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The synthesis of a major α′-mycolic acid of *Mycobacterium smegmatis*

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ABSTRACT

The synthesis of (2*R*,3*R*,*Z*)-2-docosyl-3-hydroxytetracont-21-enoic acid, a significant α′-mycolic acid of *Mycobacterium smegmatis* and other mycobacteria is reported.

1. Introduction

Mycolic acids **1** (Scheme 1) are characteristic components of mycobacteria and related taxa (Barry et al., 1998). They are defined by a *R*,*R*-β-hydroxy acid with two long chain substituents. In mycobacteria, the α-chain is a simple alkyl substituent with a chain length of either 22 or 24 carbons. In contrast, the mero-chain can contain a number of substituents—in a number of important bacteria, such as *Mycobacterium tuberculosis*, these include two or three functional groups and can be divided into α- methoxy and keto-classes of mycolic acid depending on X and Y. Some years ago, it was reported that important mycolic acids of *Mycobacterium smegmatis* are much simpler and contain just one *cis*-alkene in the mero-chain; the mixture of α′-smegmamycolates, m.p. 50–51 °C, $[\alpha]_D$+2.8, makes up some 25% of the mycolic acids of this species and the major component mycolic acid was reported to have the chain lengths identified in structure **2** ($a = b = 17$) (Etemadi et al., 1964; Krembel and Etemadi, 1966; Etemadi, 1967).

Some years later, Wong et al. separated three series of mycolic acids from *M. smegmatis*, and confirmed that one of these simply contained one alkene substituent in the mero-chain. However, they were able to separate this series into component parts by preparative HPLC and identified six major homologues of this type with slightly different chain lengths, including **2** ($a = b = 17$) (Wong and Gray, 1979; Wong et al., 1979; Gray et al., 1983). Direct analysis of the mycolic acids by MALDI-TOF mass spectrometry confirmed the presence of a series of components with molecular weights consistent with 60, 62, 64 and 66 carbons as suggested by Wong et al. (Laval et al., 2001) *Mycobacterium chelonae* contains comparable amounts of α-mycolates, containing either two *cis*-alkenes, one *cis* and one α-methyl-*trans*-alkene, or one *cis*-cyclopropane and one α-methyl-*trans*-alkene at distal and proximal positions and of shorter chain α′-mycolates containing just one *cis*-alkene corresponding to **2** ($a = 15,17$, $b = 17,19$) (Goodfellow and Minnikin, 1981; Minnikin et al., 1982). In other cases, mycobacteria such as *Mycobacterium fortuitum* (Minnikin et al., 1980, 1984) and '*Mycobacterium thamnopheos*' (Lacave et al., 1987; Daffé et al., 1988) are reported to contain mycolic acid which include two to four *cis*-alkenes. α′-mycolates containing 0–2 double bonds and 22–36 carbons have been reported in the genera *Corynebacteria*, 1–4 double bonds and 48–66 carbons in *Gordona*, 0–3 double bonds and 44–60 carbons in *Nocardia*, 0–4 double bonds and 34–48 carbons in *Rhodococcus* and 1–6 double bonds and 64–78 carbons in *Tsukmurella* (Barry et al., 1998). Unsaturated mycolic acids may be intermediates in the biosynthesis of other classes of mycolic acid (Lopez-Marin et al., 1991; Wheeler et al., 1993; Asselineau et al., 2002).

We have reported the synthesis of examples of a range of α-methoxy- and keto-mycolic acids with structures chosen to match the chain lengths and, where known the stereochemistry of major mycobacterial mycolic acids (Al Dulayymi et al., 2005, 2006, 2007; Baird and Koza, 2007; Koza et al., 2009) We now report the synthesis of the simple *cis*-alkene mycolic acid **2**.

2. Results and discussion

The aldehyde **3** (Koza et al., 2009) was chain extended by a modified Julia–Kocienski reaction with 5-(eicosane-1-sulfonyl)-1-phenyl-1H-tetrazole (prepared from 1-phenyl-1H-tetrazole-5-

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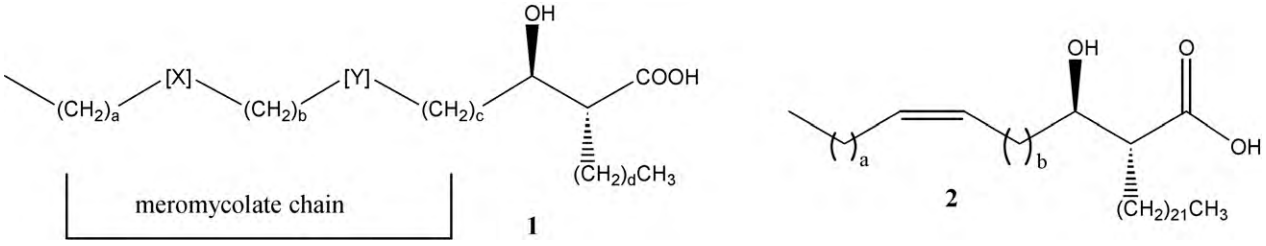


Scheme 1.

thiol and 1-bromoeicosane under standard conditions) and base, followed by hydrogenation of the derived mixture of *E* and *Z*-alkenes to give the ester **4**. Removal of the benzyl group led to alcohol **5** which was in turn oxidised to the aldehyde **6** (Scheme 2).

Reaction of aldehyde **6** with 2,2-dimethyl-propionic acid 16-(1-phenyl-1H-tetrazole-5-sulfonyl)-hexadecyl ester in the presence of lithium bis-trimethylsilylamide in a modified Julia–Kocienski reaction led to a mixture of *E* and *Z*-alkenes which was hydrogenated to give the diester **7** (Scheme 3). Deprotection of the pivolyl ester led to the alcohol **8** which was oxidized to the aldehyde **9**. Reaction of **9** with 5-(nonadecylsulfonyl)-1-phenyl-1H-tetrazole and base gave a mixture of *E* and *Z*-alkenes **11** and in 70% yield, providing comparison spectroscopic data for both isomers. In contrast, reaction with nondecyltriphenylphosphonium bromide and sodium bis-trimethylsilylamide gave the required *Z*-isomer **10** (62%).

