mmol) in dry ether (1 ml) was added and the reaction mixture was heated under reflux for two days. The solvent was then removed by rotary evaporation. Column chromatography (petrol/ethyl acetate, 10:1) gave methyl (*R*)-10-methyloctadecanoate deuteride **(113)** (33 mg, 0.10 mmol, 81 %) as a colourless oil, $[\alpha]_D^{20.0}$ -2.38 (*c* 0.86, $CHCl_3$) {Found $(M + Na)^+$: 336.3003, $C_{20}H_{39}O_2DNa$ requires: 336.2989}. This showed ν_{max}(film)/cm^{-1}: 2924 (C-H saturated), 2853 (C-H saturated), 1743 (C=O) and 1463; δ_H: 0.84 (3H, d, *J* 6.6, CH_3CH), 0.87 (2H, m, CH_2DCH_2), 1.07 (2H, m), 1.29 (25H, m), 1.62 (2H, m), 2.31 (2H, t, *J* 7.6, CH_2CH_2CO) and 3.67 (3H, s, OCH_3); δ_C: 13.7 (triplet, 13.8, 13.9), 19.7, 22.6, 24.9, 27.0, 27.1, 29.2, 29.3, 29.4, 29.5, 29.7, 29.9, 30.0, 31.9, 32.7, 34.1, 37.0, 37.1, 51.4 and 174.3.

1-Phenyl-5-(12-(tetrahydro-2*H*-pyran-2-yloxy)dodecylthio)-1*H*-terazole (132)

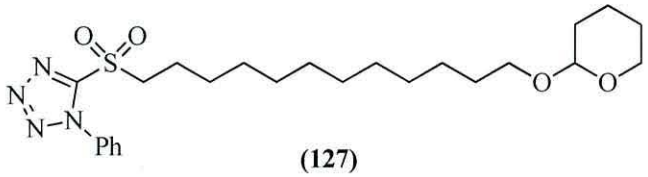


(132)

2-(12-Bromododecyloxy)tetrahydro-2*H*-pyran **(131)** (12.38 g, 35.47 mmol) was added with vigorous stirring to 1-phenyl-1*H*-tetrazole-5-thiol (6.32 g, 35.47 mmol) and anhydrous potassium carbonate (9.80 g, 70.94 mmol) in acetone (150 ml). The mixture was heated under reflux overnight when TLC showed no starting material remaining and the inorganic solids were filtered off and washed with acetone (100 ml). The organic filtrate was evaporated to give a residue to which dichloromethane (150 ml) and water (300 ml) were added. The solution was extracted with dichloromethane (3 x 300 ml) and

the combined organic extracts were dried, filtered and evaporated. Column chromatography (petrol/ethyl acetate, 5:1) gave 1-phenyl-5-(12-(tetrahydro-2*H*-pyran-2-yloxy)dodecylthio)-1*H*-terazole **(132)** (14.26 g, 33.0 mmol, 90 %) as a colourless oil. This showed ν_{max}(film)/cm^{-1}: 2927 (C-H saturated), 2853 (C-H saturated), 1597, 1500 and 1463; δ_H: 1.26 (12H, m), 1.44 (2H, m), 1.57 (8H, m), 1.72 (1H, m), 1.81 (3H, m), 3.39 (3H, m including a t, $CH_2CH(CH)O$ and CH_2CH_2S), 3.50 (1H, m, $CH_2CH(CH)O$), 3.73 (1H, dt, *J* 7.0, 9.5, $CH_2CH(CH)O$), 3.87 (1H, ddd, *J* 3.3, 7.7, 11.0, $CH_2CH(CH)O$), 4.57 (1H, t, *J* 3.5, $OCH(CH_2)O$) and 7.58 (5H, m, 5 x aromatic C*H*); δ_C: 19.7, 25.5, 26.2, 28.6, 29.0, 29.0, 29.1, 29.4, 29.4, 29.5, 29.5, 29.5, 29.6, 29.8, 30.8, 33.4, 62.4, 67.7, 98.9, 123.9, 129.8, 130.1, 133.8 and 154.5.

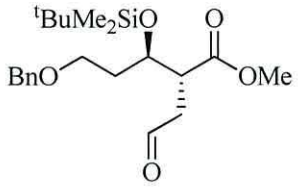
1-Phenyl-5-(12-(tetrahydro-2*H*-pyran-2-yloxy)dodecylsulfonyl)-1*H*-tetrazole (127)



(127)

A solution of ammonium molybdate (VI) tetrahydrate (19.54 g, 0.016 mol) in ice cold hydrogen peroxide (35 %, w/w, 50 ml) was added to a stirred solution of 1-phenyl-5-(12-(tetrahydro-2*H*-pyran-2-yloxy)dodecylthio)-1*H*-terazole **(132)** (14.1 g, 0.032 mol) in THF (200 ml) and IMS (400 ml) at 12 °C. The solution was stirred at 15-20 °C for 2 hours. A further solution of ammonium molybdate (VI) tetrahydrate (7.90 g, 6.4 mmol) in ice cold hydrogen peroxide (35 %, w/w, 20 ml) was added and the reaction mixture was stirred at room temperature for 18 hours. The solution was then poured into water (1 L), extracted with dichloromethane (3 x 250 ml), and the combined organic extracts were dried, filtered and evaporated. Column chromatography (petrol/ethyl acetate, 10:3) gave 1-phenyl-5-(12-(tetrahydro-2*H*-pyran-2-yloxy)dodecylsulfonyl)-1*H*-tetrazole **(127)** (11.60 g, 24.25 mmol, 77 %) as a white solid, m.p. 30-32 °C. This showed ν_{max}(film)/cm^{-1}: 2920 (C-H saturated), 2853 (C-H saturated), 1592, 1497 and 1457; δ_H: 1.29 (14H, m), 1.52 (8H, m), 1.68 (1H, m, C*H*), 1.81 (1H, m, C*H*), 1.92 (2H, m, CH_2), 3.38 (1H, dt, *J* 6.7, 9.6, $CH_2CH(CH)O$), 3.49 (1H, m, $CH_2CH(CH)O$), 3.72 (3H, m, $CH_2CH(CH)O$ and CH_2CH_2S), 3.86 (1H, ddd, *J* 3.4, 7.6, 11.1, $CH_2CH(CH)O$), 4.55 (1H, dd, *J* 3.0, 4.3, $OCH(CH_2)O$), 7.59 (3H, 3 x aromatic C*H*) and 7.67 (2H, m, 2 x aromatic C*H*); δ_C: 19.7, 21.9, 25.5, 26.2, 28.1, 28.9, 29.2, 29.4, 29.5, 29.5, 29.5, 29.7, 30.8, 56.0, 62.3, 67.7, 98.8, 125.1, 129.7, 131.4, 133.1 and 153.5.

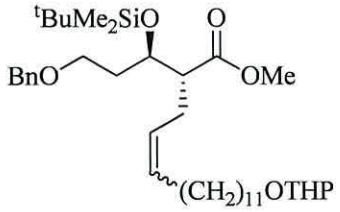
Methyl (2*R*,3*R*)-5-(benzyloxy)-3-(*tert*-butyldimethylsilyloxy)-2-(2-oxoethyl) pentanoate (116)[61]



(116)

2,6-Lutidine (1.64 g, 15.28 mmol, 1.77 ml) and osmium tetroxide 2.5 % in 2-methyl-2-propanol (1.72 ml, 0.14 mmol), followed by $NaIO_4$ (6.52 g, 30.48 mmol) were added to a stirred solution of methyl (*R*)-2-((*R*)-3-(benzyloxy)-1-(*tert*-butyldimethylsilyloxy)-propyl)pent-4-enoate **(37)**[61] (3.0 g, 7.63 mmol) in 1,4-dioxane/water (3:1, 150 ml) at room temperature. After stirring for ~2½ hours at room temperature the reaction mixture was quenched with water (200 ml) and extracted with dichloromethane (1 x 200 ml, 2 x 50 ml). The combined organic layers were washed with brine (150 ml), dried, filtered and evaporated. Column chromatography (petrol/ethyl acetate, 5:2) gave methyl (2*R*,3*R*)-5-(benzyloxy)-3-(*tert*-butyldimethylsilyloxy)-2-(2-oxoethyl) pentanoate **(116)**[61] (2.31 g, 5.86 mmol, 77 %) as a colourless oil. This showed ν_{max}(film)/cm^{-1}: 3031, 2955 (C-H saturated), 2857 (C-H saturated), 1729 (C=O) and 1462; δ_H: 0.07 (3H, s, C*H*$_3$Si), 0.08 (3H, s, C*H*$_3$Si), 0.86 (9H, s, C(C*H*$_3$)$_3$), 1.68 (2H, m, CH$_2$C*H*$_2$CH), 2.71 (1H, dd, *J* 3.3, 18.1, CHC*H*(H)CO), 2.98 (1H, dd, *J* 10.6, 18.1, CHC*H*(H)CO), 3.23 (1H, ddd, *J* 3.5, 7.9, 10.4, CHC*H*(CO)CH$_2$), 3.52 (2H, m, OC*H*$_2$CH$_2$), 3.68 (3H, s, OC*H*$_3$), 4.26 (1H, dt, *J* 4.1, 8.1, CH$_2$C*H*(O)CH), 4.47 (1H, d, *J* 12.0, OC*H*(CH)Ph), 4.50 (1H, d, *J* 11.7, OC*H*(CH)Ph), 7.28-7.36 (5H, m, 5 x aromatic C*H*) and 9.81 (1H, s, CO*H*); δ_C: -4.8, -4.7, 17.9, 19.4, 22.6, 25.7, 33.7, 40.0, 45.3, 52.0, 66.6, 68.8, 72.9, 127.5, 127.6, 128.4, 172.4 and 200.5.

Methyl (2*R*)-2-((*R*)-3-(benzyloxy)-1-(*tert*-butyldimethylsilyloxy)propyl)-16-tetrahydro-2*H*-pyran-2-yloxy)hexadec-4-enoate (117)

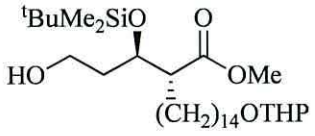


(117)

Lithium *bis*(trimethylsilyl)amide (8.63 ml, 9.15 mmol, 1.06 M) was added to a stirred solution of aldehyde **(116)** (2.31 g, 5.86 mmol) and 1-phenyl-5-(12-((tetrahydro-2*H*-pyran-

2-yloxy)dodecylsulfonyl)-1*H*-tetrazole **(127)** (3.36 g, 7.03 mmol) in dry THF (150 ml) at -10 °C. The solution turned bright yellow/orange and was left to reach room temperature and stirred overnight under N_2 (g). The reaction was quenched by the addition of a saturated aqueous solution of NH_4Cl (150 ml) at -20 °C. The reaction mixture was then extracted with petrol/ethyl acetate (1:2, 3 x 150 ml) and the combined organic layers were dried, filtered and evaporated. Column chromatography (petrol/ethyl acetate, 20:1) gave methyl (2*R*)-2-((*R*)-3-(benzyloxy)-1-(*tert*-butyldimethylsilyloxy)propyl)-16-(tetrahydro-2*H*-pyran-2-yloxy)hexadec-4-enoate **(117)** (2.86 g, 4.42 mmol, 75 %) as a colourless oil, $[\alpha]_D^{21}$ -4.38 (*c* 2.32, $CHCl_3$) {Found $(M + Na)^+$: 669.4522, $C_{38}H_{66}O_6SiNa$ requires 669.4521}. This showed ν_{max}(film)/cm^{-1}: 2927 (C-H saturated), 2854 (C-H saturated), 1737 (C=O), 1638 (C=C), 1495 and 1455; δ_H: 0.04 (6H, s (including another smaller s at 0.05), 2 x C*H*$_3$Si), 0.86 (9H, s (including another smaller s at 0.87), C(C*H*$_3$)$_3$), 1.26 (15H, m), 1.57 (8H, m), 1.72 (1H, m, C*H*), 1.83 (3H, m), 1.94 (1H, m, C*H*), 2.02 (1H, m, C*H*), 2.25 (1H, m, C*H*), 2.61 (1H, m, C*H*), 3.39 (1H, dt, *J* 6.7, 9.6, CH_2CH(CH)O), 3.50 (1H, m, CH_2CH(CH)O), 3.56 (2H, m, CH_2CH_2OBn), 3.64 (3H, s, OC*H*$_3$), 3.74 (1H, dt, *J* 6.9, 9.5, CH_2CH(H)O), 3.88 (1H, ddd, *J* 3.4, 7.6, 11.1, CH_2CH(H)O), 4.11 (1H, m, CH_2CH(O)CH_2), 4.49 (2H, s, C*H*$_2$Ph), 4.58 (1H, dd, *J* 2.7, 4.3, OC*H*(CH_2)O), 5.28 (1H, m, *H*C=CH), 5.41 (1H, m, HC=C*H*) and 7.33 (5H, m, 5 x aromatic C*H*); δ_C: -4.8 (split into 2), -4.6 (split into 2), 18.0, 19.7, 25.5, 25.7, 26.3, 29.1, 29.5, 29.5, 29.6, 29.7, 29.8, 30.4, 30.8, 32.6, 33.7, 33.7, 51.3, 51.4, 52.2, 52.3, 62.3, 63.1, 66.3, 67.7, 70.3, 70.4, 72.9, 98.8, 126.2, 126.8, 127.5, 127.6, 128.3, 131.9, 132.8, 138.5 and 174.0.

Methyl (2*R*)-2-((*R*)-1-(*tert*-butyldimethylsilyloxy)-3-hydroxypropyl)-16-(tetrahydro-2*H*-pyran-yloxy)hexadecanoate (118)

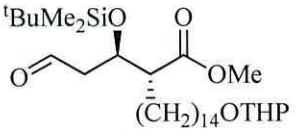


(118)

Palladium on carbon (10 %, 0.5 g) was slowly added under a stream of N_2 (g) to a stirred solution of olefin **(117)** (2.56 g, 3.96 mmol) in IMS (50 ml) and THF (10 ml). The flask was connected to a hydrogenation apparatus which was purged of any air by repeated application of vacuum followed by refilling the system with H_2 (g). The reaction was monitored by observing the amount of H_2 (g) absorbed by a burette that is part of the apparatus. When the burette reading was steady the reaction was complete. The reaction

mixture was filtered through a pad of Celite®, which was washed with copious ethyl acetate and the solvent was removed by rotary evaporation. Column chromatography (petrol/ethyl acetate, 5:1) gave methyl (2*R*)-2-((*R*)-1-(*tert*-butyldimethylsilyloxy)-3-hydroxypropyl)-16-(tetrahydro-2*H*-pyran-yloxy)hexadecanoate **(118)** (1.75 g, 3.13 mmol, 79 %) as a colourless oil, $[\alpha]_D^{23}$ -3.98 (*c* 0.93, $CHCl_3$) {Found $(M + Na)^+$: 581.4215, $C_{31}H_{62}O_6SiNa$ requires 581.4208}. This showed ν_{max}(film)/cm^{-1}: 3465 (broad O-H), 2926 (C-H saturated), 2854 (C-H saturated), 1737 (C=O) and 1463; δ_H: 0.08 (3H, s, C*H*$_3$Si, including a smaller singlet at 0.07), 0.09 (3H, s, C*H*$_3$Si, including a smaller singlet at 0.11), 0.89 (9H, s, C(C*H*$_3$)$_3$, including a smaller singlet at 0.88), 1.26 (20H, m), 1.58 (10.4H, m), 1.70-1.85 (2.7H, m), 1.89-2.02 (1.3H, m), 2.09 (0.6H, m), 2.31 (0.6H, ddd, *J* 3.2, 5.4, 8.5, CHC*H*(CO)CH$_2$), 2.64 (0.4H, ddd, *J* 3.8, 6.9, 10.9, CHC*H*(CO)CH$_2$), 3.39 (1H, dt, *J* 6.7, 9.6, CH$_2$C*H*(CH)O), 3.50 (2H, s, OC*H*$_3$), 3.51 (0.7H, m), 3.68 (1H, s, OC*H*$_3$), 3.74 (1.7H, m), 3.88 (1H, ddd, *J* 3.2, 7.9, 11.4, CH$_2$C*H*(CH)O), 4.14 (0.5H, m), 4.28 (1.2H, m), 4.53 (0.6H, ddd, *J* 4.7, 9.6, 11.2), and 4.58 (0.8H, dd, *J* 2.9, 4.4); δ_C: -5.0, -4.3, 18.0, 19.4, 19.7, 25.5, 25.7, 25.7, 26.3, 26.4, 27.2, 29.4, 29.5, 29.6, 29.6, 29.7, 29.8, 30.5, 30.8, 31.7, 32.8, 47.6, 55.0, 61.9, 62.4, 63.1, 64.7, 65.9, 67.7, 98.9, 99.8 and 173.1.

Methyl (2*R*)-2-((*R*)-1-(*tert*-butyldimethylsilyloxy)-3-oxopropyl)-16-(tetrahydro-2*H*-pyran-2-yloxy)hexadecanoate (119)

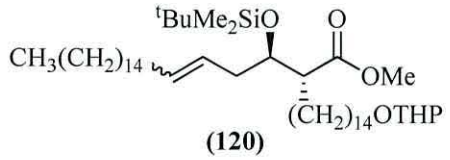


(119)

A solution of alcohol **(118)** (1.73 g, 3.10 mmol) in dichloromethane (10 ml) was added to a stirred suspension of PCC (1.67 g, 7.75 mmol) in dichloromethane (100 ml) by pipette at room temperature. The mixture was stirred at room temperature for 3 hours. When TLC showed that the reaction was complete, ethyl acetate (50 ml) was added and the mixture was filtered through a bed of silica and the solvent was evaporated. Column chromatography (petrol/ethyl acetate, 5:2) gave methyl (2*R*)-2-((*R*)-1-(*tert*-butyldimethylsilyloxy)-3-oxopropyl)-16-(tetrahydro-2*H*-pyran-2-yloxy)hexadecanoate **(119)** (1.41 g, 2.54 mmol, 82 %) as a colourless oil, $[\alpha]_D^{21}$ -7.04 (*c* 0.98, $CHCl_3$). This showed ν_{max}(film)/cm^{-1}: 2927 (C-H saturated), 2855 (C-H saturated), 1735 (C=O) and 1464; δ_H: 0.07 (3H, s, C*H*$_3$Si), 0.08 (3H, s, C*H*$_3$Si), 0.86 (9H, s, C(C*H*$_3$)$_3$), 1.28 (20H, m), 1.56 (10H, m), 1.72 (1H, m, C*H*), 1.83 (1H, m, C*H*), 2.60 (3H, m, COC*H*$_2$CH$_2$ and

CHC*H*CH_2), 3.39 (1H, dt, *J* 6.7, 9.6, OC*H*(H)CH_2), 3.51 (1H, m, OC*H*(H)CH_2), 3.69 (3H, s, C*H*$_3$O), 3.74 (1H, dt, *J* 6.9 and 9.6, OC*H*(H)CH_2), 3.88 (1H, ddd, *J* 3.3, 7.7, 11.2, OC*H*(H)CH_2), 4.43 (1H, apparent dt, CH_2C*H*(O)CH), 4.58 (1H, apparent dd, O(O)C*H*CH_2) and 9.18 (1H, dd, *J* 1.6, 2.5, CH_2CO*H*); δ_C: -4.9, -4.6, 17.9, 19.7, 25.5, 25.6, 26.3, 27.0, 27.8, 29.4, 29.5, 29.6, 29.6, 29.7, 29.8, 30.8, 48.1, 51.6, 52.3, 62.4, 67.7, 68.8, 98.9, 174.1 and 201.3. This aldehyde was used immediately in the next stage of the synthesis.

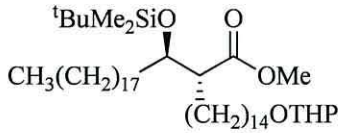
Methyl (2*R*,3*R*)-3-(*tert*-butyldimethylsilyloxy)-2-(14-(tetrahydro-2*H*-pyran-2-yloxy)tetradecyl)heincos-5-enoate (120)



(120)

Lithium *bis*(trimethylsilyl)amide (3.68 ml, 3.90 mmol, 1.06 M) was added to a stirred solution of aldehyde **(119)** (1.39 g, 2.50 mmol) and 5-(hexadecylsulfonyl)-1-phenyl-1*H*-tetrazole **(128)** (1.30 g, 3.00 mmol) in dry THF (25 ml) at -10 °C. The solution turned bright yellow/orange and was left to reach room temperature and stirred overnight under N_2 (g). The reaction was quenched by addition of a saturated aqueous solution of NH_4Cl (100 ml) at -20 °C. The reaction mixture was then extracted with petrol/ethyl acetate (1:2, 3 x 150 ml) and the combined organic layers were dried, filtered and evaporated. Column chromatography (petrol/ethyl acetate, 15:1) gave methyl (2*R*,3*R*)-3- (*tert*-butyldimethylsilyloxy)-2-(14-(tetrahydro-2*H*-pyran-2-yloxy)tetradecyl)heincos-5-enoate **(120)** (1.43 g 1.87 mmol, 75 %) as a colourless oil, $[\alpha]_D^{20}$ -13.8 (*c* 1.54, $CHCl_3$) {Found (M + Na)$^+$: 787.6606, $C_{47}H_{92}O_5SiNa$ requires 787.6606}. This showed ν_{max}(film)/cm^{-1}: 2925 (C-H saturated), 2854 (C-H saturated), 1739 (C=O), 1646 (C=C) and 1464; δ_H: 0.01-0.05 (7H, 4s, 1H-corresponding to a minor impurity, 2 x C*H*$_3$Si), 0.87 (9H, s, (including another smaller s at 0.88), C(C*H*$_3$)$_3$), 0.89 (3H, t, *J* 6.9, C*H*$_3$$CH_2$), 1.26 (46H, m), 1.58 (10H, m), 1.72 (1H, m, C*H*), 1.83 (1H, m, C*H*), 2.00 (2H, m, C*H*$_2$), 2.24 (2H, m, C*H*$_2$), 2.53 (1H, m, CHC*H*CH_2), 3.39 (1H, dt *J* 6.7, 9.6, OC*H*(H)CH_2), 3.50 (1H, m, OC*H*(H)CH_2), 3.66 (3H, s, C*H*$_3$O), 3.74 (1H, dt, *J* 6.9, 9.6, OC*H*(H)CH_2), 3.90 (2H, m, CH_2C*H*(O)CH and OC*H*(H)CH_2), 4.58 (1H, t, *J* 3.5, O(O)C*H*CH_2) and 5.44 (2H, m, HC=CH); δ_C: -5.0, -4.3 (split into 2), 14.1, 17.9, 19.7, 22.7, 25.5, 25.7, 26.2, 27.6, 27.7, 29.2, 29.3, 29.4, 29.5, 29.6, 29.6, 29.7, 29.7, 29.7, 29.7, 29.8, 30.8, 31.9, 32.8, 37.4, 51.2, 51.3, 51.5, 62.3, 67.7, 73.2, 98.8, 124.3, 124.8, 132.2, 133.8 and 175.2.

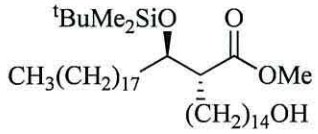
Methyl (2*R*,3*R*)-3-(*tert*-butyldimethylsilyloxy)-2-(14-(tetrahydro-2*H*-pyran-2-yloxy)tetradecyl)henicosanoate (121)



(121)

Palladium on carbon (10 %, 0.3 g) was added to a stirred solution of olefin **(120)** (1.40 g, 1.83 mmol) in IMS (20 ml) and THF (10 ml). The flask was connected to a hydrogenation apparatus which was purged of any air by repeated application of vacuum followed by refilling the system with H_2 (g). The reaction was monitored by observing the amount of H_2 (g) absorbed. When the burette reading was steady the reaction was complete. The reaction mixture was filtered through a pad of Celite®, which was washed with copious ethyl acetate. The solvent was removed by rotary evaporation. Column chromatography (petrol/ethyl acetate, 10:1) gave methyl (2*R*,3*R*)-3-(*tert*-butyldimethylsilyloxy)-2-(14-(tetrahydro-2*H*-pyran-2-yloxy)tetradecyl)henicosanoate **(121)** (1.30 g, 1.69 mmol, 92 %) as a colourless oil, $[\alpha]_D^{21.3}$ -4.15 (*c* 0.92, $CHCl_3$) {Found $(M + Na)^+$: 789.6748, $C_{47}H_{94}O_5SiNa$ requires 789.6763}. This showed ν_{max}(film)/cm^{-1}: 2924 (C-H saturated), 2853 (C-H saturated), 1740 (C=O) and 1464; δ_H: 0.02 (3H, s, C*H*$_3$Si), 0.05 (3H, s, C*H*$_3$Si), 0.87 (9H, s, C(C*H*$_3$)$_3$), 0.88 (3H, t, *J* 6.9, C*H*$_3$CH$_2$), 1.26 (50H, m), 1.57 – 1.83 (16H, m), 2.53 (1H, ddd, *J* 3.7, 7.2, 11.0, CHC*H*CH$_2$), 3.39 (1H, dt, *J* 6.7, 9.6, OC*H*(H)CH$_2$), 3.51 (1H, m, OC*H*(H)CH$_2$), 3.66 (3H, s, C*H*$_3$O), 3.74 (1H, dt, *J* 7.0, 9.6, OC*H*(H)CH$_2$), 3.91 (2H, m, CH$_2$C*H*(O)CH and OC*H*) and 4.58 (1H, dd, *J* 3.0, 4.3, O(O)C*H*CH$_2$); δ_C: -4.9, -4.4, 14.1, 18.0, 19.7, 22.7, 23.7, 25.5, 25.8, 26.3, 27.5, 27.9, 29.4, 29.5, 29.5, 29.6, 29.7, 29.7, 29.8, 29.8, 30.8, 31.9, 32.8, 33.7, 51.2, 51.6, 62.3, 67.7, 73.2, 76.6, 98.9 and 175.2.

Methyl (2*R*,3*R*)-3-(*tert*-butyldimethylsilyloxy)-2-(14-hydroxytetradecyl) henicosanoate (122)

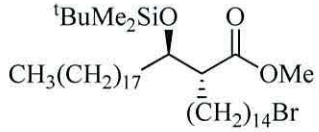


(122)

Pyridinium-*p*-toluene sulfonate (40 mg, 1.58 mmol) was added to a stirred solution of methyl ester **(121)** (485 mg, 0.633 mmol) in THF (15 ml), methanol (3 ml) and water (1 ml) at room temperature. The mixture was stirred at room temperature overnight. When

TLC showed no starting material remaining a saturated aqueous solution of $NaHCO_3$ (10 ml) and petrol/ethyl acetate (1:1, 10 ml) were added. The reaction mixture was then extracted with petrol/ethyl acetate (1:1, 3 x 25 ml) and the combined organic layers were washed with brine (20 ml), dried, filtered and evaporated. Column chromatography (petrol/ethyl acetate, 5:1) gave methyl (2*R*,3*R*)-3-(*tert*-butyldimethylsilyloxy)-2-(14-hydroxytetradecyl) henicosanoate **(122)** (352 mg, 0.516 mmol, 82 %) as a colourless oil, $[\alpha]_D^{22.4}$ -3.74 (*c* 0.95, $CHCl_3$) {Found $(M + Na)^+$: 705.6205, $C_{42}H_{86}O_4SiNa$ requires 705.6188}. This showed ν_{max}(film)/cm^{-1}: 3357 (br O-H), 2924 (C-H saturated), 2853 (C-H saturated), 1739 (C=O) and 1462; δ_H: 0.02 (3H, s, C*H*$_3$Si), 0.05 (3H, s, C*H*$_3$Si), 0.87 (9H, s, C(C*H*$_3$)$_3$), 0.88 (3H, t, *J* 7.1, C*H*$_3$CH$_2$), 1.26 (52H, m), 1.53 (9H, br m), 2.53 (1H, ddd, *J* 3.8, 7.2, 11.0, CHC*H*CH$_2$), 3.65 (2H, t, *J* 6.6, CH$_2$C*H*$_2$OH), 3.66 (3H, s, CH$_3$O) and 3.91 (1H, dt, *J* 4.7, 6.8, CH$_2$C*H*(O)CH); δ_C: -4.9, -4.3, 14.1, 18.0, 22.7, 23.7, 25.7, 25.8, 27.5, 27.9, 29.3, 29.4, 29.6, 29.6, 29.7, 29.7, 29.8, 31.9, 32.8, 33.7, 51.2, 51.6, 63.1, 73.2 and 175.2.

