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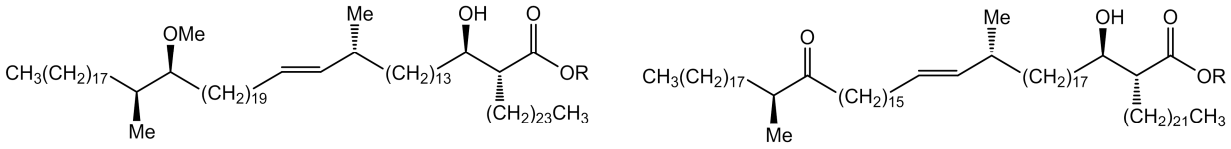

R = H, Me, sugar, glycerol

The synthesis of single enantiomers of *trans*-alkene containing mycolic acids and related sugar esters

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Abstract— Routes to single enantiomers of hydroxy, methoxy and ketomycolic acids containing an α–methyl-*trans*-alkene unit as well as related trehalose, glucose, arabinose and glycerol esters are described. In serodiagnostic assays to detect antibodies to mycobacterial lipids, the trehalose esters give higher responses to the serum of patients with culture positive tuberculosis than to that of culture negative patients.

Key words: Mycolic acid, TDM, TMM, GMM, *trans*-alkene, trehalose monomycolate, trehalose dimycolate

1. Introduction

Mycolic acids (MAs) are characteristic components of the cell envelopes of mycobacteria, often covalently bound to arabinogalactan as a penta-arabinose tetramycolate.[1-3] They are also seen as trehalose dimycolate (TDM – a potent signalling agent in the immune system),[4,5] trehalose monomycolate (TMM)[6] and other sugar esters that are not bound to the cell wall,[7-9] or as free mycolic acids.[10] MAs comprise two elements (**Figure 1**), the β-hydroxy-acid, for which d is generally 21 or 23, and the meromycolate. The latter is a long chain usually containing two groups X and Y.

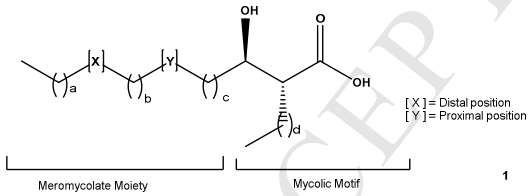
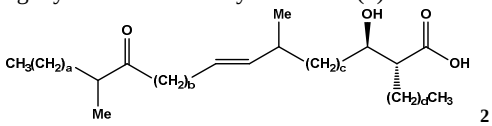


Figure 1: Typical mycolic acid structure

In the most common classes of mycobacterial MA, the two groups are both *cis*-cyclopropanes (α-MA), or group X is an α–methyl-β-methoxy or α–methyl-β-keto group (methoxy and keto-MA respectively). MAs are generally present as complex mixtures containing several different chain lengths of each class, and the detailed composition is dependent on the mycobacterium.[11,12] The presence and proportion of individual classes of MA is important for the virulence of diseases such as tuberculosis.[13,14] The MAs are themselves strongly bioactive and indeed individual synthetic MAs of different classes matching the structures of components of natural mixtures are selectively active.[15] In addition, there are a range of generally less abundant MAs in mycobacteria containing other X and Y groups; these include molecules containing an α-methyl-*trans*-alkene unit.[11,12] Keto-mycolic acids **2** with a proximal *trans*-alkene substituent and a variety of chain lengths have been reported (**Table 1**).[11, 12,16-18] In these cases, the methyl group adjacent to the alkene is on the proximal side relative to the hydroxy-acid. The methyl group in the X position of keto- and methoxy-MA and at the Y-position in α–methyl-*trans*-cyclopropane containing MA, is distal from it.[11,12]

Table 1. Typical chain lengths of major β-methyl-*trans*-alkene containing mycobacterial ketomycolic acids (**2**)



Species	a	b	c	d	Ref
Mycobacterium tuberculosis, Mycobacterium bovis, M. bovis BCG, Mycobacterium microti	17	19	15	23	11
M. tuberculosis, M. bovis, M. bovis BCG, M. microti	17	19	13	23	11
M. tuberculosis Canetti	17	17	17	23	11
Mycobacterium avium complex (MAC)	17	17	17	21	11
Mycobacterium marinum	17	15	17	21	11
Mycobacterium scrofulaceum	15	17	17	21	11
M. aurum	15	13	19	19	19
M. aurum	15	13	17	19	19

A derivative of a hydroxy-MA with a proximal α–methyl-*trans*-alkene has also been reported.[16] *Mycobacterium smegmatis* synthesizes keto-MAs containing about 33% of a *trans*-alkene. The same percentage is seen in the

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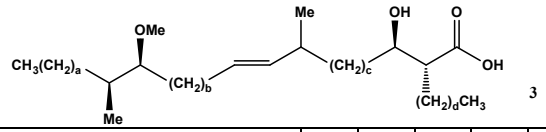
hydroxy-mycolate.[20] In addition, related methoxy-MA **3** have been reported as in **Table 2**.[11]

Table 2. Typical chain lengths of major β-methyl-*trans*-alkene containing mycobacterial methoxymycolic acids (**3**)[11]



3

Species	a	b	c	d	
M. tuberculosis, *M. bovis*, *M. bovis BCG*	17	19	13	23	
M. tuberculosis Canetti	17	19	15	23	
M. microti	17	15	17	21	

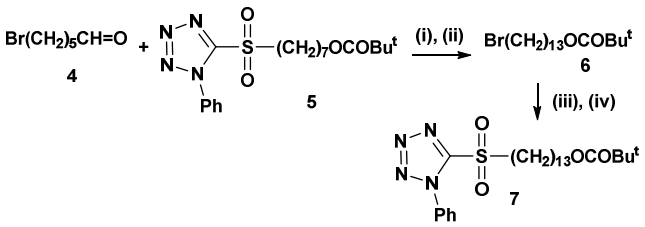
The biosynthesis of these and other MA has been studied extensively.[21-27] Labelling studies show the methyl branch of *trans*-MA from *M. tuberculosis* are exclusively on carbons derived from the 2-position of acetate, while those from *M. smegmatis*, are exclusively derived from the 1-position.[28]

Another type of mycolic acid, isolated from *M. smegmatis* and *Mycobacterium aurum*,[29] *Mycobacterium chelonei*,[30] and *Mycobacterium fortuitum*,[31] contains an α–methyl-*trans*-alkene at the proximal position and a *cis*-alkene at the distal position.[32-34] In the latter case, the specific rotation of its methyl ester has been reported to be +1.4 ($CHCl_3$),[35] while that of the acetoxy methyl ester is reported as +3 ($CHCl_3$), and that of a deacetoxy derivative of a mycolate containing only an α–methyl-*trans*-alkene chiral centre corresponds to a molecular rotation (M_D) of -19.5.[35] In addition, the specific rotation of a wax ester containing this unit has been reported to be +4.3 ($CHCl_3$).[29,36,37] This allows the contribution to the molecular rotation from the α–methyl-*trans*-alkene to be calculated, as the only other chiral centres in these cases are at the hydroxy-acid position, the contribution of which to the molecular rotation is known (+40°). This leads to a value of -25° for the α–methyl-*trans*-alkene (for 30% of methyl branched molecules); this in turn suggests this sub-unit has an (*R*)-stereochemistry, based on model compounds.[18]

As part of a study to determine the biological significance of specific MA structures,[38-44] and to confirm regio- and stereochemistry, we now report the synthesis of hydroxy-MA, keto-MA and methoxy-MA containing a α–methyl-*trans*-alkene unit. Four sugar esters and one glycerol ester were prepared from one methoxy-MA, to provide the opportunity for a systematic comparison of their effects on cytokines and chemokines, as well as their application as antigens in the serodiagnosis of tuberculosis.

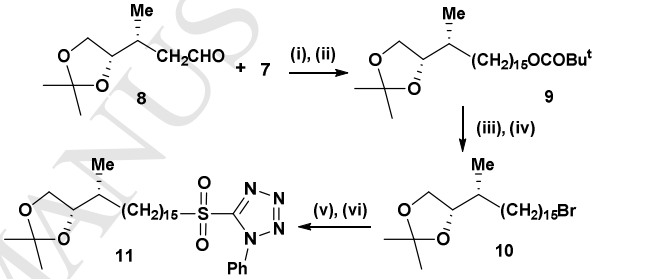
2. Results and discussion

As the first step in the synthesis of alkene-MA **23**, 6-bromohexanal (**4**) was chain extended to the ester **6** and then converted into the sulfone **7** (**Scheme 1**):



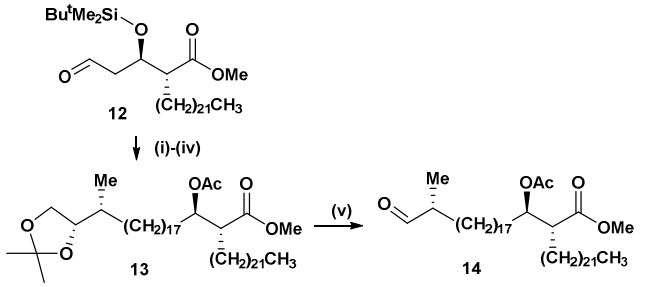
Scheme 1: (i) LiHMDS, THF (71%); (ii) H_2, Pd/C, EtOAc/EtOH (88%); (iii) 1-phenyl-1*H*-tetrazole-5-thiol, K_2CO_3, acetone (86%); (iv) H_2O_2, $(NH_4)_6Mo_7O_{24}.4H_2O$, THF/IMS (95%)

In order to fix the (*R*)-stereochemistry of the α-methyl-*trans*-alkene fragment, aldehyde **8**,[39, 45-47] was chain-extended with compound **7** and base, using a modified Julia–Kocienski reaction,[48-50] followed by hydrogenation of the derived alkenes to give **9**. The pivaloate group was removed and the primary alcohol was converted into sulfone **11** (**Scheme 2**) via the bromide **10**.



Scheme 2: (i) LiHMDS, THF (86%); (ii) H_2, Pd/C, EtOAc/EtOH (96%); (iii) KOH, THF, MeOH, H_2O (97%); (iv) *N*-bromosuccinimide, PPh_3, $NaHCO_3$, CH_2Cl_2 (92%); (v) 1-phenyl-1*H*-tetrazole-5-thiol, K_2CO_3, acetone (95%); (vi) H_2O_2, $(NH_4)_6Mo_7O_{24}.4H_2O$, THF/IMS (81%)

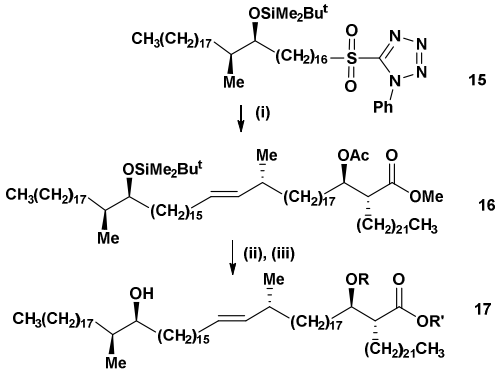
A modified Julia reaction between the aldehyde **12**,[51,66] and sulfone **11** gave a mixture of alkenes which was hydrogenated. The silyl ether was changed to an acetyl group as in **13**, in order to avoid the presence of two identical protecting groups at a later stage, and the acetal group was converted into aldehyde **14** with periodic acid (**Scheme 3**).



Scheme 3: (i) **11**, LiHMDS, THF (86%); (ii) Pd/C, EtOAc (96%); (iii) HF.pyridine, pyridine (83%); (iv) Ac_2O, pyridine (94%); (v) periodic acid (80%)

The modified Julia–Kocienski reaction using a 1-phenyl-1*H*-tetrazole sulfone and an aldehyde with potassium bis-(trimethylsilyl)amide in 1,2-dimethoxyethane is known to lead to an *E*-alkene with good stereoselectivity, especially if the sulfone or aldehyde is α-substituted.[52-54] Reaction of the sulfone **15,**[60] with aldehyde **14** gave the *trans*-alkene **16** (**Scheme 5**). It is essential that no epimerization occurs adjacent to the aldehyde during this process. There is

considerable precedent for the retention of the chirality.[53, 56-59] Removal of the silyl group gave **17** (R= Ac, R' = Me) and hydrolysis of the two esters produced the free hydroxy acid **17** (R= H, R' = H). The $[\alpha]^{21}_{D}$ of this, -2.07 ($CHCl_3$, 0.743 µmol), corresponding to M_D -26°, is in agreement with that reported for the hydroxymycolates of *M. smegmatis* (M_D -16°, though it must be noted these only contain ~30% *trans*-alkene).[18]



Scheme 4: (i) **14**, KHMDS, 1,2-dimethoxyethane (34%); (ii) HF.pyridine, THF (**17** (R= Ac, R' = Me), 91%); (iii) LiOH, MeOH, THF, H_2O (**17** (R= H, R' = H), 65%)

In the same way the enantiomer of **15**, compound **18**,[60] was converted into **19** and free hydroxy-MA **20** (**Figure 1**). The ^{1}H NMR spectra of each of these in the alkene region was identical to that reported in the literature,[20,24] and to minor signals in fractions of α-mycolates derived from *M. avium*.[55]

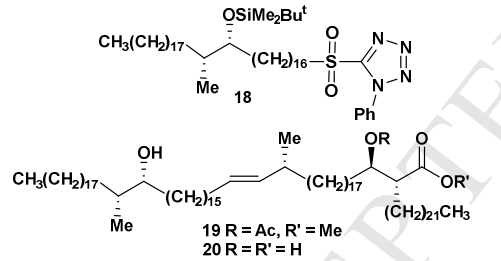
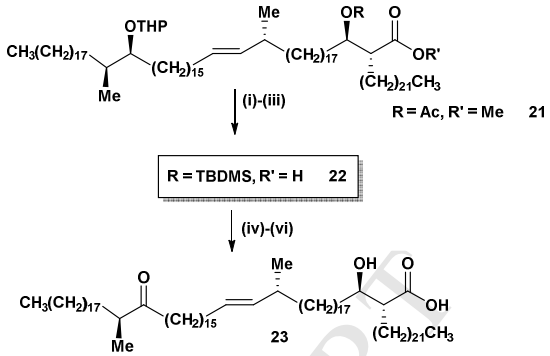


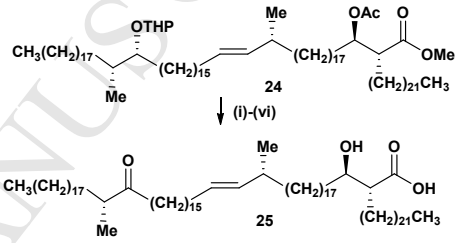
Figure 1

Oxidation of either **17** (R= Ac, R' = Me) or **19** led to the corresponding protected keto-MAs. However, attempted deprotection of these using LiOH led to epimerization at the position adjacent to the ketone. In order to avoid this, the alcohol **17** (R= Ac, R' = Me) was first protected as THP-ether **21**, followed by hydrolysis of the esters and then reprotection at the alcohol group as a silyl ether **22**. Removal of the THP-group, oxidation, and then deprotection now proceeded without epimerization,[60] leading to the free acid **23** (**Scheme 4**), matching the overall structure of the major *trans*-alkene keto-mycolate reported for *M. marinum*.[11]



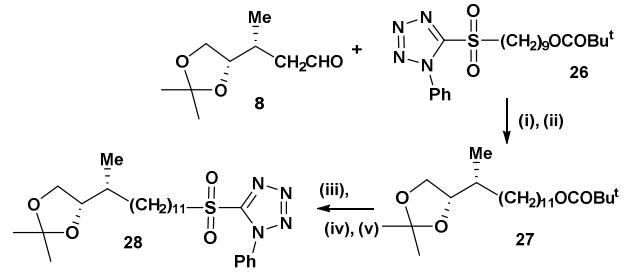
Scheme 4: (i) LiOH, MeOH, H_2O, THF (72%); (ii) TBDMSCl, imidazole, DMF; (iii) K_2CO_3, MeOH/H_2O, then $KHSO_4$, (70%); (iv) PPTS, MeOH, H_2O, THF (73%); (v) PCC (89%); (vi) HF.pyridine (44%)

In the same way compound **19** was first protected with a tetrahydropyranyl group to give **24** and then converted into the keto-MA **25** ($[\alpha]^{23}_{D}$ +1.58 ($CHCl_3$, 0.436 µmol)) **(Scheme 5)**:



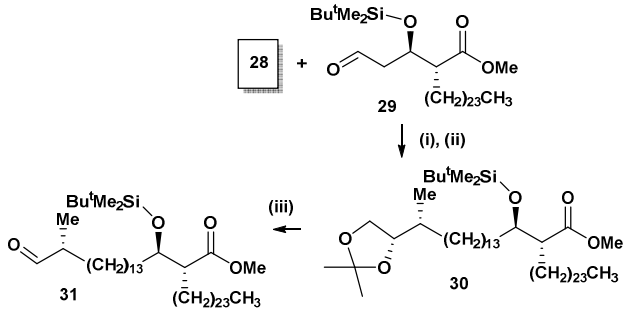
Scheme 5: (i) LiOH, MeOH, H_2O, THF (72%); (ii) TBDMSCl, imidazole, DMF; (iii) K_2CO_3, MeOH/H_2O, then $KHSO_4$, (70%); (iv) PPTS, MeOH, H_2O, THF (73%); (v) PCC (89%); (vi) HF.pyridine (44%)

The chain lengths reported for major *trans*-alkene-methoxy-MA are somewhat different from the keto-analogues. In order to prepare the methoxy-MA **3** (a = 17, b = 19, c = 13, d = 23), aldehyde **8** was treated with **26** and base, followed by hydrogenation to produce **27**, which was in turn converted into sulfone **28** by standard methods (**Scheme 6**):



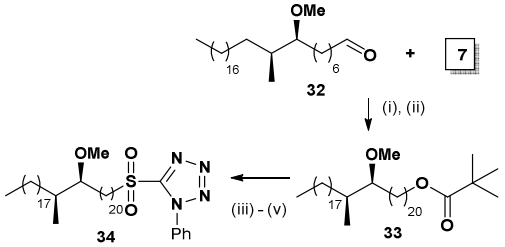
Scheme 6: (i) LiHMDS, THF (76 %); (ii) Pd/C,H_2, EtOAc (92 %); (iii) $LiAlH_4$, THF (81 %); (iv) diethyl azodicarboxylate, Ph_3P, 1-phenyl-1H-tetrazole-5-thiol (84 %); (v) $(NH_4)_6Mo_7O_{24}.4H_2O$, H_2O_2, THF/IMS (84 %).

