# *Mycobacterium alvei* (ω-1)-methoxy mycolic acids: absolute stereochemistry and synthesis

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**Abstract:** Cells of *Mycobacterium alvei* are known to contain a unique set of mycolic acids with a (ω-1)-methoxy group; although the various enzymes in the biosynthesis of other types of mycolic acid have been widely studied, the biosynthetic route to this substituent is unclear. We now define the stereochemistry of the (ω-1)-methoxy fragment as *R*, and describe the synthesis of a major *R*-(ω-1)-methoxy-mycolic acid and its sugar esters, and of two natural *M. alvei* diene mycolic acids.

## 1. Introduction

Mycolic acids (MAs) (**Figure 1**), are major constituents of the cell envelope of *Mycobacterium tuberculosis* and other mycobacteria, some of which are pathogenic to animals and humans.[1-3] Their structure and biosynthesis has been reviewed,[4-6] and a number of structural and stereochemical relationships examined.[7,8,2] Their presence is thought to be linked to the resistance of these organisms to many current antibiotics and other chemotherapeutic agents.[9] MAs are defined by a *R,R*-β-hydroxy acid unit with two long chain substituents, the so called meromycolate chain and an α-chain (**Figure 1**). [1-8] In each case 'a-d' represent long alkyl chains, and generally each *Mycobacterium* contains a mixture of several homologues. The α-chain is a simple alkyl substituent, often with d = 21 or 23, though in some species it can be shorter. In contrast, the mero-chain can contain a number of substituents – in some important bacteria, such as *Mycobacterium tuberculosis*, these include two or three functional groups. In the common classes of MA, the proximal group is often a cyclopropane or an alkene and the distal group is a cyclopropane (α-MA), an α-methoxy-β-methyl fragment (methoxy-MA) or an α-keto-β-methyl fragment (keto-MA).[7,8] While free MA are present in cells, they are most commonly found bound to the wall as penta-arabinose tetramycolates or as non-covalently bound sugar esters; among these, trehalose dimycolates (TDM) and monomycolates (TMM) are known to be potent immune signalling agents.[2]

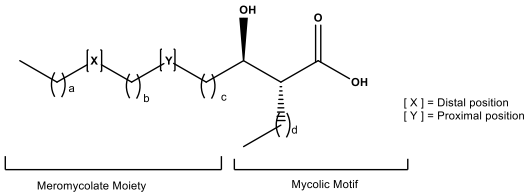



Figure 1: Typical mycolic acid structure

In the case of mycobacteria such as *M. tuberculosis*, the MA typically contain around 90 carbon atoms.[1-8] Important MAs of *Mycobacterium smegmatis* are much simpler and contain around 60 carbons with just one *cis*-alkene in the mero-chain.[10-18] MAs containing two *cis*-alkenes were also reported.[10,11] *Cis*-Alkene and diene mycolates are also present in *M. smegmatis* bound to trehalose and arabinogalactan.[19] *Mycobacterium marinum*,[20,21] *Mycobacterium ulcerans*,[21] and *Mycobacterum simiae* include mycolic acids with a single *cis*-alkene in the mero-chain.[22] *Mycobacterium chelonei* contains comparable amounts of α-mycolates, containing either two *cis*-alkenes, one *cis* and one α–methyl-*trans*-alkene, or one *cis*-cyclopropane and one α–methyl-*trans*-alkene at distal and proximal positions, of shorter chain α'-mycolates containing just one *cis*-alkene,[23-25] and diene mycolates apparently similar to those from *M. smegmatis*.[25] A di-unsaturated MA apparently essentially the same as that from *M. smegmatis* has also been isolated from *Mycobacterium phlei*,[26] as have monounsaturated compounds.[27,28] *Mycobacterium fortuitum* contains diunsaturated mycolic acids, with either *cis*- or *trans*-alkenes, the latter with a methyl-substituent on the allylic carbon. The regiochemistry of these α-methyl-*trans*-alkenes has been examined by chemical degradation, and the methyl group is found to be on the side near the hydroxy acid when the *trans*-alkene is in the proximal position of the chain, but on the side further from the hydroxy acid when in the distal position.[23] The MA of *Mycobacterium avium* include those containing two double bonds, either both in the *cis*-configuration, or with the distal position in *cis*-configuration and the proximal position in *trans*-configuration with an adjacent methyl group.[29] Diene MA have also been found in *Mycobacterium brumae* which contain two double bonds both in the *trans*-configuration.[30] Related species such as *Nocardia* and *Rhodococcus* are also reported to contain alkene and diene mycolates, although in this case the α-chain is much shorter, and the position of the alkenes in the mero-chain has not been determined.[31-34]

Some years ago a new species of rapidly growing nonphotochromogenic mycobacterium, *Mycobacterium alvei*, was described*;* it was reported that the cells contained α-MA and significant amounts of an unusual set of MAs in which there is an (ω-1)-methoxy substituent in the mero-chain, **1** (R, R' = H or Me);[35,36] (ω-1)-methoxy-MA were also reported in *Mycobacterium fortuitum*, *margeritense*, *peregrinum*, *porcinum* and *senegalense*.[37] In *M. alvei*, these were accompanied by MA with two alkene groups at positions X and Y in **Figure 1**, one of which was generally *cis*, the other *trans* with a methyl substituent on an adjacent carbon, compounds **2 (Figure 2)**. A careful analysis showed that the mixture of free MA from *M. alvei* consists of four series of homologues.[35,38] The first (series A) is

a dialkene series with carbon numbers 74, 76, 78, 80, the second (series B) a dialkene series with carbon numbers 75, 77, 79, 81, the third (series C) an (ω-1)-methoxy-diene series with carbon numbers 75, 77, 79, 81, and the fourth (series D) an (ω-1)-methoxy series with carbon numbers 76,78,80 and 82. Given that the core skeleton of MA is biosynthesised in units of two carbons, and that the introduction of a methyl-*trans*-alkene fragment or a methoxy group introduces one extra carbon, an even carbon number diene series (series A) should have two *cis* alkenes and the odd-carbon series (series B), one *cis*- and one *trans*-alkene. Series C should contain two cis-alkenes, and series D, one *cis*- and one *trans*-alkene. The fact that there are no significant corresponding peaks starting at 76 carbons in the diene MA or 77 carbons in the (ω-1)-methoxy series suggests that the content of di-*trans*-alkenes is low. The detailed structures and values of the chain lengths a – d or a' – d' in each molecule were probed by oxidative cleavage of the double bonds.[35]

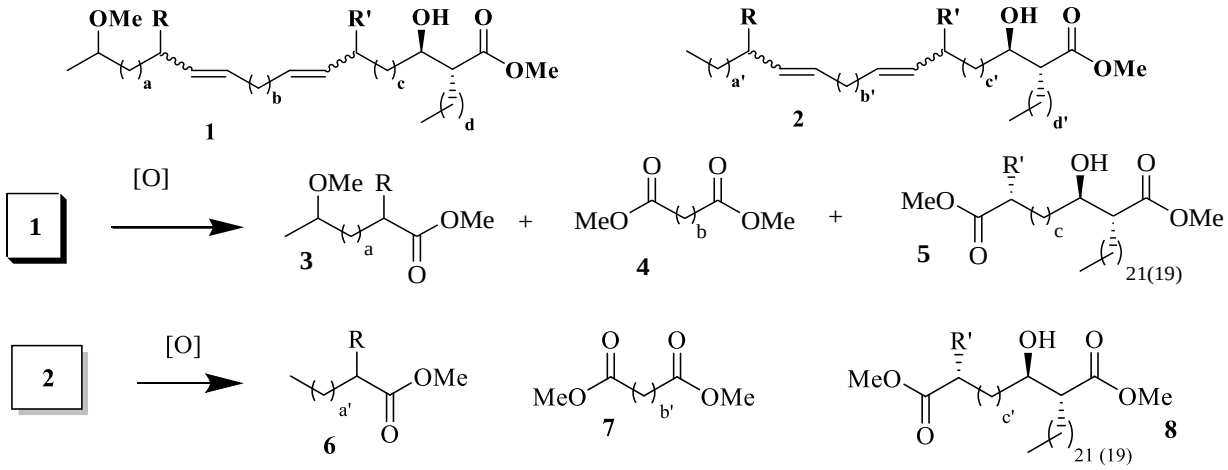



Figure 2: Reported oxidation products of *M. alvei* mycolic acids

The diesters **4** and **7** isolated from each oxidation were in each case un-branched molecules,[35] confirming that no methyl group adjacent to a *trans*-alkene in the natural products was within this chain, and had lengths of 14 – 19 methylene groups, with 12, 13 and 14 reported to be the most abundant. The monoesters **3** either had R = H and chain lengths of 16 – 18 carbons, although a minor component was methyl 2-methyl-17-methoxyoctadecanoate. Fragments **6** were straight chain esters with 16 – 20 carbons, plus methyl 2-methyloctadecanoate. Compound series **5** and **8** were very similar and in each case R' was principally a methyl group. On thermolysis, they undergo a retro-ene reaction to form a methyl ester, principally $CH_3(CH_2)_{22}CO_2Me$, together with $CH_3(CH_2)_{20}CO_2Me$, and long chain ester-aldehydes, including a compound with a methyl branch adjacent to the ester.

In addition to the MA themselves, the structures of the MA trehalose esters of *M. alvei* have been analysed and the most common chain lengths identified. Surprisingly, they are represented as having a methyl-*trans*-alkene at the distal position of the mycolic acid and a *cis*-alkene at the proximal position. Nonetheless, these molecules selectively stimulate the secretion of some pro-inflammatory cytokines of relevance in tuberculosis.[38,39] Exposure to *M. alvei* has also been linked to infections in humans and animals.[40-43]

Although the biosynthesis of alkene MA has been extensively studied,[44,45] and unsaturated mycolic acids may be intermediates in the biosynthesis of other classes of mycolic acid,[46]

little is known of the processes leading to the introduction of the (ω-1)-methoxy group, which distinguishes the MA of *M. alvei*. This mycobacterium is also differentiated from a range of other mycobacteria because it does not form cords, by a range of its activities to enzymes, and by its growth properties.[38] It was of interest therefore to prepare individual *M. alvei* (ω-1)-methoxy MA and their sugar esters, in order to allow their biochemical properties to be determined. To achieve this, it was first necessary to determine the absolute stereochemistry of the (ω-1)-methoxy group.

## 2 Results and discussion

### 2.1 Isolation and stereochemistry of the (ω-1)-methoxy fragment 3

Cells from *M. alvei* were hydrolysed as before,[35] and the resulting acids converted into the corresponding methyl esters by reaction with diazomethane. Chromatography gave a diene fraction and an (ω-1)-mycolate fraction. The *cis/trans*-alkene ratio in the (ω-1)-mycolate fraction was ca. 1.1:0.9 based on the integration of the alkene signals (Supplementary **S2**). The MALDI MS (**Figure 3**) showed a major series of homologues starting at M+Na 1163 ($C_{77}H_{152}O_4$), with the major cluster at 1191 ($C_{79}H_{156}O_4$). In addition there was a minor series (ca. 1:4) starting at 1149 ($C_{76}H_{150}O_4$) and ending at 1233. These two series can reasonably be assigned to a series with one *cis* and one methyl-*trans*-*alkene*, and a series of di-*cis*-alkenes respectively. The lack of any peaks at 1261 argues against the presence of significant quantities of di-*trans*-alkene.

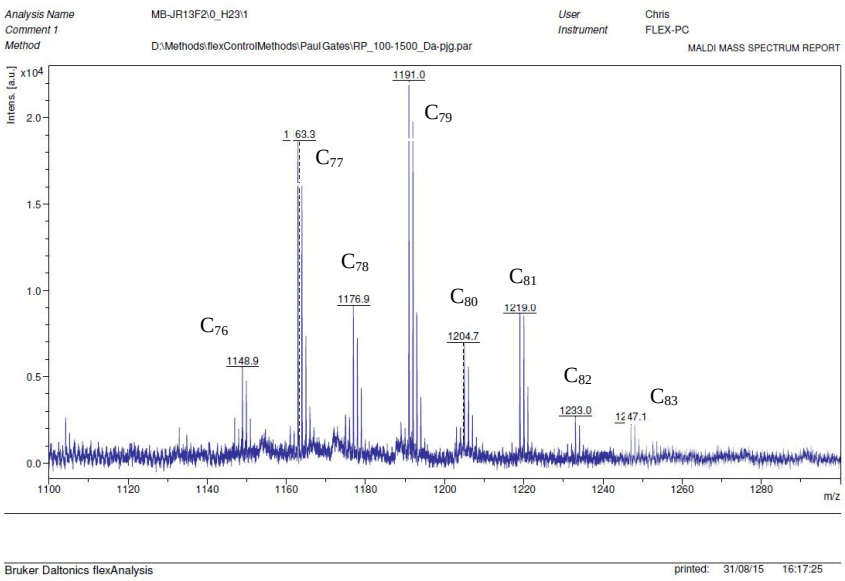
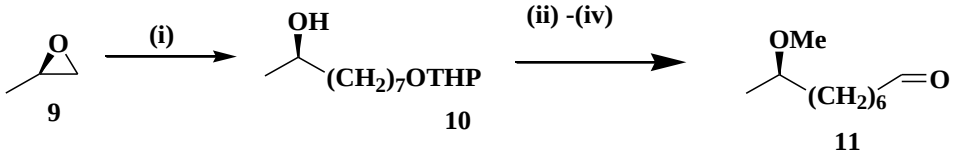



Figure 3: MALDI MS of natural methyl (ω-1)-methoxymycolate fraction (M + Na)

Oxidative cleavage of this fraction, using the method described earlier,[35] gave three major fractions. The first fraction (**S3F1**) comprised methyl esters of (ω-1)-methoxyalkanoic acids (3), with a minor impurity of dimethyl alkandioates. The presence of the $CH_3$-$CH(OCH_3)$-$CH_2$ fragment was clear from a three proton singlet at δ 3.22 for the methoxy group, partly overlapping a sextet at 3.29, together with a doublet at 1.13 for the terminal methyl group. It was reported earlier that there was no evidence in this fraction for the presence of 2-methyl

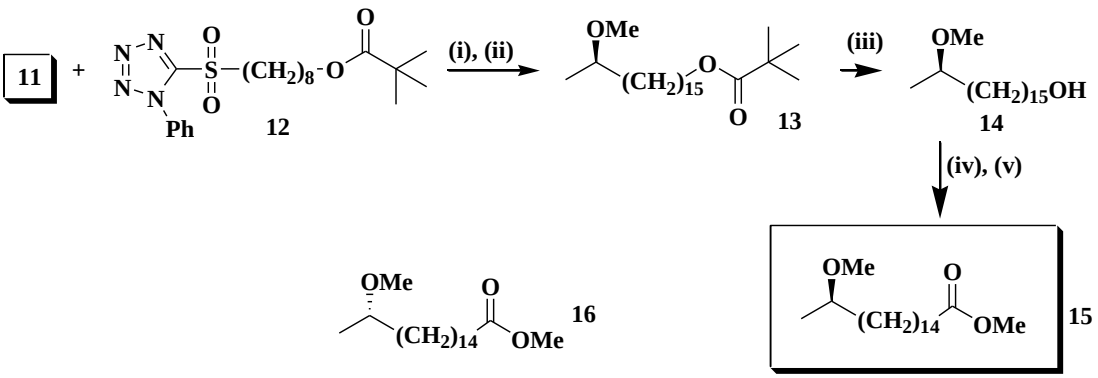
substituted esters.[35] However, in this case it was possible to see a minor sextet at δ 2.45, which matched the position of the signal for this proton in (**6**, R = Me); this could be assigned to around 3 – 5 % of this series of compounds, (**3**, R = Me). The GC-MS showed two major peaks in ratio ca. 3:2, the major isomer corresponding to synthetic $CH_3CH(OCH_3)(CH_2)_{14}COOCH_3$ (see below), the other to $CH_3CH(OCH_3)(CH_2)_{16}COOCH_3$. The mixture of compounds **3** showed $[\alpha]_D^{22}$ –1.55 (*c* 0.39, $CHCl_3$). In order to determine the absolute stereochemistry of these esters, the two enantiomers of (**3**, a=13, R = H) were prepared – it is well known that in such long chain molecules, the chiral element contributes an approximately constant amount to the molecular rotation $M_D$ of the molecule ($M_D = [\alpha]_D$ x M.W. / 100),[47] and therefore to the specific rotations of homologues; assuming all homologues in the mixture **3** have the same absolute stereochemistry, the specific rotation of a single enantiomer of the major component is therefore a good indication of the stereochemistry of the chiral element in the mixture.