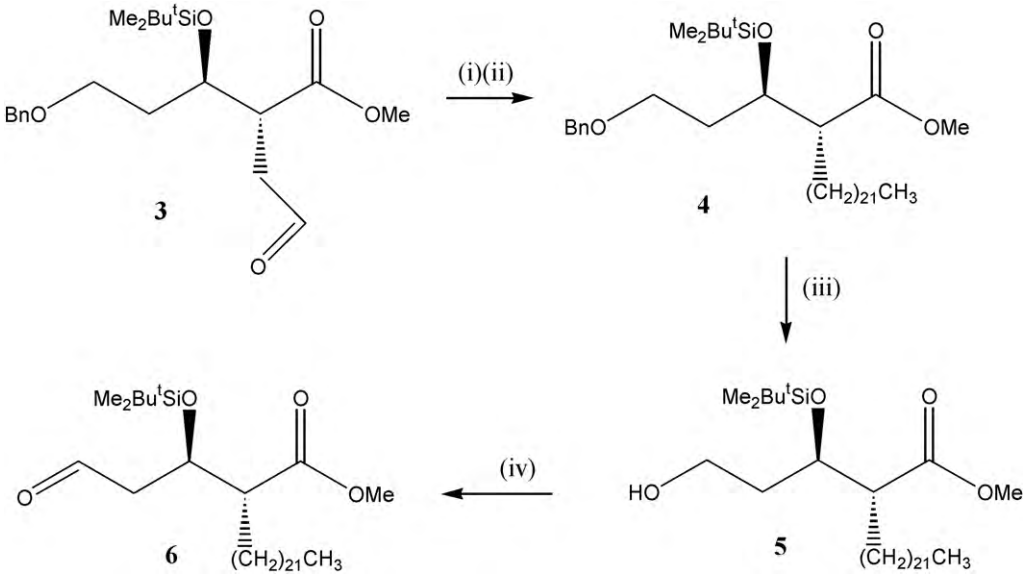
The silyl protecting group was removed by reaction with HF-pyridine to give the methyl ester **12** as a white solid, m.p. 44–46 °C with a specific rotation of +4.65 (*c* 1.83, $CHCl_3$). Removal of the methyl ester using lithium hydroxide in THF–methanol–water then gave the free mono-alkene mycolic acid **13** as a white solid, m.p. 65–66 °C which showed a specific rotation $[\alpha]_D^{21}$ of +3.4 in $CHCl_3$ corresponding to an M_D of +33 (Scheme 4).

The value for the specific rotation of **13** is in line with that reported for α′-smegmamycolates (Etemadi et al., 1964; Krembel and Etemadi, 1966; Etemadi, 1967), and with the M_D predicted on the basis of the summing of molecular rotations for individual chiral elements in the molecule, a technique which generally works well in molecules of this type where the chiral elements are separated by long chains (Quémard et al., 1997). Thus the typical M_D for only chiral element present, the *R*,*R*-β-hydroxy-acid fragment, has been reported to be around +40 (Quémard et al., 1997), while other values for natural coryno-mycolic acids are reported to be +35 (Asselineau and Asselineau, 1966) and +37.5 (Pudles and Lederer, 1951) and that for synthetic (*S*)-2-((*S*)-1-hydroxytetradecyl)eicosanoic acid is reported to be −23 (the synthetic *R*,*R*-isomer in this case has a value of +13) (Kumaraswamy and Markondaiah, 2008).