A further modified Julia reaction on the sulfone **28** and aldehyde **29**,[43] again followed by hydrogenation, then reaction with periodic acid led to aldehyde **31** (**Scheme 7**):



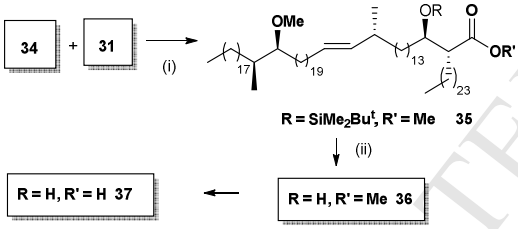
Scheme 7: (i) LiHMDS, THF (96 %); (ii) Pd/C,H_2 (96 %); (iii) periodic acid (87 %).

The *S*-methoxy-*S*-methyl substituted aldehyde **32**,[39] was chain extended to sulfone **34**, again using standard procedures (**Scheme 8**):



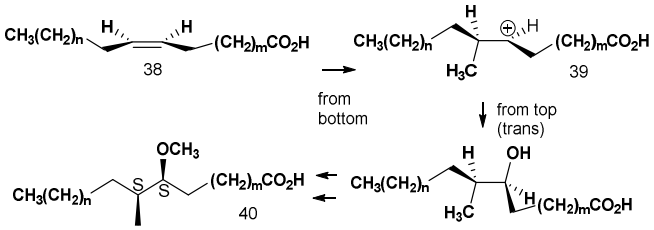
Scheme 8: (i) LiHMDS, THF, (85 %); (ii) Pd/C,H_2, (100 %); (iii) $LiAlH_4$, THF (99 %); (iv) diethyl azodicarboxylate, Ph_3P, 1-phenyl-1H-tetrazole-5-thiol (91%); (v) $(NH_4)_6Mo_7O_{24}$-$4H_2O$, H_2O_2 (35%), THF/IMS (97 %).

A modified Julia reaction between sulfone **34** and aldehyde **31** led, after deprotection, to the final MA, **37**.(**Scheme 8**)



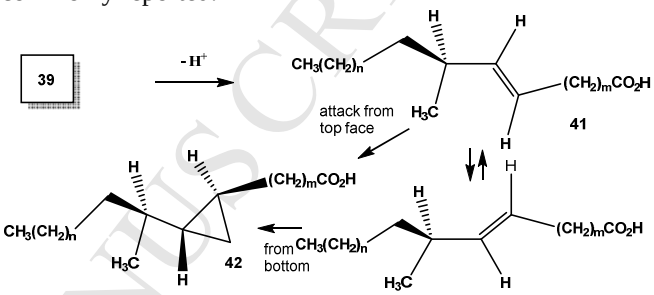
Scheme 8: (i) KHMDS, 1,2-dimethoxyethane (37 %); (ii) HF-pyridene, THF (96 %); (iii) LiOH, MeOH, H_2O, THF (82 %).

These results provide the first synthetic approaches to keto-, hydroxy- and methoxy-MAs containing a proximal (*R*)-α–methyl-*trans*-alkene unit, and a comparison of molecular rotations with those in the literature confirms the stereochemistry of this subunit. It has been proposed that MA biosynthesis involves alkylation of a *cis*-alkene by SAM (S-adenosylmethionine) to provides an intermediate cation leading to each of the other MA functionalities.[61] Although the actual mechanism may be different,[27] this provides a model by which to analyse the stereochemistry of the various classes of MA. Thus, using **38** as a model precursor, formal addition of a methyl cation to the distal carbon of the alkene from the bottom face would produce cation **39**; trapping from the top face could lead eventually to the known (*S*,*S*)-stereochemistry of **40** at the distal position. (**Scheme 9**). [16, 21]



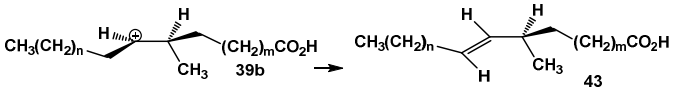
Scheme 9: Formation of S,S-methoxy-MA fragment

Elimination of a proton from **39** would lead to **41**, and in a second step to the proximal α–methyl-*trans*-cyclopropane unit **42** (**Scheme 10**). [21-26, 39] In this case, it appears that rapid cyclopropanation occurs, as the alkenes **41** are not commonly reported.



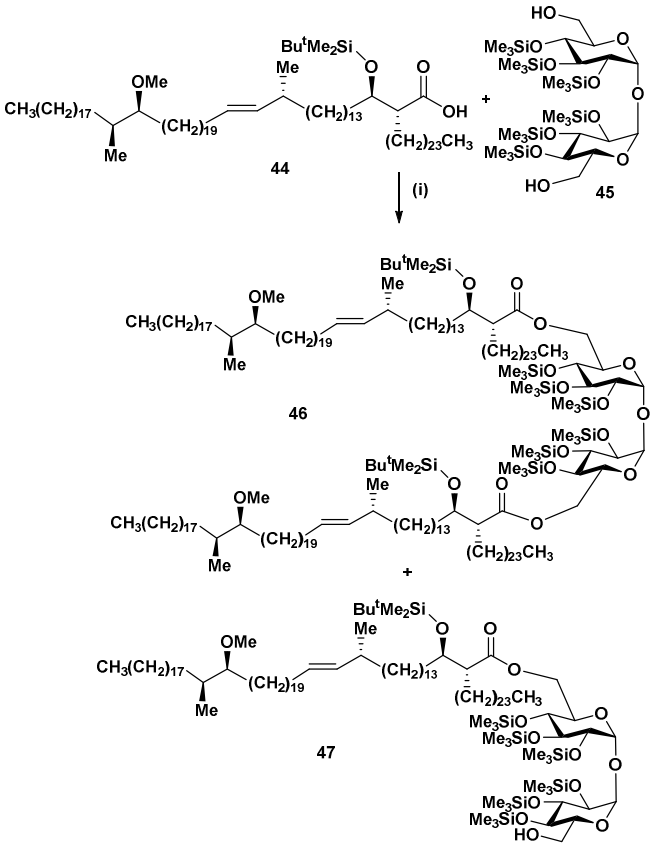
Scheme 10: Formation of *trans*-cyclopropane

In applying this to the stereochemistry of *trans*-alkene mycolates, one possibility is that alkylation of the *cis*-alkene **38**, again occurs from the bottom face, this time to the proximal carbon leading formally to **39b**; elimination of a proton would then lead to the (*R*)-α–methylalkene **43** (**Scheme 11**). In this case, it appears that cyclopropanation of the alkene does not occur, as no such regioisomers have been reported.



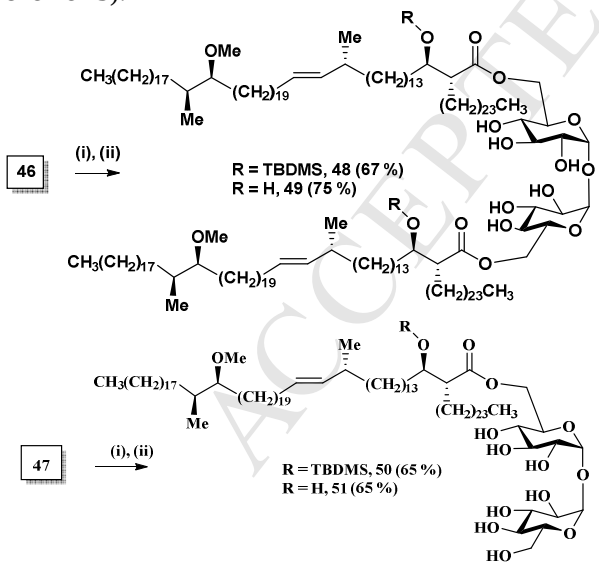
Scheme 11: Proposed stereochemistry of methylation by SAM

The biological activity of mycolic acids is often seen most strongly in their sugar esters, such as glucose mycolate (GMM), TDM and TMM.[3] In natural isolates it is difficult to identify within the complex mixtures whether particular sub-classes or chain lengths are selectively responsible for these properties. To try to understand whether *trans*-alkene mycolic acids have specific biological properties, a series of sugar esters of the methoxy-MA **37** was prepared. The acid was first reprotected at the β-hydroxy group with TBDMS to give **44** and then coupled to the protected trehalose **45**:[62]



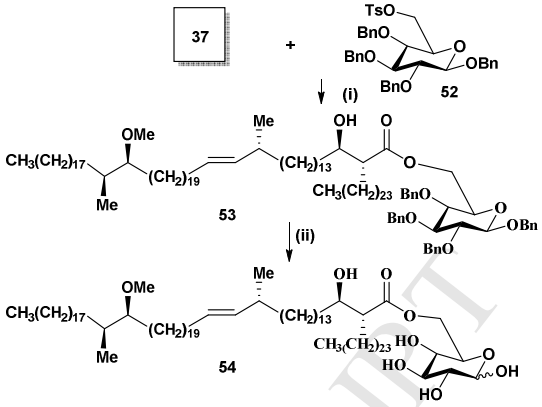
Scheme 12: (i) EDCl, 4-DMAP, CH_2Cl_2, 4 °A MS, rt, 6 days; **46** (25%), **47** (50%).

The protecting groups were then removed in two steps (**Scheme 13**):



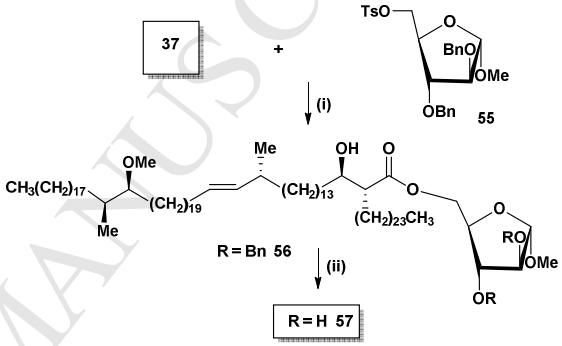
Scheme 13: (i) TBAF, THF, 5 °C 1h; (ii) Pyridine, THF, HF-pyridine complex, 43 °C, 17 h, then neutralised with aq. $NaHCO_3$

The acid **37** was also coupled to protected glucose **52** to give, after debenzyation, the GMM **54**. (**Scheme 14**)



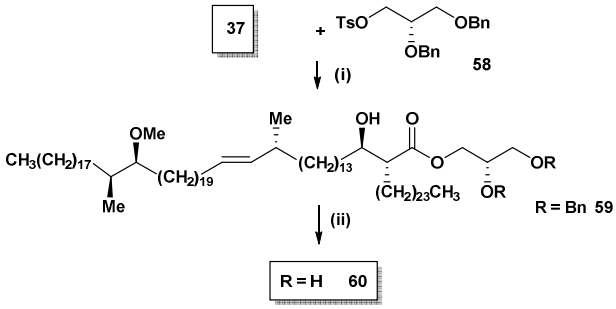
Scheme 14: (i) $CsHCO_3$, DMF:THF, 70 %, (ii) Na/NH_3, 47 %.

It was also coupled to the protected arabinose **55**. (**Scheme 15**)



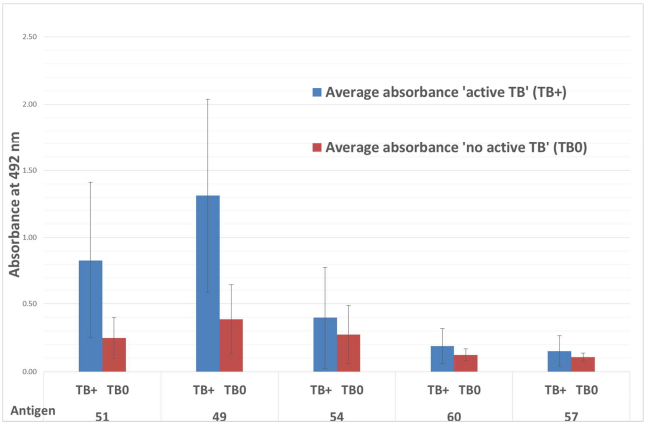
Scheme 15: (i) $CsHCO_3$, DMF:THF, 71 %, (ii) Na/NH_3, 57 %.

As a final comparison, the glycerol ester (GroMM) **60** was prepared. (**Scheme 16**).



Scheme 16: (i) $CsHCO_3$, DMF:THF, 89 %, (ii) Na/NH_3, 40 %.

Like sugar esters of other synthetic alpha-, keto- and methoxy-mycolic acids, the sugar esters of *trans*-alkene methoxy described above show strong effects on a range of cytokines and chemokines.[63] Such sugar esters are also antigenic to antibodies in serum of patients infected with active TB.[64] The *trans*-alkene sugar esters described above were each evaluated as antigens in ELISA for responses to antibodies in serum of patients infected with pulmonary tuberculosis, using modified IgG(Fc) as secondary antibody to provide a colour response. Twenty samples from a WHO Specimen Bank were used, 9 culture and smear positive for TB, 11 smear and culture negative and diagnosed as not having TB. The samples were all from patients showing symptoms of suspected TB.[65] The results are presented in **Table 1s** (Supplementary Information) and the average responses are given in **Graph 1**.



Graph 1: Average responses and standard deviations for 9 serum samples from pulmonary TB, culture and smear positive patients (TB+) and 11 from culture and smear negative patients (TB0) from the WHO TDR TB specimen Bank,[65] clinically diagnosed using standard protocols in ELISA assay using IgG(Fc) secondary antibody

Although the standard deviations within this small sample set are large, these results indicate that the synthetic alkene containing TDM **49** and TMM **51** may offer potential in the diagnosis of active TB. It is interesting that, for this series of compounds, the GMM **54**, GroMM **60** and ArMM **57** do not show significant distinction between pulmonary TB positive or negative samples. An extended study using a large serum set is now under way to evaluate these molecules compared to other synthetic sugar mycolates as antigens in diagnosis.

3. Experimental section

3.1 Chemicals were obtained from commercial suppliers (Sigma, Aldrich, and Alfa Aeser) 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 CH_2Cl_2 was dried over calcium hydride. All reagents and solvents used were of reagent grade unless otherwise stated. Silica gel (Merck 7736) and silica gel plates used for column chromatography and thin layer chromatography were obtained from Aldrich; separated components were detected using variously UV light, I_2 and phosphomolybdic acid solution in IMS followed by charring. Anhydrous magnesium sulfate was used to dry organic solutions. Infra-red (IR) spectra were carried out on a Perkin-Elmer 1600 F.T.I.R. spectrometer as liquid films or KBr disc (solid). Melting points were measured using a Gallenkamp melting point apparatus. NMR spectra were carried out on a Bruker Avance 400 or 500 spectrometer. Specific rotations were recorded in $CHCl_3$ on a POLAAR 2001 Optical Activity Polarimeter. Mass spectra were recorded on a Bruker matrix-assisted laser desorption/ ionisation-time of flight mass spectrometry (MALDI-TOF MS) values are given plus sodium to an accuracy of 1 d.p.; accurate mass values were run on a Bruker LC-MS or on MALDI-TOF MS in Bristol University.

The detailed procedures for the preparation of compounds **7** – **11** are provided in the Supplementary Information.

Serum samples, provided by the World Health Organisation from the TDR TB Specimen Bank with the necessary ethical approval,[65] were all from individuals with symptoms of suspected tuberculosis.

3.2 (*R*)-2-[(1*R*,19*R*)-1-(*tert*-Butyldimethylsilanyloxy)-19 (*R*)-2-[(1*R*,19*R*)-1-acetoxy-19-((*S*)-2,2-dimethyl[1,3]di-oxolan-4-yl)-eicosyl]tetracosanoic acid methyl ester 13

(i) LiHMDS (8.50 mL, 8.76 mmol, 1.06 M) was added to a stirred solution of ester **12** (2.62 g, 4.61 mmol)[66] and tetrazole **11** (2.78 g, 5.07 mmol) in dry THF (50 mL) at –5 °C under nitrogen. The mixture was allowed to reach r. t., stirred for 30 min. Then quenched with sat. aq. NH_4Cl (10 mL) and extracted with EtOAc and petrol (5:1, 3 × 50 mL). The combined organic layers were washed with water (25 mL), dried, and evaporated; chromatography (petrol/ether, 15:1) gave a colourless oil, methyl (*R*)-2-[(*E/Z*)-(1*R*,19*R*)-1-(*tert*-butyldimethylsilanyloxy)-19-((*S*)-2,2-dimethyl[1,3]di-oxolan-4-yleicos-3-enyl]tetracosanote (3.6 g, 86%) as a mixture of isomers in ratio 2.2:1. Palladium 10% on carbon (1.0 g) was added to a stirred solution of the alkenes (3.45 g, 3.88 mmol) in EtOAc (150 mL). Hydrogenation was carried out for 1.5 h. The solution was filtered over a bed of celite and the solvent was evaporated. Chromatography (petrol/ether, 15:1) gave a colourless oil, (*R*)-2-[(1*R*,19*R*)-1-(*tert*-butyl-dimethylsilan-yloxy)-19-((*S*)-2,2-dimethyl[1,3]-dioxolan-4-yl)eicosyl]-tetracosanoic acid methyl ester (3.2 g, 96%), $[\alpha]_D^{22}$+3.6 (*c* 0.94, $CHCl_3$) [Found $(M+H)^+$: 893.8321, $C_{56}H_{113}O_5Si$ requires: 893.8352]; δ_H (500 MHz, $CDCl_3$): 4.0 (1H, dd, *J* 6.3, 7.9 Hz), 3.93 – 3.90 (1H, m), 3.87 (1H, br q, *J* 7.0 Hz), 3.66 (3H, s), 3.61 (1H, br t, *J* 7.9 Hz), 2.53 (1H, ddd, *J* 3.8, 7.3, 11.1 Hz), 1.59 – 1.52 (4H, m), 1.41 (3H, s), 1.36 (3H, s), 1.35 – 1.26 (72H, m, v.br), 1.10 – 1.07 (1H, m), 0.97 (3H, d, *J* 6.6 Hz), 0.89 (3H, t, *J* 6.9 Hz), 0.87 (9H, s), 0.05 (3H, s), 0.03 (3H, s); δ_C (126 MHz, $CDCl_3$): 175.1, 108.5, 80.4, 76.8, 73.2, 51.6, 51.2, 36.5, 33.7, 32.7, 31.9, 29.9, 29.8, 29.7(v.br), 29.66, 29.62, 29.6, 29.58, 29.5, 29.4, 29.3, 27.8, 27.5, 27.0, 26.6, 25.7, 25.5, 23.7, 22.7, 18.0, 15.6, 14.1, –4.4, – 4.9; ν_{max}/cm^{-1}: 2925, 2854, 1741, 1465, 1368, 1253, 1165, 1068.