The (ω-1)-methoxy group was first introduced by ring opening of *R*-propylene oxide (**9)** using a copper catalysed Grignard reaction. The alcohol **10** was methylated, followed by deprotection of the acetal, and oxidation to provide the aldehyde **11 (Scheme 1)**:




**Scheme 1:** (i) $BrMg(CH_2)_6OTHP$, CuI, THF (86%); (ii) NaH, THF, MeI (96%); (iii) pTSA, THF, MeOH (86%); (iv) PCC, $CH_2Cl_2$ (80%).

Coupling of **11** with the sulfone **12** and base in a modified Julia-Kocienski reaction, followed by hydrogenation of the derived mixture of alkenes led to ester **13**, which was converted by standard transformations into ester **15 (Scheme 2)**:



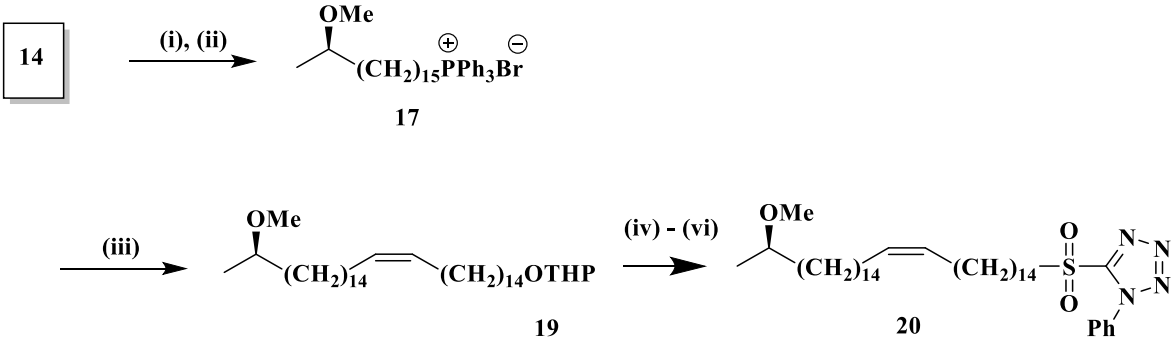

**Scheme 2:** (i) LiHMDS, THF (66%); (ii) $H_2$, Pd/C, IMS/EtOAc (99.5%); (iii) $LiAlH_4$, THF (88%); (iv) $RuCl_3.H_2O$, $NaIO_4$, acetonitrile/$CCl_4$/$H_2O$ (86%); (v) $H_2SO_4$/MeOH, reflux, 3 h, (89%).

In the same way, the enantiomer **16** was prepared from S-propylene oxide (Supplementary). The ester **15** showed $[\alpha]_D^{26}$ −1.09 (*c* 0.9, $CHCl_3$), while **16** showed $[\alpha]_D^{26}$ +1.08 (*c* 0.92, $CHCl_3$); on this basis, the natural fragment (**3**, R = H**)** and the mycolic acid from which it was derived are characterised as having an *R*-(ω-1)-methoxy stereochemistry.

Having established the stereochemistry of the novel element in the MAs **1**, it was of interest to synthesise the major isomer to enable its biochemical properties to be evaluated. First it was necessary to confirm the lengths of the other chains in the natural MA mixture. This was achieved by more detailed examination of the other fractions from its oxidation above, as described in the Supplementary Information.
On the basis of this, the target (ω-1)-methoxy-MA for synthesis was (**1**, R = H, R' = Me, a = 13, b = 13, c = 17) with an α-chain of 22 carbons total.

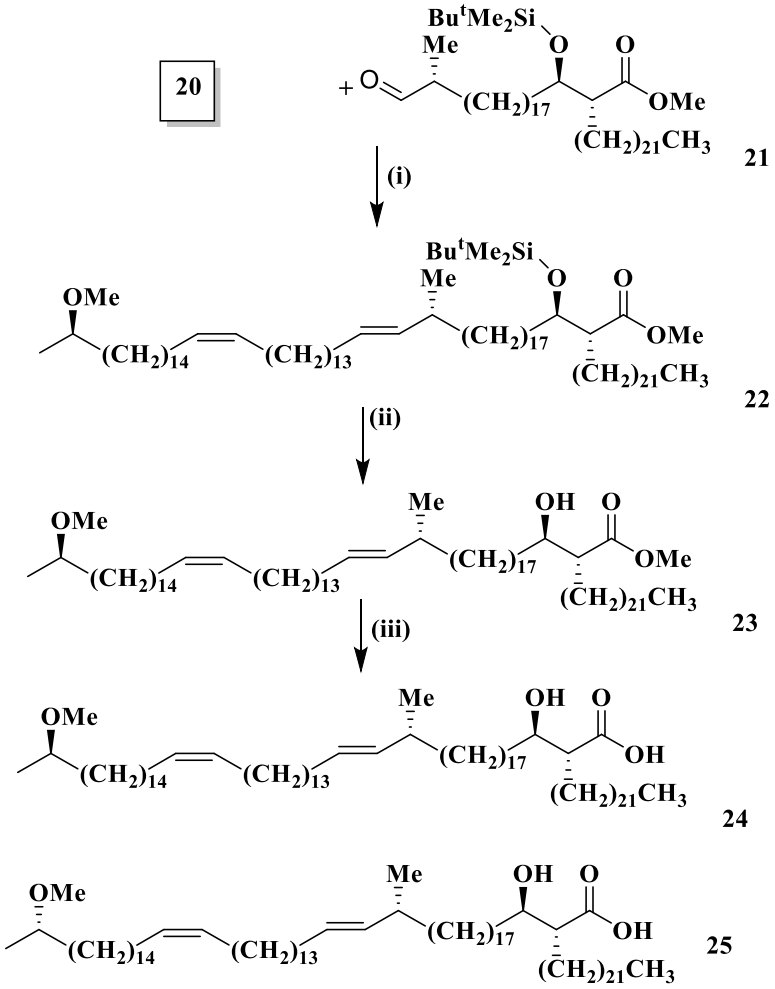
### 2.2 Synthesis of a major *R*-(ω-1)-methoxy-mycolic acid

The *R*-alcohol **14** was first brominated and then treated with triphenylphosphine to give phosphonium salt **17**. This was coupled to 15-tetrahydropyranyloxypentadecanal (**18**) in a Wittig reaction to provide the chain extended *Z*-alkene (**19**), which was further converted into the sulfone **20** using standard transformations (**Scheme 3**):




**Scheme 3:** (i) *N*-bromosuccinimide, $PPh_3$, $CH_2Cl_2$(83%); (ii) $PPh_3$, toluene (97%); (iii) $OCH(CH_2)_{14}OTHP$ (**18**), NaHMDS, THF (70%); (iv) PPTS, THF/MeOH (1:1) (94%); (v) DEAD, $PPh_3$, 1-phenyl-1*H*-tetrazole-5-thiol (94%); (vi) $H_2O_2$, $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, THF/IMS (3:1) (86%).

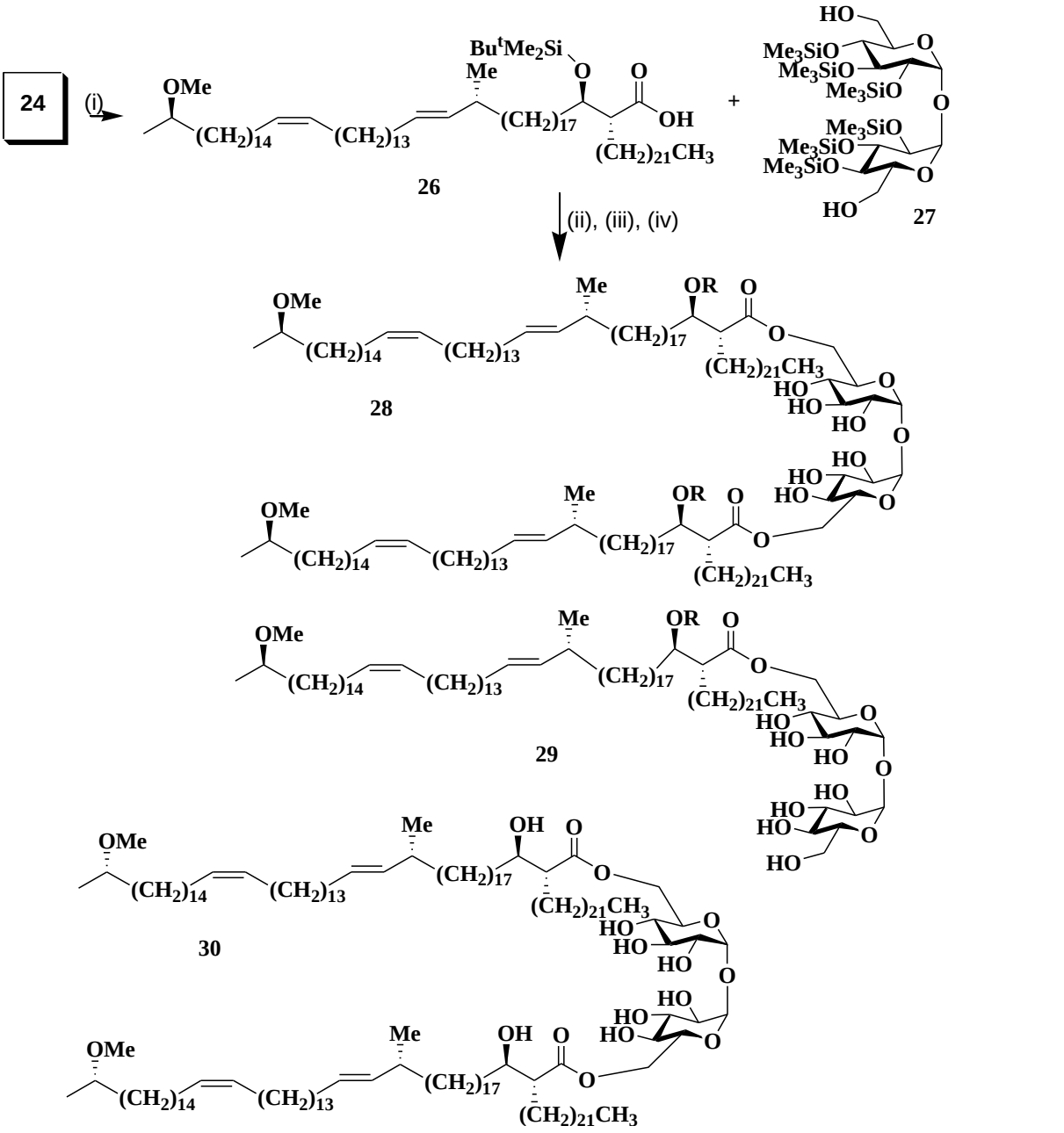
Coupling of the sulfone with the protected aldehyde **21** using KHMDS,[48,49] with retention of the absolute stereochemistry of the adjacent methyl substituent, as described for the preparation of *trans*-alkene keto- and methoxy-MA, [48] gave the diene **22**. Removal of the silyl protecting group led to the methyl ester **23**, which in turn was hydrolysed to the free acid **24 (Scheme 4)**:




**Scheme 4:** (i) KHMDS, THF (66%); (ii) pyridine, THF, HF.pyridine (66%); (iii) LiOH, THF, $H_2O$, MeOH (93%).

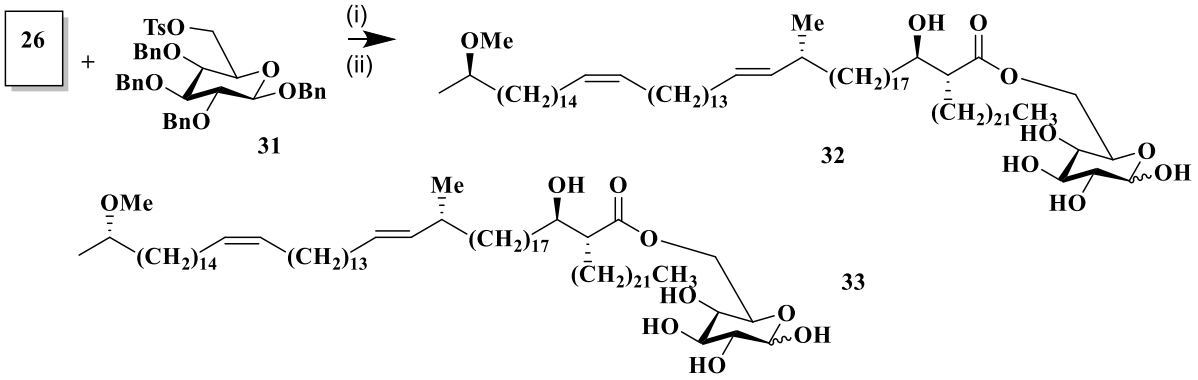
In the same way, the *S*-enantiomer of alcohol **14** was converted into the epimeric acid **25.** The (ω-1)-methoxy MA ester fraction isolated from the *M. alvei* cells had an $[\alpha]_D$ +1.25 (c 2.0 $CHCl_3$); the value for the single synthetic ester **23** was $[\alpha]_D$ +1.3 (*c* 0.99, $CHCl_3$). The *S*-methoxy epimer showed $[\alpha]_D$ +1.8 (c 1.0, $CHCl_3$). The NMR spectra of the two epimers were essentially identical, and identical to those of the natural mixture isolated from the cells. The MALDI MS of **23** matched the major isomer in the natural mixture.

The *R*-methoxy epimer was converted into a set of sugar esters. Protection of the secondary alcohol of **24** to give **26** using known procedures,[50,51] followed by coupling to the silyl protected trehalose **27** gave, after deprotection using an established procedure, the corresponding trehalose dimycolate (TDM, **28**, R = H) and monomycolate (TMM, **29**, R = H) (**Scheme 5**).



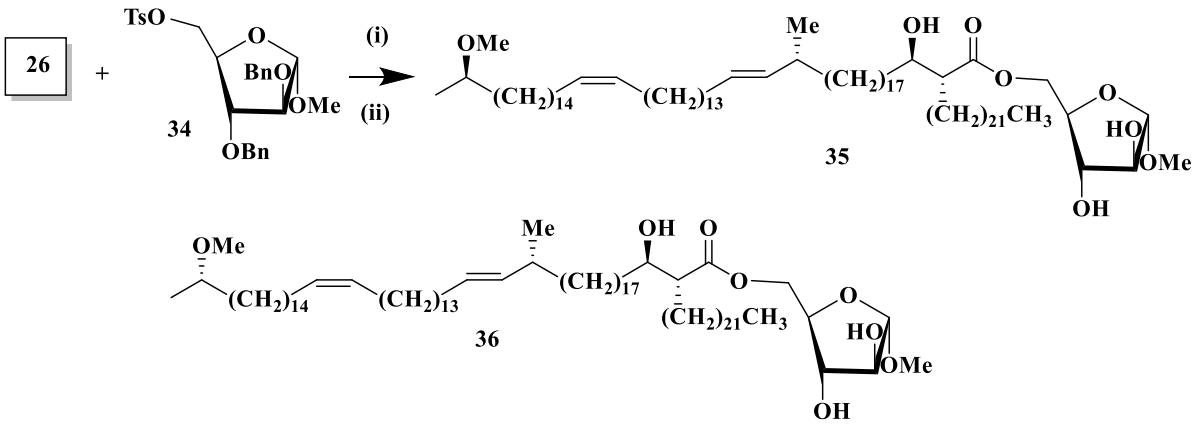

**Scheme 5:** (i) Imidazole, TBDMSCl, DMAP, DMF, toluene, then $K_2CO_3$, MeOH, THF (94%); (ii) EDCl, 4-DMAP, $CH_2Cl_2$, 4 Å molecular sieve (MS), rt, 8 days; **TDM** (21%), **TMM** (28%); (iii) TBAF, THF, 5 °C 1 h; **28** R = TBDMS (84%), **29** R = TBDMS (78 %); (iv) Pyridine, THF, HF-pyridine complex, 43 °C, 17 h, then neutralised with aq $NaHCO_3$, **28** R = H (56%), **29** R = H (29 %).