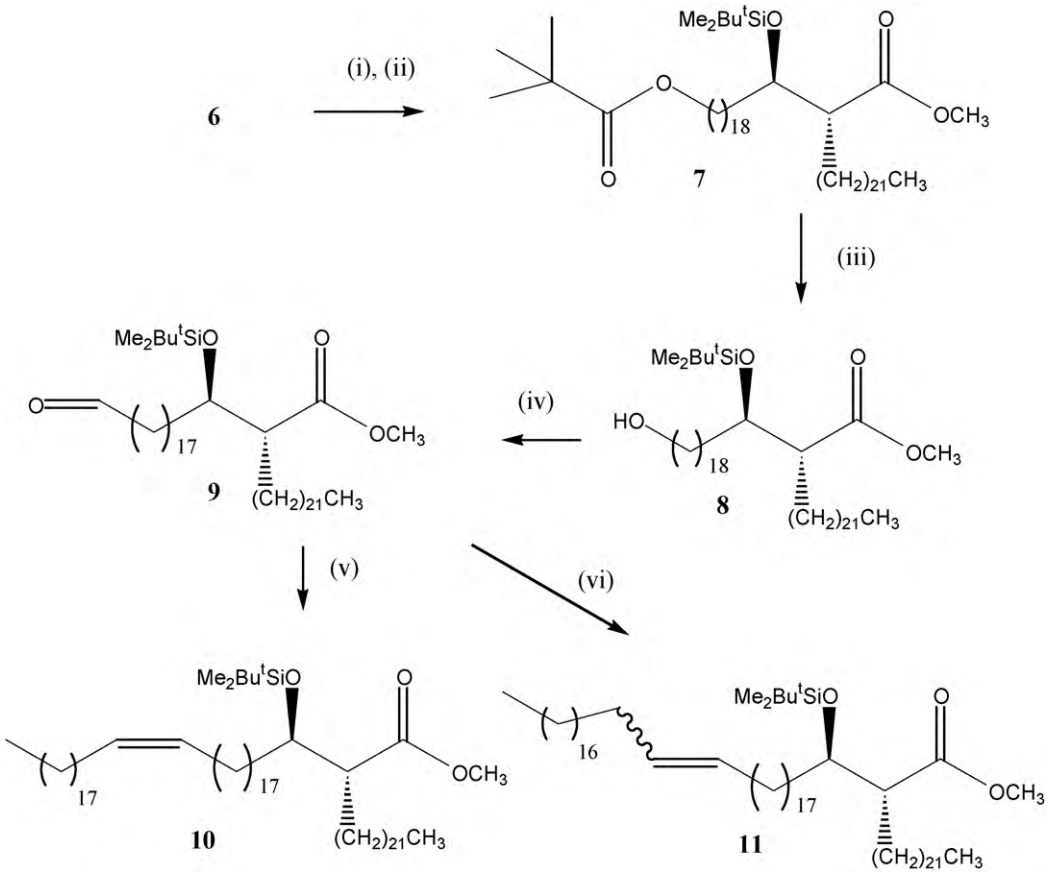
3. Experimental

3.1. General

Chemicals used were obtained from commercial suppliers or prepared from them by methods described. Solvents which had to be dry, e.g. ether (which throughout refers to diethyl ether), THF were dried over sodium wire. Petrol was of boiling point 40–60 °C. Reactions carried under inert conditions, were carried out under a slow stream of nitrogen. Those carried out at low temperatures were cooled using a bath of methylated spirit with liquid nitrogen. Silica gel (Merck 7736) and silica gel plates (Silica Gel 60 F_{254}) used for column and thin layer chromatography were obtained from Aldrich. Organic solutions were dried over anhydrous magnesium sulfate. IR spectra were carried out on a Perkin–Elmer 1600 F.T.I.R. spectrometer as liquid films. NMR spectra were recorded on Bruker AC250 or Advance 500 spectrometers. Specific rotation values were recorded in $CHCl_3$ on a POLAAR 2001 Optical Activity polarimeter. Mass spectra were recorded on a Bruker Microtof ESI instrument.



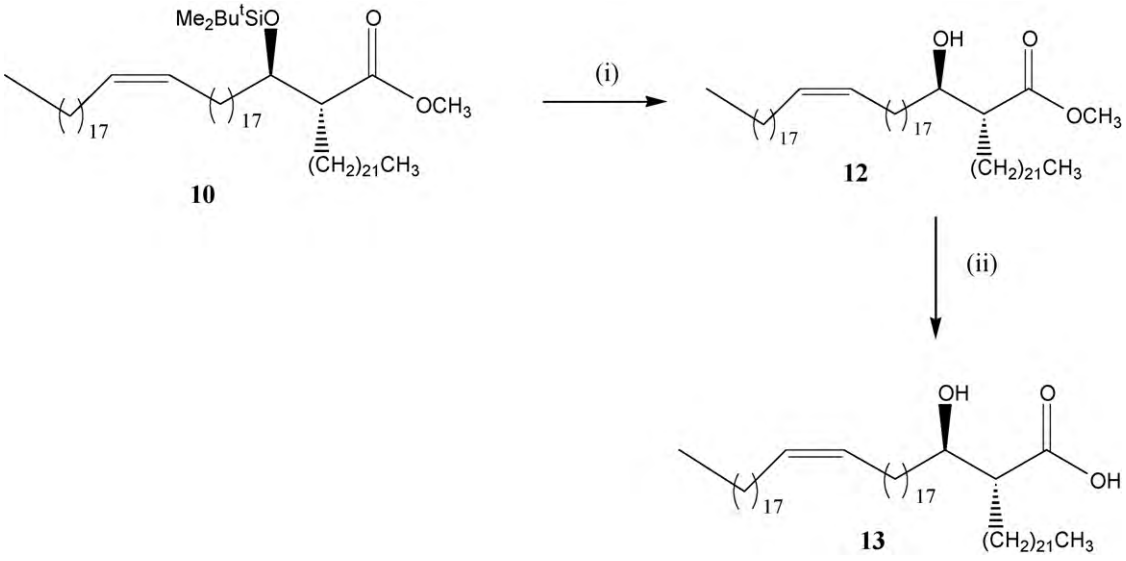
Scheme 2. (i) $LiN(SiMe_3)_2$, 5-(eicosane-1-sulfonyl)-1-phenyl-1H-tetrazole, THF, argon, −10 °C, then r.t., 1 h (79%); (ii) industrial methylated spirit (IMS), THF, Pd/C, H_2, 2 h (90%); (iii) IMS, THF, Pd/C, H_2, 3 days (90%); (iv) pyridinium chlorochromate (PCC), CH_2Cl_2 (96%).



Scheme 3. (i) $LiN(SiMe_3)_2$, 2,2-dimethyl-propionic acid 16-(1-phenyl-1H-tetrazole-5-sulfonyl)-hexadecyl ester, THF, argon, −10 °C, then r.t., 1 h (95%), (ii)) IMS, THF, Pd/C, H_2 (85%),(iii) KOH:THF:H_2O, 70 °C, 3 h (84%), (iv) PCC, CH_2Cl_2 (92%), (v) nonadecyltriphenyl-phosphonium bromide, sodium bis(trimethylsilyl)amide, THF, argon, r.t., 1 h (62%), (vi)) $LiN(SiMe_3)_2$, 5-(nonadecane-1-sulfonyl)-1-phenyl-1H-tetrazole, THF, argon, −10 °C, then r.t., 1 h (70%).

3.2. 5-Eicosylsufanyl-1-phenyl-lH-tetrazole

1-Phenyl-1H-tetrazole-5-thiol (7.76 g, 43.5 mmol), 1-bromoeicosane (12.5 g, 34.6 mmol), anhydrous potassium carbonate (15 g, 41.5 mmol), and acetone (500 ml) were mixed. The mixture was vigorously stirred and refluxed at 60 °C for 15 h when TLC indicated complete reaction of the thiol. The inorganic salts were filtered off and washed well with acetone. The acetone solution was evaporated to a small bulk and dissolved in dichloromethane (200 ml). The solution was washed with water (300 ml) and the aqueous layer was re-extracted with dichloromethane (2× 50 ml). The combined organic phases were washed with water (300 ml), dried and the solvent was evaporated. The crude product was recrystallised from acetone (90 ml)



