Chapter 3

Synthesis of a methoxy mycolic acid

3.1 Introduction

3.1.1 Why synthesize mycolic acids?

Except for the mycolic acid motif, very little is known about the stereochemistry of the other chiral centres in the mero chain. It is also not known whether the stereochemistry is important at all. There are a few unanswered questions that need investigation: does the stereochemistry influence the folding of the molecule? Is it necessary for infection, evasion of the immune system, for survival in the host or any for biological activity? Is the methoxy-MA really more important than the α- and keto-MA? Is there one specific MA responsible for all this, or is it a combination of some of them, or all of them? These are complex molecules that are found in a variety of combinations of different functional groups as well as different chain lengths. How important the chain length and the position of the functional groups are and what exactly the structural requirements for functionality and antibody recognition are, remain to be discovered.

Apart from separation of MAs by TLC and HPLC, chemical synthesis provides another way for investigating the structure of MAs. Stereochemically controlled single enantiomers and diastereomers, which can not be separated and purified from natural MAs extracts, can be synthesized and the biological activity of each can be investigated separately.

3.1.2 Previous synthesis of mycolic acids

In 1982 Huang *et al.* (80) reported the total synthesis of naturally occurring monoalkene MAs from *M. smegmatis*. They synthesized *(E)*- and *(Z)-threo*-2-docosyl-3-hydroxytetracont-21-enoate. The key feature of this synthesis was the incorporation of the 2-docosyl side chain and functionalized main chain (R) in a regiospecific manner. The mycolic acid motif was synthesized by alkylation of methyl aceto acetate at C-2 with 1-iododocosane (Figure 3.1).

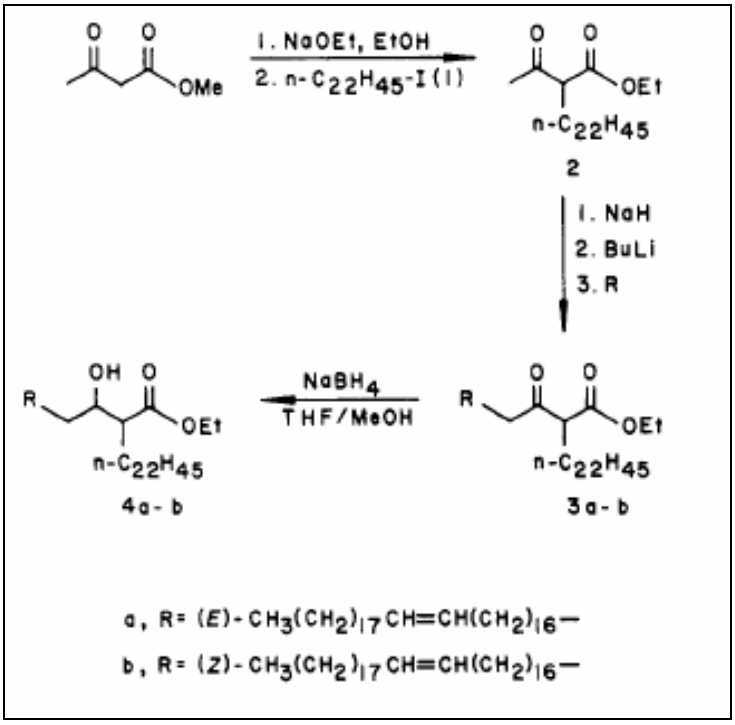


Figure 3.1: *Synthesis of a monoalkene MA from M. smegmatis (80).*

Al Dulayymi *et al.* (6, 7) described the synthesis of a single enantiomer of a major α-MA of *M. tuberculosis* (labelled MB for this study). They had a different approach to synthesizing the mycolic acid motif than reported by Huang *et al.* (80). Figure 3.2 shows how they started from a ring opening of the epoxide **(a)** with a Grignard reagent prepared from 9-bromo-nonan-1-ol tetrahydropyranyl ether which led to a single enantiomer of **(b)**. This was converted, in a few steps, to the diol **(c)**. The next step was the protection of the primary alcohol and the alkylation of the α-carbon to give the ester **(d)**. Acetylation of the β-hydroxy, deprotection and oxidation of the primary alcohol yielded the aldehyde **(e)**, which was coupled to the dicyclopropane sulfone **(f)** in a modified Julia reaction to give the protected α-MA **(g)**.

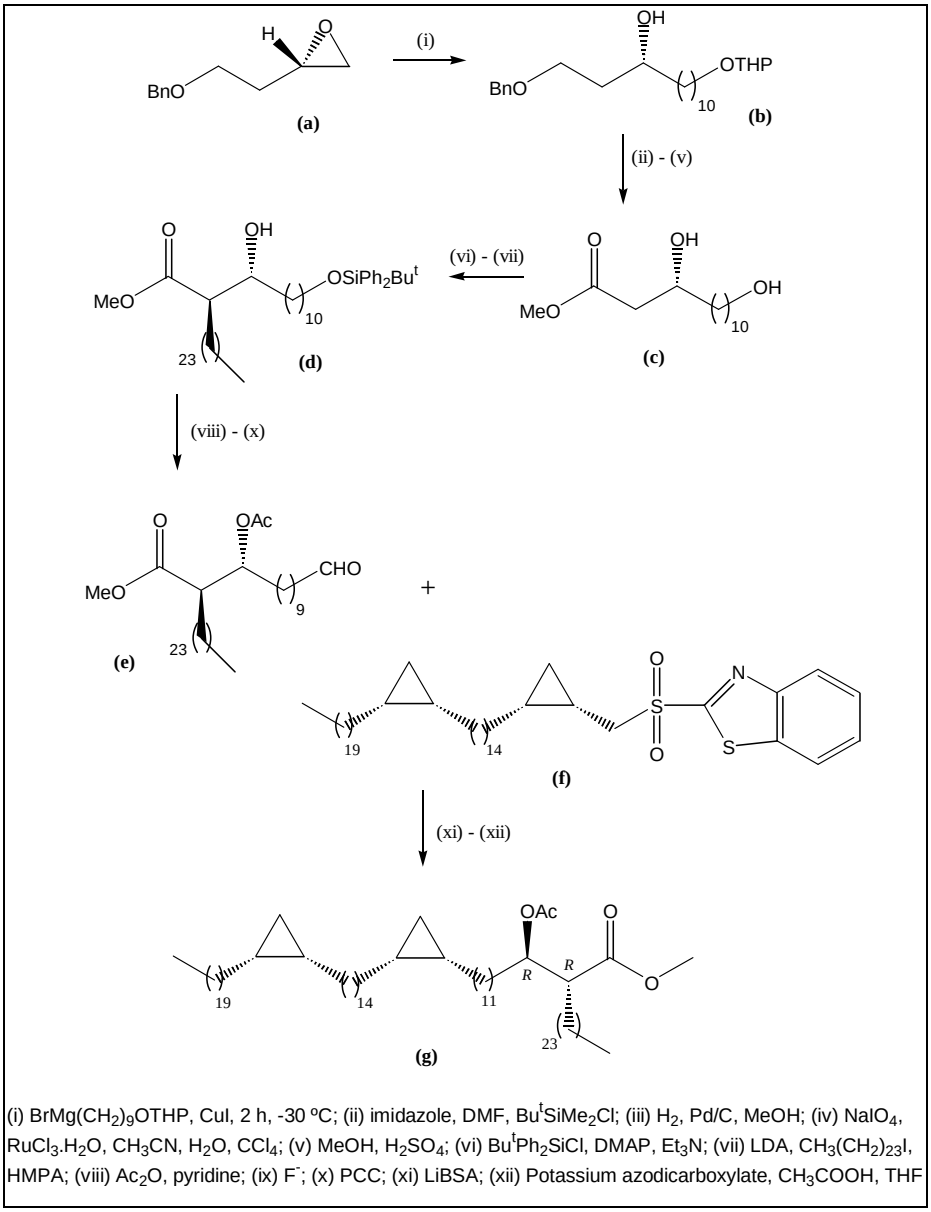


Figure 3.2: *Synthesis of protected α-MA, modified from (7).*

They also reported the synthesis of single enantiomers of a mero MA (8) and one isomer of the α-methyl-*trans*-cyclopropane unit (5) (Figure 3.3).

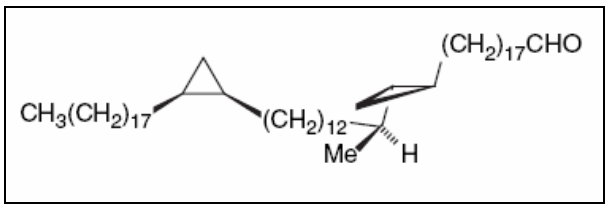


Figure 3.3: *α-Methyl-trans-cyclopropane mero aldehyde (5).*

Coxon *et al.* described the synthesis of enantiomers of lactobacillic acid and MA analogues (37, 39) containing *cis*-cyclopropanes, as well as the synthesis of methyl 5-(1'*R*,2'*S*)-(2-octadecylcyclopropane-1-yl)pentanoate and other ω-19 chiral cyclopropane fatty acids and esters related to mycobacterial MAs (38). These (shown in Figure 3.4) were assayed for inhibition of MA biosynthesis using a cell-wall preparation from *M. smegmatis*. The assay measured elongation of endogenous fatty acid precursors associated with the cell wall extracts. From all the analogues tested, **(V)** and **(XII)** showed marginal inhibition of MA biosynthesis, whereas the other all stimulated biosynthesis. Stimulation might be a result of direct incorporation of the long-chain cyclopropane compounds, but extensive investigation is necessary.

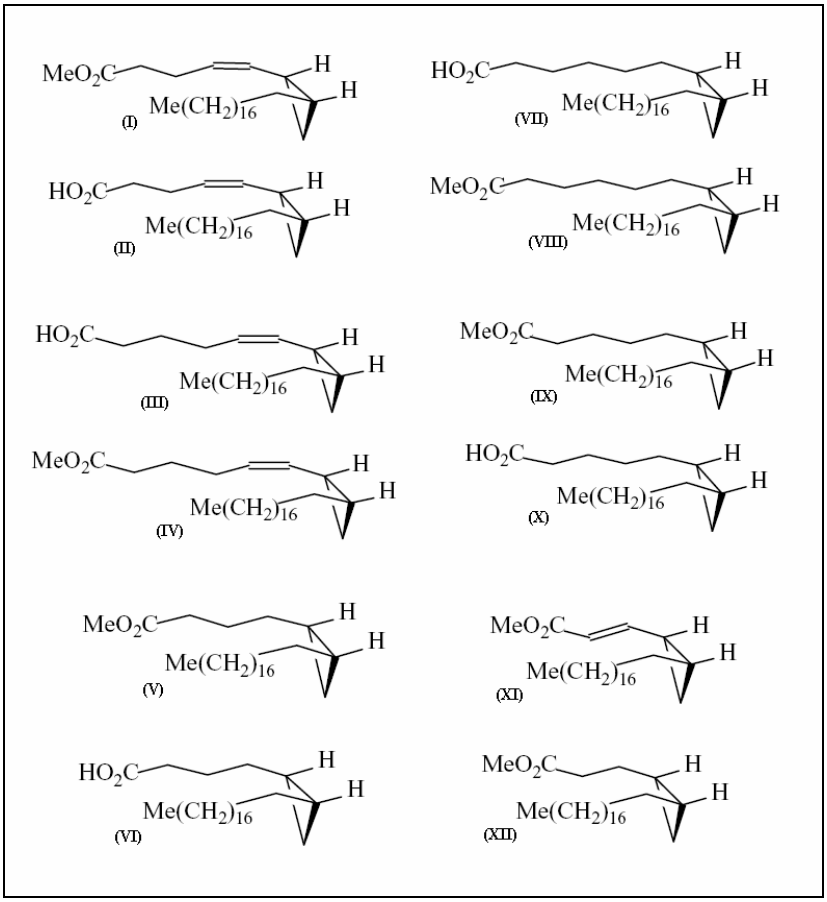


Figure 3.4: *(1*R,*2*S*) Long-chain ω-19 cyclopropane fatty acids and esters related to mycobacterial MAs (38).*

3.1.3 Cholesterol and mycolic acids

Siko (134) investigated cholesterol binding to MAs and found that if a biosensor cuvette surface was coated with natural MAs in liposomes, cholesterol accumulated onto the surface. This was not seen when empty PC liposomes were added to the MA surface (Figure 3.5).

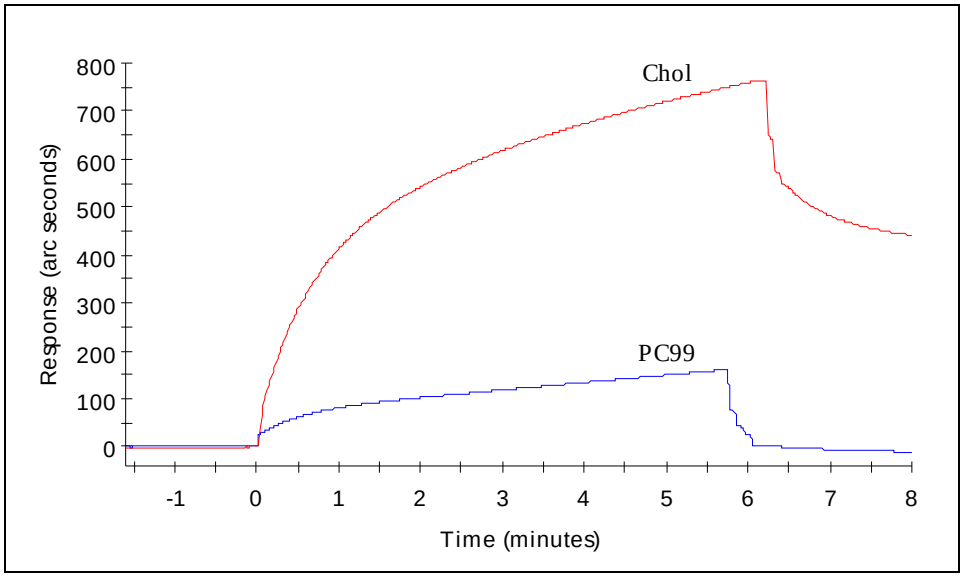


Figure 3.5: *Biosensor binding profile of cholesterol on a MA-coated surface. Chol = binding profile after addition of cholesterol/PC99 liposomes; PC99 = binding profile after adding PC99 liposomes (134).*

Based on this observation and results from ELISA, where antibody binding to MAs were also found to bind cholesterol, a possible existence of molecular relatedness between MAs and cholesterol and different MAs folding structures was investigated. Benadie (24) found that if a biosensor surface was coated with either MA or cholesterol, Amphotericin B (a known bactericide) accumulated onto the MA or cholesterol surface. This further supports a structural and functional relation between MA and cholesterol. A folded structure of the oxygenated MA species (methoxy-MAs and keto-MAs, Figure 3.6) was drawn that could explain such a possible structural relation. The methoxy-MAs could assume a folded structure in which relation to cholesterol could be seen. The methoxy-group of MAs corresponds to the hydroxyl position of cholesterol. In the folded structure with all the oxygenated groups on one side of the molecule and a hairpin bend induced by the cyclopropane moiety in the long hydrocarbon chain of MAs, a ‘mimicry’ of the structure of cholesterol appeared feasible. Keto-MAs would probably not ‘mimic’ cholesterol, as the plane introduced by the double bond between the oxygen and carbon might prevent formation of such a structure.

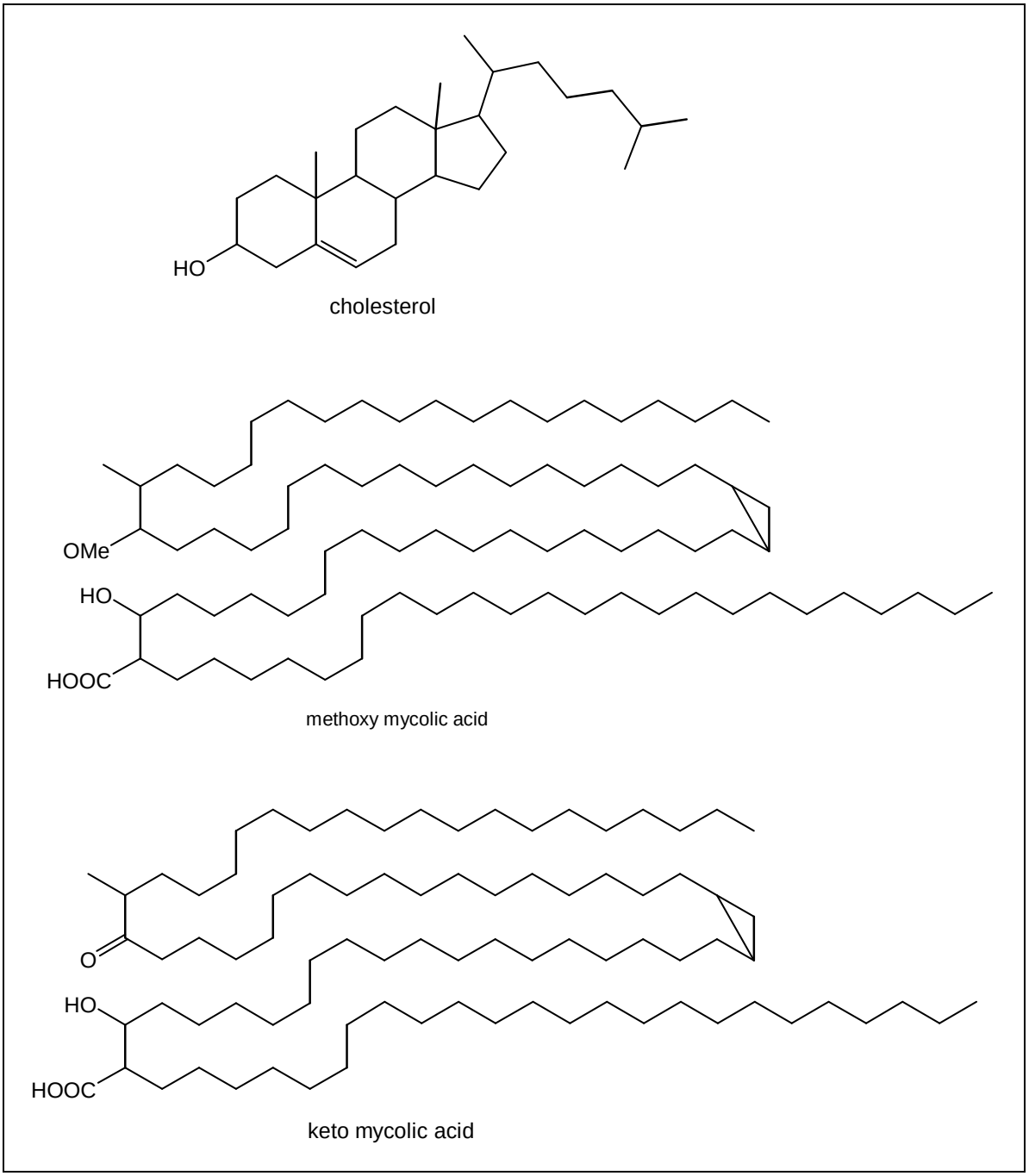
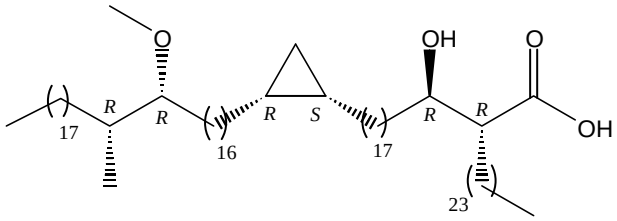


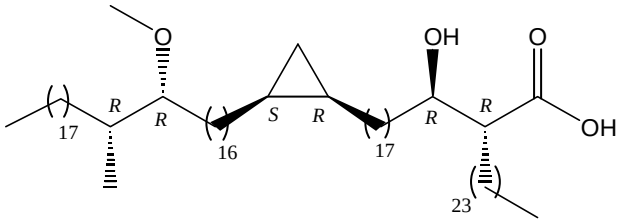
Figure 3.6: *The structures representing the possible molecular mimicry between the methoxy-MAs, keto-MAs and cholesterol (134).*

3.2 Aim

The aim was to synthesize the diastereomer (Figure 3.7 - **A**) of the previously synthesized *cis*-cyclopropane methyl methoxy-MA, synthesised by Dr J. Al Dulayymi (University of Wales, Bangor, UK) (Figure 3.7 - **B**), in order to prove the stereochemistry of the *cis*-cyclopropane and to determine the biological activity/antigenicity of different synthesized MAs.



(A)



(B)

Figure 3.7: *The two diastereomers of cis-cyclopropane methyl methoxy-MA*

3.3 Synthesis of a methoxy mycolic acid

3.3.1 Results and discussion

The MA can be decoupled to give the mycolic acid motif and the meromycolate chain. In turn, the meromycolate chain can be decoupled to give the methoxy part and the cyclopropane part as shown in Figure 3.8. The synthesis of these different parts and the coupling to get the full MA is described and shown in Figures 3.9-3.19.

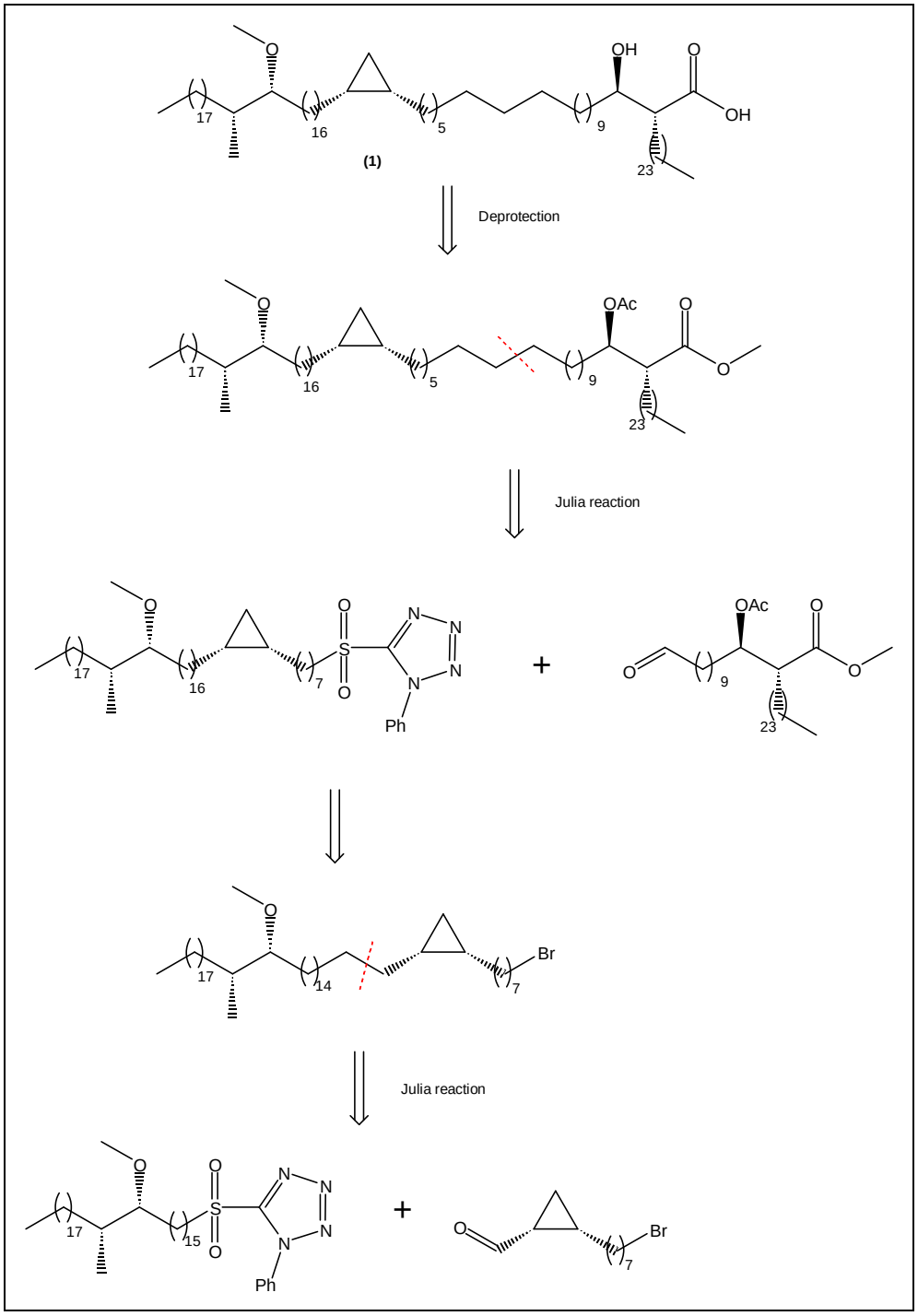


Figure 3.8: *Retrosynthesis of (R)-2-{(R)-1-acetoxy-18-(1S,2R)-2-((17R,18R)-17-methoxy-18-methylhexatri-acontyl)-cyclopropyl}-octadecyl}-hexacosanoic acid.*

To synthesize *cis*-methyl methoxy-MA **(1)**, *D*-mannitol was used as starting material. The two terminal hydroxyl groups on each end were protected by being stirred in acetone and $ZnCl_2$, then the 1,2 diol was oxidised to the aldehyde and attached to triethyl phosphonoacetate in a Wittig reaction to give the alkene **(3)**. A methyl group was added to the β-carbon in a Michael addition in dry ether at -78 °C. The reduction of the ester **(4)** using lithium aluminium hydride

in dry THF gave the alcohol **(5)**, which was oxidised using PCC in dichloromethane to give the aldehyde **(6)** as shown in Figure 3.9.

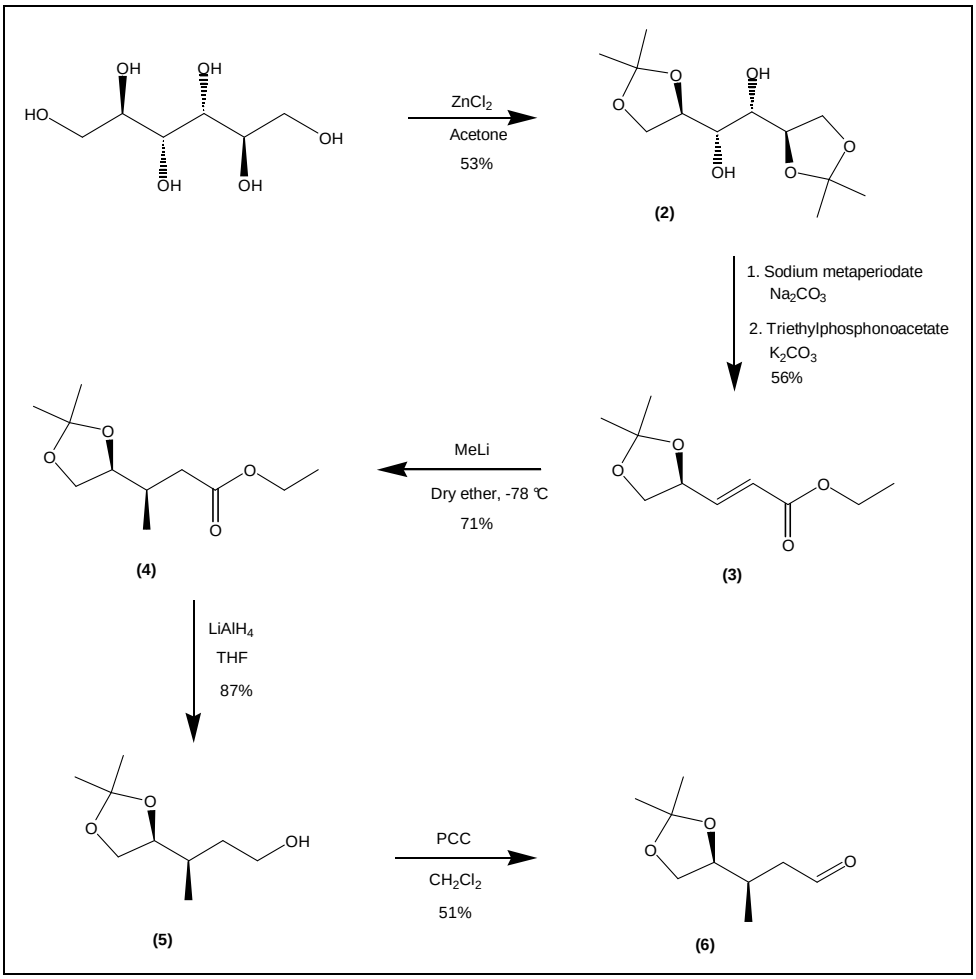


Figure 3.9: *Synthesis of (R)-3-((S)-2,2-dimethyl-[1,3]-dioxolan-4-yl)-butyraldehyde.*

The ^{1}H NMR spectrum of **(3)** showed two double doublets for the protons at the double bond at δ 6.85 (*J* 5.65, 15.75 Hz) and 6.07 (J 1.6, 15.75 Hz) respectively. In the ^{1}H NMR of **(4),** these signals disappeared and it showed a doublet at δ 0.98 (*J* 6.6 Hz) for the methyl group. The IR spectrum of the alcohol **(5)** showed a broad peak at 3426 cm^{-1} for O-H stretching. The ^{1}H NMR spectrum of the aldehyde **(6)** showed a triplet for the proton of the aldehyde at δ 9.79 (*J* 1.9 Hz) and the ^{13}C NMR spectrum included the carbonyl signal at δ 201.7.

5-(Hexadecane-1-sulfonyl)-1-phenyl-1*H*-tetrazole **(9)** was synthesized, as shown in Figure 3.10, to extend the chain length of the aldehyde **(6)** from C_2 to C_{17}. 1-Bromohexadecane **(7)** was reacted with 1-phenyl-1*H*-tetrazole-5-thiol and anhydrous potassium carbonate in acetone

to give the sulfide **(8)** which was oxidised with ammonium molybdate (VI) tetrahydrate and H_2O_2 in THF and IMS to give **(9)**.

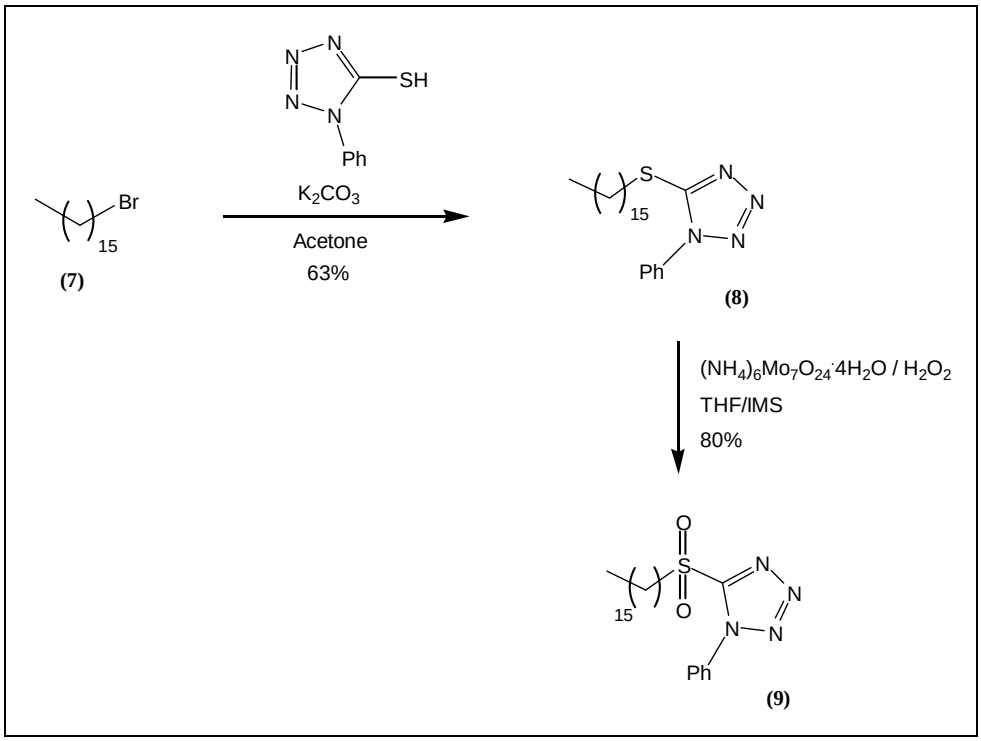


Figure 3.10: *Synthesis of 5-(Hexadecane-1-sulfonyl)-1-phenyl-1H-tetrazole.*

The ^{1}H NMR spectrum of **(9)** showed the phenyl group protons as two multiplets at δ 7.70 and 7.61, and a distorted triplet at δ 3.73 (*J* 7.85 Hz) for the two chain protons next to the sulfone.