Methyl (2*R*,3*R*)-2-(14-bromotetradecyl)-3-(*tert*-butyldimethylsilyloxy)henicosanoate (123)

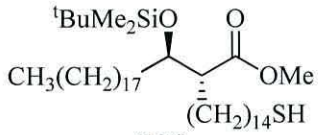


(123)

N-Bromosuccinimide (51 mg, 0.23 mmol) was added in portions to a stirred solution of alcohol **(122)** (129 mg, 0.19 mmol) and triphenylphosphine (74 mg, 0.23 mmol) in dichloromethane (10 ml) at 0 °C. The solution was allowed to warm to room temperature and stirred for 1 hour when no starting material remained. The reaction was then quenched with a saturated aqueous solution of $Na_2S_2O_5$ (20 ml). The layers were separated and the aqueous layer was re-extracted with dichloromethane (2 x 20 ml). The combined organic layers were washed with water (10 ml), dried, filtered and evaporated to give a residue. This was treated with a mixture of petrol/ethyl acetate (1:1, 20 ml) and heated under reflux for 30 minutes. The solution was filtered, washed with petrol/ethyl acetate (1:1) and the solvent evaporated. Column chromatography (petrol/ethyl acetate, 10:1) gave methyl (2*R*,3*R*)-2-(14-bromotetradecyl)-3-(*tert*-butyldimethylsilyloxy)henicosanoate **(123)** (102 mg, 0.137 mmol, 72 %) as a colourless oil, $[\alpha]_D^{20}$ -0.89 (*c* 1.13, $CHCl_3$). This showed ν_{max}(film)/cm^{-1}: 2925 (C-H saturated), 2853 (C-H saturated), 1740 (C=O) and 1463; δ_H:

0.02 (3H, s, SiCH_3), 0.05 (3H, s, SiCH_3), 0.87 (9H, s, C(CH_3)$_3$), 0.88 (3H, t, *J* 6.9, CH_3CH$_2$), 1.26 (54H, m), 1.42 (2H, m, CH_2), 1.54 (2H, m, CH_2), 1.85 (2H, p, *J* 7.2, CH$_2$CH_2CH$_2$), 2.53 (1H, ddd, *J* 3.7, 7.2, 11.0, CHC*H*(CH$_2$)CO), 3.41 (2H, t, *J* 7.0, CH$_2$CH_2Br), 3.66 (3H, s, OCH_3) and 3.91 (1H, dt, *J* 4.7, 6.6, CH$_2$C*H*(O)CH); δ_C: -5.9, -5.4, 13.1, 17.0, 21.7, 22.7, 24.7, 26.5, 26.8, 27.2, 27.8, 28.4, 28.4, 28.5, 28.6, 28.6, 28.6, 28.7, 28.7, 28.8, 30.9, 31.9, 32.7, 32.9, 50.2, 50.6, 72.2 and 174.1. No mass spectrum could be obtained for this compound.

Methyl (2*R*,3*R*)-3-(*tert*-butyldimethylsilyloxy)-2-(14-mercaptotetradecyl)-henicosanoate (124)



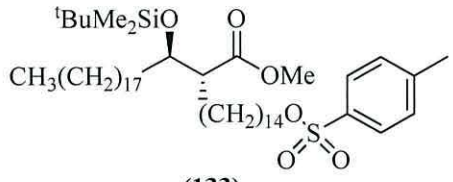
(124)

(i) A solution of thiourea (1.5 mg, 0.020 mmol) and bromide **(123)** (10 mg, 0.013 mmol) in ethanol (1 ml) was heated under reflux for 2½ hours. The solvent was evaporated and 5 M NaOH (1 ml) was added slowly whilst stirring and the reaction mixture was heated under reflux for another 2 hours. The solution was cooled in an ice bath, acidified with dilute HCl and extracted with ethyl acetate (3 x 10 ml). The combined organic layers were dried, filtered and evaporated. The residue of this reaction gave a complicated ^{1}H NMR spectrum which contained numerous spots on TLC.

(ii) The reaction was repeated using thiourea (1.5 mg, 0.020 mmol), bromide **(123)** (10 mg, 0.013 mmol) and ethanol (1 ml) which were heated under reflux overnight. The residue of this reaction gave a complicated ^{1}H NMR spectrum which contained numerous spots on TLC.

(iii) A solution of thiourea (7.5 mg, 0.099 mmol) and bromide **(123)** (10 mg, 0.013 mmol) in ethanol (1 ml) was heated to 80 °C for 16 hours. A 5 M solution of NaOH (1 ml) was added and the reaction mixture was stirred for a further 5 minutes at ambient temperature. The solution was then neutralised with dilute H_2SO_4 and stirring continued for 20 minutes at ambient temperature. The reaction mixture was extracted with dichloromethane (3 x 10 ml) and the combined organic layers were dried, filtered and evaporated. The residue of this reaction gave a complicated ^{1}H NMR spectrum which contained numerous spots on TLC.

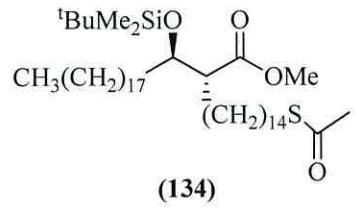
Methyl (2*R*,3*R*)-3-(*tert*-butyldimethylsilyloxy)-2-(14-(tosyloxy)tetradecyl) heicosanoate (133)



(133)

A solution of alcohol **(122)** (256 mg, 0.376 mmol) and triethylamine (1 ml) in dry dichloromethane (10 ml) was cooled to -20 °C under N_2 (g) and stirred for 30 minutes. *p*-Toluene sulfonyl chloride (86 mg, 0.451 mmol) was added in one portion. The solution was kept in the refrigerator overnight and when TLC showed no starting material remaining the solvent was removed by rotary evaporation. Column chromatography (petrol/ethyl acetate, 5:1) gave methyl (2*R*,3*R*)-3-(*tert*-butyldimethylsilyloxy)-2-(14-(tosyloxy)tetradecyl) heicosanoate **(133)** (233 mg, 0.279 mmol, 74 %) as a colourless oil, $[\alpha]_D^{20.7}$ -0.82 (*c* 0.94, $CHCl_3$) {Found $(M + Na)^+$: 859.33, $C_{49}H_{92}O_6SSiNa$ requires: 859.63}. This showed ν_{max}(film)/cm^{-1}: 2925 (C-H saturated), 2854 (C-H saturated), 1739 (C=O), 1598 (C=C aromatic) and 1464; δ_H: 0.02 (3H, s, CH_3Si), 0.05 (3H, s, CH_3Si), 0.87 (9H, s, $C(CH_3)_3$), 0.88 (3H, t, *J* 6.9, CH_3CH_2), 1.26 (54H, m), 1.63 (8H, m), 2.46 (3H, s, CH_3Ph), 2.52 (1H, ddd, *J* 3.7, 7.2, 10.8, $CHCHCH_2$), 3.66 (3H, s, CH_3O), 3.91 (1H, dt, *J* 4.7, 6.9, $CH_2CH(O)CH$), 4.02 (2H, t, *J* 6.5, CH_2CH_2O), 7.36 (2H, d, *J* 7.9, 2 x aromatic CH) and 7.80 (2H, d, *J* 8.5, 2 x aromatic CH); δ_C: -4.9, -4.3, 14.1, 14.2, 18.0, 21.0, 21.6, 22.7, 23.7, 25.3, 25.8, 27.5, 27.9, 28.8, 28.9, 29.4, 29.4, 29.5, 29.5, 29.6, 29.6, 29.6, 29.7, 29.7, 29.8, 31.9, 33.7, 51.2, 51.6, 60.4, 70.7, 73.2, 127.9, 129.8, 133.3, 144.6 and 175.1.

Methyl (2*R*,3*R*)-2-(14-(acetylthio)tetradecyl)-3-(*tert*-butyldimethylsilyloxy) henicosanoate (134)

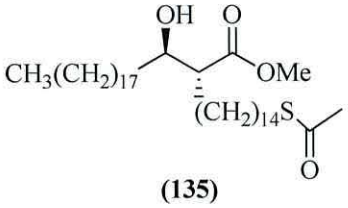


(134)

Tosylate **(133)** (231 mg, 0.276 mmol) and potassium thioacetate (126 mg, 0.111 mmol) in acetone (13 ml) were stirred at room temperature for 4 hours. When TLC showed no starting material remained the solvent was removed. Column chromatography (petrol/ethyl

acetate, 5:1) gave methyl (2*R*,3*R*)-2-(14-(acetylthio)tetradecyl)-3-(*tert*-butyldimethyl-silyloxy)henicosanoate **(134)** (154 mg, 0.208 mmol, 75 %) as a colourless oil, $[\alpha]_D^{24.2}$ -1.73 (*c* 0.47, $CHCl_3$) {Found $(M + Na)^+$: 763.5978, $C_{44}H_{88}O_4SSiNa$ requires 763.6070}. This showed ν_{max}(film)/cm^{-1}: 2926 (C-H saturated), 2847 (C-H saturated), 1737 (C=O), 1695 (C=O) and 1460; δ_H: 0.02 (3H, s, CH_3Si), 0.05 (3H, s, CH_3Si), 0.86 (9H, s, $C(CH_3)_3$), 0.88 (3H, t, *J* 7.0 CH_3CH_2), 1.26-1.55 (60H, m), 2.32 (3H, s, CH_3CO), 2.53 (1H, ddd, *J* 3.8, 7.1, 10.9, $CHCHCH_2$), 2.87 (2H, t, *J* 7.4, CH_2CH_2S), 3.66 (3H, s, CH_3O) and 3.91 (1H, dt, *J* 4.7, 6.9, $CH_2CH(O)CH$); δ_C: -4.9, -4.3, 14.1, 18.0, 22.7, 23.7, 25.8, 27.5, 27.9, 28.8, 29.1, 29.2, 29.4, 29.5, 29.5, 29.6, 29.6, 29.6 (br), 29.8, 30.6, 31.9, 33.7, 51.2, 51.6, 73.2, 175.1, 196.0.

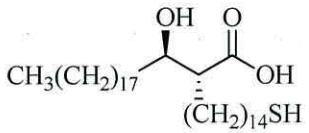
Methyl (2*R*,3*R*)-2-(14-(acetylthio)tetradecyl)-3-hydroxyhenicosanoate (135)



(135)

Thioacetate **(134)** (287 mg, 0.388 mmol) was dissolved in dry THF (6 ml) in a dry polyethylene vial under N_2 (g) at 0 °C. Pyridine (0.2 ml, 2.48 mmol) and HF.pyridine (1.5 ml, mmol) were added and the mixture stirred at 45 °C overnight. When TLC showed no starting material remaining, the mixture was added slowly to a saturated aqueous solution of $NaHCO_3$ (20 ml). The solution was extracted with petrol/ethyl acetate (1:1, 3 x 20 ml) and the combined organic layers were dried, filtered and evaporated. Column chromatography (petrol/ethyl acetate, 10:1) gave methyl (2*R*,3*R*)-2-(14-(acetylthio)tetradecyl)-3-hydroxyhenicosanoate **(135)** (199 mg, 0.325 mmol, 84 %) as a white solid, m.p. 66-68 °C, $[\alpha]_D^{24.9}$ +5.87 (*c* 0.59, $CHCl_3$) {Found $(M + Na)^+$: 649.5224, $C_{38}H_{74}O_4SiNa$ requires 649.5200}. This showed ν_{max}(film)/cm^{-1}: 3407 (br O-H), 2921 (C-H saturated), 2852 (C-H saturated), 1732 (C=O), 1688 (C=O) and 1465; δ_H: 0.88 (3H, t, *J* 6.9 CH_3CH_2), 1.26 (52H, m), 1.45 (4H, m), 1.56 (4H, m), 2.09 (1H, br s, O*H*), 2.32 (3H, s, CH_3CO), 2.44 (1H, dt, *J* 5.3, 9.3, $CHCHCH_2$), 2.86 (2H, t, *J* 7.4, CH_2CH_2S), 3.66 (1H, m, $CH_2CH(O)CH$) and 3.71 (3H, s, CH_3O); δ_C: 14.1, 22.7, 25.7, 27.4, 28.8, 29.1, 29.2, 29.3, 29.4, 29.5, 29.5, 29.6, 29.6, 29.7, 29.7 (br), 30.6, 31.9, 35.7, 51.0, 51.5, 72.3, 176.2 and 196.0.

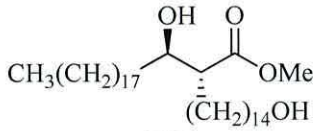
(2*R*,3*R*)-3-Hydroxy-2-(14-mercaptotetradecyl)henicosanoic acid (115)



(115)

(i) Lithium hydroxide (15 eq, 49 mg, 1.17 mmol) was added to ester **(135)** (50 mg, 0.079 mmol) in a mixture of THF (5 ml), methanol (0.5 ml) and water (0.5 ml) and the mixture was stirred at 45 °C overnight. The reaction was diluted by addition of petrol/ethyl acetate (1:1, 10 ml) and brought to pH 1 by dropwise addition of dilute HCl. The product was extracted with petrol/ethyl acetate (5:1, 5 x 15 ml) and the combined organic extracts were dried, filtered and evaporated. The residue of this reaction gave a very complicated ^{1}H NMR spectrum which contained numerous spots by TLC.

Methyl (2*R*,3*R*)-3-hydroxy-2-(14-hydroxytetradecyl)henicosanoate (136)

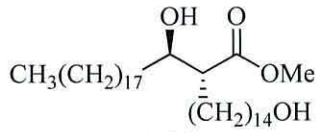


(136)

Methyl ester **(121)** (0.50 g, 0.65 mmol) was dissolved in dry THF (10 ml) in a dry polyethylene vial under N_2 (g) at 0 °C. Pyridine (0.19 g, 0.2 ml, 2.48 mmol) and HF.Pyridine (1.5 ml) were added and the mixture was stirred at 45 °C overnight. When TLC showed that no starting material remained, the mixture was added slowly to a saturated aqueous solution of $NaHCO_3$ (20 ml). The product was extracted with petrol/ethyl acetate (1:1, 2 x 25 ml) and the combined organic extracts were dried, filtered and evaporated. Column chromatography (petrol/ethyl acetate, 5:1) gave methyl (2*R*,3*R*)-3-hydroxy-2-(14-((tetrahydro-2*H*-pyran-2-yl)oxy)tetradecyl)henicosanoate **(139)** (0.12 g, 0.18 mmol, 27 %) as a white solid. This showed ν_{max}(nujol)/cm^{-1}: 3418 (broad OH), 2923 (C-H saturated), 2853 (C-H saturated), 1720 (C=O) and 1465; δ_H: 0.84 (3H, t, *J* 6.8, C*H*$_3$CH$_2$), 1.21 (54H, m), 1.49 (10H, m), 1.67 (2H, m), 1.79 (1H, m, C*H*), 2.40 (1H, dt, *J* 5.3, 9.3, CHC*H*CH$_2$), 3.34 (1H, dt, *J* 6.7, 9.6, OC*H*(H)CH$_2$), 3.46 (1H, m, OC*H*(H)CH$_2$), 3.62 (1H, m, CH$_2$C*H*(OH)C), 3.67 (3H, s, OCH$_3$), 3.68 (1H, m, OC*H*(H)CH$_2$), 3.83 (1H, ddd, *J* 3.4, 7.6, 11.1, OC*H*(H)CH$_2$) and 4.54 (1H, t, *J* 3.6, O(O)C*H*CH$_2$); δ_C: 14.1, 19.7, 22.7, 25.5, 25.7, 26.2, 27.4, 29.3, 29.4, 29.5, 29.5, 29.6, 29.7, 29.8, 30.8, 31.9, 35.7, 51.0, 51.5, 62.3, 67.7, 72.3, 98.8 and 176.2. The second fraction was methyl (2*R*,3*R*)-3-

hydroxy-2-(14-hydroxytetradecyl)henicosanoate **(136)** (0.21 g, 0.38 mmol, 58 %) as white solid {Found (M + Na)$^+$: 591.5371, $C_{36}H_{72}O_4Na$ requires 591.5328}. This showed ν_{max}(nujol)/cm^{-1}: 3387 (br O-H), 2953 (C-H saturated), 2922 (C-H saturated), 2852 (C-H saturated), 1703 (C=O) and 1463; δ_H: 0.87 (3H, t, *J* 6.9, C*H*$_3$CH$_2$), 1.24 (53H, m), 1.44 (3H, m), 1.56 (3H, m), 1.71 (1H, m, C*H*), 2.00 (2H, br s, 2 x O*H*), 2.43 (1H, dt, *J* 5.4, 9.5, CHC*H*(CH$_2$)CO), 3.63 (2H, t, *J* 6.6, CH$_2$C*H*$_2$OH), 3.65 (1H, m, CH$_2$C*H*(OH)C) and 3.70 (3H, s, OC*H*$_3$); δ_C: 14.1, 22.7, 25.7, 25.8, 27.4, 29.4, 29.4, 29.4, 29.5, 29.5, 29.6, 29.6, 29.6, 29.7, 29.7, 31.9, 32.8, 35.7, 51.0, 51.5, 63.0, 72.3 and 176.3.

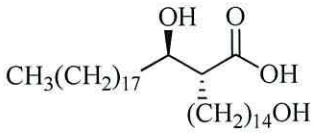
Methyl (2*R*, 3*R*)-3-hydroxy-2-(14-hydroxytetradecyl)henicosanoate (136)



(136)

Pyridinium-*p*-toluene sulfonate (35 mg, 0.443 mmol) was added to a stirred solution of ester **(139)** (116 mg, 0.177 mmol) in THF (1 ml), methanol (4 ml) and water (0.2 ml) at room temperature and the mixture was stirred overnight. When TLC showed no starting material remaining a saturated aqueous solution of $NaHCO_3$ (10 ml) and petrol/ethyl acetate (1:1, 10 ml) were added. The reaction mixture was extracted with petrol/ethyl acetate (1:1, 3 x 25 ml) and the combined organic layers were washed with brine (20 ml), dried, filtered and evaporated. Column chromatography (petrol/ethyl acetate, 5:1) gave (2*R*,3*R*)-methyl 3-hydroxy-2-(14-hydroxytetradecyl)henicosanoate **(136)** (95 mg, 0.167 mmol, 94 %) as a white solid {Found (M + Na)$^+$: 591.5243, $C_{36}H_{72}O_4Na$ requires 591.5328}. This showed ν_{max}(nujol)/cm^{-1}: 3387 (br O-H), 2953 (C-H saturated), 2922 (C-H saturated), 2852 (C-H saturated), 1703 (C=O) and 1463; δ_H: 0.87 (3H, t, *J* 6.9, C*H*$_3$CH$_2$), 1.24 (53H, m), 1.44 (3H, m), 1.56 (3H, m), 1.71 (1H, m, C*H*), 2.00 (2H, br s, 2 x O*H*), 2.43 (1H, dt, *J* 5.4, 9.5, CHC*H*(CH$_2$)CO), 3.63 (2H, t, *J* 6.6, CH$_2$C*H*$_2$OH), 3.65 (1H, m, CH$_2$C*H*(OH)C) and 3.70 (3H, s, OC*H*$_3$); δ_C: 14.1, 22.7, 25.7, 25.8, 27.4, 29.4, 29.4, 29.4, 29.5, 29.5, 29.6, 29.6, 29.6, 29.7, 29.7, 31.9, 32.8, 35.7, 51.0, 51.5, 63.0, 72.3 and 176.3.

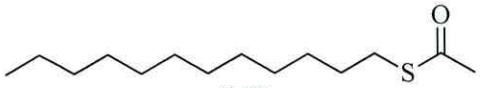
(2*R*,3*R*)-3-Hydroxy-2-(14-hydroxytetradecyl)henicosanoic acid (137)



(137)

Lithium hydroxide (457 mg, 10.88 mmol) was added to a stirred solution of ester **(136)** (309 mg, 0.544 mmol) in a mixture of THF (10 ml), water (1.5 ml) and methanol (1 ml) and the mixture was stirred at 45 °C overnight. The reaction was diluted by addition of petrol/ethyl acetate (1:1, 10 ml) and brought to pH 1 by dropwise addition of dilute HCl. The solution was extracted with petrol/ethyl acetate (5:1, 5 x 15 ml) and the combined organic extracts were dried, filtered and evaporated. Column chromatography (chloroform/methanol, 9.5:0.5) gave (2*R*,3*R*)-3-hydroxy-2-(14-hydroxytetradecyl)-henicosanoic acid **(137)** (214 mg, 0.386 mmol, 71 %) as a white solid, m.p. 91-93 °C {Found $(M + Na)^+$: 577.5107, $C_{35}H_{70}O_4Na$ requires: 577.5171}. This showed ν_{max}(nujol)/cm^{-1}: 3387 (broad OH), 2953 (C-H saturated), 2923 (C-H saturated), 2854 (C-H saturated), 1680 (C=O) and 1465; δ_H: 0.86 (3H, t, *J* 7.1, CH_3CH_2), 1.25-1.47 (53H, m), 1.51-1.63 (8H, m), 2.48 (1H, dt, *J* 5.0, 9.5, $CHCH(CH_2)CO$), 3.67 (2H, t, *J* 6.6, CH_2CH_2OH) and 3.72 (1H, m, $CH_2CH(OH)C$); δ_C(DMSO): 13.8, 22.0, 24.8, 25.5, 27.1, 27.6, 28.6, 28.7, 28.8, 28.9, 29.0, 31.2, 32.5, 33.8, 39.9, 40.1, 40.9, 41.0, 52.0, 60.7, 71.0 and 175.4.

***S*-Dodecyl ethanethioacetate (140)**

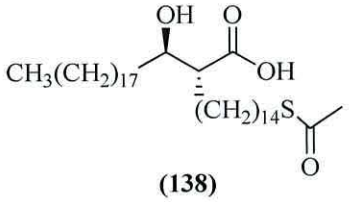


(140)

(i) DEAD (0.21 g, 0.19 ml, 1.2 mmol) was added dropwise to a solution of triphenylphosphine (0.34 g, 1.3 mmol) in dry THF (5 ml) at 0 °C, and the mixture was stirred for 30 minutes. A solution of 1-dodecanol **(141)** (0.186 g, 1.0 mmol), 12-hydroxystearic acid **(142)** (0.306 g, 1.0 mmol) and thioacetic acid (0.076 g, 0.071 ml, 1.0 mmol) in dry THF (30 ml) was then added dropwise. After stirring at 0 °C for a further 1 hour the reaction mixture was allowed to warm to room temperature and the solvent was removed by rotary evaporation. The ^1H NMR spectrum of the residue of this reaction was extremely complicated; however a triplet at 2.84 ppm suggested the formation of a thioacetate. TLC also contained numerous spots.

(ii) The above experiment was repeated using triphenylphosphine (0.53 g, 2.0 mmol) in dry THF (5 ml); DEAD (0.34 g, 0.32 ml, 2.0 mmol), 1-dodecanol **(141)** (0.186 g, 1.0 mmol), 12-hydroxystearic acid **(142)** (0.306 g, 1.0 mmol) and thioacetic acid (0.152 g, 0.142 ml, 1.0 mmol) in dry THF (30 ml). The ^{1}H NMR spectrum of the residue of this reaction was extremely complicated; however a triplet at 2.82 ppm suggested the formation of a thioacetate. TLC also contained numerous spots.

(2*R*,3*R*)-2-(14-(Acetylthio)tetradecyl)-3-hydroxyhenicosanoic acid (138)



(138)

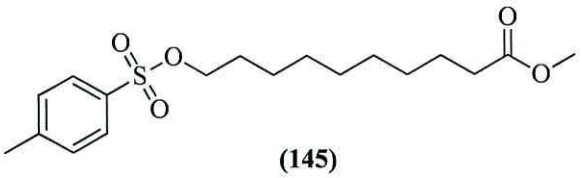
(i) DEAD (23 mg, 21 µl, 0.135 mmol) was added dropwise to a solution of triphenylphosphine (35 mg, 0.135 mmol) in dry THF (4 ml) at 0 °C and the mixture was stirred for 30 minutes. A solution of acid **(137)** (50 mg, 0.09 mmol) and thioacetic acid (10 mg, 97 µl, 0.135 mmol) in dry THF (10 ml) was then added dropwise. After stirring at 0 °C for a further 1 hour the reaction mixture was allowed to warm to room temperature and the solvent removed by rotary evaporation. The ^{1}H NMR spectrum of the residue of this reaction was extremely complicated and the TLC contained numerous spots. A triplet at 2.78 ppm and a singlet at 2.24 ppm could be identified which suggested that the thioacetate had been formed. The residue was stirred in a solution of potassium carbonate (5 ml), chloroform (3 ml) and methanol (0.5 ml). The layers were separated and the aqueous layer was acidified with dilute hydrochloric acid and extracted with chloroform (4 x 5 ml). This gave a very complicated ^{1}H NMR spectrum. Column chromatography (chloroform/methanol, 9.5:0.5) and (petrol/ethyl acetate, 5:2) also resulted in products with very complicated ^{1}H NMR spectra.

(ii) The reaction was repeated using triphenylphosphine (47 mg, 0.18 mmol) in THF (3 ml) and DEAD (31 mg, 28 µl, 0.18 mmol), acid **(137)** (50 mg, 0.09 mmol) and thioacetic acid (14 mg, 13µl, 0.18 mmol) in THF (7 ml). The ^{1}H NMR spectrum of the residue of this reaction was extremely complicated and the TLC contained numerous spots. The residue was stirred in a solution of sodium hydrogen carbonate (6 ml) and dichloromethane (6 ml), at 30 °C for four hours. The layers were separated and the aqueous layer was acidified with dilute hydrochloric acid and extracted with warmed dichloromethane (4 x 5 ml). This gave

a product with a very complicated ^{1}H NMR spectrum. Column chromatography (chloroform/methanol, 9.5:0.5) also resulted in a product with a very complicated ^{1}H NMR spectrum.

(iii) The reaction was repeated using triphenylphosphine (35 mg, 0.135 mmol) in THF (4 ml) and DEAD (23 mg, 21 µl, 0.135 mmol), acid **(137)** (50 mg, 0.09 mmol) and thioacetic acid (10 mg, 97 µl, 0.135 mmol) in THF (7 ml). The ^{1}H NMR spectrum of the residue of this reaction was extremely complicated and the TLC contained numerous spots. Column chromatography (chloroform/methanol, 9.5:0.5 (twice)) and (petrol/ethyl acetate, 5:2 (twice)) gave a relatively clean ^{1}H NMR spectrum with some impurities but only 1 mg of the residue remained.

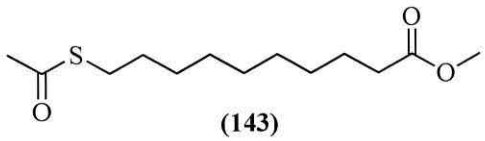
Methyl 10-(tosyloxy)decanoate (145)



(145)

A solution of methyl 10-hydroxydecanoate **(144)** (1.0 g, 4.94 mmol) and triethylamine (7.5 ml) in dry dichloromethane (50 ml) was cooled to -20 °C under N_2 (g) and stirred for 30 minutes. *p*-Toluene sulfonyl chloride (1.13 g, 5.93 mmol) was added in one portion. The solution was kept in the refrigerator overnight and when TLC showed no starting material remaining the solvent was removed by rotary evaporation. Column chromatography (petrol/ethyl acetate, 5:1) gave methyl 10-(tosyloxy)decanoate **(145)** (0.82 g, 2.30 mmol, 47 %) as a colourless oil. This showed ν_{max}(film)/cm^{-1}: 2920 (C-H saturated), 2851 (C-H saturated), 1711 (C=O) and 1464; δ_H: 1.27 (8H, m), 1.61 (6H, m), 2.30 (2H, t, *J* 7.6, CH_2CH_2CO), 2.46 (3H, s, $PhCH_3$), 3.67 (3H, s, OCH_3), 4.02 (2H, t, *J* 6.5, CH_2CH_2O), 7.36 (2H, d, *J* 8.2, 2 x aromatic C*H*) and 7.79 (2H, d, *J* 8.2, 2 x aromatic C*H*); δ_C: 21.6, 24.9, 25.3, 28.8, 28.8, 29.0, 29.1, 29.1, 29.2, 34.1, 51.4, 70.7, 127.9, 129.8, 133.3, 144.6 and 174.3.