(ii) The above ester (3.22 g, 3.61 mmol) was stirred in dry THF (30 mL) in dry polyethylene vial under nitrogen at 0 °C. Pyridine (1.2 mL) and HF.pyridine complex (5.0 mL) were added and the mixture was stirred for 18 h at 45 °C. The mixture was neutralised by slowly pouring it into sat. aq. $NaHCO_3$ (20 mL). The product was extracted with petrol/EtOAc (5:1, 3 × 10 mL), and evaporated to give a white solid. Chromatography (5:1 petrol/EtOAc) gave a white solid, methyl (R)-2-[(1R,19R)-19-((S)-2,2-dimethyl-[1,3]dioxolan-4-yl)-1-hydroxyeicosyl]tetracosanoate (2.3 g, 83%), m.p. 67 – 68 °C, $[\alpha]_D^{25}$+14.8 (*c* 0.970, $CHCl_3$) [Found $(M+H)^+$: 779.7450, $C_{50}H_{99}O_5$ requires: 779.7487]. This showed δ_H (500 MHz, $CDCl_3$): 4.0 (1H, dd, *J* 6.3, 7.9 Hz), 3.87 (1H, br q, *J* 6.9 Hz), 3.71 (3H, s), 3.68 – 3.64 (1H, m), 3.60 (1H, br t, *J* 7.7 Hz), 2.44 (1H, dt, *J* 5.4, 10.4 Hz), 1.74 – 1.26 (77H, m, v br), 1.41 (3H, s), 1.36 (3H, s), 1.11 – 1.07 (1H, m), 0.96 (3H, d, *J* 7.0 Hz), 0.88 (3H, t, *J* 7.0 Hz); δ_C (126 MHz, $CDCl_3$): 176.2, 108.5, 80.4, 72.3, 67.8, 51.5, 50.9, 36.5, 35.7, 32.7, 31.9, 29.9, 29.7(v.br), 29.65, 29.62, 29.6, 29.57, 29.55, 29.53, 29.48, 29.4, 29.3, 27.4, 27.0,

26.6, 25.7, 25.5, 22.7, 15.6, 14.1; ν_{max}/cm^{-1}: 3520, 2924, 2854, 1710, 1461, 1377, 1264, 1189, 1164.
(iii) A mixture of acetic anhydride (20 mL) and anhydrous pyridine (20 mL) was added with stirring to the above ester (2.24 g, 2.88 mmol) in dry toluene (15 mL), stirred for 20 h at r.t, then diluted with toluene (20 mL); the solvent was removed under reduced pressure to give an oil. Chromatography (petrol/EtOAc, 5:1) gave a white solid, compound **13** (2.2 g, 94%), m.p. 37 – 38 °C, $[\alpha]_D^{25}$+15.5 (*c* 1.03, $CHCl_3$) [Found $(M+Na)^+$: 843.7431, $C_{52}H_{100}NaO_6$ requires: 843.7412]; δ_H (500 MHz, $CDCl_3$): 5.11 – 5.07 (1H, m), 4.01 (1H, dd, *J* 6.0, 7.9 Hz), 3.88 (1H, br q, *J* 6.9 Hz), 3.69 (3H, s), 3.61 (1H, br t, *J* 7.7 Hz), 2.62 (1H, ddd, *J* 4.4, 6.9, 10.9 Hz), 2.04 (3H, s), 1.62 – 1.26 (76H, m, v.br), 1.41 (3H, s), 1.36 (3H, s), 1.13 – 1.06 (1H, m), 0.96 (3H, d, *J* 7.0 Hz), 0.89 (3H, t, *J* 7.0 Hz); δ_C (126 MHz, $CDCl_3$): 173.6, 170.3, 108.5, 80.4, 74.1, 67.8, 51.5, 49.6, 36.5, 32.7, 31.9, 31.7, 29.9, 29.7(v.br), 29.64, 29.61, 29.54, 29.46, 29.42, 29.4, 29.3, 28.1, 27.5, 27.0, 26.6, 25.5, 25.0, 22.7, 21.0, 15.6, 14.1; ν_{max}/cm^{-1}: 2924, 2854, 1747, 1465, 1369, 1236, 1163, 1066.

3.3 (*R*)-2-((1*R*,19*R*)-1-Acetoxy-19-methyl-20-oxoeicosyl)-tetracosanoic acid methyl ester 14

Periodic acid (1.0 g, 4.4 mmol) was added to the acetal **13** (1.20 g, 1.46 mmol) in dry ether (60 mL) at r.t. under argon and stirred for 18 h. The mixture was filtered through celite and washed with ether. The solvent was evaporated; chromatography eluting with petrol/EtOAc (4:1) gave a white solid, the title compound **14** (0.87 g, 80%), m.p. 31 – 31 °C, $[\alpha]_D^{23}$ +3.2 (*c* 0.69, $CHCl_3$) [Found $(M+Na)^+$: 771.6834, $C_{48}H_{92}NaO_5$ requires: 771.6837]. This showed δ_H (500 MHz, $CDCl_3$): 9.62 (1H, d, *J* 1.9 Hz), 5.11 – 5.07 (1H, m), 3.68 (3H, s), 2.62 (1H, ddd, *J* 4.1, 7.0, 10.4 Hz), 2.33 (1H, d sext, *J* 1.9, 7.0 Hz), 2.03 (3H, s), 1.73 – 1.26 (76H, m, v.br), 1.09 (3H, d, *J* 7.0 Hz), 0.88 (3H, t, *J* 7.0 Hz); δ_C: 205.4, 173.7, 170.3, 74.1, 51.5, 49.6, 46.3, 31.9, 31.7, 30.5, 29.7(v.br), 29.62, 29.6, 29.54, 29.5, 29.42, 29.4, 29.3, 28.1, 27.5, 26.9, 25.0, 22.7, 21.0, 14.1, 13.3; ν_{max}/cm^{-1}: 2922, 2852, 1744, 1467, 1372, 1237, 1167, 1022.

3.4 (2*R*,3*R*,21*R*,39*S*,40*S*,*E*)-2-Docosyl-3,39-dihydroxy-21,40-dimethyloctapentacont-22-enoic acid 17 (R = R' = H)

(i) Ester **14** (0.24 g, 0.32 mmol) in dry 1,2-dimethoxyethane (10 mL) was added to a stirred solution of sulfone **15** (0.34 g, 0.40 mmol)[60] in dry 1,2-dimethoxyethane (20 mL) at r.t under nitrogen. The mixture was cooled to –20 °C and KHMDS (1.04 mL, 0.520 mmol, 0.5 M in toluene) was added, then allowed to reach r.t and stirred for 1.5 h. Sat. aq. NH_4Cl (25 mL) and petrol/ ether (1:1, 50 mL) were added. The aqueous layer was re-extracted with petrol/ether (1:1, 2 × 40 mL) and the combined organic layers were evaporated. Chromatography (petrol/ether, 20:1) gave a white solid, methyl (2*R*,3*R*,21*R*,39*S*,40*S*,*E*)-3-acetoxy-39-((*tert*-butyl-dimethylsilyl)oxy)-2-docosyl-21,40-dimethyloctapenta-cont-22-enoate **16** (0.15 g, 34%), m.p. 25 – 27 °C, $[\alpha]_D^{23}$ – 3.18 (c 0.875, $CHCl_3$) [MALDI-Found $M+Na^+$: 1404.2; $C_{91}H_{180}NaO_5Si$ requires: 1404.3] which showed δ_H (500 MHz, $CDCl_3$): 5.34 (1H, dt, *J* 6.7, 15 Hz), 5.23 (1H, dd, *J* 7.5, 15 Hz), 5.09 (1H, ddd, *J* 4.1, 7.23, 8.2 Hz), 3.68 (3H, s), 3.49 (1H, dt, *J* 3.5, 6.3 Hz), 2.62 (1H, ddd, *J* 4.4, 7.0, 11.0 Hz), 2.05 (3H, s), 1.99 (2H, q, *J* 7 Hz), 1.70 – 1.20 (139H, br m including br s at 1.27), 1.08 – 1.00 (1H, m), 0.93 (3H, d, *J* 7 Hz), 0.89 (6H, t, *J* 6.7 Hz), 0.88 (9H, s), 0.80 (3H, d, *J* 7 Hz), 0.04 (3H, s), 0.03 (3H, s); δ_C (126 MHz, $CDCl_3$): 173.6, 170.3, 136.5, 128.4, 75.9, 74.1, 51.5, 49.6, 37.7, 37.2, 36.7, 33.5, 32.6, 32.5, 31.9, 31.7, 30.0, 29.9, 29.8, 29.7, 29.67, 29.62, 29.6, 29.54, 29.5, 29.43, 29.41, 29.4, 29.1, 28.1, 27.7, 27.5, 27.4, 26.0, 25.9, 25.0, 22.7, 21.0, 20.9, 18.2, 14.4, 14.1, –4.2, –4.4; υ_{max}: 2923, 2853, 1745, 1464 cm^{-1}.
(ii) Compound **16** (150 mg, 0.108 mmol) was dissolved in dry THF (8 mL) under nitrogen in a dry polyethylene vial, equipped with a rubber septum, at 0 °C. Pyridine (0.1 mL) and HF-pyridine complex (0.4 mL) were added and the mixture stirred for 18 h at 45 °C, then neutralized by slowly pouring it into sat. aq. $NaHCO_3$ (10 mL) until no more carbon dioxide was liberated. The product was extracted with petrol/ether (1:1, 3 × 25 mL), dried and evaporated to give a white solid. Chromatography (petrol/ether, 10:1) gave a white solid, *methyl (2R,3R,21R,39S,40S,E)-3-acetoxy-2-docosyl-39-hydroxy-21,40-dimethyloctapenta-cont-22-en-oate* **17** (R = Ac, R' = Me) (138 mg, 92%), $[\alpha]_D^{23}$ –3.59 (*c* 1.04, $CHCl_3$); m.p. 36 – 38 °C [MALDI-Found $M+Na^+$: 1290.2; $C_{85}H_{166}NaO_5$ requires: 1290.2]; δ_H (500 MHz, $CDCl_3$): 5.33 (1H, dt, *J* 6.6, 15.5 Hz), 5.24 (1H, dd, *J* 7.6, 15.5 Hz), 5.09 (1H, dt, *J* 3.9, *J* 8 Hz), 3.69 (3H, s), 3.53 – 3.49 (1H, m), 2.62 (1H, ddd, *J* 4.4, 7, 10.9 Hz), 2.04 (3H, s), 1.98 (2H, q, *J* 7 Hz), 1.63 –1.20 (140H, br m including br s at 1.27), 1.18 – 1.11 (1H, m), 0.94 (3H, d, *J* 6.6 Hz), 0.89 (6H, t, *J* 6.7 Hz), 0.86 (3H, d, *J* 7 Hz); δ_C (126 MHz, $CDCl_3$): 173.6, 170.3, 136.5, 128.4, 75.3, 74.1, 51.5, 49.6, 38.3, 37.2, 36.7, 34.5, 33.5, 32.6, 31.9, 31.7, 30.0, 29.9, 29.8, 29.72, 29.7, 29.63, 29.6, 29.5, 29.45, 29.44, 29.42, 29.4, 29.1, 28.1, 27.7, 27.5, 27.4, 27.3, 26.2, 25.0, 22.7, 21.0, 20.9, 18.2, 14.4, 13.6; υ_{max}: 3542, 2923, 2843, 1745, 1464 cm^{-1}.
(iii) Lithium hydroxide monohydrate (17.04 mg, 0.7102 mmol, 30 mol. equiv.) was added to a stirred solution of ester **17** (R = Ac, R' = Me) (30.0 mg, 0.0237 mmol) in THF (3 mL), methanol (0.5 mL) and water (0.5 mL) at RT. The mixture was stirred at 43 °C for 18 h, then cooled to RT and acidified with hydrochloric acid (5 %, 2 mL) and the aqueous layer was extracted with warm petrol/ether (1:1, 3 × 10 mL). The combined organic extracts were dried and evaporated; column chromatography (warm petrol/EtOAc, 5:1) gave a white solid, acid **17** (R = R' = H) (18.6 mg, 65%), $[\alpha]^{21}{}_D$ –2.1 ($CHCl_3$, 0.74 µmol) [MALDI-Found $M+Na^+$: 1234.3; $C_{82}H_{162}NaO_4$ requires: 1234.2]; δ_H (500 MHz, $CDCl_3$): 5.33 (1H, dt, *J* 6.6, 15.5 Hz), 5.23 (1H, dd, *J* 7.6, 15.5 Hz), 3.70 – 3.69 (1H, m), 3.52 – 3.51 (1H, m), 2.45 (1H, br pent, *J* 4.7 Hz), 2.05 – 2.00 (1H, m), 1.97 (2H, q, *J* 6.9 Hz), 1.79 – 1.71 (1H, m), 1.66 – 1.59 (2H, m), 1.64 – 1.23 (139H, br m, including br s at 1.26), 0.94 (3H, d, *J* 6.6 Hz), 0.89 (6H, t, *J* 7.0 Hz), 0.86 (3H, d, *J* 7.3 Hz); δ_C (126 MHz, $CDCl_3$): 177.3, 136.5, 128.5, 75.4, 72.2, 50.5, 37.3, 36.7, 34.4, 33.4, 32.6, 31.9, 30.0, 29.7, 29.6, 29.52, 29.5, 29.4, 29.1, 27.4, 22.7, 21.0, 16.6, 14.1; ν_{max}: 3534, 2922, 2854, 1751, 1466 cm^{-1}.

3.5 (2*R*,3*R*,21*R*,40*S*,*E*)-2-Docosyl-3-hydroxy-21,40-dimethyl-39-oxooctapentacont-22-enoic acid 23

(i) 2,3-Dihydropyran (0.18 mL, 2.0 mmol) and pyridinium-*p*-toluene sulfonate (120 mg, 0.0510 mmol) were added to a stirred solution of the ester **17** (R = Ac) (130 mg, 0.101 mmol) in dry CH_2Cl_2 (1 mL) under nitrogen at r.t. The mixture was stirred for 1 h, then quenched with sat. aq. $NaHCO_3$ (3 mL) and extracted with CH_2Cl_2 (2 × 15 mL). The combined organic layers were washed with water (5 mL) and evaporated to give an oil; chromatography (petrol/EtOAc, 10:1) gave a colourless oil, methyl (2*R*, 3*R*,21*R*,39*S*,40*S*,*E*)-3-acetoxy-2-docosyl-21,40-dimethyl-39-((tetrahydro-2*H*-pyran-2-yl)oxy)octapenta-cont-22-en-oate **21** (128 mg, 92%) as a mixture of diastereoisomers [MALDI-Found M+Na^+: 1374.2; $C_{90}H_{174}NaO_6$ requires: 1374.3]; δ_H (400 MHz, $CDCl_3$): 5.33 (1H, dt, *J* 6.7, 15.2 Hz), 5.24 (1H, dd, *J* 7.6, 15.2 Hz), 5.11 – 5.07 (1H, m), 4.65 (0.5H, br t, *J* 2.8 Hz), 4.62 (0.5H, br t, *J* 2.6 Hz), 3.96 – 3.89 (1H, m), 3.68 (3H, s), 3.50 – 3.44 (2H, m), 2.63 (1H, ddd, *J* 4.7, 6.7, 10.8 Hz), 2.1 – 2.0 (4H, including s at 2.03), 1.97 (2H, q, *J* 6.6 Hz), 1.86 – 1.82 (1H, m), 1.59 – 1.14 (144H, br m including br s at 1.26), 0.94 (3H, d, *J* 6.7 Hz), 0.89 (6H, t, *J* 6.6 Hz), 0.84 (3H, d, *J* 6.7 Hz); δ_C (126 MHz, $CDCl_3$): 173.7, 170.3, 136.5, 128.4, 98.5, 97.8, 81.4, 80.9, 74.1, 62.7, 62.4, 51.5, 49.6, 37.2, 36.7, 36.4, 35.1, 32.6, 32.5, 32.0, 31.9, 31.7, 31.4, 31.3, 31.2, 29.94, 29.9, 29.8, 29.7, 29.65, 29.6, 29.5, 29.42, 29.4, 29.35, 29.1, 28.1, 27.8, 27.5, 27.4, 27.3, 26.1, 25.7, 25.66, 25.6, 25.0, 22.7, 21.0, 20.9, 20.1, 19.8, 15.2, 14.9, 14.1; υ_{max}: 2924, 2853m, 2360m, 2341m, 1464 cm^{-1}.

(ii) Lithium hydroxide monohydrate (37 mg, 0.88 mmol.) was added to a stirred solution of ester **21** (120 mg, 0.088 mmol) in THF (8 mL), methanol (1.0 mL) and water (1.5 mL) at r.t. The mixture was stirred at 45 °C for 18 h, then cooled to r.t., acidified with hydrochloric acid (5 %, 2 mL pH 6) and extracted with warm petrol/EtOAc (5:2, 3 × 10 mL). The combined organic extracts were evaporated; chromatography (5:1 petrol/EtOAc) gave a white solid, (2*R*, 3*R*,21*R*,39*S*,40*S*,*E*)-2-docosyl-3-hydroxy-21,40-dimethyl-39-((tetrahydro-2*H*-pyran-2-yl)oxy)octapentacont-22-enoic acid as a mixture of diastereoisomers (100 mg, 90%) [MALDI-Found $(M+Na)^+$: 1318.1; $C_{87}H_{170}NaO_5$ requires: 1318.2]; δ_H (400 MHz, $CDCl_3$): 5.33 (1H, dt, *J* 6.44, 15.4 Hz), 5.25 (1H, dd, *J* 7.4, 15.4 Hz), 4.67 (0.5H, br t, *J* 3.1 Hz), 4.44 (0.5H, br t, *J* 3.9 Hz), 3.97 – 3.89 (1H, m), 3.71 (1H, br q, *J* 6.7 Hz), 3.55 – 3.43 (2H, m), 2.45 (1H, br dt, *J* 5.3, 8.6 Hz), 2.05 – 2.03 (1H, br, m), 1.97 (2H, q, *J* 6.5 Hz), 1.91 – 1.03 (147H, br, m), 0.93 (3H, d, *J* 6.7 Hz), 0.88 (6H, t, *J* 6.3 Hz), 0.85 (3H, d, *J* 6.9 Hz); δ_C (126 MHz, $CDCl_3$): 179.7, 136.4, 128.4, 98.4, 97.7, 81.5, 81.0, 72.1, 62.6, 62.3, 50.8, 37.2, 36.7, 35.5, 32.5, 31.9, 31.2, 29.9, 29.8, 29.7, 29.6, 29.53, 29.5, 29.4, 29.3, 29.1, 27.3, 25.7, 25.6, 22.6, 20.9, 15.1, 14.9, 14.1; υ_{max}: 3424, 2922, 2852, 2361, 1646 cm^{-1}.