The aldehyde **(6)** was reacted with 5-(hexadecane-1-sulfonyl)-1-phenyl-1H-tetrazole **(9)** in a Julia reaction using lithium bis(trimethylsilyl)amide in dry THF to give two isomers of the olefin. These both gave (*S*)-2,2-dimethyl-4-((*R*)-1-methyl-nonadecyl-[1,3]-dioxolane **(10)** after being hydrogenated by using Pd on C as a catalyst, as shown in Figure 3.11. The dioxolane group was deprotected using *p*-toluenesulfonic acid in methanol, THF and water to give the diol **(11)**. The epoxide **(12)** was formed by reacting the diol **(11)** with *p*-toluenesulfonic chloride, sodium hydroxide and cetrimide in dichloromethane and this was protected and the chain length extended in a Grignard reaction with 6-tetrahydropyranyloxyheptyl magnesium bromide and copper iodide in THF to give 2-((8*R*,9*R*)-8-hydroxy-9-methyl-heptacosyloxy)-tetrahydropyran **(13)**. The hydroxyl group was changed to a methoxy-group in a reaction with methyl iodide and sodium hydride in THF to give the methyl methoxy-compound **(14)**. The

THP group was deprotected by using *p*-toluenesulfonic acid monohydrate and the alcohol **(15)** was oxidized to the aldehyde (**16**) using PCC in dichloromethane as shown in Figure 3.11.

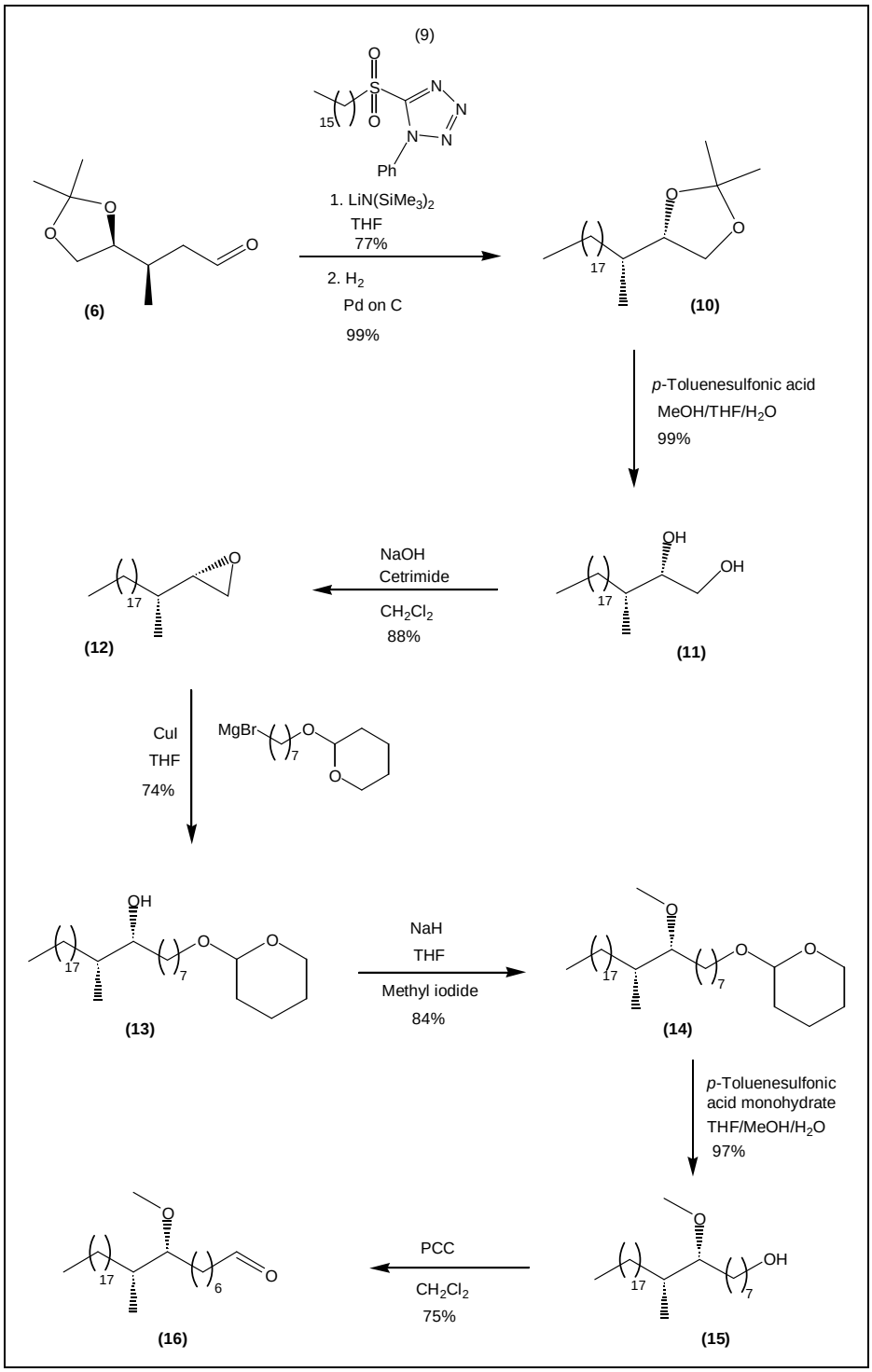


Figure 3.11: *Synthesis of (8R,9R)-8-methoxy-9-methyl-heptacosanal.*

The ^{1}H NMR spectrum of **(10)** showed two singlets at δ 1.41 and 1.36 for the two methyl groups of the protecting group which disappeared after deprotection. The IR spectrum of the

diol **(11)** showed a broad peak at 3283 cm^{-1} and that of **(13)** showed a broad peak at 3360 cm^{-1}, both for O-H stretching. The ^{1}H NMR spectrum of the methyl methoxy-compound **(14)** showed a singlet at δ 3.34 for the methyl group on the oxygen. The IR spectrum of the alcohol **(15)** showed a broad peak at 3373 cm^{-1} for the O-H stretching. The ^{1}H NMR spectrum of the aldehyde **(16)** included a triplet for the proton of the aldehyde at δ 9.79 (*J* 1.9 Hz) and the ^{13}C NMR spectrum included the carbonyl signal at δ 201.7.

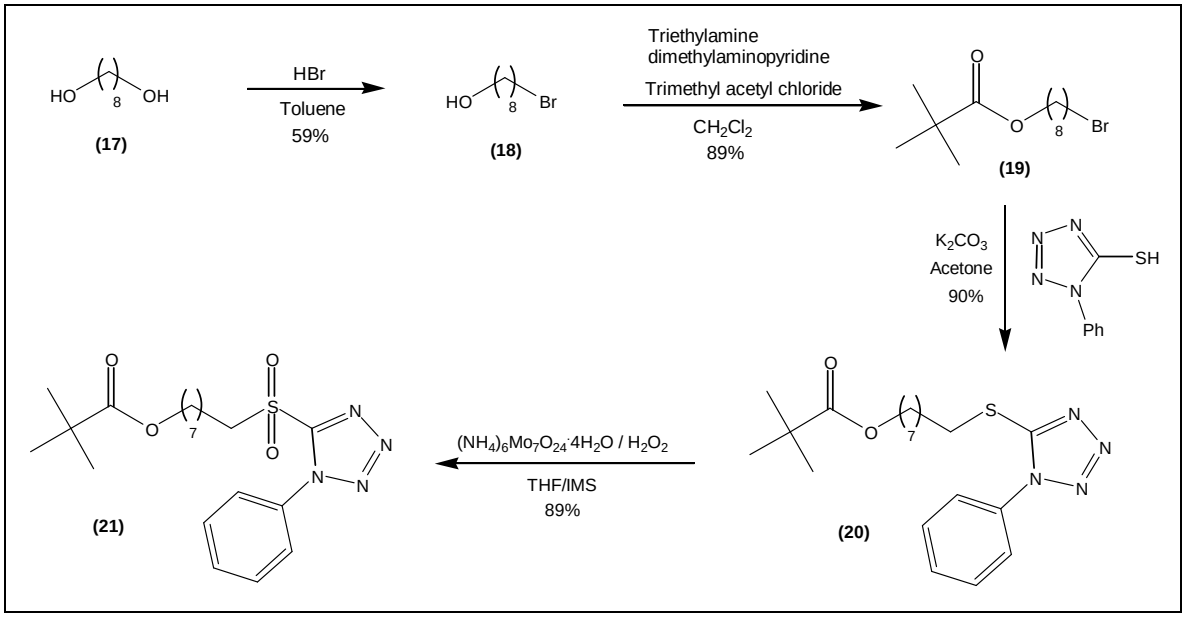


Figure 3.12: *Synthesis of 5-(1-octanolpivalate-8-sulfonyl)-1-phenyl-1H-tetrazole.*

5-(1-Octanolpivalate-8-sulfonyl)-1-phenyl-1*H*-tetrazole **(20)** was prepared to extend the chain of the aldehyde **(16)** from C_7 to C_{15}, as shown in Figure 3.12. Bromination of the diol **(17)** was done by refluxing the diol with HBr in toluene, and then the alcohol group on 8-bromo-octan-1-ol **(18)** was protected with trimethyl acetyl chloride, triethylamine and dimethylaminopyridine in dichloromethane to give 2,2-dimethyl-propionic acid 8-bromo-octyl ester **(19)**. The Julia reagent **(21)** was then prepared as described before for **(9)**.

The ^{1}H NMR spectrum of **(21)** showed the phenyl group protons as two multiplets at δ 7.69 and 7.61, a triplet for the two protons next to the oxygen at δ 4.04 (*J* 6.6 Hz), a distorted triplet at δ 3.73 (*J* 7.85 Hz) for the two protons next to the sulfone and a singlet at δ 1.19 for the three methyl groups of the protecting group. The ^{13}C NMR spectrum included the carbonyl signal at δ 178.5.

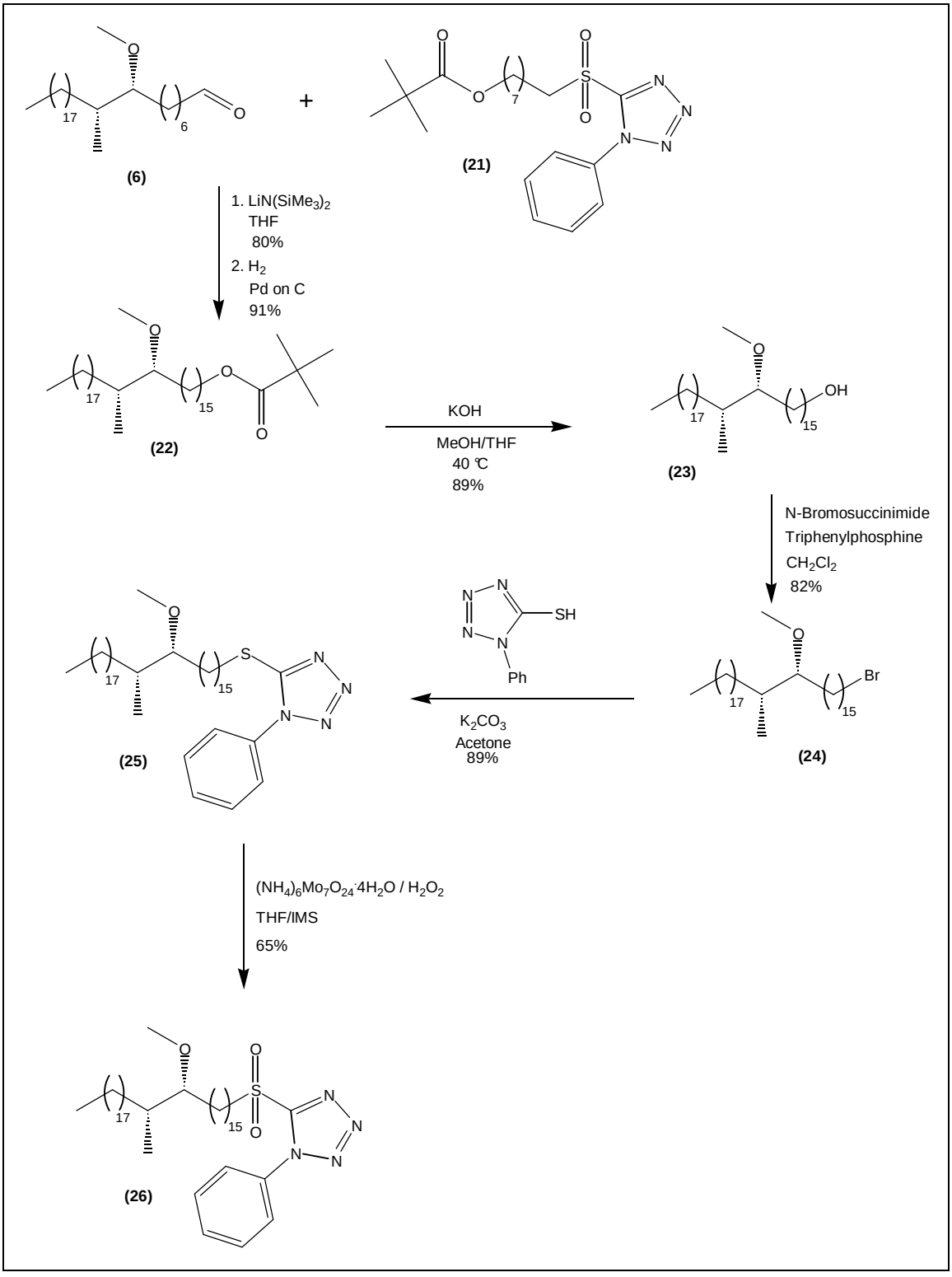


Figure 3.13: *Synthesis of 5-((16R,17R)-16-methoxy-17-methyl-pentatriacontane-1-sulfanyl)-1-phenyl-1H-tetrazole.*

The tetrazole **(21)** was then reacted with the aldehyde **(6)** using lithium bis(trimethylsilyl)amide in dry THF to give two isomers of the olefin which were saturated with hydrogen using Pd on C as catalyst to give compound **(22)**, as shown in Figure 3.13. The

ester **(22)** was hydrolised to the alcohol **(23)** using KOH in MeOH and THF, which was then brominated using *N*-bromosuccinimide and triphenylphosphine in dichloromethane to give **(24)**. The Julia reagent **(26)** was prepared as described before for **(9)**.

The ^{1}H NMR spectrum of **(22)** showed a singlet at δ 1.19 for the three methyl groups of the protecting group which disappeared after deprotection of the THP group. The IR spectrum of the alcohol **(23)** showed a broad peak at 3426 cm^{-1} for O-H stretching which disappeared after bromination **(24)**.

The addition of sodium methoxide to a mixture of methyl chloroacetate and methyl acrylate gave the *cis*- and *trans*- isomers of the cyclopropane ester **(27a, 27b)**. The *cis*-isomer was successfully separated by column chromatography and was reduced to the diol **(28)** with lithium aluminium hydride in THF. The diol was then protected with butyric anhydride to give the diester **(29)**, which was enzymatically hydrolysed to give a single enantiomer of the hydroxyl cyclopropane ester **(30)**. The Julia reagent **(33)** was prepared from the bromo cyclopropane ester **(31)** as shown in Figure 3.14.

The ^{1}H NMR spectrum of the *cis*-cyclopropane **(27b)** showed a double doublet at δ 2.08 (*J* 6.9, 8.5 Hz) and two triplet of doublets at δ 1.70 (*J* 5, 6.6, 13.2 Hz) and δ 1.26 (*J* 5.05, 8.55, 16.7 Hz) for the four protons of the cyclopropane, and a singlet at δ 3.70 for the two methyl groups of the diester, which disappeared after reduction to the alcohol. The ^{13}C NMR spectrum of showed the carbonyl signal at δ 170.21. The ^{1}H NMR spectrum of the alcohol **(30)** showed a double doublet at δ 4.46 (*J* 5.7, 12 Hz), a multiplet at δ 3.83 and a double doublet at δ 3.39 (*J* 9.15, 11.65 Hz) for the protons next to the oxygen atoms. The cyclopropane ring protons appeared as a multiplet at δ 1.30, a triplet of doublets at δ 0.84 (*J* 5.05, 8.5, 16.7 Hz) and a quartet at δ 0.22 (*J* 5.65 Hz). The IR spectrum showed a broad peak at 3426 cm^{-1} for O-H stretching and the optical rotation was +16.95. The ^{1}H NMR spectrum of the Julia reagent **(33)** showed the phenyl group protons as two multiplets at δ 7.70 and 7.63, four double doublets at δ 4.37 (*J* 5.7, 12.3 Hz), δ 4.03 (*J* 5.4, 14.85 Hz), δ 3.92 (*J* 8.2, 12 Hz) and δ 3.67 (*J* 8.85, 15.15 Hz) for the protons next to the sulfone and oxygen atoms, respectively. The protons of the cyclopropane ring appeared as a multiplet at δ 1.49, a triplet of doublets at δ 1.03 (*J* 5.7, 8.5 Hz) and a quartet at δ 0.60 (*J* 5.7 Hz).

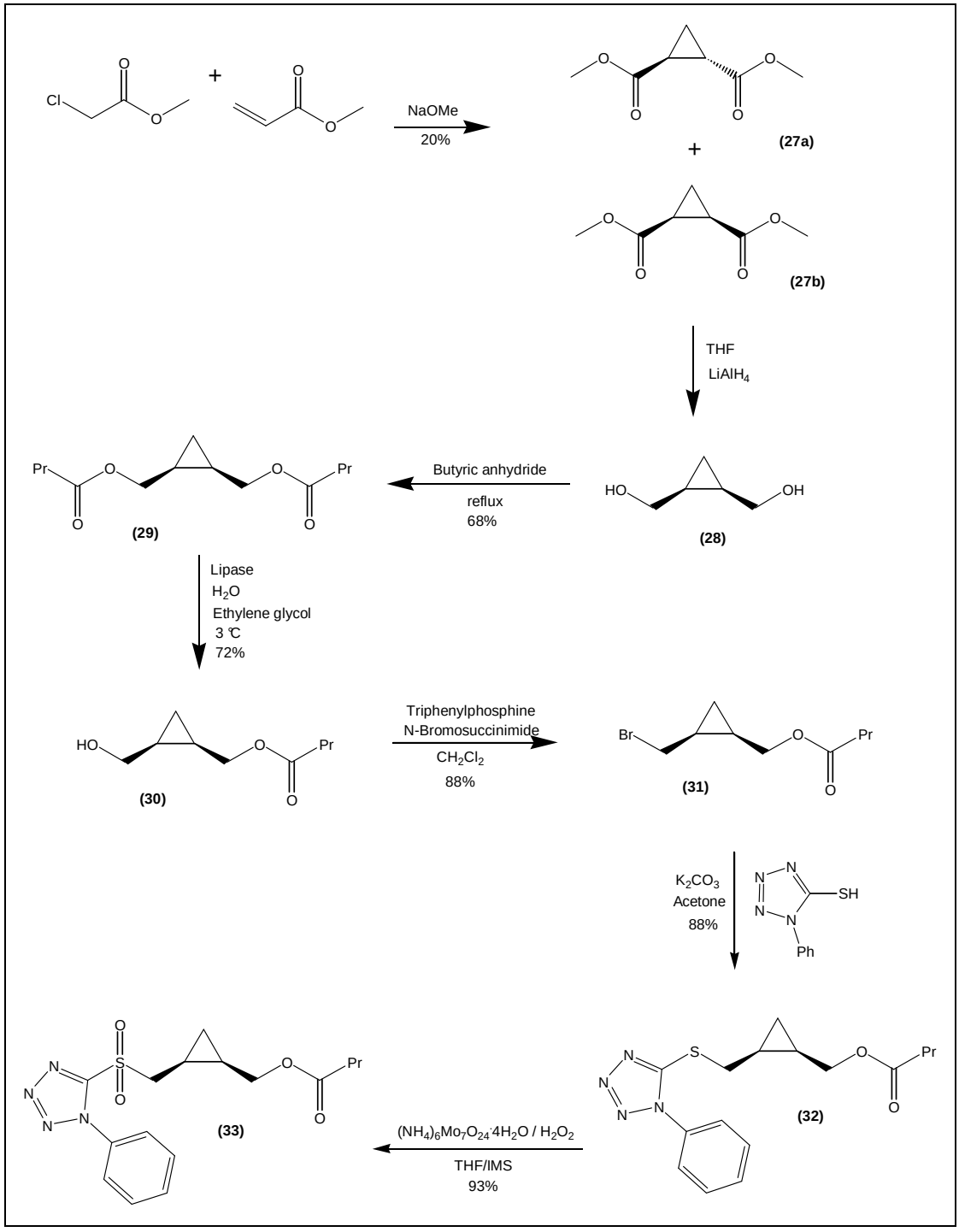


Figure 3.14: *Synthesis of butyric acid cis-2-(1-phenyl-1H-tetrazole-5-sulfonylmethyl)-cyclopropyl methyl ester.*

The Julia reagent **(33)** was coupled with 6-bromo-hexanal **(34)** to elongate the carbon chain next to the cyclopropane from C_1 to C_6 using lithium bis(trimethylsilyl)amide in dry THF to give two isomers of the olefin which were saturated with hydrogen using 2,4,6-triisopropylbenzene sulfonohydrazide to give butyric acid 2-(7-bromo-heptyl)-cyclopropyl methyl ester **(35)**, as shown in Figure 3.15. The ester **(35)** was then hydrolysed to the alcohol

(36) and then oxidised to the aldehyde **(37)** by using PCC in dichloromethane to be coupled to the Julia reagent **(26)**.

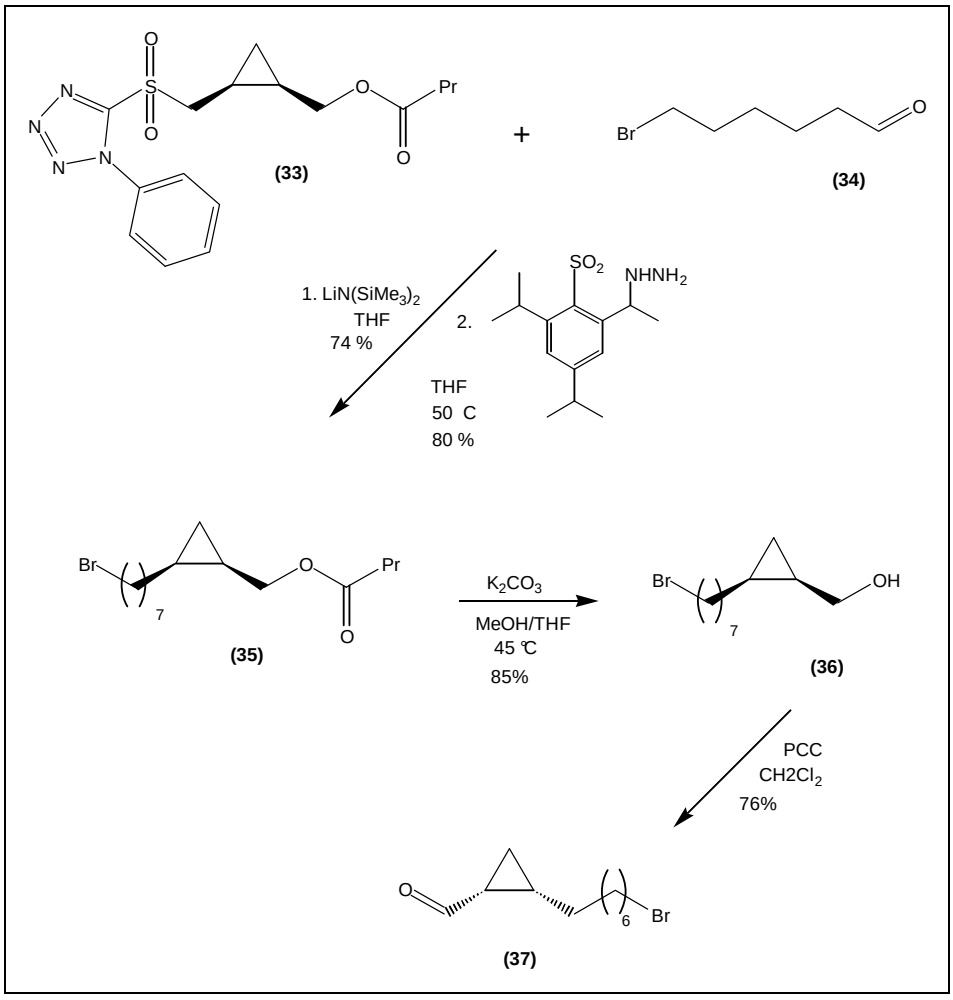


Figure 3.15: *Synthesis of 2-(7-bromo-heptyl)-cyclopropane carbaldehyde.*

The ^{1}H NMR spectrum of the bromocyclopropane ester **(35)** showed two double doublets at δ 4.19 (*J* 6.9, 11.65 Hz) and δ 3.93 (*J* 8.8, 11.65 Hz) for the two protons next to the oxygen which shifted to δ 3.65 (*J* 7.25, 11.35 Hz) and δ 3.57 (*J* 8.2, 11.35 Hz) for the two protons next to the hydroxyl group of the bromo cyclopropane alcohol **(36)**. The optical rotation for the alcohol was +12.87. The ^{1}H NMR spectrum of the aldehyde **(37)** showed the proton of the aldehyde as a doublet at δ 9.37 (*J* 5.4 Hz), a triplet at δ 170.21 (*J* 6.6 Hz) for the two protons next to the bromine and the protons of the cyclopropane appeared as a multiplet at δ 1.49, and two triplet of doublets at δ 1.24 (*J* 4.75, 7.9 Hz) and δ 1.19 (*J* 5.05, 6.6 Hz). The ^{13}C NMR spectrum included the carbonyl signal at δ 201.64 and the optical rotation was measured as +8.19 (-10.1 reported by Dr. J. Al Dulayymi for the enantiomer).

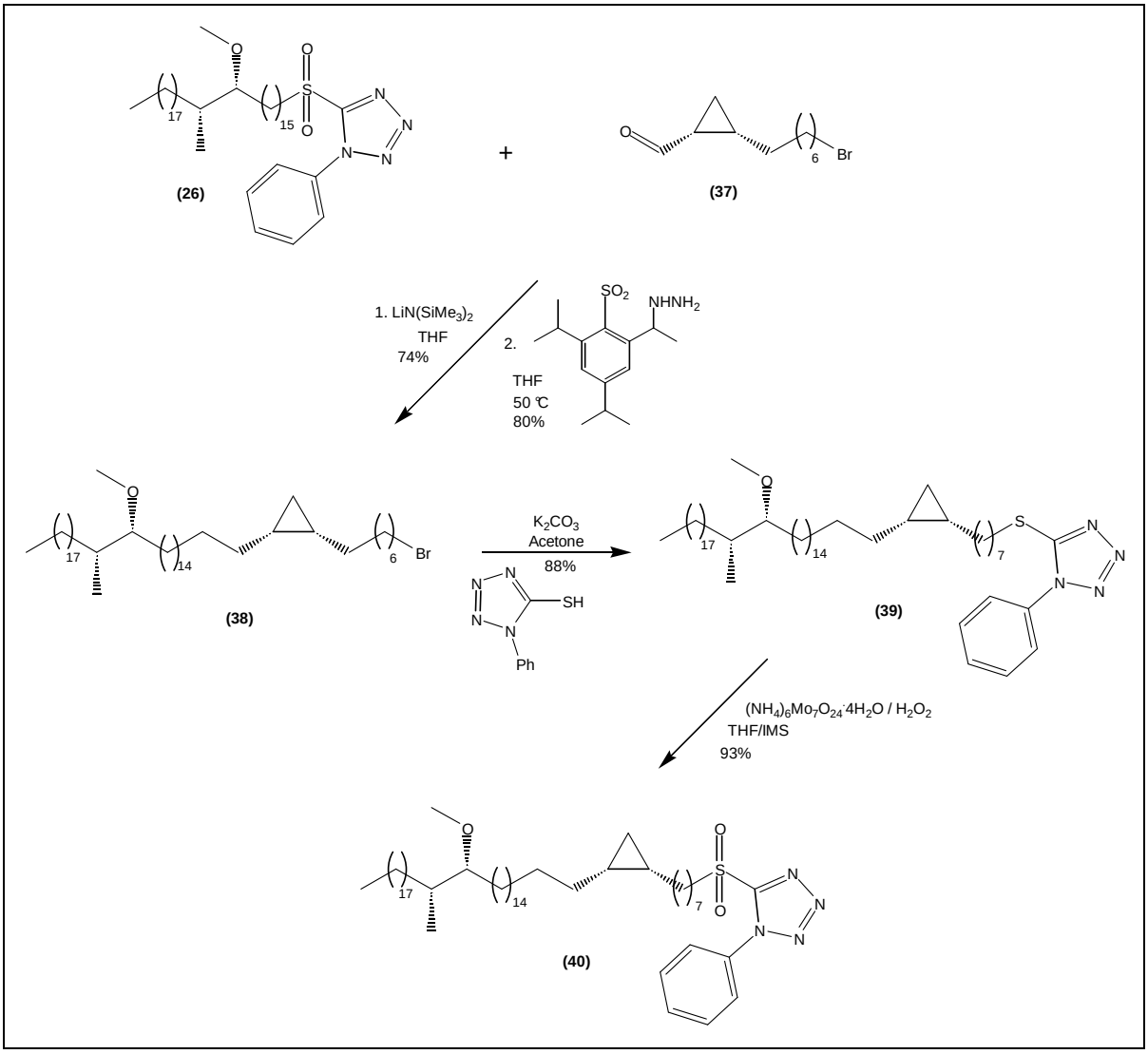


Figure 3.16: *Synthesis of 5-(7-[1R,2S]-2-((17R,18R)-17-methoxy-18-methyl-hexatriacontyl)-cyclopropyl)-heptyl sulfanyl)-1-phenyl-1H-tetrazole.*

The Julia reagent **(26)** and the aldehyde **(37)** was coupled using lithium bis(trimethylsilyl)amide in dry THF to give two isomers of the olefin which were saturated with hydrogen using 2,4,6-triisopropylbenzene sulfonohydrazide to give 1-(7-bromo-heptyl)-2-((17*R*,18*R*)-17-methoxy-18-methyl-hexatriacontyl)-cyclopropane **(38)** from which the Julia reagent **(40)** was prepared, as shown in Figure 3.16. This was prepared to be coupled to the mycolic acid motif.

The ^{1}H NMR spectrum of the Julia reagent **(40)** showed two multiplets at δ 7.71 and δ 1.70 for the protons of the phenyl group and a distorted triplet at δ 3.74 (*J* 7.9 Hz) for the two protons next to the sulfone. The methyl group on the oxygen appeared as a singlet at δ 3.35, the other

two methyl groups as a triplet at δ 0.89 (*J* 6.65 Hz) and a doublet at δ 0.86 (*J* 6.65 Hz). The cyclopropane ring protons appeared as a multiplet at δ 0.66, a triplet of doublets at δ 0.58 (*J* 3.75, 7.85 Hz) and a quartet at δ -0.32 (*J* 5.05 Hz) and the optical rotation was measured as +4.4 (+4.13 reported by Dr J. Al Dulayymi for a diastereomer).