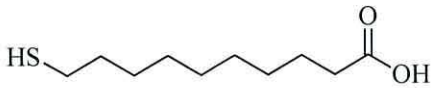
Methyl 10-(acetylthio)decanoate (143)



(143)

Methyl 10-(tosyloxy)decanoate **(145)** (0.80 g, 2.24 mmol) and potassium thioacetate (1.02 g, 8.98 mmol) in acetone (40 ml) were stirred at room temperature for 4 hours. When TLC showed no starting material remaining the solvent was removed. Column chromatography (petrol/ethyl acetate, 5:1) gave methyl 10-(acetylthio)decanoate **(143)** (0.40 g, 1.54 mmol, 69 %) as a brown oil {Found $(M + Na)^+$: 283.1217, $C_{13}H_{24}O_3SNa$ requires 283.1314}. This showed ν_{max}(film)/cm^{-1}: 2926 (C-H saturated), 2854 (C-H saturated), 1737 (C=O), 1693 (C=O) and 1437; δ_H: 1.27 (8H, m), 1.60 (6H, m), 2.30 (2H, t, *J* 7.6, CH_2CH_2CO), 2.32 (3H, s, $COCH_3$), 2.86 (2H, t, *J* 7.3, CH_2CH_2S) and 3.67 (3H, s, OCH_3); δ_C: 24.9, 28.7, 29.0, 29.1, 29.1, 29.2, 29.2, 29.5, 30.6, 34.1, 51.4, 174.3 and 196.0.

10-Mercaptodecanoic acid (146)



(146)

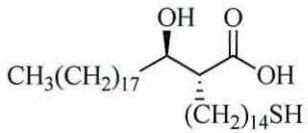
(i) Lithium hydroxide (15 eq, 0.24 g, 5.77 mmol) was added to methyl 10-(acetylthio)decanoate **(143)** (100 mg, 0.384 mmol) in a mixture of THF (10 ml), water (1 ml) and methanol (1 ml) and the mixture was stirred at 45 °C overnight. The reaction was diluted by the addition of petrol/ethyl acetate (1:1, 20 ml) and brought to pH 1 by the dropwise addition of dilute HCl. The product was extracted with petrol/ethyl acetate (5:1, 5 x 25 ml) and the combined organic extracts were dried, filtered and evaporated. The ^1H NMR spectrum of the residue of this reaction was complicated but was believed to contain the disulfide, 10,10'-disulfanediylbis(decanoic acid) **(147)** as a triplet at 2.68 ppm was observed. The residue also showed several different spots by TLC.

(ii) The reaction was repeated using lithium hydroxide (3 eq, 49 mg, 1.15 mmol) and methyl 10-(acetylthio)decanoate **(143)** (100 mg, 0.384 mmol) in THF (10 ml), water (1 ml) and methanol (0.5 ml). This resulted in a complicated ^1H NMR spectrum but, following column chromatography (petrol/ethyl acetate, 5:1), the starting material **(143)** was cleanly isolated. A mixture, which was believed to contain dimethyl 10,10'-disulfanediyl*bis*(decanoate) **(148)** and 10,10'-disulfanediyl*bis*(decanoic acid) **(147)** could not be separated.

(iii) Lithium hydroxide (4 eq, 19 mg, 0.46 mmol) dissolved in water (1.5 ml) was added dropwise to methyl 10-(acetylthio)decanoate **(143)** (30 mg, 0.12 mmol) in isopropanol (3 ml) at 23 °C under N_2 (g). The reaction mixture was stirred at 23 °C for 1 hour. The

reaction mixture was then added to water (50 ml) and the pH adjusted to pH 5 with dilute HCl. The solution was extracted with ethyl acetate (3 x 20 ml) and the combined organic extracts were dried, filtered and evaporated. The residue of this reaction was not purified but the crude ^{1}H NMR spectrum was relatively clean showing significant peaks at 2.35 (t, *J* 7.4, CH_2CH_2CO), 2.53 (q, *J* 7.5, CH_2CH_2SH) and 2.68 (t, *J* 6.7, CH_2CH_2SS) for the thiol and the disulfide. A small singlet only was seen at 3.67 ppm representing the methyl ester.

(2*R*,3*R*)-3-Hydroxy-2-(14-mercaptotetradecyl)henicosanoic acid (115)



(115)

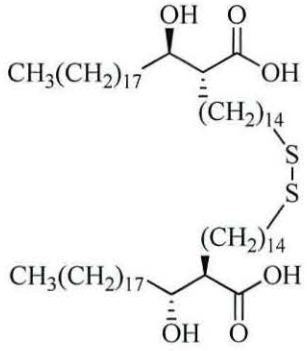
(ii) Lithium hydroxide (4 eq, 8 mg, 0.187 mmol) dissolved in water (1.5 ml) was added dropwise to methyl (2*R*,3*R*)-2-(14-(acetylthio)tetradecyl)-3-hydroxyhenicosanoate **(135)** (30 mg, 0.047 mmol) in isopropanol (3 ml) and THF (0.5 ml) at 40 °C under N_2 (g). The reaction mixture was stirred at 23 °C for 1 hour. The reaction mixture was then added to water (50 ml) and the pH adjusted to pH 5 with dilute HCl. The solution was extracted with ethyl acetate (3 x 20 ml) and the combined organic extracts were dried, filtered and evaporated. This gave the starting material, **(135)**.

(iii) Lithium hydroxide (2 eq, 1.3 mg, 0.031 mmol) was added to methyl (2*R*,3*R*)-2-(14-(acetylthio)tetradecyl)-3-hydroxyhenicosanoate **(135)** (10 mg, 0.016 mmol) in a mixture of THF (2 ml), water (0.2 ml) and methanol (0.2 ml) and the mixture was stirred at 45 °C overnight. The reaction was diluted by the addition of petrol/ethyl acetate (1:1, 10 ml) and brought to pH 1 by the dropwise addition of dilute HCl. The product was extracted with petrol/ethyl acetate (5:1, 5 x 10 ml) and the combined organic extracts were dried, filtered and evaporated. A suspected mixture of (2*R*,2'*R*,3*R*,3'*R*)-2,2'-(disulfanediyl*bis*(tetradecane-14,1-diyl))*bis*(3-hydroxyhenicosanoic acid **(149)** and (2*R*,2'*R*,3*R*,3'*R*)-dimethyl 2,2'-(disulfanediyl*bis*(tetradecane-14,1-diyl))*bis*(3-hydroxyhenicosanoate **(150)** was produced which could not be separated.

(iv) The reaction was repeated using lithium hydroxide (3 eq, 2.0 mg, 0.048 mmol) and methyl (2*R*,3*R*)-2-(14-(acetylthio)tetradecyl)-3-hydroxyhenicosanoate **(135)** (10 mg, 0.016 mmol) in THF/water/methanol (2 ml/0.2 ml/0.2 ml). A mixture of disulfide **(149)** and dimerised methyl ester **(150)** that could not be separated was believed to be produced.

Significant peaks in the crude ^{1}H NMR spectrum included two triplets at 2.69 ppm and a singlet at 3.72 ppm.

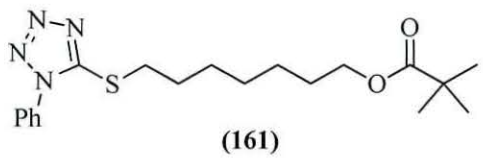
(2*R*,2'*R*,3*R*,3'*R*)-2,2'-Disulfanediyl*bis*(tetradecane-14,1-diyl))*bis*(3-hydroxyhenicosanoic acid (149)



(149)

Lithium hydroxide (4 eq, 10.7 mg, 0.255 mmol) was added to methyl (2*R*,3*R*)-2-(14-(acetylthio)tetradecyl)-3-hydroxyhenicosanoate **(135)** (40 mg, 0.064 mmol) in a mixture of THF (4 ml), water (0.4 ml) and methanol (0.4 ml) and the mixture was stirred at 45 °C overnight. The reaction was diluted by addition of petrol/ethyl acetate (1:1, 10 ml) and brought to pH 1 by dropwise addition of dilute HCl. The product was extracted with petrol/ethyl acetate (1:1, 10 ml) and the combined organic extracts were dried, filtered and evaporated. Column chromatography (petrol/ethyl acetate, 5:2) gave (2*R*,2'*R*,3*R*,3'*R*)-2,2'-disulfanediylbis(tetradecane-14,1-diyl))*bis*(3-hydroxyhenicosanoic acid **(149)** (9.3 mg, 0.016 mmol, 26 %) as a white solid, $[\alpha]_D^{20}$ -0.15 (*c* 0.23, $CHCl_3$) {Found $(M - H)^+$: 1138.3447, $C_{70}H_{137}O_6S_2$ requires 1137.9857}. This showed $\nu_{max}(CHCl_3)/cm^{-1}$: 3451 (broad OH), 2916 (C-H saturated), 2850 (C-H saturated), 1682 (C=O) and 1470; δ_H: 0.89 (3H, t, *J* 6.8, C*H*$_3$CH$_2$), 1.26 (56H, m), 1.52 (3H, m), 1.68 (4H, m), 2.47 (1H, dt, *J* 4.9, 9.6, CHC*H*CH$_2$), 2.70 (2H, t, *J* 7.3, CH$_2$C*H*$_2$S-) and 3.70 (1H, m, CH$_2$C*H*(O)CH); δ_C: 14.1, 22.6, 22.7, 25.7, 27.4, 28.4, 28.9, 29.1, 29.2, 29.4, 29.4, 29.5, 29.6, 29.6, 29.7, 29.7, 31.9, 35.5, 39.5, 51.0, 72.3 and 180.7.

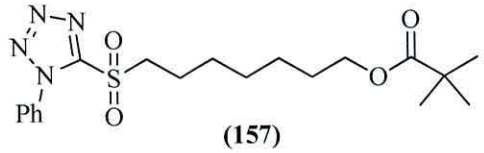
7-(1-Phenyl-1*H*-tetrazole-5-ylthio)heptyl pivalate (161)



(161)

7-Bromoheptyl pivalate **(160)** (30.78 g, 0.110 mol) was added with vigorous stirring to a stirred solution of 1-phenyl-1*H*-tetrazole-5-thiol (19.72 g, 0.110 mmol) and anhydrous potassium carbonate (30.59 g, 0.220 mol) in acetone (250 ml). The reaction was heated under reflux overnight. When TLC showed no starting material was left the inorganic solids were filtered off and washed with acetone (100 ml). The organic filtrate was evaporated to give a residue. Dichloromethane (150 ml) and water (300 ml) were added to the residue. The layers were separated and the aqueous layer was re-extracted with dichloromethane (3 x 100 ml). The combined organic layers were dried, filtered and evaporated. Column chromatography (petrol/ethyl acetate, 10:1) gave 7-(1-phenyl-1*H*-tetrazole-5-ylthio)heptyl pivalate **(161)** (32.28 g, 0.085 mol, 78 %) as a pale yellow oil. This showed ν_{max}(film)/cm^{-1}: 2933 (C-H saturated), 2857 (C-H saturated), 1723 (C=O), 1597, 1499, 1479 and 1460; δ_H: 1.19 (9H, s, C(C*H*$_3$)$_3$), 1.37 (4H, m), 1.47 (2H, m), 1.63 (2H, m), 1.84 (2H, p, *J* 7.4, CH$_2$C*H*$_2$CH$_2$O), 3.40 (2H, t, *J* 7.4, CH$_2$C*H*$_2$S), 4.05 (2H, t, *J* 6.6, CH$_2$C*H*$_2$O) and 7.57 (5H, m, 5 x aromatic C*H*); δ_C: 25.7, 27.2, 28.5, 28.5, 28.6, 29.1, 33.3, 38.7, 64.3, 123.9, 129.8, 130.1, 154.4 and 178.6.

7-(1-Phenyl-1*H*-terazol-5-ylsulfonyl)heptyl pivalate (157)

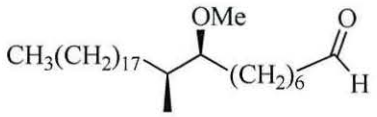


(157)

A solution of ammonium molybdate (VI) tetrahydrate (52.88 g, 0.043 mol) in ice cold hydrogen peroxide (35 %, w/w, 100 ml) was added to a stirred solution of 7-(1-phenyl-1*H*-tetrazole-5-ylthio)heptyl pivalate **(161)** (32.18 g, 0.0856 mol) in IMS (300 ml) at 12 °C and the mixture was stirred at 15-20 °C for 2/3 hours. A further solution of ammonium molybdate (VI) tetrahydrate (21.15 g, 0.017 mol) in ice cold hydrogen peroxide (35 %, w/w, 40 ml) was added and the reaction mixture was stirred at room temperature for 18 hours. The solution was poured into 1 L of water and extracted with dichloromethane (3 x 350 ml). The combined organic layers were dried, filtered and evaporated. Column chromatography (petrol/ethyl acetate, 5:2) gave 7-(1-phenyl-1*H*-terazol-5-ylsulfonyl)heptyl pivalate **(157)** (28.83 g, 0.074 mol, 86 %) as a pale yellow oil. This showed ν_{max}(film)/cm^{-1}: 2935 (C-H saturated), 2863 (C-H saturated), 1720 (C=O), 1497, 1479 and 1461; δ_H: 1.20 (9H, s, C(C*H*$_3$)$_3$), 1.40 (4H, m), 1.53 (2H, m), 1.63 (2H, p, *J* 6.8, CH$_2$C*H*$_2$CH$_2$S), 1.98 (2H, p, *J* 7.8, CH$_2$C*H*$_2$CH$_2$O), 3.75 (2H, distorted t, *J* 8.1, CH$_2$C*H*$_2$S),

4.05 (2H, t, *J* 6.5, CH_2CH_2O), 7.59-7.65 (3H, m, 3 x aromatic C*H*) and 7.69-7.71 (2H, m, 3 x aromatic C*H*); δ_C: 21.9, 25.6, 27.2, 28.0, 28.4, 28.6, 38.7, 56.0, 64.1, 125.1, 129.7, 131.4, 133.1, 153.5 and 178.6.

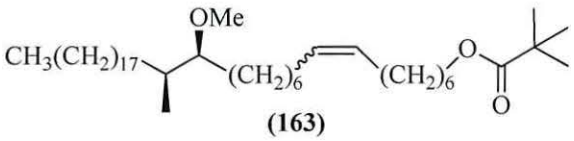
(8*S*,9*S*)-8-Methoxy-9-methylheptacosanal (162)[60]



(162)

(8*S*,9*S*)-8-Methoxy-9-methylheptacosan-1-ol **(155)** (4.0 g, 9.09 mmol) in dichloromethane (50 ml) was added with the aid of a pipette to a stirred suspension of PCC (4.90 g, 22.7 mmol) in dichloromethane (200 ml) at room temperature. The mixture was stirred at room temperature for 3 hours. When TLC showed that the reaction was complete, petrol/ethyl acetate (5:2, 150 ml) was added, the mixture was filtered through a bed of silica and the solvent evaporated. Column chromatography (petrol/ethyl acetate, 20:1) gave (8*S*,9*S*)-8-methoxy-9-methylheptacosanal **(162)**[60] (3.52 g, 8.04 mmol, 88 %) as a colourless oil. This showed ν_{max}(film)/cm^{-1}: 2923 (C-H saturated), 2856 (C-H saturated), 1728 (C=O) and 1462; δ_H: 0.86 (3H, d, *J* 7.0, CH_3CH), 0.89 (3H, t, *J* 7.0, CH_3CH_2), 1.09 (1H, m, C*H*), 1.26 (42H, m), 1.65 (2H, br p, *J* 7.1, $CH_2CH_2CH_2$), 2.43 (2H, dt, *J* 1.9, 7.4, CH_2CH_2COH), 2.95 (1H, m, $CH_2CH(OCH_3)CH_2$), 3.35 (3H, s, OCH_3) and 9.77 (1H, t, *J* 1.9, CH_2COH); δ_C: 14.1, 14.9, 22.1, 22.7, 26.0, 27.6, 29.2, 29.3, 29.6, 29.6, 29.7, 30.0, 30.4, 31.9, 32.3, 35.4, 43.9, 57.7, 85.4 and 202.7.

(15*S*,16*S*)-15-Methoxy-16-metyltetratriacont-7-enyl pivalate (163)[223]

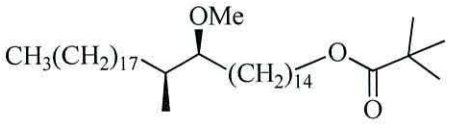


(163)

Lithium *bis*(trimethylsilyl)amide (11.8 ml, 12.46 mmol, 1.06 M) was added to a stirred solution of aldehyde **(162)** (3.50 g, 7.99 mmol) and 7-(1-phenyl-1*H*-terazol-5-ylsulfonyl)heptyl pivalate **(157)** (3.76 g, 9.58 mmol) in dry THF (50 ml) at -10 °C. The solution turned bright yellow/orange and was left to reach room temperature. The reaction mixture was stirred for 1 hour. When TLC showed that no starting material remained the reaction was quenched by addition of a saturated aqueous solution of NH_4Cl (30 ml) at -20 °C. The mixture was extracted with petrol/ethyl acetate (1:2, 3 x 100 ml) and the

combined organic layers were dried, filtered and evaporated. Column chromatography (petrol/ethyl acetate, 15:1) gave (15*S*,16*S*)-15-methoxy-16-metyltetratriacont-7-enyl pivalate **(163)**[223] (3.97 g, 6.40 mmol, 80 %) as a colourless oil, $[\alpha]_D^{23}$ –4.07 (*c* 0.66, $CHCl_3$) {Found (M + Na)$^+$: 643.5991, $C_{41}H_{80}O_3Na$ requires 643.6000}. This showed ν_{max}(film)/cm^{-1}: 2924 (C-H saturated), 2853 (C-H saturated), 1731 (C=O), 1479 and 1462; δ_H: 0.86 (3H, d, *J* 7.0, C*H*$_3$CH), 0.89 (3H, t, *J* 6.9, C*H*$_3$CH$_2$), 1.10 (1H, m, C*H*), 1.20 (9H, s, C(C*H*$_3$)$_3$), 1.27 (46H, m), 1.62 (6H, m), 1.98 (4H, m), 2.96 (1H, m, CH$_2$C*H*(OCH$_3$)CH$_2$), 3.35 (3H, s, OC*H*$_3$), 4.05 (2H, t, *J* 6.6, CH$_2$C*H*$_2$O), 5.37 (2H, m, CH$_2$C*H*=C*H*CH$_2$); δ_C: 14.1, 14.9, 22.7, 25.8, 26.1, 27.2, 27.6, 28.6, 28.7, 29.2, 29.3, 29.5, 29.6, 29.6, 29.7, 29.8, 30.0, 30.5, 31.9, 32.4, 32.5, 32.6, 35.4, 57.7, 64.4, 85.5, 129.6, 130.0, 130.0 and 130.5.

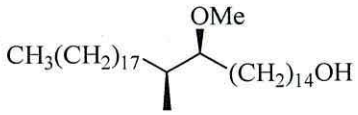
(15*S*,16*S*)-15-Methoxy-16-methyltetratriacontyl pivalate (164)[223]



(164)

Palladium on carbon (10 %, 0.3 g) was added to a stirred solution of olefin **(163)** (3.91 g, 6.31 mmol) in IMS (50 ml) and THF (20 ml). The flask was connected to a hydrogenation apparatus which was purged of any air by repeated application of vacuum followed by refilling the system with H_2 (g). The reaction was monitored by observing the amount of H_2 (g) absorbed by a burette that is part of the apparatus. When the burette reading was steady the reaction was complete. The reaction mixture was added to a solution of petrol/ethyl acetate (1:1, 100 ml) and the mixture was filtered through a pad of Celite® which was washed with copious petrol/ethyl acetate (1:1). The solvent was removed by rotary evaporation. Column chromatography (petrol/ethyl acetate, 1:1) gave (15*S*,16*S*)-15-methoxy-16-methyltetratriacontyl pivalate **(164)**[223] (3.87 g, 6.22 mmol, 98 %) as a colourless oil, $[\alpha]_D^{24}$ –5.78 (*c* 1.77, $CHCl_3$) {Found (M + Na)$^+$: 645.6152, $C_{41}H_{82}O_3Na$ requires 645.6156}. This showed ν_{max}(film)/cm^{-1}: 2924 (C-H saturated), 2854 (C-H saturated), 1731 (C=O) and 1464; δ_H: 0.86 (3H, d, *J* 6.9, C*H*$_3$CH), 0.89 (3H, t, *J* 7.0, C*H*$_3$CH$_2$), 1.11 (1H, m, C*H*), 1.20 (9H, s, C(C*H*$_3$)$_3$), 1.27 (58H, m), 1.62 (2H, br p, *J* 7.3, CH$_2$C*H*$_2$CH$_2$O), 2.96 (1H, m, CH$_2$C*H*(OCH$_3$)CH$_2$), 3.35 (3H, s, OC*H*$_3$) and 4.05 (2H, t, *J* 6.6, CH$_2$C*H*$_2$O); δ_C: 14.1, 14.9, 22.7, 25.9, 26.2, 27.2, 27.6, 28.6, 29.2, 29.4, 29.5, 29.6, 29.7, 29.9, 30.0, 30.5, 31.9, 32.4, 35.3, 38.7, 57.7, 64.5, 85.5 and 178.7.

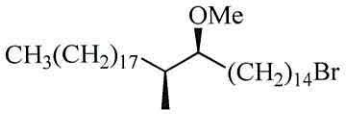
(15*S*,16*S*)-15-Methoxy-16-methyltetratriacontan-1-ol (165)[223]



(165)

Pivalate **(164)** (3.83 g, 6.16 mmol) in THF (10 ml) was added dropwise to a suspension of lithium aluminium hydride (0.35 g, 9.24 mmol) in THF (80 ml) at 0 °C. The mixture was allowed to reach room temperature and then heated under reflux for 1 hour. When TLC showed no starting material remaining the solution was quenched with a saturated aqueous solution of $Na_2SO_4.10H_2O$ at 0 °C until a white precipitate was formed. THF (30 ml) and $MgSO_4$ (20 g) were added and the solution was stirred for 30 min. The solution was filtered through a bed of Celite® and the solvent evaporated. Column chromatography (petrol/ethyl acetate, 5:1) gave (15*S*,16*S*)-15-methoxy-16-methyltetratriacontan-1-ol **(165)**[223] (3.09 g, 5.74 mmol, 93 %) as a white solid, m.p. 32-34 °C (lit m.p. 46-48 °C), $[\alpha]_D^{24}$ –12.6 (*c* 0.62, $CHCl_3$). This showed ν_{max}(nujol)/cm^{-1}: 3383 (br O-H), 2925 (C-H saturated), 2853 (C-H saturated) and 1462; δ_H: 0.86 (3H, d, *J* 7.0, C*H*$_3$CH), 0.88 (3H, t, *J* 7.0, C*H*$_3$CH$_2$), 1.11 (1H, m, C*H*), 1.26 (57H, m), 1.53 (1H, br s, O*H*), 1.57 (2H, p, *J* 7.1, CH$_2$C*H*$_2$CH$_2$O), 1.63 (1H, m, C*H*), 2.96 (1H, m, CH$_2$C*H*(OCH$_3$)CH$_2$), 3.35 (3H, s, C*H*$_3$O) and 3.65 (2H, t, *J* 6.6, CH$_2$C*H*$_2$O); δ_C: 14.1, 14.9, 22.7, 25.7, 26.2, 27.6, 29.4, 29.4, 29.6, 29.7, 29.9, 30.0, 30.5, 31.9, 32.4, 32.8, 35.3, 57.7, 63.1 and 85.5.

(15*S*,16*S*)-1-Bromo-15-methoxy-16-methyltetratriacontane (166)[223]

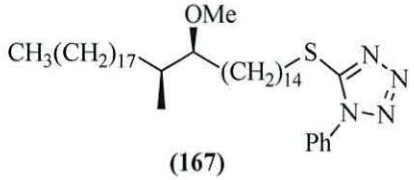


(166)

N-Bromosuccinimide (1.29 g, 7.25 mmol) was added in portions to a stirred solution of alcohol **(165)** (3.0 g, 5.58 mmol) and triphenylphosphine (2.05 g, 7.81 mmol) in dichloromethane (100 ml) at 0 °C. The reaction was stirred at room temperature for 1 hour until there was no starting material remaining. The reaction was then quenched with a saturated aqueous solution of $Na_2S_2O_5$ (100 ml). The organic layer was separated and the aqueous layer was re-extracted with dichloromethane (2 x 70 ml). The combined organic layers were washed with water (30 ml), dried, filtered and evaporated to give a residue. This was treated with a mixture of petrol/ethyl acetate (1:1, 100 ml) and heated under

reflux for 30 minutes. The mixture was filtered and the solvent was removed by rotary evaporator. Column chromatography (petrol) gave (15*S*,16*S*)-1-bromo-15-methoxy-16-methyltetratriacontane **(166)**[223] (3.08 g, 5.11 mmol, 92 %) as a white solid, m.p. 35-37 °C (lit m.p. 38-40 °C), $[\alpha]_D^{24}$ –14.8 (*c* 0.60, $CHCl_3$) {Found (M + Na)$^+$: 623.4713 and 625.4693, $C_{36}H_{73}BrONa$ requires 623.4737 and 625.4722}. This showed ν_{max}(nujol)/cm^{-1}: 2923 (C-H saturated), 2853 (C-H saturated) and 1463; δ_H: 0.86 (3H, d, *J* 7.0, CH_3CH), 0.89 (3H, t, *J* 6.9, CH_3CH_2), 1.09 (1H, m, C*H*), 1.26 (51H, m), 1.41 (6H, m), 1.63 (1H, m, C*H*), 1.86 (2H, p, *J* 7.2, $CH_2CH_2CH_2$), 2.96 (1H, m, $CH_2CH(OCH_3)CH_2$), 3.35 (3H, s, OCH_3), 3.42 (2H, t, *J* 6.8, CH_2CH_2Br); δ_C: 14.1, 14.9, 22.7, 26.2, 27.6, 28.2, 28.8, 29.4, 29.5, 29.6, 29.6, 29.7, 29.9, 30.0, 31.9, 32.4, 32.8, 34.1, 35.3, 57.7 and 85.5.