Scheme 4. (i) HF-pyridine, THF, argon, 45 °C, 18 h (87%), (ii) LiOH, THF:MeOH:H_2O (10:1:1), 40 °C, 18 h (81%).

and methanol (90 ml) to give a white solid, 5-eicosylsufanyl-1-pheny-lH-tetrazole (17.0 g, 89%), m.p. 45–46 °C {Found $[M+Na]^+$: 459.3500, $C_{27}H_{46}N_4S$ requires: 459.3516}. This showed δ_H: 7.65–7.52 (5H, m), 3.37 (2H, t, *J* 7.25 Hz), 1.78 (2H, pent., *J* 7.25 Hz), 1.43 (2H, pent., *J* 7.6 Hz), 1.34–1.22 (32H, m), 0.88 (3H, t, *J* 6.95 Hz,); δ_C: 154.5, 133.8, 130.12, 129.8, 123.9, 33.4, 31.9, 29.68, 29.65, 29.62, 29.54, 29.44, 29.3, 29.09, 28.65, 22.7, 14.1; ν_{max}: 2917, 1501, 1471, 1390, 892, 763, 686 cm^{-1}.

3.3. 5-(Eicosane-1-sulfonyl)-1-phenyl-lH-tetrazole

3-Chloroperoxybenzoic acid (9.08 g, 377 mmol) in dichloromethane (100 ml) was added to a stirred solution of 5-eicosylsulfanyl-1-phenyl-1H-tetrazole (16.82 g, 36.6 mmol) and $NaHCO_3$ (13.86 g, 164.0 mmol) in dichloromethane (100 ml) at 0 °C. The reaction mixture was stirred at r.t. overnight. The reaction was quenched with sodium hydroxide solution (200 ml, 5%) and stirred for 1 h before being extracted with dichloromethane (3 × 200 ml). The combined organic layers were dried over $MgSO_4$, filtered and evaporated to a powder. The crude product was purified by recrystallisation from methanol/acetone (1:1), to give 5-(eicosane-1-sulfonyl)-1-phenyl-1H-tetrazole as a white solid (14.96 g, 83%), m.p. 56–58 °C {Found $[M+Na]^+$: 491.3403, $C_{27}H_{46}O_2S$ requires: 491.3414}. This showed δ_H: 7.72–7.70 (2H, m), 7.64–7.60 (3H, m), 3.74 (2H, distorted t, *J* 7.91 Hz), 1.96 (2H, br, pent., *J* 7.8 Hz), 1.50 (2H, br, pent., J 7 Hz), 1.36–1.26 (32 H, m), 0.89 (3H, t, *J* 6.95 Hz); δ_C: 153.46, 133.45, 131.45, 129.72, 125.08, 56.04, 31.93, 29.7, 29.63, 29.57, 29.46, 29.36, 29.19, 28.9, 28.15, 22.69, 21.95, 14.12; ν_{max}: 2913, 2846, 1493, 1461, 1337, 1148, 763, 686 cm^{-1}.

3.4. (R)-2-[(R)-1-(tert-Butyldimethylsilanyloxy) -3-hydroxypropyl]tetracosanoic acid methyl ester (**4**)

Lithium bis(trimethylsilyl)amide (1.06 M in THF, 16.44 ml, 17.4 mmol) was added to a stirred solution of 5-benzyloxy-3-(*tert*-butyldimethylsilanyloxy)-2-(2-oxo-ethyl)pentanoic acid methyl ester **3** (Koza et al., 2009) (3.82 g, 9.68 mmol) and 5-(eicosane-1-sulfonyl)-1-phenyl-1H-tetrazole (5.7 g, 11.6 mmol) in dry THF (50 ml) at −5 °C. The reaction turned bright yellow and was left to reach r.t. and stirred for 1 h under nitrogen atmosphere, when TLC showed no starting material was left, the reaction was quenched by addition of sat. aq. NH_4Cl (10 ml). The product was extracted with petrol/ether (1:2) (3× 150 ml). The combined organic layers were dried over $MgSO_4$, filtered and evaporated, to give a crude product which was purified by column chromotography over silica gel, eluting with petrol/ether (20:1) to give a colourless oil (*E*/*Z*)-(*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilanyloxy)-3-benzyloxy-propyl]-tetracos-4-enoic acid methyl ester as a mixture in ratio 2:1 (4.92 g, 79%) {Found $[M+H]^+$: 659.5409, $C_{41}H_{74}O_4Si$ requires: 659.5426}. (i) Palladium 10% on carbon (2.5 g) was added to a stirred solution of (*E*/*Z*)-(*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilanyloxy)-3-benzyloxypropyl]tetracos-4-enoic acid methyl ester (5.5 g, 8.34 mmol) in IMS (60 ml) and THF (60 ml) under a hydrogen atmosphere. Hydrogenation was carried out for 2 h. The solution was filtered over a bed of celite and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ether (2:1) to give (*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilanyloxy)-3-benzyloxypropyl]tetracosanoic acid methyl ester **4** as a colourless oil (4.96 g, 90%), $[\alpha]_D^{23}$ −5.42 (c 1.13, $CHCl_3$) {Found $[M+H]^+$: 661.5570, $C_{41}H_{76}O_4Si$ requires: 661.5586} which showed δ_H: 7.33–7.31 (4H, m), 7.28–7.25 (1H, m), 4.44 (2H, br., s), 4.13–4.06 (1H, m), 3.65 (3H, s), 3.59–3.54 (2H, m), 2.52 (1H, ddd, *J* 10.4, 6.6, 3.8 Hz), 1.82 (2H, q, *J* 6.6 Hz), 1.64–1.51 (2H, m), 1.43–1.15 (40H, br., m), 0.85 (3H, t, *J* 7 Hz), 0.82 (9H, s), 0.05 (3H, s), 0.04 (3H, s); δ_C: 174.65, 138.46, 128.25, 127.5, 127.41, 72.86, 70.71, 66.12, 52.00, 51.19, 33.64, 31.92, 29.69, 29.66, 29.65, 29.63, 29.56, 29.43, 29.35, 27.86, 27.22, 25.7, 22.7, 17.9, 14.08,−4.62,−4.93; ν_{max}/cm^{-1}: 2928, 2856, 1738, 1665, 1361, 1254, 1192, 1168, 1102. (ii) Palladium 10% on carbon (0.5 g) was added to a stirred solution of the methyl ester from (i) (4.96 g, 7.52 mmol) in IMS (60 ml) and THF (60 ml) under a hydrogen atmosphere. Hydrogenation was carried out for 3 days. The solution was filtered over a bed of celite and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol/ether (2:1) to give (*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilanyloxy)-3-hydroxypropyl]tetracosanoic acid methyl ester **5** as a white solid (4.77 g, 90%), m.p. 35–37 °C, $[\alpha]_D^{28}$ −1.76 (c 0.81, $CHCl_3$) {Found $[M+H]^+$: 571.5101, $C_{34}H_{71}O_4Si$ requires: 571.5116}. This showed δ_H: 4.29 (1H, td, *J* 6, 4.4 Hz), 3.70–3.66 (2H, m) 3.56 (3H, s), 2.66 (1H, ddd, 10.5, 6.65, 3.8 Hz), 1.98 (1H, br, s), 1.69–1.65 (2H, m), 1.44–1.39 (1H, m), 1.38–1.32 (1H, m), 1.21–1.08 (40H, m), 0.78–0.72 (12H, including a singlet at δ 0.77), 0.00 (3H, s), −0.04 (3H, s); δ_C: 174.66, 72.01, 59.47, 51.39, 35.23, 31.92, 29.69, 29.66, 29.64, 29.61, 29.56, 29.55, 29.43, 29.34, 27.85, 27.16, 25.68, 22.67, 17.84, 14.08, −4.35, −4.99; ν_{max}: 3462, 2921, 2857, 1740, 1464, 1362, 1255, 1196, 1166, 1092, 908, 837, 776, 735 cm^{-1}.