To synthesize the mycolic acid motif, 8-bromo-octan-1-ol was used as starting material, the alcohol was protected with THP in dry dichloromethane and the bromide was replaced with iodide while being stirred in acetone and $NaHCO_3$ to give **(41)**. This was coupled with propagyl alcohol in liquid ammonia to extend the carbon chain from C_8 to C_{11} to give two isomers of the olefin which were saturated with hydrogen using nickel acetate tetrahydrate to give the alcohol **(42),** which was protected with trimethyl acetyl chloride in dichloromethane to give the ester **(43)** as shown in Figure 3.17. The ester **(43)** was then hydrolysed to the alcohol **(44)** by using *p*-toluenesulfonic acid monohydrate in THF, methanol and water. The alcohol was then oxidised to the aldehyde **(45)** by using PCC in dichloromethane to be coupled to (methoxycarbonylmethylene)-triphenylphosphorane as shown in Figure 3.17.

The ^{1}H NMR spectrum of the alcohol **(44)** showed a singlet at δ 1.19 for the three methyl groups of the protecting group and the IR spectrum showed a broad peak at 3384 cm^{-1} for O-H stretching. The ^{1}H NMR spectrum of the aldehyde **(45)** showed the proton of the aldehyde as a triplet at δ 9.76 (*J* 1.55 Hz) and a singlet at δ 1.19 for the three methyl groups of the protecting group. The ^{13}C NMR spectrum included the carbonyl signals at δ 202.83 for the aldehyde and at δ 178.62 for the protecting group.

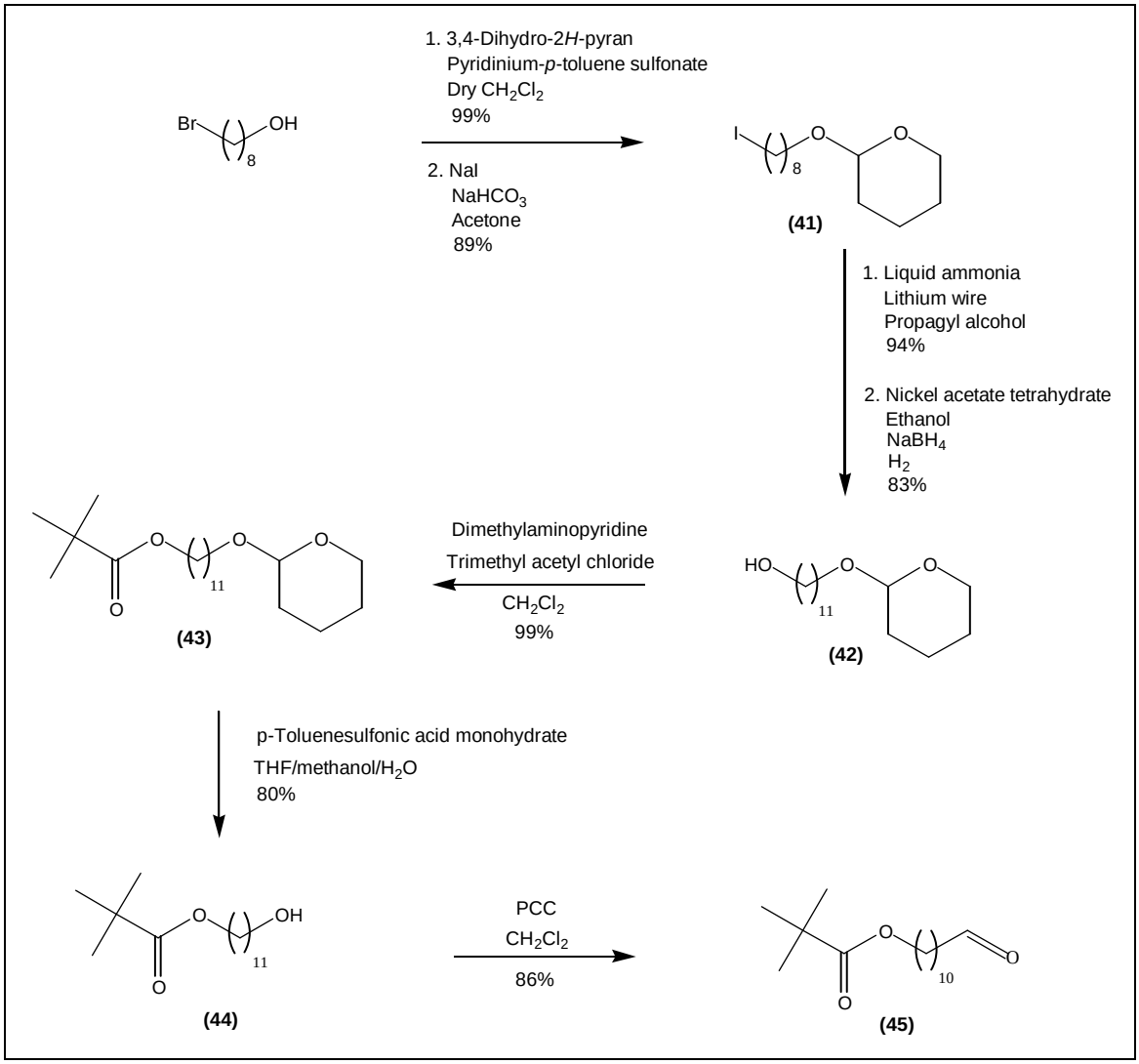


Figure 3.17: *Synthesis of 2,2-dimethyl-propionic acid-11-oxo-undecyl ester.*

The aldehyde **(45)** was coupled to (methoxycarbonylmethylene) triphenylphosphorane to give 13-(2,2-dimethyl-propionyloxy)-tridec-2-enoic acid methyl ester **(46)** which was oxidized using $(DHQD)_2PHAL$, K_3Fe_6, potassium carbonate, osmium tetroxide and methane sulfone amide to give the diol **(47)**. This was reacted with thionyl chloride in CCl_4 and then oxidized with $NaIO_4$ and ruthenium trichloride hydrate to give the cyclic sulfone ester **(48),** which was stirred in DMAC and sodium borohydrate to give the alcohol ester **(49)**. The acidic proton between the hydroxyl and the carbonyl groups was removed with freshly prepared LDA and coupled to allylic iodide to give the allyl ester **(50)**, as shown in Figure 3.18.

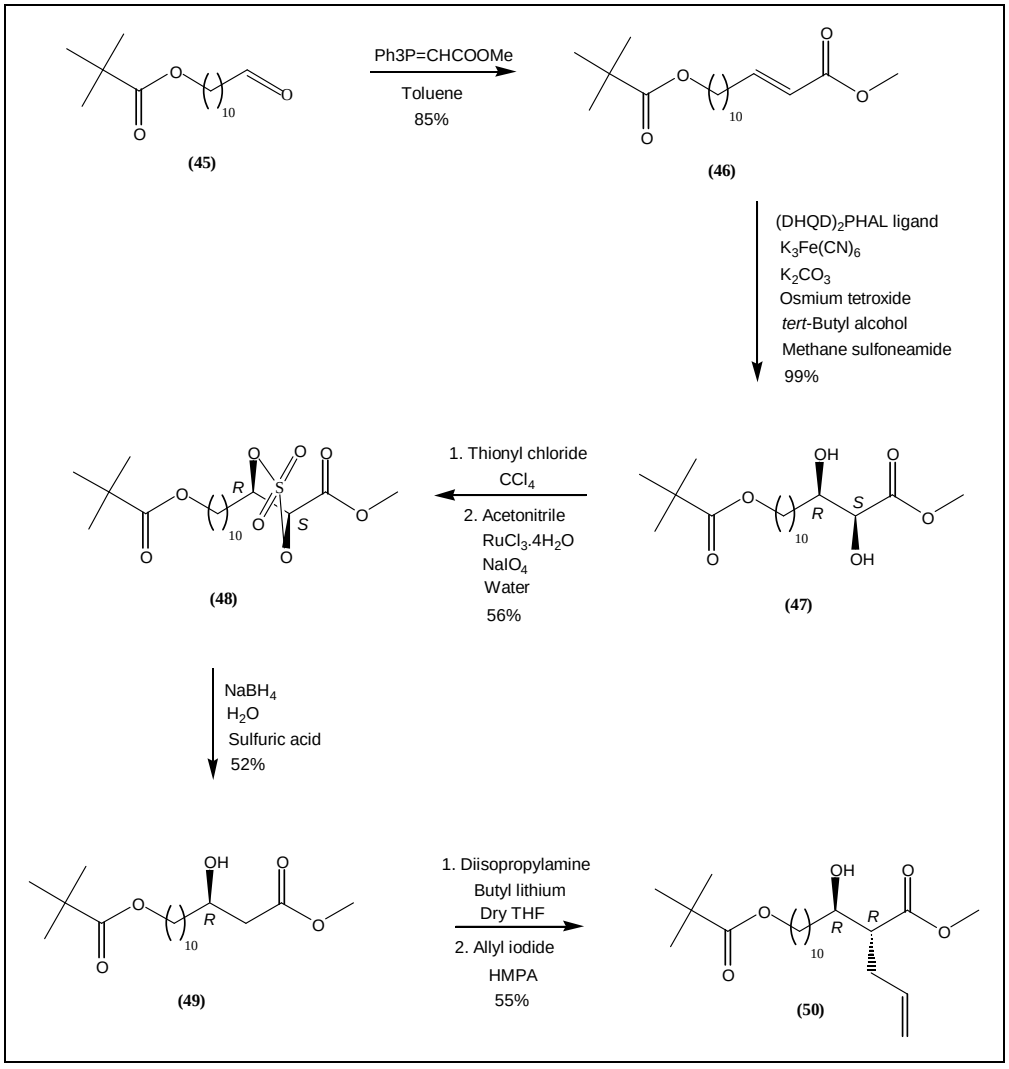


Figure 3.18: *Synthesis of (2R,3R)-2-allyl-[11-(2,2-dimethyl-propionyloxy)-1-hydroxy-undecyl]-pent-4-enoic acid methyl ester.*

The ^{1}H NMR spectrum of **(46)** showed the two protons of the double bond as two double triplets at δ 6.79 (*J* 6.95, 15.45 Hz) and at δ 5.81 (*J* 1.6, 15.45 Hz) which disappeared after the oxidation to the diol **(47)**. IR spectrum of **(47)** showed a broad peak at 3449 cm^{-1} for the OH-stretching. The ^{1}H NMR spectrum of the cyclic sulfone **(48)** showed the two protons next to the oxygens bound to the sulfone as a double of triplets at δ 4.95 (*J* 5.05, 7.25 Hz) and a doublet at δ 4.89 (*J* 7.25 Hz). The ^{1}H NMR spectrum of the alcohol **(54)** showed two double doublets for the two protons between the hydroxyl group and the carbonyl group at δ 2.51 (*J* 3.15, 16.4 Hz) and δ 2.41 (*J* 9.15, 16.5 Hz) and the IR spectrum showed a broad peak at 3517 cm^{-1} for OH-stretching. The ^{1}H NMR spectrum of the allylic ester **(50)** showed a multiplet at δ 5.75, a double doublet at δ 5.11 (*J* 0.95, 17 Hz) and a broad doublet at δ 5.05 (*J* 10.05 Hz) for

the three protons of the double bond. The IR spectrum showed a peak at 3524 cm^{-1} for OH-stretching and a peak at 1642 cm^{-1} for the double bond.

Methyl (2*R*,3*R*)-3-acetoxy-2-tetracosanyl-13-hydroxytridecanoate **(51)** (kindly provided by Dr J. Al Dulayymi, University of Wales, Bangor, UK) was oxidised to the aldehyde **(52)** by using PCC in dichloromethane. This was coupled to the Julia reagent **(40)** using lithium bis(trimethylsilyl)amide in dry THF to give two isomers of the olefin, which were saturated with hydrogen using dipotassium azodicarboxylate to give the full protected methoxy-MA **(53)** The final step was the elimination of the OAc and the methyl group to give the final product **(1)** as shown in Figure 3.19.

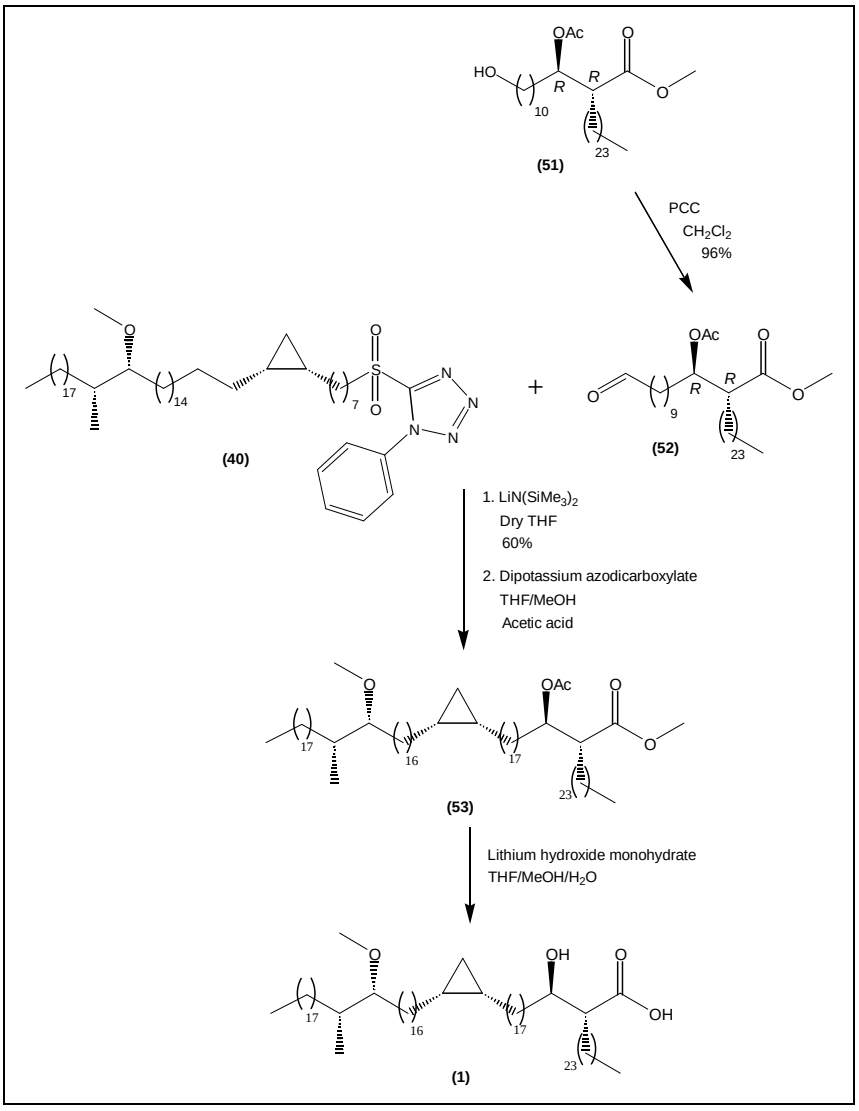


Figure 3.19: *Synthesis of (R)-2-{(R)-1-hydroxy-18-(1S,2R)-2-((17R,18R)-17-methoxy-18-methyl hexatri acontyl)-cyclopropyl}-octadecyl-hexacosanoic acid.*

The ^{1}H NMR spectrum of the protected MA **(53)** showed double triplets at δ 5.09 (J 3.8, 7.9 Hz) for the proton next to the OAc group and two singlets at δ 3.69 and δ 3.35 for the protons of the two methoxy groups. The proton next to the methoxy-group appeared as a multiplet at δ 2.96, the α proton appeared as a double double doublet at δ 2.62 (J 4.4, 6.95, 14.8 Hz) and the protons of the methyl group of the OAc group appeared as a singlet at δ 2.04. The spectrum also showed a triplet at δ 0.89 (*J* 6.6 Hz) for the six protons of the two terminal methyl groups and a doublet at δ 0.85 (*J* 6.9 Hz) for the methyl group next to the methoxy in the meromycolate chain. The cyclopropane ring protons appeared as a multiplet at δ 0.65, a triplet of doublets at δ 0.58 (*J* 4.1, 7.9 Hz) and a quartet at δ -0.32 (*J* 5.05 Hz). The ^{13}C NMR spectrum included two carbonyl signals at δ 173.65 and δ 170.33. The optical rotation was measured as +7.69 (+7.17 reported by Dr. J. Al Dulayymi for a diastereomer). In the ^{1}H NMR spectrum for the final MA **(1)**, the double of triplets at δ 5.09 (J 3.8, 7.9 Hz) for the proton next to the OAc group, and the singlet at δ 3.69 for the protons of the methyl ester group disappeared. Instead, the proton next to the β-OH group appeared as a multiplet at δ 2.99-2.95. The optical rotation was measured as +6.95.

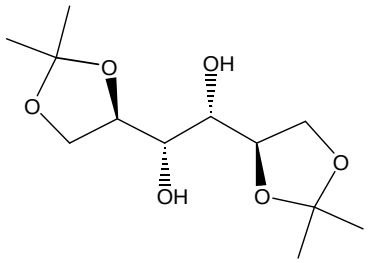
3.3.2 Experimental

General considerations

All chemicals were purchased form Lancaster Synthesis Ltd. (UK), Aldrich Chemical Co. Ltd. (UK), or Avocado Chemical Co. Ltd (UK). Diethyl ether and THF were distilled over sodium and benzophenone under a nitrogen atmosphere, while dichloromethane was distilled over calcium hydride. Distilled solvents were used within one day. Organic solutions were dried over anhydrous magnesium sulfate. Bulk solvents were removed under vacuum at 14 mm Hg and residual traces of solvent were finally removed at 0.1 mm Hg. All glassware used in anhydrous reactions were dried for not less than 5 hours at 250 °C. Column chromatography was done under medium pressure using silica gel (particle size 33-70 μm) from DBH Chemicals (UK); thin layer chromatography (TLC) was carried out on pre-coated Kieselgel 60 F254 (Art. 5554, Merck, UK) plates. Routine gas liquid chromatography (GLC) was performed using a temperature programmable Hewlett-Packard (Agilent) 5890 Gas Chromatograph with manual injection. The carrier gas was 5.0 grade helium with a column head pressure of 100 KPa supplied by Air Products plc (UK). The column was Rtx-5 supplied by Restek Corporation (USA). The phase thickness was 25 μm, the column internal diameter 0.31 mm and the column length 15 m. Optical rotations of compounds were measured in solutions of

chloroform at known concentration using a Polar 2001 Automatic Polarimeter, with the assistance of Dr J. Al Dulayymi (University of Wales, Bangor, UK). Infra-red spectra were recorded as KBr disc (solid) or thin films (liquid) on NaCl windows using a Perkin Elmer 1600 series FT-IR spectrometer. NMR spectra were recorded either on a Bruker AC 250 spectrometer with 5 mm dual probe or on Bruker Advance 500 spectrometer with 5 mm BBO probe. Compounds analysed were solutions in denatured chloroform ($CDCl_3$), unless indicated differently. All chemical shifts are quoted in δ relative to the trace resonance of protonated chloroform (δ 7.27 ppm) and $CDCl_3$ (δ 77.0 ppm). Low resolution mass spectra using electron impact (EI) were measured at 70 eV on a Hewlett-Packard (Agilent) 5970 quadrupole mass selective detector where the Gas Chromatograph was a Hewlett-Packard (Agilent) 5890 Gas Chromatograph with a 5975 auto sampler. It contained a Rtx-5 column supplied by Restek Corporation (USA). The phase thickness was 25 μm, the column diameter 0.25 mm and the column length 25 m.

3.3.2.1 Preparation of 1,2,5,6-di-O-isopropylidene-D-mannitol (2)



D-Mannitol **(1)** (30.31 g, 166.38 mmol) was added to a stirring solution of zinc chloride (60.75 g, 445.74 mmol, 2.7 mol eq.) in acetone (300 ml) at RT and the mixture was stirred for 18 hours at RT. Potassium carbonate (50.23 g, 363.43 mmol) in water (50 ml) was added to the reaction mixture resulting in the precipitation of a white crystalline solid (zinc chloride and potassium carbonate). The mixture was vacuum filtered, the precipitate washed with dichloromethane (4 x 100 ml) and the solvent evaporated to yield a viscous oil. This oil was dissolved in dichloromethane (200 ml) and washed with water (2 x 75 ml). The organic layer was separated and washed with brine (150 ml), dried and evaporated to give a white solid. The crude product was recrystallised from ethyl acetate (60 ml) and petroleum ether (300 ml) to give *1,2,5,6-di-O-isopropylidene-D-mannitol* **(2)** as a white solid (18.76 g, 43%).

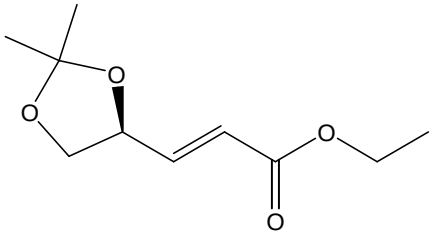
Physical properties:

ν_{max}/cm^{-1}: 3288, 2987, 1371, 1208, 1157, 1065

δ_H (500 MHz, $CDCl_3$): 4.21 (2H, q, *J* 6.3 Hz), 4.13 (2H, dd, *J* 6.6, 8.5 Hz), 3.98 (2H, dd, *J* 5.65, 8.5 Hz), 3.76 (2H, t, *J* 6 Hz), 2.55 (2H, d, *J* 6.3 Hz), 1.43 (6H, s), 1.37 (6H, s)

δ_C (500 MHz, $CDCl_3$): 109.39, 76.37(-), 71.29(-), 66.73(+), 26.70(-), 25.18(-), [+ = CH_2, - = CH, CH_3]

3.3.2.2 Preparation of ethyl-4,5-O-isopropylidene-(S)-4,5-dihydroxy-2-pentanoate (3)



A solution of sodium metaperiodate (18.38 g, 85.92 mmol, 1.20 mol eq.) in water (100 ml) was added to a stirring solution of sodium carbonate (9.59 g, 90.48 mmol, 1.25 mol eq.) and 1,2,5,6-di-O-isopropylidene-D-mannitol **(2)** (18.76 g, 71.60 mmol) in water (150 ml) at 5 °C (the temperature was kept below 5 °C). The reaction mixture was allowed to reach RT and stirred for 1 hour. The mixture was then cooled to 0 °C and triethylphosphonoacetate (34.3 ml, 30.45 g, 136.04 mmol) and potassium carbonate (37.60 g, 272.08 mmol) in water (50 ml) was added and the mixture stirred for 20 hours at RT. The product was extracted with dichloromethane (3 x 200 ml) and the combined organic layers were washed with brine (200 ml), dried and evaporated to give a oily residue which was purified by column chromatography on silica gel eluted with petroleum ether/diethyl ether (5:1) to give *ethyl-4,5-O-isopropylidene-(S)-4,5-dihydroxy-2-pentanoate* **(3)** (15.10 g, 56%) (109).

Physical properties:

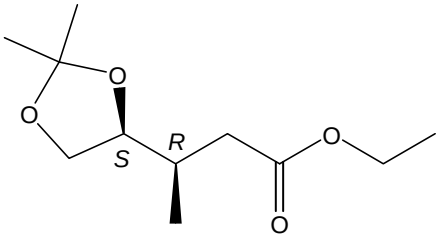
ν_{max}/cm^{-1}: 2985, 2937, 2875, 1725, 1662

δ_H (500 MHz, $CDCl_3$): 6.85 (1H, dd, *J* 5.65, 15.75 Hz), 6.07 (1H, dd, *J* 1.6, 15.75 Hz), 4.64 (1H, dd, *J* 1.25, 6.9 Hz), 4.17 (3H, m including q, *J* 7.25 Hz), 3.65 (1H, dd, *J* 7.55, 8.2 Hz), 1.42 (3H, s), 1.38 (3H, s), 1.27 (3H, t, *J* 7.25 Hz)

δ_C (500 MHz, $CDCl_3$): 165.9, 144.5(-), 122.4(-), 110.1, 74.9(-), 68.7(+), 60.5(+), 26.4(-), 25.6(-), 14.1(-), [+ = CH_2, - = CH, CH_3]

$[\alpha]_D^{24}$: +40.4 ° (c = 1.09, $CHCl_3$)

3.3.2.3 Preparation of (R)-3-((S)-2,2-dimethyl-[1,3]-dioxolan-4-yl)-butyric acid ethyl ester (4)



Methyl lithium (81 ml, 120 mmol, 1.50 M, 2 mol eq.) was added to a stirring solution of 3-(2,2-dimethyl-(1,3)-dioxolan-4-yl)-acrylic acid **(3)** (12 g, 60 mmol) in dry diethyl ether (300 ml) at -78 °C under nitrogen atmosphere. The reaction mixture was stirred at -78 °C for 2½ hours and allowed to reach -60 °C. Water was then added (10 ml) and after 5 minutes a saturated solution of ammonium chloride (80 ml), where upon the temperature rose to -40 °C. The cooling bath was removed, the mixture was allowed to reach 0 °C and the reaction was quenched with water (100 ml). The organic layer was separated and the aqueous layer extracted with diethyl ether (2 x 100 ml). The combined organic layers were washed with brine (2 x 150 ml), dried and evaporated to give a yellow oily residue, which was purified by column chromatography on silica gel eluted with petroleum ether/diethyl ether (4:1) to give *(R)-3-((S)-2,2-dimethyl-[1,3]-dioxolan-4-yl)-butyric acid ethyl ester* **(4)** (9.17 g, 71%) (109).

Physical properties:

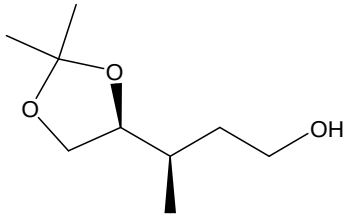
ν_{max}/cm^{-1}: 2984, 2980, 1732, 1012

δ_H (500 MHz, $CDCl_3$): 4.12 (2H, q, *J* 7.25 Hz), 3.99 (2H, m), 3.61 (1H, t, *J* 6.6), 2.38 (1H, dd, *J* 4.75, 14.85 Hz), 2.19 (1H, m), 2.11 (1H, dd, *J* 8.8, 14.8 Hz), 1.38 (3H, s), 1.32 (3H, s), 1.24 (3H, t, *J* 7.25 Hz), 0.98 (3H, d, *J* 6.6 Hz)

δ_C (500 MHz, $CDCl_3$): 172.57, 108.85, 78.74(+), 66.71(-), 60.31(-), 37.51(-), 32.94(+), 26.31(+), 25.19(+), 15.33(+), 14.16(+), [- = CH_2, + = CH, CH_3]

$[\alpha]_D^{22}$: +10.29 ° (c = 2.02, $CHCl_3$)

3.3.2.4 Preparation of (R)-3-((S)-2,2-dimethyl-[1,3]-dioxolan-4-yl)-butan-1-ol (5)

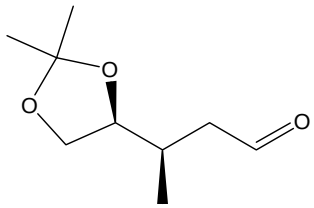


(*R*)-3-((*S*)-2,2-Dimethyl-[1,3]dioxolan-4-yl)-butyric acid ethyl ester **(4)** (6.90 g, 31.94 mmol) in dry THF (30 ml) was added drop-wise over a period of 15 minutes to a suspension of lithium aluminium hydride (1.46 g, 38.33 mmol) in dry THF (160 ml) under nitrogen atmosphere at RT. The reaction mixture was refluxed for 1 hour. When TLC showed that no more starting material was left, the mixture was cooled to RT and carefully quenched with freshly prepared saturated aqueous sodium sulfate decahydrate (10 ml) until a white precipitate was formed. This was followed by addition of magnesium sulfate (10 g). The mixture was stirred vigorously for 10 minutes, filtered through a pad of celite and washed thoroughly with THF (2 x 50 ml). The combined organic layers were evaporated to give a residue which was dissolved in a mixture of petroleum ether/diethyl ether (1:1, 150 ml) and triethylamine (2 drops). The solution was dried by stirring for one hour over anhydrous potassium carbonate. Filtration yielded a solution which was concentrated by evaporation to yield a colourless liquid of *(R)-3-((S)-2,2-dimethyl-[1,3]-dioxolan-4-yl)-butan-1-ol* **(5)** (4.8 g, 87%) (124).

Physical properties:

ν_{max}/cm^{-1}:	3423, 2933
δH (500 MHz, CDCl3):	3.99 (2H, m), 3.72 (1H, m), 3.64 (2H, m), 2.08 (1H, broad s), 1.82 (1H, m), 1.64 (1H, m), 1.45-1.37 (4H, m including s), 1.34 (3H, m), 0.97 (3H, dd, *J* 2.55, 6.95 Hz)
δC (500 MHz, CDCl3):	108.75, 79.69(+), 67.20(-), 60.35(-), 35.68(-), 32.81(+), 26.42(+), 25.32(+), 15.17(+), [- = CH_2, + = CH, CH_3]
$[\alpha]_D^{22}$:	+16.1 ° (c = 1.35, $CHCl_3$); *reported as* $[\alpha]_D^{22}$*: +16.8 ° (*$CHCl_3$*) by Ryosuke et al. (124)*

3.3.2.5 Preparation of (R)-3-((S)-2,2-dimethyl-[1,3]-dioxolan-4-yl)-butyraldehyde (6)

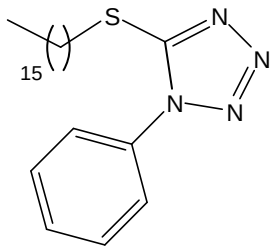


(*R*)-3-((*S*)-2,2-Dimethyl-[1,3]-dioxolan-4-yl)-butan-1-ol **(5)** (6.10 g, 35.06 mmol) in dichloromethane (30 ml) was added to a stirring suspension of pyridinium chlorochromate (16.07 g, 92.00 mmol) in dichloromethane (500 ml) at RT. The mixture was refluxed for 30 minutes. When TLC indicated that no starting material was left, the mixture was cooled down and poured into diethyl ether (200 ml), filtered through a pad of silica gel and washed thoroughly with diethyl ether. The filtrate was evaporated to give a residue which was purified by column chromatography on silica gel eluted with petroleum ether (BP 40-60 °C)/diethyl ether (1:1) to give *(R)-3-((S)-2,2-dimethyl-[1,3]-dioxolan-4-yl)-butyraldehyde* **(6)** as a colourless oil (3.05 g, 51%) (36).

Physical properties:

ν_{max}/cm^{-1}:	2985, 1725, 1215, 1066
δ_H (500 MHz, $CDCl_3$):	9.79 (1H, t, *J* 1.9 Hz), 4.09 (1H, m), 4.00 (1H, dd, *J* 6.6, 8.2 Hz), 3.65 (1H, dd, *J* 7.3, 8.2 Hz), 2.57 (1H, m), 2.40 (1H, m), 2.29 (1H, m), 1.43 (3H, s), 1.36 (3H, s), 1.01 (3H, d, *J* 6.9 Hz)
δ_C (500 MHz, $CDCl_3$):	201.7(-), 109.1, 78.6(-), 66.3(+), 46.6(+), 30.4(-), 26.2(-), 25.1(-), 15.5(-), [+ = CH_2, - = CH, CH_3]
$[\alpha]_D^{22}$:	+8.27 ° (c = 1.44, $CHCl_3$)

*3.3.2.6 Preparation of 5-hexadecylsulfanyl-1-phenyl-1*H*-tetrazole (8)*

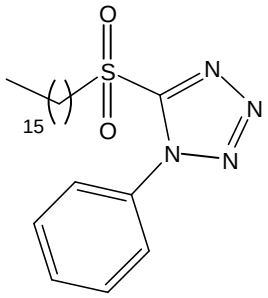


1-Bromohexadecane **(7)** (16.20 g, 53.05 mmol) was added to a stirring solution of 1-phenyl-1*H*-tetrazole-5-thiol (9.69 g, 54.37 mmol) and anhydrous potassium carbonate (15.24 g, 110.27 mmol) in acetone (165 ml). The mixture was vigorously stirred and refluxed for 2½ hours. When TLC showed that no starting material was left, the inorganic salts were filtered off and washed with acetone. The acetone solution was evaporated to a small bulk and dissolved in dichloromethane (150 ml). The solution was washed with water (300 ml), the organic layer separated and the aqueous layer re-extracted with dichloromethane (2 x 50 ml). The combined organic phases were washed with water (300 ml), dried and the solvent evaporated to give a solid. This was dissolved in acetone (50 ml) and diluted with methanol (100 ml). The mixture was left at ambient temperature for 1 hour and then at 0 °C for 1 hour. A white solid crystallised out; this was filtered off and washed with cold acetone/methanol (1:2) to give a white solid of *5-hexadecylsulfanyl-1-phenyl-1H-tetrazole* **(8)** (13.92 g, 63%).