(iii) Imidazole (510 mg, 0.756 mmol) was added to a stirred solution of the above acid (98.0 mg, 0.076 mmol) in dry DMF (1.5 mL) and dry toluene (2.5 mL) at RT followed by addition of TBDMSCl (0.110 mg, 0.756 mmol) and DMAP (14.0 mg, 0.115 mmol). The mixture was heated to 70 °C for 18 h; it was then diluted with petrol/EtOAc (1:1, 15 mL) and sat. aq. $NaHCO_3$ (3 mL). The aqueous layer was re-extracted with petrol/EtOAc (3 × 15 mL), and evaporated. The residue was dissolved in THF (6 mL), methanol (1.0 mL) and water (1.5 mL); to this, potassium carbonate (300 mg) was added and the mixture stirred at 45 °C for 2 h. The mixture was diluted with petrol/EtOAc (1:1, 10 mL) and water (1 mL) and acidified with potassium hydrogen sulfate to pH 6. The aqueous layer was re-extracted with petrol/EtOAc (5:2, 2 × 10 mL), the combined organic layers were dried and evaporated. Chromatography (petrol/EtOAc, 20:1) gave a colourless oil, (2*R*,3*R*,21*R*,39*S*, 40*S*,*E*)-3-((*tert*-butyldi-methylsilyl)-oxy)-2-docosyl-21,40-dimethyl-39-((tetra-hydro-2*H*-pyran-2-yl)oxy)octapentacont-22-enoic acid **22** (84 mg, 84%) as a mixture of diastereisomers [MALDI-Found M+Na^+: 1433.5; $C_{93}H_{184}NaO_5Si$ requires: 1433.5]; δ_H (400 MHz, $CDCl_3$): 5.34 (1H, dt, *J* 6.6, 15.2 Hz), 5.24 (1H, dd, *J* 7.6, 15.2 Hz), 4.67 (0.5H, br d, *J* 2.9 Hz), 4.62 (0.5H, br t, *J* 4.5 Hz), 3.96 – 3.89 (1H, m), 3.86 – 3.82 (1H, m), 3.50 – 3.42 (2H, m), 2.55 – 2.51 (1H, ddd, *J* 3.3, 5.6, 12.6 Hz), 2.07 – 2.01 (1H, m), 1.97 (2H, q, *J* 7.3 Hz), 1.85 – 1.80 (1H, m), 1.75 – 1.1 (145H, br m), 0.93 (3H, d, *J* 6.8 Hz), 0.90 (9H, s), 0.87 (6H, t, *J* 6.6 Hz), 0.84 (3H, d, *J* 7.0 Hz), 0.14 (3H, s), 0.13 (3H, s); δ_C (126 MHz, $CDCl_3$): 171.0, 136.4, 128.3, 98.5, 97.8, 81.4, 80.9, 73.5, 62.7, 62.4, 50.7, 37.2, 36.7, 36.4, 35.1, 34.9, 32.6, 32.4, 32.0, 31.9, 31.4, 31.2, 31.17, 30.1, 30.0, 29.82, 29.8, 29.73, 29.7, 29.6, 29.57, 29.52, 29.5, 29.42, 29.4, 29.1, 29.0, 27.7, 27.4, 27.38, 26.3, 25.8, 25.7, 25.2, 22.73, 22.7, 22.6, 21.1, 20.9, 20.4, 19.4, 18.8, 17.8, 14.3, 14.1, 13.6, 11.4, 4.3, –4.9; υ_{max}: 3425, 2924, 2853, 2362, 1702, 1464 cm^{-1}.

(iv) Pyridinium-*p*-toluene sulfonate (PPTS) (128 mg, 0.588 mmol) was added to a stirred solution of the acid **22** (83 mg, 0.058 mmol) in THF (3 mL), and MeOH (0.5 mL) and the mixture stirred at 40 °C for 6 h, then sat. aq. $NaHCO_3$ (0.2 mL) was added and the product extracted with petrol/EtOAc (1:1, 3 × 10 mL). The combined organic layers were evaporated to give a crude oil; chromatography (petrol/ EtOAc, 10:1) gave a colourless oil, (2*R*,3*R*,21*R*,39*S*,40*S*,*E*)-3-((*tert*-butyldimethylsilyl)oxy)-2-docosyl-39-hydroxy-21, 40-dimethyloctapentacont-22-enoic acid (76 mg, 97%) [Found $(M+Na)^+$: 1348.6; $C_{88}H_{176}NaO_4Si$ requires: 1348.3]; δ_H (400 MHz, $CDCl_3$): 5.34 (1H, dt, *J* 6.4, 15.3 Hz), 5.24 (1H, dd, *J* , 7.4, 15.3 Hz), 3.85 (1H, br, dd, *J* 6.1, 9.8 Hz), 3.51 (1H, dt, *J* 4.5, 8.8 Hz), 2.53 (1H, ddd, *J* 3.8, 5.4, 9.3 Hz), 2.03 (1H, m), 1.97 (2H, q, *J* 6.6 Hz), 1.73 – 1.62 (1H, m), 1.43 – 1.26 (140H, br m including, s at 1.26), 0.94 (3H, d, *J* 6.7 Hz), 0.92 (9H, s), 0.88 (6H, t, *J* 6.8 Hz), 0.86 (3H, d, *J* 6.6 Hz), 0.13 (3H, s), 0.12 (3H, s); δ_C (126 MHz, $CDCl_3$): 177.7, 136.5, 128.4, 75.3, 73.7, 50.0, 41.4, 38.2, 37.3, 36.7, 35.8, 34.5, 33.4, 32.6, 31.9, 31.6, 30.1, 30.0, 29.82, 29.8, 29.73, 29.7, 29.64, 29.6, 29.52, 29.5, 29.44, 29.4, 29.1, 29.0, 27.7, 27.4, 27.37, 26.3, 25.8, 25.7, 25.2, 22.72, 22.7, 22.6, 21.1, 20.9, 20.4, 19.4, 18.8, 17.9, 14.3, 14.2, 14.1, 13.6, 11.4, –4.2, –4.9; υ_{max}: 3424, 2923, 2852, 1709, 1464 cm^{-1}.

(v) The above acid (75 mg, 0.056 mmol), in CH_2Cl_2 (10 mL), was added to a stirred suspension of PCC (36 mg, 0.16 mmol) in CH_2Cl_2 (2 mL) at r.t. and stirred for 1 h. The solvent was evaporated; chromatography (petrol/EtOAc, 5:1) gave a semi solid, (2*R*,3*R*,21*R*,40*S*, *E*)-3-((*tert*-butyldimethylsilyl)oxy)-2-docosyl-21,40-dimethyl-39-oxo-

octapentacont-22-enoic acid (61 mg, 82%) [MALDI-Found $(M+Na)^+$: 1346.3; $C_{88}H_{174}NaO_4Si$ requires: 1346.3]; δ_H (400 MHz, $CDCl_3$): 5.34 (1H, dt, *J* 6.4, 15.2 Hz), 5.24 (1H, dd, *J* 7.4, 15.3 Hz), 3.86 (1H, br q, *J* 6.1 Hz), 2.57 – 2.46 (2H, m), 2.41 (2H, dt, *J*, 1.9, 7.3 Hz), 2.08 – 2.00 (1H, m), 1.97 (2H, q, *J* 6.7 Hz), 1.70 – 1.59 (2H, m), 1.60 – 1.49 (4H, m), 1.28 (131H, s), 1.05 (3H, d, *J* 6.9 Hz), 0.95 (3H, d, *J* 6.7 Hz), 0.91(9H, s), 0.88 (6H, t, *J* 6.6 Hz), 0.12 (3H, s), 0.11 (3H, s); δ_C (126 MHz, $CDCl_3$): 215.3, 178.0, 136.5, 128.4, 73.7, 50.0, 46.3, 41.2, 37.3, 36.7, 35.8, 33.1, 32.6, 31.9, 29.8, 29.72, 29.7, 29.64, 29.6, 29.58, 29.54, 29.5, 29.48, 29.46, 29.44, 29.4, 29.3, 29.22, 29.2, 27.4, 27.32, 27.3, 25.7, 25.2, 23.7, 22.7, 20.1, 17.9, 16.4, 14.1, –4.2, –4.9; υ_{max}: 3419, 2925, 2854, 2360, 2341, 1711, 1464 cm^{-1}.

(vi) The above acid (10 mg, 0.0075 mmol) was dissolved in dry THF (4 mL) under nitrogen in a dry polyethylene vial equipped with a rubber septum at 0 °C. Pyridine (0.1 mL) and HF-pyridine complex (0.32 mL, 0.022 mmol.) were added and the mixture stirred for 18h at 45 °C then neutralised by slowly pouring into sat. aq. $NaHCO_3$ (3 mL) until no more carbon dioxide was liberated. The product was extracted with petrol/ether (1:1, 3 × 10 mL), and evaporated to give a white solid. Chromatography (petrol/EtOAc, 5:1) gave a white solid, compound **23** (9.0 mg, 98%) [MALDI-Found $(M+Na)^+$: 1232.2; $C_{82}H_{160}O_4Na$ requires: 1232.2]; δ_H (500 MHz, $CDCl_3$): 5.32 (1H, dt, *J* 6.7, 15.5 Hz), 5.24 (1H, dd, *J* 7.6, 15.5 Hz), 3.73 (1H, m), 2.50 – 2.45 (2H, m), 2.42 (2H, t, *J* 7.0 Hz), 2.02 (1H, m), 1.96 (2H, q, *J* 6.8 Hz), 1.74 (2H, m), 1.65 – 1.51 (10H, m), 1.26 (126H, br m), 1.05 (3H, d, *J* 7.0 Hz), 0.95 (3H, d, *J* 7.0 Hz), 0.89 (6H, t, *J* 6.7 Hz); δ_C: 215.5, 177.9, 136.5, 128.4, 72.2, 50.6, 46.4, 41.2, 37.3, 36.7, 35.6, 33.1, 32.6, 31.9, 29.8, 29.7, 29.64, 29.6, 29.54, 29.51, 29.5, 29.43, 29.4, 29.3, 29.1, 28.9, 27.4, 27.3, 25.7, 23.7, 22.7, 22.6, 21.0, 19.4, 16.4, 14.1; υ_{max}: 3420, 3019, 2926, 2855, 1521, 1420, 1215 cm^{-1}.

3.6 (2*R*,3*R*,21*R*,40*R*,*E*)-2-Docosyl-3-hydroxy-21,40-dimethyl-39-oxooctapentacont-22-enoic acid 25

(i) Lithium hydroxide monohydrate (52.8 mg, 1.55 mmol, 30 mol.equiv.) was added to a stirred solution of ester **24** (70.0 mg, 0.0520 mmol) (see Supplementary Information) in THF (5 mL), methanol (0.5 mL) and water (0.5 mL) at RT. The mixture was stirred at 43 °C for 18 h, then cooled to RT and acidified with hydrochloric acid (5%, 2 mL) and the aqueous layer extracted with warm petrol/ether (1:1, 3 × 10 mL). The combined organic extracts were dried and evaporated; chromatography eluting with petrol/EtOAc (5:2) gave a semi solid, (2*R*,3*R*,21*R*, 39*R*,40*R*,*E*)-2-docosyl-3-hydroxy-21,40-dimethyl-39-((tetrahydro-2*H*-pyran-2-yl)oxy)octapenta-cont-22-enoic acid (46.0 mg, 72%), $[\alpha]^{22}_D$ +1.75 (*c* 1.19 µmol, $CHCl_3$) [MALDI-Found $(M+Na)^+$: 1318.1; $C_{87}H_{170}NaO_5$ requires: 1318.3]; which showed δ_H (500 MHz, $CDCl_3$): 5.33 (1H, dt, *J* 6.7, 15.2 Hz), 5.24 (1H, dd, *J* 7.6, 15.2 Hz), 4.67 (0.5H, br t, *J* 3.1 Hz), 4.44 (0.5H, br t, *J* 3.8 Hz), 3.97 – 3.89 (1H, m), 3.71 (1H, br q, *J* 6.7 Hz), 3.55 – 3.43 (2H, m), 2.49 – 2.42 (1H, m), 2.05 – 2.03 (1H, m), 1.97 (2H, q, *J* 7.3 Hz), 1.88 – 1.82 (1H, m), 1.63 – 1.21 (146H, br m including br s at 1.26), 0.94 (3H, d, *J* 6.7 Hz), 0.89 (6H, t, *J* 6.6 Hz), 0.84 (3H, d, *J* 6.7 Hz); δ_C (126 MHz, $CDCl_3$): 177.5, 145.5, 136.5, 72.2, 67.4, 60.4, 50.5, 37.3, 36.7, 35.6, 34.5, 32.6, 31.9, 30.9, 30.3, 30.1, 30.0, 29.9, 29.7, 29.6, 29.5, 29.42, 29.4, 29.3, 29.12, 29.1, 28.9, 28.1, 27.3, 26.1, 25.8, 22.7, 22.6, 22.3, 21.1, 21.0, 20.4, 19.4, 14.2, 14.11, 14.06; υ_{max}: 3424, 2922, 2852, 2361, 1646 cm^{-1}.

(ii) Imidazole (22.7 mg, 0.333 mmol, 10 mol. equiv.) was added to a stirred solution of the above acid (45 mg, 0.033 mmol) in dry DMF (0.2 mL) at r. t. followed by addition of TBDMSCl (50.0 mg, 0.333 mmol, 10 mol. equiv.) and DMAP (4.01 mg, 0.0333 mmol). The mixture was heated to 70 °C for 18 h, then diluted with petrol/EtOAc (1:1, 15 mL) and sat. aq. $NaHCO_3$ (3 mL). The aqueous layer was re-extracted with petrol/EtOAc (3 × 15 mL), and the solvent evaporated. The residue was dissolved in THF (5 mL), methanol (0.5 mL) and water (0.5 mL); to this, potassium carbonate (100 mg) was added and the mixture stirred at 45 °C for 6 h, then diluted with petrol/EtOAc (1:1, 10 mL) and water (1 mL) and acidified with potassium hydrogen sulfate to pH 2. The aqueous layer was re-extracted with petrol/EtOAc (1:1, 2 × 10 mL), and the combined organic layers dried and the solvent evaporated. Chromatography (petrol/EtOAc, 20:1) gave a colourless oil, (2*R*,3*R*,21*R*,39*R*, 40*R*,*E*)-3-((*tert*-butyl-dimethylsilyl)oxy)-2-docosyl-21,40-dimethyl-39-((tetra-hydro-2*H*-pyran-2-yl)oxy)octapenta-cont-22-enoic acid (34 mg, 70%), $[\alpha]^{23}_D$+1.58 (*c* 0.436 µmol, $CHCl_3$) [MALDI-Found $(M+Na)^+$: 1432.3; $C_{93}H_{184}NaO_5Si$ requires: 1432.3]; δ_H (500 MHz, $CDCl_3$): 5.34 (1H, dt, *J* 6.6, 15.1 Hz), 5.24 (1H, dd, *J* 7.6, 15.1 Hz), 4.67 (0.5H, br t, *J* 3.0 Hz), 4.44 (0.5H, br t, *J* 3.8 Hz), 3.97 – 3.89 (1H, m), 3.71 (1H, br q, *J* 6.7 Hz), 3.57 – 3.42 (2H, m), 2.55 – 2.51 (1H, m), 2.05 – 2.03 (1H, m), 1.97 (2H, q, *J* 7.0 Hz), 1.88 – 1.82 (1H, m), 1.70 – 1. 31 (145H, br m including br s at 1.26), 0.94 (3H, d, *J* 7.0 Hz), 0.93 (9H, s), 0.89 (6H, t, *J* 6.6 Hz), 0.84 (3H, d, *J* 7.0 Hz), 0.15 (3H, s), 0.14 (3H, s); δ_C (126 MHz, $CDCl_3$): 177.4, 136.6, 128.3, 98.5, 75.3, 73.7, 67.4, 49.9, 41.7, 39.7, 37.6, 36.7, 35.8, 34.8, 33.3, 32.6, 31.9, 31.6, 30.1, 30.0, 29.82, 29.8, 29.73, 29.7, 29.61, 29.6, 29.52, 29.5, 29.42, 29.4, 29.1, 29.0, 27.7, 27.41, 27.4, 26.3, 25.8, 25.7, 25.2, 22.73, 22.69, 22.6, 21.1, 20.9, 20.4, 19.4, 18.8, 17.8, 14.3, 14.1, 13.6, 11.4, –4.3, –4.9; υ_{max}: 3425, 2924, 2853, 2362, 1702, 1464 cm^{-1}.

(iii) PPTS (29.6 mg, 0.118 mmol) was added to a stirred solution of the above acid (17.3 mg, 0.0118 mmol) in THF (1 mL), methanol (0.1 mL) and water (0.1 mL) and the mixture refluxed for 2.5 h followed by stirring at 47 °C for 24 h, then sat. aq. $NaHCO_3$ (0.2 mL) was added and the product extracted with petrol/EtOAc (1:1, 3 × 5 mL). The combined organic layers were evaporated to give an oil; chromatography eluting with petrol/EtOAc (10:1) gave a colourless oil, (2*R*,3*R*,21*R*,39*R*,40*R*,*E*)-3-((*tert*-butyldi-methylsilyl)-oxy)-2-docosyl-39-hydroxy-21,40-dimethyl-octapentacont-22-enoic acid (12 mg, 73%), $[\alpha]^{23}_D$+1.46 (*c* 1.43 µmol, $CHCl_3$) [MALDI-Found $(M+Na)^+$: 1348.5; $C_{88}H_{176}NaO_4Si$ requires: 1348.3]; δ_H (500 MHz, $CDCl_3$): 5.31 (1H, dt, *J* 6.6, 15.3 Hz), 5.24 (1H, dd, *J* 7.6, 15.3 Hz), 3.83 (1H, dist. pent, *J* 2.3 Hz), 3.52 – 3.49 (1H, m), 2.55 – 2.51 (1H, m), 2.05 – 2.03 (1H, m), 1.97 (2H, q, *J* 6.6 Hz), 1.63 – 1.22 (141H, br m including br s at 1.26), 0.97 (3H, d, *J* 6.7 Hz), 0.93 (9H, s), 0.89 (3H, d, *J* 7.3 Hz), 0.88 (6H, t, *J* 6.6 Hz), 0.15 (3H, s), 0.14 (3H, s); δ_C (126 MHz, $CDCl_3$): 177.7, 136.5, 128.4, 75.3, 73.7, 50.0, 41.4, 38.2,

37.3, 36.7, 35.8, 34.5, 33.4, 32.6, 31.9, 31.6, 30.1, 30.0, 29.82, 29.8, 29.73, 29.7, 29.64, 29.6, 29.52, 29.5, 29.44, 29.4, 29.1, 29.0, 27.7, 27.4, 27.37, 26.3, 25.8, 25.7, 25.2, 22.72, 22.7, 22.6, 21.1, 20.9, 20.4, 19.4, 18.8, 17.9, 14.3, 14.2, 14.1, 13.6, 11.4, –4.2, –4.9; υ_{max}: 3424, 2923, 2852, 1709, 1464 cm^{-1}.