3.5. (R)-2-[(R)-1-(tert-Butyldimethylsilanyloxy) -3-oxopropyl]tetracosanoic acid methyl ester (**6**)

(*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-3-hydroxypropyl]tetracosanoic acid methyl ester (2.01 g, 3.51 mmol) in dichloromethane (25 ml) was added in portions at r.t. to a stirred solution of PCC (1.89 g, 12.29 mmol) in dichloromethane (100 ml). During the addition a black colour appeared. The reaction was stirred at r.t. for 2 h, when TLC showed complete reaction, then ether (300 ml) was added and filtered through a bed of silica gel. The solvent was evaporated and the crude product was purified by column chromatography eluting with petrol/ether (4:1) to give (*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilanyloxy)-3-oxopropyl]tetracosanoic acid methyl ester **6** (1.92 g, 96%) as a colourless oil, $[\alpha]_D^{26}$ −4.98 (c 1.23, $CHCl_3$) {Found $(M+Na)^+$: 591.4774. $C_{34}H_{68}NaO_4Si$ requires: 591.4779}; which showed δ_H: 9.72 (1H, t, *J* 2.2 Hz), 4.36 (1H, br., q, *J* 6 Hz), 3.59 (3H, s), 2.59 –2.49 (3H, m), 1.57–1.48 (1H, m), 1.46–1.4 (1H, m), 1.35–1.13 (40H, m), 0.80 (3H, t, *J* 7 Hz), 0.78 (9H, s), 0.002 (3H, s), −0.008 (3H, s); δ_C: 201.06, 174.26, 173.95, 68.79, 52.22, 51.43, 48.07, 31.90, 29.68, 29.64, 29.60. 29.52, 29.47, 29.36, 29.34, 27.72, 27.00, 25.58, 22.66, 17.83, 14.06, −4.69, −4.96; ν_{max}: 2924, 2856, 1734, 1466, 1362, 1255, 1198, 1167, 590, 477 cm^{-1}.

3.6. 2,2-Dimethylpropionic acid 16-(1-phenyl-1H-tetrazol-5-ylsulfanyl)hexadecyl ester

1-Phenyl-1H-tetrazole-5-thiol (3.66 g, 20.5 mmol), 2,2-dimethylpropionic acid 16-bromohexadecyl ester (8.34 g, 20.5 mmol), anhydrous potassium carbonate (4.26 g, 30.8 mmol), and acetone (70 ml) were mixed. The mixture was vigorously stirred and refluxed at 60 °C for 15 h when TLC showed no starting material was left, the inorganic salts were filtered off and washed well with acetone. The acetone solution was evaporated to a small bulk and dissolved in dichloromethane (100 ml). The solution was washed with water (100 ml) and the aqueous layer was re-extracted with dichloromethane (2× 50 ml). The combined organic phases were washed with water (300 ml), dried and the solvent was evaporated. The crude product was purified by column chromatography eluting with petrol:ethyl acetate (5:1) to give 2,2-dimethylpropionic acid 16-(1-phenyl-1H-tetrazol-5-ylsulfanyl)hexadecyl ester (8.01 g, 77%) (Found $[M+Na]^+$: 525.3356. $C_{28}H_{46}N_4O_2SNa$ requires: 525.3234) which showed δ_H: 7.53–7.45

(5H, m), 3.98 (2H, t, *J* 6.6 Hz), 3.33 (2H, t, *J* 7.55 Hz), 1.76 (2H, pent., *J* 7.9 Hz), 1.55 (2H, pent., *J* 6.6 Hz),1.38 (2H, pent., *J* 5.95 Hz), 1.20–1.17(22H, br. m), 1.34 (9H, s); δ_C 178.33, 154.27, 133.63, 129.84, 129.57, 123.64, 64.23, 38.7, 33.17, 29.45, 29.43, 29.42, 29.36, 29.32, 29.25, 29.03, 28.92, 28.84, 28.45, 28.43, 27.00 25.72; ν_{max}: 2923, 2849, 1723, 1597, 1499, 1479, 1386, 1282, 1156, 760, 693 cm^{-1}.