Physical properties:

ν_{max}/cm^{-1}:	2917, 1501, 1091, 759
m.p.	48-50 ºC
δ_H (500 MHz, $CDCl_3$):	7.56 (5H, m), 3.40 (2H, t, *J* 7.55 Hz), 1.82 (2H, pent, *J* 7.25 Hz), 1.44 (2H, pent, *J* 6.95 Hz), 1.35-1.24 (24H, m including s), 0.88 (3H, t, *J* 6.95 Hz)
δ_C (500 MHz, $CDCl_3$):	154.46, 133.82, 129.98(-), 129.70(-), 123.83(-), 33.39(+), 31.88(+), 29.64(+), 29.62(+), 29.60(+), 29.57(+), 29.49(+), 29.39(+), 29.30(+), 29.08(+), 28.99(+), 28.60(+), 22.64(+), 14.04(-), [+ = CH_2, - = CH, CH_3]

*3.3.2.7 Preparation of 5-(hexadecane-1-sulfonyl)-1-phenyl-1*H*-tetrazole (9)*

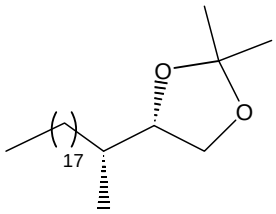


A solution of ammonium molybdate (VI) tetrahydrate (18.90 g, 15.31 mmol) in ice cold H_2O_2 35% (w/w, 50 ml) was added to a stirring solution of 5-hexadecylsulfanyl-1-phenyl-1*H*-tetrazole **(8)** (13.50 g, 33.58 mmol) in THF (150 ml) and IMS (300 ml) at 12 °C and stirred at RT for 2 hours. A further solution of ammonium molybdate (VI) tetrahydrate (7.20 g, 5.83 mmol) in ice cold H_2O_2 35% (w/w, 20 ml) was added and the mixture was stirred at RT for 18 hours. The mixture was poured into 3 L of water and extracted with dichloromethane (3 x 400 ml). The combined organic phases were washed with water (2 x 500 ml), dried and the solvent was evaporated. The residue was dissolved in methanol (200 ml) and the mixture left at RT for 1 hour and then at 0 °C for 1 hour. A white solid crystallised out; this was filtered off and washed with cold methanol to give a white solid of *5-(hexadecane-1-sulfonyl)-1-phenyl-1H-tetrazole* **(9)** (12.42 g, 80%).

Physical properties:

ν_{max}/cm^{-1}:	2918, 1470, 1343, 1154, 770
m.p.	65-67 ºC
δ_H (500 MHz, $CDCl_3$):	7.70 (2H, m), 7.61 (3H, m), 3.73 (2H, distorted t, *J* 7.85 Hz), 1.96 (2H, m), 1.50 (2H, pent, *J* 6.95 Hz), 1.34-1.25 (24H, m including s), 0.89 (3H, t, *J* 6.9 Hz)
δ_C (500 MHz, $CDCl_3$):	153.56, 133.11, 131.39(+), 129.66(+), 125.09(+), 56.06(-), 31.89(-), 29.65(-), 29.64(-), 29.62(-), 29.59(-), 29.52(-), 29.42(-), 29.31(-), 29.16(-), 28.87(-), 28.12(-), 22.64(-), 21.92(-), 14.05(+), [- = CH_2, + = CH, CH_3]

3.3.2.8 Preparation of (S)-2,2-dimethyl-4-((R)-1-methyl-nonadecyl)-[1,3]-dioxolane (10)



Lithium bis(trimethylsilyl)amide (27 ml, 28.60 mmol, 1.06 M) was added drop-wise to a stirring solution of (*R*)-3-((*S*)-2,2dimethyl-[1,3]-dioxolan-4-yl)-butyraldehyde **(6)** (3.05 g, 17.73 mmol) and 5-(hexadecane-1-sulfonyl)-1-phenyl-1*H*-tetrazole **(9)** (9.30 g, 20.17 mmol) in dry THF (130 ml) under nitrogen at - 2 °C. The reaction mixture was allowed to reach RT and stirred for 16 hours, then quenched with water (100 ml) and petroleum ether/diethyl ether (1:1, 100 ml). The organic layer was separated and the aqueous layer re-extracted with petroleum ether/diethyl ether (1:1, 2 x 50 ml). The combined organic layers were washed with brine (2 x 100 ml), dried and evaporated to give a thick oil which was purified by column chromatography on silica gel eluting with petroleum ether/diethyl ether (10:0.5) to give *(S)-2,2-dimethyl-4-((E,Z)-(R)-1-methyl-nonadec-3-enyl)-[1,3]-dioxolane* (5.18 g, 77%).

Palladium on charcoal (10%, 1.20 g) was added to a stirring solution of (*S*)-2,2-dimethyl-4-((*E,Z*)-(*R*)-1-methyl-nonadec-3-enyl)-[1,3]-dioxolane (5.18 g, 13.63 mmol) in IMS (125 ml) and methanol (30 ml). The mixture was stirred while being hydrogenated under hydrogen atmosphere. When no more hydrogen was absorbed the mixture was filtered through a pad of celite and washed with warmed ethyl acetate (100 ml). The clear colourless filtrate was evaporated at 14 mm Hg to give a white solid of *(S)-2,2-dimethyl-4-((R)-1-methyl-nonadecyl-[1,3]-dioxolane* **(10)** (5.18 g, 99%).

Physical properties:

ν_{max}/cm^{-1}: 2919, 2851, 1467, 1076, 856

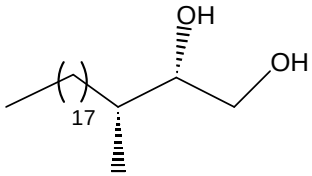
δ_H (500 MHz, $CDCl_3$): 4.01 (1H, dd, *J* 5.95, 7.55 Hz), 3.87 (1H, q, *J* 7.2 Hz), 3.61 (1H, t, *J* 7.55 Hz), 1.55 (1H, m), 1.41 (3H, s), 1.36 (3H, s), 1.34-1.20 (33H, m including s), 1.09 (1H, m), 0.97 (3H, d, *J* 6.6 Hz), 0.89 (3H, t, *J* 6.6 Hz)

δ_C (500 MHz, $CDCl_3$): 108.50, 80.42(-), 67.82(+), 36.51(-), 32.76(+), 31.93(+), 29.87(+), 29.69(+), 29.66(+), 29.64(+), 29.61(+), 29.36(+),

26.99(+), 26.64(-), 25.54(-), 22.68(+), 15.61(-), 14.09(-), [+ = CH_2, - = CH, CH_3]

$[\alpha]_D^{22}$: *reported as +23.01 ° (c = 0.62, $CHCl_3$)*

3.3.2.9 Preparation of (2S,3R)-3-methyl-henicosane-1,2-diol (11)



p-Toluenesulfonic acid (0.48 g, 2.51 mmol, 1.90 mol eq.) was added to a stirring solution of (*S*)-2,2-dimethyl-4-((*R*)-1-methyl-nonadecyl)-[1,3]-dioxolane **(10)** (5.04 g, 13.19 mmol) in THF (35 ml), methanol (50 ml) and water (5 ml) at RT. The reaction mixture was refluxed for 3½ hours. When TLC showed that no starting material was left, the solvent was evaporated and the residue diluted with petroleum ether/diethyl ether (1:1, 150 ml). Then a solution of saturated sodium bicarbonate (50 ml) was added, the organic layer separated and the aqueous layer re-extracted with diethyl ether (2 x 300 ml). The combined organic layers were washed with brine (250 ml), dried and evaporated to give a white solid of *(2S,3R)-3-methyl-henicosane-1,2-diol* **(11)** (4.58 g, >99%).

Physical properties:

ν_{max}/cm^{-1}: 3420

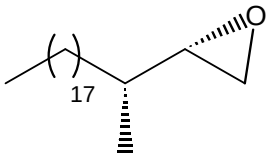
m.p. 67-68 ºC

δ_H (500 MHz, $CDCl_3$): 3.69 (1H, m), 3.59 (2H, m), 2.04 (1H, d, *J* 4.1 Hz), 1.95 (1H, dd, *J* 4.8, 7.0 Hz), 1.57 (1H, m), 1.28 (34 H, m), 0.95 (3H, d, *J* 7.0 Hz), 0.90 (3H, t, *J* 7.0 Hz)

δ_C (500 MHz, $CDCl_3$): 75.75(-), 65.15(+), 35.71(-), 32.98(+), 31.91(+), 29.85(+), 29.69(+), 29.68(+), 29.35(+), 27.10(+), 22.68(+), 14.55(-), 14.10(-), [+ = CH_2, - = CH, CH_3]

$[\alpha]_D^{22}$: *reported as +12.7 ° (c = 1.11, $CHCl_3$*

3.3.2.10 Preparation of (S)-2-((R)-1-methyl-nonadecyl)-oxirane (12)

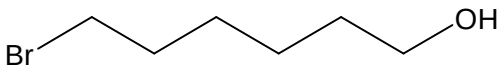


Sodium hydroxide solution (50%, 17.5 ml) was added to a vigorously stirring solution of (2*S*,3*R*)-3-methyl-henicosane-1,2-diol **(11)** (4.58 g, 13.39 mmol) and cetrimide (0.50 g) in dichloromethane (200 ml) at RT. To this, a solution of *p*-toluenesulfonyl chloride (3.15 g, 16.35 mmol) in dichloromethane (20 ml) was added over 10 minutes. The mixture was stirred for 30 minutes at RT and when TLC showed that no starting material was left, the mixture was quenched with water (150 ml). The organic layer was separated and the aqueous layer extracted with dichloromethane (2 x 50 ml). The combined organic layers were washed with water (100 ml), dried and evaporated to give a residue which was purified by column chromatography on silica gel eluting with petroleum ether/diethyl ether (10:0.5) to give *(S)-2-((R)-1-methyl-nonadecyl)-oxirane* **(12)** as a white solid (3.82 g, 88%).

Physical properties:

ν_{max}/cm^{-1}:	2919, 1473, 1261, 892, 729
m.p.	45-47 ºC
δ_H (500 MHz, $CDCl_3$):	2.78 (1H, dd, *J* 3.8, 4.75 Hz), 2.68 (1H, m), 2.54 (1H, dd, *J* 2.8, 5.0 Hz), 1.32-1.24 (35H, m including s), 1.03 (3H, d, *J* 6 Hz), 0.89 (3H, t, *J* 6.6 Hz)
δ_C (500 MHz, $CDCl_3$):	57.17(-), 47.03(+), 36.23(-), 33.57(+), 31.92(+), 29.87(+), 29.69(+), 29.65(+), 29.63(+), 29.58(+), 29.36(+), 27.12(+), 22.68(+), 17.13(-), 14.11(-), [+ = CH_2, - = CH, CH_3]
$[\alpha]_D^{22}$:	*reported as +0.3 ° (c = 1.01, $CHCl_3$)*

3.3.2.11 Preparation of 6-bromo-hexan-1-ol



HBr (48%, 44.51 g, 30 ml, 0.55 mol) was added to a stirring solution of 1,6-hexanediol (25 g, 0.21 mol) in toluene (300 ml). The mixture was refluxed overnight, cooled down and the toluene was evaporated. A saturated solution of $NaHCO_3$ (200 ml) was added and the mixture was extracted with dichloromethane (3 x 150 ml). The combined organic layers were dried and evaporated, the product was purified by column chromatography on silica gel eluting with petroleum ether/diethyl ether (5:1, then 1:1) to give *6-bromohexan-1-ol* (26.83 g, 71%) (108).

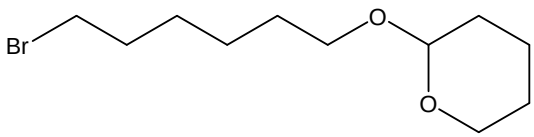
Physical properties:

ν_{max}/cm^{-1}: 3357, 2937, 1638, 1054

δ_H (500 MHz, $CDCl_3$): 3.56 (2H, t, *J* 6.6 Hz), 3.36 (2H, t, *J* 6.95 Hz), 2.63 (1H, s), 1.82 (2H, pent, *J* 6.95 Hz), 1.51 (2H, pent, *J* 6.95 Hz), 1.41 (2H, pent, *J* 6.95 Hz), 1.33 (2H, m)

δ_C (500 MHz, $CDCl_3$): 62.27(+), 33.74(+), 32.53(+), 32.23(+), 27.75(+), 24.76(+), [+ = CH_2, - = CH, CH_3]

3.3.2.12 Preparation of 1-bromo-6-tetrahydropyranyloxynonane



3,4-Dihydro-2*H*-pyran (26.50 g, 0.32 mol, 2.1 mol eq.) and pyridinium-*p*-toluene sulfonate (3 g) were added to a stirring solution of 6-bromo-hexan-1-ol (26.80 g, 0.15 mol) in dry dichloromethane (250 ml) under nitrogen at RT. The reaction was stirred at RT for 3 hours. When TLC showed that no starting material was left, the reaction mixture was filtered through a pad of silica gel and washed with dichloromethane (200 ml). The dichloromethane was evaporated and the residue purified by column chromatography on silica gel eluted with petroleum ether/diethyl ether (9:1) to give *1-bromo-6-tetrahydropyranyloxynonane* (38.08 g, 93%) (108).

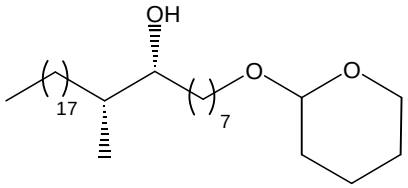
Physical properties:

ν_{max}/cm^{-1}: 2939, 2865, 1440, 1120

δ_H (500 MHz, $CDCl_3$): 4.54 (1H, m), 3.83 (1H, m), 3.71 (1H, ddd, *J* 6.6, 9.45, 16.4 Hz), 3.47 (1H, m), 3.38 (2H, t, *J* 6.6 Hz), 3.34 (1H, m), 1.89-1.76 (3H, m), 1.71-1.64 (1H, m), 1.60-1.33 (10H, m)

δ_C (500 MHz, $CDCl_3$): 98.73(-), 67.25(+), 62.21(+), 33.70(+), 32.63(+), 30.65(+), 29.43(+), 27.88(+), 25.34(+), 25.35(+), 19.57(+), [+ = CH_2, - = CH, CH_3]

*3.3.2.13 Preparation of (8**R**,9**R**)-9-methyl-1-(tetrahydropyran-2-yloxy)-heptacosan-8-ol (13)*



A solution of 1-bromo-6-tetrahydropyranyloxynonane (9.38 g, 35.39 mmol) in dry THF (25 ml) was added drop wise to a suspension of magnesium turnings (1.70 g, 70.83 mmol) in dry THF (30 ml) under nitrogen. The mixture was refluxed for 1 hour, then cooled down to RT and added drop wise to a stirring solution of purified copper iodide (0.59 g, 3.11 mmol) in dry THF (40 ml) at -30 °C. After 30 min a solution of (*S*)-2-((*R*)-1-methyl-nonadecyl)-oxirane **(12)** (3.82 g, 11.79 mmol) in dry THF (30 ml) was added drop-wise. The reaction mixture was stirred for another 2 ½ hours, then allowed to reach RT and stirred overnight. The mixture was quenched with saturated aqueous ammonium chloride (100 ml) and extracted with diethyl ether (3 x 200 ml). The combined organic layers were washed with brine (250 ml), dried and evaporated to give a colourless oil which was purified by column chromatography on silica gel eluted with petroleum ether/diethyl ether (9:1 changing to 3:1, 2:1 and 1:1) to give *(8*R,9R*)-9-methyl-1-(tetrahydropyran-2-yloxy)-heptacosan-8-ol* as a colouless oil **(13)** (4.45 g, 74%).

Physical properties:

ν_{max}/cm^{-1}: 3450, 2921, 2851, 1465, 1033

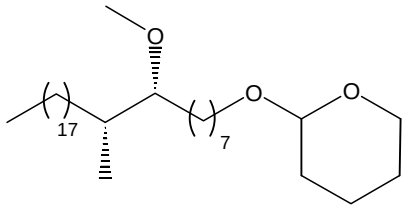
δ_H (500 MHz, $CDCl_3$): 4.57 (1H, m), 3.87 (1H, m), 3.73 (1H, ddd, *J* 6.9, 9.75, 16.7 Hz), 3.39 (2H, m), 3.38 (1H, ddd, *J* 6.6, 9.45, 16.05 Hz), 1.83 (1H, m), 1.72 (1H, m), 1.63-1.49 (6H, m), 1.42 (4H, m), 1.33 (7H, m),

1.29-1.20 (33H, m including s), 0.88 (3H, t, *J* 6.6 Hz), 0.85 (3H, d, *J* 6.65 Hz)

δ_C (500 MHz, $CDCl_3$): 98.80(-), 75.13(-), 67.61(+), 62.27(+), 38.17(-), 34.43(+), 33.33(+), 31.90(+), 30.75(+), 29.24(+), 29.71(+), 29.67(+), 29.63(+), 29.44(+), 29.33(+), 27.40(+), 26.21(+), 26.18(+), 25.48(+), 22.66(+), 22.58(+), 19.66(+), 14.08(-), 13.54(-), [+ = CH_2, - = CH, CH_3]

$[\alpha]_D^{22}$: *reported as +9.15 (c = 1.28, $CHCl_3$)*

*3.3.2.14 Preparation of 2-((8**R**,9**R**)-8-methoxy-9-methyl-heptacosyloxy)-tetrahydropyran (14)*



Sodium hydride (1.42 g, 58.1 mmol, 60% dispersion) was washed with petroleum ether (3 x 20 ml) and then suspended in dry THF (35 ml). The suspension was cooled to 5 °C and (8*R*,9*R*)-9-methyl-1-(tetrahydropyran-2-yloxy)-heptacosan-8-ol **(13)** (4.45 g, 8.73 mmol) in THF (35 ml) was added over 5 minutes. After 15 minutes methyl iodide (7.31 g, 51.5 mmol) was added. The mixture was stirred for 16 hours at RT. When TLC showed that no starting material was left, saturated ammonium chloride solution (50 ml) was added carefully followed by the addition of diethyl ether (100 ml). The organic layer was separated and the aqueous layer re-extracted with petroleum ether/diethyl ether (1:1, 2 x 50 ml). The combined organic layers were washed with brine (2 x 80 ml), dried and evaporated to give a residue which was purified by column chromatography on silica gel eluted with petroleum ether/diethyl ether (10:2) to give *2-((8R,9R)-8-methoxy-9-methyl-heptacosyloxy)-tetrahydropyran* **(14)** as a pale yellow oil (3.83 g, 84%).

Physical properties:

ν_{max}/cm^{-1}: 2928, 2846, 1077

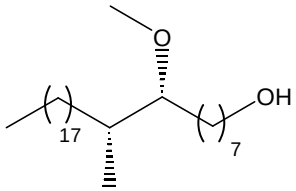
δ_H (500 MHz, $CDCl_3$): 4.60 (1H, t, *J* 2.3 Hz), 3.80 (1H, m), 3.74 (1H, dt, *J* 7, 9.45 Hz), 3.52 (1H, m), 3.40 (1H, dt, *J* 6.6, 9.45 Hz), 3.35 (3H, s), 2.97

(1H, m), 1.90-1.82 (1H, m), 1.76-1.70 (1H, m), 1.68-1.50 (6H, m), 1.45-1.25 (44H, m), 1.10 (1H, m), 0.91 (3H, t, *J* 7 Hz), 0.86 (3H, d, *J* 6.65 Hz)

δ_C (500 MHz, $CDCl_3$): 98.84, 85.43, 67.67, 62.30, 57.70, 35.30, 32.35, 31.91, 30.82, 30.47, 30.01, 29.89, 29.76, 29.70, 29.67, 29.50, 29.42, 27.59, 26.20, 26.14, 25.48, 22.17, 19.70, 14.89, 14.13, [+ = CH_2, - = CH, CH_3]

$[\alpha]_D^{22}$: *reported as +8.76 ° (c = 1.45, $CHCl_3$)*

3.3.2.15 Preparation of (8R,9R)-8-methoxy-9-methyl-heptacosan-1-ol (15)



p-Toluenesulfonic acid monohydrate (0.36 g, 1.89 mmol) was added to a stirring solution of 2-((8*R*,9*R*)-8-methoxy-9-methyl-heptacosyloxy)-tetrahydro-pyran **(14)** (3.80 g, 7.25 mmol) in THF (20 ml), methanol (70 ml) and water (1 ml) at RT. The reaction mixture was refluxed for 30 min. When TLC showed that no starting material was left, the solution was evaporated to approximately half of the volume and diluted with a saturated solution of sodium bicarbonate (50 ml). A mixture of petroleum ether/diethyl ether (1:1, 150 ml) was added and extracted. The organic layer was separated and the aqueous layer re-extracted with petroleum ether/diethyl ether (1:1, 2 x 50 ml). The combined organic layers were washed with brine (100 ml), dried and evaporated to give a residue which was purified by column chromatography on silica gel eluted with petroleum ether/diethyl ether (5:2) to give *(8R,9R)-8-methoxy-9-methyl-heptacosan-1-ol* **(15)** as a colourless liquid, which solidified later (3.23 g, 97%).

Physical properties:

ν_{max}/cm^{-1}: 3463, 2912, 2846, 1078

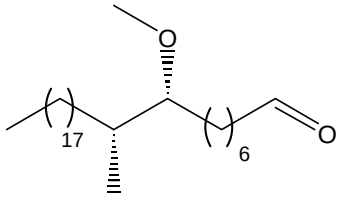
m.p. 33-35 ºC

δ_H (500 MHz, $CDCl_3$): 3.63 (2H, t *J* 6.6 Hz), 3.33 (3H, s), 2.95 (1H, m), 1.54 (4H, m), 1.45-1.20 (44H, m including s), 0.88 (3H, t, *J* 6.3 Hz), 0.84 (3H, d, *J* 6.9 Hz)

δ_C (500 MHz, $CDCl_3$): 85.46(-), 62.94(+), 57.66(-), 35.37(-), 32.78(+), 32.35(+), 31.91(+), 30.49(+), 29.97(+), 29.86(+), 29.68(+), 29.63(+), 29.42(+), 29.33(+), 27.56(+), 26.09(+), 25.71(+), 22.66(+), 14.87(-), 14.05(-), [+ = CH_2, - = CH, CH_3]

$[\alpha]_D^{26}$: +9.88 °, (c = 1.29, $CHCl_3$); *reported as* $[\alpha]_D^{22}$*: +11.05 ° (c = 1.19)*

3.3.2.16 Preparation of (8R,9R)-8-methoxy-9-methyl-heptacosanal (16)



(8*R*,9*R*)-8-Methoxy-9-methyl-heptacosan-1-ol **(15)** (3.1 g, 7.05 mmol) in dichloromethane (30 ml) was added to a stirring suspension of pyridinium chlorochromate (3.8 g, 17.6 mmol) in dichloromethane (200 ml) at RT. The mixture was stirred vigorously and refluxed for 3 hours. When TLC showed that no starting material was left, the mixture was cooled down and poured into diethyl ether (200 ml) and filtered through a pad of silica gel, washed with diethyl ether and the filtrate evaporated to give a residue that was purified by column chromatography on silica gel eluted with petroleum ether/diethyl ether (10:1) to give *(8R,9R)-8-methoxy-9-methyl-heptacosanal* **(16)** as a colourless liquid (2.31 g, 75%).

Physical properties:

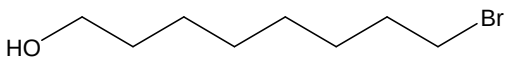
ν_{max}/cm^{-1}: 2924, 2850, 1729, 1465, 1097

δ_H (500 MHz, $CDCl_3$): 9.78 (1H, s), 3.40 (3H, s), 2.96 (1H, m), 2.44 (2H, t, *J* 7.25 Hz), 1.65 (4H, m), 1.45-1.25 (40H, m), 1.10 (1H, m), 0.88 (3H, t, *J* 6.3 Hz), 0.85 (3H, d, *J* 6.6 Hz)

δ_C (500 MHz, $CDCl_3$): 202.88, 85, 50, 57.70, 43.91, 35.29, 32.25, 30.00, 29.72, 29.66, 29.63, 29.17, 27.58, 25.97, 22.69, 22.10, 14.94, 14.11, [+ = CH_2, - = CH, CH_3]

$[\alpha]_D^{22}$: *reported as +11.6 ° (c = 1.16, $CHCl_3$)*

3.3.2.17 Preparation of 8-bromo-octan-1-ol (18)



HBr (48%, 34.79 g, 25 ml, 0.43 mol) was added to a stirring solution of 1,8-octanediol **(17)** (25 g, 0.17 mol) in toluene (350 ml). The mixture was refluxed overnight, cooled down and the toluene was evaporated. A saturated solution of $NaHCO_3$ (200 ml) was added and the mixture was then extracted with dichloromethane (3 x 150 ml). The combined organic layers were dried and evaporated and the product was purified by column chromatography on silica gel eluted with petroleum ether/diethyl ether (1:2) to give *8-bromo-octan-1-ol* **(18)** (21.2 g, 59%) (108).

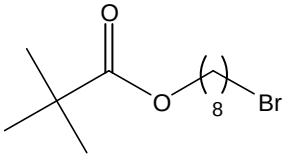
Physical properties:

ν_{max}/cm^{-1}: 3353, 2928, 2855,

δ_H (500 MHz, $CDCl_3$): 3.59 (2H, m), 3.38 (2H, m), 1.84 (2H, m), 1.54 (2H, m), 1.42 (2H, m), 1.32 (6H, broad s)

δ_C (500 MHz, $CDCl_3$): 62.72(+), 33.82(+), 32.69(+), 32.58(+), 29.11(+), 28.60(+), 27.99(+), 25.54(+), [+ = CH_2, - = CH, CH_3]

3.3.2.18 Preparation of 2,2-dimethyl-propionic acid 8-bromo-octyl ester (19)



A solution of trimethyl acetyl chloride (14.47 g, 120 mmol, 1.20 mol eq.) in dichloromethane (45 ml) was added over 15 minutes to a stirring solution of bromo alcohol **(18)** (21 g, 100 mmol), triethylamine (42 ml, 300 mmol, 3 mol eq.) and 4-dimethylaminopyridine (0.25 g, 2 mmol) in dichloromethane (160 ml). The reaction mixture was stirred overnight at RT. Diluted hydrochloric acid (150 ml, 5%) was added and the organic layer separated and washed with diluted hydrochloric acid (1 x 100 ml) and then with brine (2 x 200 ml), dried and evaporated. The residue was dissolved in petroleum ether (200 ml), filtered through a pad of silica gel and washed with petroleum ether (50 ml). The silica gel pad was washed with petroleum

ether/diethyl ether (1:1, 150 ml) and the solvent evaporated to give *2,2-dimethyl-propionic acid 8-bromo-octyl ester* **(19)** as a colourless oil (26.09 g, 89%).

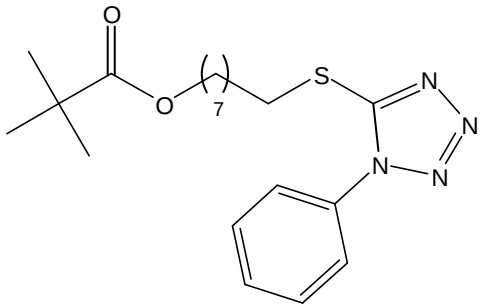
Physical properties:

ν_{max}/cm^{-1}: 2930, 2857, 1810, 1728

δ_H (500 MHz, $CDCl_3$): 4.03, (2H, t, *J* 6.6 Hz), 3.38 (2H, t, *J* 6.65 Hz), 1.84 (2H, pent, *J* 6.95 Hz), 1.61 (2H, m), 1.42 (2H, m), 1.32 (6H, m), 1.18 (9H, s)

δ_C (500 MHz, $CDCl_3$): 178.45, 64.25(-), 38.64, 33.71(-), 32.69(-), 28.94(-), 28.55(-), 28.51(-), 27.98(-), 27.13(+), 26.44(+), 25.73(-), 22.53(+), [- = CH_2, + = CH, CH_3]

*3.3.2.19 Preparation of 5-(1-octanolpivalate-8-sulfanyl)-1-phenyl-1*H*-tetrazole (20)*



2,2-Dimethyl-propionic acid 8-bromo-octyl ester **(19)** (26 g, 88.73 mmol) was added to a stirring solution of 1-phenyl-1*H*-tetrazole-5-thiol (17.40 g, 97.63 mmol, 1.10 mol eq.) and anhydrous potassium carbonate (25.80 g, 186.67 mmol, 2.1 mol eq.) in acetone (300 ml) and stirred overnight at RT. When TLC showed no starting material was left, the mixture was added to water (1L) and extracted with dichloromethane (1 x 150 ml, 2 x 25 ml). The combined organic layers were washed with brine (2 x 200 ml), dried and evaporated to give a dark yellow oil which was purified by column chromatography on silica gel eluted with petroleum ether/diethyl ether (7:2.5) to give *5-(1-octanolpivalate-8-sulfanyl)-1-phenyl-1H-tetrazole* **(20)** (31 g, 90%).

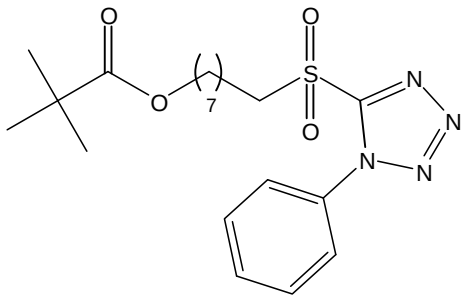
Physical properties:

ν_{max}/cm^{-1}: 2931, 2856, 1725

δ_H (500 MHz, $CDCl_3$): 7.55 (5H, m), 4.02 (2H, t, *J* 6.65 Hz), 3.37 (2H, t, *J* 7.55 Hz), 1.81 (2H, pent, *J* 7.25 Hz), 1.59 (2H, m), 1.43 (2H, m), 1.31 (6H, m), 1.17 (9H, s)

δ_C (500 MHz, $CDCl_3$): 178.51, 154.38, 133.65, 129.97(+), 129.67(+), 123.74(+), 64.23(-), 38.62, 33.20(-), 28.96(-), 28.92(-), 28.79(-), 28.45(-), 28.44(-), 27.11(+), 25.70(-), 22.52(+), 14.23(+), 11.33(+), [- = CH_2, + = CH, CH_3]

*3.3.2.20 Preparation of 5-(1-octanolpivalate-8-sulfonyl)-1-phenyl-1*H*-tetrazole (21)*



A solution of ammonium molybdate(VI)tetrahydrate (24.50 g, 19.88 mmol) in ice cold H_2O_2 35% (w/w, 48 ml) was added to a stirring solution of 5-(1-octanolpivalate-8-sulfanyl)-1-phenyl-1*H*-tetrazole **(20)** (15.54 g, 39.75 mmol) in THF (225 ml) and IMS (420 ml) at 10 °C and stirred at RT for 2 hours. A further solution of ammonium molybdate(VI)tetrahydrate (12.90 g) in ice cold H_2O_2 35% (w/w, 30 ml) was added and the mixture was stirred overnight at RT. The mixture was poured into water (1.8 ℓ) and extracted with dichloromethane (1 x 200 ml, 3 x 30 ml). The combined organic layers were washed with water (1 x 500 ml), dried and evaporated to give a colourless oil which was purified by column chromatography on silica gel eluted with petroleum ether/diethyl ether (3:1, changing to 2:1 and then to 1:1) to give *5-(1-octanolpivalate-8-sulfonyl)-1-phenyl-1H-tetrazole* **(21)** (14.93 g, 89%).