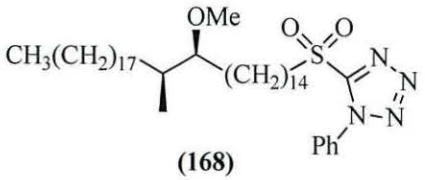
5-((15*S*,16*R*)-15-Methoxy-16-methyltetratriacontylthio)-1-phenyl-1*H*-tetrazole (167)[223]



(167)

Bromide **(166)** (3.05 g, 5.07 mmol) was added with vigorous stirring to 1–phenyl-1*H*-tetrazole-5-thiol (0.90 g, 5.07 mmol) and anhydrous potassium carbonate (1.40 g, 10.1 mmol) in acetone (30 ml). The reaction mixture was heated under reflux overnight. When TLC showed no starting material remaining the inorganic solids were filtered off and washed with acetone (100 ml). The organic filtrate was evaporated to give a residue to which dichloromethane (50 ml) and water (50 ml) were added. The mixture was re-extracted with dichloromethane (3 x 50 ml) and the combined organic layers were dried, filtered and evaporated. Column chromatography (petrol/ethyl acetate, 10:1) gave 5-((15*S*,16*R*)-15-methoxy-16-methyltetratriacontylthio)-1-phenyl-1*H*-tetrazole **(167)**[223] (3.16 g, 4.52 mmol, 89 %) as a white solid, m.p. 25-27 °C, $[\alpha]_D^{18}$ –6.19 (*c* 0.92, $CHCl_3$) {Found (M + Na)$^+$: 721.5784, $C_{43}H_{78}N_4OSNa$ requires 721.5789}. This showed ν_{max}(film)/cm^{-1}: 2922 (C-H saturated), 2853 (C-H saturated) and 1463; δ_H: 0.86 (3H, d, *J* 6.6, CH_3CH), 0.89 (3H, t, *J* 6.9, CH_3CH_2), 1.10 (1H, m, C*H*), 1.26 (51H, m), 1.43 (6H, m), 1.62 (1H, m, C*H*), 1.82 (2H, p, *J* 7.5, $CH_2CH_2CH_2$), 2.96 (1H, m, $CH_2CH(OCH_3)CH_2$), 3.35 (3H, s, CH_3O), 3.40 (2H, t, *J* 7.3, CH_2CH_2S) and 7.58 (5H, m, 5 x aromatic C*H*); δ_C: 14.1, 14.9, 22.7, 26.2, 27.6, 28.6, 29.0, 29.1, 29.3, 29.4, 29.5, 29.6, 29.7, 29.9, 30.0, 30.5, 31.9, 32.4, 33.4, 35.4, 57.7, 85.5, 123.3, 128.6, 129.7, 133.8 and 154.5.

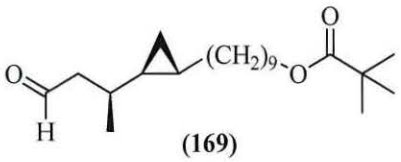
5-((15*S*,16*R*)-15-Methoxy-16-methyltetratriacontylsulfonyl)-1-phenyl-1*H*-tetrazole (168)



(168)

A solution of ammonium molybdate (VI) tetrahydrate (2.67 g, 2.16 mmol) in ice cold hydrogen peroxide (35 %, w/w, 25 ml) was added to a stirred solution of sulfide **(167)** (3.02 g, 4.32 mmol) in IMS (20 ml) and THF (20 ml) at 12 °C. The solution was stirred at 15-20 °C for 2 to 3 hours and a further solution of ammonium molybdate (VI) tetrahydrate (1.06 g, 0.86 mmol) in ice cold hydrogen peroxide (10 ml) was added. The reaction mixture was stirred at room temperature for a further 3 days. The reaction mixture was then poured into water (300 ml) and extracted with dichloromethane (3 x 50 ml). The combined organic layers were dried, filtered and evaporated. Column chromatography (petrol/ethyl acetate, 10:1) gave 5-((15*S*,16*R*)-15-methoxy-16-methyltetratriacontyl-sulfonyl)-1-phenyl-1*H*-tetrazole **(168)**[223] (2.46 g, 3.37 mmol, 78 %) as a white solid, m.p. 43-45 °C (lit m.p. 42-44 °C), $[\alpha]_D^{18}$ –10.5 (*c* 0.59, $CHCl_3$) {Found $(M + Na)^+$: 753.5674, $C_{43}H_{78}N_4O_3SNa$ requires 753.5687}. This showed ν_{max}(film)/cm^{-1}: 2923 (C-H saturated), 2853 (C-H saturated) and 1462; δ_H: 0.86 (3H, d, *J* 7.0, C*H*$_3$CH), 0.89 (3H, t, *J* 6.9, C*H*$_3$CH$_2$), 1.09 (2H, m, C*H*$_2$), 1.26 (54H, m), 1.50 (2H, p, *J* 7.5, CH$_2$C*H*$_2$CH$_2$), 1.63 (1H, m, C*H*), 1.96 (2H, br p, *J* 7.7, CH$_2$C*H*$_2$CH$_2$), 2.96 (1H, m, CH$_2$C*H*(OCH$_3$)CH$_2$), 3.35 (3H, s, C*H*$_3$O), 3.74 (2H, distorted t, *J* 7.9, CH$_2$C*H*$_2$S), 7.63 (3H, m, 3 x aromatic C*H*) and 7.70 (2H, m, 2 x aromatic C*H*); δ_C: 14.1, 14.9, 22.0, 22.7, 26.2, 27.6, 28.1, 28.9, 29.2, 29.4, 29.5, 29.6, 29.6, 29.7, 29.9, 30.0, 30.5, 31.9, 32.4, 35.3, 38.9, 56.0, 57.7, 85.4, 125.1, 129.7 and 131.5.

9-((1*S*,2*R*)-2-((*S*)-4-Oxobutan-2-yl)cyclopropyl)nonyl pivalate (169)[223]

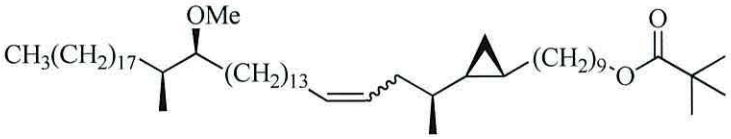


(169)

A solution of 9-((1*S*,1*R*)-2-((*S*)-4-hydroxybutan-2-yl)cyclopropyl)nonyl pivalate **(156)** (1.20 g, 3.53 mmol) in dichloromethane (10 ml) was added with the aid of a pipette to a stirred suspension of PCC (2.28 g, 10.59 mmol) in dichloromethane (70 ml) at room

temperature. The mixture was stirred at room temperature for ~3 hours. When TLC showed that the reaction was complete, petrol/ethyl acetate (10:1, 200 ml) was added and the mixture was filtered through a bed of silica. The solvent was removed by rotary evaporation. Column chromatography (petrol/ethyl acetate, 10:1) gave 9-((1*S*,2*R*)-2-((*S*)-4-oxobutan-2-yl)cyclopropyl)nonyl pivalate **(169)**[223] (1.11 g, 3.28 mmol, 93 %) as a colourless oil. This showed ν_{max}(film)/cm^{-1}: 2923 (C-H saturated), 2876 (C-H saturated), 1724 (C=O) and 1463; δ_H: 0.19-0.36 (3H, m, 3 x cyclopropane C*H*), 0.46-0.52 (1H, m, cyclopropane C*H*), 1.16 (2H, m, CH_2), 1.04 (3H, d, *J* 7.0, CH_3CH), 1.20 (9H, s, C(CH_3)$_3$), 1.27 (14H, m), 1.62 (2H, p, *J* 6.9, $CH_2CH_2CH_2$), 2.36 (1H, ddd, *J* 2.6, 7.6, 15.8, COC*H*(H)CH_2), 2.50 (1H, ddd, *J* 2.5, 6.1, 15.9, COC*H*(H)CH_2), 4.05 (2H, t, *J* 6.8, CH_2CH_2O) and 9.79 (1H, t, *J* 2.4, CH_2CO*H*); δ_C: 11.4, 18.8, 20.0, 25.6, 25.9, 27.2, 28.6, 29.1, 29.2, 29.4, 29.5, 29.5, 29.6, 33.9, 34.1, 38.7, 51.4, 64.5, 178.7 and 202.9.

9-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-Methoxy-20-methyloctatriacont-4-en-2-yl)cyclopropyl)nonyl pivalate (170)[223]

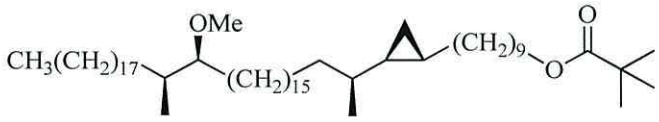


(170)

Lithium *bis*(trimethylsilyl)amide (4.1 ml, 4.33 mmol, 1.06 M) was added to a stirred solution of 9-((1*S*,2*R*)-2-((*S*)-4-oxobutan-2-yl)cyclopropyl)nonyl pivalate **(169)** (1.10 g, 3.25 mmol) and 5-((15*S*,16*R*)-15-methoxy-16-methyltetratriacontylsulfonyl)-1-phenyl-1*H*-tetrazole **(168)** (2.43 g, 3.33 mmol) in dry THF (30 ml) at -10 °C. The solution turned bright yellow/orange and was left to reach room temperature and stirred under N_2 (g) for 1 hour. When TLC showed no starting material remaining the reaction was quenched by addition of a saturated aqueous solution of NH_4Cl (20 ml) at -20 °C. The solution was extracted with petrol/ethyl acetate (1:1, 3 x 20 ml) and the combined organic layers were dried, filtered and evaporated. Column chromatography (petrol/ethyl acetate, 20:1) gave 9-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacont-4-en-2-yl)cyclopropyl)nonyl pivalate **(170)**[223] (1.96 g, 2.33 mmol, 72 %) as a colourless oil, $[\alpha]_D^{19}$ –6.25 (*c* 1.6, $CHCl_3$) {Found (M + Na)$^+$: 865.8424, $C_{57}H_{110}O_3Na$ requires: 865.8353}. This showed ν_{max}(film)/cm^{-1}: 2927 (C-H saturated), 2856 (C-H saturated), 1720 (C=O), 1651 (C=C) and 1465; δ_H: 0.11-0.29 (3H, m, 3 x cyclopropane C*H*), 0.43-0.58 (1H, m, cyclopropane C*H*), 0.74 (1H, m C*H*), 0.86 (3H, d, *J* 7.0, CH_3CH), 0.89 (3H, t, *J* 7.9, CH_3CH_2), 0.90 (3H, d, *J*

6.6, C*H*$_3$CH), 1.29 (80H, m, including a s at 1.20), 1.62 (4H, distorted p, *J* 6.6), 1.98 (3H, m), 2.14 (1H, m, C*H*), 2.96 (1H, m, CH$_2$C*H*(OCH$_3$)CH$_2$), 3.35 (3H, s, OC*H*$_3$), 4.05 (2H, t, *J* 6.6, CH$_2$C*H*$_2$O) and 5.39 (2H, m, CH$_2$C*H*=C*H*CH$_2$); δ_C: 10.8, 14.1, 14.9, 18.6, 19.2, 22.7, 25.7, 25.8, 25.9, 26.2, 27.2, 27.6, 28.6, 29.2, 29.3, 29.4, 29.4, 29.5, 29.6, 29.6, 29.6, 29.7, 29.8, 29.9, 30.0, 30.5, 31.9, 32.4, 32.7, 34.4, 34.7, 35.3, 38.7, 38.8, 40.3, 57.7, 64.5, 85.4, 128.4, 128.8, 130.4, 131.4 and 178.7.

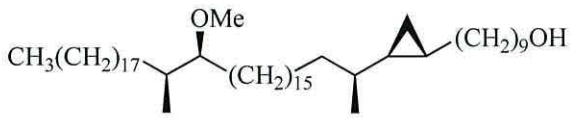
9-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-Methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonyl pivalate (171)[223]



(171)

Dipotassium azodicarboxylate was added in excess to a stirred solution of olefin **(170)** (1.94 g, 2.30 mmol) in dry THF (25 ml) and methanol (7 ml) at 0 °C under N_2 (g). A solution of acetic acid (2 ml) in dry THF (4 ml) was added dropwise in small portions at 0 °C throughout the day. Leaving the mixture overnight resulted in the yellow colour being replaced by a white colour. Further dipotassium azodicarboxylate, followed by more of the acetic acid in THF solution was added at 0 °C the following day. After stirring overnight, again more dipotassiumazodicarboxylate was added, followed by more of the solution of acetic acid in THF. After stirring for 24 hours, the reaction was quenched by adding the reaction mixture in small portions to a saturated aqueous solution of $NaHCO_3$ (20 ml). The product was extracted with petrol/ethyl acetate (5:2, 3 x 30 ml) and the combined organic layers were dried, filtered and evaporated. Column chromatography (petrol/ethyl acetate, 20:1) gave 9-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonyl pivalate **(171)**[223] (1.79 g, 2.19 mmol, 92 %) as a colourless oil, $[\alpha]_D^{24}$ –7.25 (*c* 1.1, $CHCl_3$). This showed ν_{max}(film)/cm^{-1}: 2922 (C-H saturated), 2856 (C-H satuarted), 1720 (C=O) and 1463; δ_H: 0.09-0.21 (3H, m, 3 x cyclopropane C*H*), 0.41-0.48 (1H, m, cyclopropane C*H*), 0.66 (1H, m, C*H*), 0.86 (3H, d, *J* 6.7, C*H*$_3$CH), 0.87 (3H, t, *J* 7.0, C*H*$_3$CH$_2$), 0.90 (3H, d, *J* 6.7, C*H*$_3$CH), 1.20 (9H, s, C(C*H*$_3$)$_3$), 1.26 (72H, m), 1.56 (10H, m), 2.52 (1H, m, C*H*), 2.96 (1H, m, CH$_2$C*H*(OCH$_3$)CH$_2$), 3.35 (3H, s, OC*H*$_3$), 4.05 (2H, t, *J* 6.8, CH$_2$C*H*$_2$O); δ_C: 10.8, 14.1, 14.9, 15.2, 18.6, 19.5, 22.7, 25.8, 25.9, 26.1, 26.2, 27.2, 27.6, 29.3, 29.4, 29.5, 29.6, 29.7, 29.8, 29.9, 30.0, 30.5, 31.9, 32.4, 32.7, 34.7, 35.3, 38.8, 40.3, 57.7, 63.2, 64.5, 85.4 and 178.7.

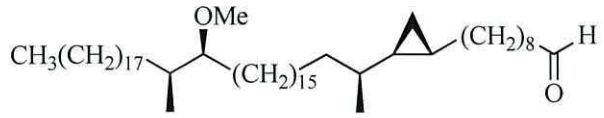
9-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-Methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonan-1-ol (172)[223]



(172)

Pivalate **(171)** (1.77 g, 2.10 mmol) in THF (5 ml) was added dropwise to a stirred suspension of lithium aluminium hydride (0.119 g, 3.15 mmol) in THF (25 ml) at 0 °C. The mixture was allowed to reach room temperature and heated under reflux for 1 hour. When TLC showed no starting material remaining the solution was quenched with a saturated aqueous solution of $Na_2SO_4.10H_2O$ (20 ml) at 0 °C until a white precipitate was formed. THF (50 ml) and $MgSO_4$ (25 g) was added and the solution was stirred for 30 minutes, filtered through a bed of Celite® and the solvent evaporated. Column chromatography (petrol/ethyl acetate, 10:1) gave 9-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonan-1-ol **(172)**[223] (1.39 g, 1.83 mmol, 87 %) as a white solid, m.p. 37-38 °C, $[\alpha]_D^{20}$ –5.89 (*c* 1.17, $CHCl_3$) {Found $(M + Na)^+$: 783.6184, $C_{52}H_{104}O_2Na$ requires: 783.7934}. This showed ν_{max}(film)/cm^{-1}: 3356 (br O-H), 2924 (C-H saturated), 2853 (C-H saturated) and 1461; δ_H: 0.09-0.22 (3H, m, 3 x cyclopropane C*H*), 0.41-0.48 (1H, m, cyclopropane C*H*), 0.66 (1H, m, C*H*), 0.86 (3H, d, *J* 7.0, CH_3CH), 0.89 (3H, t, *J* 6.9, CH_3CH_2), 0.91 (3H, d, *J* 6.6, CH_3CH), 1.26 (74H, m), 1.58 (10H, m), 2.96 (1H, m, $CH_2CH(OCH_3)CH_2$), 3.35 (3H, s, OCH_3) and 3.64 (2H, t, *J* 6.8, CH_2CH_2O); δ_C: 19.5, 22.7, 25.9, 26.2, 27.2, 27.6, 29.3, 29.4, 29.5, 29.6, 29.7, 29.9, 30.0, 30.5, 31.9, 32.4, 32.7, 34.5, 35.4, 37.3, 38.8, 57.7, 63.2, and 85.4.

9-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-Methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonanal (153)[223]

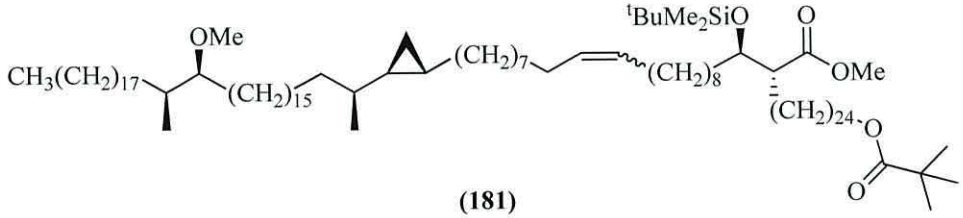


(153)

9-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-Methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonan-1-ol **(172)** (0.60 g, 0.79 mmol) in dichloromethane (5 ml) was added by pipette to a stirred suspension of PCC (0.51 g, 2.37 mmol) in dichloromethane (20 ml) at room temperature. The mixture was stirred at room temperature for 3 hours. When TLC showed that the

reaction was complete, petrol/ethyl acetate (5:1, 60 ml) was added. The mixture was filtered through a bed of silica and the solvent was evaporated. Column chromatography (petrol/ethyl acetate, 20:1) gave 9-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonanal **(153)**[223] (0.51 g, 0.67 mmol, 85 %) as a colourless oil, $[\alpha]_D^{24}$ –5.25 (*c* 1.01, $CHCl_3$). This showed $\nu_{max}(CHCl_3)/cm^{-1}$: 3434 (br O-H), 2923 (C-H saturated), 2853 (C-H saturated), 1728 (C=O), 1638 and 1465; δ_H: 0.09-0.22 (3H, m, 3 x cyclopropane C*H*), 0.41-0.48 (1H, m, cyclopropane C*H*), 0.66 (1H, m, C*H*), 0.86 (3H, d, *J* 6.7, CH_3CH), 0.89 (3H, t, *J* 6.8, CH_3CH_2), 0.91 (3H, d, *J* 6.6, CH_3CH_2), 1.26 (77H, m), 1.64 (4H, m), 2.42 (2H, dt, *J* 1.4, 7.3, CH_2CH_2COH), 2.96 (1H, m, $CH_2CH(OCH_3)CH_2$), 3.35 (3H, s, OCH_3) and 9.77 (1H, br s, CH_2COH); δ_C: 10.5, 14.1, 14.9, 18.6, 19.7, 22.1, 22.7, 26.2, 26.2, 27.3, 27.6, 29.2, 29.4, 29.5, 29.5, 29.6, 29.7, 29.8, 29.9, 30.0, 30.1, 30.5, 31.9, 32.4, 34.5, 35.3, 37.4, 38.1, 43.9, 57.7, 85.5 and 202.9.

Methyl (*R*)-2-((*R*)-1-(*tert*-butyldimethylsilyloxy)-19-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonadec-10-enyl)-26-(pivaloyloxy)hexacosanoate (181)

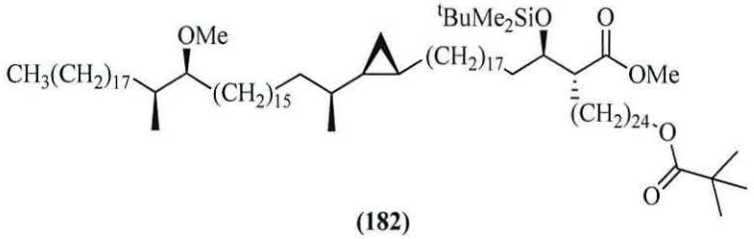


(181)

Lithium *bis*(trimethylsilyl)amide (0.96 ml, 1.02 mmol, 1.06 M) was added to a stirred solution of 9-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonanal **(153)** (0.494 g, 0.651 mmol) and methyl (*R*)-2-((*R*)-1-(*tert*-butyldimethylsilyloxy)-10-(1-phenyl-1-tetrazol-5-ylsulfonyl)decyl)-26-(pivaloyloxy)hexacosanoate **(154)** (0.773 g, 0.781 mmol) in dry THF (15 ml) at 0-5 °C. The solution turned bright yellow/orange and was left to reach room temperature and stirred for 1 hour under N_2 (g). When TLC showed no starting material remaining the reaction was quenched by the addition of a saturated aqueous solution of NH_4Cl (10 ml) at -20 °C. The mixture was extracted with petrol/ethyl acetate (1:1, 3 x 15 ml) and the combined organic layers were dried, filtered and evaporated. Column chromatography (petrol/ethyl acetate, 20:1) gave methyl (*R*)-2-((*R*)-1-(*tert*-butyldimethylsilyloxy)-19-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonadec-10-enyl)-26-(pivaloyloxy)hexacosanoate **(181)** (0.849 g, 0.558 mmol, 86 %) as a

colourless oil, $[\alpha]_D^{23}$ –8.54 (*c* 1.19, $CHCl_3$). This showed ν_{max}(film)/cm^{-1}: 2923 (C-H), 2853 (C-H), 1732 (C=O) and 1463; δ_H: 0.02 (3H, s, SiC*H*$_3$), 0.05 (3H, s, SiC*H*$_3$), 0.09-0.22 (3H, m, 3 x cyclopropane C*H*), 0.41-0.48 (1H, m, cyclopropane C*H*), 0.68 (1H, m, C*H*), 0.83-0.90 (23H, m, including a singlet at 0.87, SiC(C*H*$_3$)$_3$), 1.26 (151H, m, including a singlet at 1.20), 1.62 (5H, m), 1.96 (2H, m, C*H*$_2$), 2.02 (1H, m, C*H*), 2.53 (1H, ddd, *J* 3.6, 7.2, 11.0, CHC*H*(CH$_2$)CO), 2.96 (1H, m, CH$_2$C*H*(OCH$_3$)CH$_2$), 3.35 (3H, s, OC*H*$_3$), 3.66 (3H, s, OC*H*$_3$), 3.91 (1H, m, CH$_2$C*H*(O)CH), 4.05 (2H, t, *J* 6.6, CH$_2$C*H*$_2$O) and 5.37 (2H, m, CH$_2$C*H*=C*H*CH$_2$); -4.9, -4.4, 10.5, 14.1, 14.9, 18.0, 18.6, 19.7, 22.6, 22.7, 23.7, 25.8, 25.9, 26.1, 26.2, 27.2, 27.3, 27.5, 27.6, 27.8, 28.6, 29.1, 29.2, 29.2, 29.3, 29.4, 29.5, 29.5, 29.6, 29.6, 29.7, 29.7, 29.8, 30.0, 30.0, 30.1, 30.5, 31.9, 32.4, 32.6, 33.7, 34.5, 35.3, 37.4, 38.1, 51.2, 51.6, 57.7, 64.5, 73.2, 85.5, 129.8, 129.9, 130.3, 130.4, 143.2, 175.2 and 178.7. No mass spectrum could be obtained for the sample.

Methyl (*R*)-2-((*R*)-1-(*tert*-butyldimethylsilyloxy)-19-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonadecyl)-26-(pivaloyloxy)hexacosanoate (182)

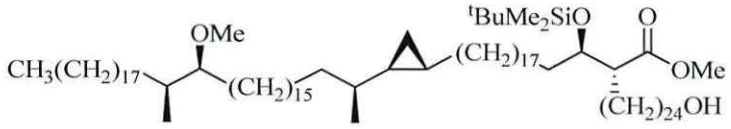


(182)

Dipotassiumazodicarboxylate was added in excess to a stirred solution of olefin **(181)** (0.840 g, 0.552 mmol) in dry THF (10 ml) and methanol (5 ml) at 0 °C under N_2 (g). Acetic acid (2 ml) in dry THF (4 ml) was added dropwise in small portions throughout the day at 0 °C. The following morning further dipotassium azodicarboxylate followed by more of the solution of acetic acid in THF was added. Again, after stirring overnight, more dipotassiumazodicarboxylate was added, followed by more of the solution of acetic acid in THF. After stirring for a further 24 h the reaction was quenched by adding it in small portions to a saturated solution of aqueous $NaHCO_3$ (15 ml). The mixture was extracted with petrol/ethyl acetate (5:2, 3 x 25 ml) and the combined organic layers were dried, filtered and evaporated. Column chromatography (petrol/ethyl acetate, 20:1) gave methyl (*R*)-2-((*R*)-1-(*tert*-butyldimethylsilyloxy)-19-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonadecyl)-26-(pivaloyloxy)hexacosanoate **(182)** (0.727 g, 0.477 mmol, 86 %) as a colourless oil, $[\alpha]_D^{21}$ –6.52 (*c* 0.87, $CHCl_3$). This showed

ν_{max}(film)/cm^{-1}: 2924 (C-H saturated), 2853 (C-H saturated), 1733 (C=O) and 1464; δ_H: 0.02 (3H, s, SiCH_3), 0.05 (3H, s, SiCH_3), 0.10-0.22 (3H, m, 3 x cyclopropane C*H*), 0.42-0.47 (1H, m, cyclopropane C*H*), 0.67 (1H, m, C*H*), 0.89 (23H, m, including a singlet at 0.87), 1.26 (147H, m), 1.62 (6H, m), 2.53 (1H, ddd, *J* 3.7, 7.2, 10.9, CHC*H*(CH_2)CO), 2.96 (1H, m, CHC*H*(OCH_3)CH_2), 3.35 (3H, s, OCH_3), 3.66 (3H, s, COOCH_3), 3.91 (1H, dt, *J* 4.7, 7.0, CH_2C*H*(O)CH) and 4.05 (2H, t, *J* 6.6, CH_2CH_2O); δ_C: -4.9, -4.4, 10.5, 11.4, 14.1, 14.9, 18.0, 18.6, 19.7, 22.6, 22.7, 25.7, 25.9, 26.2, 2, 27.2, 27.3, 27.5, 27.6, 28.6, 29.1, 29.2, 29.4, 29.5, 29.5, 29.6, 29.6, 29.6, 29.7, 29.8, 29.9, 30.0, 30.1, 30.5, 31.9, 32.4, 34.5, 35.3, 37.4, 38.1, 38.7, 51.2, 51.6, 57.7, 64.5, 73.2, 85.5, 143.2, 175.2 and 178.7. No mass spectrum could be obtained for this sample.

Methyl (*R*)-2-((*R*)-1-(*tert*-butyldimethylsilyloxy)-19-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonadecyl)-26-hydroxyhexacosanoate (183)

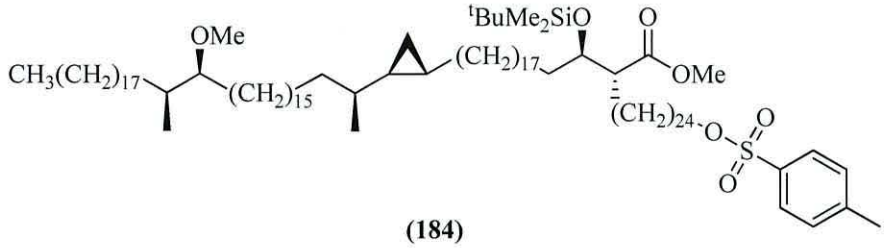


(183)

Pivalate **(182)** (0.71 g, 0.47 mmol) was added to a stirred solution of potassium hydroxide (0.39 g, 6.99 mmol) in THF (10 ml), methanol (10 ml) and water (1 ml). The mixture was heated under reflux at 70 °C and monitored by TLC. After ~3 hours, when TLC showed no starting material remaining the reaction was quenched with water (10 ml) and extracted with ethyl acetate (3 x 15 ml). The combined organic extracts were dried, filtered and evaporated. Column chromatography (petrol/ethyl acetate, 10:1) gave methyl (*R*)-2-((*R*)-1-(*tert*-butyldimethylsilyloxy)-19-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonadecyl)-26-hydroxyhexacosanoate **(183)** (0.583 g, 0.41 mmol, 86 %) as a white solid, m.p. 27-28 °C, $[\alpha]_D^{21}$ –4.54 (*c* 0.69, $CHCl_3$) {Found (M + Na)$^+$: 1462.45, $C_{95}H_{190}O_5SiNa$ requires: 1462.43}. This showed ν_{max}(nujol)/cm^{-1}: 3424 (broad O-H), 2923 (C-H saturated), 1853 (C-H saturated), 1741 (C=O), 1719 (C=O) and 1463; δ_H: 0.02 (3H, s, SiCH_3), 0.05 (3H, s, SiCH_3), 0.10-0.21 (3H, m, 3 x cyclopropane C*H*), 0.41-0.48 (1H, m, cyclopropane C*H*), 0.66 (1H, m, C*H*), 0.86 (3H, d, *J* 7.0, CH_3CH), 0.87 (9H, s, SiC(CH_3)$_3$), 0.89 (3H, t, *J* 7.1, CH_3CH_2), 0.91 (3H, d, *J* 6.6, CH_3CH), 1.58 (9H, br m), 1.26 (141H, m), 2.53 (1H, ddd, *J* 3.6, 7.2, 10.9, CHC*H*(CH_2)CO), 2.96 (1H, m, CHC*H*(OCH_3)CH_2), 3.35 (3H, s, OCH_3), 3.65 (2H, t, *J* 6.6, CH_2CH_2O), 3.66 (3H, s,

OCH_3) and 3.91 (1H, dt, *J* 4.8, 6.6, $CH_2CH(O)CH$); δ_C: -4.9, -4.4, 10.5, 14.1, 14.9, 18.0, 18.6, 19.7, 22.7, 23.7, 25.5, 25.8, 26.1, 26.2, 27.3, 27.5, 27.6, 27.8, 28.3, 29.1, 29.4, 29.4, 29.5, 29.5, 29.6, 29.6, 29.7, 29.8, 29.9, 30.0, 30.1, 30.5, 31.9, 32.4, 32.8, 33.7, 34.5, 35.3, 37.4, 38.1, 51.2, 51.6, 57.7, 63.1, 72.4, 73.3, 85.5, 143.2, 175.2 and 176.3.