3.7. 2,2-Dimethylpropionic acid 16-(1-phenyl-1H-tetrazole-5-sulfonyl)-hexadecyl ester

Ammonium molybdate (VI) tetrahydrate (8.52 g, 6.9 mmol) in 35% H_2O_2 (23 ml), prepared and cooled in an ice bath, was added to a stirred solution of 2,2-dimethylpropionic acid 16-(1-phenyl-1*H*-tetrazol-5-ylsulfanyl)-hexadecyl ester (7.71 g, 15.3 mmol) in IMS (100 ml) and THF (40 ml) at 10 °C. After 2 h at room temperature, a further solution of ammonium molybdate (VI) tetrahydrate (8.52 g, 6.9 mmol) in 35% H_2O_2 (20 ml) was added and left overnight. The mixture was poured into water (1L) and extracted with dichloromethane (2 × 400 ml). The combined organic layers were dried and evaporated to give a solid residue which was purified by re-crystallised from petrol giving 2,2-dimethylpropionic acid 16-(1-phenyl-1H-tetrazole-5-sulfonyl)hexadecyl ester (6.72 g, 81%) (Found $[M+Na]^+$: 557.3132. $C_{28}H_{46}N_4O_4SNa$ requires: 557.3132) which showed δ_H: 7.71–7.69 (2H, m), 7.64–7.61 (3H, m), 4.05 (2H, t, *J* 6.6 Hz), 3.75 (2H, distorted t, *J* 7.9 Hz), 1.95 (2H, pent., *J* 7.8 Hz), 1.62 (2H, pent., *J* 6.65 Hz), 1.5 (2H, pent., *J* 6.65 Hz), 1.35–1.25 (22H, m), 1.2 (9H, s); δ_C: 178.67, 153.51, 133.07, 131.46, 129.72, 125.08, 64.23, 56.03, 38.73, 29.62, 29.56, 29.52, 29.46, 29.23, 29.2, 28.9, 28.82, 28.62, 28.15, 27.22, 25.91, 21.95; ν_{max}: 2928, 2864, 1728, 1498, 1480, 1462, 1344, 1284, 1155, 762, 688 cm^{-1}.

3.8. (R)-2-[(R)–1-(tert-Butyldimethylsilanyloxy)-19-(2, 2-dimethylpropionyloxy)-nonadecyl]-tetracosanoic acid methyl ester (**7**)

(i) Lithium bis(trimethyl silyl)amide (6.1 ml, 6.65 mmol, 1.06 M) was added to a stirred solution of (*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilanyloxy)-3-oxopropyl]tetracosanoic acid methyl ester (1.66 g, 2.91 mmol) and 2,2-dimethylpropionic acid 16-(1-phenyl-1H-tetrazole-5-sulfonyl)-hexadecyl ester (2.34 g, 4.37 mmol) in dry THF (30 ml) at −5 °C. The reaction turned bright yellow and was left to reach r.t. and stirred for 1 h under nitrogen, when TLC showed no starting material was left. The reaction was quenched by addition of sat.aq NH_4Cl at 0 °C. The product was extracted with petrol/ethyl acetate (10:1) (3× 50 ml). The combined organic layers were dried over $MgSO_4$, filtered and evaporated; the crude product was purified by column chromotography over silica gel, eluting with petrol/ethyl acetate (10:1) to give (*E,Z*)-(*R*)- 2-((*R*-1-(*tert*-butyldimethylsilyloxy)-19-(pivaloyloxy)nonadec-3-enyl)tetracosanoic acid methyl ester (2.45 g, 95%) as a 2:1 mixture of isomers; (ii) Palladium on 10% carbon (0.5 g, 10%) and the ester from (i) (2.1 g; 2.39 mmol), in IMS (50 ml) and THF (20 ml) was stirred under hydrogen atmosphere. Work up as before and the product was purified by column chromatography eluting with petrol:ethyl acetate (10:1) to give (*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilanyloxy)-19-(2,2-dimethylpropionyloxy)nonadecyl]tetracosanoic acid methyl ester **7** as a colourless oil (1.8 g, 85%), $[\alpha]_D^{20}$ −11.19 (c 1.51, $CHCl_3$) (Found $[M+Na]^+$: 901.8007 $C_{55}H_{110}O_5SiNa$ requires: 901.8015). This showed δ_H: 4.04 (2H, t, *J* 6.6 Hz), 3.9 (1H, br. td, *J* 7, 4.75 Hz), 3.65 (3H, s), 2.53 (1H, ddd, *J* 10.7, 6.9, 3.45 Hz), 1.64–1.59 (2H, m), 1.57–1.24 (74H, m), 1.2 (9H, s), 0.89 (3H, t, *J* 7 Hz), 0.86 (9H, s), 0.04 (3H, s), 0.02 (3H, s); δ_C: 178.57, 175.06, 73.24, 64.44, 51.59, 51.15, 38.71, 33.71, 31.92, 29.82, 29.69, 29.65, 29.59, 29.57, 29.52, 29.43, 29.35, 29.23, 28.63, 27.83, 27.48, 27.19, 25.91, 25.76, 23.75, 22.67, 17.97, 14.08, −4.37, −4.92; ν_{max}: 2924, 2856, 1734, 1466, 1362, 1255, 1198, 1167, 590, 477 cm^{-1}.