Using a modification of established methods,[55] protected MA **26** was also converted into the glucose monomycolate (GMM, **32**), deprotection as well as coupling occurring in the first step, while the *S*-epimer was converted into **33** (**Scheme 6**):




**Scheme 6:** (i) $CsHCO_3$, DMF:THF (64%); (ii) $Na/NH_3$, 1,4-dioxane (22%).

Again, using a modification of the published procedure,[56] the arabinose ester **35** and its epimer **36** were prepared from protected MA **26** (**Scheme 7**):

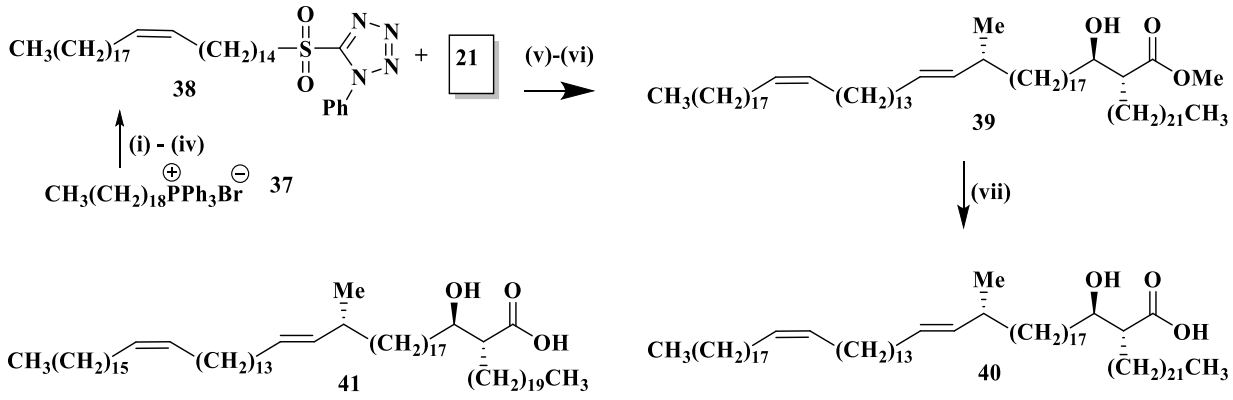



**Scheme 7:** (i) $CsHCO_3$, DMF:THF (70%); (ii) $Na/NH_3$, 1,4-dioxane (38%).

This provides a set of synthetic (ω-1)-methoxy-MA and sugar esters for evaluation of their effects on immune signals and their value as antigens. In order to provide a baseline for the possible effects of the (ω-1)-methoxy group, a major component of the natural diene mixture lacking this group was also synthesised. This first required a more detailed analysis of the major chain lengths and substitution patterns in the second fraction, **2**, of the natural mixture.

### 2.3 Synthesis of two homologues of *cis,trans*-diene MA of M. alvei

A more detailed analysis of the oxidation products of the diene mycolic acids is provided in the Supplementary Inofrmtion. On the basis of this, the major chain lengths in the (ω-1)-diene and simple diene fractions are summarised in Table S1. Although the MALDI MS of the natural mixture was very complex (**S1**), on the basis of the above, and matching the chain lengths b, c and d of **24**, the diene acid **40** was prepared using the same approach as before (**Scheme 8**). Using a similar approach, the diene acid **41**corresponding in chain lengths to the likely lowest homologue of the natural diene (Table S1) was also prepared; this has also been reported as a component of *M. brumae* cells.[35]




**Scheme 8:** (i) O=CH(CH$_2$)$_{14}$OTHP (**18**), NaHMDS, THF (43%); (ii) PPTS, THF/MeOH (5:2) (88%); (iii) DEAD, PPh$_3$, 1-phenyl-1*H*-tetrazole-5-thiol, THF (89%); (iv) $H_2O_2$, $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, THF/IMS (2:1) (57%); (v) KHMDS, THF (52%); (vi) pyridine, THF, HF.pyridine (96%); (vii) LiOH, THF, MeOH, $H_2O$ (98%).

Initial ELISA studies of the effects of the TDMs **28** and **30** as antigens in the diagnosis of infection by a related non-tuberculous mycobacterium in human serum showed that, although both distinguished infected from non-infected serum, compound **28**, derived from the natural *R*-(ω-1)-methoxy mycolic acid gave a slightly better distinction. A fuller analysis of the biological properties will be provided elsewhere.

## 3. Conclusion

The stereochemistry of the unusual (ω-1)-methoxy mycolic acids present in *M. alvei* has been established a being 53*R*. A major contributor to the natural mixture of homologues, and the epimeric 53*S* compound, as well as corresponding sugar esters, have been synthesised and the specific rotation of the 53*R* methyl ester matches that of the natural mixture of homologues. In order to establish whether the (ω-1)-methoxy group has a significant effect on the biochemical properties of *M. alvei*, a related diene mycolic acid with a proximal *trans*-alkene and distal *cis*-alkene, corresponding to one component of the natural extract, was also synthesised.

## 4. Experimental section

### 4.1 General

Chemicals used were obtained from commercial suppliers (Sigma-Aldrich and Alfa Aesar) or prepared from them by the methods described. Solvents which were required to be dry, e.g. ether, tetrahydrofuran were dried over sodium wire and benzophenone under nitrogen, while dichloromethane was dried over calcium hydride. Petroleum spirit (petrol) was of boiling point 40 – 60 °C. Reagents and solvents were of reagent grade unless otherwise stated. Silica gel (Merck 7736) and silica gel used for column and thin layer chromatography were obtained from Fisher Scientific; separated components were detected using UV light, $I_2$ or phosphomolybdic acid solution in IMS followed by charring. Anhydrous $MgSO_4$ was used to dry organic solutions. Infrared (IR) spectra were carried out on a Bruker Alpha ART-IR spectrometer. Products were colourless oil unless otherwise stated. NMR spectra were carried

out on a Bruker Avance 400 or 500 spectrometers. $[\alpha]_D$ values were recorded in $CHCl_3$ or MeOH on a POLAAR 3001 polarimeter. Accurate MALDI-TOF mass spectra were recorded on a Bruker ultrafleXtreme2 at Bristol University. NMR, MS and GC data are provided in Supplemenary File 1. Experimental data for the synthesis of the diene mycolic acids **40** and **41** are presented in Supplementary File 2. Data for the synthesis of the S-methoxydiene are in Supplementary File 3.

### 4.2 Separation of alkene and (ω-1)-methoxymycolates[35]

Cells that had been harvested from agar plates (2 g)[38] were suspended in 5 % KOH (1.25 g) in benzene / methanol (25 mL, 2:8 vol) and heated under reflux overnight. The solution was then acidified with 20% $H_2SO_4$ and the free MA were extracted with ether. Esterification with excess diazomethane gave the crude methyl mycolates, which were separated by column chromatography, eluting with petrol / ethyl acetate (10:1) to give 2 fractions.

The first fraction (**S1** 50 mg) was an unsaturated mycolate with a *cis* and *trans* double bond in a ratio of 1.1:0.9, $[\alpha]_D^{22}$ +1.4 (*c* 1.99, $CHCl_3$) showed $\delta_H$ (400 MHz; $CDCl_3$): 5.30 – 5.29 (3H, m), 5.23 (1H, br. dd, *J* 7.4, 15.4 Hz), 3.71 (3H, s), 3.68 – 3.60 (1H, m), 2.43 (1H, br. dt, *J* 5.4, 9.1 Hz), 2.40 (1H – OH, d, *J* 8.2 Hz), 2.06 – 1.90 (7H, m), 1.75 – 1.65 (1H, m), 1.64 – 1.55 (1H, m), 1.50 – 1.15 (127H, m), 1.13 – 1.05 (1H, m), 0.94 (3H, d, *J* 6.7 Hz), 0.88 (6H, t, *J* 6.5 Hz); $\delta_C$ (101 MHz; $CDCl_3$): 176.2, 136.5, 129.9, 128.42, 72.3, 51.5, 50.9, 37.2, 36.7, 35.7, 32.6, 31.9, 29.8, 29.7, 29.67, 29.6, 29.57, 29.54, 29.5, 29.4, 29.36, 29.1, 27.4, 27.35, 27.2, 25.7, 22.7, 20.95, 14.1;

The second fraction (**S2**, 23 mg) $[\alpha]_D^{22}$ +1.25 (*c* 2.04, $CHCl_3$) showed $\delta_H$ (400 MHz; $CDCl_3$): 5.38 – 5.29 (3H, m), 5.23 (1H, br. dd, *J* 7.4, 15.4 Hz), 3.71 (3H, s), 3.68 – 3.62 (1H, m), 3.31 (3H, s), 3.30 – 3.24 (1H, br. pentet, *J* 6.0 Hz), 2.43 (1H, br. dt, *J* 5.3, 9.1 Hz), 2.40 (1H – OH, d, *J* 8.2 Hz), 2.10 – 1.93 (7H, m), 1.75 – 1.65 (1H, m), 1.62 – 1.55 (1H, m), 1.50 – 1.15 (122H, m), 1.11 (3H, d, *J* 6.1 Hz) , 0.93 (3H, d, *J* 6.7 Hz), 0.88 (6H, t, *J* 6.6 Hz); $\delta_C$ (101 MHz; $CDCl_3$): 176.2, 136.5, 129.9, 128.4, 76.9, 72.3, 55.9, 51.5, 50.9, 37.2, 36.7, 36.3, 35.7, 32.6, 31.9, 29.8, 29.7, 29.6, 29.54, 29.5, 29.4, 29.36, 29.3, 29.1, 27.4, 27.35, 27.2, 25.7, 25.5, 22.7, 20.9, 19.0, 14.1.

### 4.3 Oxidative cleavage of diene mycolates of (ω-1)-methoxymycolate fraction[35]

A solution of methyl ω-methoxymycolates (21 mg), in benzene (1.0 mL) was added to a mixture of *t*-butanol (9 mL), aqueous sodium carbonate (0.02 M, 2.5 mL), distilled water (1.2 mL) and sodium periodate (82 mg), potassium permanganate (8.0 mg) in water (5.4 mL). The mixture was stirred at rt for 64 h, acidified with 2M aq. HCl then extracted with diethyl ether, dried and evaporated. The extract was methylated with diazomethane and columned with petrol / ethyl acetate (10:1) to give three fractions. The first was an oil (3.9 mg), $[\alpha]_D^{22}$ – 1.55 (*c* 0.39, $CHCl_3$) which showed $\delta_H$ (400 MHz; $CDCl_3$): 3.67 (3H, s), 3.32 (3H, s), 3.27 (1H, sextet, *J* 6.1 Hz), 2.45 (1H, m, minor isomer), 2.31 (2H, t, *J* 7.5 Hz), 1.66 – 1.48 (5H, m), 1.40 – 1.20 (22H, m), 1.13 (3H, d, *J* 6.1 Hz); $\delta_C$ (101 MHz; $CDCl_3$): 174.4, 76.9, 55.9, 51.4, 36.3, 34.1, 29.8, 29.63, 29.58, 29.4, 29.2, 29.1, 25.4, 24.9, 19.0 (**S3F1**).

The second fraction (5.0 mg) showed $\delta_H$ (400 MHz; $CDCl_3$): 3.67 (6H, s), 2.30 (4H, t, *J* 7.5 Hz), 1.65 – 1.57 (6H, m), 1.35 – 1.20 (16H, m); $\delta_C$ (101 MHz; $CDCl_3$): 174.4, 51.4, 34.1, 29.7, 29.6, 29.5, 29.4, 29.2, 29.1, 24.9 (**S3F2**).
The third fraction (8 mg) showed $\delta_H$ (400 MHz; $CDCl_3$): 3.70 (3H, s), 3.67 (3H, s), 3.60 (1H, m), 2.46 – 2.41 (2H, m), 2.30 (2H, t, *J* 7.5 Hz, minor isomer), 1.74 – 1.50 (8H, m), 1.50 – 1.35 (5H, m), 1.32 – 1.20 (64H, m), 1.14 (3H, d, *J* 7.0 Hz), 0.88 (3H, t, *J* 6.7 Hz); $\delta_C$ (101 MHz; $CDCl_3$): 175.2, 71.3, 50.5, 50.4, 49.9, 34.7, 32.8, 30.9, 28.7, 28.6, 28.57, 28.53, 28.5, 28.4, 28.35, 26.4, 21.7, 16.0, 13.1 (**S3F4**).

### 4.4 (*2R*)-9-((Tetrahydro-*2H*-2-yl)oxy)nonan-2-ol (10)

A solution of 2-(6-bromohexyloxy)tetrahydro-*2H*-pyran[31] (68.5 g, 0.258 mol) in dry THF (50 mL) was added dropwise to a suspension of magnesium turnings (18.9 g, 0.774 mol) in dry THF (120 mL) under nitrogen. The mixture was refluxed for 1 h, then cooled to rt and added dropwise to a stirred suspension of copper iodide (12.3 g, 0.0645 mol) in dry THF (150 mL) at – 30 °C. After 30 min, (*R*)-(+)-propylene oxide (6.0 g, 0.10 mol) in dry THF (30 mL) was added dropwise at − 30 °C. The reaction was kept at − 30 °C for 3 h, then allowed to reach 0 °C before slowly quenching with sat. aq. ammonium chloride (100 mL), allowed to reach rt, and extracted with ether (300 mL). The aqueous layer was re-extracted with ether (2 × 200 mL). The combined organic layers were washed with water (50 mL) and dried. The solvent was evaporated to give a colourless oil. Chromatography on silica gel eluting with petrol/ether (5:1) gave (*2R*)-9-((tetrahydro-*2H*-2-yl)oxy)nonan-2-ol (**10**) (22 g, 86%), $[\alpha]_D^{23}$ − 4.8 (*c* 1.14, MeOH) {Found $[M+NH_4]^+$: 262.2374; $C_{14}H_{32}O_3N$ requires: 262.2377} which showed $\delta_H$ (400 MHz, $CDCl_3$): 4.58 (1H, br. s), 3.92 − 3.84 (1H, m), 3.81 − 3.77 (1H, m), 3.73 (1H, dt, *J* 7.1, 9.4 Hz), 3.53 − 3.47 (1H, m), 3.38 (1H, dt, *J* 6.3, 9.4 Hz), 1.86 − 1.80 (1H, m), 1.75 − 1.69 (1H, m), 1.64 − 1.50 (7H, m), 1.46 − 1.27 (10H, m), 1.19 (3H, d, *J* 6.2 Hz); $\delta_C$ (101 MHz, $CDCl_3$): 98.8, 68.1, 67.6, 62.3, 39.3, 30.7, 29.7, 29.5, 29.4, 26.1, 25.7, 25.5, 23.4, 19.7; $\nu_{max}$ /$cm^{-1}$: 3411, 2927, 2854, 1454.