(iv) The above acid (18.1 mg, 0.0131 mmol), in CH_2Cl_2 (1 mL) was added to a stirred suspension of PCC (8.47 mg, 0.0393 mmol, 3 mol. equiv.) in CH_2Cl_2 (2 mL) at RT. The mixture was stirred for 1 h. The solvent was evaporated; chromatography (petrol/EtOAc, 5:1) gave a white solid, (2*R*, 3*R*,21*R*,40*R*,*E*)-3-((*tert*-butyldimethylsilyl)oxy)-2-docosyl-21,40-dimethyl-39-oxooctapentacont-22-enoic acid (16.0 mg, 89%), $[\alpha]_D^{23}$+3.70 (*c* 0.457 µmol, $CHCl_3$) [MALDI-Found $(M+Na)^+$: 1346.4; $C_{88}H_{174}NaO_4Si$ requires: 1346.3]; δ_H (500 MHz, $CDCl_3$): 5.33 (1H, dt, *J* 6.0, 15.3 Hz), 5.24 (1H, dd, *J* 7.6, 15.3 Hz), 3.85 (1H, m), 2.56 – 2.52 (1H, m), 2.50 (1H, br q, *J* 6.9 Hz), 2.41 (2H, dt, *J*, 1.9, 7.3 Hz), 2.05 – 2.00 (1H, m), 1.97 (2H, q, *J* 6.6 Hz), 1.75 – 1.68 (2H, m), 1.64 – 1.21 (135H, br m including br s at 1.26), 1.05 (3H, d, *J* 6.6 Hz), 0.95 (3H, d, *J* 7.0 Hz), 0.93 (9H, s), 0.89 (6H, t, *J* 6.6 Hz), 0.15 (3H, s), 0.14 (3H, s); δ_C (126 MHz, $CDCl_3$): 215.3, 178.0, 136.5, 128.4, 73.7, 50.0, 46.3, 41.2, 37.3, 36.7, 35.8, 33.1, 32.6, 31.9, 29.8, 29.72, 29.7, 29.64, 29.61, 29.6, 29.54, 29.5, 29.48, 29.46, 29.44, 29.4, 29.3, 29.23, 29.2, 27.4, 27.32, 27.3, 25.7, 25.2, 23.7, 22.7, 20.1, 17.9, 16.4, 14.1, –4.2, – 4.9; v_{max}: 3419, 2925, 2854, 2360, 2341, 1711, 1464 cm^{-1}.

(v) A dry polyethylene vial equipped with a rubber septum, was charged with the above acid (15 mg, 0.011 mmol) in dry THF (1 mL) under nitrogen at 0 °C. Pyridine (0.1 mL) and HF-pyridine complex (0.05 mL, 0.03263 mmol, 3 mol. equiv.) were added and the mixture stirred for 18h at 43 °C, then neutralised by slowly pouring it into sat. aq. $NaHCO_3$ (3 mL) until no more carbon dioxide was liberated. The product was extracted with petrol/ether (1:1, 3 × 10 mL), and evaporated to give a solid. Chromatography (petrol/ EtOAc, 5:1) gave a white solid, (2*R*,3*R*,21*R*,40*R*,*E*)-2-docosyl-3-hydroxy-21,40-dimethyl-39-oxooctapentacont-22-enoic acid **25** (6.0 mg, 44%), $[\alpha]_D^{23}$+2.90 (*c* 0.471 µmol, $CHCl_3$) [MALDI-Found $(M+Na)^+$: 1232.3; $C_{82}H_{160}NaO_4$ requires: 1233.1]; δ_H (500 MHz, $CDCl_3$): 5.32 (1H, td, *J* 6.7, 15.5 Hz), 5.24 (1H, dd, *J* 7.6, 15.5 Hz), 3.72 (1H, m), 2.50 – 2.45 (2H, m), 2.42 (2H, dt, *J* 1.6, 7.0 Hz), 2.02 (1H, m), 1.97 (2H, q, *J* 7.0 Hz), 1.65 – 1.17 (138H, br m, including br s at 1.26), 1.05 (3H, d, *J* 7.0 Hz), 0.95 (3H, d, *J* 7.0 Hz), 0.89 (6H, t, *J* 7.0 Hz); δ_C (126 MHz, $CDCl_3$): 215.5, 177.9, 136.5, 128.4, 72.2, 50.6, 46.4, 41.2, 37.3, 36.7, 35.6, 33.1, 32.6, 31.9, 29.8, 29.7, 29.64, 29.6, 29.54, 29.51, 29.5, 29.43, 29.4, 29.3, 29.1, 28.9, 27.4, 27.3, 25.7, 23.7, 22.7, 22.6, 21.0, 19.4, 16.4, 14.1; υ_{max}: 3420, 3019, 2926, 2855, 1521, 1420, 1215 cm^{-1}.

3.7 (2*R*,3*R*,21*R*,39*R*,40*R*,*E*)-2-Docosyl-3,39-dihydroxy-21,40-dimethyloctapentacont-22-enoic acid 20 (R = R' = H)

Lithium hydroxide monohydrate (12.0 mg, 0.355 mmol, 30 mol. equiv.) was added with stirring to ester **19** (15.0 mg, 0.0118 mmol) (see Supplementary Information) in THF (2 mL), methanol (0.2 mL) and water (0.2 mL) at RT. The mixture was stirred at 43 °C for 18 h, cooled to RT, then acidified with hydrochloric acid (5 %, 2 mL) and the aqueous layer extracted with warm petrol/ether (1:1, 3 × 10 mL). The combined organic extracts were evaporated; chromatography (petrol/EtOAc, 5:1) gave a white solid, (2*R*,3*R*,21*R*,39*R*,40*R*,*E*)-2-docosyl-3,39-dihydroxy-21,40-dimethyloctapentacont-22-enoic acid **(20,** R = R' = H) (8.6 mg, 59%), $[\alpha]^{20}_D$ +1.67 (*c* 1.29 µmol, $CHCl_3$,) [Found $(M+Na)^+$: 1234.5; $C_{82}H_{162}NaO_4$ requires: 1234.2]; δ_H (500 MHz, $CDCl_3$): 5.33 (1H, dt, *J* 6.6, 15.5 Hz), 5.23 (1H, dd, *J* 7.6, 15.5 Hz), 3.70 – 3.69 (1H, m), 3.52 – 3.51 (1H, m), 2.45 (1H, br pent, *J* 4.7 Hz), 2.05 – 2.00 (1H, m), 1.97 (2H, q, *J* 6.9 Hz), 1.79 – 1.71 (1H, m), 1.66 – 1.59 (2H, m), 1.64 – 1.23 (139H, br m, including br s at 1.26), 0.94 (3H, d, *J* 6.6 Hz), 0.89 (6H, t, *J* 7.0 Hz), 0.86 (3H, d, *J* 7.3 Hz); δ_C (126 MHz, $CDCl_3$): 177.3, 136.5, 128.5, 75.4, 72.2, 50.5, 37.3, 36.7, 34.4, 33.4, 32.6, 31.9, 30.0, 29.7, 29.6, 29.52, 29.5, 29.4, 29.1, 27.4, 22.7, 21.0, 16.6, 14.1; υ_{max}: 3534, 2922, 2854, 1751, 1466 cm^{-1}.

3.8 (*R*)-12-((*S*)-2,2-Dimethyl-1,3-dioxolan-4-yl)tridecyl pivaloate 27

LiHMDS (87.70 mL, 92.96 mmol, 1.06 M) was added to a stirred solution of 2,2-dimethylpropionic acid 9-(2-phenyl-2*H*-pentazol-1-sulfonyl)nonyl ester **26** (27.03 g, 61.97 mmol)[43] and (*R*)-3-((*S*)-aldehyde **8** (8.20 g, 47.6 mmol)[39, 45-47] in dry THF (200 mL) at –10 °C under nitrogen, then allowed to reach r.t. and stirred for 2 h. Petrol/ether (1:1, 100 mL) and sat. aq. NH_4Cl (100 mL) were added, the organic layer was separated and the aq. layer was extracted with petrol/ether (1:1, 2 × 100 mL). The combined organic layers were evaporated. Chromatography (petrol/ether, 50:1) gave a colourless oil, (*E/Z*)(*R*)-12-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)tridec-9-en-1-yl pivaloate (14 g, 76%) in ratio (2.5:1). Palladium 10 % on carbon (1 g) was stirred with the above alkenes (14 g, 36 mmol) in IMS (200 mL) under hydrogen for 2 h. The solution was filtered on a bed of celite and evaporated to give a colourless oil of the title compound **27** (13 g, 92%),$[\alpha]_D^{26}$ +16.1 (c 1.14, $CHCl_3$) [Found $[M - CH_3]^+$: 369.2989, $C_{22}H_{41}O_4$ requires: 369.2989]; δ_H (400 MHz, $CDCl_3$): 4.05 (2H, t, *J* 6.6 Hz), 4.0 (1H, dd, *J* 6.3, 7.9 Hz), 3.87 (1H, br q, *J* 6.9 Hz), 3.60 (1H, br t, *J 7.9* Hz), 1.62 (2H, quintet, *J* 6.6 Hz,), 1.40 (3H, s), 1.35 (3H, s), 1.32 – 1.26 (18H, m), 1.18 (9H, s), 1.1 – 1.05 (1H, m), 0.96 (3H, d, *J* 6.6 Hz). δ_C (126 MHz, $CDCl_3$) : 178.6, 108.5, 80.4, 67.8, 64.5, 38.7, 36.5, 32.7, 29.9, 29.7, 29.6, 29.59, 29.54, 29.50, 29.2, 28.6, 27.2, 27.0, 26.6, 25.9, 25.5, 15.6; υ_{max}/cm^{-1}: 2926, 2855, 1731, 1463, 1368, 1284, 1158.

3.9 5-(((*R*)-12-((*S*)-2,2-Dimethyl-1,3-dioxolan-4-yl)tridecyl)sulfonyl)-1-phenyl-1*H*-tetrazole 28

(i) A solution of (*R*)-12-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)tridecyl pivaloate **27** (12.7 g, 33.1 mmol) in dry THF (100 mL) was added slowly to a stirred suspension of $LiAlH_4$ (1.88 g, 49.6 mmol) at –20 °C under nitrogen. The mixture was heated under reflux for 1h, then quenched with sat. aq. Na_2SO_4 (10 mL) at –10 °C, stirred until a white precipitate formed, then $MgSO_4$ (20 g) was added. The precipitate was filtered through celite and washed with THF (2 × 50 mL). Column chromatography (5:1 petrol/ ether) gave a colourless oil, *(R)-12-((S)-2,2-dimethyl-1,3-dioxolan-4-yl)-tridecan-1-ol* (8.0 g, 81%), $[\alpha]_D^{23}$ +19.3 (c 0.95, $CHCl_3$) [Found $[M–CH_3]^+$: 285.2443, $C_{17}H_{33}O_3$

requires: 285.2425]; δ_H (400 MHz, $CDCl_3$)*:* 3.84 (1H, dd, *J* 6.2, 7.7 Hz), 3.77 (1H, br q, *J* 7.0 Hz), 3.50 (1H, br t, *J* 7.7 Hz), 3.41 (2H, t, *J* 6.6 Hz,), 1.52 – 1.47 (1H, m), 1.45 (3H, s), 1.43 – 1.40 (1H, m), 1.36 (3H, s), 1.34 –1.15 (20H, m), 1.01 (3H, d, *J* 6.9 Hz); δ_C (126 MHz, $CDCl_3$): 108.7, 80.6, 68.2, 62.7, 37.0, 33.3, 33.2, 30.4, 30.2, 30.18, 30.15, 30.14, 30.12, 30.0, 27.4, 27.0, 26.3, 25.9, 15.9; υ_{max}/cm^{-1}: 3395, 2926, 2853, 1466, 1377, 1266, 1205, 1155, 1058.

(ii) Diethyl azodicarboxylate (6.1, 35 mmol) in dry THF (2 mL) was added to a stirred solution of 1-phenyl-1*H*-tetrazole-5-thiol (6.20 g, 35.0 mmol), Ph_3P (9.12 g, 35.0 mmol) and (*R*)-12-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)tridecan-1-ol (8.00 g, 27.0 mmol) in dry THF (50 mL) at 0 ^oC. The mixture was vigorously stirred at r. t. for 16 h. The solvent was evaporated and the residue was diluted with petrol/EtOAc (10:1), refluxed for 0.5 h then filtered, washed with petrol/EtOAc and the filtrate evaporated. Chromatography (petrol/ether, 5:1) gave a white solid, *5*-(((*R*)-12-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)tridecyl)thio)-1-phenyl-1H-tetrazole (10.0 g, 84%), $[\alpha]_D^{22}$ +13.6 (c 0.88, $CHCl_3$) [Found $[M–CH_3]^+$: 445.2635, $C_{24}H_{37}N_4O_2S$ requires: 445.2637]; δ_H (500 MHz, $CDCl_3$): 7.60 – 7.52 (5H, m), 4.0 (1H, dd, *J* 6.3, 7.9 Hz), 3.87 (1H, br q, *J* 7.1 Hz), 3.60 (1H, br t, *J* 7.6 Hz), 3.39 (2H, t, *J* 7.4 Hz,), 1.82 (2H, quintet, *J* 7.3 Hz), 1.58 – 1.53 (1H, m), 1.46 – 1.41 (2H, m), 1.40 (3H, s), 1.35 (3H, s), 1.33 – 1.25 (15H, m), 1.11 – 1.04 (1H, m), 0.96 (3H, d, *J* 6.6 Hz); δ_C (126 MHz, $CDCl_3$) : 154.5, 133.8, 130.0, 129.7, 123.8, 108.4, 80.4, 67.8, 36.5, 33.3, 32.7, 29.8, 29.6, 29.59, 29.5, 29.1, 29.0, 28.6, 27.0, 26.6, 25.5, 15.6; ν_{max}/cm^{-1}: 2925, 2854, 1598, 1501, 1464, 1380, 1245, 1161, 1065.

(iii) A solution of ammonium molybdate (VI) tetrahydrate (13 g, 10 mmol) in 35 % H_2O_2 (40 mL) was cooled in an ice bath and added to the above tetrazole (10.3 g, 22.0 mmol) in THF (100 mL) and IMS (250 mL) at 10 ^oC and stirred at r.t for 2 h. A further solution of ammonium molybdate (6.9 g, 5.6 mmol) in 35 % H_2O_2 (20 mL) was added and the mixture was stirred at r.t. for 18 h. The mixture was poured into water (1.2 L) and extracted with CH_2Cl_2 (1 × 200 mL, 3 × 30 mL). The combined organic phases were washed with water (500 mL), dried and evaporated. Chromatography (petrol/ether, 5:1) gave a white solid, the title compound **28** (9.2 g, 84%), $[\alpha]_D^{25}$ +12.6 (c 1.01, $CHCl_3$) [Found $[M – CH_3]^+$: 477.2523, $C_{24}H_{37}N_4O_4S$ requires: 477.2531]; δ_H (500 MHz, $CDCl_3$): 7.71 – 7.69 (2H, m), 7.65 – 7.59 (3H, m), 4.00 (1H, dd, *J* 6.3, 7.9 Hz), 3.87 (1H, br q, *J* 7.0 Hz), 3.75 – 3.72 (2H, m), 3.60 (1H, br t, *J* 7.9 Hz), 1.99 – 1.92 (2H, m), 1.53 – 1.47 (2H, m), 1.41 (3H, s), 1.35 (3H, s), 1.32 – 1.26 (16H, m), 1.11 – 1.04 (1H, m), 0.96 (3H, d, *J* 6.6 Hz); δ_C (126 MHz, $CDCl_3$): 153.5, 133.0, 131.4, 129.7, 125.0, 108.5, 80.4, 67.8, 56.0, 36.5, 33.7, 29.8, 29.6, 29.58, 29.5, 29.4, 29.2, 28.9, 28.1, 27.0, 26.6, 25.5, 21.9, 15.6; υ_{max}/cm^{-1}: 3069, 2917, 2854, 1594, 1498, 1473, 1356, 1259, 1209, 1150, 1064.

3.10 Methyl (*R*)-2-((1*R*,15*R*)-1-((*tert*-butyldimethylsilyl)-oxy)-15-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)hexadecyl)-hexacosanoate 30

LiHMDS (9.3 mL, 9.9 mmol, 1.06 M) was added to a stirred solution of sulfone **28** (3.23 g, 6.57 mmol) and ester **29** (3.27 g, 5.49 mmol)[43] in dry THF (50 mL) at –10 ^oC under nitrogen. The mixture was allowed to reach r. t., stirred for 2 h, then quenched with sat. aq. NH_4Cl (100 mL) and diluted with petrol/ether (100 mL). The organic phase was separated and the aqueous layer was extracted with petrol/ether (1:1, 2 × 100 mL). The combined organic layers were dried and tevaporated. Chromatography (15:1 petrol/ether) gave a colourless oil, methyl (*E/Z*)-(2*R*)-2-((1*R*,15*R*)-1-((*tert*-butyldimethylsilyl)oxy)-15-(2,2-dimethyl-1,3-dioxolan-4-yl)hexadec-3-en-1-yl)hexacosanoate (4.5 g, 96%) as a 2.5: 1 mixture. Palladium (10% on carbon, 1.0 g) was added to the above alkenes (4.5 g, 5.21 mmol) in EtOAc (150 mL) under hydrogen and stirred for 1.5 h. The solution was filtered over celite and evaporated. Chromatography (petrol/ether, 15:1) gave a colourless oil, the title compound **30** (4.3 g, 96%), $[\alpha]_D^{22}$ +3.6 (*c* 0.94, $CHCl_3$) [Found $[M+Na]^+$: 887.7857, $C_{54}H_{108}NaO_5Si$ requires: 887.7863]; δ_H: 4.0 (1H, dd, *J* 6.3, 7.9 Hz), 3.93 – 3.90 (1H, m), 3.87 (1H, br q, *J* 7.0 Hz), 3.66 (3H, s), 3.61 (1H, br t, *J* 7.9 Hz), 2.53 (1H, ddd, *J* 3.8, 7.3, 11.1 Hz), 1.59 –1.52 (4H, m), 1.41 (3H, s), 1.36 (3H, s), 1.35-1.26 (68H, m, v. br), 1.10 – 1.07 (1H, m), 0.97 (3H, d, *J* 6.6 Hz), 0.89 (3H, t, *J* 6.9 Hz), 0.87 (9H, s), 0.05 (3H, s), 0.03 (3H, s); δ_C (126 MHz, $CDCl_3$): 175.1, 108.5, 80.4, 73.2, 76.8, 51.6, 51.2, 36.5, 33.7, 32.7, 31.9, 29.9, 29.8, 29.7, 29.66, 29.62, 29.60, 29.58, 29.5, 29.4, 29.3, 27.8, 27.5, 27.0, 26.6, 25.7, 25.5, 23.7, 22.7, 18.0, 15.6, 14.1, –4.4, –4.9; ν_{max}/cm^{-1}: 2925, 2854, 1741, 1465, 1368, 1253, 1165, 1068.