3.9. (R)-2-[(R)-1-(tert-Butyldimethylsilanyloxy) -19-hydroxynonadecyl]tetracosanoic acid methyl ester (**8**)

(*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-19-(2,2-dimethylpropionyloxy)nonadecyl]-tetracosanoic acid methyl ester (1.81 g, 2.07 mmol) was added to a stirred solution of potassium hydroxide (1.72 g, 30.8 mmol) dissolved in THF:MeOH:H_2O (10:10:1, 21 ml). The mixture was refluxed at 70 °C and monitored by TLC. After 3 h, the TLC showed no starting material was left and the reaction was cooled down, quenched with water and extracted with ethyl acetate (3 × 100 ml). The combined organic layers were dried and the solvent was evaporated. The product was purified by column chromatography eluting with petrol/ether (2:1 and then 1:1) to give a semi-solid (*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilanyloxy)-19-hydroxynonadecyl]tetracosanoic acid methyl ester **8** (1.38 g, 84%), $[\alpha]_D^{20}$ −11.19 (c 1.51, $CHCl_3$) (Found $[M+Na]^+$: 817.7401. $C_{50}H_{102}O_4SiNa$ requires: 817.7440). This showed δ_H: 3.89 (1H, td, *J* 7.25, 4.7 Hz), 3.63 (3H, s), 3.61 (2H, t *J* 6.6 Hz), 2.51 (1H, ddd, *J* 11.05, 7.25, 3.8 Hz), 1.7 (1H, br, s), 1.54 (2H, pent., *J* 6.3 Hz), 1.23 (74H, br, s), 0.86 (3H, t *J* 6.9 Hz), 0.84 (9H, br, s), 0.02 (3H, s), 0.001(3H, s); δ_C: 175.07, 73.18, 62.91, 60.30, 51.52, 33.64, 31.78, 31.89, 29.77, 29.66, 29.61, 29.58, 29.53, 29.51, 29.42, 29.39, 29.32, 27.77, 27.45, 26.86, 22.64, 14.03, −4.43, −5.00; ν_{max}: 3384, 2923, 2853, 1741, 1464, 1361, 1254, 1195, 1166, 1070, 836, 775, 720 cm^{-1}.

3.10. (R)-2-[(R)-1-(tert-Butyldimethylsilanyloxy) -19-oxononadecyl]tetracosanoic acid methyl ester

(*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilanyloxy)-19-hydroxynonadecyl]tetracosanoic acid methyl ester (0.25 g, 0.314 mmol) in dichloromethane (5 ml) was added in portions at r.t. to a stirred solution of PCC (0.17 g, 0.785 mmol) in dichloromethane (30 ml). During the addition a black colour appeared. The reaction was stirred at r.t. for 2 h, when TLC showed the reaction was complete. The mixture was poured into petrol/ethyl acetate (50 ml, 10:1) and filtered through a bed of silica gel. The solvent was evaporated and the product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give (*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilanyloxy)-19-oxononadecyl]tetracosanoic acid methyl ester as a colourless oil (0.23 g, 92%), $[\alpha]_D^{24}$ −1.09 (c 1.35, $CHCl_3$) (Found $[M+Na]^+$: 816.48, $C_{50}H_{100}O_4SiNa$ requires: 816.41 (MALDI)); this showed δ_H: 9.77 (1H, t, *J* 1.9 Hz), 3.92 (1H, br.td, *J* 6.6, 4.75 Hz), 3.66 (3H, s), 2.55 (1H, ddd, *J* 10.7, 6.95, 3.5 Hz), 2.44 (2H, dt, *J* 7.55, 1.9 Hz), 1.63 (2H, pent., *J* 7 Hz), 1.55–1.20 (72H, br. m), 0.88 (3H, t, *J* 6.6 Hz), 0.87 (9H, s), 0.04 (3H, s), 0.02 (3H, s); δ_C: 202.9, 175.13, 73.22, 51.58, 51.21, 43.91, 33.68, 31.92, 29.82, 29.69, 29.65, 29.58, 29.43, 29.35, 29.17, 27.83, 27.48, 25.76, 23.71, 22.67, 22.09, 17.97, 14.10, −4.37, −4.93; ν_{max}: 2924, 2853, 1741, 1464, 1372, 1249, 1167, 1048, 836, 775 cm^{-1}.

3.11. Methyl (2R,3R,Z)-3-(tert-butyldimethylsilyloxy) -2-docosyltetracont-21-enoate (**10**)

Sodium bis(trimethylsilyl)amide (1.75 ml, 1.57 mmol, 1.0 M in THF) was added to a stirred solution of nonadecyltriphenylphosphonium bromide (0.476 g, 0.76 mmol) at room temperature under nitrogen atmosphere. The mixture was stirred for 30 min then (*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilanyloxy)-19-oxononadecyl]tetracosanoic acid methyl ester (0.31 g, 0.39 mmol)

in dry THF (15 ml) was added. The mixture was stirred for 3 h when TLC showed no starting material was left, then the reaction was quenched with sat. aq. NH_4Cl (10 ml) and the product was extracted with petrol:ethyl acetate (20:1) (3 × 20 ml). The combined organic layers were dried over $MgSO_4$, filtered and the solvent was evaporated. The product was purified by chromatography over silica gel, eluting with petrol/ethyl acetate (40:1) to give methyl (2*R*,3*R*,*Z*)-3-(*tert*-butyldimethylsilyloxy)-2-docosyltetracont-21-enoate **10** (0.255 g, 62%), $[\alpha]_D^{24}$ −2.45 (c 1.18, $CHCl_3$) (Found $[M+Na]^+$: 1066.0319. $C_{69}H_{138}O_3Si$ Na requires: 1066.0307); this showed δ_H: 5.35 (2H, dt, *J* 11.65, 5.65 Hz), 3.91 (1H, br.td, *J* 7, 4.4 Hz), 3.66 (3H, s), 2.53 (1H, ddd, *J* 11.05, 7.25, 3.8 Hz), 2.02 (4H, br.q, *J* 7 Hz), 1.6–1.2 (106H, br. m), 0.89 (6H, t, *J* 6.6 Hz), 0.87 (9H, s), 0.05 (3H, s), 0.02 (3H, s); δ_C 175.14, 129.89, 73.22, 51.57, 51.21, 33.67, 31.92, 29.83, 29.77, 29.70, 29.61, 29.58, 29.56, 29.44, 29.36, 29.31, 27.83, 27.50, 27.21, 25.75, 23.68, 22.69, 17.97, 14.11, −4.37, −4.93; ν_{max}: 2923, 2852, 1741, 1468, 1437, 1361, 1179, 1120, 836, 775, 720, 695 cm^{-1}.