### 4.5 2-(((*R*)-8-Methoxynonyl) oxy)tetrahydro-*2H*-pyran

Sodium hydride (24.9 g, 0.622 mol, 60 % dispersion, 7 eq.) was washed with petrol (3 × 20 mL) and then suspended in dry THF (160 mL), cooled to 5 °C and (*2R*)-9-((tetrahydro-*2H*-pyran-2-yl)oxy)nonan-2-ol (**10**)[52] (21.7 g, 0.088 mol) in THF (100 mL) was added over 15 min. The mixture stirred for 15 min, then methyl iodide (75.7 g, 0.533 mol, 6 eq.) was added, and stirred for 16 h at rt. The mixture was then cooled to 0 °C and quenched slowly with sat. aq. ammonium chloride (50 mL), followed by ether (200 mL); the aqueous layer was extracted with petrol/ethyl acetate (2 × 100 mL, 1:1). The combined organic layers were washed with brine (2 × 80 mL), dried and evaporated to give a residue; chromatography on silica gel eluting with petrol/ethyl acetate (10:1) gave 2-(((*R*)-8-methoxynonyl)oxy)tetrahydro-*2H*-pyran (21 g, 96%), $[\alpha]_D^{23}$ –1.2 (*c* 1.9, MeOH) {Found $[M+NH_4]^+$: 276.2535; $C_{15}H_{34}O_3N$ requires: 276.2533} which showed $\delta_H$ (400 MHz, $CDCl_3$): 4.55 (1H, br. s), 3.87 − 3.82 (1H, m), 3.71 (1H, dt, *J* 6.9, 9.4 Hz), 3.52 − 3.45 (1H, m), 3.36 (1H, dt, *J* 6.7, 9.4 Hz),

3.29 (3H, s), 3.25 (1H, pent, *J* 6.0 Hz), 1.84 − 1.77 (1H, m), 1.72 − 1.66 (1H, m), 1.58 − 1.47 (8H, m), 1.31 − 1.28 (8H, m), 1.1 (3H, d, *J* 6.1 Hz); $\delta_C$ (101 MHz, $CDCl_3$): 98.8, 76.8, 67.6, 62.3, 55.9, 36.3, 30.7, 29.7, 29.6, 29.4, 26.1, 25.4, 25.3, 19.6, 18.9; $\nu_{max}/cm^{-1}$: 2928, 2855, 1464.

### 4.6 (*R*)-8-Methoxynonan-1-ol

Pyridinium *p*-toluenesulfonate (6.9 g, 0.03 mol) was added to a stirred solution of 2-(((*R*)-8-methoxynonyl)oxy)tetrahydro-*2H*-pyran (35.3 g, 0.136 mol) in methanol/THF (200 mL, 1:1) and stirred at 50 °C for 3 h, when sat. aq. $NaHCO_3$ (25 mL) and water (20 mL) were added and the mixture was extracted with ether ( 3 × 150 mL). The combined organic layers were dried, evaporated and the product was purified by column chromatography, eluting with petrol/ethyl acetate (5:1) to give a colourless oil, (*R*)-8-methoxynonan-1-ol (22 g, 86%), $[\alpha]_D^{23}$ –5.2 (*c* 1.9, MeOH) {Found $[M+H]^+$: 175.1693; $C_{10}H_{23}O_2$ requires: 175.1693} which showed $\delta_H$ (400 MHz, $CDCl_3$): 3.64 (2H, t, *J* 6.6 Hz), 3.31 (3H, s), 3.27 (1H, pent, *J* 6.0 Hz), 1.58 − 1.49 (4H, m), 1.45 − 1.28 (9H, br. m ), 1.12 (3H, d, *J* 6.1 Hz); $\delta_C$ (101 MHz, $CDCl_3$): 76.9, 62.9, 55.9, 36.3, 32.7, 29.7, 29.3, 25.6, 25.3, 18.9; $\nu_{max}/cm^{-1}$: 3360, 2927, 2855,1463.

### 4.7 (*R*)-8-Methoxynonanal (11)

(*R*)-8-Methoxynonan-1-ol (25.2 g, 0.015 mol) in DCM (50 mL) was added to a vigorously stirred suspension of pyridinium chlorochromate (62.6 g, 0.29 mol) in DCM (500 mL) at rt. The mixture was stirred for 2 h, then poured into petrol/ethyl acetate (200 mL, 5:1). The solution was filtered through a pad of silica, washed with petrol/ethyl acetate (200 mL, 5:1) and the filtrate was evaporated. Chromatography on silica gel eluting with petrol/ ethyl acetate (5:1) gave (*R*)-8-methoxynonanal (**11**) (20 g, 80%), $[\alpha]_D^{18}$ –3.4 (*c* 1.8 , MeOH) {Found $[M+NH_4]^+$: 190.1807; $C_{10}H_{24}O_2N$ requires: 190.1802} which showed $\delta_H$ (400 $MH_Z$, $CDCl_3$): 9.77 (1H, t, *J* 1.9 Hz), 3.31 (3H, s), 3.27 (1H, pent, *J* 6.0 Hz), 2.43 (2H, td, *J* 1.9, 7.4 Hz), 1.66 − 1.61 (2H, m), 1.58 – 1.48 (1H, m), 1.40 − 1.30 (7H, m), 1.12 (3H, d, *J* 6.1 Hz); $\delta_C$ (101 MHz, $CDCl_3$): 202.9, 76.7, 55.9, 43.8, 36.2, 29.4, 29.1, 25.2, 22.0, 18.9 ; $\nu_{max}/cm^{-1}$: 2930, 2857, 1724, 1463.

### 4.8 (*R*)-16-Methoxyheptadecyl pivalate (13)

i. Lithium *bis*(trimethylsilyl)amide (84.0 mL, 0.0594 mol, 1.06 M) was added dropwise to a stirred solution of (*R*)-8-methoxynonanal (**11**) (8.5 g, 0.088 mol) and 8-((1-phenyl-*1H*-tetrazol-5-yl)sulfonyl)octyl pivalate[53] (**12**) (25.0 g, 0.059 mol) in dry THF (100 mL) under nitrogen at −2 °C. The mixture was allowed to reach r.t, stirred for 2 h, then quenched with sat.aq. ammonium chloride (50 mL) and petrol/ethyl acetate (1:1), (300 mL). The aqueous layer was re-extracted with petrol/ethyl acetate (1:1, 2 × 50 mL). The combined organic layers were washed with sat. aq. sodium chloride (2 × 100 mL), dried and evaporated to give a thick oil. Chromatography on silica eluting with petrol/ethyl acetate (20:1) gave (*E/Z*)-(*R*)-

16-methoxyheptadec-8-en-1-yl pivalate (12.0 g, 66%) as a mixture of isomers in ratio 2:1, $[\alpha]_D^{29}$–1.0 (*c* 2.3, $CHCl_3$) {Found $[M+H]^+$: 369.3358; $C_{23}H_{45}O_3$ requires: 369.3363} which showed $\delta_H$ (500 MHz,$CDCl_3$, major isomer): 5.42 – 5.37 (2H, m), 4.04 (2H, t, *J* 6.6 Hz), 3.31 (3H, s), 3.27 (1H, br. pent, *J* 6.0 Hz), 1.98 – 1.92 (4H, m), 1.62 ( 2H, br. pent, *J* 6.5 Hz), 1.45 – 1.24 (18H, m), 1.20 (9H, s), 1.12 (3H, d, *J* 6.1 Hz); $\delta_H$ (500 MHz,$CDCl_3$, minor isomer): 5.36 – 5.32 (2H, m), 2.03 – 2.01 (4H, m), 1.54 – 1.48 (2H, m); the remaining signals were obscured by the major isomer; $\delta_C$ (101 MHz,$CDCl_3$ for the two isomers): 178.6, 130.4, 130.2, 129.9, 129.8, 76.8, 64.4, 55.9, 36.3, 32.6, 32.5, 29.7, 29.6, 29.6, 29.56, 29.5, 29.14, 29.1, 29.0, 28.9, 28.6, 27.2, 25.9, 25.85, 25.4, 18.9; $\nu_{max}/cm^{-1}$: 2926, 2854,1729, 1479.

ii. Palladium 10 % on carbon (1.5 g) was stirred with the above alkenes (12.0 g) in IMS (100 mL) and ethyl acetate (250 mL) under hydrogen. After 3 h, the reaction was quenched with two drops of water then filtered through a bed of celite and the solvent was evaporated to give a colourless oil, (*R*)-16-methoxyheptadecyl pivalate (**13**) (12.0 g, 99.5%), $[\alpha]_D^{29}$ –1.33 (*c* 2.02, $CHCl_3$) {Found $[M+H]^+$: 371.3516; $C_{23}H_{47}O_3$ requires: 371.3520} which showed $\delta_H$ (500 MHz, $CDCl_3$); 4.05 (2H, t, *J* 6.6 Hz), 3.32 (3H, s), 3.28 (1H, br. pent, *J* 5.9 Hz), 1.62 (2H, pent, *J* 6.5 Hz), 1.34 − 1.26 (26H, m), 1.20 (9H, s), 1.12 (3H, d, *J* 6.1 Hz); $\delta_C$ (101 MHz, $CDCl_3$): 178.6, 76.9, 64.5, 55.9, 36.3, 29.8, 29.7, 29.6, 29.54, 29.5, 29.2, 28.6, 27.2, 25.9, 25.4, 19.0; $\nu_{max}/cm^{-1}$: 2923, 2853, 1729, 1479.

### 4.9 (*R*)-16-Methoxyheptadecan-1-ol (14)

A solution of pivalate (**13**) (11.8 g, 0.0319 mol) in dry THF (50 mL) was added slowly to a stirred suspension of $LiAlH_4$ (1.81g, 0.0479 mol) in dry THF (150 mL) at – 20 °C under nitrogen, allowed to reach rt and then refluxed for 1 h. The mixture was cooled to −20 °C and quenched with sat. aq. sodium sulfate (40 mL) and THF (60 mL), stirred at rt until a white precipitate had formed, then filtered through a bed of silica and celite. The filtrate was evaporated and the product purified by column chromatography, eluting with petrol/ethyl acetate (5:1) to give a white solid, (*R*)-16-methoxyheptadecan-1-ol (**14**) (8.0 g, 88%), m.p. 46 − 48 °C, $[\alpha]_D^{29}$ –1.9 (*c* 1.59, $CHCl_3$) {Found $[M+H]^+$: 287.2945; $C_{18}H_{39}O_2$ requires: 287.2945} which showed $\delta_H$ (500 MHz, $CDCl_3$): 3.65 (2H, q, *J* 6.4 Hz), 3.32 (3H, s), 3.27 (1H, br. pent, *J* 6.0 Hz ), 1.63 − 1.50 (3H, m), 1.43 − 1.20 (26H, m), 1.12 (3H, d, *J* 6.1 Hz); $\delta_C$ (101 MHz, $CDCl_3$): 76.9, 63.0, 55.9, 36.3, 32.8, 29.8, 29.6, 29.59, 29.5, 29.4, 25.7, 25.4, 19.0; $\nu_{max}/ cm^{-1}$: 3432, 2916, 2847, 1471.

### 4.10 Methyl (*R*)-16-methoxyheptadecanoate (15)

Alcohol **14** (0.50 g, 1.7 mmol) in $CCl_4$ ( 2.8 mL) was added over 30 min at r.t. to a stirred solution of sodium periodate (1.12 g, 5.24 mmol) and ruthenium(III) chloride hydrate (4.70 mg, 0.0227 mmol) in acetonitrile (5.6 mL), $CCl_4$ (2.8 mL) and water (8.4 mL), then stirred for 16 h. The mixture was concentrated *in vacuo* then quenched with $H_2SO_4$ (20 mL, 2 N) and extracted with ethyl acetate (2 × 50 mL). The combined organic layers were dried and

evaporated. Column chromatography, eluting with petrol/ethyl acetate (5:2) gave (*R*)-16-methoxyheptadecanoic acid (0.45 g, 86%). Conc. $H_2SO_4$ (3 drops) was added to a stirred solution of the acid ( 0.45 g, 1.5 mmol) in methanol (15 mL) and the mixture was refluxed for 3 h at 92 °C. The solution was evaporated and the residue was diluted with a sat.aq. $NaHCO_3$ (10 mL). The product was extracted with petrol/ethyl acetate (2 × 30 mL 1:1), dried and evaporated, then purified by column chromatography, eluting with petrol/ethyl acetate (5:1) to give a white solid, methyl (*R*)-16-methoxyheptadecanoate (0.45 g, 89.4%), m.p. 33 − 35 °C, $[\alpha]_D^{26}$ −1.09 (*c* 0.9, $CHCl_3$); which showed $\delta_H$ (400 MHz, $CDCl_3$): 3.67 (3H, s), 3.31 (3H, s), 3.27 (1H, pent, *J* 6.0 Hz), 2.30 (2H,t, *J* 7.5 Hz), 1.66 − 1.56 (2H, m), 1.50 − 1.46 (1H, m), 1.43 − 1.20 (23H, m), 1.12 (3H, d, *J* 6.1 Hz); $\delta_C$ (101 MHz, $CDCl_3$): 174.4, 76.8, 55.9, 51.4, 36.4, 34.1, 29.8, 29.6, 29.5, 29.4, 29.3, 29.1, 25.5, 24.9, 19.0; $\nu_{max}/cm^{-1}$: 2917, 2849, 1733, 1463.

### 4.11 Methyl (*S*)-16-methoxyoctadecanoate (16)

i. A solution of (*S*)-16-methoxyheptadecyl pivalate (Supplementary) (17.5 g, 0.0473 mol) in dry THF (60 mL) was added slowly to a stirred suspension of $LiAlH_4$ (2.7 g, 0.071 mol) in dry THF (150 mL) at – 20 °C under nitrogen, allowed to reach room temperature and then refluxed for 1 h. Work up and purification as above gave a colourless oil, (*S*)-16-methoxy-heptadecan-1-ol (11.8 g, 84%), $[\alpha]_D^{29}$ +2.1 (*c* 1.53, $CHCl_3$) {Found $[M+H]^+$: 287.2946; $C_{18}H_{39}O_2$ requires: 287.2945} which showed $\delta_H$ (500 MHz, $CDCl_3$): 3.65 (2H, q, *J* 6.4 Hz), 3.32 (3H, s), 3.27 (1H, br. pent, *J* 6.0 Hz), 1.63 – 1.49 (3H, m), 1.43 – 1.20 (26H, m), 1.12 (3H, d, *J* 6.1 Hz); $\delta_C$ (101 MHz, $CDCl_3$): 76.9, 63.1, 55.9, 36.3, 32.8, 29.8, 29.64, 29.6, 29.5, 29.4, 25.7, 25.5, 19.0; $\nu_{max}/cm^{-1}$: 3433, 2916, 2847, 1471.

ii A solution of the alcohol (0.40 g, 1.4 mmol) in $CCl_4$ (2.0 mL) was added over 30 min. at r.t. to a stirred solution of sodium periodate (0.89 g, 4.2 mmol) and ruthenium(III) chloride hydrate (3.7 mg, 0.0182 mmol) in acetonitrile (5.0 mL), $CCl_4$ (2.0 mL) and water (8.0 mL). The mixture was stirred for an additional 16 h, then concentrated *in vacuo*. Work up and purification as for the *R*-isomer gave (*S*)-16-methoxyheptadecanoic acid (0.4 g, 95%). Conc.$H_2SO_4$ (3 drops) was added to a stirred solution of the acid (0.40 g, 1.3 mmol) in methanol (15 mL) and refluxed for 3 h at 92 °C. Work up and purification as above gave a white solid, methyl (*S*)-16-methoxyheptadecanoate **16** (0.35 g, 83.7%), m.p 32 − 34 °C, $[\alpha]_D^{26}$ +1.08 (*c* 0.92, $CHCl_3$) which showed $\delta_H$ (400 MHz, $CDCl_3$): 3.67 (3H, s), 3.31 (3H, s), 3.27 (1H, pent, *J* 6.0 Hz), 2.30 (2H, t, *J* 7.5 Hz), 1.63 − 1.56 (2H, m), 1.52 − 1.47 (1H, m), 1.35 − 1.22 (23H, m), 1.12 (3H, d, *J* 6.1 Hz); $\delta_C$ (101 MHz, $CDCl_3$): 174.4, 76.9, 55.9, 51.4, 36.3, 34.1, 29.8, 29.6, 29.5, 29.4, 29.3, 29.1, 25.5, 24.9, 19.0; $\nu_{max}/cm^{-1}$: 2914, 2849, 1772, 1428.