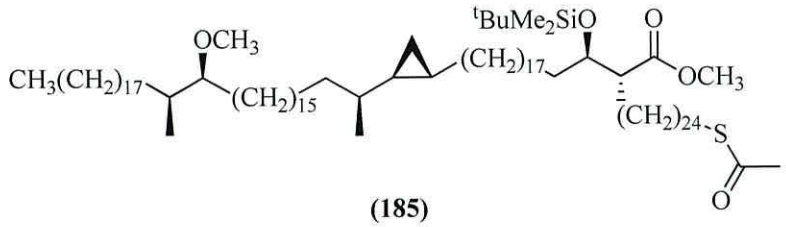
Methyl (*R*)-2-((*R*)-1-(*tert*-butyldimethylsilyl)oxy)-19-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonadecyl)-26-(tosyloxy)hexacosanoate (184)



(184)

Alcohol **(183)** (0.474 g, 0.327 mmol) and triethylamine (2 ml) in dry dichloromethane (25 ml) was cooled to -20 °C under N_2 (g) and stirred for 30 minutes, followed by the addition of *p*-toluene sulfonyl chloride (0.081 g, 0.425 mmol) in one portion. The solution was kept in the refrigerator overnight. When TLC showed no starting material remaining the solvent was evaporated. Column chromatography (petrol/ethyl acetate, 10:1) gave methyl (*R*)-2-((*R*)-1-(*tert*-butyldimethylsilyl)oxy)-19-((1S,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonadecyl)-26-(tosyloxy) hexacosanoate **(184)** (0.333 g, 0.211 mmol, 65 %) as a colourless oil, $[\alpha]_D^{23}$ -5.03 (*c* 0.63, $CHCl_3$) {Found $(M + Na)^+$: 1618.15, $C_{102}H_{196}O_7SSiNa$ requires: 1616.44}. This showed ν_{max}(film)/cm^{-1}: 2923 (C-H saturated), 2853 (C-H saturated), 1740 (C=O) and 1464; δ_H: 0.02 (3H, s, $SiCH_3$), 0.05 (3H, s, $SiCH_3$), 0.09-0.21 (3H, m, 3 x cyclopropane C*H*), 0.42-0.47 (1H, m, cyclopropane C*H*), 0.66 (1H, m, C*H*), 0.86 (3H, d, *J* 6.7, CH_3CH), 0.87 (9H, s, $SiC(CH_3)_3$), 0.89 (3H, t, *J* 7.2, CH_3CH_2), 0.91 (3H, d, *J* 6.6, CH_3CH), 1.26 (143H, m), 1.63 (6H, m), 2.46 (3H, s, $PhCH_3$), 2.53 (1H, ddd, *J* 3.6, 7.2, 10.9, $CHCH(CH_2)CO$), 2.96 (1H, m, $CHCH(OCH_3)CH_2$), 3.35 (3H, s, OCH_3), 3.66 (3H, s, OCH_3), 3.91 (1H, dt, *J* 4.8, 6.6, $CH_2CH(O)CH$), 4.03 (2H, t, *J* 6.5, CH_2CH_2O), 7.36 (2H, d, *J* 7.9, 2 x aromatic C*H*) and 7.80 (2H, d, *J* 8.2, 2 x aromatic C*H*); δ_C: -4.9, -4.4, 10.5, 14.1, 14.9, 18.0, 18.6, 19.7, 21.6, 22.7, 23.7, 25.3, 25.8, 26.1, 26.2, 27.3, 27.5, 27.6, 27.8, 28.8, 28.9, 29.3, 29.4, 29.5, 29.5, 29.6, 29.6, 29.7, 29.7, 29.7, 29.9, 30.0, 30.0, 30.1, 30.5, 31.9, 32.4, 33.7, 34.5, 35.3, 37.4, 38.1, 51.2, 51.6, 57.7, 60.4, 70.7, 73.2, 85.5, 127.9, 129.8, 133.3, 144.5 and 175.1.

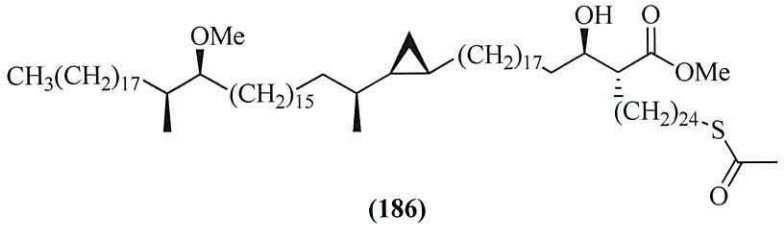
Methyl (*R*)-26-(acetylthio)-2-((*R*)-1-(*tert*-butyldimethylsilyloxy)-19-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonadecyl)hexacosanoate (185)



(185)

A solution of tosylate **(184)** (0.399 g, 0.251 mmol) and potassium thioacetate (0.115 g, 1.003 mmol) in acetone (15 ml) was stirred at room temperature overnight. When TLC showed that no starting material remained the solvent was evaporated. Column chromatography (petrol/ethyl acetate, 20:1) gave methyl (*R*)-26-(acetylthio)-2-((*R*)-1-(*tert*-butyldimethylsilyloxy)-19-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonadecyl)hexacosanoate **(185)** (0.227 g, 0.152 mmol, 61 %) as a colourless oil, $[\alpha]_D^{24}$ – 4.43 (*c* 0.73, $CHCl_3$) {Found $(M + Na)^+$: 1520.42, $C_{97}H_{192}O_5SSiNa$ requires: 1520.42}. This showed ν_{max}(film)/cm^{-1}: 2921 (C-H saturated), 2851 (C-H saturated), 1731 (C=O), 1643 (C=O) and 1463; δ_H: 0.02 (3H, s, $SiCH_3$), 0.04 (3H, s, $SiCH_3$), 0.09-0.20 (3H, m, 3 x cyclopropane C*H*), 0.41-0.48 (1H, m, cyclopropane C*H*), 0.65 (1H, m, C*H*), 0.85 (3H, d, *J* 7.0, CH_3CH), 0.86 (9H, s, $SiC(CH_3)_3$), 0.88 (3H, t, *J* 6.6, CH_3CH_2), 0.90 (3H, d, *J* 6.9, CH_3CH), 1.26 (143H, m), 1.55 (6H, m), 2.31 (3H, s, $COCH_3$), 2.52 (1H, ddd, *J* 3.6, 7.3, 10.9, $CHCH(CH_2)CO$), 2.85 (2H, t, *J* 7.4, CH_2CH_2S), 2.96 (1H, m, $CHCH(OCH_3)CH_2$), 3.33 (3H, s, OCH_3), 3.65 (3H, s, OCH_3) and 3.90 (1H, dt, *J* 4.7, 7.1, $CH_2CH(O)CH$); δ_C: -4.9, -4.3, 10.5, 14.1, 14.9, 17.9, 18.6, 19.7, 22.7, 23.7, 25.7, 26.1, 26.2, 27.3, 27.5, 27.6, 27.8, 28.8, 29.1, 29.4, 29.5, 29.5, 29.5, 29.6 , 29.6, 29.6, 29.6, 29.7, 29.7, 29.7, 29.8, 30.0, 30.0, 30.1, 30.4, 30.5, 30.6, 31.9, 32.4, 32.6, 32.8, 32.8, 33.7, 34.5, 35.3, 37.4, 37.7, 38.1, 51.2, 51.6, 57.7, 71.0, 73.2, 73.4, 85.4, 175.1 and 195.9.

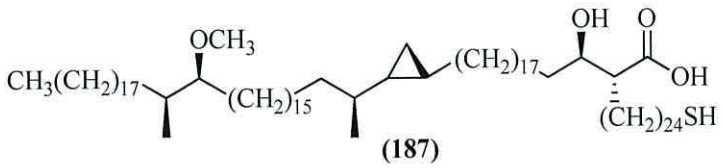
Methyl (*R*)-26-(acetylthio)-2-((*R*)-1-hydroxy-19-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonadecyl)hexacosanoate (186)



(186)

Thioacetate **(185)** (50 mg, 0.0333 mmol) was dissolved in dry THF (4 ml) in a dry polyethylene vial under N_2 (g) at 0 °C. Pyridine (98.2 mg, 7.77 mmol, 0.1 ml) and HF.Pyridine (88 mg, 0.8 ml) were added and the mixture was stirred at 45 °C overnight. When TLC showed no starting material remaining, the mixture was added slowly to a saturated aqueous solution of $NaHCO_3$ (10 ml). The solution was extracted with petrol/ethyl acetate (1:1, 3 x 15 ml) and the combined organic extracts were dried, filtered and evaporated. Column chromatography (petrol/ethyl acetate, 10:1) gave methyl (*R*)-26-(acetylthio)-2-((*R*)-1-hydroxy-19-((1*S*,2*R*)-2-((2*S*,19*S*,20S)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonadecyl)hexacosanoate **(186)** (41.1 mg, 0.0299 mmol, 90 %) as a white solid, m.p. 41-43 °C, $[\alpha]_D^{21}$ -2.27 (*c* 2.14, $CHCl_3$) {Found $(M + Na)^+$: 1406.33, $C_{91}H_{178}O_5SNa$ requires: 1406.33}. This showed ν_{max}(nujol)/cm^{-1}: 3418 (broad OH), 2922 (C-H saturated), 2851 (C-H saturated), 1709 (C=O), 1687 (C=O) and 1465; δ_H: 0.09-0.21 (3H, m, 3 x cyclopropane C*H*), 0.41-0.48 (1H, m, cyclopropane C*H*), 0.66 (1H, m, C*H*), 0.86 (3H, d, *J* 6.9, C*H*$_3$CH), 0.89 (3H, t, *J* 6.9, C*H*$_3$CH$_2$), 0.91 (3H, d, *J* 6.6, C*H*$_3$CH), 1.26 (142H, m), 1.58 (8H, m), 2.33 (3H, s, COC*H*$_3$), 2.45 (1H, dt, *J* 5.4, 9.1 CHC*H*(CH$_2$)CO), 2.87 (2H, t, *J* 7.4, CH$_2$C*H*$_2$S), 2.96 (1H, m, CH$_2$C*H*(OCH$_3$)CH$_2$), 3.35 (3H, s, OC*H*$_3$), 3.66 (1H, m, CH$_2$C*H*(OH)CH$_2$) and 3.72 (3H, s, COOC*H*$_3$); δ_C: 10.5, 14.1, 14.9, 18.6, 19.7, 22.7, 25.7, 26.1, 26.2, 27.3, 27.4, 27.6, 28.8, 29.1, 29.2, 29.4, 29.4, 29.5, 29.6, 29.6, 29.6, 29.7, 29.7, 29.9, 30.0, 30.1, 30.1, 30.5, 30.6, 31.9, 32.4, 34.5, 35.3, 35.7, 37.4, 38.1, 50.9, 51.5, 57.7, 72.3, 76.6, 85.5, 176.3 and 196.1.

(*R*)-2-((*R*)-1-Hydroxy-19-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonadecyl)-26-mercaptohexacosanoic acid (187)

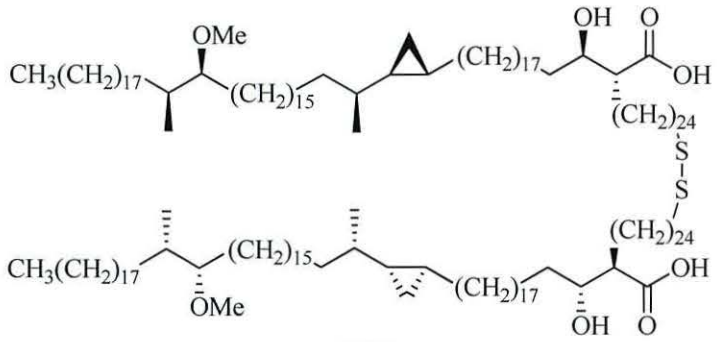


(187)

Lithium hydroxide (14 mg, 0.33 mmol) was added to a stirred solution of methyl (*R*)-26-(acetylthio)-2-((*R*)-1-hydroxy-19-((1*S*,2*R*)-2-((2*S*,19*S*,20S)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonadecyl)hexacosanoate **(186)** (31 mg, 0.023 mmol) in a mixture of THF (3 ml), water (0.3 ml) and methanol (0.3 ml). The reaction mixture was then stirred at 45 °C overnight. The reaction was diluted by addition of petrol/ethyl acetate (1:1, 4 ml) and brought to pH 3 by dropwise addition of dilute HCl.

The product was extracted with petrol/ethyl acetate (1:1, 5 x 15 ml) and the combined organic extracts were dried, filtered and evaporated. The crude ^{1}H NMR spectrum was extremely complicated and it contained numerous spots on TLC.

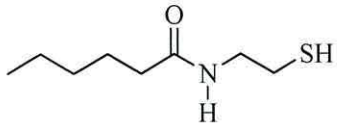
(*S*,*S*,*S*,*R*,*S*,*R*,2*R*,2*R*')-26-26'-Disulfanediyl*bis*(2-((*R*)-1-hydroxy-19-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonadecyl)hexacosanoic acid (152)



(152)

Methyl (*R*)-26-(acetylthio)-2-((*R*)-1-hydroxy-19-((1*S*,2*R*)-2-((2*S*,19*S*,20S)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonadecyl)hexacosanoate **(186)** (14 mg, 0.010 mmol) was suspended in a 5 % aqueous solution of TBAH (2 ml) and the solution was heated to 100 °C overnight. After this time TLC showed that the reaction was complete. The solution was cooled to room temperature, acidified to pH 1 with 1 M HCl and extracted with diethyl ether (3 x 15 ml). The combined organic layers were dried, filtered and the solvent evaporated. Column chromatography (chloroform/methanol, 10:1) gave (*S*,*S*,*S*,*R*,*S*,*R*,2*R*,2*R*')-26-26'-disulfanediyl*bis*(2-((*R*)-1-hydroxy-19-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonadecyl)hexacosanoic acid **(152)** (7.7 mg, 0.0058 mmol, 58 %) as a white solid, $[\alpha]_D^{22}$ -2.78 (*c* 0.77, $CHCl_3$). This showed ν_{max}(film)/cm^{-1}: 3423 (broad O-H), 2924 (C-H saturated), 2852 (C-H saturated) 1718 (C=O) and 1465; δ_H: 0.08-0.20 (3H, m, 3 x cyclopropane C*H*), 0.44 (1H, m, cyclopropane C*H*), 0.66 (1H, m, C*H*), 0.86 (3H, d, *J* 6.9, C*H*$_3$CH), 0.89 (3H, t, *J* 7.0, C*H*$_3$CH$_2$), 0.90 (3H, d, *J* 6.6, C*H*$_3$CH), 1.26 (144H, m), 1.67 (8H, m), 2.47 (1H, dt, *J* 5.4, 9.1 CHC*H*(CH$_2$)CO), 2.69 (2H, t, *J* 7.4, CH$_2$C*H*$_2$S), 2.97 (1H, m, CH$_2$C*H*(OCH$_3$)CH$_2$), 3.35 (3H, s, OC*H*$_3$) and 3.91 (1H, m, CH$_2$C*H*(OH)CH$_2$); δ_C: 8.6, 10.5, 14.1, 14.9, 18.6, 19.77, 22.7, 26.1, 27.3, 27.6, 28.5, 29.2, 29.4, 29.5, 29.9, 30.0, 30.1, 30.5, 30.9, 31.9, 32.4, 34.5, 35.3, 37.4, 38.1, 39.4, 45.3, 50.9, 57.9 and 85.5.

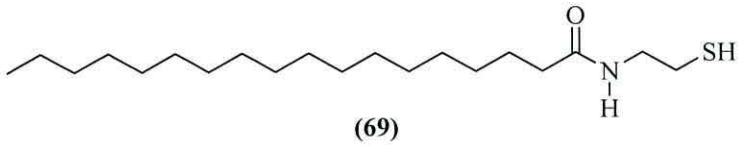
_N_-(2-Mercaptoethyl)hexanamide (189)



(189)

2,5-Dioxopyrrolidin-1-yl hexanoate **(191)** (1.0 g, 4.69 mmol) and 2-mercaptoethylamine hydrochloride (0.80 g, 7.04 mmol) were dissolved in dry dichloromethane (40 ml). Triethylamine (1.43 g, 1.96 ml, 14.1 mmol) that was dissolved in dry dichloromethane (5 ml) was added to the solution and the reaction was allowed to stir overnight at room temperature. The following morning ethyl acetate (20 ml) and ethanol (10 ml) were added. The organic solution was washed with dilute acid (3 x 15 ml), water (2 x 15 ml), dried, filtered and evaporated. Column chromatography (petrol/ethyl acetate, 1:1) gave *N*-(2-mercaptoethyl)hexanamide **(189)** (536 mg, 3.06 mmol, 65 %) as a white solid, m.p. 28-30 °C {Found $(M + Na)^+$: 198.0927, $C_8H_{17}NOSNa$ requires: 198.0928}. This showed ν_{max}(nujol)/cm^{-1}: 3301 (N-H), 2955 (C-H saturated), 2857 (C-H saturated), 2729 (weak S-H), 1635 (C=O), 1544 and 1465; δ_H: 0.90 (3H, t, *J* 7.0, CH_3CH_2), 1.32 (4H, m), 1.65 (2H, p, *J* 7.5, $CH_2CH_2CH_2$), 2.21 (2H, t, *J* 7.6, CH_2CH_2CO), 2.68 (2H, dt, *J* 6.4 and 8.4, $NHCH_2CH_2SH$), 3.44 (2H, q, *J* 6.2, $NHCH_2CH_2SH$) and 5.96 (1H, br s, N*H*); δ_C: 13.9, 22.4, 24.7, 25.4, 31.4, 36.7, 43.3 and 173.4.

_N_-(2-Mercaptoethyl)stearamide (69)[231]

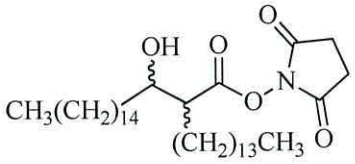


(69)

The procedure used for the synthesis of **(189)** was repeated using 2,5-dioxopyrrolidin-1-yl stearate **(188)** (5.29 g, 13.9 mmol) and 2-mercaptoethylamine hydrochloride (2.36 g, 20.8 mmol) in dry dichloromethane (140 ml) and triethylamine (4.20 g, 41.6 mmol) in dry dichloromethane (5 ml). Recrystallisation (chloroform) gave *N*-(2-mercaptoethyl)stearamide **(69)** (2.66 g, 7.76 mmol, 56 %) as a white solid, m.p. 63-65 °C {Found $(M + Na)^+$: 366.2818, $C_{20}H_{41}NOSNa$ requires: 366.2806}. This showed ν_{max}(nujol)/cm^{-1}: 3300 (N-H), 2920 (C-H saturated), 2853 (C-H saturated), 1640 (C=O), 1550 and 1464; δ_H: 0.89 (3H, t, *J* 6.9, CH_3CH_2), 1.26 (28H, m), 1.64 (2H, m), 2.20 (2H, t, *J* 7.6, CH_2CH_2CO), 2.68 (2H, dt, *J* 6.4 and 8.4, $NHCH_2CH_2SH$), 3.45 (2H, q, *J* 6.2,

NHCH_2CH_2SH) and 5.83 (1H, br s, NH); δ_C: 14.1, 22.7, 24.8, 25.7, 29.3, 29.4, 29.5, 29.6, 29.7, 29.7, 31.9, 36.8, 42.2 and 173.3.

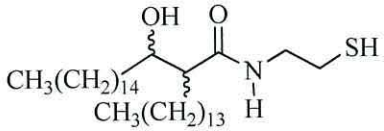
2,5-Diocopyrrolidin-1-yl 3-hydroxy-2-tetradecyloctadecanoate (193)



(193)

3-Hydroxy-2-tetradecyloctadecanoic acid **(192)** (100 mg, 0.202 mmol) was added to a solution of *N*-hydroxysuccinimide (23 mg, 0.202 mmol) in dry ethyl acetate (8 ml). A solution of DCC (42 mg, 0.202 mmol) in dry ethyl acetate (1.5 ml) was added and the reaction mixture was stirred overnight at room temperature. DCU was removed by filtration and the filtrate was concentrated under reduced pressure. Column chromatography (petrol/ethyl acetate, 5:1) gave 2,5-diocopyrrolidin-1-yl 3-hydroxy-2-tetradecyloctadecanoate **(193)** (81 mg, 0.136 mmol, 68 %) as a white solid, m.p. 63-65 °C {Found $(M + Na)^+$: 616.4940, $C_{36}H_{67}O_5NNa$ requires 616.4916}. This showed ν_{max}(nujol)/cm^{-1}: 2919 (C-H saturated), 2850 (C-H saturated), 1814 (C=O), 1785 (C=O), 1727 (C=O) and 1468; δ_H: 0.88 (6H, t, *J* 7.0, 2 x CH_3CH$_2$), 1.25 (46H, m), 1.40 (3H, m), 1.51 (2H, m), 1.63 (2H, m), 1.79 (1H, m, CH), 2.23 (1H, br s, OH), 2.73 (1H, dt, *J* 5.3 and 10.5, CHCH(CH$_2$)CO), 2.85 (4H, d, *J* 7.0, COCH_2CH_2CO) and 3.79 (1H, m, CH$_2$CH(O)CH); δ_C: 14.1, 22.7, 25.6, 25.6, 27.2, 28.9, 29.3, 29.4, 29.4, 29.5, 29.5, 29.6, 29.6, 29.6, 29.7, 29.7, 31.9, 34.9, 50.5, 72.5, 169.3 (split into 2 – 169.2) and 169.4.

3-Hydroxy-*N*-(2-merccaptoethyl)-2-tetradecyloctadecanamide (194)



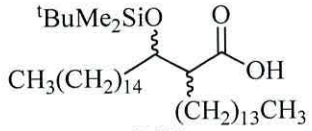
(194)

(i) *N*-hydroxysuccinimide ester **(193)** (90 mg, 0.152 mmol) and 2-mercaptoethylamine hydrochloride (26 mg, 0.228 mmol) were suspended in dry dichloromethane (5 ml). Triethylamine (46 mg, 0.455 mmol) that was dissolved in dry dichloromethane (2 ml) was added to the solution. The reaction was allowed to stir for 2.5 hours and ethyl acetate

(15 ml) and ethanol (10 ml) were added to the cooled solution. The organic solution was then washed with dilute acid (3 x 20 ml), water (2 x 20 ml), dried, filtered and evaporated. This gave mainly the starting material, 2,5-diocopyrrolidin-1-yl 3-hydroxy-2-tetradecyloctadecanoate **(193)**.

(ii) The above reaction was repeated using *N*-hydroxysuccinimide ester **(193)** (30 mg, 0.05 mmol) and 2-mercaptoethylamine hydrochloride (8.6 mg, 0.076 mmol) in dry dichloromethane (5 ml) and triethylamine (15 mg, 0.152 mmol) in dry dichloromethane (2 ml) and was stirred overnight. This gave mainly the starting material, 2,5-diocopyrrolidin-1-yl 3-hydroxy-2-tetradecyloctadecanoate **(193)**.

3-((*tert*-Butyldimethylsilyl)oxy)-2-tetradecyloctadecanoic acid (195)

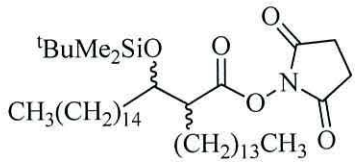


(195)

Imidazole (137 mg, 2.0 mmol) was added to a stirred solution of 3-hydroxy-2-tetradecyloctadecanoic acid **(192)** (100 mg, 0.20 mmol) in dry DMF (2 ml) and dry toluene (3 ml) at room temperature followed by the addition of *tert*-butyldimethylsilylchloride (304 mg, 2.0 mmol) and 4-dimethylaminopyridine (24 mg, 0.20 mmol). The reaction was stirred at 70 °C for 24 hours followed by at room temperature for 18 hours. When TLC showed no starting material remaining the solvent was removed under high vacuum and the residue was diluted with petrol/ethyl acetate (10:1, 25 ml) and water (10 ml). The organic layer was separated and the aqueous layer was extracted with petrol/ethyl acetate (2 x 30 ml). The combined organic layers were washed with water, dried, filtered and evaporated to give a colourless residue. The residue was dissolved in THF (7 ml), water (1 ml) and methanol (1 ml) and to this potassium carbonate (0.15 g, 1.1 mmol) was added. The reaction mixture was then stirred at 45 °C for 18 hours. The mixture was diluted with petrol/ethyl acetate (10:1, 20 ml) and water (2 ml) then acidified with potassium hydrogen sulfate to pH 2. The organic layer was separated and the aqueous layer was extracted with petrol/ethyl acetate (10:1, 2 x 20 ml). The combined organic layers were washed with water, dried, filtered and evaporated. Column chromatography (petrol/ethyl acetate, 5:1) gave 3-((*tert*-butyldimethylsilyl)oxy)-2-tetradecyloctadecanoic acid **(195)** (101 mg, 0.165 mmol, 83 %) as a yellow oil. This showed ν_{max}(nujol)/cm^{-1}: 3347 (broad OH), 2924 (C-H saturated), 2853 (C-H saturated) 1707 (C=O) and 1463; δ_H: 0.12 (3H, s, CH_3Si), 0.13

(3H, s, CH_3Si), 0.88 (6H, t, J 6.9, 2 x CH_3CH$_2$), 0.92 (9H, s, C(CH_3)$_3$), 1.26 (50H, m), 1.53 (3H, m), 1.64 (1H, m), 2.53 (1H, ddd, J 3.9, 5.5, 9.5, CHCH(CH$_2$)CO) and 3.38 (1H, m, CH$_2$CH(O)CH); δ_C: -4.9, -4.2, 14.1, 17.9, 22.7, 24.8, 25.7, 27.5, 29.4, 29.4, 29.5, 29.5, 29.5, 29.6, 29.6, 29.7, 29.7, 31.9, 35.4, 50.4, 73.6 and 176.4. No mass spectrum could be obtained for this sample.