3.11 Methyl (*R*)-2-((1*R*,15*R*)-1-((*tert*-butyldimethylsilyl)-oxy)-15-methyl-16-oxohexadecyl)hexacosanoate 31

Periodic acid (0.40 g, 1.7 mmol) was added to a stirred solution of ester **30** (0.50 g, 0.58 mmol) in dry ether (35 mL) at r.t. under nitrogen and stirred 18 h. The mixture was filtered through celite and washed with ether (20 mL). The solvent was evaporated and the product was purified by column chromatography eluting with petrol/EtOAc (5:1) to give a white solid, the title compound **31** (0.4 g, 87%) [Found $[M–^tBu]^+$: 735.6679; $C_{46}H_{91}O_4Si$ requires: 735.6687]; δ_H (500 MHz, $CDCl_3$): 9.77 (1H, t, *J* 1.9 Hz), 5.11 – 5.07 (1H, m), 3.92 – 3.89 (1H, m), 3.66 (3H, s), 2.52 (1H, ddd, *J* 3.8, 7.0, 11.0 Hz), 2.42 (2H, dt, *J* 1.9, 7.6 Hz), 1.63 (2H, pent, *J* 6.3 Hz), 1.55 – 1.22 (68H, m), 1.09 (3H, d, *J* 7.0 Hz), 0.88 (3H, t, *J* 7 Hz), 0.87 (9H, s), 0.05 (3H, s), 0.027 (3H, s); δ_C (126 MHz, CDCl3): 202.8, 175.1, 73.2, 51.6, 51.2, 43.9, 33.7, 31.9, 29.8, 29.7, 29.65, 29.6, 29.5, 29.4, 29.35, 29.3, 29.2, 27.9, 27.5, 25.8, 23.8, 22.7, 22.1, 18.0,14.1, –4.4, –4.9; ν_{max}/cm^{-1}: 2924, 2853, 1738, 1464.

3.12 (21*S*,22*S*)-21-Methoxy-22-methyltetracontyl pivaloate 33

LiHMDS (1.1 mL, 1.0 mmol, 1.06 M) was added dropwise to a stirred solution of (8*S*, 9*S*)-8-methoxy-9-methyl-heptacosanal **32** (0.34 g, 0.77 mmol)[39] and 2,2-dimethyl-propionic acid 13-(1-phenyl-1*H*-tetrazole-5-sulfonyl)-tridecyl ester **7** (0.42 g, 0.93 mmol) in dry THF (15 mL) under nitrogen at –15 ^oC. The solution was allowed to reach r.t. and stirred for 2 h. Work up as in 3.10 and chromatography (petrol/EtOAc, 20:1) gave a colourless oil, (21*S*,22*S*,*E*)-21-methoxy-22-methyltetracont-13-en-1-yl pivaloate (0.46 g, 85%) in ratio 2.6:1. Palladium 10% on

carbon (0.5 g) was added to a stirred solution of the alkene (0.46 g, 0.65 mmol) in IMS (15 mL) and THF (5 mL) under hydrogen. Hydrogenation was carried out for 1 h. The mixture was filtered over celite and the solvent was evaporated. Chromatography eluting with petrol/EtOAc (20:1) to give a colourless oil, of the title compound **33** (0.46g, 100 %), $[\alpha]_D^{22}$ –6.5 (c 1.50, $CHCl_3$) [Found $[M+Na]^+$: 729.7102, $C_{47}H_{94}NaO_3$ requires: 729.7100]; δ_H: (500 MHz, $CDCl_3$): 4.05 (2H, t, *J* 6.7 Hz), 3.34 (3H, s), 2.97 – 2.94 (1H, m), 1.62 (2H, pent, *J* 6.7 Hz), 1.46 – 1.22 (70H, m), 1.20 (9H, s), 1.12 – 1.06 (1H, m), 0.88 (3H, t, *J* 6.7 Hz), 0.85 (3H, d, *J* 7.0 Hz); δ_C (126 MHz, $CDCl_3$): 178.5, 85.4, 64.4, 57.7, 38.7, 35.3, 32.4, 31.9, 30.8, 30.5, 30.0, 29.9, 29.7, 29.69, 29.66, 29.6, 29.5, 29.4, 29.2, 28.6, 27.6, 27.2, 26.2, 25.9, 22.7, 14.8, 14.1; ν_{max}/cm^{-1}: 2923, 1731,1154.

3.13 5-(((21*S*,22*S*)-21-Methoxy-22-methyltetracontyl)-sulfonyl)-1-phenyl-1*H*-tetrazole 34

(i) A solution of pivaloate **33** (0.75 g, 1.06 mmol) in HPLC grad THF (15 mL) was added to a stirred solution of LAH (0.2 g, 1.6 mmol) in THF (35 mL) at –20 °C under argon. The mixture was allowed to warm and refluxed for 1h, then sat. aq. sodium sulfate was added at –20 °C until a white precipitate had formed and THF (20 mL) was added. The mixture was stirred at r.t. for 30 min, filtered through celite and evaporated. Chromatography (petrol/EtOAc, 10:1 then 5:1) gave a white solid**,** (21*S*,22*S*)-21-methoxy-22-methyl-tetracontan-1-ol (0.65 g, 99 %), mp. 46 – 48 °C, $[\alpha]_D^{22}$ – 8.21 (c 1.21, $CHCl_3$) [Found $[M–H]^+$: 621.6545, $C_{42}H_{85}O_2$ requires: 621.6549]; δ_H: (500 MHz, $CDCl_3$): 3.66 (2H, t, *J* 6.6 Hz), 3.36 (3H, s), 2.99 – 2.96 (1H, m), 1.67 – 1.64 (1H, m), 1.59 (2H, pent, *J* 6.6 Hz), 1.49 (1H, br s), 1.48 – 1.22 (69H, m), 1.14 – 1.07 (1H, m), 0.90 (3H, t, *J* 7.0 Hz), 0.87 (3H, d, *J* 6.7 Hz); δ_C (126 MHz, $CDCl_3$): 85.5, 63.1, 57.7, 35.3, 32.8, 32.4, 31.9, 30.5, 30.0, 29.9, 29.7, 29.68, 29.63, 29.5, 29.4, 27.6, 26.2, 25.7, 22.7, 14.9, 14.1; ν_{max}/cm^{-1}: 3373, 2921, 1098,1076.

(ii) DEAD (0.27 g, 1.6 mmol) in dry THF (2 mL) was added to a stirred solution of 1-phenyl-1*H*-tetrazole-5-thiol (0.28 g, 1.6 mmol), Ph_3P (0.41 g, 1.6 mmol) and the above alcohol (0.75 g, 1.2 mmol) in dry THF (25 mL) at 0 °C, The mixture was vigorously stirred and refluxed for 2.5 h, then 5:1 petroleum/EtOAc (100 mL) was added. The mixture filtered through celite and the solvent was evaporated. Chroma-tography (petrol/ether, 5:1) gave a white solid, 5-(((21*S*, 22*S*)-21-methoxy-22-methyltetracontyl)thio)-1-methyl-1H-tetrazole (0.80 g, 91%), $[\alpha]_D^{22}$ –6.4 (c 1.2, $CHCl_3$) [MALDI–Found $(M+Na)^+$: 805.3, $C_{49}H_{90}NaN_4OS$ requires: 805.6]; δ_H: (500 MHz, $CDCl_3$): 7.65 – 7.61 (5H, m), 3.66 (2H, t, *J* 6.3 Hz), 3.36 (3H, s), 2.98 – 2.96 (1H, m), 1.97 (2H, br pent, *J* 7.5 Hz), 1.68 – 1.61 (2H, m), 1.52 (2H, br pent, *J* 7.5 Hz), 1.45 – 1.22 (66H, m), 1.15 – 1.07 (1H, m), 0.90 (3H, t, *J* 6.6 Hz), 0.87 (3H, d, *J* 6.9 Hz); δ_C: (126 MHz, $CDCl_3$): 153.5, 133.1, 131.5, 129.7, 125.1, 85.5, 57.7, 56.0, 35.3, 32.4, 31.9, 30.5, 30.0, 29.97, 29.7, 29.65, 29.6, 29.5, 29.4, 29.2, 28.9, 28.2, 27.6, 26.2, 22.7, 21.9, 14.9, 14.1; ν_{max}/cm^{-1}: 3072, 2917, 2853, 1343, 1096.

(iii) To a stirred solution of the above tetrazole (0.80 g, 1.0 mmol) and $NaHCO_3$ (0.34, 4.1 mmol) in CH_2Cl_2 (50 mL) was added m-chloroperbenzoic acid (0.41 g, 2.35 mmol) at r.t. and stirred for 24 h. The mixture was quenched with 10% NaOH solution (25 mL) and vigorously stirred for 2 h. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (2 × 50 mL). The combined organic layers were washed with water, dried and evaporated. Chromatography (petroleum/ether, 10:1) gave a white solid, sulfone **34** (0.81 g, 97%), mp.: 44 – 46 °C; $[\alpha]_D^{22}$ – 6.19 (c 1.39, $CHCl_3$) [MALDI–Found: $[M+Na]^+$: 837.3, $C_{49}H_{90}N_4NaO_3S$ requires: 837.6]; δ_H (500 MHz, CDCl3): 7.73 – 7.71 (2H, m), 7.65 – 7.61 (3H, m), 3.75 (2H, distorted t, *J* 7.9 Hz), 3.36 (3H, s), 2.98 – 2.96 (1H, m), 1.97 (2H, br pent, *J* 7.5 Hz), 1.68 –1.61 (2H, m), 1.52 (2H, br pent, *J* 7.5 Hz), 1.45 – 1.22 (66H, m), 1.15 – 1.07 (1H, m), 0.90 (3H, t, *J* 6.6 Hz), 0.87 (3H, d, *J* 6.9 Hz); δ_C (126 MHz, CDCl3): 153.5, 133.1, 131.5, 129.7, 125.1, 85.5, 57.7, 56.0, 35.3, 32.4, 31.9, 30.5, 30.0, 29.9, 29.7, 29.65, 29.6, 29.5, 29.4, 29.2, 28.9, 28.2, 27.6, 26.2, 22.7, 21.9, 14.9, 14.1; ν_{max}: 2924, 2849, 1343, 1157, 1096 cm^{-1}.

3.14 (2*R*,3*R*,17*R*,39*S*,40*S*,*E*)-3-Hydroxy-39-methoxy-17,40-dimethyl-2-tetracosyloctapentacont-18-enoic acid 37

(i) Ester **31** (0.30 g, 0.38 mmol) in dry 1,2-dimethoxyethane (60 mL) was added to stirred solution of sulfone **34** (0.34 g, 0.42 mmol) in dry 1,2-dimethoxyethane (25 mL) under nitrogen at RT. The mixture was cooled to –20 °C and potassium bis (trimethylsilyl)amide (1.08 mL, 0.42 mmol, 0.5 M in toluene) was added and the mixture allowed to reach r.t. and then stirred for 1.5 h. Sat. aq. NH_4Cl (30 mL) and petrol /ether (1:1, 55 mL) were added. The aqueous layer was extracted with petrol/ether (1:1, 2 × 45 mL). The combined organic layers were evaporated. Chromatography (petrol/ether, 18:1) gave a semi solid, methyl (2*R*,3*R*,17*R*, 39*S*,40*S*,*E*)-3-((*tert*-butyldimethylsilyl)oxy)-39-methoxy-17,40-dimethyl-2-tetracosyloctapentacont-18-enoate **35** (0.18 g, 37%) [MALDI–Found $[M+Na]^+$: 1404.3; $C_{92}H_{184}NaO_4Si$ requires: 1404.3]; δ_H: 5.33 (1H, dt, *J* 6.36, 15.0 Hz), 5.24 (1H, dd, *J* 7.4, 15.0 Hz), 3.9 (1H, td, *J* 4.3, 6.8 Hz), 3.65 (3H, s), 3.35 (3H, s), 2.98 – 2.94 (1H, m), 2.53 (1H, ddd, *J* 3.7, 7.0, 10.8 Hz), 2.05 – 2.0 (1H, m), 1.97 (2H, q, *J* 6.9 Hz), 1.68 – 1.26 (142H, m, v.br), 1.08 – 1.01 (1H, m), 0.94 (3H, d, *J* 7.0 Hz), 0.90 (6H, t, *J* 6.7 Hz), 0.87 (9H, s), 0.85 (3H, d, *J* 7.0 Hz), 0.52 (3H, s), 0.03 (3H, s); δ_C: (101 MHz, $CDCl_3$): 175.2, 136.5, 128.4, 85.4, 73.2, 57.7, 51.6, 51.2, 37.3, 36.7, 35.3, 33.7, 32.6, 32.4, 31.9, 30.5, 30.0, 29.9, 29.84, 29.8, 29.7, 29.66, 29.6, 29.5, 29.45, 29.4, 29.1, 27.8, 27.6, 27.5, 27.4, 26.2, 25.8, 23.7, 22.7, 20.9, 14.9, 14.1, –4.4, –4.9; ν_{max}/cm^{-1}: 2924, 2854, 1741, 1465, 1377, 1254, 1099.

(ii) The ester **35** (0.70 mg, 0.05 mmol) was dissolved in dry THF (10 mL) in a dry polyethylene vial under nitrogen at r.t. and stirred. Pyridine (0.07 mL) and HF–pyridine complex (0.5 mL) were added; the mixture was stirred for 17 h. at 42 °C, neutralized with sat. aq. $NaHCO_3$, then extracted with petrol/EtOAc (1:5, 3 × 50 mL). The combined organic layers were washed with brine, dried and evaporated; chromatography (petrol/EtOAc, 20:1) gave a white solid, methyl (2*R*,3*R*,17*R*,39*S*, 40*S*,*E*)-3-hydroxy-39-methoxy-17,40-dimethyl-2-tetracosyloctapentacont-18-en-oate **36** (0.62 g, 96%), m.p.: 34 – 36 °C [MALDI–Found

$[M+Na]^+$: 1290.3; $C_{86}H_{170}NaO_4$ requires: 1290.2]; δ_H: 5.33 (1H, dt, *J* 6.4, 15.0 Hz), 5.24 (1H, dd, *J* 7.5, 15.0 Hz), 3.72 (3H, s), 3.68 – 3.63 (1H, m), 3.35 (3H, s), 2.98 – 2.93 (1H, m), 2.46 – 2.41 (1H, td, *J* 5.4, 9.1 Hz), 2.05 – 2.0 (1H, m), 1.97 (2H, br q, *J* 7 Hz), 1.76 – 1.68 (1H, m), 1.65 – 1.5 (12H, m), 1.48 – 1.2 (128H, m), 1.15 – 1.05 (1H, m), 0.95 (3H, d, *J* 6.7 Hz), 0.89 (6 H, t, *J* 7.1 Hz), 0.86 (3H, d, *J* 6.8 Hz), 1.96 (2H, br q, *J* 7 Hz); δ_C (101 MHz, $CDCl_3$): 176.2, 136.5, 128.4, 85.4, 72.3, 57.7, 51.5, 50.9, 37.2, 36.7, 35.7, 35.3, 32.6, 32.4, 31.9, 30.5, 30.0, 29.9, 29.8, 29.7, 29.6, 29.56, 29.5, 29.4, 29.36, 29.1, 27.6, 27.4, 27.36, 26.2, 25.7, 22.7, 20.9, 14.9, 14.1; ν_{max}/cm^{-1}: 3431, 2917, 2850, 1739, 1466, 1099.

(iii) Lithium hydroxide monohydrate (0.31 g, 7.3 mmol) was added with stirring to ester **36** (0.62 g, 0.49 mmol) in THF (15 mL), methanol (1.3 mL) and water (1.5 mL) at r. t. The mixture was stirred at 43 °C for 18 h. The mixture was cooled to r.t. and acidified with HCl (5%, 2 mL) and the aqueous layer was extracted with warm petroleum/ether (1:1, 3 × 50 mL). The combined organic layers were dried and evaporated; chromatography (warm petroleum/EtOAc, 7:2) gave a white solid, compound **37** (0.50, 82%), m.p.: 43 – 45 °C [MALDI–Found $[M+Na]^+$: 1276.2861; $C_{85}H_{168}NaO_4$ requires: 1276.2835]; δ_H (500 MHz, CDCl3): 5.33 (1H, dt, *J* 6.4, 15.2 Hz), 5.24 (1H, dd, *J* 7.4, 15.2 Hz), 3.73 – 3.70 (1H, m), 3.35 (3H, s), 2.98 – 2.96 (1H, m), 2.49 – 2.44 (1H, m), 2.0 –1.97 (1H, m), 1.96 (2H, br q, *J* 6.7 Hz), 1.77 – 1.70 (1H, m), 1.67 – 1.57 (2H, m), 1.55 – 1.15 (141 H, m), 1.14 – 1.05 (1H, m), 0.94 (3H, d, *J* 6.7 Hz), 0.89 (6H, t, *J* 6.5 Hz), 0.85 (3H, d, *J* 6.9 Hz); δ_C (101 MHz, $CDCl_3$): 179.5, 136.5, 128.4, 85.6, 72.1, 57.7, 50.7, 37.3, 36.7, 35.6, 35.3, 32.6, 32.4, 31.9, 30.5, 29.9, 29.7, 29.5, 29.4, 29.36, 29.1, 27.6, 27.4, 22.4, 21.0, 14.9, 14.1; ν_{max}/cm^{-1}: 3470, 2917, 2854, 1726, 1689, 1471, 1392, 1202, 1099, 968, 887, 839.

3.15 (2*R*,3*R*,19*R*,41*S*,42*S*,*E*)-3-((*tert*-Butyldimethylsilyl)-oxy)-41-methoxy-19,42-dimethyl-2-tetracosylhexacont-20-enoic acid 44

Imidazole (0.125 g, 1.80 mmol) was added to a stirred solution of acid **37** (0.23 g, 0.18 mmol) in dry DMF (1.5 mL) and dry toluene (2.5 mL) at r. t. followed by the addition of TBDMSCl (0.27 g, 1.8 mmol) and DMAP (10 mg). The mixture was stirred at 70 °C for 24 h. The solvent was evaporated under reduced pressure and the residue was diluted with EtOAc (50 mL) and water (20 mL). The organic layer was separated and the aqueous layer was extracted with EtOAc (2 × 30 mL). The combined organic layers were washed with water, dried and evaporated to give a colourless oil. The residue was dissolved in THF (8 mL) and n-Bu_4NOH (0.9 mL, 4%) and stirred for 10 min. at r. t. then diluted with water (10 mL) and extracted with EtOAc (2 × 20 mL), dried and evaporated to give a residue; column chromatography (petroleum/EtOAc, 20:1) gave a colourless oil, the title compound **44** (0.25 g, 96%) [MALDI–Found $[M+Na]^+$: 1390.3; $C_{91}H_{182}NaO_4Si$ requires: 1390.3]; δ_H (500 MHz, $CDCl_3$): 5.33 (1H, dt, *J* 6.4, 15.0 Hz), 5.25 (1H, dd, *J* 7.5, 15.0 Hz), 3.86 – 3.80 (1H, m), 3.35 (3H, s), 2.99 – 2.94 (1H, m), 2.57 – 2.51 (1H, m), 2.07 – 2.01 (1H, m), 1.96 (2H, br q, *J* 6.8 Hz), 1.73 – 1.68 (1H, m), 1.65 –1.54 (4H, m), 1.5 – 1.2 (138H, m), 1.1 – 1.0 (1H, m), 0.95 (3H, s), 0.94 (9H, s), 0.89 (6H, t, *J* 6.7 Hz), 0.85 (3H, d, *J* 6.9 Hz), 0.16 (3H, s), 0.14 (3H, s); δ_C (101 MHz, $CDCl_3$): 174.9, 136.5, 128.4, 85.5, 73.7, 57.7, 50.0, 37.3, 36.7, 35.8, 35.3, 32.6, 32.4, 31.9, 30.5, 30.1, 30.0, 29.9, 29.8, 29.7, 29.63, 29.6, 29.54, 29.5, 29.4, 29.36, 29.1, 27.6, 27.4, 27.36, 26.2, 25.9, 25.7, 25.5, 25.1, 22.7, 20.9, 17.9, 14.9, 14.1, –4.3, –4.9; ν_{max}: 2922, 2852, 1709, 1467, 1362, 1253, 1180, 1101, 836, cm^{-1}.