### 4.12 (*R*)-(16-Methoxyheptadecyl)triphenylphosphonium bromide (17)

i. Triphenylphosphine (25.1 g, 0.0956 mol) was added to a stirred solution of (*R*)-16-methoxyheptadecan-1-ol (**14**) (22.8 g, 0.079 mol) in DCM (350 mL), followed by addition of $NaHCO_3$ (0.5 g). The mixture was cooled to 10 °C then *N*-bromosuccinimide (21.3 g, 0.0119 mol) was added in portions and stirred at r.t. for 1 h. Sat.aq. sodium metabisulphate (150 mL) was added and the product was extracted with DCM (2 × 200 mL). The combined organic layers were dried and evaporated and the residue was refluxed in petrol/ethyl acetate (50:1) for 1 h. The triphenyl phosphonium oxide was filtered on a pad of celite and washed with petrol/ethyl acetate (50:1), the solvent was evaporated. Column chromatography, eluting with petrol/ethyl acetate (50:1) gave (*R*)-1-bromo-16-methoxyheptadecane (23.0 g, 83%), $[\alpha]_D^{29}$ – 1.11 (*c* 1.97, $CHCl_3$) {Found $[M+H]^+$: 349.2087; $C_{18}H_{38}OBr$ requires: 349.2101} which showed $\delta_H$ (500 MHz, $CDCl_3$): 3.41 (2H, t, *J* 6.8 Hz), 3.32 (3H, s), 3.27 (1H, br. pent, *J* 6.0 Hz), 1.86 (2H, pent, *J* 6.9 Hz), 1.42 (2H, pent, *J* 6.8 Hz), 1.34 − 1.23 (25H, br. m), 1.12 (3H, d, *J* 6.1 Hz); $\delta_C$ (101 MHz, $CDCl_3$): 76.9, 55.9, 36.3, 34.0, 32.8, 29.8, 29.63, 29.6, 29.5, 29.4, 28.8, 28.2, 25.4, 19.0 ; $\nu_{max}/cm^{-1}$: 2921, 2852, 1465.

ii. The (*R*)-1-bromide (22.5 g, 0.064 mol) was added to a stirred solution of triphenyl phosphine (50.7 g, 0.193 mol) in toluene (120 mL), refluxed for 120 h, then the solvent was evaporated. The residue was treated with petroleum ether (150 mL) and stirred for 30 min, then decanted; column chromatography, eluting with $CHCl_3$/MeOH (10:1) gave a white solid, (*R*)-(16-methoxyheptadecyl)triphenylphosphonium bromide (**17**) (38 g, 97%); $[\alpha]_D^{29}$ −1.0 (*c* 1.9, $CHCl_3$) {Found $[M–Br]^+$: 531.3737; $C_{36}H_{52}OP$ requires: 531.3750} which showed $\delta_H$ (500 MHz, $CDCl_3$): 7.88 − 7.84 (6H, m), 7.80 − 7.77 (3H, m), 7.72 − 7.68 (6H, m), 3.89 − 3.79 (2H, m), 3.31 (3H, s), 3.27 (1H, br. pent, *J* 6.0 Hz), 1.68 − 1.58 (4H, m), 1.57 − 1.46 (1H, m), 1.42 − 1.31 (2H, m), 1.29 − 1.15 (21H, m), 1.11 (3H, d, *J* 6.1 Hz); $\delta_C$ (101 MHz, $CDCl_3$): 135.0, 134.9, 133.6, 133.5, 130.5, 130.4, 118.7, 117.9, 76.8, 55.8, 36.2, 30.4, 30.3, 29.7, 29.54, 29.5, 29.49, 29.4, 29.14, 29.1, 25.4, 22.9, 22.6, 22.5, 22.46, 18.9; $\nu_{max}/cm^{-1}$: 2922, 2853, 1467.

### 4.13 15-((Tetrahydro-2*H*-pyran-2-yl)oxy)pentadecanal (18)

A solution of 15-((tetrahydro-2*H*-pyran-2-yl)oxy)pentadecan-1-ol[54] (12.5 g, 0.0381 mol) in DCM (40 mL) was added to a stirred solution of PCC (18.1 g, 0.0838 mol) in DCM (350 mL), when a black colour appeared. The mixture was stirred for 3 h, then poured into petrol/ethyl acetate (200 mL 10:1). The solution was filtered through a pad of silica, washed with petrol/ethyl acetate (200 mL), then evaporated. Chromatography on silica gel eluting with petrol/ethyl acetate (5:1) gave 15-((tetrahydro-2*H*-pyran-2-yl)oxy)penta-decanal **(18)** (11.7 g, 94%) which showed $\delta_H$ (500 MHz, $CDCl_3$): 9.76 (1H, t, *J* 1.6 Hz), 4.58 (1H, m), 3.93 – 3.85 (1H, m), 3.74 (1H, dt, *J* 6.9, 9.5 Hz), 3.57 – 3.45 (1H, m), 3.39 (1H, dt, *J* 6.7, 9.5 Hz), 2.42 (2H, td, *J* 1.6, 7.3 Hz), 1.90 – 1.79 (1H, m), 1.77 – 1.69 (1H, m), 1.64 – 1.50 (10H, m), 1.40 – 1.19 (18H, m); $\delta_C$ (101 MHz, $CDCl_3$): 202.9, 98.8, 67.7, 62.3, 43.9, 30.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.1, 26.2, 25.5, 22.1, 19.7; $\nu_{max}/ cm^{-1}$: 2926, 2854, 1727, 1462.

### 4.14 2-(((*R*,*Z*)-31-methoxydotriacont-15-en-1-yl)oxy)tetrahydro-2*H*-pyran (19)

Sodium *bis*(trimethylsilyl)amide (67.6 mL, 0.0676 mol, 1.0 M in THF) was added to a stirred solution of the phosphonium salt **(17)** (24.3 g, 0.0398 mol) and aldehyde **(18)** (11.8 g, 0.0361 mol) in dry THF (150 mL) at – 35 °C under nitrogen. The mixture was stirred for 30 min, allowed to reach rt for 30 min, then cooled and quenched with sat. aq. $NH_4Cl$ (20 mL). The product was extracted with 10:1 petrol/ethyl acetate (3 × 100 mL). The combined organic layers were washed with water (100 mL), brine (50 mL), dried and the solvent was evaporated. Column chromatography, eluting with petrol/ethyl acetate (20:1) gave 2-(((*R*,*Z*)-31-methoxydotriacont-15-en-1-yl)oxy)tetrahydro-2*H*-pyran **(20)** (14.6 g, 70%), $[\alpha]_D^{29}$ -3.8 (*c* 1.06, $CHCl_3$) {Found $[M+NH_4]^+$: 596.5970; $C_{38}H_{78}O_3N$ requires: 596.5976} which showed $\delta_H$ (500 MHz, $CDCl_3$): 5.35 ( 2H, pent, *J* 10.4 Hz), 4.61 – 4.57 (1H, m), 3.94 – 3.85 (1H, m), 3.74 (1H, dt, *J* 6.9, 9.5 Hz), 3.54 – 3.46 (1H, m), 3.39 (1H, dt, *J* 6.7, 9.5 Hz) 3.32 (3H, s), 3.27 (1H, br. pent, *J* 6.0 Hz), 2.05 – 1.98 (4H, m), 1.89 – 1.80 (1H, m), 1.75 – 1.69 (1H, m ), 1.63 – 1.49 (8H, m ), 1.39 – 1.23 (46H, m), 1.12 (3H, d, *J* 6.1 Hz); $\delta_C$ (101 MHz, $CDCl_3$): 129.9, 98.8, 76.9, 67.7, 62.3, 55.9, 36.3, 30.8, 29.8, 29.7, 29.66, 29.6, 29.56, 29.5, 29.3, 27.2, 26.2, 25.5, 25.4, 19.7, 19.0; $\nu_{max}/cm^{-1}$: 2918, 2851, 1471, 1199.

### 4.15 *R,Z*-5-((31-methoxydotriacont-15-en-1-yl)sulfonyl)-1-phenyl-1*H*-tetrazole (20)

i Pyridinum-*p*-toluenesulfonate (2.50 g, 9.94 mmol) was added to a stirred solution of compound **(20)** (11.5 g, 19.8 mmol) in THF (100 mL) and methanol (100 mL), stirred at 50 °C for 5 h, then sat. aq. $NaHCO_3$ (30 mL) and water (20 mL) were added and the product was extracted with ethyl acetate (3 × 100 mL). The combined organic layers were washed with water (150 mL), dried, and the solvent was evaporated. Chromatography, eluting with petrol/ethyl acetate (10:1) gave a white solid, (*R,Z)*-31-methoxydotriacont-15-en-1-ol (9.2 g, 94%), m.p. 47 − 49 °C, $[\alpha]_D^{29}$–3.4 (*c* 1.01, $CHCl_3$) {Found $[M+H]^+$: 495.5133; $C_{33}H_{67}O_2$ requires: 495.5136} which showed $\delta_H$ (500 MHz, $CHCl_3$): 5.35 (2H, pent, *J* 10.4 Hz), 3.64 (2H, t, *J* 6.6 Hz), 3.32 (3H, s), 3.27 (1H, br. pent, *J* 6.0 Hz), 2.02 (4H, q, *J* 6.5 Hz), 1.57 (4H, m), 1.40 – 1.22 (47H, m), 1.12 (3H, d, *J* 6.1 Hz); $\delta_C$ (101 MHz, $CDCl_3$): 129.9, 76.9, 63.1, 55.9, 36.3, 32.8, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 27.2, 25.7, 25.5, 19.0; $\nu_{max}/cm^{-1}$: 3437, 2914, 2847, 1470, 1199.

iii Diethyl azodicarboxylate (4.1 g, 0.0234 mol) in dry THF (10 mL) was added to a stirred solution of the above alcohol (8.9 g, 0.018 mol), triphenylphosphine (6.61 g, 0.0252 mol) and 1-phenyl-1*H*-tetrazole-5-thiol (4.17 g, 0.0234 mol) in dry THF (120 mL) at 0 °C under nitrogen. The mixture was allowed to reach rt and then stirred for 1.5 h; the solvent was evaporated, and the residue was treated with petrol/ethyl acetate (10:1, 150 mL) then refluxed for 30 min. The mixture was allowed to reach rt, filtered through celite and the filtrate evaporated to give a residue; chromatography, eluting with petrol/ethyl acetate (10:1) gave a white solid, (*R,Z*)-5-((31-methoxydotriacont-15-en-1-yl)thio)-1-phenyl-1*H*-1,2,3-triazole (11.0 g, 94%), m.p. 41 − 43 °C, $[\alpha]_D^{29}$− 4.8 (*c* 1.22, $CHCl_3$) {Found $[M+H]^+$: 655.5337; $C_{40}H_{71}N_4SO$ requires: 655.5343} which showed $\delta_H$ (500 MHz, $CDCl_3$): 7.62 − 7.53 (5H, m), 5.35 (2H, pent, *J* 10.9 Hz), 3.40 (2H, t, *J* 7.4 Hz), 3.32 (3H, s), 3.27 (1H, br. pent, *J* 6.0 Hz), 2.02 (4H, q, *J* 5.7 Hz), 1.82 (2H, pent, *J* 7.4 Hz), 1.60 – 1.50 (1H, m), 1.44 (1H, pent, *J* 6.7 Hz), 1.38 − 1.21 (46H, m), 1.12 (3H, d, *J* 6.1 Hz); $\delta_C$ (101 MHz, $CDCl_3$): 130.0, 129.9, 129.8,

123.9, 76.9, 55.9, 36.3, 33.4, 29.8, 29.7, 29.6, 29.5, 29.3, 29.1, 29.0, 28.7, 27.2, 25.5, 19.0; $\nu_{max}/cm^{-1}$: 2911, 2848, 1742, 1471, 1116.

iii Ammonium molybdate (VI) tetrahydrate (3.2 g, 0.0030 mol) dissolved in cold hydrogen peroxide (35%, 10 mL) was gradually added to a stirred solution of the above triazole (5.6 g, 0.0085 mol) in IMS (30 mL) and THF (60 mL) at 0 °C. The mixture was allowed to reach rt and stirred for 2 h, then further ammonium molybdate (VI) tetrahydrate (1.6 g, 0.00090 mol) in cold hydrogen peroxide (35%, 5 mL) was added, stirred for 6 h, poured into water (100 mL) and extracted with DCM (3 × 50 mL). The combined organic layers were dried and evaporated to give a residue. The $^1$H NMR showed a mixture of starting material, product and sulphoxide; the reaction was repeated 4 times, when TLC showed no starting material was left. Column chromatography, eluting with petrol/ethyl acetate (5:1) gave a white solid, (*R*,*Z*)-5-((31-methoxydotriacont-15-en-1-yl)sulfonyl)-1-phenyl-1*H*-tetrazole **(21)** (5.0 g, 86%), m.p. 45 − 47 °C, $[\alpha]_D^{29}$–5.57 (*c* 1.04, $CHCl_3$) {Found $[M+H]^+$: 687.5243; $C_{40}H_{71}N_4O_3S$ requires: 687.5241}, which showed $\delta_H$ (500 MHz, $CDCl_3$): 7.75 – 7.69 (2H, m), 7.68 – 7.59 (3H, m), 5.35 (2H, pent, *J* 10.9 Hz), 3.74 (2H, t, *J* 7.9 Hz), 3.32 (3H, s), 3.27 (1H, br. pent, *J* 6.0 Hz), 2.02 (4H, q, *J* 5.4 Hz), 1.97 – 1.92 (2H, m), 1.50 (3H, pent, *J* 6.6 Hz), 1.38 – 1.21 (45H, m), 1.12 (3H, t, *J* 6.8 Hz); $\delta_C$ (101 MHz, $CDCl_3$): 131.5, 129.9, 129.7, 125.1, 76.9, 56.0, 55.9, 36.3, 29.8, 29.7, 29.6, 29.5, 29.3, 29.2, 28.9, 28.2, 27.2, 25.5, 21.9, 19.0; $\nu_{max}/cm^{-1}$: 2915, 2849, 1593, 1471.

### 4.16 Methyl (*R*)-2-((1*R*,19*R*)-1-((tert-butyldimethylsilyl)oxy)-19-methyl-20-oxoeicosyl)-tetracosanoate (21)

Periodic acid (2.5 g, 0.01 mol) was added to a stirred solution of methyl (*R*)-2-((1*R*,2*R*,19*R*)-1-(*tert*-butyldimethylsilyloxy)-19-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)eicosyl)tetracosanoate (prepared as described in the literature, $[\alpha]_D^{28}$ +3.1 (*c* 0.85, $CHCl_3$), *lit.* $[\alpha]_D^{22}$ +3.6 (*c* 0.94, $CHCl_3$)[48] (2.0 g, 0.002 mol) in ether/THF (5:2, 112 mL) at r.t. under nitrogen and stirred for 3 h, then filtered through a bed of celite and washed with ether/THF (50 mL). The solvent was evaporated and column chromatography, eluting with petrol/ethyl acetate (5:1) gave methyl-(*R*)-2-((1*R*,19*R*)-1-((*tert*-butyldimethylsilyl)oxy)-19-methyl-20-oxoeicosyl)tetracosanoate **(21)** (1.7 g, 90%) {Found $[M + Na^+]$: 843.7589; $C_{52}H_{104}O_4Si$ Na requires: 843.7596}, $[\alpha]_D^{28}$ –9.7 (*c* 0.88, $CHCl_3$) which showed $\delta_H$ (500 MHz, $CDCl_3$): 9.62 (1H, d, *J* 2.0 Hz), 3.90 (1H, br. td, *J* 4.6, 6.8 Hz), 3.66 (3H, s), 2.53 (1H, ddd, *J* 3.7, 7.2, 10.9 Hz), 2.43 (1H, br. qd *J* 2.0, 7.0 Hz), 1.78 – 1.63 (1H, m), 1.59 – 1.15 (75H, m), 1.09 (3H, d, *J* 7.0 Hz), 0.88 (3H, t, *J* 7.1 Hz), 0.86 (9H, s), 0.04 (3H, s), 0.02 (3H, s); $\delta_C$ (101 MHz, $CDCl_3$): 205.4, 175.2, 73.2, 51.6, 51.2, 33.7, 31.9, 30.5, 29.8, 29.7, 29.6, 29.5, 29.46, 29.4, 29.3, 27.8, 27.5, 26.9, 25.8, 23.7, 22.7, 17.9, 14.1, 13.3, – 4.4, – 4.9; $\nu_{max}/cm^{-1}$: 2921, 2852, 1737, 1463.