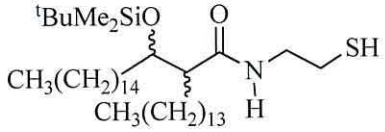
2,5-Dioxopyrrolidin-1-yl 3-((*tert*-butyldimethylsilyl)oxy)-2-tetradecyloctadecanoate (196)



(196)

3-((*tert*-Butyldimethylsilyl)oxy)-2-tetradecyloctadecanoic acid **(195)** (98 mg, 0.16 mmol) was added to a solution of *N*-hydroxysuccinimide (18.7 mg, 0.16 mmol) in dry ethyl acetate (8 ml). A solution of DCC (33.8 mg, 0.164 mmol) in dry ethyl acetate (2 ml) was added and the reaction mixture was stirred overnight at room temperature. DCU was removed by filtration and the filtrate was concentrated under reduced pressure. Column chromatography (petrol/ethyl acetate, 5:1) gave 2,5-dioxopyrrolidin-1-yl 3-((*tert*-butyldimethylsilyl)oxy)-2-tetradecyloctadecanoate **(196)** (79 mg, 0.11 mmol, 70 %) as a white solid. This showed ν_{max}(nujol)/cm^{-1}: 2924 (C-H saturated), 2854 (C-H saturated), 1813 (C=O), 1787 (C=O), 1745 (C=O) and 1465; δ_H: 0.07 (3H, s, SiCH_3), 0.08 (3H, s, SiCH_3), 0.88 (6H, t, J 7.3, 2 x CH_3CH$_2$), 0.90 (9H, s, C(CH_3)$_3$), 1.26 (48H, m) 1.40-1.76 (6H, m), 2.81 (5H, m, COCH_2CH_2CO and CHCH(CH$_2$)CO) and 4.03 (1H, q, J 5.4, CH$_2$CH(O)CH); δ_C: -4.7, -4.5, 14.1, 18.0, 22.7, 25.2, 25.6, 25.8, 26.6, 27.6, 29.4, 29.6, 29.6, 29.7, 29.7, 29.7, 31.9, 33.6, 49.3, 72.6, 169.0 and 169.1. No mass spectrum could be obtained for this sample.

3-((*tert*-Butyldimethylsilyl)oxy)-*N*-(2-mercaptoethyl)-2-tetradecyloctadecanamide (197)

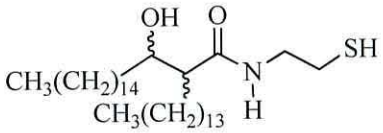


(197)

(i) *N*-hydroxysuccinimide ester **(196)** (31 mg, 0.04 mmol) and 2-mercaptoethylamine hydrochloride (8 mg, 0.065 mmol) were suspended in dry dichloromethane (5 ml). Triethylamine (13 mg, 18 μl, 0.130 mmol) that was dissolved in dry dichloromethane (2 ml) was added to the solution. The reaction was allowed to stir overnight at room temperature. The following morning ethyl acetate (10 ml) and ethanol (3 ml) were added. The organic solution was washed with dilute acid (3 x 10 ml), water (2 x 10 ml), dried, filtered and evaporated. This gave mainly the starting material, **(196)**.

(ii) The reaction was repeated as above using *N*-hydroxysuccinimide ester **(196)** (48 mg, 0.069 mmol) and 2-mercaptoethylamine hydrochloride (12 mg, 0.104 mmol) in dry dichloromethane (4 ml) and triethylamine (21 mg, 29 ml, 0.207 mmol) in dry dichloromethane (2 ml) and the mixture was stirred at room temperature for 1 week. This gave mainly the starting material, **(196)**.

3-Hydroxy-*N*-(2-mercaptoethyl)-2-tetradecyloctadecanamide (194)



(194)

(iii) *N*-hydroxysuccinimide ester **(193)** (19.7 mg, 0.033 mmol) and 2-mercaptoethylamine hydrochloride (5.7 mg, 0.050 mmol) were suspended in dry dichloromethane (2 ml). Triethylamine (10.1 mg, 0.099 mmol) that was dissolved in dry dichloromethane (1 ml) was added to the solution and the mixture was heated under reflux for 3 nights. Additional 2-mercaptoethylamine hydrochloride (5.7 mg, 0.050 mmol) was then added and the solution was again heated under reflux for a further 3 nights. Ethyl acetate (10 ml) and ethanol (3 ml) were added to the cooled mixture. The organic solution was then washed with dilute acid (3 x 10 ml), water (2 x 10 ml), dried, filtered and evaporated. Column chromatography (petrol/ethyl acetate, 5:2) gave 3-hydroxy-*N*-(2-merccaptoethyl)-2-tetradecyloctadecanamide **(194)** (14.4 mg, 0.026 mmol, 79 %) as a white solid {Found (M + Na)$^+$: 578.4859, $C_{34}H_{69}NO_2SNa$ requires: 578.4947}. This showed $\nu_{max}(CHCl_3)/cm^{-1}$: 3298 (broad OH/NH), 2919 (C-H saturated), 2850 (C-H saturated) and 1642 (C=O); δ_H: 0.88 (6H, m, 2 x C*H*$_3$CH$_2$), 1.26 (49H, m), 1.43 (3H, m), 1.57 (2H, m), 1.77 (2H, m), 2.10 (1H, dt *J* 4.7, 9.3, CHC*H*(CH$_2$)CO), 2.70 (2H, m, CH$_2$C*H*$_2$S), 3.47 (2H, q, *J* 6.2, CH$_2$C*H*$_2$NH), 3.66 (1H, m, CH$_2$C*H*(O)CH) and 6.18 (1H, t, *J* 5.7, N*H*CH$_2$); δ_C: 14.1, 14.3,

19.4, 22.6, 22.7, 24.7, 26.0, 27.7, 28.9, 29.4, 29.5, 29.6, 29.7, 29.7, 30.5, 31.9, 36.0, 41.4, 42.0, 52.2, 60.4, 72.6 and 175.6.

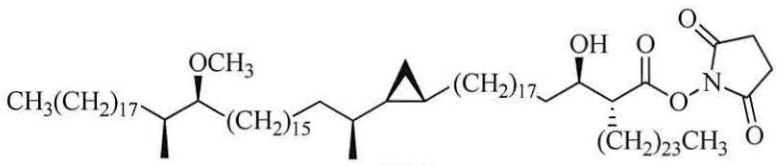
The *N*-hydroxysuccinimide ester of natural mycolic acid (199)

Natural mycolic acid **(198)** (18 mg, 0.0138 mmol) was added to a solution of *N*-hydroxy succinimide (2 mg, 0.0138 mmol) in dry ethyl acetate (2 ml). A solution of DCC (3 mg, 0.0138 mmol) in dry ethyl acetate (1 ml) was added and the reaction mixture was stirred overnight at room temperature. DCU was removed by filtration and the filtrate was concentrated under reduced pressure. Column chromatography (petrol/ethyl acetate, 5:2) gave the *N*-hydroxysuccinimide ester of natural mycolic acid **(199)** (11.7 mg, 0.0084 mmol, 61 %). Due to the presence of numerous different mycolic acids in the natural mixture the ^{1}H NMR spectrum of the residue of this reaction was extremely complicated. TLC also contained numerous spots.

The *N*-(2-mercaptoethyl)amide of natural mycolic acid (200)

The *N*-hydroxysuccinimide ester of natural mycolic acid **(199)** (11.3 mg, 0.0081 mmol) and 2-mercaptoethylamine hydrochloride (1.5 mg, 0.0121 mmol) were suspended in dry dichloromethane (2 ml). Triethylamine (2.5 mg, 3.4 µl, 0.0242 mmol) that was dissolved in dry dichloromethane (1 ml) was added to the solution. The reaction was allowed to heat under reflux for 3 nights and more 2-mercaptoethylamine hydrochloride (1.5 mg, 0.0121 mmol) was added. The solution was again heated under reflux for a further 3 nights. Ethyl acetate (15 ml) and ethanol (10 ml) were added to the cooled solution. The organic solution was then washed with dilute acid (3 x 20 ml), water (2 x 20 ml), dried, filtered and evaporated. Column chromatography (petrol/ethyl acetate, 5:2) gave the *N*-(2-mercaptoethyl)amide of natural mycolic acid **(200)** (5.5 mg, 0.0040 mmol, 50 %) as a white solid. Due to the presence of numerous different mycolic acids in the natural mixture the ^{1}H NMR spectrum of the residue of this reaction was extremely complicated. TLC also contained numerous spots.

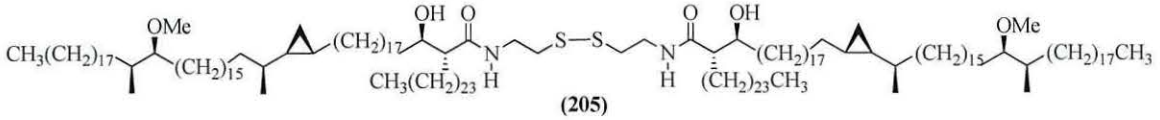
(*R*)-2,5-Dioxopyrrolidin-1-yl 2-((*R*)-1-hydroxy-19-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonadecyl)hexacosanoate (203)



(203)

(*R*)-2-((*R*)-1-Hydroxy-19-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonadecyl)hexacosanoic acid **(68)** (25 mg, 0.0193 mmol) was added to a solution of *N*-hydroxy succinimide (2.2 mg, 0.0193 mmol) in ethyl acetate (2 ml). A solution of DCC (4.0 mg, 0.0193 mmol) in dry ethyl acetate (1 ml) was then added and the reaction mixture was stirred overnight at room temperature. DCU was removed by filtration and the filtrate was concentrated under reduced pressure. Column chromatography (petrol/ethyl acetate, 5:1) gave (*R*)-2,5-dioxopyrrolidin-1-yl 2-((*R*)-1-hydroxy-19-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonadecyl)hexacosanoate **(203)** (10.3 mg, 0.0074 mmol, 38 %) as a white solid; $[\alpha]_D^{18}$ -1.32 (*c* 1.01, $CHCl_3$). This showed $\nu_{max}(CHCl_3)/cm^{-1}$: 3420 (broad O-H), 2918 (C-H saturated), 2850 (C-H saturated), 1810 (C=O), 1780 (C=O), 1739 (C=O) and 1467; δ_H: 0.10-0.20 (3H, m, 3 x cyclopropane C*H*), 0.45 (1H, m, cyclopropane C*H*), 0.66 (1H, m, C*H*), 0.85-0.91 (12H, m, 2 x CH_3CH$_2$ and 2 x CH_3CH), 1.26 (140H, m), 1.63 (9H, m), 1.81 (1H, m), 2.49 (1H, m, CHC*H*(CH$_2$)CO), 2.86 (4H, d, *J* 6.3, COCH_2CH_2CO), 2.96 (1H, m, CH$_2$C*H*(OCH$_3$)CH$_2$), 3.35 (3H, s, OCH_3) and 3.80 (1H, br s, CH$_2$C*H*(OH)CH$_2$); δ_C: 8.5, 12.1, 12.9, 16.7, 17.7, 20.7, 23.6, 23.6, 24.2, 24.2, 25.2, 25.3, 25.6, 26.9, 27.0, 27.4, 27.4, 27.4, 27.5, 27.6, 27.6, 27.6, 27.7, 27.7, 27.7, 27.8, 28.0, 28.0, 28.1, 28.4, 28.5, 30.0, 30.4, 32.5, 33.0, 33.3, 35.5, 36.2, 48.5, 55.7, 58.4, 70.6, 74.5, 83.5, 167.1, 167.3 and 167.9. No mass spectrum could be pbtained for this sample.

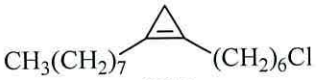
(S)-2-((S)-1-Hydroxy-19-((1R,2S)-2-((2R,19R,20R)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonadecyl)-N-(2-((2-((R)-2-((R)-1-hydroxy-19-((1S,2R)-2-((2S,19S,20S)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonadecyl)hexacosanamido)ethyl)disulfanyl)ethyl)hexacosanamide (205)



(205)

N-hydroxsuccinimide ester **(203)** (8.3 mg, 0.00597 mmol) and 2-mercaptoethylamine hydrochloride (1.0 mg, 0.00895 mmol) were suspended in dry dichloromethane (2 ml). Triethylamine (1.8 mg, 0.0179 mmol) that was dissolved in dry dichloromethane (1 ml) was added to the solution. The reaction was allowed to heat under reflux for 3 nights and more 2-mercaptoethylamine hydrochloride (1.0 mg, 0.00895 mmol) was added. The solution was again heated under reflux for a further 3 nights. Ethyl acetate (10 ml) and ethanol (5 ml) were added to the cooled solution and the organic solution was then washed with dilute acid (3 x 10 ml), water (2 x 10 ml), dried, filtered and evaporated. Column chromatography (petrol/ethyl acetate, 5:1) gave (*S*)-2-((*S*)-1-hydroxy-19-((1*R*,2*S*)-2-((2*R*,19*R*,20*R*)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonadecyl)-*N*-(2-((2-((*R*)-2-((*R*)-1-hydroxy-19-((1*S*,2*R*)-2-((2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl)cyclopropyl)nonadecyl)hexacosanamido)ethyl)disulfanyl)ethyl)hexacosanamide **(205)** (3.7 mg, 0.0014 mmol, 23 %) as a white solid, $[\alpha]_D^{19}$ -0.29 (*c* 0.23, $CHCl_3$). This showed $\nu_{max}(CHCl_3)/cm^{-1}$: 3425 (broad OH), 2919 (C-H saturated), 2850 (C-H saturated), 1645 (C=O) and 1467; δ_H: 0.05-0.19 (3H, m, 3 x cyclopropane C*H*), 0.46 (1H, m, cyclopropane C*H*), 0.68 (1H, m, C*H*), 0.85-0.91 (12H, m, 2 x CH_3CH_2 and 2 x CH_3CH), 1.26 (132H, m), 1.58 (20H, br m), 2.85 (2H, t, *J* 6.6, CH_2CH_2S), 2.97 (1H, m, $CH_2CH(OCH_3)CH_2$), 3.35 (3H, s, OCH_3), 3.59 (2H, m, CH_2CH_2NH), 3.66 (1H, m, $CH_2CH(OH)CH_2$) and 6.76 (1H, t, *J* 5.6, $NHCH_2$); δ_C: 11.3, 14.1, 14.2, 18.7, 19.3, 20.4, 21.0, 22.5, 22.6, 25.6, 27.6, 28.8, 29.0, 29.7, 30.8, 33.7, 41.3, 60.3, 60.4, 67.9 and 171.0. No mass spectrum could be obtained for this sample.

1-(6-Chloro-hexyl)-2-octyl-cyclopropene (215)



(215)

n-Butyllithium (1.6 M, 22.9 ml, 36.7 mmol) was added dropwise to a stirred solution of 1,1,2-tribromo-2-octyl-cyclopropane **(208)** (5.7 g, 14.8 mmol) in dry ether (50 ml) under N_2 (g) at -78 °C. The mixture was allowed to reach room temperature, stirred for 30 minutes and then cooled to 0 °C. HMPA (5.2 g, 5.1 ml, 29.2 mmol) was added dropwise followed by 1-chloro-6-iodo-hexane **(211)** (3.28 g, 13.3 mmol). The mixture was stirred overnight at room temperature and then cooled to 0 °C. Water (50 ml) was added and the product was extracted with ether (2 x 100 ml). The combined organic layers were washed with water (2 x 20 ml), dried and evaporated. Column chromatography (petrol)

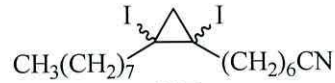
gave 1-(6-chloro-hexyl)-2-octyl-cyclopropene **(215)** (1.41 g, 5.2 mmol, 39 %) as a colourless oil. This showed ν_{max}(film)/cm^{-1}: 2927 (C-H saturated), 2856 (C-H saturated) and 1462; δ_H: 0.78 (2H, s, cyclopropene CH_2), 0.89 (3H, t, *J* 6.9, CH_3CH$_2$), 1.30 (12H, m), 1.46 (2H, m), 1.56 (4H, m), 1.78 (2H, p, *J* 7.1, CH$_2$CH_2CH$_2$Cl), 2.39 (4H, app 2t, CH$_2$CH_2C=CCH_2CH$_2$) and 3.54 (2H, t, *J* 6.8, CH$_2$CH_2Cl); δ_C: 7.4, 14.1, 22.3, 26.0, 26.7, 27.2, 27.4, 28.3, 28.6, 29.3, 29.4, 31.9, 32.6, 45.1, 109.1 and 109.6. Data was consistent with that in the literature.[142]

7-(2-Octyl-cycloprop-1-enyl)-heptanenitrile (216)

(216)

Sodium cyanide (0.64 g, 13.1 mmol) was added to a stirred solution of 1-(6-chloro-hexyl)-2-octyl-cyclopropene **(215)** (1.18 g, 4.3 mmol) in DMSO (20 ml) and the solution was stirred at 60 °C for 6 hours. Water (15 ml) was added to the cooled solution and the mixture was extracted with ether (2 x 100 ml). The combined organic layers were washed with saturated aqueous NH_4Cl (30 ml), dried, filtered and evaporated. Column chromatography (petrol/ether, 5:1) gave 7-(2-octyl-cycloprop-1-enyl)-heptanenitrile **(216)** (0.87 g, 3.3 mmol, 78 %) as a yellow oil {Found (M + H)$^+$: 262.2533, $C_{18}H_{32}N$ requires: 262.2535}. This showed ν_{max}(film)/cm^{-1}: 2927 (C-H saturated), 2856 (C-H saturated), 2246 (C≡N), 1871 (cyclopropene C=C) and 1463; δ_H: 0.77 (2H, s, cyclopropene CH_2), 0.88 (3H, t, *J* 6.9, CH_3CH$_2$), 1.29 (10H, m), 1.38 (2H, m), 1.52 (6H, m), 1.67 (2H, p, *J* 7.4, CH$_2$CH_2CH$_2$), 2.34 (2H, t, *J* 7.1, CH$_2$CH_2CN) and 2.39 (4H, app 2t, CH$_2$CH_2C=CCH_2CH$_2$); δ_C: 7.4, 14.1, 17.1, 22.3, 25.3, 25.8, 26.0, 27.0, 27.4, 28.5, 29.3, 29.4, 29.4, 31.9, 108.9, 109.8 and 119.8.

7-(1,2-Diiodo-2-octyl-cyclopropyl)-heptanenitrile (217)

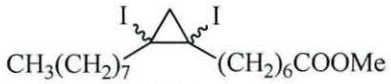


(217)

Iodine (0.83 g, 3.3 mmol) in dry ether (100 ml) was added dropwise to a stirred solution of 7-(2-octyl-cycloprop-1-enyl)-heptanenitrile **(216)** (0.74 g, 2.8 mmol) in dry ether (100 ml) at -90 °C. The mixture was allowed to reach room temperature and stirred for 1 hour. Once

TLC showed that no starting material remained, the reaction was quenched with a saturated aqueous solution of $Na_2S_2O_3$ (30 ml) to remove any excess iodine. The aqueous layer was extracted with ether (2 x 50 ml) and the combined organic layers were washed with water (2 x 20 ml), dried, filtered and evaporated. Column chromatography (petrol/ether, 5:1) gave 7-(1,2-diiodo-2-octyl-cyclopropyl)-heptanenitrile **(217)** (1.14 g, 2.2 mmol, 78 %) as a yellow oil {Found $(M + Na)^+$: 538.0416, $C_{18}H_{31}NI_2Na$ requires 538.0448}. This showed ν_{max}(film)/cm^{-1}: 2927 (C-H saturated), 2854 (C-H saturated), 2246 (C≡N) and 1462; δ_H: 0.90 (3H, t, *J* 6.9, CH_3CH_2), 1.30 (12H, m), 1.44 (2H, m), 1.55 (4H, m), 1.73 (6H, m), 2.13 (2H, m) and 2.38 (2H, t, *J* 7.1, CH_2CH_2CN); δ_C: 14.1, 17.1, 22.7, 23.9, 24.3, 25.3, 27.9, 28.6, 28.8, 29.3, 29.4, 29.5, 29.8, 31.9, 36.1, 50.5, 50.7 and 119.8.

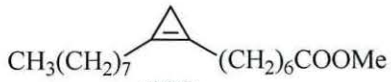
7-(1,2-Diiodo-2-octyl-cyclopropyl)-heptanoic acid methyl ester (218)



(218)

Gaseous HCl was bubbled through a solution of 2:1 dry ether/methanol (30 ml) and the solution was cooled to -79 °C. 7-(1,2-Diiodo-2-octyl-cyclopropyl)-heptanenitrile **(217)** (0.96 g, 1.9 mmol) in dry ether (20 ml) was added to the mixture. The solution was allowed to warm to room temperature and stirred overnight. The reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (100 ml). The solution was neutralised to pH 7 and extracted with ether (3 x 100 ml). The combined organic layers were dried, filtered and evaporated. Column chromatography (petrol/ether, 5:1) gave 7-(1,2-diiodo-2-octyl-cyclopropyl)-heptanoic acid methyl ester **(218)** (0.46 g, 0.84 mmol, 45 %) as a yellow oil {Found $(M + Na)^+$: 571.0514, $C_{19}H_{34}O_2I_2Na$ requires 571.0550}. This showed ν_{max}(film)/cm^{-1}: 2927 (C-H saturated), 2854 (C-H saturated), 1741 (C=O) and 1462; δ_H: 0.89 (3H, t, *J* 6.9, CH_3CH_2), 1.28 (16H, m), 1.56 (2H, m), 1.66 (4H, m), 1.77 (2H, m), 2.10 (2H, m), 2.33 (2H, t, *J* 7.6, CH_2CH_2CO) and 3.68 (3H, s, CH_3COO); δ_C: 14.1, 22.7, 24.2, 24.4, 24.9, 28.4, 28.8, 19.1, 29.3, 29.5, 29.6, 29.8, 31.9, 34.1, 36.1, 50.6, 50.7, 51.5 and 174.3.

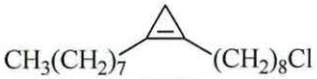
7-(2-Octyl-cycloprop-1-enyl)-heptanoic acid methyl ester (206)



(206)

n-Butyllithium (1.6 M, 0.49 ml, 0.78 mmol) was added dropwise to a stirred solution of 7-(1,2-diiodo-2-octyl-cyclopropyl)-heptanoic acid methyl ester **(218)** (0.39 g, 0.71 mmol) in dry ether (10 ml) at -90 °C. The temperature was allowed to reach -70 °C and water (1 ml) was added dropwise. The reaction mixture was extracted with ether (2 x 20 ml) and the combined organic layers were washed with water (10 ml), dried, filtered and evaporated. Column chromatography (petrol/ether, 5:1) gave 7-(2-Octyl-cycloprop-1-enyl)-heptanoic acid methyl ester **(206)** (0.15 g, 0.51 mmol, 72 %) as a yellow oil {Found $(M + Na)^+$: 317.2442, $C_{19}H_{34}O_2Na$ requires 317.2457}. This showed ν_{max}(film)/cm^{-1}: 2926 (C-H saturated), 2856 (C-H saturated), 1871 (cyclopropene C=C), 1743 (C=O) and 1462; δ_H: 0.77 (2H, s, cyclopropene C*H*$_2$), 0.89 (3H, t, *J* 6.9, C*H*$_3$CH$_2$), 1.29 (14H, m), 1.55 (4H, m), 1.63 (2H, m), 2.31 (2H, t, *J* 7.6, CH$_2$C*H*$_2$COO), 2.38 (4H, app 2t, CH$_2$C*H*$_2$C=CC*H*$_2$CH$_2$) and 3.67 (3H, s, C*H*$_3$COO); δ_C: 7.4, 14.1, 22.7, 24.9, 25.9, 26.0, 27.2, 27.4, 28.9, 29.0, 29.3, 29.4, 31.9, 34.0, 51.4, 109.1, 109.5, 174.3.

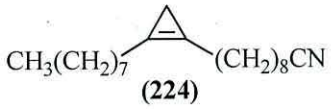
1-(8-Chloro-octyl)-2-octyl-cyclopropene (223)



(223)

n-Butyllithium (1.6 M, 22.1 ml, 35.2 mmol) was added dropwise to a stirred solution of 1,1,2-tribromo-2-octyl cyclopropane **(208)** (5.5 g, 14.2 mmol) in dry ether (50 ml) under N_2 (g) at -78 °C. The mixture was allowed to reach room temperature, stirred for 30 minutes and then cooled to 0 °C. HMPA (5.08 g, 4.94 ml, 28.4 mmol) was added dropwise followed by 1-chloro-8-iodo-octane **(219)** (3.5 g, 12.8 mmol). The mixture was stirred overnight at room temperature and then cooled to 0°C. Water (50 ml) was added and the solution was extracted with ether (2 x 100 ml). The combined organic layers were washed with water (2 x 20 ml), dried, filtered and evaporated. Column chromatography (petrol) gave 1-(8-chloro-octyl)-2-octyl-cyclopropene **(223)** (1.81 g, 6.06 mmol, 43 %) as a colourless oil. This showed ν_{max}(film)/cm^{-1}: 2927 (C-H saturated), 2856 (C-H saturated), 1872 (cyclopropene C=C) and 1464; δ_H: 0.78 (2H, s, cyclopropene C*H*$_2$), 0.89 (3H, t, *J* 6.9, C*H*$_3$CH$_2$), 1.32 (16H, m), 1.43 (2H, m), 1.55 (4H, m), 1.78 (2H, p, *J* 7.1, CH$_2$C*H*$_2$CH$_2$Cl), 2.39 (4H, app 2t, *J* 7.1, CH$_2$C*H*$_2$C=CC*H*$_2$CH$_2$) and 3.54 (2H, t, *J* 6.8, CH$_2$C*H*$_2$Cl); δ_C: 7.4, 14.1, 22.7, 26.0, 26.1, 26.9, 27.3, 27.4, 28.9, 29.2, 29.3, 29.3, 29.4, 31.9, 32.7, 45.1, 109.2 and 109.4. No mass spectrum could be obtained for this sample.

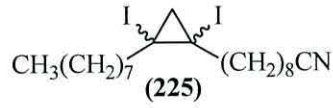
9-(2-Octyl-cycloprop-1-enyl)-nonanenitrile (224)



(224)

Sodium cyanide (0.81 g, 16.6 mmol) was added to a stirred solution of 1-(8-chloro-octyl)-2-octyl-cyclopropene **(223)** (1.65 g, 5.5 mmol) in DMSO (25 ml). The mixture was heated to 60 °C for 6 hours or until the TLC showed no starting material remaining. Water (15 ml) was added to the cooled mixture and the product extracted with ether (2 x 100 ml). The combined organic layers were washed with a saturated aqueous solution of NH_4Cl (30 ml), dried, filtered and evaporated. Column chromatography (petrol/ether, 5:1) gave 9-(2-octyl-cycloprop-1-enyl)-nonanenitrile **(224)** (1.16 g, 4.0 mmol, 73 %) as a yellow oil {Found $(M + H)^+$: 290.2842, $C_{20}H_{36}N$ requires 290.2842}. This showed ν_{max}(film)/cm^{-1}: 2928 (C-H saturated), 2854 (C-H saturated), 2247 (C≡N) and 1462; δ_H: 0.77 (2H, s, cyclopropene CH_2), 0.89 (3H, t, *J* 6.7, CH_3CH_2), 1.29 (16H, m), 1.45 (2H, m), 1.54 (4H, m), 1.66 (2H, p, *J* 7.4, $CH_2CH_2CH_2CN$), 2.34 (2H, t, *J* 7.1 CH_2CH_2CN) and 2.38 (4H, app 2t, *J* 7.1, $CH_2CH_2C{=}CCH_2CH_2$); δ_C: 7.4, 14.1, 17.1, 22.7, 25.4, 26.0, 26.1, 27.3, 27.4, 28.7, 28.8, 29.1, 29.2, 29.3, 29.4, 31.9, 109.2, 109.5 and 119.8.