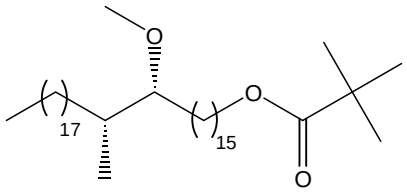
Physical properties:

ν_{max}/cm^{-1}: 2934, 2860, 1723

δ_H (500 MHz, $CDCl_3$): 7.69 (2H, m), 7.61 (3H, m), 4.04 (2H, t, *J* 6.6 Hz), 3.73 (2H, distorted t, *J* 7.85 Hz), 1.95 (2H, m), 1.61 (2H, m), 1.50 (2H, m), 1.34 (6H, m), 1.19 (9H, s)

δ_C (500 MHz, $CDCl_3$): 178.53, 153.42, 132.97, 131.39(+), 129.64(+), 125.00(+), 64.19(-), 55.87(-), 38.65, 28.72(-), 28.45(-), 27.97(-), 27.13(+), 25.68(-), 21.87(-), 15.20(+), [- = CH_2, + = CH, CH_3]

*3.3.2.21 Preparation of 2,2-dimethyl-propionic acid-(16**R**,17**R**)-16-methoxy-17-methyl-pentatriacontyl ester (22)*



Lithium bis(trimethylsilyl)amide (9.88 ml, 10.50 mmol, 1.06 M) was added drop-wise to a stirring solution of (8R,9R)-8-methoxy-9-methyl-heptacosanal **(6)** (2.30 g, 5.24 mmol) and 5-(1-octanolpivalate-8-sulfonyl)-1-phenyl-1*H*-tetrazole **(21)** (3.32 g, 7.86 mmol) in dry THF (100 ml) under nitrogen at -2 °C. The reaction mixture was allowed to reach RT and stirred for 16 hours, then quenched with saturated solution of ammonium chloride (50 ml) and petroleum ether/diethyl ether (1:1, 100 ml). The organic layer was separated and the aqueous layer re-extracted with petroleum ether/diethyl ether (1:1, 2 x 50 ml). The combined organic layers were washed with brine (2 x 100 ml), dried over magnesium sulfate and evaporated to give a thick oil, which was purified by column chromatography on silica gel eluted with petroleum ether/diethyl ether (10:0.3) to give *(E/Z)-2,2-dimethyl-propionic acid-(16R, 17R)-16-methoxy-17-methyl-pentatriacont-8-enyl ester* as a colourless liquid (2.65 g, 80%).

Physical properties:

δ_H (500 MHz, $CDCl_3$): 5.39 (1H, m), 5.35 (1H, m), 4.05 (2H, t, *J* 6.6 Hz), 3.34 (3H, s), 2.96 (1H, m), 1.99 (4H, m), 1.63 (4H, m), 1.37-1.24 (48 H, m including s), 1.20 (9H, s), 0.88 (3H, t, *J* 6.95 Hz), 0.85 (3H, d, *J* 6.9 Hz)

δ_C (500 MHz, $CDCl_3$): 178.56, 130.41(-), 130.21(-), 85.45(-), 64.41(+), 57.68(-), 38.70, 35.38(-), 32.57(+), 32.53(+), 32.39(+), 31.92(+), 30.52(+), 29.69(+), 29.35(+), 29.17(+), 29.07(+), 29.00(+), 28.62(+), 27.58(+), 27.20(-), 26.14(-), 25.87(+), 22.67(+), 14.88(-), 14.07(-), [+ = CH_2, - = CH, CH_3]

Palladium on charcoal (10%, 0.70 g) was added to a stirring solution of *(E/Z)-2,2-dimethyl-propionic acid-(16R, 17R)-16-methoxy-17-methyl-pentatriacont-8-enyl ester* (2.60 g, 4.11 mmol) in IMS (100 ml) and ethyl acetate (25 ml). The mixture was stirred while being hydrogenated under hydrogen atmosphere. When no more hydrogen was absorbed the mixture

was filtered through a pad of celite and washed with warmed ethanol (100 ml). The clear colourless filtrate was evaporated to give a white solid of *2,2-dimethyl-propionic acid-(16R, 17R)-16-methoxy-17-methyl-pentatriacontyl ester* **(22)** as a colourless oil (2.36 g, 91%).

Physical properties:

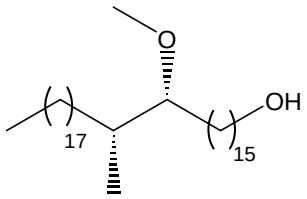
ν_{max}/cm^{-1}: 2922, 2852, 1731, 1155

δ_H (500 MHz, $CDCl_3$): 4.04 (2H, t, *J* 6.65 Hz), 3.34 (3H, s), 2.95 (1H, m), 1.33-1.23 (56 H, m including s), 1.19 (9H, s), 0.88 (3H, t, *J* 6.95 Hz), 0.85 (3H, d, *J* 6.9 Hz)

δ_C (500 MHz, $CDCl_3$): 178.56, 85.44(-), 64.43(+), 57.67(-), 38.70, 35.37(-), 32.40(+), 31.91(+), 30.51(+), 29.96(+), 29.92(+), 29.68(+), 29.55(+), 29.51(+), 29.34(+), 29.22(+), 28.62(+), 27.56(+), 27.18(-), 26.16(+), 25.90(+), 22.66(+), 20.97(-), 14.86(-), 14.17(-), 14.07(-), [+ = CH_2, - = CH, CH_3]

$[\alpha]_D^{24}$: +6.08 °, (c = 1.19, $CHCl_3$); *reported as* $[\alpha]_D^{22}$*: +6.8 ° (c = 1.49, $CHCl_3$)*

3.3.2.22 Preparation of (16R,17R)-16-methoxy-17-methyl-pentatriacontan-1-ol (23)

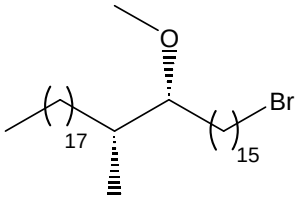


Potassium hydroxide (0.84 g, 14.97 mmol) in methanol (20 ml) was added to a stirring solution of (16*R*,17*R*)-1-*tert*-butoxy-16-methoxy-17-methyl-pentatriacontane **(22)** (2.35 g, 3.71 mmol) in THF (50 ml) at RT. The reaction mixture was stirred overnight at 40 °C. When TLC showed that no starting material was left, the reaction was quenched with water (100 ml) and a mixture of petroleum ether/diethyl ether (1:1, 100 ml). The organic layer was separated and the aqueous layer re-extracted with petroleum ether/diethyl ether (2 x 50 ml). The combined organic layers were washed with brine (60 ml), dried and evaporated to give a white solid which was purified by column chromatography on silica gel eluted with petroleum ether/diethyl ether (2:1) to give a white solid of *(16R,17R)-16-methoxy-17-methyl-pentatriacontan-1-ol* **(23)** (1.81 g, 89%).

Physical properties:

ν_{max}/cm^{-1}:	3373, 2921, 1098, 1076
m.p.	46-48 ºC
δ_H (500 MHz, $CDCl_3$):	3.65 (2H, q, *J* 6.6 Hz), 3.35 (3H, s), 2.96 (1H, m), 1.64 (1H, m), 1.58, (6H, pent, J 6.9 Hz), 1.4-1.24 (54H, m including s), 1.10 (1H, m), 0.89 (3H, t *J* 6.95 Hz), 0.85 (3H, d, *J* 6.95 Hz)
δ_C (500 MHz, $CDCl_3$):	85.45(-), 63.10(+), 57.71(-), 35.30(-), 32.81(+), 32.34(+), 31.92(+), 30.46(+), 29.98(+), 29.93(+), 29.69(+), 29.60(+), 29.42(+), 29.36(+), 27.56(+), 26.16(+), 25.73(+), 22.68(+), 14.88(-), 14.11(-), [+ = CH_2, - = CH, CH_3]
$[\alpha]_D^{24}$:	+7.76 °, (c = 1.03, $CHCl_3$); *reported as* $[\alpha]_D^{22}$*: +7.9 ° (c = 1.40, $CHCl_3$)*

*3.3.2.23 Preparation of (16**R**,17**R**)-1-bromo-16-methoxy-17-methyl-pentatriacontane (24)*

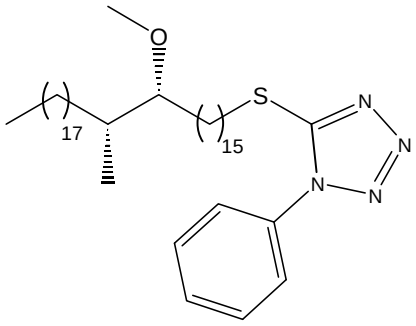


N-Bromosuccinimide (0.75 g, 4.21 mmol, 1.3 mol eq.) was added in portions over 15 minutes to a stirring solution of (16*R*,17*R*)-16-methoxy-17-methyl-pentatriacontan-1-ol **(23)** (1.80 g, 3.26 mmol) and triphenylphosphine (0.94 g, 3.58 mmol, 1.1 mol eq.) in dichloromethane (50 ml) at 0 °C. The reaction mixture was stirred at RT for 1 hour. When TLC showed that no starting material was left, the reaction was quenched with saturated solution of sodium meta-bisulfite (50 ml). The organic layer was separated and the aqueous layer re-extracted with dichloromethane (2 x 30 ml). The combined organic layers were washed with water, dried and evaporated to give a residue which was treated with a mixture of petroleum ether/diethyl ether (1:1, 100 ml). The mixture was refluxed for 30 minutes. The triphenylphosphine oxide was filtered off and washed with petroleum ether/diethyl ether (50 ml). The filtrate was evaporated and the residue was purified by column chromatography on silica gel eluted with petroleum ether/diethyl ether (10:0.2) to give *(16R,17R)-1-bromo-16-methoxy-17-methyl-pentatriacontane* **(24)** as a white solid (1.65 g, 82%).

Physical properties:

ν_{max}/cm^{-1}:	2929, 2849, 1099,717
m.p.	38-40 ºC
δ_H (500 MHz, $CDCl_3$):	3.42 (2H, t, *J* 6.9 Hz), 3.35 (3H, s), 2.96 (1H, m), 1.86 (2H, pent, *J* 6.95 Hz), 1.64 (1H, m), 1.45-1.2 (60 H, m including s), 0.89 (3H, t, *J* 6.6 Hz), 0.85 (3H, d, *J* 6.95 Hz)
δ_C (500 MHz, $CDCl_3$):	85.44(-), 57.71(-), 35.29(-), 34.06(+), 32.84(+), 32.34(+), 31.92(+), 30.46(+), 29.98(+), 29.93(+), 29.70(+), 29.62(+), 29.54(+), 29.44(+), 29.36(+), 28.77(+), 28.18(+), 27.57(+), 26.16(+), 22.69(+), 14.89(-), 14.12(-), [+ = CH_2, - = CH, CH_3]
$[\alpha]_D^{20}$:	+5.98 °, (c = 1.06, $CHCl_3$); *reported as* $[\alpha]_D^{22}$*: +6.5 ° (c = 1.16, $CHCl_3$)*

*3.3.2.24 Preparation of 5-((16**R**,17**R**)-16-methoxy-17-methyl-pentatriacontyl-1- sulfanyl)-1-phenyl-1**H**-tetrazole (25)*



(16*R*,17*R*)-1-Bromo-16-methoxy-17-methyl-pentatriacontane **(24)** (1.60 g, 2.60 mmol) was added to a stirring solution of 1-phenyl-1*H*-tetrazol-5-thiol (0.51 g, 2.86 mmol) and potassium carbonate (1.44 g, 10.42 mmol) in acetone (60 ml) at RT. After 23 hours of stirring, TLC still showed that starting material was left. A further amount of 1-phenyl-1*H*-tetrazol-5-thiol (0.2 g) was added and the mixture was stirred overnight at RT. Then the solvent was evaporated and the residue diluted with a mixture of petroleum ether/diethyl ether (1:1, 150 ml) and water (100 ml). The organic layer was separated and the aqueous layer re-extracted with petroleum ether/diethyl ether (2 x 50 ml). The combined organic layers were dried and evaporated to give a pale yellow viscous oil which was purified by column chromatography on silica gel eluting

with petroleum ether/diethyl ether (10:1) to give *5-((16R,17R)-16-methoxy-17-methyl-pentatriacontyl-1-sulfanyl)-1-phenyl-1H-tetrazole* **(25)** as colourless viscous oil (1.65 g, 89%).

Physical properties:

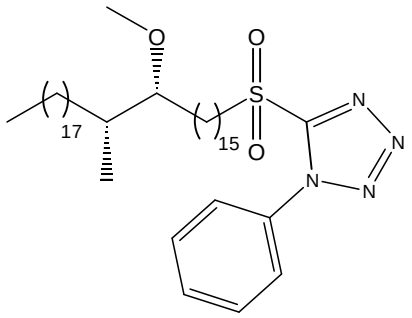
ν_{max}/cm^{-1}: 2928, 2861, 1097

δ_H (500 MHz, $CDCl_3$): 7.58 (5H, m), 3.41 (2H, t, *J* 7.25 Hz), 3.35 (3H, s), 2.96 (1H, m), 1.83 (2H, pent, *J* 7.25 Hz), 1.63 (1H, m), 1.47-1.20 (62H, m including s), 1.10 (1H, m), 0.89 (3H, t, *J* 6.65 Hz), 0.85 (3H, d, *J* 6.95 Hz)

δ_C (500 MHz, $CDCl_3$): 130.03(-), 129.75(-), 123.88(-), 85.48(-), 57.72 (-), 35.40(-), 33.42(+), 32.42(+), 31.93(+), 30.54(+), 29.98(+), 29.95(+), 29.70(+), 29.63(+), 29.55(+), 29.44(+), 29.36(+), 29.11(+), 29.04(+), 28.66(+), 27.58(+), 26.19(+), 22.68(+), 14.89(-), 14.10(-), [+ = CH_2, - = CH, CH_3]

$[\alpha]_D^{18}$: +6.79 °, (c = 1.03, $CHCl_3$); *reported as* $[\alpha]_D^{22}$*: +6.18 (c = 1.12, $CHCl_3$)*

*3.3.2.25 Preparation of 5-((16*R*,17*R*)-16-methoxy-17-methyl-pentatriacontane-1-sulfonyl)-1-phenyl-1*H*-tetrazole (26)*



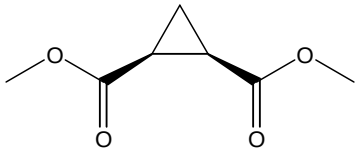
A solution of ammonium heptamolybdate(VI)tetrahydrate (2.48 g, 2.30 mmol, 1.1 mol eq.) in ice cold H_2O_2 35% (w/w, 11 ml) was added to a stirring solution of 5-((16*R*,17*R*)-16-methoxy-17-methyl-pentatriacontyl-1-sulfanyl)-1-phenyl-1*H*-tetrazole **(25)** (1.62 g, 2.28 mmol) in THF (30 ml) and IMS (70 ml) at 5 °C and stirred at RT for 1 hour. A further solution of ammonium heptamolybdate(VI)tetrahydrate (1.42 g, 1.15 mmol) in ice cold H_2O_2 35% (w/w, 5 ml) was added and the mixture was stirred overnight at RT. Dichloromethane (60 ml) and water (300 ml) was added, the organic layer separated and the aqueous layer re-extracted with

dichloromethane (2 x 30 ml). The combined organic layers were washed with water, dried and evaporated to give a residue which was purified by column chromatography on silica gel eluting with petroleum ether/diethyl ether (10:1) to give *5-((16R,17R)-16-methoxy-17-methyl-pentatriacontane-1-sulfonyl)-1-phenyl-1H-tetrazole* **(26)** as a white solid (1.1 g, 65%).

Physical properties:

ν_{max}/cm^{-1}:	2924, 2849, 1343, 1157, 1096
m.p.	42-44 ºC
δ_H (500 MHz, $CDCl_3$):	7.71 (2H, m), 7.61 (3H, m), 3.74 (2H, distorted t, *J* 7.85 Hz), 3.34 (3H, s), 2.96 (1H, m), 1.96 (2H, m), 1.63 (1H, m), 1.50-1.20 (63H, m including s), 0.89 (3H, t, *J* 6.65 Hz), 0.86 (3H, d, *J* 6.6 Hz)
δ_C (500 MHz, $CDCl_3$):	153.56, 133.11, 131.44(-), 129.70(-), 125.10(-), 85.48(-), 57.72(-), 56.07(+), 35.40(-), 33.42(+), 32.42(+), 31.93(+), 30.54(+), 29.98(+), 29.95(+), 29.70(+), 29.66(+), 29.57(+), 29.47(+), 29.36(+), 29.19(+), 29.11(+), 28.90(+), 28.66(+), 28.16(+), 27.58(+), 26.19(+), 22.68(+), 21.20(+), 14.89(-), 14.10(-), [+ = CH_2, - = CH, CH_3]
$[\alpha]_D^{26}$:	+5.46 °, (c = 1.08, $CHCl_3$); *reported as* $[\alpha]_D^{22}$*: +5.65 ° (c = 1.90, $CHCl_3$)*

3.3.2.26 Preparation of cis*-cyclopropane-1,2-dicarboxylic acid dimethyl ester (27b)*



Sodium methoxide (25.60 g, 0.66 mol) was added to a stirring mixture of methylacrylate (100 ml, 1.54 mol) and methylchloroacetate (41.50 ml, 0.66 mol) in an ice bath at 20-32 °C over 1 hour. The reaction is strongly exothermic. The reaction mixture was stirred at 18-25 °C for 1 hour. Water (150 ml) was added, stirred for 10 minutes and the organic phase washed with brine (2 x 50 ml) and the combined aqueous phases were re-extracted with dichloromethane (2 x 50 ml). The combined organic phases were dried and the solvent evaporated to give a pale yellow oil. This oil was distilled at 1 mm Hg, and collected in four fractions. The fractions

were analysed by GLC. The programme: initial temperature was 80 °C for 1 minute, increase rate of 20 °C/minute up to 240 °C, and then for 1 minute at 240 °C. The oven temperature was 80 °C. Peaks observed were: at 0.94 minutes the *trans*-ester, at 1.15 minutes the *cis*-ester, at 1.55, 2.20, 1.93, 2.51, 3.09, 4.04 and 5.06 minutes high boiling point impurities. The rough composition of the different fractions was as follow:

Fraction	Yield	Boiling point	*trans*-ester	*cis*-ester	impurities
1	71 g	< 40 °C	0	0	0.2
2	40 g	40-70 °C	1.6	2.5	0.2
3	8.73 g	70-80 °C	0.1	4.2	0.7
4	1.2 g	80-95 °C	0.02	2.3	1.5

The fractions containing the *cis*-stereoisomer were separated by column chromatography on silica gel eluted with petroleum ether/diethyl ether (9:1) to give a colourless oil, *cis-cyclopropane-1,2-dicaboxylic acid-dimethylester* **(27b)** (32.74 g, 31%) (68).

Physical properties:

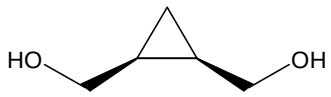
ν_{max}/cm^{-1}: 3626, 3003, 2955, 1720

δ_H (500 MHz, $CDCl_3$): 3.70 (6H, s), 2.08 (2H, dd, *J* 6.9, 8.5 Hz), 1.70 (1H, ddd, *J* 5, 6.6, 13.2 Hz), 1.26 (1H, dt, *J* 5.05, 8.55 Hz)

δ_C (500 MHz, $CDCl_3$): 170.21, 51.98(+), 21.23(+), 11.64(-), [- = CH_2, + = CH, CH_3]

$[\alpha]_D^{26}$: +0.18 ° (c = 1.51, $CHCl_3$)

*3.3.2.27 Preparation of (*cis*-2-hydroxymethylcyclopropyl)-methanol (28)*



$LiAlH_4$ (0.2 g) was added to stirring THF (300 ml) at -20 °C under nitrogen to check the dryness of the THF. Then $LiAlH_4$ (9.00 g, 237.09 mmol, 2 mol eq.) was added. A solution of *cis*-cyclopropane-1,2-dicaboxylic acid dimethyl ester **(27b)** (18.73 g, 118.55 mmol) in THF (100 ml) was added drop-wise at -20 °C and the mixture refluxed for 1 hour. A freshly prepared saturated solution of sodium sulfate decahydrate (30 ml) was added at -20 °C and

stirred for 30 minutes at RT. The mixture was filtered through a pad of silica gel, dried and the solvent evaporated to give a colourless oil of *(cis-2-hydroxymethylcyclopropyl)-methanol* **(28)** (11.42 g, 95%) (68).

Physical properties:

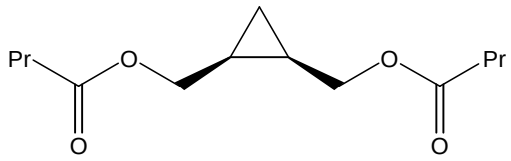
ν_{max}/cm^{-1}: 3320, 3003, 2885

δ_H (500 MHz, $CDCl_3$): 4.09 (2H, m), 3.24 (2H, m), 1.28 (2H, m), 0.80 (1H, m), 0.20 (1H, m)

δ_C (500 MHz, $CDCl_3$): 62.95(+), 17.48(-), 8.49(+), [+ = CH_2, - = CH, CH_3]

$[\alpha]_D^{26}$: -0.2 ° (c = 1.15, $CHCl_3$)

3.3.2.28 Preparation of butyric acid cis-*2-butyryloxymethylcyclopropylmethyl ester (29)*



Butyric anhydride (37.53 g, 237.25 mmol, 2.2 mol eq.) was added to (*cis*-2-hydroxymethylcyclopropyl)-methanol **(28)** (11.00 g, 107.84 mmol). The mixture was refluxed at 140 °C for 1 hour and then cooled to RT. Dichloromethane (200 ml) and sodium hydroxide solution (14 g in 200 ml water) were added and then extracted. The aqueous layer was re-extracted with dichloromethane (2 x 50 ml) and the combined organic layers were washed with sodium bicarbonate solution (100 ml). The organic phase was dried, the solvent evaporated and the excess butyric anhydride distilled at high vacuum. The crude product was purified by column chromatography eluted with petroleum ether/diethyl ether (5:1) to give a colourless oil of *butyric acid cis-2-butyryloxymethylcyclopropylmethyl ester* **(29)** (23.69 g, 91%) (68).

Physical properties:

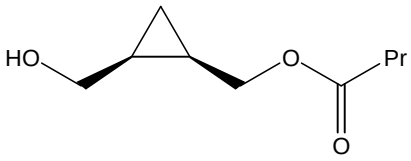
ν_{max}/cm^{-1}: 2966, 2877, 1725

δ_H (500 MHz, $CDCl_3$): 4.25 (2H, dd, *J* 6.9, 11.95 Hz), 3.97 (2H, dd, *J* 7.85, 11.95), 2.30 (4H, t, *J* 7.55 Hz), 1.67 (4H, sextet, *J* 7.25 Hz), 1.33 (2H, m), 0.96 (6H, t, *J* 7.25 Hz), 0.90 (1H, dt, *J* 5.05, 8.2 Hz), 0.35 (1H, q, *J* 5.65 Hz)

δ_C (500 MHz, $CDCl_3$): 173.52, 64.12(+), 36.15(+), 18.40(+), 14.65(-), 13.59(-), 8.58(+), [+ = CH_2, - = CH, CH_3]

$[\alpha]_D^{26}$: +0.2 ° (c = 1.65, $CHCl_3$)

3.3.2.29 Preparation of butyric acid cis-*2-hydroxymethylcyclopropylmethyl ester (30)*



Lipase (1 g, PPL (Pig pancreas lipase), type II) was added to a flask fitted with a glass pH electrode which had been accurately calibrated, containing a gently stirring solution of distilled water (161 ml) and ethylene glycol (41 ml) at 3 °C under a steady stream of nitrogen. Butyric acid *cis*-2-butyryloxymethylcyclopropylmethyl ester **(29)** (10.50 g, 43.38 mmol) was then added. When hydrolysis began, pH decreased due to the formation of butyric acid. The pH was brought back to 6.5 by the careful addition of sodium hydroxide (1M) whilst maintaining the temperature at 3 °C. More lipase (0.75 g) was added to the reaction mixture after 1 hour and sodium hydroxide solution was added drop-wise during the reaction to keep the pH at 6.5. The total added sodium hydroxide solution was 45 ml and it took 4 ¼ hours. The mixture was filtered through a pad of celite; the pad of celite was then washed with water (25 ml) and then ether (50 ml). A saturated solution of sodium bicarbonate (60 ml) was added, pH 8.44, and then neutralised by ammonium chloride solution to pH 7.4. The mixture was extracted with ether (2 x 300 ml) and the combined organic layers were dried. The solvent was evaporated and the crude product was purified by column chromatography on silica gel eluted with petroleum ether/diethyl ether (1:1) to give a colourless oil of *butyric acid cis-2-hydroxymethylcyclopropylmethyl ester* **(30)** (5.39 g, 72%) (68).

Physical properties:

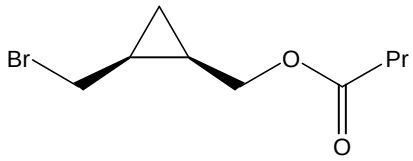
ν_{max}/cm^{-1}: 3417, 2966, 1732, 1186, 1046

δ_H (500 MHz, $CDCl_3$): 4.46 (1H, dd, *J* 5.7, 12 Hz), 3.83 (2H, m), 3.39 (1H, dd, *J* 9.15, 11.65 Hz), 2.30 (2H, t, *J* 7.55 Hz), 2.21 (1H, broad s), 1.65 (2H, sextet, *J* 7.25 Hz), 1.30 (2H, m), 0.94 (3H, t, *J* 7.55 Hz), 0.84 (1H, dt, *J* 5.05, 8.5 Hz), 0.22 (1H, q, *J* 5.65 Hz)

δ_C (500 MHz, $CDCl_3$): 173.62, 64.37(+), 62.46(+), 36.20(+), 18.58(-), 18.37(+), 14.36(-), 13.56(-), 7.68(+), [+ = CH_2, - = CH, CH_3]

$[\alpha]_D^{26}$: +19.35 ° (c = 1.33, $CHCl_3$), *reported as* $[\alpha]_D^{22}$*: +18.2 ° (c = 1.58,* $CHCl_3$*) by Grandjean et al. (68)*

3.3.2.30 Preparation of butyric acid cis*-2-bromomethylcyclopropylmethyl ester (31)*



Triphenylphosphine (8.78 g, 33.43 mmol, 1.15 mol eq.) and butyric acid *cis*-2-hydroxymethylcyclopropylmethyl ester **(30)** (5 g, 29.07 mmol) were dissolved in dichloromethane (150 ml) at RT and then cooled to 0 °C. *N*-bromosuccinimide (6.57 g, 36.92 mmol, 1.27 mol eq.) was added portion wise over 20 minutes at 0-4 °C. The mixture was allowed to reach RT and then stirred for 1 hour. When TLC showed that no starting material was left, a saturated solution of sodium bisulfate (140 ml) was added and the mixture was extracted. The aqueous layer was re-extracted with dichloromethane (2 x 50 ml) and the combined organic layers were washed with water (100 ml). The solution was dried and the solvent evaporated. Petroleum ether/diethyl ether (1:1, 250 ml) was added and the mixture stirred for 30 minutes at RT. The triphenylphosphonium oxide was filtered off and washed thoroughly with a mixture of petroleum ether/diethyl ether (1:1). The solvent was evaporated and the crude product purified by column chromatography on silica gel eluted with petroleum ether/diethyl ether (4:1) to give a colourless oil of *butyric acid cis-2-bromomethylcyclopropylmethyl ester* **(31)** (6 g, 88%).

Physical properties:

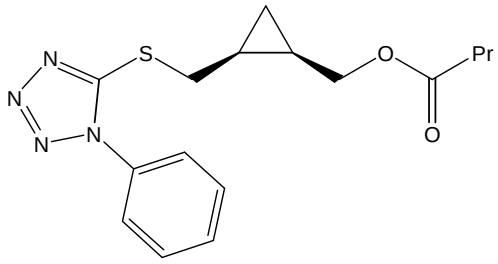
ν_{max}/cm^{-1}: 2966, 2876, 1734, 1181, 986

δ_H (500 MHz, $CDCl_3$): 4.22 (1H, dd, *J* 6.95, 12 Hz), 4.03 (1H, dd, *J* 8.5, 12.3 Hz), 3.51 (1H, dd, *J* 7.55, 10.4 Hz), 3.38 (1H, dd, *J* 8.2, 10.4 Hz), 2.29 (2H, t, *J* 7.6 Hz), 1.65 (2H, sextet, *J* 7.25 Hz), 1.48 (2H, m), 1.01 (1H, dt, *J* 5.35, 8.2 Hz), 0.94 (3H, t, *J* 7.6 Hz), 0.36 (1H, q, *J* 5.65 Hz)

δ_C (500 MHz, $CDCl_3$): 173.52, 63.31(+), 36.13(+), 33.96(+), 19.29(-), 18.36(+), 17.96(-), 13.59(-), 12.49(+), [+ = CH_2, - = CH, CH_3]

$[\alpha]_D^{24}$: -9.69 ° (c = 1.37, $CHCl_3$); *reported as* $[\alpha]_D^{23}$*: -10.6 ° (c = 0.81,* $CHCl_3$*)*

*3.3.2.31 Preparation of butyric acid (1*R*,2*S*)-2-(1-phenyl-1*H*-tetrazol-5-ylsulfanylmethyl)-cyclopropyl methyl ester (32)*



Butyric acid *cis*-2-bromomethylcyclopropylmethyl ester **(31)** (8.00 g, 34.04 mmol) was added to a stirring solution of 1-phenyl-1H-tetrazol-5-thiol (6.67 g, 37.44 mmol) and potassium carbonate (9.89 g, 71.48 mmol) in acetone (250 ml) at RT and the reaction mixture was stirred for 19 hours at RT. When TLC showed that no starting material was left, the mixture was added to water (1.5 L) and extracted with dichloromethane (250 ml). The aqueous layer was re-extracted with dichloromethane (2 x 80 ml) and the combined organic layers were washed with brine (2 x 200 ml), dried and evaporated. The crude product was purified by column chromatography on silica gel eluted with petroleum ether/diethyl ether (2:1) to give a pale yellow oil of *butyric acid (1*R*,2*S*)-2-(1-phenyl-1H-tetrazol-5-ylsulfanylmethyl)-cyclopropylmethyl ester* **(32)** (10.95 g, 88%).

Physical properties:

Found M + Na^+: 355.1185, $C_{16}H_{20}N_4NaO_2S$ requires: 355.1199

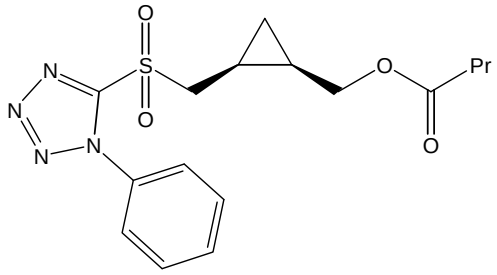
ν_{max}/cm^{-1}: 2965, 2875, 1732, 1500, 1174, 983, 763

δ_H (500 MHz, $CDCl_3$): 7.57 (5H, m), 4.34 (1H, dd, *J* 6.6, 12.25 Hz), 3.96 (1H, dd, *J* 9.15, 11.95 Hz), 3.59 (1H, dd, *J* 7.85, 13.25 Hz), 3.43 (1H, dd, *J* 7.9, 13.55 Hz), 2.27 (2H, t, *J* 7.55 Hz), 1.64 (2H, sextet, *J* 7.55 Hz), 1.51 (1H, m), 1.41 (1H, m), 0.97 (1H, dt, *J* 5.4, 8.2 Hz), 0.93 (3H, t, *J* 7.25 Hz), 0.40 (1H, q, *J* 5.7 Hz)

δ_C (500 MHz, $CDCl_3$): 173.45, 154.25, 133.81, 130.05(+), 129.73(+), 123.84(+), 63.72(-), 36.20(-), 34.18(-), 18.38(-), 16.39(+), 15.53(+), 13.61(+), 10.96(-), [- = CH_2, + = CH, CH_3]

$[\alpha]_D^{24}$: -1.28 ° (c = 1.14, $CHCl_3$); *reported as* $[\alpha]_D^{22}$*: -1.2 ° (c = 1.06, $CHCl_3$)*

*3.3.2.32 Preparation of butyric acid (1*R*,2*S*)-2-(1-phenyl-1*H*-tetrazole-5-sulfonylmethyl)-cyclopropyl methyl ester (33)*



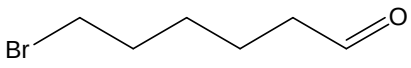
A solution of ammonium heptamolybdate(VI)tetrahydrate (18.59 g, 15.06 mmol) in ice cold H_2O_2 35% (w/w, 50 ml) was added to a stirring solution of butyric acid (1*R*,2*S*)-2-(1-phenyl-1*H*-tetrazol-5-ylsulfanylmethyl)-cyclopropylmethyl ester **(32)** (10.00 g, 30.12 mmol) in THF (125 ml) and IMS (250 ml) at 10 °C and stirred at RT for 2 hours. A further solution of ammonium heptamolybdate(VI)tetrahydrate (5.03 g, 4.07 mmol) in ice cold H_2O_2 35% (w/w, 15 ml) was added and the mixture stirred for 19 hours at RT. The reaction mixture was poured into water (1.5 L) and extracted with dichloromethane (1 x 300 ml, 2 x 80 ml). The combined organic layers were washed with water (700 ml), dried and evaporated to give a residue which was purified by column chromatography on silica gel eluted with petroleum ether/diethyl ether (2:1) to give a thick pale yellow oil of *butyric acid (1*R*,2*S*)-2-(1-phenyl-1H-tetrazole-5-sulfonylmethyl)-cyclopropylmethyl ester* **(33)** (10.18 g, 93%).