### 4.17 Methyl (2*R*, 3*R*, 21*R*, 22*E*, 37*Z*, 53*R*)-3-((*tert*-butyldimethylsilyl)oxy)-2-docosyl-53-methoxy-21-methyltetrapentaconta-22, 37-dienoate (22)

Potassium *bis*(trimethylsilyl)amide (7.8 mL, 0.003 mol, 0.5 M in toluene) was added with stirring to compound **(21)** (1.65 g, 0.002 mol ) and (*R*,*Z*)-5-((31-methoxydotriacont-15-en-1-yl)sulfonyl)-1-phenyl-1H-tetrazole **(20)** (1.59 g, 0.003 mol) in dry THF (60 mL ) at –20 °C. The reaction turned bright yellow and was left to reach rt and stirred for 10 min under

nitrogen, then cooled to –5 °C, diluted with ethyl acetate (20 mL) and quenched with sat. aq. $NH_4Cl$ (10 mL). The product was extracted with ethyl acetate (3 × 150 mL). The combined organic layers were washed with water (100 mL), dried and evaporated. Chromatography over silica gel, eluting with petrol/ether (20/1) gave the title compound **(22)** (1.7 g, 66%), $[\alpha]_D^{24}$ –3.6 (*c* 0.94, $CHCl_3$) {Found [M+$Na^+$]: 1304.2618; $C_{85}H_{168}O_4SiNa$ requires:1304.2604}, which showed $\delta_H$ (500 MHz, $CDCl_3$): 5.40 – 5.30 (3H, m), 5.24 (1H, br. dd, *J* 7.5, 15.2 Hz), 3.90 (1H, br. dt, *J* 4.7, 6.5 Hz), 3.66 (3H, s), 3.32 (3H, s), 3.27 (1H, pent, *J* 6.0 Hz), 2.53 (1H, ddd, *J* 3.7, 7.0, 10.8 Hz), 2.10 – 1.90 (7H, m), 1.60 – 1.17 (124H, v.br. m), 1.12 (3H, d, *J* 6.1 Hz), 0.94 (3H, d, *J* 6.7 Hz), 0.88 (3H, t, *J* 6.8 Hz), 0.86 (9H, s), 0.04 (3H, s), 0.02 (3H, s); $\delta_C$ (101 MHz, $CDCl_3$): 175.2, 136.5, 129.9, 128.4, 76.9, 73.2, 55.9, 51.6, 51.2, 37.2, 36.7, 36.3, 33.7, 32.6, 31.9, 29. 8, 29.7, 29.6, 29.5, 29.3, 27.8, 27.5, 27.2, 25.8, 25.5, 23.7, 22.7, 20.9, 19.0, 14.1, – 4.4, – 4.9; $\nu_{max}/cm^{-1}$: 2920, 2851, 1740, 1463, 1370.

### 4.18 Methyl (2*R*,3*R*,21*R*,22*E*,37*Z*,53*R*)-2-docosyl-3-hydroxy-53-methoxy-21-methyltetrapenta-conta-22,37-dienoate (23)

The dienoate **(22)** (2.1 g, 1.6mmol) was dissolved in dry THF (22 mL) in a dry polyethylene vial equipped with a rubber septum, followed by addition of pyridine (0.3 mL) at rt under nitrogen. The mixture was cooled to 0 °C, when hydrogen fluoride-pyridine complex (~70%, 2 mL) was added dropwise. The mixture was stirred at 43 °C for 18 h, then added slowly to sat. aq. $NaHCO_3$ and stirred until no more $CO_2$ was liberated. The product was extracted with petrol/ethyl acetate (1:1, 3 × 30 mL), then the combined organic layers were dried, and evaporated; chromatography, eluting with petrol/ethyl acetate (15:1, then 5:1) gave a white solid, the title compound **(23)** (1.9 g, 66%), m.p. 51 − 53 °C, $[\alpha]_D^{24}$ +1.3 (*c* 0.99, $CHCl_3$) {Found [M+$Na^+$]: 1190.1752; $C_{79}H_{154}O_4Na$ requires: 1190.1739} which showed $\delta_H$ (400 MHz, $CDCl_3$): 5.42 – 5.29 (3H, m), 5.23 (1H, br. dd, *J* 7.4, 15.3 Hz), 3.71 (3H, s), 3.66 – 3.62 (1H, m), 3.32 (3H, s), 3.27 (1H, br. pent, *J* 6.0 Hz), 2.44 (1H, dt, *J* 5.3, 9.2 Hz), 2.09 – 1.94 (7H, m), 1.76 – 1.65 (1H, m), 1.62 – 1.16 (124H, m, v. br. m), 1.12 (3H, d, *J* 6.1 Hz), 0.94 (3H, d, *J* 7.0 Hz), 0.88 (3H, t, *J* 7.0 Hz); $\delta_C$ (101 MHz, $CDCl_3$): 176.2, 136.5, 129.9, 128.4, 76.7, 72.3, 55.9, 51.5, 50.9, 37.2, 36.7, 36.3, 35.7, 32.6, 32.9, 29.8, 29.7, 29.6, 29.54, 29.5, 29.4, 29.36. 29.3, 29.1, 27.4, 27.3, 27.2, 25.7, 25.5, 22.7, 20.9, 19.0, 14.1; $\nu_{max}/cm^{-1}$: 3518, 2916, 2848, 1709, 1461, 1374.

### 4.19 (2*R*,3*R*,21*R*,22*E*,37*Z*,53*R*)-2-docosyl-3-hydroxy-53-methoxy-21-methyltetrapentaconta-22, 37-dienoic acid (24)

Lithium hydroxide monohydrate (0.46 g, 11.1 mmol) was added to a stirred solution of dienoate **(23)** (1.3 g, 1.11 mmol) in THF (20 mL), water (2.6 mL) and MeOH (2 mL). The mixture was heated at 45 °C for 16 h, then diluted with petrol/ethyl acetate (5:1, 20 mL), and acidified with sat. aq. $KHSO_4$. The product was extracted with warm petrol/ethyl acetate (5:1, 3 × 50 mL), and the combined organic layers were dried and evaporated. Chromatography, eluting with petrol/ethyl acetate (15:1 then 5:1) gave a white solid, the title acid **(24)** (1.2 g, 93%), m.p. 62 − 64 °C, $[\alpha]_D^{23}$ +0.86 (*c* 0.81, $CHCl_3$) {MALDI-Found [M–H]$^+$: 1152.1615;

$C_{78}H_{151}O_4$ requires: 1152.1612}, which showed $\delta_H$ (400 MHz, $CDCl_3$): 5.39 – 5.29 (3H, m), 5.24 (1H, br. dd, *J* 7.4, 15.3 Hz), 3.75 – 3.62 (1H, br. m), 3.32 (3H, s), 3.27 (1H, br. pent, *J* 6.0 Hz), 2.49 – 2.33 (1H, br. m), 2.06 – 1.99 (7H, m), 1.43 – 1.16 (126H, v. br. m), 1.12 (3H, d, *J* 6.1 Hz), 0.94 (3H, d, *J* 7.0 Hz), 0.88 (3H, t, *J* 7.0 Hz); $\delta_C$ (101 MHz, $CDCl_3$): 180.0, 136.5, 129.9, 128.4, 76.9, 72.2, 55.8, 37.3, 36.7, 36.3, 35.5, 32.6, 31.9, 29.8, 29.76, 29.72, 29.7, 29.6, 29.5, 29.4, 29.3, 27.4, 27.2, 25.8, 25.5, 22.7, 20.9, 19.0, 14.1; $v_{max}/cm^{-1}$: 3272, 2915, 2848, 1711, 1467, 1372.

### 4.20 (2*R*,3*R*,21*R*,22*E*,37*Z*,53*S*)-2-docosyl-3-hydroxy-53-methoxy-21-methyltetrapentaconta-22, 37-dienoic acid (25)

Lithium hydroxide monohydrate (0.28 g, 6.0 mmol) was added to a stirred solution of methyl (2*R*,3*R*,21*R*,22*E*,37*Z*,53*S*)-2-docosyl-3-hydroxy-53-methoxy-21-methyltetrapentaconta-22,37-dienoate **(Supplementary)** (0.8 g, 0.6 mmol) in THF (16 mL), water (1.5 mL) and MeOH (1 mL). The mixture was heated at 45 ºC for 16 h, then worked up as above to give a white solid, the title acid **(25)** (0.56 g, 71%), m.p. 61 − 63 °C, $[\alpha]_D^{24}$ +0.89 (*c* 0.78 $CHCl_3$) {MALDI-Found $[M–H]^+$: 1152.1608; $C_{78}H_{151}O_4$ requires: 1152.1612} which showed $\delta_H$ (400 MHz, $CDCl_3$): 5.41 – 5.30 (3H, m), 5.24 (1H, br. dd, *J* 7.4, 15.3 Hz), 3.75 – 3.65 (1H, br. m), 3.33 (3H, s), 3.27 (1H, br. pent, *J* 6.0 Hz), 2.45 – 2.35 (1H, br. m), 2.10 – 1.91 (7H, m), 1.45 – 1.19 (126H, v. br. m), 1.12 (3H, d, *J* 6.1 Hz), 0.94 (3H, d, *J* 7.0 Hz), 0.88 (3H, t, *J* 7.0 Hz); $\delta_C$ (101 MHz, $CDCl_3$): 180.0, 136.5, 129.9, 128.4, 76.9, 72.3, 55.9, 37.3, 36.7, 36.3, 32.6, 31.9, 29.8, 29.76, 29.72, 29.7, 29.6, 29.5, 29.4, 29.3, 27.4, 27.2, 25.8, 25.5, 22.7, 20.9, 19.0, 14.1; $v_{max}/cm^{-1}$: 3272, 2915, 2848, 1711, 1467, 1371.

### 4.21 (2*R*,3*R*,21*R*,22*E*,37*Z*,53*R*)-3-((*tert*-Butyldimethylsilyl)oxy)-2-docosyl-53-methoxy-21-methyl-tetrapentaconta-22,37-dienoic acid (26)

Imidazole (0.62 g, 91.8 mmol) was added to a stirred solution of acid **(24)** (1.06 g, 9.1 mmol) in dry DMF (7 mL) and dry toluene (7 mL) at rt followed by the addition of *tert*-butyl-dimethylsilylchloride (1.4 g, 91.8 mmol) and 4-DMAP (0.11 g). The mixture was stirred at 70 °C for 24 h. The solvent was removed under high vacuum and the residue was diluted with petrol/ethyl acetate (1:1, 50 mL) and water (20 mL). The aqueous layer was re-extracted with petrol/ethyl acetate (1:1, 3 × 20 mL). The combined organic layers were washed with water, dried and evaporated to give a colourless oil. This was dissolved in THF (40 mL), and aq. tetra-*n*-butyl ammonium hydroxide (26 mL, 5%) was added slowly. The mixture was stirred at r.t. for 3 h, diluted with petrol/ethyl acetate (1:1, 30 mL) and water (2 mL) then acidified with $KHSO_4$ to pH 2. The aqueous layer was re-extracted with petrol/ethyl acetate (1:1, 20 mL). The combined organic layers were washed with water, dried and evaporated to give a residue. Column chromatography, eluting with petrol/ethyl acetate (15:1) gave the acid **(26)** (1.09 g, 94%), $[\alpha]_D^{28}$ –1.02 (c 0.78, $CHCl_3$) {MALDI-Found $[M – H]^+$: 1266.2480; $C_{84}H_{165}O_4Si$ requires: 1266.2477} which showed $\delta_H$ (400 MHz, $CDCl_3$): 5.41 – 5.29 (3H, m), 5.24 (1H, br. dd, *J* 7.4, 15.2 Hz), 3.83 (1H, ddd, *J* 3.0, 5.6, 7.6 Hz), 3.32 (3H, s), 3.27 (1H, br. pent, *J* 6.0 Hz), 2.53 (1H, ddd, *J* 2.7, 5.9, 8.8 Hz), 2.09 – 1.92 (7H, m), 1.79 – 1.65 (1H, m), 1.64 – 1.48 (7H, m), 1.45 – 1.20 (117H, v. br. m), 1.12 (3H, d, *J* 6.1 Hz), 0.96 – 0.92 (12H,

including s at 0.93), 0.88 (3H, t, *J* 7.0 Hz), 0.15 (3H, s), 0.14 (3H, s); $\delta_C$ (101 MHz, $CDCl_3$): 175.9, 136.5, 129.9, 128.4, 76.9, 73.6, 55.9, 50.3, 41.3, 37.2, 36.7, 36.3, 35.5, 32.6, 31.9, 29. 8, 29.7, 29.6, 29.5, 27.4, 27.2, 25.7, 25.6, 25.5, 24.9, 22.7, 22.6, 20.9, 19.0, 17.9, 14.1, – 4.3, – 4.9; $\nu_{max}/cm^{-1}$: 3688, 2918, 2850, 1708, 1463, 1370.

**4.22 6,6'-*bis*-*O*-(2*R*,3*R*,21*R*,22*E*,37*Z*,53*R*)-3-((*tert*-Butyldimethylsilyl)oxy)-2-docosyl-53-meyhoxy-21-methyltetrapentaconta-22,37-dienoic-2,3,4,2',3',4'-hexakis-*O*-(trimethylsilyl)-α,α'-trehalose and 6-*O*-(2*R*,3*R*,21*R*,22*E*,37*Z*,53*R*)-3-((*tert*-butyldimethylsilyl)oxy)-2-docosyl-53-methoxy-21-methyltetrapentaconta-22,37-dienoic-2,3,4,2',3',4'-hexakis-*O*-(trimethylsilyl)-α,α'-trehalose**

1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (0.277 g, 1.44 mmol) and DMAP (0.176 g, 1.44 mmol) were added to a stirred solution of (**26)** (0.412 g, 0.325 mmol) and 2,3,4,2',3',4'-hexakis-*O*-(trimethylsilyl)α,α'-trehalose **(27)**[51] (0.14 g, 0.18 mmol) and powdered 4 A˚ molecular sieves in dry dichloromethane (4 mL) at rt under nitrogen. The mixture was stirred for 8 days then diluted with dichloromethane (5 mL) and silica gel (1 g) was added. The mixture was evaporated under reduced pressure to give a residue; chromatography, eluting with petrol/ethyl acetate (20:1) gave a first fraction, 6,6'-*bis*-*O*-(2*R*,3*R*,21*R*,22*E*,37*Z*,53*R*)-3-((*tert*-butyldimethylsilyl)oxy)-2-docosyl-53-methoxy-21-methyl-tetrapentaconta-22,37-dienoic-2,3,4,2',3',4'-hexakis-*O*-(trimethylsilyl)-α,α'-trehalose (**TDM)** as a colourless thick oil (0.22 g, 21%), $[\alpha]_D^{20}$ +23 (*c* 0.98, $CHCl_3$) {Found $[M+Na]^+$: 3297.8398; $C_{198}H_{398}O_{17}Si_8Na$ requires: 3297.8375} which showed $\delta_H$ (400 MHz, $CDCl_3$): 5.40 – 5.29 (6H, m), 5.24 (2H, br. dd, *J* 7.4, 15.3 Hz), 4.85 (2H, br. d, *J* 3.0 Hz), 4.37 (2H, br. d, *J* 10.0 Hz), 4.04 – 3.97 (4H, m), 3.96 – 3.93 (2H, m), 3.89 (2H, br. t, *J* 8.8 Hz), 3.52 (2H, br. t, *J* 9.0 Hz), 3.38 (2H, dd, *J* 3.0, 9.0 Hz), 3.32 (6H, s), 3.27 (2H, br. pent, *J* 6.1 Hz), 2.57 – 2.53 (2H, m), 2.06 – 1.94 (14H, m), 1.66 – 1.16 (248H, m), 1.12 (6H, d, *J* 6.7 Hz), 0.94 (6H, d, *J* 6.7 Hz), 0.94 – 0.85 (24H, including t at 0.90, *J* 6.6 Hz, and s at 0.88), 0.16 (18H, s), 0.14 (18H, s), 0.13 (18H, s), 0.06 (12H, s); $\delta_C$ (101 MHz, $CDCl_3$): 173.8, 136.5, 129.9, 128.4, 94.8, 76.9, 73.5, 73.4, 72.8, 71.8, 70.7, 62.4, 55.9, 51.8, 37.3, 36.7, 36.4, 33.4, 32.6, 31.9, 29.8, 29.7, 29.6, 29.55, 29.5, 29.4, 29.1, 27.4, 27.2, 25.8, 25.5, 25.1, 22.7, 20.9, 19.0, 14.1, 1.09, 0.94, 0.15, – 4.5, – 4.6 ; $\nu_{max}/cm^{-1}$; 3667, 2922, 2852, 1739, 1463.