9-(1,2-Diiodo-2-octyl-cyclopropyl)-nonanenitrile (225)

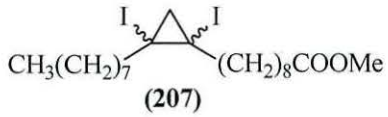


(225)

Iodine (1.0 g, 3.9 mmol) in dry ether (150 ml) was added dropwise to a stirred solution of 9-(2-octyl-cycloprop-1-enyl)-nonanenitrile **(224)** (0.99 g, 3.4 mmol) in dry ether (100 ml) at -90 °C. The mixture was allowed to reach room temperature and stirred for 1 hour. Once the TLC showed no starting material remaining, the reaction was quenched with a saturated solution of aqueous $Na_2S_2O_3$ (50 ml) to remove any excess iodine. The aqueous layer was extracted with ether (2 x 100 ml) and the combined organic layers were washed with water (2 x 25 ml), dried, filtered and evaporated. Column chromatography (petrol/ether, 5:1) gave 9-(1,2-diiodo-2-octyl-cyclopropyl)-nonanenitrile **(225)** (1.53 g, 2.8 mmol, 83 %) as an orange oil {Found $(M + Na)^+$: 566.0747, $C_{20}H_{35}NI_2Na$ requires 566.0761}. This showed ν_{max}(film)/cm^{-1}: 2928 (C-H saturated), 2854 (C-H saturated), 2246 (C≡N) and 1463; δ_H: 0.89 (3H, t, *J* 6.8, CH_3CH_2), 1.34 (18H, m), 1.53 (4H, m), 1.67 (4H, m), 1.77 (2H, m), 2.10 (2H, m), and 2.34 (2H, t, *J* 7.1 CH_2CH_2CN); δ_C: 14.1, 17.2, 22.6,

22.7, 24.3, 24.5, 25.4, 28.6, 28.7, 28.8, 29.2, 29.3, 29.5, 29.7, 29.8, 31.9, 36.1, 50.6, 50.7 and 119.8.

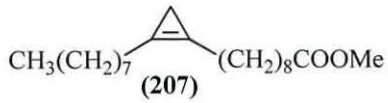
9-(1,2-Diiodo-2-octyl-cyclopropyl)-nonanoic acid methyl ester (207)



(207)

Gaseous HCl was bubbled through a solution of 2:1 dry ether/methanol (30 ml) and the solution was cooled to -79 °C. 9-(1,2-Diiodo-2-octyl-cyclopropyl)-nonanenitrile **(225)** (1.31 g, 2.4 mmol) in dry ether (20 ml) was added to the mixture. The solution was allowed to warm to room temperature and stirred overnight. A saturated aqueous solution of $NaHCO_3$ (100 ml) was added slowly. The solution was neutralised to pH 7 and extracted with ether (3 x 100 ml). The combined organic layers were dried, filtered and evaporated. Column chromatography (petrol/ether, 5:1) gave 9-(1,2-diiodo-2-octyl-cyclopropyl)-nonanoic acid methyl ester **(226)** (0.85 g, 1.5 mmol, 61 %) as a yellow oil {Found $(M + Na)^+$: 599.0826, $C_{21}H_{38}O_2I_2Na$ requires 599.0863}. This showed ν_{max}(film)/cm^{-1}: 2927 (C-H saturated), 2854 (C-H saturated), 1714 (C=O) and 1462; δ_H: 0.89 (3H, t, *J* 6.8, CH_3CH_2), 1.31 (20H, m), 1.65 (6H, m), 1.78 (2H, m), 2.11 (2H, m), 2.33 (2H, t, *J* 7.6, CH_2CH_2COO) and 3.69 (3H, s, $COOCH_3$); δ_C: 14.1, 22.6, 22.7, 24.4, 24.5, 25.0, 28.7, 28.8, 29.2, 29.3, 29.4, 29.5, 29.7, 29.8, 31.9, 34.1, 36.1, 50.7, 50.7, 51.5 and 174.3.

9-(2-Octyl-cycloprop-1-enyl)-nonanoic acid methyl ester (207)

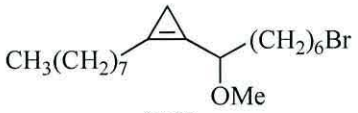


(207)

n-Butyllithium (1.6 M, 1.3 ml, 2.1 mmol) was added dropwise to a stirred solution of 9-(1,2-diiodo-2-octyl-cyclopropyl)-nonanoic acid methyl ester **(226)** (0.61 g, 1.9 mmol) in dry ether (10 ml) at -90 °C. The temperature was allowed to reach -70 °C and water (1 ml) was added dropwise. The product was extracted with ether (2 x 20 ml) and the combined organic layers were washed with water (10 ml), dried, filtered and evaporated. Column chromatography (petrol/ether, 5:1) gave 9-(2-octyl-cycloprop-1-enyl)-nonanoic acid methyl ester **(207)** (0.18 g, 0.56 mmol, 29 %) as a colourless oil {Found $(M + Na)^+$: 345.2752, $C_{21}H_{38}O_2Na$ requires 345.2770}. This showed ν_{max}(film)/cm^{-1}: 2926 (C-H

saturated), 2854 (C-H saturated), 1869 (cyclopropene C=C), 1744 (C=O) and 1464; δ_H: 0.77 (2H, s, cyclopropene CH_2), 0.89 (3H, t, *J* 7.0, CH_3CH$_2$), 1.31 (18H, m), 1.54 (4H, m), 1.63 (2H, m), 2.31 (2H, t, *J* 7.6, CH$_2$CH_2COO), 2.38 (4H, app 2t, CH$_2$CH_2C=CCH_2CH$_2$) and 3.67 (3H, s, COOCH_3); δ_C: 7.4, 14.1, 22.7, 25.0, 26.0, 26.1, 27.3, 27.4, 29.1, 29.2, 29.2, 29.3, 29.3, 29.4, 31.9, 34.1, 51.4, 109.3, 109.4 and 174.3.

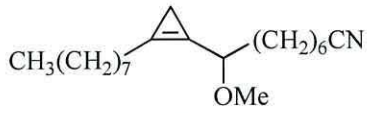
(±)-1-(7-Bromo-1-methoxyheptyl)-2-octyl-cyclopropene (229)



(229)

n-Butyllithium (1.6 M, 26.9 ml, 43.2 mmol) was added dropwise to a stirred solution of 1,1,2-tribromo-2-octyl cyclopropane **(208)** (6.75 g, 17.2 mmol) in dry ether (60 ml) under N_2 (g) at -78 °C. The mixture was allowed to reach room temperature, stirred for 30 min and then cooled to 0 °C. HMPA (6.18 g, 6.0 ml, 34.5 mmol) was added dropwise followed by 7-bromo-heptanal **(228)** (3.0 g, 15.5 mmol). The mixture was stirred for 3 hours and iodomethane (24.51 g, 10.76 ml, 172.7 mmol) was added. The mixture was stirred overnight at room temperature, cooled to 0 °C and water (70 ml) was added. The solution was extracted with ether (2 x 100 ml) and the combined organic layers washed with water (2 x 20 ml), dried, filtered and evaporated. Column chromatography (petrol/ether, 5:1) gave (±)-1-(7-bromo-1-methoxy-heptyl)-2-octyl-cyclopropene **(229)** (3.24 g, 9.0 mmol, 53 %) as a colourless oil. This showed ν_{max}(film)/cm^{-1}: 2925 (C-H saturated), 2854 (C-H saturated) and 1463; δ_H: 0.88 (3H, t, *J* 6.9, CH_3CH$_2$), 0.91 (1H, d, *J* 8.5, cyclopropene C*H*(H)), 0.95 (1H, d, *J* 8.5, cyclopropene C*H*(H)), 1.30 (16 H, m), 1.57 (2H, p, *J* 7.3, CH$_2$CH_2CH$_2$), 1.68 (2H, m, CH_2), 1.81 (2H, m, CH_2), 2.45 (2H, dt, *J* 0.6 and 7.3, CH$_2$CH_2CCH(H)), 3.18 (2H, t, *J* 7.0, CH$_2$CH_2Br), 3.31 (3H, s, CH_3O) and 4.19 (1H, t, *J* 6.3, CH$_3$OC*H*CH$_2$); δ_C: 7.0, 7.5, 14.1, 22.7, 25.1, 26.1, 27.2, 28.5, 29.2, 29.3, 29.4, 30.4, 31.9, 33.3, 33.4, 26.6, 76.3, 108.6 and 114.1. No mass spectrum could be obtained for this sample.

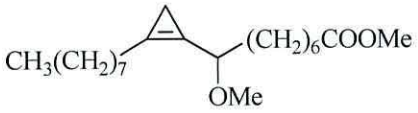
(±)-8-Methoxy-8-(2-octyl-cycloprop-1-enyl)-octanenitrile (230)



(230)

Sodium cyanide (0.97 g, 19.8 mmol) was added to a stirred solution of (±)-1-(7-bromo-1-methoxy-heptyl)-2-octyl-cyclopropene **(229)** (2.97 g, 8.27 mmol) in DMSO (40 ml). The mixture was heated to 60 °C for 3 hours or until the TLC showed no starting material remaining. Water (15 ml) was added to the cooled solution and the product was extracted with ether (2 x 100 ml). The combined organic layers were washed with a saturated aqueous solution of NH_4Cl (30 ml), dried, filtered and evaporated. Column chromatography (petrol/ether, 5:2) gave (±)-8-methoxy-8-(2-octyl-cycloprop-1-enyl)-octanenitrile **(230)** (1.85 g, 6.1 mmol, 73 %) as a yellow oil {Found $(M + Na)^+$: 328.2595, $C_{20}H_{35}ONNa$ requires 328.2616}. This showed ν_{max}(film)/cm^{-1}: 2925 (C-H saturated), 2854 (C-H saturated), 2246 (C≡N), 1865 (cyclopropene C=C) and 1465; δ_H: 0.89 (3H, t, *J* 6.8, C*H*$_3$CH$_2$), 0.93 (1H, d, *J* 8.5, cyclopropene C*H*(H)), 0.96 (1H, d, *J* 8.2, cyclopropene C*H*(H)), 1.30 (14H), 1.46 (2H, m), 1.58 (2H, m), 1.68 (4H, m), 2.33 (2H, t, *J* 7.1, CH$_2$C*H*$_2$CN), 2.45 (2H, t, *J* 7.3, CH$_2$C*H*$_2$C=C), 3.32 (3H, s, C*H*$_3$O) and 4.20 (1H, t, *J* 6.6, CH$_3$OC*H*CH$_2$); δ_C: 7.5, 14.1, 17.1, 22.7, 25.0, 25.3, 26.1, 27.2, 28.6, 28.7, 29.3, 39.4, 29.4, 31.8, 33.3, 56.6, 76.3, 108.6, 114.2 and 119.8.

(±)-8-Methoxy-8-(2-octyl-cycloprop-1-enyl)-octanoic acid methyl ester (227)

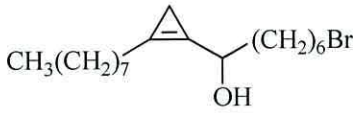


(227)

Sodium hydroxide (0.39 g, 9.6 mmol) was dissolved in a mixture of ethanol (3.5 ml) and water (0.5 ml). (±)-8-Methoxy-8-(2-octyl-cycloprop-1-enyl)-octanenitrile **(230)** (0.5 g, 1.6 mmol) was then added and the mixture heated under reflux under N_2 (g) for 8 hours. The ethanol was evaporated and to the residue a 0.1 M TBAH solution (40 ml) was added. Dichloromethane (40 ml) and iodomethane (2.3 g, 1.02 ml, 16.4 mmol) were then added and the mixture was stirred for 12 hours. The reaction mixture was separated and the organic layer was washed with water (3 x 20 ml) and brine (20 ml). The organic solution was dried, filtered and evaporated. Column chromatography (petrol/ethyl acetate, 5:1) gave (±)-8-methoxy-8-(2-octyl-cycloprop-1-enyl)-octanoic acid methyl ester **(227)** (0.52 g, 1.5 mmol, 93 %) as a colourless oil {Found $(M + Na)^+$: 361.2679, $C_{21}H_{38}O_3Na$ requires 361.27119}. This showed ν_{max}(film)/cm^{-1}: 2927 (C-H saturated), 2855 (C-H saturated), 1863 (cyclopropene C=C), 1743 (C=O) and 1464; δ_H: 0.88 (3H, t, *J* 6.9, C*H*$_3$CH$_2$), 0.90 (1H, d, *J* 8.5, cyclopropene C*H*(H)), 0.92 (1H, d, *J* 8.5, cyclopropene C*H*(H)), 1.31 (16H,

m), 1.61 (6H, m), 2.29 (2H, t, *J* 7.6, CH_2CH_2COO), 2.44 (2H, t, *J* 7.25, $CH_2CH_2C{=}C$), 3.30 (3H, s, OCH_3), 3.66 (3H, s, $COOCH_3$) and 4.19 (1H, t, *J* 6.5, CH_3OCHCH_2); δ_C: 7.5, 14.1, 22.6, 24.9, 25.2, 26.1, 27.2, 29.1, 29.2, 29.3, 29.3, 29.4, 31.9, 33.3, 34.0, 51.4, 56.5, 76.4, 108.6, 114.0 and 174.2.

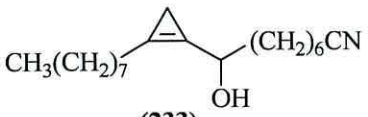
(±)-7-Bromo-1-(2-octyl-cycloprop-1-enyl)-heptan-1-ol (232)[241]



(232)

n-Butyllithium (1.6 M, 30.58 ml, 48.9 mmol) was added dropwise to a stirred solution of 1,1,2-tribromo-2-octyl cyclopropane **(208)** (7.65 g, 19.6 mmol) in dry ether (60 ml) under N_2 (g) at -78 °C. The mixture was allowed to reach room temperature, stirred for 30 min and cooled to 0 °C. HMPA (7.0 g, 6.8 ml, 39.1 mmol) was added dropwise followed by 7-bromo-heptanal (3.4 g, 17.6 mmol). The mixture was stirred overnight at room temperature, cooled to 0 °C and water (50 ml) was added. The reaction mixture was extracted with ether (2 x 100 ml) and the combined organic layers were washed with water (2 x 20 ml), dried, filtered and evaporated. Column chromatography (petrol/ether, 5:1) gave (±)-7-bromo-1-(2-octyl-cycloprop-1-enyl)-heptan-1-ol **(232)**[241] (2.42 g, 7.0 mmol, 54 %) as a colourless oil. This showed ν_{max}(film)/cm^{-1}: 3356 (br O-H), 2930 (C-H saturated), 2854 (C-H saturated), 1868 (cyclopropene C=C) and 1464; δ_H: 0.89 (3H, t, *J* 6.9, CH_3CH_2), 0.95 (2H, s, cyclopropene CH_2), 1.38 (17H, m), 1.57 (2H, m), 1.69 (2H, m), 1.87 (2H, p, *J* 7.2, $CH_2CH_2CH_2$), 2.44 (2H, dt, *J* 1.1 and 7.3, $CH_2CH_2CCH(H)$), 3.41 (2H, t, *J* 6.9, CH_2CH_2Br) and 4.61 (1H, t, *J* 6.3, $OHCHCH_2$); δ_C: 7.5, 14.1, 22.7, 25.0, 25.9, 27.4, 28.1, 28.6, 29.2, 29.3, 29.4, 31.9, 32.7, 33.8, 35.6, 67.8, 110.4 and 112.6.

(±)-8-Hydroxy-8-(2-octyl-cycloprop-1-enyl)-octanenitrile (233)[241]

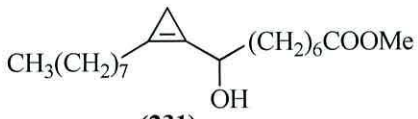


(233)

Sodium cyanide (1.28 g, 26.1 mmol) was added to a stirred solution of (±)-7-bromo-1-(2-octyl-cycloprop-1-enyl)-heptan-1-ol **(232)** (3.0 g, 8.7 mmol) in DMSO (40 ml). The mixture was heated to 60 °C for 3 hours or until the TLC showed no starting material

remaining. Water (15 ml) was added to the cooled solution and the product extracted with ether (2 x 100 ml). The combined organic layers were washed with a saturated aqueous solution of NH_4Cl (30 ml), dried, filtered and evaporated. Column chromatography (petrol/ether, 5:4) gave (±)-8-hydroxy-8-(2-octyl-cycloprop-1-enyl)-octanenitrile **(233)**[241] (1.19 g, 4.1 mmol, 70 %) as a yellow oil {Found $(M + Na)^+$: 314.2460, $C_{19}H_{33}ONNa$ requires 314.2460}. This showed ν_{max}(film)/cm^{-1}: 3443 (br O-H), 2931 (C-H saturated), 2854 (C-H saturated), 2247 (C≡N), 1868 (cyclopropene C=C) and 1465; δ_H: 0.88 (3H, t, *J* 6.9, CH_3CH_2), 0.94 (2H, s, cyclopropene CH_2), 1.39 (17H, m), 1.57 (2H, m), 1.68 (4H, m), 2.34 (2H, t, *J* 7.3, CH_2CH_2CN), 2.44 (2H, dt, *J* 1.1, 7.4, $CH_2CH_2CCH(H)$) and 4.61 (1H, t, *J* 6.1, $OHCHCH_2$); δ_C: 7.5, 14.1, 17.1, 22.6, 24.9, 25.3, 25.9, 27.4, 28.6, 28.7, 29.2, 29.3, 29.4, 31.9, 35.6, 67.8, 110.4, 112.7 and 119.7.

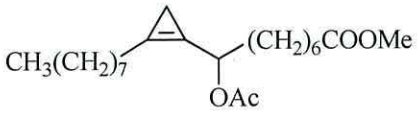
(±)-8-Hydroxy-8-(2-octylcycloprop-1-enyl)-octanoic acid methyl ester (231)[241]



(231)

Sodium hydroxide (0.88 g, 21.9 mmol) was dissolved in a mixture of ethanol (7 ml) and water (1 ml). (±)-8-Hydroxy-8-(2-octyl-cycloprop-1-enyl)-octanenitrile **(233)** (1.1 g, 3.78 mmol) was then added and the mixture heated under reflux under N_2 (g) for 8 hours. The ethanol was evaporated and to the residue a 0.1 M TBAH solution (80 ml) was added. Dichloromethane (80 ml) and iodomethane (5.37 g, 2.36 ml, 37.8 mmol) were then added and the mixture was stirred for 12 hours. The reaction mixture was separated and the organic layer was washed with water (3 x 30 ml) and brine (30 ml). The organic solution was dried, filtered and evaporated. Column chromatography (petrol/ethyl acetate, 6:4) gave (±)-8-hydroxy-8-(2-octylcycloprop-1-enyl)-octanoic acid methyl ester **(231)**[241] (0.81 g, 2.5 mmol, 66 %) as a yellow oil. This showed ν_{max}(film)/cm^{-1}: 3430 (br O-H), 2928 (C-H saturated), 2857 (C-H saturated), 1869 (cyclopropene C=C), 1742 (C=O) and 1462; δ_H: 0.88 (3H, t, *J* 6.9, CH_3CH_2), 0.93 (2H, s, cyclopropene CH_2), 1.32 (16 H, m), 1.62 (6H, m), 1.77 (1H, br s, O*H*), 2.30 (2H, t, *J* 7.5, CH_2CH_2C=C), 2.43 (2H, dt, *J* 0.95, 7.2, CH_2CH_2CHOH), 3.66 (3H, s, $COOCH_3$) and 4.59 (1H, t, *J* 6.4, CH_2CHOH); δ_C: 7.5, 14.1, 22.7, 24.9, 25.0, 25.9, 27.4, 29.1, 29.1, 29.3, 29.3, 29.4, 31.9, 34.1, 35.7, 51.4, 67.9, 110.4, 112.5 and 174.3.

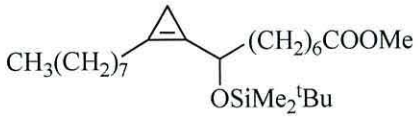
(±)-8-Acetoxy-8-(2-octylcycloprop-1-enyl)octanoic acid methyl ester (234)



(234)

Pyridine (0.24 g, 0.25 ml, 3.08 mmol) and acetic anhydride (0.31 g, 0.29 ml, 3.08 mmol) were added to a stirred solution of (±)-8-hydroxy-8-(2-octylcycloprop-1-enyl)octanodecanoic acid methyl ester **(231)** (0.20 g, 0.617 mmol) in dry toluene (10 ml) at room temperature under N_2 (g). The mixture was stirred overnight. The solvent was then evaporated and dilute acid was added to the residue. The solution was extracted with dichloromethane (3 x 20 ml), dried, filtered and evaporated. Column chromatography (petrol/ether, 7:3) gave (±)-8-acetoxy-8-(2-octylcycloprop-1-enyl)octanoic acid methyl ester **(234)** (0.22 g, 0.601 mmol, 97 %) as a yellow oil {Found $(M + Na)^+$: 389.2689, $C_{22}H_{38}O_4Na$ requires 389.2668}. This showed ν_{max}(film)/cm^{-1}: 2928 (C-H saturated), 2856 (C-H saturated), 1873 (cyclopropene C=C), 1742 (C=O) and 1463; δ_H: 0.86 (3H, t, *J* 6.9, C*H*$_3$CH$_2$), 0.92 (1H, d, *J* 8.5, cyclopropene C*H*(H)), 0.95 (1H, d, *J* 8.7, cyclopropene C*H*(H)), 1.31 (16H, m), 1.53 (2H, m), 1.60 (2H, m), 1.74 (2H, m), 2.06 (3H, s, COC*H*$_3$), 2.28 (2H, t, *J* 7.6, CH$_2$C*H*$_2$C=C), 2.39 (2H, dt, *J* 1.25, 7.25, AcOCHC*H*$_2$CH$_2$), 3.65 (3H, s, COOC*H*$_3$) and 5.65 (1H, t, *J* 6.5, AcOC*H*CH$_2$); δ_C: 8.4, 14.1, 21.1, 22.6, 24.8, 24.9, 25.9, 27.2, 29.0, 29.2, 29.3, 31.8, 32.6, 34.0, 51.4, 70.3, 108.0, 114.2, 170.4 and 174.1.

(±)-8-(*tert*-Butyldimethylsilyloxy)-8-(2-octylcycloprop-1-enyl)octanoic acid methyl ester (235)

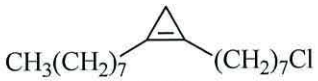


(235)

Imidazole (0.10 g, 1.5 mmol) was added to a stirred solution of (±)-8-hydroxy-8-(2-octylcycloprop-1-enyl)-octanoic acid methyl ester **(231)** (0.20 g, 0.617 mmol) in dry DMF (10 ml) at room temperature. The solution was cooled to 0 °C and *tert*-butyldimethylchlorosilane (0.12 g, 0.80 mmol) was added. The cooling bath was removed and the reaction mixture was stirred at 45 °C for 20 hours. A few crystals of DMAP, imidazole (0.10 g, 0.15 mmol) and *tert*-butyldimethylchlorosilane (0.12 g, 0.80 mmol)

were added to the solution and the temperature was increased to 70 °C. The reaction mixture was then stirred for another 20 hours. Once TLC showed complete reaction, the DMF was removed by flash distillation. Water (30 ml) was then added and the product was extracted with dichloromethane (3 x 30 ml). The combined organic layers were washed with water (25 ml), dried, filtered and evaporated. Column chromatography (petrol/ether, 7:3) gave (±)-8-(*tert*-butyldimethylsilyloxy)-8-(2-octylcycloprop-1-enyl)octanoic acid methyl ester **(235)** (0.17 g, 0.387 mmol, 63 %) as a yellow oil {Found $(M + Na)^+$: 461.3425, $C_{26}H_{50}O_3SiNa$ requires 461.3421}. This showed ν_{max}(film)/cm^{-1}: 2929 (C-H saturated), 2854 (C-H saturated), 1870 (cyclopropene C=C), 1744 (C=O) and 1463; δ_H: 0.03 (3H, s, CH_3Si), 0.05 (3H, s, CH_3Si), 0.89 (14H, m, cyclopropene CH_2, $C(CH_3)_3$, CH_3CH_2), 1.29 (16H, m), 1.54 (2H, m), 1.62 (4H, m), 2.29 (2H, t, *J* 7.5, CH_2CH_2C=C), 2.40 (2H, t, *J* 7.1, SiOCHCH_2CH_2), 3.65 (3H, s, COOCH_3), 4.59 (1H, t, *J* 6.3, CH_2CHOSi); δ_C: -5.1, -4.6, 8.0, 14.1, 18.3, 22.7, 24.9, 25.1, 25.8, 25.8, 27.2, 29.1, 29.2, 29.3, 29.4, 29.4, 31.9, 34.0, 36.2, 51.3, 68.5, 111.1, 111.3 and 174.2.

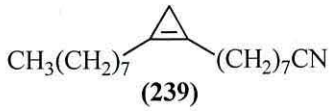
1-(7-Chloroheptyl)-2-octylcycloprop-1-ene (238)



(238)

n-Butyllithium (1.6 M, 26.7 ml, 42.71 mmol) was added dropwise to a stirred solution of 1,1,2-tribromo-2-octyl cyclopropane **(208)** (6.68 g, 17.1 mmol) in dry ether (50 ml) under N_2 (g) at -78 °C. The mixture was allowed to reach room temperature, stirred for 30 min and then cooled to 0 °C. HMPA (6.11 g, 5.9 ml, 34.17 mmol) was added dropwise followed by 1-chloro-7-iodo-heptane **(236)** (3.4 g, 17.6 mmol). The mixture was stirred overnight at room temperature, cooled to 0°C and water (50 ml) was added. The solution was extracted with ether (2 x 100 ml) and the combined organic layers were washed with water (2 x 20 ml), dried, filtered and evaporated. Column chromatography (petrol/ether, 5:1) gave 1-(7-chloroheptyl)-2-octylcycloprop-1-ene **(238)** (2.02 g, 7.1 mmol, 42 %) as a colourless oil. This showed ν_{max}(film)/cm^{-1}: 2927 (C-H saturated), 2856 (C-H saturated), 1871 (cyclopropene C=C) and 1463; δ_H: 0.78 (2H, s, cyclopropene CH_2), 0.89 (3H, t, *J* 6.9, CH_3CH_2), 1.30 (14H, m), 1.44 (2H, m), 1.55 (4H, m), 1.78 (2H, p, *J* 7.2, $CH_2CH_2CH_2Cl$), 2.38 (4H, app dt, CH_2CH_2C=CCH_2CH_2) and 3.54 (2H, t, *J* 6.8, CH_2CH_2Cl); δ_C: 7.4, 14.1, 22.7, 25.9, 26.0, 26.8, 27.3, 27.4, 28.7, 29.2, 29.3, 29.4, 31.9, 32.6, 45.1, 109.2 and 109.5. No mass spectrum could be obtained for this sample.

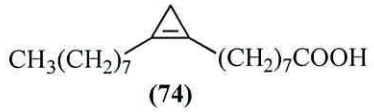
8-(2-Octylcycloprop-1-enyl)octanenitrile (239)



(239)

Sodium cyanide (1.02 g, 20.8 mmol) was added to a stirred solution of 1-(7-chloroheptyl)-2-octylcycloprop-1-ene **(238)** (1.92 g, 6.8 mmol) in DMSO (40 ml). The mixture was heated to 60 °C for 4 hours or until the TLC showed no starting material remaining. Water (15 ml) was added to the cooled solution and the solution was extracted with ether (2 x 100 ml). The combined organic layers were washed with a saturated aqueous solution of NH_4Cl (30 ml), dried, filtered and evaporated. Column chromatography (petrol/ether, 5:4) gave 8-(2-octylcycloprop-1-enyl)octanenitrile **(239)** (1.53 g, 5.6 mmol, 81 %) as a yellow oil. This showed ν_{max}(film)/cm^{-1}: 2928 (C-H saturated), 2853 (C-H saturated), 2246 (C≡N), 1870 (cyclopropene C=C) and 1461; δ_H: 0.77 (2H, s, cyclopropene CH_2), 0.89 (3H, t, *J* 6.9, CH_3CH$_2$), 1.30 (14H, m), 1.46 (2H, m), 1.55 (4H, m), 1.67 (2H, p, *J* 7.4, CH$_2$CH_2CH$_2$CN), 2.34 (2H, t, *J* 7.3, CH$_2$CH_2CN) and 2.38 (4H, m, CH$_2$CH_2C=CCH_2CH$_2$); δ_C: 7.4, 14.1, 17.1, 22.7, 25.4, 25.9, 26.0, 27.2, 27.4, 28.6, 28.6, 29.0, 29.3, 29.4, 31.9, 109.1, 109.6 and 119.8. No mass specreum could be obtained for this sample.