3.16 6,6'-Bis-*O*-(2*R*,3*R*,17*R*,39*S*,40*S*,*E*)-3-((*tert*-butyldimethylsilyl)oxy)-39-methoxy-17,40-dimethyl-2-tetracosylocta-pentacont-18-enoic-2,3,4,2',3',4'-hexakis-*O*-(trimethylsilyl)-α,α'-trehalose 46 and 6-*O*-(2*R*,3*R*,17*R*,39*S*,40*S*,*E*)-3-((*tert*-butyldimethylsilyl)oxy)-39-methoxy-17,40-dimethyl-2-tetracosylocta-pentacont-18-enoic-2,3,4,2',3',4'-hexakis-*O*-(trimethylsilyl)-α,α'-trehalose 47

1-(3-Dimethylaminopropyl)-3-ethylcarbodiimidehydrochloride (EDCI) (0.044 g, 0.230 mmol) and DMAP (0.028 g, 0.23 mmol) were added to a stirred solution of acid **44** (0.090 g, 0.065 mmol), protected α,α'-trehalose **45** (0.022 g, 0.028 mmol)[67] and powdered 4 Å molecular sieve in dry CH_2Cl_2 (1 mL) at r. t. under nitrogen. The mixture was stirred for 6 days, diluted with CH_2Cl_2 (3 mL) and silica (5 g), then the solvent was evaporated. Chromatography (petroleum/EtOAc, 20:1) gave a first fraction, the TDM **46** (0.228 g, 25%), $[\alpha]_D^{22}$ +19 (c 1.2, $CHCl_3$) [MALDI–Found $[M+Na]^+$: 3496.4; $C_{212}H_{430}NaO_{17}Si_8$ requires: 3496.0]; δ_H (400 MHz, $CDCl_3$): 5.35 (2H, dt, *J* 6.4, 15.3 Hz), 5.25 (2H, br dd, *J* 7.4, 15.3 Hz), 4.85 (2H, br d, *J* 3.0 Hz), 4.36 (2H, br d, *J* 10.8 Hz), 4.01 (2H, br tt, *J* 3.1, 10.8 Hz), 3.94 (2H, br pent, *J* 5.1 Hz), 3.9 (2H, br t, *J* 8.8 Hz), 3.52 (2H, br t, *J* 9.0 Hz), 3.38 (2H, dd, *J* 2.9, 9.3 Hz), 3.34 (6H, s), 2.96 (2H, br pent, *J* 4.2 Hz), 2.57 – 2.53 (2H, m), 2.03 (2H, br pent, *J* 6.5 Hz), 1.96 (4H, br q, *J* 6.7 Hz), 1.7 – 1.2 (292H, m), 1.13 – 1.07 (2H, m), 0.9 (6H, d, *J* 6.7 Hz), 0.89 (12H, t, *J* 6.6 Hz), 0.88 (18H, s), 0.16 (18H, s), 0.15 (18H, s), 0.14 (18H, s), 0.06 (12H, s); δ_C (101 MHz, $CDCl_3$): 173.8, 136.5, 128.4, 94.8, 85.4, 73.5, 73.4, 72.8, 71.8, 70.7, 62.4, 57.7, 51.9, 37.3, 36.7, 35.3, 32.6, 32.4, 31.9, 30.5, 30.0, 29.95, 29.9, 29.7, 29.66, 29.6, 29.5, 29.4, 29.2, 27.6, 27.4, 26.2, 25.8, 22.7, 20.9, 18.0, 14.9, 14.1, 1.1, 0.9, 0.2, –4.5, –4.7; ν_{max}: 2924, 2853, 1743, 1466, 1377, 1251, 1163, 1100 cm^{-1}. The second fraction was TMM **47** (0.072 g, 50%), $[\alpha]_D^{22}$ +32 (c 1.1, $CHCl_3$) [MALDI–Found $[M+Na]^+$: 2146.7; $C_{121}H_{250}NaO_{14}Si_7$ requires: 2146.7]; δ_H (500 MHz, $CDCl_3$): 5.34 (1H, dt, *J* 6.4, 15.3 Hz), 5.24 (1H, 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* 2.0, 11.8 Hz), 4.08 (1H, dd, *J* 4.1, 11.8 Hz), 4.0 (1H, td, *J* 2.2, 9.5 Hz), 3.96 – 3.92 (2H, m), 3.89 (1H, dd, *J* 5.2, 8.9 Hz), 3.84 (1H, td, *J* 3.0, 9.4 Hz), 3.73 – 3.64 (2H, m), 3.5 (1H, dd, *J* 3.7, 9.0 Hz), 3.49 (1H, dd, *J* 3.7, 9.0 Hz), 3.47 (1H, dd, *J* 3.7, 9.0 Hz), 3.43 (1H, dd, *J* 3.0, 9.3 Hz), 3.4 (1H, dd, *J* 3.0, 9.3 Hz), 3.35 (3H, s), 2.96 (1H, br pent, *J* 4.2 Hz), 2.56 (1H, ddd, *J* 3.36, 5.4, 9.7 Hz), 2.1 – 2.0 (1H, m), 1.96 (2H, br q, *J* 6.7 Hz), 1.72 (1H, br t, *J* 5.2, 6.9 Hz), 1.67 – 1.57 (3H, m), 1.5 – 1.15 (138H, m), 1.3 – 1.07 (1H, m), 0.94 (3H, d, *J* 6.7 Hz), 0.89 (6H, t, *J* 6.6 Hz), 0.87 (9H, s), 0.85 (3H, d, *J* 7.0 Hz), 0.17 (9H, s), 0.16 (9H, s), 0.15 (9H, s), 0.148 (18H, s), 0.12 (9H, s), 0.06 (3H, s), 0.055 (3H, s); δ_C (101 MHz, $CDCl_3$): 174.1, 136.5, 128.4, 94.5, 94.4, 85.4, 73.4, 73.3, 72.9, 72.8, 72.7, 72.0, 71.4, 70.7, 62.5, 61.7, 57.7, 51.8, 37.3, 36.7, 35.3, 33.4, 32.6,

32.3, 31.9, 30.5, 30.0, 29.9, 29.8, 29.7, 29.66, 29.5, 29.4, 29.2, 28.1, 27.6, 27.4, 26.4, 26.2, 25.8, 24.8, 22.7, 20.9, 18.0, 14.9, 14.1, 1.1, 1.0, 0.9, 0.85, 0.2, 0.04, –4.5, –4.7; ν_{max}: 3608, 2925, 2854, 1743, 1466, 1251, 1100, 1077, 844 cm^{-1}.

3.17 6,6'-Bis-*O*-(2*R*,3*R*,17*R*,39*S*,40*S*,*E*)-3-hydroxy-39-methoxy-17,40-dimethyl-2-tetracosylocta-pentacont-18-enoic-*α*,*α*'-trehalose 49

(i) n-Bu_4NF (0.1 mL, 0.1 mmol, 1 M) was added to a stirred solution of diester **46** (0.06 g, 0.017 mmol) in dry THF (5 mL) at 5 ^oC under nitrogen, allowed to reach r.t., stirred for 1 h, then evaporated under reduced pressure. Chroma-tography ($CHCl_3$/MeOH, 10:1) gave 6,6'-bis-*O*-(2*R*,3*R*,17*R*, 39*S*,40*S*,*E*)-3-((*tert*-butyldimethylsilyl)oxy)-39-methoxy-17,40-dimethyl-2-tetracosyloctapentacont-18-enoic-α,α'-trehalose **48** (0.035 g, 67%), $[\alpha]_D^{22}$ +19.5 (c 0.50, $CHCl_3$) [MALDI–Found $[M+Na]^+$: 3063.9; $C_{194}H_{382}NaO_{17}Si_2$ requires: 3063.8]; δ_H (500 MHz, $CDCl_3$ + few drops of CD_3OD): 5.27 (2H, dt, *J* 6.5, 15.3 Hz), 5.18 (2H, dd, *J* 7.4, 15.3 Hz), 5.04 (2H, d, *J* 3.4 Hz), 4.3 (2H, br dd, *J* 3.8, 12.0 Hz), 4.2 (2H, br d, *J* 10.84 Hz), 3.91 (2H, br d, *J* 9.4 Hz), 3.85 (2H, br q, *J* 5.0 Hz), 3.76 (2H, br t, *J* 9.3 Hz), 3.44 (2H, dd, *J* 3.4, 9.6 Hz), 3.32 (2H, br m), 3.3 (6H, s), 2.94 – 2.91 (2H, m), 2.53 – 2.48 (2H, m), 1.97 (2H, br pent, *J* 6.3 Hz), 1.91 (2H, br q, *J* 6.8 Hz), 1.57 – 1.1 (292H, m), 1.07 – 1.0 (2H, m), 0.88 (6H, d, *J* 6.7 Hz), 0.84 (12H, t, *J* 6.3 Hz), 0.81 (18H, s), 0.8 (6H, d, *J* 7.3 Hz), –0.003 (6H, s), –0.023 (6H, s); δ_C (101 MHz, $CDCl_3$ + CD_3OD): 175.2, 136.4, 128.3, 93.5, 85.5, 73.2, 73.1, 71.6, 70.2, 69.9, 62.9, 57.6, 51.59, 37.1, 36.57, 35.2, 33.5, 32.5, 32.3, 31.8, 30.4, 29.83, 29.8, 29.7, 29.63, 29.6, 29.57, 29.4, 29.2, 29.0, 27.7, 27.4, 27.3, 26.9, 26.0, 25.7, 25.6, 24.1, 22.6, 20.8, 17.8, 14.7, 13.9, –4.6, –5.0; ν_{max}: 3400, 2924, 2853, 1742, 1464, 1100 cm^{-1}.

(ii) A dry polyethylene vial equipped with a rubber septum was charged with **48** (0.022 g, 0.007 mmol) and pyridine (0.05 mL) in dry THF (1.3 mL) and stirred at r. t. under argon. To it was added hydrogen fluoride-pyridine complex (~70% HF, 0.2 mL). The mixture was stirred at 43 ^oC for 17 h, then poured slowly into sat. aq. $NaHCO_3$ until no more CO_2 was liberated. The product was extracted with $CHCl_3$ (3 × 50 mL); the combined organic layers were evaporated to give a residue. Chromatography ($CHCl_3$/MeOH, 10:1) gave compound **49** (0.015, 75%), $[\alpha]_D^{22}$ +18 (c 1.0, $CHCl_3$) [Found $[M+Na]^+$: 283.6751; $C_{182}H_{354}NaO_{17}$ requires: 2835.6728]; δ_H (500 MHz, $CDCl_3$ + few drops of CD_3OD): 5.3 (2H, dt, *J* 6.2, 15.6 Hz), 5.21 (2H, dd, *J* 7.2, 15.6 Hz), 5.0 (2H, br d, *J* 3.4 Hz), 4.68 (2H, br d, *J* 11.9 Hz), 4.23 (2H, br t, *J* 9.4 Hz), 4.0 – 3.94 (2H, m), 3.74 (2H, br t, *J* 9.3 Hz), 3.67 – 3.62 (2H, m), 3.5 (2H, dd, *J* 2.8, 9.6 Hz), 3.32 (6H, s), 3.21 (2H, t, *J* 9.3 Hz), 2.96 – 2.93 (2H, m), 2.4 (2H, m), 1.97 – 2.02 (2H, m), 1.93 (4H, br q, *J* 6.7 Hz), 1.65 – 1.01 (294H, m), 0.91 (6H, d, *J* 6.7 Hz), 0.86 (12H, t , *J* 6.7 Hz), 0.82 (6H, d, *J* 7 Hz); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 175.2, 136.2, 128.2, 94.0, 85.5, 72.8, 72.3, 71.3, 70.6, 69.8, 63.5, 57.4, 52.4, 37.0, 36.5, 35.1, 34.6, 32.3, 32.1, 31.7, 30.2, 29.7, 29.6, 29.6, 29.4, 29.3, 29.3, 29.2, 29.1, 29.0, 28.9, 27.2, 27.1, 27.1, 25.8, 25.1, 22.4, 20.6, 14.5, 13.8; υ_{max} 3584, 3395, 2923, 2853, 1732, 1464, 1376 cm^{-1}.

3.18 6-*O*-(2*R*,3*R*,17*R*, 39*S*,40*S*,*E*)-3-Hydroxy-39-methoxy -17,40-dimethyl-2-tetracosylocta-pentacont-18-enoic-*α*,*α*'-trehalose 51

(i) n-Bu_4NF (0.16 mL, 0.16 mmol, 1M) was added with stirring to monoester **47** (0.058 g, 0.027 mmol) in dry THF (2 mL) at 5 ^oC under nitrogen, stirred at r.t. for 1 h, then worked up and purified as before to give 6-*O*-(2*R*,3*R*,17*R*, 39*S*,40*S*,*E*)-3-((*tert*-butyldimethylsilyl)oxy)-39-methoxy-17,40-dimethyl-2-tetracosyloctapentacont-18-enoic-α,α'-trehalose **50** (0.030, 65%) as a colourless oil, $[\alpha]_D^{22}$ +39.5 (c 0.75, $CHCl_3$) [MALDI–Found $[M+Na]^+$: 1714.2; $C_{103}H_{202}NaO_{14}Si$ requires: 1714.4]; δ_H (500 MHz, $CDCl_3$ + few drop of CD_3OD): 5.28 (1H, dt, *J* 6.5, 15.5 Hz), 5.19 (1H, br dd, *J* 7.6, 15.5 Hz), 5.05 (2H, br t, *J* 3.5 Hz), 4.3 (1H, dd, *J* 4.6, 12.3 Hz), 4.23 (1H, dd, *J* 2.3, 12.3 Hz), 3.9 (1H, br d, *J* 8.3 Hz), 3.87 – 3.84 (1H, m), 3.81 – 3.71 (4H, m), 3.65 (1H, dd, *J* 5.2, 11.6 Hz), 3.47 (1H, dd, *J* 3.9, 5.9 Hz), 3.46 (1H, dd, *J* 5.9, 9.7 Hz), 3.3 (3H, s), 2.93 – 2.91 (1H, m), 2.6 – 2.48 (1H, m), 1.99 – 1.95 (1H, m), 1.91 (2H, br q, *J* 7.0 Hz), 1.6 – 1.55 (1H, m), 1.45 – 1.15 (150H, m), 1.07 – 1.03 (1H, m), 0.89 (3H, d, *J* 6.7 Hz), 0.83 (6H, t, *J* 7.0 Hz), 0.81 (9H, s), 0.8 (3H, d, *J* 7.0 Hz), –0.003 (3H, s), –0.024 (3H, s); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 174.9, 136.4, 128.3, 93.5, 93.4, 85.5, 73.2, 72.2, 71.7, 70.7, 70.3, 70.0, 62.7, 62.0, 58.7, 57.6, 52.1, 51.7, 50.1, 37.2, 36.6, 35.2, 32.5, 32.3, 31.8, 30.4, 29.8, 29.8, 29.7, 29.7, 29.6, 29.5, 29.4, 29.2, 29.0, 27.4, 27.3, 26.0, 25.7, 25.1, 23.8, 22.6, 20.8, 20.0, 19.6, 17.9, 14.7, 14.0, 13.4, 13.38, –4.6, –5.0; υ_{max}: 3903, 3368, 2918, 2850, 1733, 1468, 1378, 1253, 1148, 1102, 1078, 1051, 992, 941, 836, 775, 721 cm^{-1}.

(ii) Compound **50** (0.025 g, 0.015 mmol) and pyridine (0.05 mL) in dry THF (3 mL) in a dry polyethylene vial equipped with a rubber septum was stirred at r. t. under argon, and hydrogen fluoride-pyridine complex (~70% HF, 0.25 mL) was added. The mixture was stirred at 43 ^oC for 17 h, then worked up and purified as above to give the title compound **51** (0.015, 65%), $[\alpha]_D^{22}$ +34 (c 0.52, $CHCl_3$) [MALDI–Found $[M+Na]^+$: 1600.3879; $C_{97}H_{188}NaO_{14}$ requires: 1600.3897]; δ_H (400 MHz, $CDCl_3$): 5.26 (1H, dt, *J* 6.6, 15.2 Hz), 5.17 (1H, br dd, *J* 7.4, 15.2 Hz), 5.03 (1H, d, *J* 3.2 Hz), 4.98 (1H, d, *J* 3.3 Hz), 4.61 (1H, br d, *J* 11 Hz), 4.18 (1H, br t, *J* 7.9 Hz), 3.95 (1H, dd, *J* 7.8, 12.0 Hz), 3.87 – 3.82 (1H, m), 3.79 – 3.71 (2H, m), 3.62 –3.55 (2H, m), 3.5 (1H, dd, *J* 3.2, 9.8 Hz), 3.44 (1H, dd, *J* 3.3, 9.8 Hz), 3.32 (8H, br s), 3.28 (3H, s), 3.23 (1H, br t, *J* 9.5 Hz), 3.18 (1H, br t, *J* 9.6 Hz), 2.93 – 2.89 (1H, m), 2.38 –2.33 (1H, m), 1.99 – 1.94 (1H, m), 1.9 (2H, br q, *J* 6.8 Hz), 1.57 – 1.10 (143H, m), 1.06 – 0.98 (1H, m), 0.87 (3H, d, *J* 6.7 Hz), 0.81 (6H, t, *J* 6.4 Hz), 0.78 (3H, d, *J* 7.1 Hz); δ_C (101 MHz, $CDCl_3$): 175.4, 136.3, 128.3, 94.6, 85.5, 72.6, 72.4, 72.3, 71.5, 71.2, 71.1, 70.8, 69.9, 64.2, 62.3, 62.1, 57.5, 52.3, 37.1, 36.5, 35.2, 34.6, 32.4, 32.2, 31.8, 30.3, 29.8, 29.7, 29.7, 29.5, 29.4, 29.3, 29.2, 29.0, 27.3, 27.2, 27.1, 25.9, 25.1, 22.5, 20.7, 14.6, 13.9; υ_{max}: 3364, 2918, 2850, 1728, 1467, 1148, 992 cm^{-1}.