The second fraction, 6-*O*-(2*R*,3*R*,21*R*,22*E*,37*Z*,53*R*)-3-((*tert*-butyldimethylsilyl)oxy)-2-docosyl-53-methoxy-21-methyltetrapentaconta-22,37-dienoic-2,3,4,2',3',4'-hexakis-*O*-(trimethylsilyl)-α,α'-trehalose (**TMM**) was a thick oil (0.18 g, 28%), $[\alpha]_D^{25}$ +38 (*c* 0.92, $CHCl_3$) {Found $[M+Na]^+$: 2047.5918; $C_{114}H_{234}O_{14}Si_7Na$ requires: 2047.5901} which showed $\delta_H$ (400 MHz, $CDCl_3$): 5.40 – 5.29 (3H, m), 5.24 (1H, br. dd, *J* 7.4, 15.3 Hz), 4.91 (1H, d, *J* 3.0 Hz), 4.84 (1H, d, *J* 3.0 Hz), 4.35 (1H, dd, *J* 2.2, 11.8 Hz), 4.07 (1H, dd, *J* 4.2, 11.8 Hz), 3.98 (1H, dt, *J* 2.2, 6.0 Hz), 3.99 – 3.87 (3H, including td, *J* 5.8, 9.9 Hz), 3.84 (1H, dt, *J* 3.4, 6.6 Hz), 3.75 – 3.65 (2H, m), 3.48 (2H, br. td, *J* 4.4, 9.2 Hz), 3.42 (1H, dd, *J* 3.0, 9.2 Hz), 3.39 (1H, dd, *J* 3.0, 9.2 Hz), 3.32 (3H, s), 3.27 (1H, br. pent, *J* 6.0 Hz), 2.59 – 2.53 (1H, m), 2.10 – 1.92 (7H, m), 1.76 – 1.68 (1H, m), 1.56 – 1.17 (124H, m), 1.12 (3H, d, *J* 6.1 Hz), 0.94 (3H d, *J* 6.6 Hz), 0.91 – 0.86 (12H, including t at 0.90, *J* 6.0 Hz, and s at 0.87), 0.17 (9H, s), 0.16 (9H, s), 0.15 (9H, s), 0.14

(18H, s), 0.12 (9H, s), 0.06 (3H, s), 0.05 (3H, s); $\delta_C$ (101 MHz, $CDCl_3$): 174.1, 136.5, 129.9, 128.4, 94.5, 94.4, 76.9, 73.4, 73.3, 72.9, 72.8, 72.7, 71.9, 71.4, 70.7, 62.5, 61.7, 55.9, 51.8, 37.3, 36.7, 36.4, 33.4, 32.6, 31.9, 29.8, 29.78, 29.7, 29.6, 29.58, 29.5, 29.4, 29.3, 29.1, 28.1, 27.4, 27.2, 26.4, 25.8, 25.5, 24.8, 22.7, 20.9, 19.0, 14.1, 1.05, 1.00, 0.92, 0.84, 0.17, 0.04, – 4.5, – 4.7; $\nu_{max}/cm^{-1}$; 2923, 2853, 1733, 1463.

### 4.23 6,6'-*bis*-*O*-(2*R*,3*R*,21*R*,22*E*,37*Z*,53*R*)-3-Hydroxy-2-docosyl-53-methoxy-21-methyltetrapentaconta-22,37-dienoic-α,α'-trehalose (28)

(i) Tetrabutylammonium fluoride (TBAF) (0.41 mL, 0.41 mmol, 1.0 M) was added to a stirred solution of the above **TDM** (0.19 g, 0.058 mmol) in dry THF (10 mL) under nitrogen. The mixture was stirred for 1 h at rt, then evaporated to give a residue, which was purified by column chromatography, eluting with $CHCl_3$/MeOH (10:1) to give 6,6'-*bis*-*O*-(2*R*,3*R*,21*R*,22*E*,37*Z*,53*R*)-3-((*tert*-butyldimethylsilyl)oxy)-2-docosyl-53-methoxy-21-methyltetrapentaconta-22,37-dienoic-α,α'-trehalose as a colourless thick oil (0.14 g, 84%), $[\alpha]_D^{28}$ +13 (*c* 0.80, $CHCl_3$) {Found $[M+Na]^+$: 2865.6033; $C_{180}H_{350}O_{17}Si_2Na$ requires: 2865.6015} which showed $\delta_H$ (400 MHz, $CDCl_3$ + few drops of $CD_3OD$): 5.35 – 5.25 (6H, m), 5.19 (2H, dd, *J* 7.3, 15.4 Hz), 5.05 (2H, br. d, *J* 3.2 Hz), 4.33 (2H, br. dd, *J* 4.7, 12.0 Hz), 4.20 (2H, br. d, *J* 12.0 Hz), 3.98 – 3.85 (4H, m), 3.74 (2H, br. t, *J* 9.2 Hz), 3.44 (2H, dd, *J* 3.2, 9.2 Hz), 3.32 – 3.23 (10H, including s at 3.27 for the methoxy groups), 2.83 (6H, br. s for OH groups), 2.59 – 2.46 (2H, m), 2.05 – 1.86 (14H, m), 1.60 – 1.11 (254H, m), 1.08 (6H, d, *J* 6.1 Hz), 0.89 (6H, d, *J* 6.7 Hz), 0.85 – 0.75 (12H, including t at 0.84, *J* 6.0 Hz, and s at 0.81), 0.003 (9H, s), 0.02 (9H, s); $\delta_C$ (101MHz, $CDCl_3$ + few drops of $CD_3OD$): 175.2, 136.4, 129.8, 128.3, 93.4, 76.9, 73.2, 71.7, 70.5, 69.9, 62.9, 55.8, 51.6, 37.2, 36.6, 36.1, 33.6, 32.5, 31.8, 29.7, 29.62, 29.6, 29.5, 29.3, 27.7, 27.3, 27.1, 26.9, 25.8, 25.7, 25.4, 24.2, 22.6, 20.8, 18.9, 17.9, 14.0, – 4.6, – 4.9; $\nu_{max}/cm^{-1}$: 3383, 2920, 2851, 1739, 1463.

(ii) The above product (0.12 g, 0.042 mmol) was dissolved in dry THF (12 mL) in a small dry polyethylene vial equipped with a rubber septum, followed by addition of pyridine (0.1 mL) at r.t under nitrogen. The mixture was cooled to 5 °C, and then hydrogen fluoride-pyridine complex (~70%, 1.2 mL) was added dropwise. The mixture was stirred at 45 °C for 17 h, when the excess of the HF was neutralized with sat. aq. sodium hydrogen carbonate (10 mL), and the product was extracted with $CHCl_3$ (3 × 30 mL). The combined organic layers were dried and evaporated. Column chromatography eluting with $CHCl_3$/MeOH (15:1) gave a yellowish semi-solid, the title compound **(28)** (0.061 g, 56%), $[\alpha]_D^{21}$ +28.6 (*c* 1.35, $CHCl_3$) {Found $[M+Na]^+$: 2635.4224; $C_{168}H_{322}O_{17}Na$ requires: 2635.4238} which showed $\delta_H$ ($CDCl_3$ + few drops of $CD_3OD$): 5.44 – 5.28 (6H, m), 5.22 (2H, br. dd, *J* 7.4, 15.4 Hz), 4.99 (2H, br. d, *J* 3.3 Hz), 4.81 (2H, br. d, *J* 11.0 Hz), 4.35 (2H, br. t, *J* 9.8 Hz), 3.85 (2H, br. t, *J* 11.0 Hz), 3.75 (2H, br. t, *J* 9.2 Hz), 3.72 – 3.65 (2H, br. m), 3.52 (2H, dd, *J* 3.3, 9.8 Hz), 3.30 (6H, s), 3.26 (2H, br. pent, *J* 6.0 Hz), 3.19 (2H, br. t, *J* 9.2 Hz), 2.43 – 2.37 (2H, m), 2.04 – 1.92 (14H, m), 1.78 (8H, s ), 1.65 – 1.15 (248H, m), 1.10 (6H, d, *J* 6.1 Hz), 0.92 (6H, d, *J* 6.7 Hz),

0.86 (6H, t, *J* 6.5 Hz); $\delta_C$ (101 MHz, $CDCl_3$ + few drops of $CD_3OD$): 175.5, 136.4, 129.8, 128.3, 95.2, 77.0, 72.5, 72.3, 71.5, 71.1, 69.7, 64.6, 55.7, 52.1, 37.1, 36.6, 36.1, 34.6, 32.5, 31.8, 29.6, 29.5, 29.4, 29.36, 29.26, 29.2, 29.0, 27.3, 27.2, 27.1, 25.3, 25.1, 22.6, 20.8, 18.8, 13.9; $\nu_{max}/cm^{-1}$: 3371, 2917, 2849, 1721, 1466.

### 4.24 6-*O*-(2*R*,3*R*,21*R*,22*E*,37*Z*,53*R*)-3-hydroxy-2-docosyl-53-methoxy-21-methyltetrapentaconta-22, 37-dienoic-α,α'-trehalose (29)

(i) TBAF (0.45 mL, 0.45 mmol, 1.0 M) was added to a stirred solution of the above **TMM** (0.13 g, 0.064 mmol) in dry THF (6 mL) under nitrogen, stirred for 12 h, then evaporated; column chromatography, eluting with $CHCl_3$/MeOH (10:1) gave 6-*O*-(2*R*,3*R*,21*R*,22*E*,37*Z*, 53*R*)-3-((*tert*-butyldimethylsilyl)oxy)-2-docosyl-53-methoxy-21-methyltetrapentaconta-22,37-dienoic-α,α'-trehalose **(29)** as a colourless thick oil (0.080 g, 78%), $[\alpha]_D^{25}$ +26 (*c* 0.65, $CHCl_3$) {Found $[M+Na]^+$: 1614.3504; $C_{96}H_{186}O_{14}SiNa$ requires: 1614.3519} which showed $\delta_H$ (400 MHz, $CDCl_3$ + few drops of $CD_3OD$): 5.32 – 5.26 (3H, m), 5.18 (1H, br. dd, *J* 7.4, 15.4 Hz), 5.03 (2H, br. s), 4.30 – 4.20 (2H, m), 3.95 – 3.71 (6H, m), 3.68 – 3.60 (1H, m), 3.48 – 3.40 (2H, m), 3.34 – 3.20 (6H, including s at 3.27), 2.52 – 2.47 (1H, m), 2.02 – 1.87 (7H, m), 1.50 – 1.10 (131H, m), 1.06 (3H, d, *J* 6.1 Hz), 0.87 (3H, d, *J* 6.6 Hz), 0.82 (3H, t, *J* 6.3 Hz), 0.79 (9H, s), – 0.016 (3H, s), – 0.038 (3H, s); $\delta_C$ (101 MHz, $CDCl_3$ + few drops of $CD_3OD$): 175.1, 136.3, 130.2, 129.8, 128.3, 93.5, 93.4, 76.9, 73.1, 73.0, 72.6, 72.1, 71.5, 70.7, 70.2, 69.9, 62.7, 61.9, 55.7, 51.6, 37.1, 36.6, 36.0, 33.5, 32.4, 31.8, 29.7, 29.6, 29.56, 29.4, 29.39, 29.2, 29.17, 29.0, 27.6, 27.2, 27.1, 26.9, 25.6, 25.3, 24.1, 22.5, 20.8, 18.8, 13.9, – 4.7, – 5.1; $\nu_{max}/cm^{-1}$: 3352, 2919, 2850, 1733, 1465, 940.

(ii) The above product (0.075 g, 0.047 mmol) was dissolved in dry THF (10 mL) in a small dry polyethylene vial equipped with a rubber septum, followed by addition of pyridine (0.1 mL) at r.t under nitrogen. The mixture cooled to 5 °C, and then hydrogen fluoride-pyridine complex (~70%, 1.0 mL) was added dropwise. The mixture was stirred at 45 °C for 17 h, when the excess of HF was neutralized with sat. aq. $NaHCO_3$ (10 mL), and extracted with $CHCl_3$ (3 × 30 mL). The combined organic layers were dried and evaporated. Column chromatography eluting with $CHCl_3$/MeOH (15:1) gave a yellowish semi-solid, the title compound **(29)** (0.020 g, 29%), $[\alpha]_D^{25}$ + 43 (*c* 0.30, $CHCl_3$) {Found $[M+Na]^+$:1500.2639; $C_{90}H_{172}O_{14}Na$ requires: 1500.2646} which showed $\delta_H$ (400 MHz, $CDCl_3$ + few drops of $CD_3OD$): 5.35 – 5.24 (3H, m), 5.18 (1H, dd, *J* 7.3, 15.5 Hz), 5.05 (1H, d, *J* 2.9 Hz), 4.98 (1H, d, *J* 3.1 Hz), 4.66 (1H, br. d, *J* 10.4 Hz), 4.22 (1H, br. t, *J* 10.4 Hz), 3.93 (1H, br. t, *J* 10.3 Hz), 3.91 – 3.70 (4H, m), 3.65 – 3.50 (4H, m), 3.46 (1H, br. d, *J* 10.8 Hz), 3.25 – 3.15 (5H, including s at 3.2 for methoxy group), 2.40 – 2.32 (1H, m), 2.0 – 1.85 (7H, m), 1.60 – 1.10 (132H, m), 1.07 (3H, d, *J* 6.1 Hz), 0.86 (3H, d, *J* 6.7 Hz), 0.83 (3H, t, *J* 6.4 Hz); $\delta_C$ (101 MHz, $CDCl_3$ + few drops of $CD_3OD$): 175.5, 136.3, 129.8, 128.3, 94.8, 94.7, 77.1, 72.6, 72.5, 72.4, 72.3, 71.5, 71.2, 71.1, 70.9, 69.9, 64.4, 62.1, 55.7, 52.3, 37.1, 36.6, 36.1, 34.6, 32.5,

31.8, 29.6, 29.5, 29.4, 29.3, 29.23, 29.2, 29.0, 27.2, 27.17, 27.1, 25.3, 25.0, 22.6, 20.8, 18.8, 13.9; $\nu_{max}/cm^{-1}$: 3344, 2918, 1850, 1718, 1467.