Physical properties:

Found M + Na^+: 387.1079, $C_{16}H_{20}N_4NaO_4S$ requires: 387.1097

ν_{max}/cm^{-1}:	2967, 1731, 1343, 1153, 765
δ_H (500 MHz, $CDCl_3$):	7.70 (2H, m), 7.63 (3H, m), 4.37 (1H, dd, *J* 5.7, 12.3 Hz), 4.03 (1H, dd, *J* 5.4, 14.85 Hz), 3.92 (1H, dd, *J* 8.2, 12 Hz), 3.67 (1H, dd, *J* 8.85, 15.15 Hz), 2.31 (2H, t, *J* 7.55 Hz), 1.67 (2H, sextet, *J* 7.55 Hz), 1.49 (2H, m), 1.03 (1H, dt, *J* 5.7, 8.5 Hz), 0.97 (3H, t, *J* 7.55 Hz), 0.60 (1H, q, *J* 5.7 Hz)
δ_C (500 MHz, $CDCl_3$):	173.33, 153.70, 133.14, 131.45(+), 129.68(+), 125.17(+), 63.37(-), 56.76(-), 36.13(-), 18.41(-), 14.73(+), 13.61(+), 9.70(-), 8.68(+), [- = CH_2, + = CH, CH_3]
$[\alpha]_D^{24}$:	+44.22 ° (c = 1.10, $CHCl_3$); *reported as* $[\alpha]_D^{23}$*: +52.7 ° (c = 1.45, $CHCl_3$)*

3.3.2.33 Preparation of 6-bromo-hexanal (34)



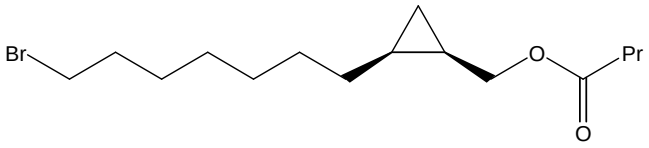
6-Bromo-hexan-1-ol (10.00 g, 55.25 mmol, see Preparation of 6-Bromo-hexan-1-ol, section 3.3.2.11) in dichloromethane (30 ml) was added to a stirring suspension of pyridinium chlorochromate (29.95 g, 138.94 mmol) in dichloromethane (300 ml) at RT. The mixture was stirred for 3 hours at RT. When TLC showed no starting material was left, the mixture was poured into diethyl ether (500 ml) and filtered through a pad of silica gel, then washed well with diethyl ether. The filtrate was evaporated to give a residue which was purified by column chromatography on silica gel eluted with petroleum ether/diethyl ether (5:1, then 1:1) to give *6-bromo-hexanal* **(34)** as a colourless oil (6.31 g, 64%).

Physical properties:

δ_H (500 MHz, $CDCl_3$): 9.77 (1H, t, *J* 1.25 Hz), 3.40 (2H, t, *J* 6.9 Hz), 2.46 (2H, dt, *J* 7.25, 1.25 Hz), 1.88 (2H, pent, *J* 6.6 Hz), 1.66 (2H, pent, *J* 7.25 Hz), 1.48 (2H, m)

δ_C (500 MHz, $CDCl_3$): 201.96(+), 43.59 (-), 33.23 (-), 32.42 (-), 27.64(-), 21.16(-), [- = CH_2, + = CH, CH_3]

*3.3.2.34 Preparation of butyric acid (1**R**,2**S**)-2-(7-bromo-heptyl)-cyclopropyl methyl ester (35)*



Lithium bis(trimethylsilyl)amide (46.66 ml, 49.56 mmol, 1.06 M, 2 mol eq.) was added drop-wise to a stirring solution of butyric acid (1*R*,2*S*)-2-(1-phenyl-1*H*-tetrazole-5-sulfonylmethyl)-cyclopropylmethyl ester **(33)** (9.00 g, 24.73 mmol) and 6-bromo-hexanal **(34)** (5.31 g, 29.67 mmol, 1.2 mol eq.) in dry THF (130 ml) under nitrogen at - 20 °C. The reaction mixture was allowed to reach RT and was then stirred for 16 hours. The reaction was quenched with water (100 ml) and petroleum ether/diethyl ether (1:1, 100 ml). The organic layer was separated and the aqueous layer re-extracted with petroleum ether/diethyl ether (1:1, 2 x 50 ml). The combined organic layers were washed with brine (2 x 100 ml), dried and evaporated to give a

thick dark yellow oil which was purified by column chromatography on silica gel eluted with petroleum ether/diethyl ether (9:1) to give *butyric acid 2-((E/Z)-7-bromo-hept-1-enyl)-cyclopropylmethyl ester* (5.80 g, 74%).

Butyric acid 2-((*E*/*Z*)-7-bromo-hept-1-enyl)-cyclopropylmethyl ester (5.75 g, 18.14 mmol) was dissolved in THF (150 ml). 2,4,6-Triisopropylbenzene sulfonohydrazide (14.88 g, 49.88 mmol, 2.75 mol eq.) was added and the reaction mixture was stirred at 50 °C for 20 hours. A further 2,4,6-triisopropylbenzene sulfonohydrazide (3.7 g, 12.40 mmol, 0.68 mol eq.) was added and the reaction was stirred at 50 °C for 22 hours. The mixture was diluted with petroleum ether/diethyl ether (1:1, 200 ml) and a solution of NaOH in water (100 ml, 2%) and extracted. The aqueous layer was re-extracted with petroleum ether/diethyl ether (1:1, 2 x 50 ml) and the combined organic layers were washed with water (100 ml), dried and evaporated. ^{1}H NMR showed that there was still some starting material left. The hydrogenation was repeated: Butyric acid 2-((*E*/*Z*)-7-bromo-hept-1-enyl)-cyclopropylmethyl ester (5.75 g, 18.14 mmol) was dissolved in THF (70 ml). 2,4,6-Triisopropylbenzene sulfonohydrazide (5.4 g, 18.14 mmol, 1 mol eq.) was added and the reaction mixture was stirred at 50 °C for 3 hours. A further 2,4,6-triisopropylbenzene sulfonohydrazide (5.4 g, 18.14 mmol, 1 mol eq.) was added and the reaction was stirred at 50 °C for 24 hours. The mixture was diluted with petroleum ether/diethyl ether (1:1, 100 ml) and a solution of NaOH in water (200 ml, 2%) and extracted. The organic layer was separated and the aqueous layer re-extracted with petroleum ether/diethyl ether (1:1, 2 x 100 ml). The combined organic layers were washed with water (150 ml), dried and evaporated. ^{1}H NMR showed that there was no more starting material left. The crude product was purified by column chromatography on silica gel eluting with petroleum ether/diethyl ether (10:1) to give *butyric acid (1*R,*2*S*)-2-(7-bromo-heptyl)-cyclopropylmethyl ester* **(35)** as a colourless oil (4.61 g, 80%).

Physical properties:

Found M + Na^{+}: 341.1021, $C_{15}H_{27}BrNaO_2$ requires: 341.1087

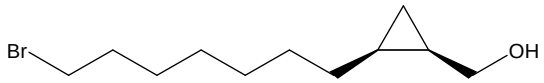
ν_{max}/cm^{-1}: 3066, 2962, 2855, 1734

δ_H (500 MHz, $CDCl_3$): 4.19 (1H, dd, *J* 6.9, 11.65 Hz), 3.93 (1H, dd, *J* 8.8, 11.65), 3.41 (2H, t, *J* 6.95 Hz), 2.30 (2H, t, *J* 7.25 Hz), 1.86 (2H, pent, *J* 6.9 Hz), 1.67 (2H, sextet, *J* 7.25 Hz), 1.4 (5H, m), 1.32 (4H, m), 1.23 (1H, m), 1.13 (1H, m), 0.96 (3H, t, *J* 7.25 Hz), 0.87 (1H, m), 0.74 (1H, dt, *J* 4.75, 8.55 Hz), 0.02 (1H, q, *J* 5.4 Hz)

δ_C (500 MHz, $CDCl_3$): 173.81, 65.07(+), 36.32(+), 33.93(+), 32.79(+), 29.81(+), 29.28(+), 28.74(+), 28.53(+), 28.12(+), 18.50(+), 16.17(-), 14.17(-), 13.66(-), 9.77(+) [+ = CH_2, - = CH, CH_3]

$[\alpha]_D^{24}$: +10.03 ° (c = 1.50, $CHCl_3$); *reported as* $[\alpha]_D^{23}$*: -14.31 ° (c = 1.2, $CHCl_3$)*

*3.3.2.35 Preparation of (1*R*,2*S*)-2-(7-bromo-heptyl)-cyclopropyl methanol (36)*



Anhydrous potassium carbonate (5.17 g, 37.39 mmol, 2.65 mol eq.) was added to a stirring solution of butyric acid (1*R*,2*S*)-2-(7-bromo-heptyl)-cyclopropylmethyl ester **(35)** (4.5 g, 14.11 mmol) in methanol (30 ml) and THF (20 ml) at room temperature. The reaction was stirred for 4 hours at 45 °C. When TLC showed that no starting material was left, the mixture was diluted with water (200 ml) and diethyl ether (100 ml). The organic layer was separated and the aqueous layer re-extracted with diethyl ether (2 x 50 ml). The combined organic layers were washed with brine, dried and evaporated to give an oily residue which was purified by column chromatography on silica gel eluting with petroleum ether/diethyl ether (5:1) to give (1*R*,2*S*)-*2-(7-bromo-heptyl)-cyclopropyl-methanol* **(36)** (2.97 g, 85%).

Physical properties:

Found M + Na^+: 271.0623, $C_{11}H_{21}BrNaO$ requires: 271.0668

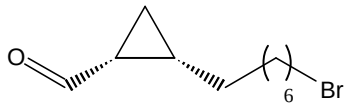
ν_{max}/cm^{-1}: 3345, 2934, 2859

δ_H (500 MHz, $CDCl_3$): 3.65 (1H, dd, *J* 7.25, 11.35 Hz), 3.57 (1H, dd, *J* 8.2, 11.35 Hz), 3.41 (2H, t, *J* 6.95 Hz), 1.86 (2H, pent, *J* 6.95 Hz), 1.43 (6H, m), 1.33 (4H, m), 1.22 (1H, m), 1.10 (1H, m), 0.86 (1H, m), 0.71 (1H, dt, *J* 4.7, 8.5 Hz), -0.03 (1H, q, *J* 5.05 Hz)

δ_C (500 MHz, $CDCl_3$): 63.27(+), 33.97(+), 32.78(+), 29.98(+), 29.28(+), 28.70(+), 28.47(+), 28.10(+), 18.13(-), 16.09(-), 9.45(+), [+ = CH_2, - = CH, CH_3]

$[\alpha]_D^{24}$: +12.87 ° (c = 1.65, $CHCl_3$); *reported as* $[\alpha]_D^{23}$*: -18.9 ° (c = 1.04, $CHCl_3$)*

3.3.2.36 Preparation of (1S,2R)-2-(7-bromo-heptyl)-cyclopropane carbaldehyde (37)



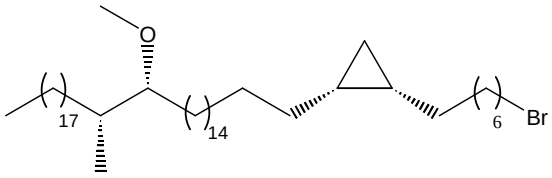
(1*R*,2*S*)-2-(7-Bromo-heptyl)-cyclopropyl-methanol **(36)** (1.0 g, 4.02 mmol) in dichloromethane (10 ml) was added to a stirring suspension of pyridinium chlorochromate (2.17 g, 10.05 mmol, 2.5 mol eq.) in dichloromethane (35 ml) at RT. The mixture was stirred for 3 hours at RT and when TLC showed that no starting material was left, the mixture was poured into diethyl ether (200 ml) and filtered through a pad of silica gel, then washed thoroughly with diethyl ether and the filtrate evaporated to give a residue which was purified by column chromatography on silica gel eluted with petroleum ether/diethyl ether (5:2) to give *(1*S,*2*R*)-2-(7-bromo-heptyl)-cyclopropane carbaldehyde* **(37)** as a colourless oil (0.75 g, 76%).

Physical properties:

Found M + Na^+: 269.04, $C_{11}H_{19}BrNaO$ requires: 269.0511

ν_{max}/cm^{-1}:	3436, 2928, 2855, 1704
δ_H (500 MHz, $CDCl_3$):	9.37 (1H, d, *J* 5.4 Hz), 3.41 (2H, t, *J* 6.6 Hz), 1.87 (3H, m), 1.60 (1H, m), 1.49 (2H, m), 1.41 (3H, m), 1.32 (5H, m), 1.24 (1H, dt, *J* 4.75, 7.9 Hz), 1.19 (1H, dt, *J* 5.05, 6.6 Hz)
δ_C (500 MHz, $CDCl_3$):	201.64(-), 33.90(+), 32.71(+), 29.79(+), 28.98(+), 28.59(+), 28.05(+), 28.02(+), 27.71(-), 24.75(-), 14.71(+), [+ = CH_2, - = CH, CH_3]
$[\alpha]_D^{24}$:	+8.19 ° (c = 1.28, $CHCl_3$); *reported as* $[\alpha]_D^{23}$*: -10.1 ° (c = 1.62, $CHCl_3$)*

3.3.2.37 Preparation of (1S,2R)-1-(7-bromo-heptyl)-2-((17R,18R)-17-methoxy-18-methyl-hexatriacontyl)-cyclopropane (38)



Lithium bis(trimethylsilyl)amide (46.70 ml, 49.56 mmol, 1.06 M, 2 mol eq.) was added drop wise to a stirring solution of (1*S*,2*R*)-2-(7-bromo-heptyl)-cyclopropane carbaldehyde **(37)** (5.31 g, 29.67 mmol) and 5-((16*R*,17*R*)-16-methoxy-17-methyl-pentatriacontane-1-sulfanyl)-1-phenyl-1*H*-tetrazole **(26)** (9 g, 24.73 mmol, 1.2 mol eq.) in dry THF (50 ml) under nitrogen at -2 °C. The reaction mixture was allowed to reach RT and stirred for 1 hour. When TLC showed that no starting material was left, the reaction mixture was quenched with a saturated solution of ammonium chloride (100 ml) and petroleum ether/diethyl ether (1:1, 100 ml). The organic layer was separated and the aqueous layer re-extracted with petroleum ether/diethyl ether (1:1, 2 x 50 ml). The combined organic layers were washed with brine (100 ml), dried and evaporated to give a pale yellow oil which was purified by column chromatography on silica gel eluted with petroleum ether/diethyl ether (9:1) to give (1*S*,2*R*)-*1-(7-bromo-heptyl)-2-((E/Z)-17-methoxy-18-methyl-hexatriacontyl)-cyclopropane* as a colourless oil (5.80 g, 74%).

Physical properties:

δ_H (500 MHz, $CDCl_3$): 5.52 (1H, m), 5.17 (1H, dd, J 8.55, 15.45 Hz), 3.41 (2H, t, *J* 6.95 Hz), 3.35 (3H, s), 2.96 (1H, m), 2.01 (2H, q, *J* 6.95 Hz), 1.87 (2H, pent, *J* 6.9 Hz), 1.64 (1H, m), 1.46-1.20 (70H, m including s), 1.11 (2H, m), 0.89 (3H, t, *J* 6.95 Hz), 0.85 (3H, d, *J* 6.95 Hz), 0.80 (1H, dt, *J* 4.4, 8.5 Hz), 0.12 (1H, q, *J* 5.35 Hz)

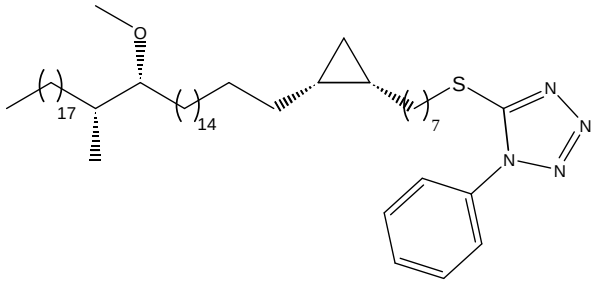
δ_C (500 MHz, $CDCl_3$): 127.84(-), 125.56(-), 85.45(-), 57.71(-), 35.36(-), 33.95(+), 32.85(+), 32.74(+), 32.39(+), 31.93(+), 30.51(+), 29.98(+), 29.94(+), 29.79(+), 29.70(+), 29.62(+), 29.56(+), 29.36(+), 29.30(+), 29.17(+), 29.07(+),28.87(+), 28.77(+), 28.17(+), 27.58(+), 26.17(+), 22.69(+), 18.43(-), 18.20(-), 14.88(-), 14.10(-), 12.30(+), [+ = CH_2, - = CH, CH_3]

2,4,6-Triisopropylbenzene sulfonohydrazide (14.88 g, 49.88 mmol, 2.75 mol eq.) was added to a stirring solution of (1*S*,2*R*)-1-(7-bromo-heptyl)-2-((E/Z)-17-methoxy-18-methyl-hexatriacontyl)-cyclopropane (5.75 g, 18.14 mmol) in THF (150 ml) at RT. The reaction mixture was stirred at 52 °C for 3 hours, followed by the addition of another mole equivalent of 2,4,6-triisopropylbenzene sulfonohydrazide (5.41 g, 18.14 mmol) and stirred under the same conditions for another 24 hours. The mixture was diluted with petroleum ether/diethyl ether (1:1, 100 ml) and a solution of NaOH in water (100 ml, 2%) and extracted. The organic layer was separated and the aqueous layer was re-extracted with petroleum ether/diethyl ether (1:1, 2 x 50 ml) and the combined organic layers were washed with brine (100 ml), dried, filtered through a pad of silica gel and washed with petroleum ether. The filtrate was evaporated to give a colourless residue. ^{1}H NMR showed that there was still some starting material left and the same procedure was repeated. The crude product was purified by column chromatography on silica gel eluting with petroleum ether/diethyl ether (10:1) to give (1*S*,2*R*)-*1-(7-bromo-heptyl)-2-(17-methoxy-18-methyl-hexatriacontyl)-cyclopropane* **(38)** as a colourless oil (4.61 g, 80%).

Physical properties:

ν_{max}/cm^{-1}:	2922, 1464, 1098, 720
δ_H (500 MHz, $CDCl_3$):	3.42 (2H, t, *J* 6.95 Hz), 3.35 (3H, s), 2.96 (1H, m), 1.87 (2H, pent, *J* 6.9 Hz), 1.64 (1H, m), 1.48-1.2 (73H, m including s), 1.18-1.06 (4H, m), 0.89 (3H, t, *J* 6.95 Hz), 0.86 (3H, d, *J* 6.95 Hz), 0.66 (2H, m), 0.57 (1H, dt, *J* 4.1, 7.9 Hz), -0.32 (1H, q, *J* 5.35 Hz)
δ_C (500 MHz, $CDCl_3$):	85.45(-), 57.71(-), 35.35(-), 34.01(-), 32.86(+), 32.39(+), 31.92(+), 30.50(+), 30.22(+), 30.07(+), 29.98(+), 29.94(+), 29.70(+), 29.41(+), 29.36(+), 28.82(+), 28.72(+), 28.65(+), 28.19(+), 27.58(+), 26.16(+), 24.10(-), 22.69(+), 15.78(-), 15.72(-), 14.88(-), 14.10(-), 10.93(+), [+ = CH_2, - = CH, CH_3]
$[\alpha]_D^{24}$:	+5.17 ° (c = 1.44, $CHCl_3$); *reported as* $[\alpha]_D^{22}$*: +5.5 ° (c = 1.29, $CHCl_3$)*

3.3.2.38 Preparation of 5-(7-[1S,2R]-2-((17R,18R)-17-methoxy-18-methyl-hexatriacontyl)-cyclopropyl)-heptyl sulfanyl)-1-phenyl-1H-tetrazole (39)



1-(7-Bromo-heptyl)-2-((17*R*,18*R*)-17-methoxy-18-methyl-hexatriacontyl)-cyclopropane **(38)** (0.5 g, 0.65 mmol) in THF (10 ml) was added to a stirring solution of 1-phenyl-1*H*-tetrazol-5-thiol (0.13 g, 0.72 mmol, 1.1 mol eq.) and potassium carbonate (0.35 g, 2.50 mmol, 3.8 mol eq.) in acetone (30 ml) at RT. The reaction mixture was stirred for 5 hours at 40 °C and then at RT for 16 hours. When TLC showed that no starting material was left, the mixture was diluted with water (50 ml) and dichloromethane (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 brine (2 x 200 ml), dried and evaporated to give an oil which solidified later. The crude product was purified by column chromatography on silica gel eluted with petroleum ether/diethyl ether (2:1) to give a oil of *5-(7-[1S,2R]-2-((17R,18R)-17-methoxy-18-methyl-hexatriacontyl)-cyclopropyl)-heptyl sulfanyl)-1-phenyl-1H-tetrazole* **(39)** (10.95 g, 88%).

Physical properties:

Found M + Na^+: 887.7434, $C_{55}H_{100}N_4NaOS$ requires: 887.7510

ν_{max}/cm^{-1}: 2926, 2851, 1509, 1464, 1097

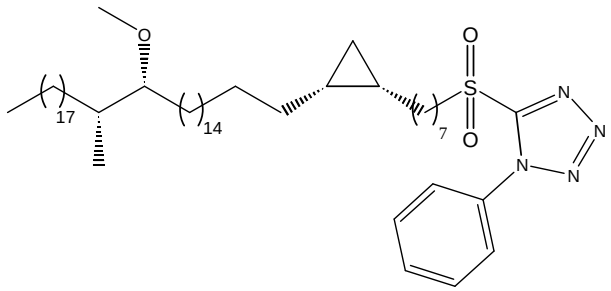
δ_H (500 MHz, $CDCl_3$): 7.57 (5H, m), 3.41 (2H, t, *J* 7.55 Hz), 3.35 (3H, s), 2.96 (1H, m), 1.83 (2H, pent, *J* 7.25 Hz), 1.63 (1H, m), 1.45-1.25 (72H, m including s), 1.13 (4H, m), 0.89 (3H, t, *J* 6.6 Hz), 0.85 (3H, d, *J* 7.05 Hz), 0.65 (2H, m), 0.57 (1H, dt, *J* 4.1, 8.2 Hz), -0.33 (1H, q, *J* 5.05 Hz)

δ_C (500 MHz, $CDCl_3$): 130.03(-), 129.75(-), 123.87(-), 85.45(-), 57.71(-), 35.35(-), 33.40(+), 32.38(+), 31.92(+), 30.50(+), 30.22(+), 30.08(+), 29.98(+), 29.94(+), 29.75(+), 29.70(+), 29.41(+), 29.36(+), 29.09(+), 28.72(+), 28.64(+), 27.57(+), 26.16(+), 22.69(+),

15.76(-), 15.70(-), 14.88(-), 14.10(-), 10.93(+), [+ = CH_2, - = CH, CH_3]

$[\alpha]_D^{25}$: +4.44 ° (c = 1.07, $CHCl_3$); *reported as* $[\alpha]_D^{22}$*: +3.9 ° (c = 1.21, $CHCl_3$)*

3.3.2.39 Preparation of 5-(7-[1S,2R]-2-((17R,18R)-17-methoxy-18-methyl-hexatriacontyl)-cyclopropyl)-heptyl sulfonyl)-1-phenyl-1H-tetrazole (40)



A solution of ammonium heptamolybdate(VI)tetrahydrate (0.36 g, 0.29 mmol, 0.5 mol eq.) in ice cold H_2O_2 35% (w/w, 5 ml) was added drop-wise to a stirring solution of 5-(7-[1*R*,2*S*]-2-((17*R*,18*R*)-17-methoxy-18-methyl-hexatriacontyl)-cyclopropyl)-heptyl sulfanyl)-1-phenyl-1*H*-tetrazole **(39)** (0.50 g, 0.58 mmol) in THF (20 ml) and IMS (20 ml) at 5 °C and stirred at RT for 1 hour, then 3 more identical solutions of ammonium heptamolybdate(VI)tetrahydrate in ice cold H_2O_2 35% (w/w) were added over the next hour. The reaction mixture was stirred for 16 hours at RT and then diluted with water (100 ml) and dichloromethane (50 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 brine (100 ml), dried and evaporated to give a residue which was purified by column chromatography on silica gel eluting with petroleum ether/diethyl ether (5:1) to give an oil of *5-(7-[1S,2R]-2-((17R,18R)-17-methoxy-18-methyl-hexatriacontyl)-cyclopropyl)-heptyl sulfonyl)-1-phenyl-1H-tetrazole* **(40)** (10.18 g, 93%).

Physical properties:

Found M + Na^+: 919.7339, $C_{55}H_{100}N_4NaO_3S$ requires: 919.6469

ν_{max}/cm^{-1}: 2908, 1596, 1499, 1464, 1343, 1153, 1099, 760

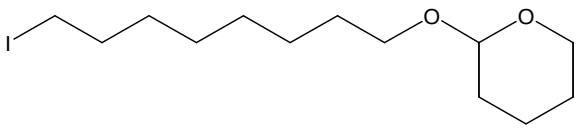
δ_H (500 MHz, $CDCl_3$): 7.71 (2H, m), 7.63 (3H, m), 3.74 (2H, distorted t, *J* 7.9 Hz), 3.35 (3H, s), 2.96 (1H, m), 1.97 (2H, pent, *J* 7.55 Hz), 1.63 (1H, m), 1.52 (2H, m), 1.48-1.2 (70H, m including s), 1.13 (4H, m), 0.89

(3H, t, *J* 6.65 Hz), 0.86 (3H, d, *J* 6.65 Hz), 0.66 (2H, m), 0.58 (1H, dt, *J* 3.75, 7.85 Hz), -0.32 (1H, q, *J* 5.05 Hz)

δ_C (500 MHz, $CDCl_3$): 131.44(-), 129.70(-), 125.08(-), 85.45(-), 57.71(-), 56.05(+), 35.35(-), 31.92(+), 30.49(+), 30.22(+), 29.99(+), 29.94(+), 29.76(+), 29.69(+), 29.36(+), 29.16(+), 28.96(+), 28.73(+), 28.59(+), 28.16(+), 27.57(+), 26.16(+), 25.07(+), 22.68(+), 21.95(+), 15.76(-), 15.66(-), 14.88(-), 14.10(-), 10.93(+), [+ = CH_2, - = CH, CH_3]

$[\alpha]_D^{25}$: +4.40 ° (c = 1.0, $CHCl_3$); *reported as* $[\alpha]_D^{23}$*: +4.13 ° (c = 1.45, $CHCl_3$)*

3.3.2.40 Preparation of 2-(8-iodo-octyloxy)-tetrahydro-pyran (41)



3,4-Dihydro-2*H*-pyran (29.67 g, 0.35 mol, 1.5 mol eq.) and pyridinium-*p*-toluene sulfonate (3.02 g, 0.012 mol, 0.05 mol eq.) were added to a stirring solution of 8-bromo-octan-1-ol (49.14 g, 0.235 mol, section 3.3.2.17) in dry dichloromethane (300 ml) under nitrogen at 5-10 °C. The reaction was stirred for 2 hours at RT, when TLC showed no starting material was left, the reaction mixture was washed with saturated solution of $NaHCO_3$ (200 ml), and then extracted with dichloromethane (3 x 200 ml). The organic layer was dried and evaporated to give *2-(8-bromo-octyloxy)-tetrahydro-pyran* (68.91 g, >99%).

NaI (109 g, mol, 3 mol eq.) was dissolved in acetone (400 ml) and stirred at RT. 2-(8-bromo-octyloxy)-tetrahydro-pyran (68.91 g, 0.235mol) was added and then $NaHCO_3$ (19.77 g, 1 mol eq.) was added. The reaction mixture was refluxed for 3 hours and then stirred overnight at RT. The reaction mixture was cooled down and the solvent was evaporated. The residue was diluted with water (400 ml) and dichloromethane (300 ml), the organic layer was separated and the aqueous layer re-extracted with dichloromethane (2 x 200 ml). The organic layer was dried and evaporated to give a yellow residue which was purified by column chromatography on silica gel eluted with petroleum ether/diethyl ether (10:1) to givean oil of *2-(8-iodo-octyloxy)-*

tetrahydro-pyran **(41)** (71.47 g, 89%). A few drops of triethylamine were added to the silica gel when the column was packed.

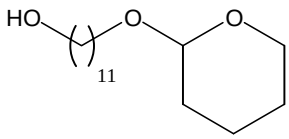
Physical properties:

ν_{max}/cm^{-1}: 2936, 2854

δ_H (500 MHz, $CDCl_3$): 4.57 (1H, t, *J* 2.5 Hz), 3.86 (1H, m), 3.72 (1H, ddd, *J* 6.95, 9.45, 16.4 Hz), 3.49 (1H, m), 3.38 (1H, ddd, *J* 6.6, 9.45, 16.05 Hz), 3.18 (2H, t, *J* 7.25 Hz), 1.82 (3H, m), 1.71 (1H, m), 1.63-1.48 (6H, m), 1.42-1.28 (8H, m)

δ_C (500 MHz, $CDCl_3$): 98.82(-), 67.56(+), 62.32(+), 33.49(+), 30.75(+), 30.40(+), 29.66(+), 29.20(+), 28.43(+), 26.12(+), 25.48(+), 19.67(+), 7.17(+), [+ = CH_2, - = CH, CH_3]

3.3.2.41 Preparation of 11-(tetrahydro-pyran-2-yloxy)-undecan-1-ol (42)



Ammonia gas was condensated to liquid ammonia into a 1 L flask (500 ml) and mechanically stirred while lithium wire (4.93 g, 0.68 mol, 2.5 mol eq. of alcohol) was cut up in tiny pieces and added one by one. The reaction mixture was stirred for 20 minutes where after propagyl alcohol (15.5 g, 0.27 mol, 1.3 mol eq.) in dry ether (30 ml) was added drop-wise and then stirred for another 40 minutes. 2-(8-Iodo-octyloxy)-tetrahydro-pyran **(41)** (71.00 g, 0.21 mol) in dry ether (30 ml) was added, the reaction mixture was stirred for 5 hours while being kept cool with liquid nitrogen. Then the stirring was stopped and the mixture was left overnight at RT for the ammonia to evaporate. Ether (700 ml) and sulfuric acid (100 ml, 10%) was added to the residue and stirred until the residue was dissolved. The ether layer was decanted off, more ether and sulfuric acid was added and the reaction mixture stirred. The ether layer was then decanted again, water was added and the mixture extracted with ether (2 x 250 ml). The combined organic layers were washed with water (500 ml), brine (500 ml) and $NaHCO_3$ (250 ml). The product was then dried and evaporated to give a dark oil of crude *11-(tetrahydro-pyran-2-yloxy)-undec-2-yn-1-ol* (52.36 g, 94%).