3.19 6-*O*-(2*R*,3*R*,17*R*,39*S*,40*S*, *E*)-3-Hydroxy-39-methoxy -17,40-dimethyl-2-tetracosyloctapentacont-18-enoate-*α*/β-D-glucopyranoside 54

(i) The acid **37** (0.054 g, 0.043 mmol) was dissolved in a mixture of THF and DMF (THF-DMF, 5:1, 1.5 mL) at r. t.

then warmed gently until all had dissolved. Dry $CsHCO_3$ (0.072 mg, 0.371 mmol) was added to a stirred solution and the mixture was left at r. t. for 1 h. then tosylate **52** (0.33 g, 0.047 mmol) was added. The mixture was stirred at 70 ^oC for 18 h then quenched with water (5 mL). The product was extracted with CH_2Cl_2 (3 × 60 mL) and the combined organic layers were dried and evaporated. Chromatography (petroleum/EtOAc, 10:1) gave benzyl 2,3,4-tri-*O*-benzyl-6-*O*-(2*R*,3*R*,17*R*,39*S*,40*S*,*E*)-3-hydroxy-39-methoxy-17,40-dimethyl-2-tetracosyloctapentacont-18-enoate-β-D-glucopyranoside **53** (0.056, 70%), $[\alpha]_D^{22}$ +15 (c 1.1, $CHCl_3$) [MALDI–Found $[M+Na]^+$: 1798.6; $C_{119}H_{202}NaO_9$ requires: 1798.5]; δ_H (400 MHz, $CDCl_3$): 7.4 –7.26 (20H, m), 5.34 (1H, dt, *J* 6.4, 15.4 Hz), 5.24 (1H, dd, *J* 7.4, 15.4 Hz), 4.97 (1H, d, *J* 11.0 Hz), 4.95 (1H, d, *J* 11.0 Hz), 4.93 (1H, d, *J* 11.0 Hz), 4.9 (1H, d, *J* 12 Hz), 4.78 (1H, d, *J* 11 Hz), 4.71 (1H, d, *J* 11 Hz), 4.64 (1H, d, *J* 12 Hz), 4.61 (1H, d, *J* 11 Hz), 4.54 (1H, br t, *J* 6.4 Hz), 4.52 (1H, br s), 4.22 (1H, dd, *J* 4.6, 11 Hz), 3.67 (2H, br t *J* 8.7 Hz), 3.54 – 3.47 (3H, m), 3.35 (3H, s), 2.96 (1H, br pent, *J* 4.2 Hz), 2.5 – 2.46 (2H, m), 2.06 – 2.01 (1H, m), 1.97 (2H, br q, *J* 6.7 Hz), 1.8 – 1.7 (1H, m), 1.68 – 1.55 (3H, m), 1.5 – 1.2 (138H, m), 1.15 – 1.05 (1H, m), 0.94 (3H, d, *J* 6.7 Hz), 0.89 (6H, t, *J* 7 Hz), 0.85 (3H, d, *J* 6.7 Hz); δ_C (101 MHz, $CDCl_3$): 175.2, 138.4, 138.2, 137.7, 137.1, 136.5, 128.5, 128.4, 128.35, 128.1, 128.0, 127.97, 127.9, 127.85, 127.7, 102.3, 85.4, 84.5, 82.3, 77.8, 75.8, 75.1, 74.9, 72.8, 72.3, 71.1, 62.9, 57.7, 51.3, 37.2, 36.7, 35.6, 35.3, 32.6, 32.3, 31.9, 30.5, 30.0, 29.9, 29.8, 29.7, 29.66, 29.6, 29.58, 29.53, 29.5, 29.4, 29.1, 27.6, 27.5, 27.4, 26.2, 25.9, 22.7, 20.9, 14.9, 14.1; υ_{max}: 3487, 2920, 2851, 1738, 1466, 1072, 727, 696 cm^{-1}.

(ii) Sodium (~50 mg) was added to liq. ammonia (100 mL) until the blue colour persisted. The glucopyranoside **53** (48 mg, 0.027 mmol) in 1,4-dioxane (2 mL) was added; the mixture was stirred for 4 – 5 min, when the blue colour disappeared, then quenched with sat. aq. NH_4Cl (5 mL) and ether (30 mL). The ammonia was allowed to evaporate; the aqueous layer was extracted with ether (2 × 30 mL). The combined organic layers were evaporated. Chromatography ($CHCl_3$/MeOH, 10:1) gave a 6:4 α,β-mixture of compound **54** (18 mg, 47%), $[\alpha]_D^{22}$ +28 (c 0.50, $CHCl_3$) [MALDI–Found $[M+Na]^+$: 1438.3385; $C_{91}H_{178}NaO_9$ requires: 1438.3363]; δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 5.28 (1H, dt, *J* 6.4, 15.2 Hz), 5.18 (1H, br dd, *J* 7.4, 15.2 Hz), 5.1 (0.6H, d, *J* 3.8 Hz, H–1α), 4.46 (0.4H, d, *J* 7.8 Hz, H–1β), 4.4 (1H, br d, *J* 11.8 Hz, H–6α,β), 4.24 (1H, br dd, *J* 5.8, 11.8 Hz, H–6α,β), 3.96 – 3.92 (0.6H, ddd, *J* 2.2, 5.6, 9.8 Hz, H–5α), 3.67 – 3.58 (1.6H, H–4α + CH-OH MA), 3.48 – 3.44 (0.4H, ddd, *J* 2.2, 5.6, 9.0 Hz, H–5β), 3.43 – 3.26 (5H, H–2,3α, H–3,4β and s at 3.3 for OCH_3), 3.17 (0.4H, br t, *J* 8.4 Hz), 2.95 – 2.91 (1H, m), 2.41 – 2.34 (1H, m), 2.0 – 1.95 (1H, m), 1.9 (2H, br q, *J* 6.7 Hz), 1.62 – 1.1 (146H, m), 1.07 – 1.0 (2H, m), 0.88 (3H, d, *J* 6.7 Hz), 0.83 (6H, t, *J* 6.6 Hz), 0.8 (3H, d, *J* 7 Hz); δ_C for α, β isomers (400 MHz, $CDCl_3$ + few drops of CD_3OD): 175.1, 136.4, 128.2, 96.6, 92.2, 85.5, 76.1, 74.5, 73.6, 72.4, 72.2, 70.5, 70.3, 69.2, 63.5, 57.5, 52.7, 52.5, 37.1, 36.6, 35.2, 35.0, 32.4, 32.2, 31.8, 30.4, 29.8, 29.76, 29.7, 29.6, 29.5, 29.41, 29.4, 29.3, 29.2, 29.1, 29.0, 27.4, 27.2, 26.0, 22.5, 20.8, 14.7, 13.9; υ_{max}: 3400, 2923, 2852, 1720, 1466, 1376, 1100 cm^{-1}. Starting material (12 mg) was recovered.

3.20 Methyl 5-*O*-[(2*R*,3*R*,17*R*,39*S*,40*S*, *E*)-3-hydroxy-39-methoxy-17,40-dimethyl-2-tetracosyloctapentacont-18-enoate]-α-D-arabinofuranoside 57

(i) The acid **37** (0.056g, 0.044 mmol) was dissolved in THF and DMF (5:1, 1.5 mL) at r. t., then warmed gently until all had dissolved. Dry $CsHCO_3$ (0.061mg, 0.313mmol) was added and stirred at r.t. for 1 h, followed by the arabinofuranoside **55** (0.033g, 0.066 mmol)[68] in THF/ DMF (0.5 mL). The mixture was stirred at 70 ^oC for 18 h, quenched with water (3 mL), then extracted with CH_2Cl_2 (3 × 30 mL) and evaporated. Chromatography (petroleum/ EtOAc, 10:1) gave methyl 2,3-di-*O*-benzyl-5-*O*-[(2*R*,3*R*, 17*R*,39*S*,40*S*,*E*)-3-hydroxy-39-methoxy-17,40-di-methyl-2-tetracosyloctapentacont-18-enoate]-α-D-arabinofuranoside **56** (0.049g, 71%) as a colourless thick oil, $[\alpha]_D^{22}$ +21 (c 0.51, $CHCl_3$) [MALDI–Found $[M+Na]^+$: 1602.3; $C_{105}H_{190}NaO_8$ requires: 1602.4]; δ_H (400 MHz, $CDCl_3$): 7.4 – 7.28 (10H, m), 5.34 (1H, dt, *J* 6.3, 15.4 Hz), 5.25 (1H, dd, *J* 7.4, 15.4 Hz), 4.92 (1H, br s), 4.57 (1H, d, *J* 12 Hz), 4.56 (1H, d, *J* 11.8 Hz), 4.5 (1H, d, *J* 12.0 Hz), 4.48 (1H, d, *J* 11.8 Hz), 4.33 – 4.26 (2H, m), 4.24 – 4.2.0 (1H, m), 3.99 (1H, d, *J* 2.1 Hz), 3.84 (1H, dd, *J* 2.6, 6.5 Hz), 3.65 – 3.62 (1H, m), 3.37 (3H, s), 3.35 (3H, s), 2.98 – 2.94 (1H, m), 2.43 (1H, tt, *J* 5.5, 9.1 Hz), 2.06 – 2.02 (1H, m), 1.97 (2H, br q, *J* 6.7 Hz), 1.7 – 1.5 (5H, m), 1.45 – 1.2 (137H, m), 1.11 – 1.04 (2H, m), 0.94 (3H, d, *J* 6.7 Hz), 0.89 (6H, t, *J* 6.6 Hz), 0.85 (3H, d, *J* 6.9 Hz); δ_C (101 MHz, $CDCl_3$): 175.0, 137.4, 137.2, 136.4, 128.5, 128.46, 128.4, 128.0, 127.9, 127.86, 107.2, 87.8, 85.4, 83.7, 79.4, 72.4, 72.2, 72.1, 63.5, 57.7, 54.9, 51.5, 37.2, 36.7, 35.5, 35.3, 32.6, 32.3, 31.9, 30.5, 30.0, 29.9, 29.7, 29.6, 29.5, 29.4, 29.35, 29.1, 27.6, 27.4, 27.36, 26.2, 25.8, 22.7, 20.9, 14.9, 14.1; υ_{max}: 2923, 2853, 1738, 1465, 1100 cm^{-1}.

(ii) Sodium (50 mg) was added to liq. ammonia (100 mL) as above. Arabinofuranoside **56** (42 mg, 0.026 mmol) in 1,4-dioxane (2 mL) was added and stirred for 4 – 5 min, when the blue colour disappeared, then quenched with sat. aq. NH_4Cl (5 mL) and ether (30 mL). Work up as before and chromatography ($CHCl_3$/MeOH, 10:1) gave compound **57** as a thick oil (21 mg, 57%), $[\alpha]_D^{22}$ +26 (c 0.70, $CHCl_3$) [MALDI–Found $[M+Na]^+$: 1422.3430; $C_{91}H_{178}NaO_8$ requires: 1422.3414]. This showed δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 5.28 (1H, dt, *J* 6.4, 15.2 Hz), 5.2 (1H, dd, *J* 7.4, 15.2 Hz), 4.78 (1H, br s), 4.33 (1H, dd, *J* 4.6, 11.7 Hz), 4.28 (1H, dd, *J* 5, 11.7 Hz), 4.08 (1H, br q, *J* 4.9 Hz), 3.96 (1H, dd, *J* 1.3, 2.8 Hz), 3.85 (1H, dd, *J* 2.9, 5.0 Hz), 3.65 – 3.60 (1H, m), 3.35 (3H, s), 3.3 (3H, s), 2.95 – 2.93 (1H, m), 2.39 (1H, ddd, *J* 4.8, 7.4, 10.2 Hz), 2.01 – 1.96 (1H, m), 1.91 (2H, br q, *J* 6.6 Hz), 1.62 – 1.03 (146H, m), 0.89 (3H, d, *J* 6.7 Hz), 0.84 (6H, t, *J* 6.6 Hz), 0.8 (3H, d, *J* 7.0 Hz); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 175.0, 136.4, 128.3, 108.8, 85.5, 81.9, 81.2, 78.0, 72.4, 63.4, 57.6, 55.0, 52.6, 37.1, 36.6, 35.2, 34.9, 32.4, 32.2, 31.8, 30.4, 29.8, 29.78, 29.7, 29.6, 29.5, 29.48, 29.4, 29.3, 29.2, 20.0, 27.4, 27.3, 27.2, 26.0, 25.3, 22.6, 20.8, 14.7, 14.0; υ_{max}: 3400, 2925, 2854, 1724, 1466, 1215, 1094 cm^{-1}. Starting material (10.2 mg) was recovered.

3.21 (*S*)-2,3-Dihydroxypropyl (2*R*,3*R*,17*R*,39*S*,40*S*,*E*)-3-hydroxy-39-methoxy-17,40-dimethyl-2-tetracosyloctapentacont-18-enoate 60

(i) $CsHCO_3$ (0.061 g, 0.313 mmol) was added to a solution of tosylate **58** (0.030 g, 0.067 mmol)[69] and acid **37** (0.056 g, 0.044 mmol) in dry DMF:THF (1:5, 2 mL) at r. t. and stirred at 70 °C for two days. The suspension was diluted with EtOAc (20 mL) and water (10 mL) and the aqueous layer was extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with water (10 mL) and brine (10 mL), dried and evaporated to give a thick oil. Column chromatography (10:1 hexane/EtOAc) gave (*S*)-2,3-bis-(benzyloxy)propyl (2*R*,3*R*,17*R*,39*S*,40*S*,*E*)-3-hydroxy-39-methoxy-17,40-dimethyl-2-tetracosyloctapentacont-18-enoate **59** (0.060, 89%) [MALDI–Found $[M+Na]^+$: 1530.3; $C_{102}H_{186}NaO_6$ requires: 1530.4]. This showed δ_H (400 MHz, $CDCl_3$): 7.4 – 7.28 (10H, m), 5.34 (1H, dt, *J* 6.4, 15.3 Hz), 5.25 (1H, dd, *J* 7.4, 15.3 Hz), 4.7 (1H, d, *J* 12.0 Hz), 4.65 (1H, d, *J* 12.0 Hz), 4.6 (2H, br s), 4.44 (1H, dd, *J* 4, 11.6 Hz), 4.23 (1H, dd, *J* 5.4, 11.6 Hz), 3.84 (1H, br pent, *J* 5.4 Hz), 3.64 – 3.57 (3H, m), 3.36 (3H, s), 2.99 – 2.96 (1H, m), 2.5 (1H, br d, *J* 5.9 Hz), 2.44 (1H, br td, *J* 5.4, 9.8 Hz), 2.1–2.03 (1H, m), 1.98 (2H, br q, *J* 6.7 Hz), 1.73 – 1.2 (142H, br m), 1.15 – 1.07 (1H, m), 0.96 (3H, d, *J* 6.7 Hz), 0.9 (6H, t, *J* 6.7 Hz), 0.87 (3H, d, *J* 6.9 Hz); δ_C (101 MHz, $CDCl_3$): 175.4, 138.0, 137.9, 136.5, 128.4, 128.35, 127.73, 127.7, 127.6, 85.4, 75.8, 73.5, 72.3, 72.1, 69.6, 63.5, 57.7, 51.4, 37.2, 36.7, 35.5, 35.3, 32.6, 32.4, 31.9, 30.5, 30.0, 29.9, 29.8, 29.7, 29.66, 29.6, 29.5, 29.44, 29.4, 29.1, 27.6, 27.5, 27.4, 26.2, 25.8, 22.7, 20.9, 14.9, 14.1; υ_{max}: 3474, 2924, 2853, 1735, 1466, 1370, 1100, 968, 908 cm^{-1}.

(ii) Sodium (~50 mg) was added to liq. ammonia (100 mL) as above. Ester **59** (52 mg, 0.034 mmol) in 1,4-dioxane (2 mL) was added. The mixture was stirred for 4 – 5 min., when the blue colour disappeared, then quenched with NH_4Cl (5 mL) and ether (30 mL). Work up as before and chroma-tography ($CHCl_3$/MeOH, 10:1) gave compound **60** (18 mg, 40%) [MALDI–Found $[M+Na]^+$: 1350.3215; $C_{88}H_{174}NaO_6$ requires: 1350.3203]; δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 5.33 (1H, dt, *J* 6.4, 15.3 Hz), 5.23 (1H, dd, *J* 7.4, 15.3 Hz), 4.27 (1H, dd, *J* 4.2, 11.5 Hz), 4.21 (1H, dd, *J* 6.4, 11.5 Hz), 3.96 – 3.91 (1H, m), 3.71 – 3.65 (2H, m), 3.61 (1H, br dd, *J* 5.5, 11.5 Hz), 3.34 (3H, s), 2.97 – 2.94 (1H, br, m), 2.48 –2.42 (1H, m), 2.06-2.01 (1H, m), 1.96 (2H, br q, *J* 6.6 Hz), 1.71 – 1.05 (146 H, m), 0.93 (3H, d, *J* 6.7 Hz), 0.88 (6 H, t, *J* 6.6 Hz), 0.84 (3H, d, *J* 6.9 Hz); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 175.5, 136.4, 128.3, 85.5, 72.6, 69.8, 65.1, 63.0, 57.6, 52.5, 37.1, 36.6, 35.2, 35.0, 32.5, 32.3, 31.8, 30.4, 29.9, 29.8, 29.7, 29.6, 29.55, 29.52, 29.5, 29.4, 29.3, 29.25, 29.0, 27.4, 27.36, 27.3, 26.0, 25.3, 22.6, 20.8, 14.7, 14.0; υ_{max}: 3434, 2922, 2853, 1722, 1465, 1099 cm^{-1}. Starting material (10 mg) was recovered.

3.22 ELISA method

ELISA were carried out on 96-well flat-bottomed polystyrene micro-plates. Antigens were dissolved in hexane to give a concentration 15 µg/ml. 50 µl of this solution was added to each well, and the solvent was left to evaporate at r. t.. Control wells were coated with hexane (50 µl / well) only. Blocking was done by adding 400 µl of 0.5 % casein/PBS buffer (pH = 7.4) to each well, and the plates were incubated at 25 ºC for 30 minutes. The buffer was aspirated and any excess flicked out until the plates were dry. Serum (1 in 40 dilution in casein/PBS buffer) (50 µl / well) was added and incubated at 25 ºC for 1 h. The plates were washed with 400 µl casein/PBS buffer 3 times using an automatic washer, and any excess flicked out onto a paper towel until dry. Secondary antibody (anti-human IgG (Fc specific) peroxidise conjugated antibody produced in goat (Aldrich) (diluted to 1:2000 in casein/PBS buffer) (50 µl / well) was added, and incubated at 25 ºC for 30 minutes. The plates were again washed 3 times with 400 µl casein/PBS buffer using an automatic washer, and any excess was again flicked out. OPD substrate (*o*-phenylenediamine, 1 mg/ml, 50 µl / well) and H_2O_2 (0.8 mg/ml) in 0.1 M citrate buffer) was added, and the plates were incubated for 30 minutes at 25 ºC. The colour reaction was terminated by adding 2.5 M H_2SO_4 (50 µl / well), and the absorbance measured at 492nm.

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