### 4.25 6-*O*-(2*R*,3*R*,21*R*,22*E*,37*Z*,53*R*)-2-Docosyl-3-hydroxy-53-methoxy-21-methytetrapentaconta-22, 37-dienoate-β-D-glucopyranoside (32)

(i) (2*R*,3*R*,21*R*,22*E*,37*Z*,53*R*)-3-((*tert*-Butyldimethylsilyl)oxy)-2-docosyl-53-methoxy-21-methyltetrapentaconta-22,37-dienoic acid **(26)** (100 mg, 0.078 mmol) was dissolved in a mixture of THF and DMF (5:1, 3 mL) at rt, and then gently heated until all had dissolved. Dry cesium hydrogen carbonate (76.6 mg, 0.39 mmol) and benzyl-2,3,4-tri-*O*-benzyl-6-*O*-tosyl-β-D-glucopyranoside **(31)**[55] (82.1 mg, 0.011 mmol) were added. The mixture was stirred at 70 °C for 18 h, then the volatiles were evaporated. Chromatography, eluting with petrol/ethyl acetate (5:1) gave benzyl-2,3,4-tri-*O*-benzyl-6-*O*-(2*R*,3*R*,21*R*,22*E*,37*Z*,53*R*)-2-docosyl-3-hydroxy-53-methoxy-21-methytetrapentaconta-22,37-dienoate-β-D-glucopyranoside (0.085 g, 64%), $[\alpha]_D^{28}$ +13 (*c* 0.80, $CHCl_3$) {Found $[M+H]^+$: 1676.4192; $C_{112}H_{187}O_9$ requires: 1676.4175} which showed $\delta_H$ (400 MHz, $CDCl_3$): 7.40 – 7.28 (20H, m), 5.40 – 5.29 (3H, m), 5.24 (1H, br. dd, *J* 7.4, 15.3 Hz), 4.96 (1H, d, *J* 11.0 Hz), 4.95 (1H, d, *J* 11.0 Hz), 4.94 (1H, br. s), 4.90 (1H, d, *J* 12.0 Hz), 4.80 (1H, d, *J* 11.0 Hz), 4.71 (1H, d, *J* 11.0 Hz), 4.63 (1H, d, *J* 12.0 Hz), 4.60 (1H, d, *J* 12.0 Hz), 4.56 – 4.52 (2H, m), 4.22 (1H, dd, *J* 4.7, 12.0 Hz), 3.70 – 3.60 (2H, m), 3.56 – 3.48 (3H, m), 3.32 (3H, s), 3.27 (1H, br. pent, *J* 6.0 Hz), 2.53 – 2.43 (2H, including d, *J* 7.9 Hz, OH), 2.10 – 1.91 (7H, m), 1.80 – 1.67 (1H, m), 1.65 – 1.60 (1H, m), 1.59 – 1.43 (7H, m), 1.40 – 1.18 (115H, m), 1.12 (3H, d, *J* 6.1 Hz), 0.94 (3H, d, *J* 6.6 Hz), 0.89 (3H, t, *J* 6.8); $\delta_C$ (101 MHz, $CDCl_3$): 175.2, 138.4, 138.2, 137.7, 137.1, 136.4, 130.4, 129.9, 128.5, 128.4, 128.37, 128.3, 128.1, 128.0, 127.9, 127.85, 127.8, 127.6,102.3, 84.5, 82.3, 77.8, 76.9, 75.1, 74.9, 72.8, 72.3, 71.1, 62.8, 55.9, 51.3, 37.2, 36.7, 36.3, 35.6, 32.6, 31.9, 29.8, 29.7, 29.65, 29.6, 29.59, 29.5, 29.3, 29.4, 29.35, 29.3, 29.1, 27.5, 27.3, 27.2, 25.9, 25.4, 22.7, 20.9, 19.0, 14.1; $\nu_{max}/cm^{-1}$; 3664, 2921, 2851, 1735, 1454.

(ii) Liquid ammonia (50 mL) was condensed into a two neck flask (100 mL) under a liquid nitrogen/methylated spirit condenser protected by a soda lime guard tube. Sodium (~ 70 mg) was added until the blue colour of the solution persisted. The above product (80.0 mg, 0.04 mmol) in 1,4-dioxane (5 mL) was added and the mixture was stirred for 4-5 min until the blue colour disappeared. The reaction was quenched with $NH_4Cl$ (10 mL) and ether (30 mL) was added. The ammonia was allowed to evaporate, and the organic layer was separated and the aqueous layer re-extracted with ether (2 × 30 mL). The combined organic layers were dried and evaporated. Column chromatography on silica gel, eluting with $CHCl_3$/MeOH (10:1) gave the title compound **(32)** (0.014 g, 22%), $[\alpha]_D^{24}$ +12 (*c* 0.78 $CHCl_3$) {Found $[M+Na]^+$: 1338.2118; $C_{84}H_{162}O_9Na$ requires: 1338.2111} which showed $\delta_H$ (400 MHz, $CDCl_3$ + few drops of $CD_3OD$); (α, β isomers) in ratio 0.6:0.4: 5.35 – 5.25 (3H, m), 5.19 (1H, br. dd, *J* 7.2, 15.1 Hz), 5.12 (0.6H, br. d, *J* 3.8 Hz, $H_1$ –α), 4.47 (0.4H, d, *J* 7.7 Hz, $H_1$ – β), 4.42 (1H, br. dd, *J* 2.3, 11.7 Hz, $H_6$ –α, β), 4.26 (1H, br. dd, *J* 5.2, 11.7 Hz, $H_6$ –α, β), 3.95 (0.6H, br. ddd, *J* 3.0, 5.6, 7.0 Hz, $H_5$ –α), 3.67 – 3.59 (1.6H, m, $H_4$ –α + CH – OH mycolic

acid), 3.50 – 3.45 (0.4H, m, $H_5$ –β), 3.40 – 3.30 (2H, m, $H_{2,3}$ –α + $H_{3,4}$ –β), 3.31 – 3.23 (4H, including s at 3.27 for methoxy group), 3.18 (0.4H, t, *J* 7.6 Hz, $H_2$ –β), 2.42 – 2.34 (1H, m), 2.05 – 1.85 (7H, m), 1.70 – 1.12 (129H, m), 1.08 (3H, d, *J* 6.1 Hz), 0.90 (3H, d, *J* 6.7 Hz), 0.83 (3H, t, *J* 7.0 Hz); $\delta_C$(101 MHz, $CDCl_3$ + few drops of $CD_3OD$); (α, β isomers): 175.2, 136.4, 129.8, 128.3, 96.5 (C-1β), 92.2 (C-1α), 77.0, 76.0, 74.4, 73.6, 73.4, 72.5, 72.1, 70.5, 70.2, 69.2, 63.6, 63.4, 55.7, 52.7, 52.6, 37.1, 36.6, 36.1, 34.9, 32.5, 31.8, 29.6, 29.57, 29.5, 29.3, 29.24, 29.2, 29.1, 29.09, 29.06, 29.0, 27.3, 27.25, 27.1, 25.3, 22.6, 20.8, 18.8, 14.0; $\nu_{max}/cm^{-1}$; 3344, 2917, 2849, 1719, 1466.

### 4.26 Methyl-5-*O*-[(2*R*,3*R*,21*R*,22*E*,37*Z*,53*R*)-2-docosyl-3-hydroxy-53-methoxy-21-methyltetrapentaconta-22,37-dienoate]-α-D-arabinofuranoside (35)

(i) Dry cesium hydrogen carbonate (65.0 mg, 0.33 mmol) was added to a stirred solution of arabinofuranoside (**34**)[56] (50.0 mg, 0.10 mmol) and acid **(26)** (0.085 g, 0.067 mmol) in dry THF and DMF (5:1, 3 mL) at rt. The mixture was brought to 70 °C and stirred at this temperature for 18 h, then the volatiles were evaporated. Column chromatography, eluting with petrol/ethyl acetate (5:1) gave methyl 2,3-di-*O*-benzyl-5-*O*-[(2*R*,3*R*,21*R*,22*E*,37*Z*,53*R*)-2-docosyl-3-hydroxy-53-methoxy-21-methyltetrapenta-conta-22,37-dienoate]-α-D-arabinofuranoside (0.056 g, 57%), $[\alpha]_D^{24}$ +17 (*c* 0.75, $CHCl_3$) {Found $[M+Na]^+$: 1503.3155; $C_{98}H_{174}O_8Na$ requires: 1503.3135} which showed $\delta_H$ (400 MHz, $CDCl_3$) 7.39 – 7.28 (10H, m), 5.40 – 5.29 (3H, m), 5.24 (1H, br. dd, *J* 7.4, 15.2 Hz), 4.92 (1H, br. s), 4.57 (1H, d, *J* 12.0 Hz), 4.56 (1H, d, *J* 12.0 Hz), 4.49 (1H, d, *J* 11.8 Hz), 4.47 (1H, d, *J* 11.8 Hz), 4.30 (1H, br. d *J* 12.0. Hz), 4.28 (1H, br. d, *J* 12.0), 4.21 (1H, br. td, *J* 4.6, 6.4 Hz), 3.99 (1H, dd, *J* 0.6, 2.4 Hz), 3.84 (1H, dd, *J* 2.7, 6.7 Hz), 3.67 – 3.59 (1H, m), 3.37 (3H, s), 3.32 (3H, s), 3.27 (1H, br. pent, *J* 6.2 Hz), 2.51 (1H, d, *J* 8.2 Hz), 2.43 (1H, dt, *J* 5.9, 9.1 Hz), 2.10 – 1.91 (7H, m), 1.75 – 1.60 (1H, m), 1.59 – 1.15 (123 H, m), 1.12 (3H, d, *J* 6.4 Hz), 0.94 (3H, d, *J* 6.7 Hz), 0.88 (3H, t, *J* 6.5 Hz); $\delta_C$ (101 MHz, $CDCl_3$): 175.0, 137.4, 137.3, 136.4, 129.9, 128.5, 128.45, 128.4, 127.9, 127.93, 127.91, 127.8, 107.2, 87.9, 83.7, 79.4, 76.9, 72.4, 72.1, 72.0, 63.5, 55.9, 54.9, 51.5, 37.2, 36.7, 36.3, 35.5, 32.6, 31.9, 29.8, 29.7, 29.6, 29.56, 29.5, 29.4, 29.3, 27.4, 27.3, 27.2, 25.7, 25.4, 22.7, 20.9, 19.0, 14.1; $\nu_{max}/cm^{-1}$; 3658, 2920, 2851, 1734, 1455.

(ii) Liquid ammonia (50 mL) was condensed into a two neck flask (100 mL) under a liquid nitrogen/methylated spirit condenser protected by a soda lime guard tube. Sodium (~70 mg) was added until the blue colour persisted. The above product (0.052 g, 0.035 mmol) in 1,4-dioxane (5 mL) was added and the mixture was stirred for 4-5 min until the blue colour disappeared. The reaction was quenched with sat.aq. $NH_4Cl$ (5 mL) and ether (20 mL). The ammonia was allowed to evaporate and the organic layer was separated and the aqueous layer re-extracted with ether (2 × 30 mL). The combined organic layers were dried and evaporated. Column chromatography, eluting with $CHCl_3$/MeOH (20:1) gave the title compound **(35)** (0.0145 g, 32%) $[\alpha]_D^{21}$ +15 (*c* 0.89, $CHCl_3$) {Found $[M+Na]^+$: 1322.2173; $C_{84}H_{162}O_8Na$ requires:1322.2162} which showed $\delta_H$ (400 MHz, $CDCl_3$ + few drops of $CD_3OD$): 5.36 – 5.25 (3H, m), 5.18 (1H, br. dd, *J* 7.4, 15.2 Hz), 4.78 (1H, br. s), 4.32 (1H, dd, *J* 4.5, 11.7 Hz), 4.28 (1H, dd, *J* 5.0, 11.7 Hz), 4.07 (1H, br. q, *J* 4.8 Hz), 3.96 (1H, br. s), 3.84 (1H, br. q, *J* 2.8 Hz), 3.65 – 3.52 (1H, m), 3.34 (3H, s), 3.28 – 3.20 (4H, including s at 3.27 for methoxy group),

2.43 – 2.34 (1H, m), 2.02 – 1.89 ( 7H, m), 1.68 – 1.11 (127H, m), 1.08 (3H, d, *J* 6.1 Hz), 0.88 (3H, d, *J* 6.7 Hz), 0.83 (3H, t, *J* 7.1 Hz); $\delta_C$ (101 MHz, $CDCl_3$ + few drops of $CD_3OD$): 175.1, 136.4, 129.8, 128.3, 108.8, 81.8, 81.2, 77.9, 77.0, 72.4, 63.4, 55.7, 54.9, 52.6, 37.1, 36.6, 36.1, 34.9, 32.5, 31.8, 29.8, 29.6, 29.5, 29.4, 29.3, 29.2, 29.19, 29.14, 29.1. 29.05, 29.0, 27.3, 27.2, 27.1, 25.3, 22.6, 20.8, 18.8, 14.0; $\nu_{max}/cm^{-1}$; 3458, 2917, 2850, 1721, 1466.

**4.27 Oxidative cleavage of diene mycolates**

A solution of methyl α'-mycolates (3.2, fraction 1, 8 mg), in benzene (1.5 mL) was added to a mixture of *t*-butanol (18 mL), aq. sodium carbonate (0.02 M, 5 mL), distilled water (2.4 mL) and sodium periodate (164 mg), potassium permanganate (20 mg) in water (10.8 mL). The mixture was stirred at rt for 64 h, acidified with 2M aq. HCl, then extracted with diethyl ether (3 × 30 mL). The combined organic layers were dried and evaporated. The crude extract was methylated with diazomethane and columned with petrol / ethyl acetate (10:1) to give a first fraction (10 mg) which showed $\delta_H$ (400 MHz; $CDCl_3$): 3.67 (3H, s), 2.43 (1H, sextet, *J* 7.2 Hz), 2.30 (2H, t, *J* 7.5 Hz), 1.62 (2H, pentet, *J* 6.5 Hz), 1.45 – 1.25 (30H, m), 1.40 (3H, d, *J* 7.0 Hz), 0.89 (3H, t, *J* 7.0 Hz); $\delta_C$ (101 MHz; $CDCl_3$): 51.4, 39.5, 34.1, 33.82, 31.9, 30.3, 29.7, 29.66, 29.65, 29.64, 29.6, 29.5, 29.48, 29.4, 29.3, 29.1, 27.2, 24.96, 22.68, 17.1, 14.1 (**S4F1**).

The second fraction (18 mg) which showed $\delta_H$ (400 MHz; $CDCl_3$): 3.72 (3H, s), 3.67 (3H, s), 3.66 – 3.63 (1H, m), 2.50 – 2.40 (1H, m), 2.40 (1H, br. d, *J* 8.2 Hz), 2.30 (2H, t, *J* 7.5 Hz), 1.75 – 1.60 (3H, m), 1.50 – 1.35 (4H, m), 1.33 – 1.20 (69H, m), 1.14 (3H, d, *J* 7.0 Hz), 0.89 (3H, t, *J* 6.7 Hz); $\delta_C$ (101 MHz; $CDCl_3$): 176.2, 72.3, 51.5, 51.4, 50.9, 39.4, 35.7, 34.1, 33.8, 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 29.25, 29.1, 27.4, 27.2, 25.7, 24.9, 22.7, 17.05, 14.1 (**S4F3**).

The third fraction (12 mg) which showed $\delta_H$ (400 MHz; $CDCl_3$): 3.67 (6H, s), 2.30 (4H, t, *J* 7.5 Hz), 1.64 – 1.57 (10H, m), 1.28 – 1.26 (26H, m); $\delta_C$ (101 MHz; $CDCl_3$): 174.3, 51.5, 34.13, 30.9, 29.7, 29.63, 29.6, 29.57, 29.54, 29.4, 29.37, 29.3, 29.2, 28.3, 25.7, 25.0, 22.7 (**S4F2**).

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