8-(2-Octylcycloprop-1-enyl)octanoic acid (74)



(74)

Sodium hydroxide (1.05 g, 26.4 mmol) was dissolved in a mixture of ethanol (7 ml) and water (1 ml). 8-(2-Octylcycloprop-1-enyl)octanenitrile **(239)** (1.25 g, 4.5 mmol) was then added and the mixture heated under reflux under N_2 (g) for 8 hours. The ethanol was evaporated and to the residue a 0.1 M TBAH solution (80 ml) was added. Dichloromethane (80 ml) was then added and the mixture was stirred for 12 hours. The solution was acidified to ~pH 3 with aqueous potassium hydrogen sulfate. The mixture was separated and the organic solution was washed with water (3 x 30 ml) and the solution was dried, filtered and evaporated. Column chromatography (petrol/ethyl acetate, 5:2) gave 8-(2-octylcycloprop-1-enyl)octanoic acid **(74)** (0.82 g, 2.8 mmol, 63 %) as a colourless oil. This showed ν_{max}(film)/cm^{-1}: 3027 (br O-H), 2926 (C-H saturated), 2850 (C-H saturated), 1707 (C=O) and 1458; δ_H: 0.77 (2H, s, cyclopropene CH_2), 0.89 (3H, t, *J* 6.8, CH_3CH$_2$), 1.33 (16 H, m), 1.54 (4H, m), 1.64 (2H, m, CH_2), 2.35 (2H, t, *J* 7.6, CH$_2$CH_2CO) and 2.38 (4H, t, *J*

7.1, $CH_2CH_2C{=}CCH_2CH_2$); δ_C: 7.4, 14.1, 22.7, 24.6, 25.9, 26.0, 27.3, 27.4, 29.0, 29.1, 29.2, 29.3, 29.4, 31.9, 34.0, 109.2, 109.5 and 179.8. No mass spectrum could be obtained for this sample.

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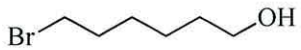
Appendices

6-Bromo-hexan-1-ol (213)[246]



(213)

Aqueous HBr (48%) (30 ml) was added to a stirred solution of 1,6-hexanediol **(212)** (25.0 g, 0.21 mol) in toluene (200 ml) and the solution was heated under reflux overnight. The layers were separated and the aqueous layer was extracted with toluene (2 x 100 ml). The combined organic extracts were dried, filtered and evaporated. Column chromatography (petrol/ether, 1:1) gave 6-bromo-hexan-1-ol **(213)**[246] (29.6 g, 0.16 mol, 78 %) as a yellow oil. This showed ν_{max}(film)/cm^{-1}: 3345 (br O-H), 2934 (C-H saturated), 2859 (C-H saturated) and 1460; δ_H: 1.39 (2H, p, *J* 6.9, $CH_2CH_2CH_2$), 1.47 (2H, p, *J* 7.3, $CH_2CH_2CH_2$), 1.58 (2H, p, *J* 6.9, $CH_2CH_2CH_2$), 1.71 (1H, br s, O*H*), 1.87 (2H, p, *J* 6.9, $CH_2CH_2CH_2$), 3.41 (2H, t, *J* 6.9, CH_2CH_2Br), 3.64 (2H, m, CH_2OH); δ_C: 24.9, 27.9, 32.0, 32.7, 33.8 and 62.7.

7-Bromo-heptan-1-ol (159)[246]

(157)

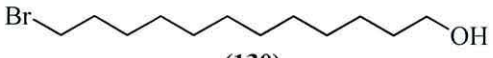
The procedure used for the synthesis of **(213)** was repeated using 1,7-heptanediol **(158)** (25.0 g, 0.19 mol), toluene (200 ml) and aqueous HBr (48%) (30 ml). Column chromatography (petrol/ether, 1:1) gave 7-bromo-heptan-1-ol **(159)**[246] (29.8 g, 0.15 mol, 79 %) as a yellow oil. This showed ν_{max}(film)/cm^{-1}: 3344 (br O-H), 2933 (C-H saturated), 2855 (C-H saturated) and 1463; δ_H: 1.36 (4H, m), 1.45 (2H, m), 1.53 (1H, br s, O*H*), 1.57 (2H, p, *J* 6.9, $CH_2CH_2CH_2Br$), 1.86 (2H, p, *J* 7.2, $CH_2CH_2CH_2OH$), 3.40 (2H, t, *J* 6.8, CH_2CH_2Br) and 3.63 (2H, t, *J* 6.6, CH_2CH_2OH); δ_C: 25.6, 28.1, 28.5, 32.6, 32.7, 33.9 and 62.9.

8-Bromo-octan-1-ol (221)[246]

(221)

The procedure used for the synthesis of **(213)** was repeated using 1,8-octanediol **(220)** (25.0 g, 0.17 mol), toluene (200 ml) and aqueous HBr (48%) (30 ml). Column chromatography (petrol/ether, 1:3) gave 8-bromo-octan-1-ol **(221)**[246] (28.9 g, 0.14 mol, 82 %) as a yellow oil. This showed ν_{max}(film)/cm^{-1}: 3333 (br O-H), 2930 (C-H saturated), 2855 (C-H saturated) and 1463; δ_H: 1.32 (6H, m), 1.42 (2H, p, *J* 7.3, $CH_2CH_2CH_2$), 1.56 (2H, p, *J* 6.9, $CH_2CH_2CH_2Br$), 1.72 (1H, br s, O*H*), 1.84 (2H, p, *J* 7.2, $CH_2CH_2CH_2OH$), 3.39 (2H, t, *J* 6.9, CH_2CH_2Br) and 3.61 (2H, t, *J* 6.6, CH_2CH_2OH); δ_C: 25.6, 28.0, 28.7, 29.2, 32.7, 32.8, 34.0 and 62.9.

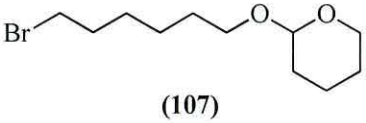
12-Bromododecan-1-ol (130)[246]



(130)

The procedure used for the synthesis of **(213)** was repeated using 1,12 dodecanediol **(129)** (25.0 g, 0.094 mmol), toluene (200 ml) and aqueous HBr (48%) (30 ml). Column chromatography (petrol/ethyl acetate, 5:1) gave 12-bromododecan-1-ol **(130)**[246] (19.73 g, 0.075 mmol, 80 %) as a white solid, m.p. 29-30 °C (lit m.p. 34-36 °C). This showed ν_{max}(nujol)/cm^{-1}: 3300 (broad O-H), 2924 (C-H saturated), 2853 (C-H saturated) and 1461; δ_H: 1.28 (14H, m), 1.42 (3H, br m), 1.57 (2H, p, *J* 6.9, $CH_2CH_2CH_2$), 1.86 (2H, p, *J* 7.2, $CH_2CH_2CH_2$), 3.41 (2H, t, *J* 7.0, CH_2CH_2Br) and 3.65 (2H, t, *J* 6.6, CH_2CH_2OH); δ_C: 25.7, 28.2, 28.7, 29.4, 29.5, 29.5, 29.6, 32.8, 32.8, 34.1 and 63.1.

2-(6-Bromohexyloxy)tetrahydro-2*H*-pyran (107)[247]

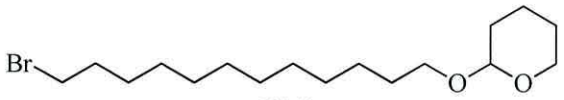


(107)

2,3-Dihydropyran (63.1 g, 0.75 mol, 63.6 ml) and pyridinium *p*-toluenesulfonate (3.5 g, 0.014 mol) were added to a stirred solution of 6-bromohexan-1-ol **(209)** (64.72 g, 0.36 mol) in dry dichloromethane (400 ml) under N_2 (g) at room temperature. The reaction was stirred for 3 hours or until the TLC showed no starting material remaining. The

reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (300 ml). The aqueous layer was extracted with dichloromethane (200 ml). The combined organic layers were washed with water (200 ml), dried, filtered and evaporated. Flash distillation gave 2-(6-bromohexyloxy)tetrahydro-2*H*-pyran **(107)**[247] (153.7 g, 0.58 mol, 77 %) as a colourless oil. This showed ν_{max}(film)/cm^{-1}: 2938 (C-H saturated), 2864 (C-H saturated) and 1453; δ_H: 1.51 (10H, m), 1.71 (1H, m, C*H*), 1.87 (3H, m), 3.41 (3H, m, OC*H*(H)CH_2 and CH_2CH_2Br), 3.50 (1H, m, OC*H*(H)CH_2), 3.74 (1H, dt, *J* 6.7, 9.6, OC*H*(H)CH_2), 3.86 (1H, m, OC*H*(H)CH_2) and 4.57 (1H, apparent br dd, O_2CHCH_2); δ_C: 19.7, 25.4, 25.5, 28.0, 29.6, 30.8, 32.8, 33.9, 62.4, 67.4 and 98.9.

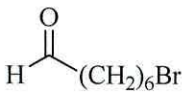
2-(12-Bromododecyloxy)tetrahydro-2*H*-pyran (131)[248]



(131)

The procedure used for the synthesis of **(107)** was repeated using 2,3-dihydropyran (7.04 g, 0.084 mol, 7.6 ml), pyridinium-*p*-toluene sulfonate (1.0 g, 3.98 mmol) and 12-bromododecan-1-ol **(130)** (10.57 g, 0.039 mmol) in dry dichloromethane (150 ml). Column chromatography (petrol/ethyl acetate, 20:1) gave 2-(12-bromododecyloxy)tetrahydro-2*H*-pyran **(131)**[248] (12.49 g, 0.036 mol, 92 %) as a colourless oil. This showed ν_{max}(film)/cm^{-1}: 2924 (C-H saturated), 2853 (C-H saturated) and 1641; δ_H: 1.27 (14H, m), 1.41 (2H, m), 1.56 (6H, m), 1.70 (1H, m), 1.85 (3H, m), 3.39 (3H, m, CH_2CH(CH)O and CH_2CH_2Br), 3.49 (1H, m, CH_2CH(CH)O), 3.73 (1H, dt, *J* 6.9, 9.6, CH_2CH(CH)O), 3.87 (1H, ddd, *J* 3.2, 7.7, 11.0, CH_2CH(CH)O), and 4.57 (1H, t, *J* 3.5, OC*H*(CH_2)O); δ_C: 19.7, 25.5, 26.2, 28.2, 28.8, 29.4, 29.5, 29.5, 29.5, 29.6, 29.8, 30.8, 32.8, 34.0, 62.3, 67.7 and 98.9.

7-Bromo-heptanal (228)

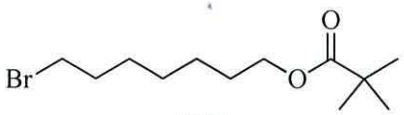


(228)

7-Bromo-heptan-1-ol **(257)** (3.25 g, 16.7 mmol) in dichloromethane (20 ml) was added dropwise to a stirred suspension of PCC (7.18 g, 33.4 mmol) in dichloromethane (100 ml). The mixture was then stirred for 2 hours. The solution was diluted with ether (400 ml),

filtered through silica and the solvent removed by rotary evaporation. Column chromatography (petrol/ether, 5:2) gave 7-bromo-heptanal **(228)** (2.51 g, 13.0 mmol, 78 %) as a colourless oil. This showed ν_{max}(film)/cm^{-1}: 2932 (C-H saturated), 2856 (C-H saturated), 1725 (C=O) and 1461; δ_H: 1.36 (2H, m), 1.47 (2H, m), 1.65 (2H, p, *J* 7.5, $CH_2CH_2CH_2$), 1.86 (2H, p, *J* 7.2, $CH_2CH_2CH_2Br$), 2.44 (2H, dt, *J* 1.7, 7.3, $CH_2CH_2C(O)H$), 3.41 (2H, t, *J* 6.8, CH_2CH_2Br) and 9.77 (1H, t, *J* 1.8, $CH_2C(O)H$); δ_C: 21.8, 27.9, 28.3, 32.5, 33.7, 43.7 and 202.5. Data were consistent with that in the literature.[241]

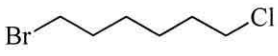
7-Bromoheptyl pivalate (160)



(158)

To a solution of 7-bromoheptanol **(159)** (25.22 g, 0.129 mol) in dichloromethane (100 ml), trimethylacetylchloride (18.71 g, 19.12 ml, 0.155 mol) in dichloromethane (80 ml) was added. The reaction mixture was placed in a water bath and triethylamine (26.17 g, 36.0 ml, 0.259 mol) was added slowly dropwise (some fuming observed). This was followed by the addition of DMAP (1.58 g, 0.0129 mol) and the reaction mixture was stirred overnight. The reaction mixture was washed with water (30 ml) and the organic solution was dried, filtered and evaporated. Column chromatography (petrol) gave 7-bromoheptyl pivalate **(160)** (30.88 g, 0.111 mol, 86 %) as a pale yellow oil. This showed ν_{max}(film)/cm^{-1}: 2931 (C-H saturated), 2859 (C-H saturated), 1720 (C=O), 1541, 1480 and 1460; δ_H: 1.21 (9H, s, $C(CH_3)_3$), 1.37 (4H, m), 1.45 (2H, m), 1.63 (2H, p, *J* 6.9, $CH_2CH_2CH_2Br$), 1.86 (2H, p, *J* 7.2, $CH_2CH_2CH_2O$), 3.40 (2H, t, *J* 6.8, CH_2CH_2Br) and 4.05 (2H, t, *J* 6.6, CH_2CH_2O); δ_C: 25.7, 27.2, 28.0, 28.3, 28.5, 32.7, 33.7, 38.7, 64.3 and 178.6.

1-Bromo-6-chloro-hexane (214)

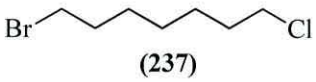


(214)

N-Chlorosuccinimide (11.5 g, 82.6 mmol) was added in portions to a stirred solution of 6-bromo-hexan-1-ol **(213)** (10.0 g, 55.2 mmol) and triphenylphosphine (21.6 g, 82.6 mmol)

in dichloromethane (150 ml) at 0 °C. The reaction mixture was stirred at room temperature for 1 hour or until TLC showed no starting material remaining. The reaction mixture was quenched with a saturated aqueous solution of $Na_2S_2O_5$ (100 ml). The organic layer was separated and the aqueous layer was re-extracted with dichloromethane (2 x 50 ml). The combined organic layers were washed with water, dried and evaporated to give a residue. This was treated with a mixture of petrol/ether (1:1) (150 ml) and heated under reflux for 30 minutes. The triphenylphosphine oxide was then filtered off, washed with petrol/ether (1:1) and the filtrate evaporated. Flash distillation gave 1-bromo-6-chloro-hexane **(214)** (7.63 g, 38.2 mmol, 68 %) as a colourless oil. This showed ν_{max}(film)/cm^{-1}: 2937 (C-H saturated), 2860 (C-H saturated) and 1460; δ_H: 1.47 (4H, m), 1.79 (2H, p, *J* 6.8, $CH_2CH_2CH_2Br$), 1.88 (2H, p, *J* 6.8, $CH_2CH_2CH_2Cl$), 3.42 (2H, t, *J* 6.6, CH_2CH_2Br) and 3.55 (2H, t, *J* 6.6, CH_2CH_2Cl); δ_C: 26.0, 27.4, 32.4, 32.6, 33.7 and 44.9. Data were consistent with those in the literature.[249]

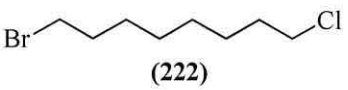
1-Bromo-6-chloro-heptane (237)



(237)

The procedure used for the synthesis of **(214)** was repeated using 6-bromo-heptan-1-ol **(157)** (7.0 g, 35.9 mmol), triphenylphosphine (14.1 g, 53.9 mmol) and *N*-chlorosuccinimide (7.2 g, 53.9 mmol) in dichloromethane (150 ml) at 0 °C. Column chromatography (petrol) gave 1-bromo-6-chloro-heptane **(237)** (5.3 g, 24.7 mmol, 68 %) as a colourless oil. This showed ν_{max}(film)/cm^{-1}: 2936 (C-H saturated), 2858 (C-H saturated) and 1461; δ_H: 1.35 (2H, m), 1.47 (4H, br p, *J* 7.4, $CH_2CH_2CH_2CH_2CH_2CH_2$), 1.79 (2H, p, *J* 7.1, $CH_2CH_2CH_2Br$), 1.87 (2H, p, *J* 7.1, $CH_2CH_2CH_2Cl$), 3.42 (2H, t, *J* 6.8, CH_2CH_2Br) and 3.54 (2H, t, *J* 6.8, CH_2CH_2Cl); δ_C: 26.7, 27.9, 28.0, 32.5, 32.6, 33.8 and 45.0.

1-Bromo-8-chloro-octane (222)

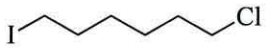


(222)

The procedure used for the synthesis of **(214)** was repeated using 8-bromo-octan-1-ol **(221)** (9.2 g, 44.0 mmol), triphenylphosphine (17.3 g, 66.0 mmol) and *N*-chlorosuccinimide

(8.8 g, 66.0 mmol) in dichloromethane (150 ml). Flash distillation gave 1-bromo-8-chloro-octane **(222)** (7.26 g, 31.9 mmol, 73 %) as a colourless oil. This showed ν_{max}(film)/cm^{-1}: 2932 (C-H saturated), 2856 (C-H saturated) and 1463; δ_H: 1.33 (4H, m), 1.44 (4H, p, *J* 6.8, $CH_2CH_2CH_2CH_2CH_2CH_2$), 1.77 (2H, p, *J* 7.1, $BrCH_2CH_2CH_2$), 1.86 (2H, p, *J* 7.2, $ClCH_2CH_2CH_2$), 3.41 (2H, t, *J* 6.8, $BrCH_2CH_2$) and 3.53 (2H, t, *J* 6.8, $ClCH_2CH_2$); δ_C: 26.8, 28.1, 28.6, 28.7, 32.6, 32.8, 33.9 and 45.1. Data was consistent to that in the literature.[250]

1-Chloro-6-iodo-hexane (211)



(211)

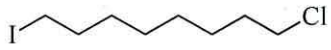
Sodium iodide (3.01 g, 20.1 mmol) and sodium bicarbonate (1.69 g, 20.1 mmol) were added to a stirred solution of 1-bromo-6-chloro-hexane **(214)** (4.00 g, 20.1 mmol) in acetone (100 ml) and the solution was heated under reflux for 2 hours. The solution was left to cool and the solvent was evaporated. Column chromatography (petrol) gave 1-chloro-6-iodo-hexane **(211)** (3.64 g, 14.8 mmol, 74 %) as a colourless oil. This showed ν_{max}(film)/cm^{-1}: 2933 (C-H saturated), 2857 (C-H saturated) and 1459; δ_H: 1.44 (4H, m), 1.79 (2H, p, *J* 6.9, $CH_2CH_2CH_2I$), 1.84 (2H, p, *J* 6.6, $CH_2CH_2CH_2Cl$), 3.19 (2H, t, *J* 6.8, CH_2CH_2I) and 3.54 (2H, t, *J* 7.0, CH_2CH_2Cl); δ_C: 6.8, 25.8, 29.8, 32.4, 33.3 and 44.9. The spectroscopic data were consistent to those in the literature.[251]

1-Chloro-7-iodo-heptane (236)

(236)

The procedure used for the synthesis of **(211)** was repeated using 1-bromo-7-chloro-heptane **(237)** (4.66 g, 22.0 mmol), acetone (100 ml), sodium iodide (3.27 g, 22.0 mmol) and sodium bicarbonate (1.84 g, 22.0 mmol). Column chromatography (petrol) gave 1-chloro-7-iod o-heptane **(236)** (4.35 g, 17.7 mmol, 76 %) as a colourless oil. This showed ν_{max}(film)/cm^{-1}: 2926 (C-H saturated), 2857 (C-H saturated) and 1463; δ_H: 1.36 (2H, m), 1.44 (4H, m), 1.81 (4H, m), 3.20 (2H, t, *J* 7.1, CH_2CH_2I) and 3.54 (2H, t, *J* 6.8, CH_2CH_2Cl); δ_C: 7.0, 26.7, 27.8, 30.3, 32.5, 33.4 and 45.0.

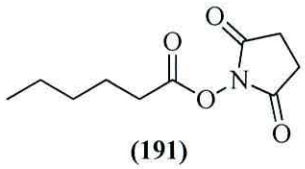
1-Chloro-8-iodo-octane (219)



(219)

The procedure used for the synthesis of **(211)** was repeated using 1-bromo-8-chloro-octane **(222)** (6.84 g, 30.1 mmol), acetone (100 ml), sodium iodide (4.51 g, 30.1 mmol) and sodium bicarbonate (2.53 g, 30.1 mmol). Column chromatography (petrol) gave 1-chloro-8-iodo-octane **(219)** (7.45 g, 27.2 mmol, 90 %) as a colourless oil. This showed ν_{max}(film)/cm^{-1}: 2990 (C-H saturated), 2923 (C-H saturated), 2854 (C-H saturated) and 1462; δ_H: 1.33 (4H, m), 1.42 (4H, m), 1.77 (2H, p, *J* 7.2, $ICH_2CH_2CH_2CH_2$), 1.82 (2H, p, *J* 7.2, $ClCH_2CH_2CH_2CH_2$), 3.19 (2H , t, *J* 7.0, $ICH_2CH_2CH_2CH_2$) and 3.53 (2H, t, *J* 6.6, $ClCH_2CH_2CH_2CH_2$); δ_C: 7.2, 26.8, 28.4, 28.7, 30.4, 32.6, 33.5 and 45.1.

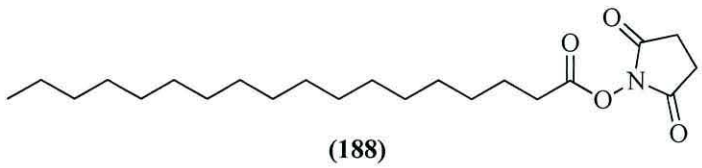
2,5-Dioxopyrrolidin-1-yl hexanoate (191)[252]



(191)

Hexanoic acid **(190)** (3.48 g, 3.79 ml, 30.0 mmol) was added to a solution of *N*-hydroxy succinimide (3.45 g, 30.0 mmol) in dry ethyl acetate (60 ml). A solution of DCC (6.18 g, 30 mmol) in dry ethyl acetate (10 ml) was added and the reaction mixture was stirred overnight at room temperature. DCU was removed by filtration and the filtrate was concentrated under reduced pressure. Column chromatography (petrol/ethyl acetate, 5:2) gave 2,5-dioxopyrrolidin-1-yl hexanoate **(191)**[252] (5.01 g, 21.7 mmol, 72 %) as a white solid, m.p. 48-50 °C. This showed ν_{max}(nujol)/cm^{-1} : 2923 (C-H saturated), 2853 (C-H saturated), 1816 (C=O), 1789 (C=O), 1739 (C=O) and 1463; δ_H: 0.92 (3H, t, *J* 7.1, $CH_3CH_2CH_2CH_2$), 1.38 (4H, m, 2 x CH_2), 1.76 (2H, p, *J* 7.5, $CH_2CH_2CH_2CH_2$), 2.61 (2H, t, *J* 7.4, $COCH_2CH_2CH_2CH_2$) and 2.84 (4H, br d, *J* 5.05, $COCH_2CH_2CO$); δ_C: 13.8, 22.2, 24.3, 25.6, 30.9, 168.7 and 169.1.

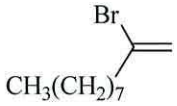
2,5-Dioxopyrrolidin-1-yl stearate (188)[230]



(188)

The procedure used for the synthesis of **(191)** was repeated using stearic acid **(20)** (8.53 g, 30.0 mmol) and *N*-hydroxy succinimide (3.45 g, 30.0 mmol) in dry ethyl acetate (130 ml) and DCC (6.18 g, 30 mmol) in dry ethyl acetate (10 ml). Recrystallisation (ethanol) gave 2,5-dioxopyrrolidin-1-yl stearate **(188)**[230] (8.51 g, 22.3 mmol, 74 %) as a white solid, m.p. 67-68 °C. This showed ν_{max}(nujol)/cm^{-1}: 2923 (C-H saturated), 2853 (C-H saturated), 1822 (C=O), 1725 (C=O), 1655 (C=O), 1518 and 1462; δ_H: 0.89 (3H, t, *J* 6.9, C*H*$_3$CH$_2$), 1.26 (26H, m), 1.42 (2H, m), 1.75 (2H, p, *J* 7.5, CH$_2$C*H*$_2$CH$_2$), 2.61 (2H, t, *J* 7.6, COC*H*$_2$CH$_2$) and 2.84 (4H, br s, COC*H*$_2$C*H*$_2$CO); δ_C: 14.1, 22.7, 24.6, 25.6, 28.8, 29.1, 29.3, 29.5, 29.6, 29.6, 29.7, 30.9, 31.9, 168.7 and 169.1.

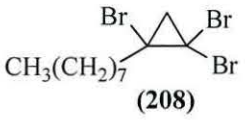
2 Bromodec-1-ene (210)[235]



(210)

A solution of tetraethylammonium bromide (41.8 g, 0.20 mol) in dichloromethane (300 ml) was cooled on ice. Dry HBr (14.64 g, 0.18 mol) was bubbled into the cooled solution until 1 equivalent had been absorbed. The solution was allowed to reach room temperature and 1 decyne **(209)** (25.0 g, 0.18 mol) was added. The solution was stirred at room temperature for 1 hour. The solution was then heated under reflux for 2 hours, cooled to room temperature and poured into ether (500 ml). The precipitated salts were filtered off on a bed of silica and the filter cake washed with ether. The solvent was removed by rotary evaporation. Flash distillation gave 2-bromo dec-1-ene **(210)**[235] (26.9 g, 0.12 mol, 68 %) as a colourless oil. This showed ν_{max}(film)/cm^{-1}: 2929 (C-H saturated), 2856 (C-H saturated), 1631 (C=C) and 1455; δ_H: 0.89 (3H, t, *J* 6.9, C*H*$_3$CH$_2$), 1.29-1.30 (10H, m), 1.50-1.58 (2H, p, *J* 7.6, CH$_2$C*H*$_2$CH$_2$C(Br)=CH$_2$), 2.41 (2H, dt, *J* 0.8, 7.4, CH$_2$C*H*$_2$C(Br)), 5.38 (1H, d, *J* 1.3, BrC=C*H*(H)) and 5.55 (1H, d, *J* 0.95, BrC=C*H*(H)); δ_C: 14.1, 22.6, 27.9, 28.4, 29.2, 29.3, 31.8, 41.4, 116.2 and 134.9.

1,1,2-Tribromo-2-octyl-cyclopropane (208)[133]



(208)

Sodium hydroxide (48.0 g, 1.2 mmol) in water (48 ml) was added in portions to a rapidly stirred mixture of 2 bromo dec-1-ene **(210)** (26.32 g, 0.12 mol), bromoform (60.75 g, 21.5 ml, 0.24 mol) and cetrimide (3 g) in dichloromethane (50 ml). The mixture was stirred vigorously at room temperature overnight or until there was no starting material remaining. The reaction mixture was diluted with brine, extracted with dichloromethane (3 x 200 ml), dried, filtered and evaporated. Petrol/ether (7:3, 200 ml) was added to precipitate the cetrimide. The solution was then filtered and the solvent removed by rotary evaporation. Column chromatography (petrol) gave 1,1,2-tribromo-2-octyl-cylopropane **(208)**[133] (42.0 g, 0.11 mol, 89 %) as a yellow oil. This showed ν_{max}(film)/cm^{-1}: 2925 (C-H saturated), 2854 (C-H saturated) and 1464; δ_H: 0.89 (3H, t, *J* 6.9, C*H*$_3$CH$_2$), 1.31 (10H, m), 1.71 (2H, m, C*H*$_2$CH$_2$CBr), 1.84 (1H, d, *J* 9.2, C*H*(H)CBr$_2$), 1.97 (2H, m, C*H*(H)CBrC*H*(H)CBr$_2$) and 2.05 (1H, ddd, *J* 5.4, 10.9, 14.7, CH$_2$C*H*(H)CBr); δ_C: 14.1, 22.7, 27.7, 28.9, 29.2, 29.4, 31.8, 33.2, 38.1, 41.8 and 45.9.