Physical properties:

δ_H (500 MHz, $CDCl_3$): 4.57 (1H, t, *J* 2.55 Hz), 4.24 (2H, s), 3.87 (1H, m), 3.73 (1H, m) 3.48 (1H, m), 3.38 (1H, m), 2.20 (2H, m), 1.91 (1H, broad s), 1.82 (1H, m), 1.70 (1H, m), 1.62-1.48 (8H, m), 1.40-1.28 (8H, m)

δ_C (500 MHz, $CDCl_3$): 98.83(-), 86.39, 78.39, 67.64(+), 62.32(+), 51.28(+), 30.73(+), 29.65(+), 29.24(+), 28.95(+), 28.69(+), 28.50(+), 26.12(+), 25.46(+), 19.65(+), 18.67(+), [+ = CH_2, - = CH, CH_3]

Nickel acetate tetrahydrate (10.20 g, 0.04 mol, 0.2 mol eq.) was stirred in ethanol (400 ml) while the flask was being filled with hydrogen. Sodium borohydride (1.81 g, 0.04 mol, 0.2 mol eq.) in ethanol (60 ml) was added and the mixture was stirred for 30 minutes at RT. 11-(Tetrahydro-pyran-2-yloxy)-undec-2-yn-1-ol (52.36 g, 0.21 mol) in ethanol (50 ml) was added and the reaction mixture stirred while being hydrogenated under hydrogen atmosphere. When no more hydrogen was absorbed, the mixture was filtrated through a pad of celite on silica gel and washed with ether (triethyl amine was added to the silica gel). Water and dichloromethane was added and the organic layer was separated. The aqueous layer was re-extracted with dichloromethane (2 x 300 ml). The combined organic layers were dried and evaporated to give a dark residue which was purified by column chromatography on silica gel eluted with petroleum ether/diethyl ether (5:2, then 1:1) to give a yellow oil of *11-(tetrahydro-pyran-2-yloxy)-undecan-1-ol* **(42)** (43.85 g, 83%).

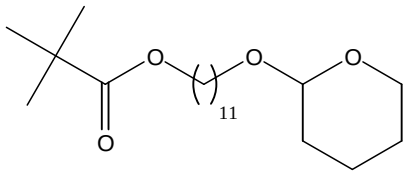
Physical properties:

ν_{max}/cm^{-1}: 3416, 2940

δ_H (500 MHz, $CDCl_3$): 4.58 (1H, t, *J* 2.85 Hz), 3.88 (1H, m), 3.73 (1H, ddd, *J* 6.9, 9.45, 16.4 Hz), 3.64 (2H, ddd, *J* 5.65, 6.6, 13.25 Hz), 3.50 (1H, m), 3.38 (1H, ddd, *J* 6.6, 9.45, 16.05 Hz), 1.83 (1H, m), 1.71 (1H, m), 1.66 (1H, s), 1.62-1.50 (8H, m), 1.38-1.26 (14H, m)

δ_C (500 MHz, $CDCl_3$): 98.82(-), 67.67(+), 63.02(+), 62.31(+), 32.79(+), 30.77(+), 29.72(+), 29.55(+), 29.52(+), 29.48(+), 29.44(+), 29.38(+), 26.20(+), 25.71(+), 25.49(+), 19.67(+) [+ = CH_2, - = CH, CH_3]

3.3.2.42 Preparation of crude 2,2-dimethyl-propionic acid-11-(tetrahydro-pyran-2-yloxy)-undecyl ester (43)



11-(Tetrahydro-pyran-2-yloxy)-undecan-1-ol **(42)** (43.66 g, 0.16 mol) and triethyl amine (24.49 g, 0.24 mol, 1.5 mol eq.) was stirred in dichloromethane (400 ml). 4-Dimethylaminopyridine (1.00 g, 0.01 mol) was added and the mixture was cooled to 5 °C. Trimethyl acetyl chloride (25.3 g, 0.21 mol, 1.3 mol eq.) was added slowly and the reaction mixture was stirred at RT for 3 hours. When TLC showed that no starting material was left, the reaction was quenched with water (500 ml) and dichloromethane. The organic layer was separated and the aqueous layer re-extracted with dichloromethane (2 x 150 ml). The combined organic layers were washed with water, dried and evaporated to give a yellow oil of crude *2,2-dimethyl-propionic acid-11-(tetrahydro-pyran-2-yloxy)-undecyl ester* **(43)** (57.32 g, >99%).

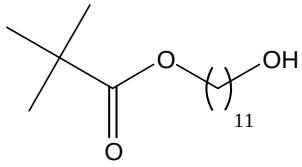
Physical properties:

ν_{max}/cm^{-1}: 2932, 2854, 1811, 1728

δ_H (500 MHz, $CDCl_3$): 4.57 (1H, t, *J* 2.55 Hz), 4.04 (2H, t, *J* 6.6 Hz), 3.87 (1H, m), 3.72 (1H, ddd, *J* 6.95, 9.45, 16.4 Hz), 3.50 (1H, m), 3.38 (1H, ddd, *J* 6.6, 9.45, 16.05 Hz), 1.83 (1H, m), 1.71 (1H, m), 1.63-1.50 (8H, m), 1.37-1.25 (14H, m), 1.19 (9H, s)

δ_C (500 MHz, $CDCl_3$): 178.60, 98.80(+), 67.64(-), 64.42(-), 62.29(-), 40.15(+), 39.09(-), 38.68(-), 30.75(-), 29.72(-), 29.51(-), 29.45(-), 29.44(-), 29.18(-), 28.58(-), 27.17(+), 26.47(+), 26.20(-), 25.87(-), 25.48(-), 19.66(-), [- = CH_2, + = CH, CH_3]

3.3.2.43 Preparation of 2,2-dimethyl-propionic acid-11-hydroxy-undecyl ester (44)



p-Toluenesulfonic acid monohydrate (3.00 g, 0.016 mol, 0.1 mol eq.) was added to a stirring solution of 2,2-dimethyl-propionic acid 11-(tetrahydro-pyran-2-yloxy)-undecyl ester **(43)** (57.32 g, 0.16 mol) in THF (100 ml), methanol (150 ml) and water (2 ml) at RT. The reaction mixture was stirred at RT for 5 hours. When TLC showed that no starting material was left, the mixture was diluted with a saturated solution of sodium bicarbonate (300 ml) and dichloromethane (300 ml). The organic layer was separated and the aqueous layer was re-extracted with dichloromethane (2 x 250ml). The combined organic layers were dried and evaporated to give a residue which was purified by column chromatography on silica gel eluted with petroleum ether/diethyl ether (5:2) to give *2,2-dimethyl-propionic acid-11-hydroxy-undecyl ester* **(44)** as a yellow oil (35.10 g, 80%).

Physical properties:

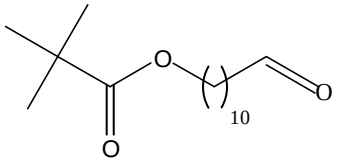
Found M + Na^+: 295.2238, $C_{16}H_{32}NaO_3$ requires: 295.2244

ν_{max}/cm^{-1}: 3384, 2929, 2855, 1731

δ_H (500 MHz, $CDCl_3$): 4.05 (2H, t, *J* 6.65 Hz), 3.64 (2H, t, *J* 6.6 Hz), 1.69 (1H, broad s), 1.65-1.54 (2H, m), 1.38-1.25 (14H, m), 1.19 (9H, s), 0.86 (2H, m)

δ_C (500 MHz, $CDCl_3$): 178.67, 64.44(-), 63.05(-), 38.71(-), 32.77(-), 29.53(-), 29.45(-), 29.44(-), 29.38(-), 29.18(-), 28.59(-), 27.18(+), 25.87(-), 25.71(-), 22.59(-), [- = CH_2, + = CH, CH_3]

3.3.2.44 Preparation of 2,2-dimethyl-propionic acid-11-oxo-undecyl ester (45)



2,2-Dimethyl-propionic acid 11-hydroxy-undecyl ester **(44)** (17.50 g, 64.30 mmol) in dichloromethane (50 ml) was added to a stirring suspension of pyridinium chlorochromate (28.27 g, 128.60 mmol, 2 mol eq.) in dichloromethane (500 ml) at RT. The mixture was stirred for 3 hours at RT. When TLC showed that no starting material was left, the mixture was poured into diethyl ether (1 L), filtered through a pad of celite on silica gel, then washed thoroughly with diethyl ether. The filtrate was evaporated to give a residue which was purified by column chromatography on silica gel eluted with petroleum ether/diethyl ether (5:2) to give *2,2-dimethyl-propionic acid-11-oxo-undecyl ester* **(45)** as a yellow oil (15 g, 86%).

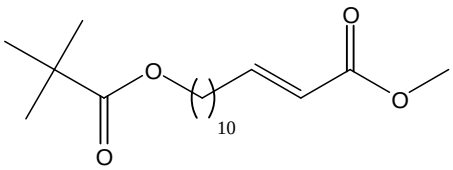
Physical properties:

ν_{max}/cm^{-1}: 2928, 1729, 1458, 1286

δ_H (500 MHz, $CDCl_3$): 9.76 (1H, t, *J* 1.55 Hz), 4.04 (2H, t, *J* 6.65 Hz), 2.41 (2H, dt, *J* 7.55, 1.9 Hz), 1.61 (4H, m), 1.37-1.25 (12H, m), 1.19 (9H, s)

δ_C (500 MHz, $CDCl_3$): 202.83(+), 178.62(-), 64.39(-), 43.86(-), 38.70(-), 29.38(-), 29.27(-), 29.14(-), 29.12(-), 28.57(-), 27.17(+), 25.85(-), 22.04(-), [- = CH_2, + = CH, CH_3]

3.3.2.45 Preparation of 13-(2,2-dimethyl-propionyloxy)-tridec-2-enoic acid methyl ester (46)



(Methoxycarbonylmethylene) triphenylphosphorane (43.31 g, 0.13 mol) was added to a stirring solution of 2,2-dimethyl-propionic acid 11-oxo-undecyl ester **(45)** (30.00 g, 0.11 mol) in toluene (500 ml). The reaction mixture was stirred overnight. The toluene was subsequently evaporated to give a residue which was diluted with petroleum ether/diethyl ether (5:2, 500 ml) and refluxed for 30 minutes. The mixture was filtrated and the precipitate washed with

petroleum ether/diethyl ether (5:2). The filtrate was evaporated to give a residue which was again diluted with petroleum ether/diethyl ether (5:2, 500 ml) and refluxed for 30 minutes. The mixture was then filtrated, the precipitate washed with petroleum ether/ diethyl ether (5:2) and the filtrate evaporated. The crude product was purified on silica gel gel eluted with petroleum ether/diethyl ether (5:2) to give a colourless oil of *13-(2,2-dimethyl-propionyloxy)-tridec-2-enoic acid methyl ester* **(46)** (31 g, 85%).

Physical properties:

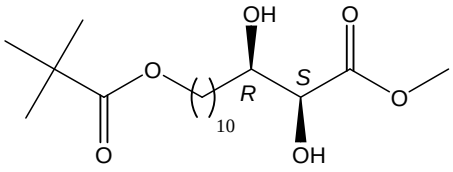
Found M + Na^+: 349.2339, $C_{19}H_{34}NaO_4$ requires: 349.2349

ν_{max}/cm^{-1}: 2928, 2855, 1729, 1158

δ_H (500 MHz, $CDCl_3$): 6.97 (1H, dt, *J* 6.95, 15.45 Hz), 5.81 (1H, dt, *J* 1.6, 15.45 Hz), 4.04 (2H, t, *J* 6.6 Hz), 3.72 (3H, s), 2.19 (2H, m), 1.61 (2H, pent, *J* 6.6 Hz), 1.45 (2H, m), 1.37-1.25 (14H, m), 1.19 (9H, s)

δ_C (500 MHz, $CDCl_3$): 178.61, 167.16, 149.74(+), 120.80(+), 64.41(-), 51.32(+), 38.70(-), 32.17(-), 29.42(-), 29.37(-), 29.30(-), 29.16(-), 29.07(-), 28.58(-), 27.98(-), 27.18(+), 25.87(-), [- = CH_2, + = CH, CH_3]

*3.3.2.46 Preparation of (2**S**,3**R**)-13-(2,2-dimethyl-propionyloxy)-2,3-dihydroxy-tridecanoic acid methyl ester (47)*



$(DHQD)_2PHAL$ ligand (0.55 g, 0.70 mmol, 0.01 mol eq.), K_3Fe_6 (69.27 g, 0.21 mol, 3 mol eq.), K_2CO_3 (29.08 g, 0.21 mol, 3 mol eq.) and OsO_4 (2.8 ml, 2.81 mmol, 0.04 mol eq.; 2.5 wt % solution in 2-methyl-2-propanol) were dissolved in a mixture of water and 2-methyl-2-propanol (800 ml, 1:1) at RT. $MeSO_2NH_2$ (6.67 g, 0.07 mol, 1 mol eq.) was added and the mixture was cooled to 2 °C while being stirred vigorously. 13-(2,2-Dimethyl-propionyloxy)-tridec-2-enoic acid methyl ester **(46)** (23 g, 0.07 mol) was added at 2 °C. The reaction was stirred at 2-4 °C for 8 hours. When TLC showed that no starting material was left, sodium metabisulfite (20 g, mol) was carefully added. The mixture was allowed to reach RT and stirred for 45 min, then extracted with dichloromethane (3 x 500 ml), dried and evaporated. The crude product was purified on column chromatography on silica gel eluted with petroleum

ether/ethyl acetate (5:1, then 1:1) to give *(2S,3R)-13-(2,2-dimethyl-propionyloxy)-2,3-dihydroxy-tridecanoic acid methyl ester* **(47)** as a colourless oil (25.60 g, >99%).

Physical properties:

Found M + Na^+: 383.2395, $C_{19}H_{36}NaO_6$ requires: 383.2404

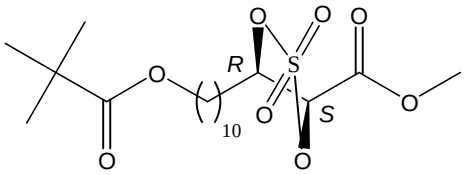
ν_{max}/cm^{-1}: 3449, 2930, 2855, 1729

δ_H (500 MHz, $CDCl_3$): 4.11 (1H, m), 4.04 (2H, t, *J* 6.6 Hz), 3.89 (1H, m), 3.83 (3H, s), 3.08 (1H, broad s), 2.04 (1H, s), 1.61 (4H, m), 1.46 (1H, m), 1.38-1.23 (12 H, m), 1.19 (9H, s)

δ_C (500 MHz, $CDCl_3$): 178.66, 174.08, 73.07(+), 72.46(+), 64.44(-), 52.77(+), 38.71(-), 33.71(-), 29.45(-), 29.43(-), 29.41(-), 29.17(-), 28.58(-), 27.18(+), 25.87(-), 25.67(-), [- = CH_2, + = CH, CH_3]

$[\alpha]_D^{26}$: +9.70, (c = 1.34, $CHCl_3$)

3.3.2.47 Preparation of (2S,3R)-5-[10-(2,2-dimethyl-propionyloxy)-decyl]-2,2-dioxo-2λ⁶-[1,3,2]-dioxathiolane-4-carboxylic acid methyl ester (48)



(2*S*,3*R*)-13-(2,2-Dimethyl-propionyloxy)-2,3-dihydroxy-tridecanoic acid methyl ester **(47)** (25.50 g, 70.44 mmol) was dissolved in CCl_4 (120 ml). Thionyl chloride (11.26 ml, 154.90 mmol, 2.2 mol eq.) was added and the mixture was vigorously refluxed for 2 hours. After cooling, the solution was carefully diluted with CH_3CN (120 ml), followed by the addition of ruthenium trichloride hydrate (0.73 g, 3.52 mmol, 0.05 mol eq.) and $NaIO_4$ (22.6 g, 105.66 mmol, 1.5 mol eq.). Water (180 ml) was then added drop-wise. The mixture was stirred overnight and then poured into diethyl ether (600 ml). The organic layer was separated and the aqueous layer re-extracted with diethyl ether (2 x 200 ml). The combined organic layers were washed with water (100 ml), saturated solution of sodium bicarbonate (100 ml) and brine (100 ml), then dried and evaporated to give a dark residue. Sodium thiosulfate pentahydrate was added to neutralise the iodine and the mixture was extracted with dichloromethane (2 x 200 ml). The organic layer was dried and evaporated. TLC showed that there was still starting material left and the reaction was repeated by addition of CCl_4 (60 ml), acetonitrile (60 ml),

ruthenium trichloride (0.5 g), $NaIO_4$ (15 g) and water (90 ml). The reaction mixture was stirred at RT and montored by TLC. After 30 min TLC showed that no starting material was left and the mixture was diluted with diethyl ether (400 ml). The organic layer was separated and the aqueous layer re-extracted with diethyl ether (2 x 100 ml). The combined organic layers were washed as described, dried and evaporated to give a very dark residue which was purified by column chromatography on silica gel eluted with petroleum ether/diethyl ether (1:1) to give a colourless oil of *(2S,3R)-5-[10-(2,2-dimethyl-propionyloxy)-decyl]-2,2-dioxo-2λ^6-[1,3,2]-dioxathiolane-4-carboxylic acid methyl ester* **(48)** (16.73 g, 56%).

Physical properties:

Found M + Na^+: 445.1857, $C_{19}H_{34}NaO_8S$ requires: 445.1867

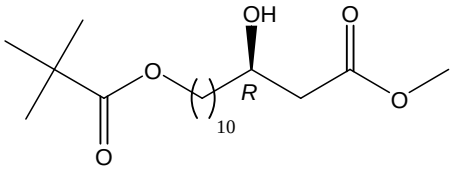
ν_{max}/cm^{-1}: 2930, 2856, 1774, 1724

δ_H (500 MHz, $CDCl_3$): 4.95 (1H, dt, *J* 5.05, 7.25 Hz), 4.89 (1H, d, *J* 7.25 Hz), 4.05 (2H, t, *J* 6.6 Hz), 3.90 (3H, s), 1.98 (2H, m), 1.64-1.45 (4H, m), 1.58-1.45 (2H, m), 1.39-1.26 (12H, m), 1.20 (9H, s)

δ_C (500 MHz, $CDCl_3$): 178.63, 165.38, 84.06(+), 79.81(+), 64.39(-), 53.67(+), 38.71(-), 32.96(-), 29.36(-), 29.26(-), 29.15(-), 29.12(-), 28.81(-), 28.57(-), 27.18(+), 25.84(-), 24.76(-), [- = CH_2, + = CH, CH_3]

$[\alpha]_D^{26}$: +31.17 º (c = 1.03, $CHCl_3$)

3.3.2.48 Preparation of (R)-13-(2,2-dimethyl-propionyloxy)-3-hydroxy-tridecanoic acid methyl ester (49)



(2*S*,3*R*)-5-[10-(2,2-Dimethyl-propionyloxy)-decyl]-2,2-dioxo-2λ^6-[1,3,2]-dioxathiolane-4-carboxylic acid methyl ester **(48)** (22 g, 51.88 mmol) was dissolved in DMAC (300 ml) and NaBH4 (2.26 g, 59.67 mmol, 1.15 mol eq.) was slowly added at 0 °C. The reaction mixture was stirred at RT for 1 hour. When TLC showed that no starting material was left, the solvent was distilled under high vacuum. The residue was diluted with THF (250 ml) and water (0.5 ml) and concentrated sulfuric acid (1.35 ml) added. The mixture was stirred for 1 hour where after sodium metabisulfate (25 g) was added and stirred for another 30 minutes. This was then

filtered through a pad of silica gel and washed with THF. The filtrate was evaporated to give a residue which was purified by column chromatography on silica gel eluting with petroleum ether/ethyl acetate (10:3) to give *(R)-13-(2,2-dimethyl-propionyloxy)-3-hydroxy-tridecanoic acid methyl ester* (9.20 g, 52%).

Physical properties:

Found M + Na^+: 367.2427, $C_{19}H_{36}NaO_5$ requires: 367.2455

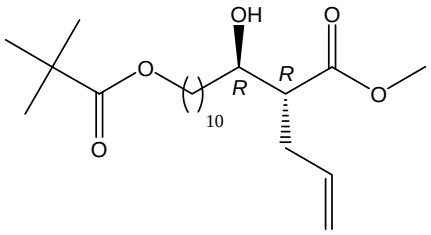
ν_{max}/cm^{-1}: 3517, 2922, 2854, 1732

δ_H (500 MHz, $CDCl_3$): 4.04 (2H, t, *J* 6.6 Hz), 3.99 (1H, m), 3.71 (3H, s), 2.51 (1H, dd, *J* 3.15, 16.4 Hz), 2.41 (1H, dd, *J* 9.15, 16.4 Hz), 1.61 (2H, pent, *J* 6.6 Hz), 1.53 (1H, m), 1.43 (2H, m), 1.37-1.25 (5H, m), 1.19 (9H, s)

δ_C (500 MHz, $CDCl_3$): 178.61, 173.42, 67.99(+), 64.41(-), 51.66(+), 41.12(-), 38.68(-), 36.50(-), 29.46(-), 29.44(-), 29.42(-), 29.41(-), 29.15(-), 28.57(-), 27.16(+), 25.85(-), 25.42(-), [- = CH_2, + = CH, CH_3]

$[\alpha]_D^{26}$: -10.0 º (c = 1.23, $CHCl_3$)

*3.3.2.49 Preparation of (2**R**,3**R**)-2-allyl-[11-(2,2-dimethyl-propionyloxy)-1-hydroxy-undecyl]-pent-4-enoic acid methyl ester (50)*



Butyl lithium (3.05 ml, 6.40 mmol, 2.2 mol eq.) was added to a stirred solution of diisopropylamine (0.65 g, 6.40 mmol, 2.2 mol eq.) in dry THF (40 ml) under nitrogen at -78 °C. The reaction mixture was allowed to reach RT and was re-cooled to -78 °C before (*R*)-13-(2,2-dimethyl-propionyloxy)-3-hydroxy-tridecanoic acid methyl ester **(49)** (1 g, 2.91 mmol) in dry THF (20 ml) was added drop-wise. The reaction mixture was allowed to slowly warm to 0 °C in the cold bath over 2 hours and then re-cooled to -65 °C before allylic iodide (0.32 ml, 3.49 mmol, 1.2 mol eq.) and HMPA (1.01 ml, 5.81 mmol, 2 mol eq.) in dry THF (2 ml) was added drop-wise. The reaction mixture was allowed to slowly warm to -5 °C in the cold bath over 2 hours and the reaction monitored by TLC. When TLC showed that little starting

material was left, ammonium chloride (10 ml) was added and the product extracted with diethyl ether/ethyl acetate (1:1, 3 x 50 ml). The combined organic layers were washed with brine (50 ml), dried and evaporated to give a residue which was purified by column chromatography on silica gel eluted with petroleum ether/ethyl acetate (4:1) to give *(2R,3R)-2-allyl-[11-(2,2-dimethyl-propionyloxy)-1-hydroxy-undecyl]-pent-4-enoic acid methyl ester* **(50)** as a pale yellow oil (614 mg, 55%).

Physical properties:

Found M + Na^+: 407.2751, $C_{22}H_{40}NaO_5$ requires: 407.2768

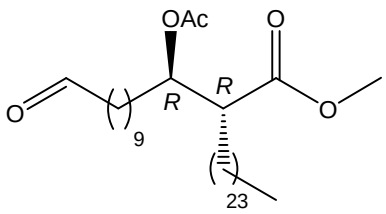
ν_{max}/cm^{-1}: 3524, 2930, 2854, 1736, 1642

δ_H (500 MHz, $CDCl_3$): 5.75 (1H, m), 5.11 (1H, dd, J 0.95, 17 Hz), 5.05 (1H, br d, *J* 10.05 Hz), 4.05 (2H, t, *J* 6.65 Hz), 3.71 (3H, s), 2.55 (1H, m), 2.50-2.38 (2H, m), 1.62 (3H, pent, *J* 6.3 Hz), 1.50-1.42 (4H, m), 1.37-1.25 (16H, m), 1.20 (9H, s)

δ_C (500 MHz, $CDCl_3$): 178.65, 175.33, 134.89(+), 117.18(+), 71.78(+), 64.44(-), 51.56(+), 50.54(+), 38.72(-), 35.57(-), 33.81(-), 29.50(-), 29.49(-), 29.47(-), 29.45(-), 29.20(-), 28.61(-), 27.21(+), 25.89(-), 25.71(-), [- = CH_2, + = CH, CH_3]

$[\alpha]_D^{25}$: +1.08, (c = 1.15, $CHCl_3$); *reported as* $[\alpha]_D^{19}$*: +1.94 (c = 1.19, $CHCl_3$)*

3.3.2.50 Preparation of (1R, 2R)-1-acetoxy-11-oxoundecyl hexacosanoic acid methyl ester (52)



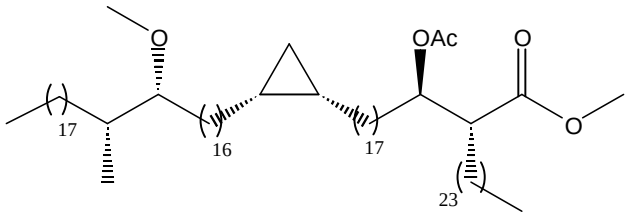
Methyl (2*R*,3*R*)-3-acetoxy-2-tetracosanyl-13-hydroxytridecanoate **(51)** (0.18 g, 0.286 mmol, kindly provided by Dr J. Al Dulayymi, University of Wales, Bangor, UK) in dichloromethane (5 ml) was added to a stirring suspension of pyridinium chlorochromate (2.50 g, 1.13 mmol, 4 mol eq.) in dichloromethane (15 ml) at RT. The mixture was stirred for 1 hour at RT. When TLC showed that no starting material was left, the mixture was poured into diethyl ether (200

ml) and filtered through a pad of celite on silica gel, then washed thoroughly with diethyl ether. The filtrate was evaporated to give a residue which was purified by column chromatography on silica gel eluted with petroleum ether/diethyl ether (5:2) to give *(1R, 2R)-1-acetoxy-11-oxoundecyl hexacosanoic acid methyl ester* **(52)** as an oil (0.17 g, 96%).

Physical properties:

ν_{max}/cm^{-1}:	2917, 2849, 1741
δ_H (250 MHz, $CDCl_3$):	9.73 (1H, t, *J* 1.8 Hz), 5.03 (1H, ddd, *J* 4.2, 7, 11.3 Hz), 3.64 (3H, s), 2.57 (1H, ddd, *J* 4.2, 6.7, 10.7 Hz), 2.38 (2H, dt, *J* 1.8, 7.2 Hz), 2.03 (3H, s), 1.95-1.08 (62H, br s), 0.88 (3H, t, *J* 7 Hz)
δ_C (250 MHz, $CDCl_3$):	202.9, 173.6, 170.3, 74.1, 51.5, 49.6, 43.9, 31.9, 31.7, 29.7, 29.3, 29.1, 28.1, 27.5, 25.0, 22.6, 22.0, 21.0, 14.1, [- = CH_2, + = CH, CH_3]
$[\alpha]_D^{22}$:	*reported as +9.8 ° (c = 1.06, $CHCl_3$)*

3.3.2.51 Preparation of (R)-2-{(R)-1-acetoxy-18-[(1S,2R)-2-((17R,18R)-17-methoxy-18-methyl hexatriacontyl)-cyclopropyl]-octadecyl}-hexacosanoic acid methyl ester (53)



Lithium bis(trimethylsilyl)amide (0.45 ml, 0.427 mmol, 1.06 M, 1.3 mol eq. to sulfone) was added drop wise to a stirring solution of (1*R*,2*R*)-1-acetoxy-11-oxoundecyl hexacosanoic acid methyl ester **(52)** (0.174 g, 0.274 mmol) and 5-(7-[1*S*,2*R*]-2-((17*R*,18*R*)-17-Methoxy-18-methyl-hexatriacontyl)-cyclopropyl)-heptyl sulfonyl)-1-phenyl-1*H*-tetrazole **(40)** (0.30 g, 0.33 mmol, 1.2 mol eq.) in dry THF (10 ml) under nitrogen at - 10 °C. The reaction mixture was allowed to reach RT and was then stirred for 1 hour. When TLC showed that no starting material was left, the reaction mixture was quenched with a saturated solution of ammonium chloride (5 ml) and diethyl ether (10 ml). The organic layer was separated and aqueous layer was re-extracted with diethyl ether (3 x 10 ml). The combined organic layers were washed with brine (20 ml), dried and evaporated to give a white solid which was purified by column chromatography on silica gel eluting with petroleum ether/diethyl ether (10:1) to give *(R)-2-*

{(E/Z)-(R)-1-acetoxy-18-(1S,2R)-2-((17R,18R)-17-methoxy-18-methyl hexatriacontyl)-cyclopropyl}-octadec-11-enyl}-hexacosanoic acid methyl ester (0.22 g, 60%).

(*R*)-2-{(*E/Z*)-(*R*)-1-Acetoxy-18-(1*S*,2*R*)-2-((17*R*,18*R*)-17-methoxy-18-methyl hexatriacontyl)-cyclopropyl}-octadec-11-enyl}-hexacosanoic acid methyl ester (0.215 g, 0.165 mmol) was stirred in THF (10 ml) and methanol (5 ml), cooled to 5 °C and excess of dipotassium azodicarboxylate was added. Acetic acid (3 ml) in methanol (2 ml) was added drop-wise to the reaction mixture over 3 hours at RT. After 6 hours another portion of dipotassium azodicarboxylate was added, followed by the addition of acetic acid (1ml) in methanol (1 ml) and this was repeated 12 hours later. The reaction was stirred for a further 20 hours and then poured into ammonium chloride (20 ml). A mixture of petroleum ether/diethyl ether (1:1, 50 ml) was added, the organic layer was separated and the aqueous layer was re-extracted with petroleum ether/diethyl ether (1:1, 2 x 30 ml). The combined organic layers were dried and evaporated. The procedure was repeated again for another 24 hours to give a white solid which was purified by column chromatography on silica gel eluting with petroleum ether/ diethyl ether (5:1) to give *(R)-2-{(R)-1-acetoxy-18-(1S,2R)-2-((17R,18R)-17-methoxy-18-methyl hexatriacontyl)-cyclopropyl}-octadecyl-hexacosanoic acid methyl ester* **(53)** (194 mg, 90%).

Physical properties:

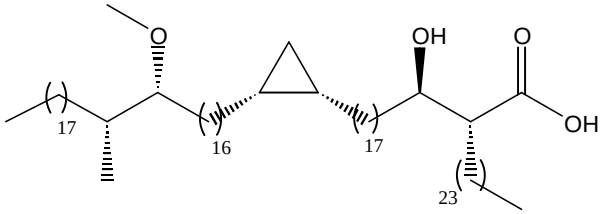
ν_{max}/cm^{-1}: 2920, 1743, 1469, 1375, 1237, 1162, 719

δ_H (500 MHz, $CDCl_3$): 5.09 (1H, dt, *J* 3.8, 7.9 Hz), 3.69 (3H, s), 3.35 (3H, s), 2.96 (1H, m), 2.62 (1H, ddd, *J* 4.4, 6.95, 14.8 Hz), 2.04 (3H, s), 1.68-1.58 (4H, m), 1.56-1.48 (1H, m), 1.46-1.20 (138 H, m including s), 1.18-1.05 (4H, m), 0.89, (6H, t, J 6.6 Hz), 0.85 (3H, d, J 6.9 Hz), 0.65 (2H, m), 0.57 (1H, dt, *J* 4.1, 7.9 Hz), -0.32 (1H, q, *J* 5.05 Hz)

δ_C (500 MHz, $CDCl_3$): 173.65, 170.33, 85.45(-), 74.11(-), 57.70(-), 51.52(-), 49.59(-), 35.35(-), 32.39(+), 31.92(+), 31.72(+), 30.500(-), 30.32(+), 30.22(+), 29.98(+), 29.94(+), 29.70(+), 29.65(+), 29.56(+), 29.47(+), 29.44(+), 29.40(+), 29.36(+), 28.72(+), 28.12(+), 27.58(+), 27.47(+), 26.16(+), 24.99(+), 22.68(+), 22.61(+), 21.01(-), 15.78(-), 14.88(-), 14.10(-), 10.91(+), [+ = CH_2, - = CH, CH_3]

$[\alpha]_D^{25}$: +7.69 ° (c = 1.04, $CHCl_3$); *reported as* $[\alpha]_D^{22}$*: +7.17 ° (c = 1.32, $CHCl_3$)*

*3.3.2.52 Preparation of (**R**)-2-{(**R**)-1-hydroxy-18-[(1**S**,2**R**)-2-((17**R**,18**R**)-17-methoxy-18 methyl hexatriacontyl)-cyclopropyl]-octadecyl}-hexacosanoic acid (1)*



Lithium hydroxide monohydrate (22 mg, 0.535 mmol) was added to a stirring solution of (*R*)-2-{(*R*)-1-acetoxy-18-[(1*S*,2*R*)-2-((17*R*,18*R*)-17-methoxy-18-methylhexatriacontyl)-cyclopropyl]-octadecyl}-hexacosnoic acid methyl ester **(53)** (50 mg, 0.038 mmol) in tetrahydrofuran (8 ml), methanol (1 ml) and water (0.5 ml) at RT. The reaction mixture was stirred at 43 ^oC for 24 hrs. When TLC showed that no starting material was left, the reaction was cooled to room temperature and diluted with petroleum ether/diethyl ether (1:1, 30 ml) and ammonium chloride (5 ml) and acidified with 5% HCl. The organic layer was separated and the aqueous layer extracted with petroleum ether/diethyl ether (1:1, 2 x 20ml). The combined organic layers were dried and evaporated to give a white solid which was purified by column chromatography on silica gel eluted with petroleum ether/ethyl acetate (5:2) to give *(R)-2-{(R)-1-hydroxy-18-[(1S,2R)-2-((17R,18R)-17-methoxy-18-methylhexatri-acontyl)-cyclopropyl]-octadecyl}-hexacosnoic acid* **(1)** (32 mg, 67%).

Physical properties:

Found M – H^+: 1252.2804, $C_{85}H_{167}O_4$ requires: 1252.2859

ν_{max}/cm^{-1}: 3516, 2917, 2850, 1718, 1462

m.p. 59-61 ºC

δ_H (500 MHz, $CDCl_3$): 3.73-3.70 (1H, m), 3.35 (3H, s), 2.99-2.95 (1H, m), 2.48-2.44 (1H, m), 1.79-1.71 (1H, m), 1.67-1.61 (2H, m), 1.55-1.08 (145H, m), 0.90-0.83 (9H, including a t, *J* 7 Hz and a d, *J* 7 Hz), 0.68-0.63 (2H, m), 0.56 (1H, br, dt, *J* 4.05, 8.15 Hz), -0.32 (1H, br, q, *J* 5.05 Hz)

δ_C(500 MHz, $CDCl_3$): 178.84, 85.56(-), 72.11(-), 57.67(-), 50.71(-), 35.55(+), 35.33(-), 32.35(+), 31.92(+), 30.47(+), 30.31(+), 30.22(+), 29.97(+), 29.93(+), 29.70(+), 29.66(+), 29.61(+), 29.58(+), 29.50(+), 29.42(+), 29.36(+), 28.71(+), 27.56(+), 27.33(+), 26.14(+), 25.72(+), 22.68(+), 15.77(-), 14.89(-), 14.11(-), 10.90(+), [+ = CH_2, - = CH, CH_3].

$[\alpha]_D^{25}$: +6.95 ^{o} (c = 1.05, $CHCl_3$)

3.4 Biological activity/antigenicity of different synthetic mycolic acids

3.4.1 Results and discussion

The ability to synthesize complex molecules like MAs is in itself quite a challenge, especially if the size, hydrophobicity, restricted solubility properties, huge variety and stereochemical features of these molecules are taken into acount. To be able to select the correct structure amongst the natural MAs and then synthesize it in such a way that it displays the same biological activity as the natural product, eg. so perfectly that these molecules are also recognised by TB antibodies the way the natural MAs are, is the ultimate achievement sought to clearly understand how the molecules are put together in nature and for what reason. Prof Baird and his group (University of Wales, Bangor, UK) are doing pioneering work on the stereochemically synthesis of MAs and have synthesized a few different MAs, including a α-MA, a keto-MA and a couple of different diastereomers of methoxy-MA (6-8), the latter in which I had the opportunity to participate.

As shown in Chapter 2 (section *2.4.1*), antibodies in TB^+ and TB^- patient serum recognised the natural MA as well as the methoxy-MA subtype. This led to other questions: How important is the stereochemistry of the functional groups in the methoxy-MA for recognition by antibodies? The only way to determine this is to synthetize a particular enantiomer of one particular MA subtype that is recognised by human antibodies and then to synthesize structural variants of this basic structure that probes particular structural aspects in a systematic way to interrogate every structural aspect of MA that may play a role in biological activity. The first challenge was to synthesise a synthetic MA that was identical in structure to the natural MA of *M. tuberculosis* and to test it for antigenicity against human TB patient serum. For the antigenicity/biological activity assay, different synthetic methoxy-MAs were kindly provided by Dr J. Al Dulayymi (University of Wales, Bangor, UK) in addition to the synthesized

methoxy-MA described in this chapter. Apart from the methoxy-MA, different synthetic keto- and hydroxy-MAs were kindly provided by Gani Koza (University of Wales, Bangor, UK). For antigenicity determination, the same ELISA assay was followed that was used in Chapter 2 for the determination of activity of the natural MA and their sub-types.

The exact stereochemistry of the cyclopropane group in the mero chain of natural MA is not known. The two possibilities of *cis*-stereochemistry around the cyclopropane group (*S,R* and *R,S*) were included in two of the methoxy-MAs to determine if one would be favoured above the other by antibodies in TB^+ serum. The stereochemistry of the methyl methoxy-group in the distal position in natural MA is thought to be *S,S*. NMR analyses showed that the stereochemistry is definitely either *S,S* or *R,R*, but not *S,R* or *R,S*. The different stereochemistries around the two loci of methoxy-MA, represented in the three synthetic variants that were tested are shown in Figure 3.20.

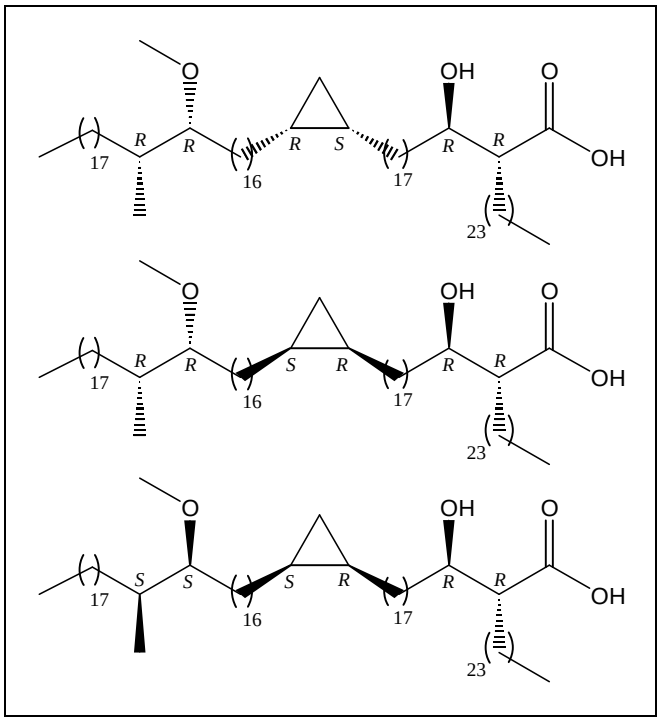


Figure 3.20: *The different diastereomers of synthetic methoxy-MAs tested for antigenicity against TB patient serum.*

Figure 3.21 shows the structures of the synthetic keto- and hydroxy-MAs that were also included in the antigenicity assay. It is known that there is a small amount of *trans*-cyclopropane MAs present in the natural MA. Should the synthetic keto- and hydroxy-MA be recognized by patient antibodies, it would be interesting to learn how either the *cis*- or the

trans-cyclopropane influences this. Although, hydroxy-MA is not present in *M. tuberculosis*, it was included in this assay to see if the oxygenated group at that locus in the mero chain was sufficient to be recognized by antibodies in TB^{+} serum.

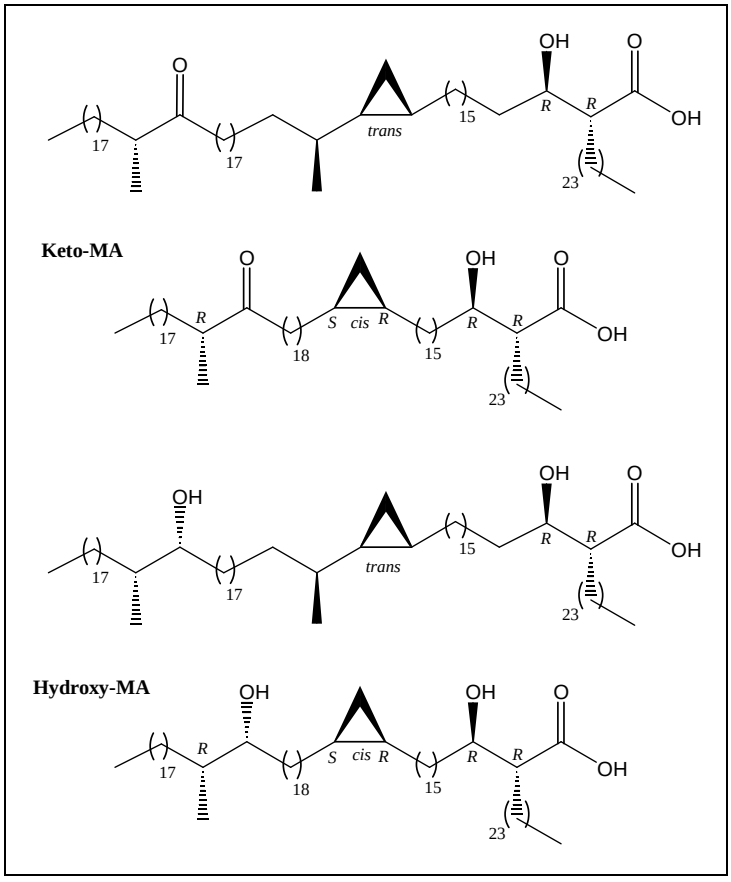


Figure 3.21: *Synthetic keto- and hydroxy-MAs*

To prove that the interaction between sera and MAs are specific, a synthetic alpha-MA (7), was O-acetylated at the β-OH and methylated at the carboxylic acid (Figure 3.22) and was included in the assay as a negative control.

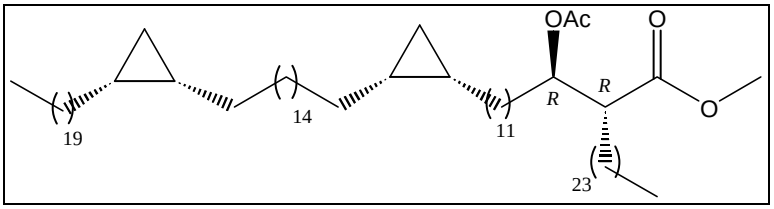


Figure 3.22: *Synthetic acetylated alpha-MA methyl ester (MB)*

This compound was chosen because it does not have a hydrogen donor group, which decreases the possibility of hydrogen bonds with antibodies. Moreover, the protection of the polar groups

in the mycolic motif will also discourage the "W" structural arrangement which is already less feasible for the alpha-MAs subclass. Therefore, this change in the three dimensional structure should disrupt its hydrophobic interactions with antibodies against MAs (145, 146).

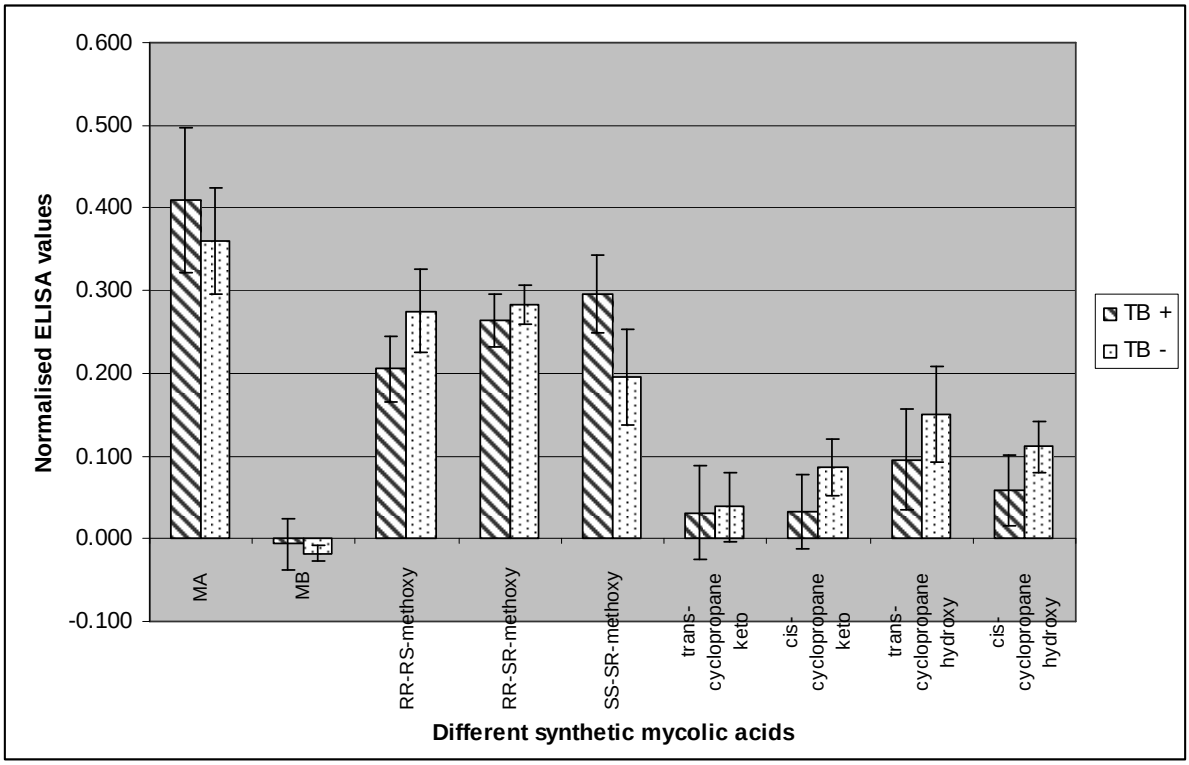


Figure 3.23: *ELISA results of human antibody binding to natural MA (MA) and various synthetic MA structures. Antigens are natural MA isolate (MA, n=92 for positive serum, n=30 for negative serum); a synthetic protected α-MA (MB, n=19 for positive serum, n=5 for negative serum); a few different diastereomers of a synthetic methoxy-MA, of which RR/SS describes the stereochemistry around the distal methoxy-methyl group, and RS/SR describes the stereochemistry around the proximal cis-cyclopropane group respectively, i.e. RR-RS-methoxy (n=20 for positive serum, n=8 for negative serum), RR-SR-methoxy (n=15 for positive serum, n=5 for negative serum), SS-SR-methoxy (n=20 for positive serum, n=5 for negative serum); trans-cyclopropane-keto-MA (n=20 for positive serum, n=8 for negative serum) and cis-cyclopropane-keto-MA (n=20 for positive serum, n=7 for negative serum); and trans-cyclopropane-hydroxy-MA (n=20 for positive serum, n=8 for negative serum) and cis-cyclopropane-hydroxy-MA (n=20 for positive serum, n=8 for negative serum). The source of the antibodies was sera collected from either TB positive or TB negative South African hospitalised patients of various adult age groups. (Values are given as a mean ± standard deviation).*

Figure 3.23 summarises the ELISA results of the different synthetic MAs. It must be noted that although the stereochemistry of these at the hydroxyl acid part is the same as that in the natural material, the stereochemistry at the other functional parts may or may not be as in the natural MA.

Antibodies in TB^+ patient serum recognised the natural MA, and a number of the deprotected synthetic MAs. All of these were also recognised by serum from TB^- patients, as was seen before with the different subclasses of natural MA (Fig 2.21, Chapter 2). There were, however, no significant differences in the way that the various MAs were recognised by sera from TB^+ and TB^- patients. However, as seen in Fig 3.23 this is reversed and is statistically significant ($P < 0.01$) only for the synthetic *SS*-*SR*-methoxy-MA, which is recognised preferentially by TB positive serum. This synthetic diastereomer is also the one that closest approximates the signal strength of antibody binding to natural MA by TB positive patient sera. One can therefore conclude that, *SS*-*SR*-methoxy-MA is the best antigen for use in a serodiagnostic assay for tuberculosis that is based on free MAs as antigen. In addition, *SS*-*SR*-methoxy-MA may well represent one of the antigenically active components that occurs in natural MA and that elicit specific antibody production in patients with TB. *RR*-*RS*-methoxy-MA was also recognised significantly better by TB^- serum compared to the TB^+ serum ($P < 0.01$). It might be that this diastereomer could be used to distinguish better between TB^+ and TB^- sera than with the natural MAs, but more sera will have to be analyzed to substantiate this.

Similar to the negative control antigen, MB, the *trans*-, *cis*-cyclopropane-keto-MA, and *cis*-cyclopropane-hydroxy-MA were also not recognised by TB^+ patient antibodies, but *trans*-cyclopropane-hydroxy-MA was recognised weakly. This demonstrates that the antigenicity of MA is not only dependent on the mycolic motif, but that the mero chain is critical in the manifestation of biological activity. In terms of the oxygenated groups in the mero chain, the methoxy is by far the most active, while some activity can be retained with a hydroxy group substituting for methoxy. Irrespective of whether the keto- and hydroxy-MA represent the natural material, the data clearly demonstrates the that structure of the mero chain is importance for antigenicity. By comparing the resolution in signals of TB^- and TB^+ sera between *SS*-*SR*-methoxy-MA to *RR*-*RS*- and *RR*-*SR*-MA it is clear that the stereochemistry of the mero chain plays a decisive role in their antigenicity. The nature of the stereochemistry of the keto- and the hydroxyl groups are not known and therefore more structures need to be analyzed.

I show, for the first time, that synthetic, stereochemically and diastereomerically pure MAs showed biological activity and were recognised by TB^+ patient serum. Surprisingly, these were also recognised by TB^- patient serum. One reason could be that cross-reactivity with cholesterol antibodies might play a role. It is also possible that there are antibodies to other

mycobacteria involving MAs, eg the saprophytic types. There may also be other unrecognised explanations.

The hydrophobicity of both MA and cholesterol and their apparent molecular relation (discussed in 1.1.7.4 and 3.1.3) suggests that the two entities could snugly fit together, stabilized by hydrophobic forces. The specificity of MA for attracting cholesterol is determined more by the type of oxygenated group (methoxy preferred) and its stereochemistry (*RR* preferred), than by the stereochemistry of the cyclopropane group in the mero chain. These two structural properties appear to be critical for defining the structural similarity of part of the MA structure to cholesterol.

The similarity in structure of MA to cholesterol may have a profound effect on the virulence of *M. tuberculosis,* in particular to their mechanism of entry into the host macrophage and their sustenance, as referred to in Chapter 1 (section 1.1.2). This applies especially to the ability of MA to attract cholesterol. To determine whether MA, in comparison to methylated MA (mMA) and synthetic protected alpha-MA (MB) attracts cholesterol, a biosensor cuvette was coated with MA-, mMA-, or MB-containing liposomes. Sensorgrams were generated by exposing the coated surface to cholesterol-containing liposomes. This was done with a resonant mirror biosensor as described by Siko (134). The resonant mirror biosensor works on the principle that the angle at which light is internally reflected from a sensing surface supported on a dielectric resonant layer, changes as mass accumulates onto it (41). This allows real time analyses of dynamic molecular binding of soluble ligates to ligands that are immobilised on the sensing surface (31). Figure 3.24 shows the coating of MA to the cuvette surface of more than 2 000 arc seconds, and then binding of cholesterol. MB could be coated to a similar degree of efficiency, while mMA coated even better at 3 000 arc seconds.

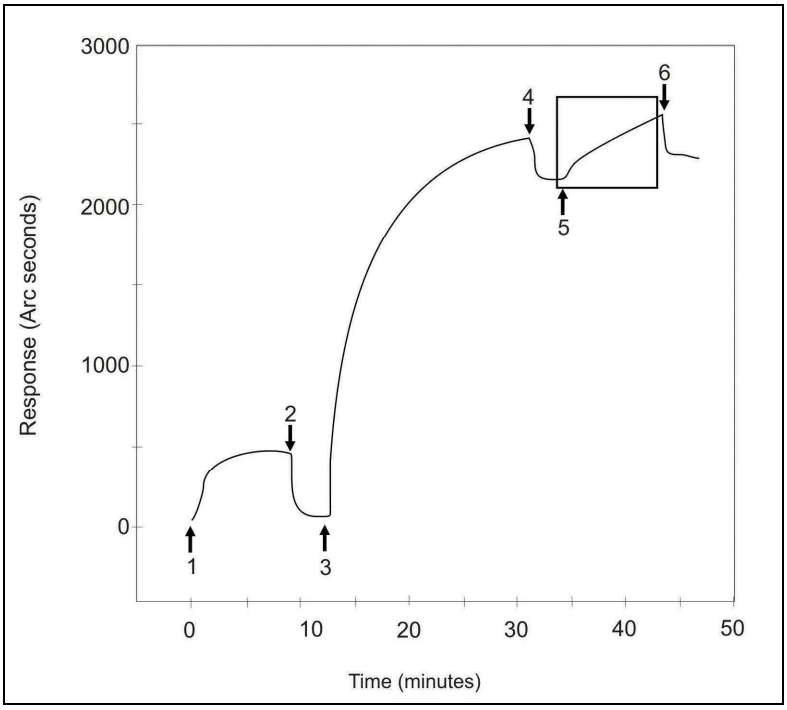


Figure 3.24: *A typical IAsys sensorgram to monitor binding of cholesterol to MA. 1: Activation of surface with CPC, 2: PBS/AE wash step, 3: addition of MA liposomes, 4: PBS/AE wash step, 5: addition of cholesterol liposomes, 6: PBS/AE wash step.*

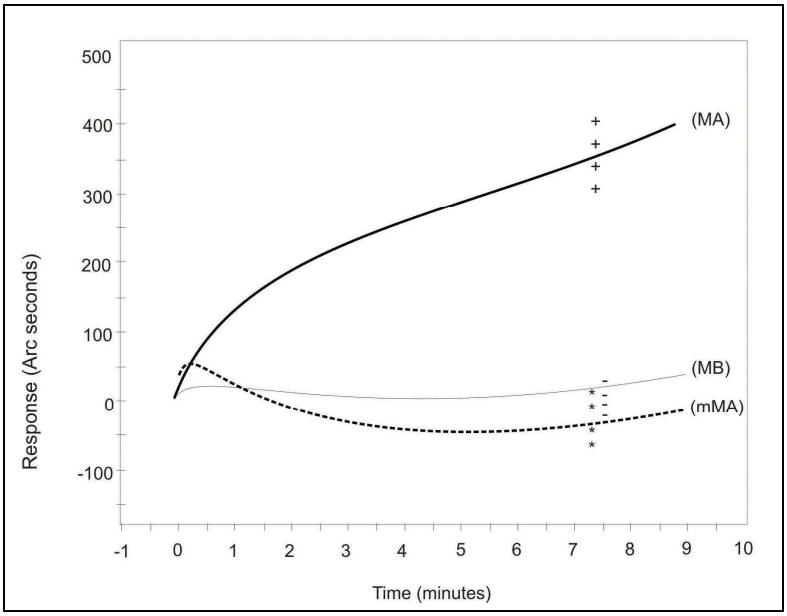


Figure 3.25: *Biosensor binding curves (the framed part of Figure 3.24) of cholesterol attraction to immobilized natural MAs (MA, thick line / +), MA methyl esters (mMA, dashed line / *) or acetylated synthetic alpha-MA methyl ester (MB, thin line / -). Each line represents the typical curve of five repeats with the exact end points of each indicated after 7.5 minutes exposure.*

Figure 3.25 shows that the MA-liposomes coated cuvette surface accumulated cholesterol from the solution, but that the MA methyl esters and the acetylated synthetic MA methyl ester (MB) were unable to do so (Student's t-test showed no significant difference between mMA and MB ($P > 0.1$), while the difference in binding of cholesterol to MA and MB was significant ($P < 0.001$).

It is clear that the attraction of cholesterol to MA is determined by the fine structure of the ligand-receptor pair. The loss of antigenicity and cholesterol binding capability of the methyl esters of MAs might be due to the requirement of free carboxylic acid to stabilize a conformational folding of the long meromycolate chain to which cholesterol can bind. Retzinger *et al*. (118) and Villeneuve *et al*. (145, 146) proposed that the long MA mero chain is kinked and folded in the lipid bilayer as three tightly packed bends of hydrocarbon. Similar folded conformations of oxygenated MA are proposed that can explain experimental observations obtained in different studies (70). The alignment of the acyl chains by folding may allow hydrophobic interactions by intra-molecular stacking that is enabled by the hairpin bend induced by the proximal *cis*-cyclopropane group. Methoxy-MA may bind better to cholesterol than keto-MA by retaining a tetrahedral structure around the oxygenated carbon, homologous to the structural architecture around the 3-hydroxy group of cholesterol. The carboxylic acid part of the molecule plays an important role in its antigenicity for both TB^+ and TB^- patient antibodies.

3.4.2 Materials and methods

3.4.2.1 Mycolic acids used as antigens in ELISA

For the antigenicity/cholesterol binding activity assay, different synthetic MAs were kindly provided by Dr J. Al Dulayymi and Gani Koza (University of Wales, Bangor, UK) in addition to the synthesized methoxy-MA described in Chapter 3 (6, 7, 90).

3.4.2.1.1 Natural mycolic acids

Mycobacterial MAs were isolated from a culture of *M. tuberculosis* H37Rv as described by Goodrum *et al.* (67), as a mixture of α-, keto- and methoxy-MAs (Figure 1.7).

3.4.2.1.2 Synthetic acetylated alpha mycolic acid methyl ester (MB)

An α-MA was synthesized by Al Dulayymi *et al.* (7) and was O-acetylated at the β-OH and methylated at the α-carboxylic acid.

3.4.2.1.3 Synthetic methoxy-MA (RR-RS-methoxy)

Deprotected as described in section 3.3.2.52.

3.4.2.1.4 Synthetic methoxy-MA (RR-SR-methoxy)

Demethylated and kindly provided by Dr J. Al Dulayymi (University of Wales, Bangor, UK).

3.4.2.1.5 Synthetic methoxy-MA (SS-SR-methoxy)

Deprotected and kindly provided by Dr J. Al Dulayymi (University of Wales, Bangor, UK).

3.4.2.1.6 Synthetic trans-*cyclopropane-keto-MA*

Synthesized and kindly provided by Gani Koza (University of Wales, Bangor, UK).

3.4.2.1.7 Synthetic cis-*cyclopropane-keto-MA*

Synthesized and kindly provided by Gani Koza (University of Wales, Bangor, UK).

3.4.2.1.8 Synthetic trans-*cyclopropane-hydroxy-MA*

Synthesized and kindly provided by Gani Koza (University of Wales, Bangor, UK).

3.4.2.1.9 Synthetic cis-*cyclopropane-hydroxy-MA*

Synthesized and kindly provided by Gani Koza (University of Wales, Bangor, UK).

3.4.2.2 Reagents and apparatus used in ELISA

As describe above in section 2.4.2.2 to 2.4.2.7.

3.4.2.3 Reagents and apparatus used in Biosensor assay

Natural mycolic acid (MA): Mycobacterial MAs were isolated from a culture of *M. tuberculosis* H37Rv as described by Goodrum *et al.* (67), as a mixture of α-, keto- and methoxy-MAs (Figure 1.7).

Synthetic α-mycolic acid (MB): Synthesized and kindly provided by Dr J. Al Dulayymi (University of Wales, Bangor, UK) (7).

Phosphatidyl choline-99 (PC): stock solution of 100 mg/ml in chloroform (99%, Sigma, USA).
Cholesterol (5-cholesten-3β-ol, Standard for chromatography, Sigma, St. Louis, MD, USA): stock solution of 100 mg/ml in chloroform.

Phosphate buffered saline (PBS): 20 x PBS stock was prepared by dissolving sodium chloride (160 g; 99%, Merck, SA), potassium chloride (4 g; 99%, Merck, SA), di-hydrogen potassium phosphate (4 g; 99%, Merck, SA) and di-sodium hydrogen phosphate (23 g; 99%, Merck, SA) in double distilled de-ionized water to a final volume of 1000 ml.

1 x PBS: 50 ml 20 x PBS in 950 ml dddH_2O. The pH of the solution was adjusted to 7.4 with 1 M NaOH.

PBS/Azide-EDTA (PBS/AE): EDTA (1 mM, Sigma, USA) and sodium azide (0.025% m/v, Sigma, USA) in 1 x PBS.

Saline: 0.9% NaCl (Merck, SA).
Potassium hydroxide (KOH): 12.5 M (Merck, SA).
Ethanol (EtOH): 95% (Saarchem, SA).
Cetyl-pyridinium chloride (CPC): 0.02 mg/ml in PBS/AE (Sigma, St. Louis, MD, USA).
IAsys resonant mirror biosensor: IAsys Affinity Sensors, Bar Hill, Cambridge, UK.
Sonicator: Branson Sonifier B-30, USA.

3.4.2.4 Preparation of liposomes containing mycolic acids, synthetic acetylated α-mycolic acid methyl ester (MB) or cholesterol

For the preparation of MA containing liposomes, PC (90 µl) was added to an amber glass vial containing MA (1 mg), mixed well to dissolve the MA, dried at 80 ˚C under a stream of N_2, and then sonicated in saline (2 ml) for 2 minutes at RT. The MB containing liposomes and the 'empty' PC liposomes were made in the same way, MB liposomes with 1 mg of MB, and PC liposomes with omission of the MAs. For the cholesterol-containing liposomes, PC (60 µl) and cholesterol (30 µl) were added to an amber glass vial without MA, mixed well, dried,

suspended in saline (2 ml) and sonicated as above. The liposomes were divided into 200 µl aliquots, freeze-dried and stored at -20 ˚C until used. Before use, the liposomes were reconstituted with PBS/AE (2 ml), heated at 80 ˚C for 15 minutes and then sonified as above. The final liposome concentration came to 500 µg/ml.

3.4.2.5 Measurements of interaction between MA, MB and cholesterol

IAsys software was used to set the IAsys affinity biosensor at a data-sampling interval of 0.4 s, temperature of 25 ^{0}C and stirring rate of 75%. Prior to use, the wells were regenerated by washing 5 times with EtOH (50 µl), for 30 seconds, followed by washing 7 times with PBS/AE (70 µl). The surface was then treated 5 times with KOH (50 µl) for 1 minute and finally washing 7 times with PBS/AE (70 µl). PBS/AE (60 µl) was pipetted into each well of the cuvette to obtain a stable baseline for 1 minute. The PBS/AE was subsequently aspirated and the surface activated with CPC (50 µl) for 10 minutes. This was followed by washing five times with PBS/AE (60 µl) and then substituting with PBS/AE (25 µl) for a new baseline before immobilization of MA-containing or MB-containing liposomes (25 µl) to the surface for 20 minutes. The immobilized liposomes were then washed 5 times with PBS/AE (60 µl) and again substituted with PBS/AE (25 µl) before the cholesterol-containing liposomes (25 µl) were added. Direct interaction between the immobilized MAs and cholesterol was monitored for 10 minutes, after which the cuvette was washed 3 times with 60 µl PBS/AE and regenerated as before.