3.12. Methyl (2R,3R,E/Z)-3-(tert-butyldimethylsilyloxy) -2-docosyltetracont-21-enoate (**11**)

The same procedure was followed as above in order to couple aldehyde (0.19 g, 2.44 mmol) and 5-(nonadecane-1-sulfonyl)-1-phenyl-1H-tetrazole (0.17 g, 3.66 mmol) using lithium bis(trimethylsilyl)amide (0.5 ml, 5.38 mmol, 0.5 ml), which was added at −10 °C. The product was purified by column chromatography eluting with petrol/ethyl acetate (40:1), to give the title compounds **11** (0.18 g, 70%) (Found $[M+Na]^+$: 1066.0248. $C_{69}H_{138}O_3SiNa$ requires: 1066.0308). This showed δ_H: (major isomer) 5.4–5.38 (2H, m), 3.86 (1H, br.dt, *J* 6.65, 4.7 Hz), 3.61 (3H, s), 2.48 (1H, ddd, *J* 11, 7.25, 3.75 Hz), 1.93–1.90 (4H, br.q, *J* 6 Hz), 1.5–1.15 (104H, m), 0.84 (6H, t, *J* 7 Hz), 0.82 (9H, s), 0.00 (3H, s), −0.025 (3H, s); (minor isomer) 5.36–5.34 (2H, m), 1.97 (4H, br.q, *J* 6.65 Hz) (the remaining signals obscured by the major isomer); δ_C (both isomers): 175.14, 130.36 (*trans* isomer), 129.89 (*cis* isomer), 73.22, 51.57, 51.2, 33.68, 32.6, 31.92, 29.83, 29.78, 29.7, 29.66, 29.6, 29.58, 29.57, 29.54, 29.53, 29.44, 29.36, 29.32, 29.2, 27.82, 27.5, 27.2, 25.75, 23.69, 22.68, 22.6, 17.97, 14.1, −4.38, −4.94; ν_{max}: 2922, 2851, 2360, 1741, 1464, 1361, 1252, 1193, 1165, 1070, 1005, 965, 835, 774, 719 cm^{-1}.

3.13. Methyl (2R,3R,Z)-2-docosyl-3-hydroxytetracont-21-enoate (**12**)

(*Z*)-(*R*)-3-(*tert*-Butyldimethylsilanyloxy)-octatriacont-20-enoic-2-tetracosanoic acid methyl ester (0.4 g, 4.1 mmol) was stirred in dry THF (10 ml) in dry polyethylene vial under nitrogen atmosphere at r.t. Pyridine (0.3 ml) and HF-pyridine (1 ml) were added and the mixture was stirred for 18 h at 40 °C. The reaction was diluted with petrol/ethyl acetate (1:1, 10 ml) and neutralized with sat. aq. $NaHCO_3$. The mixture was separated and the aqueous layer was re-extracted with petrol/ethyl acetate (1:1, 2 × 20 ml). The combined organic layers were washed with brine, dried and the solvent was evaporated. Chromatography eluting with petrol/ethyl acetate (10:1) gave methyl (2*R*,3*R*,*Z*)-2-docosyl-3-hydroxytetracont-21-enoate **12** as a white solid (0.32 g, 87%), m.p. 44–46 °C, $[\alpha]_D^{20}$ +4.65 (*c* 1.83, $CHCl_3$) (Found $[M+Na]^+$: 951.9458, $C_{63}H_{124}NaO_3$ requires: 951.9443), which showed δ_H: 5.31–5.24 (2H, m), 3.71 (3H, s), 3.60–3.58 (1H, m), 2.38 (1H, br.td, *J* 9.15, 5.35 Hz), 1.94 (4H, q, *J* 6.6 Hz), 1.66–1.61 (1H, m), 1.56–1.48 (4H, m), 1.42–1.2 (102H, br. m), 0.89 (6H, t, *J* 6.6 Hz); δ_C: 176.21, 129.89, 72.31, 51.48, 50.95, 35.70, 31.92, 29.77, 29.69, 29.60, 29.57, 29.49, 29.42, 29.35, 29.32, 27.42, 27.21, 25.72, 22.67, 14.09; ν_{max}: 3516, 2917, 2849, 1713, 1463, 1169, 719 cm^{-1}.

3.14. (2R,3R,Z)-2-Docosyl-3-hydroxytetracont-21-enoic acid (**13**)

Lithium hydroxide monohydrate (0.3 g, 5.24 mmol) was added to a stirred solution of methyl ester **12** (0.32 g, 0.3 mmol) in THF (10 ml), methanol (1 ml) and water (1.5 ml) at r.t. The mixture was stirred at 40 °C for 18 h, when TLC showed no starting material was left. The reaction mixture was cooled down to room temperature and diluted with petrol/ethyl acetate (5:2, 10 ml) and acidified to pH = 1 with 5% HCl. The product was extracted with petrol/ethyl acetate (5:2) (3 × 10 ml), and the combined organic layers were dried over $MgSO_4$ and evaporated to give a crude product which was purified by column chromatography eluting with petrol:ethyl acetate (7:2) to give (2*R*,3*R*,*Z*)-2-docosyl-3-hydroxytetracont-21-enoic acid (0.26 g, 81%) **13** as a white solid, m.p. 65–66 °C, $[\alpha]_D^{21}$ +3.43 (c 0.97, $CHCl_3$) (Found $[M+Na]^+$: 937.91, $C_{62}H_{122}NaO_3$ requires: 937.93 (MALDI)) which showed δ_H: 5.34–5.25 (2H, m), 3.70 (1H, dt, *J* 8.2, 4.95 Hz), 2.43 (1H, td, *J* 8.8, 5.35 Hz), 1.97 (4H, q, *J* 6.65 Hz), 1.64–1.48 (16H, m), 1.21 (92H, br.s), 0.85 (6H, t *J* 6.6 Hz); δ_C: 175.14, 129.90, 72.17, 50.52, 35.56, 31.92, 29.77, 29.70, 29.66, 29.57, 29.48, 29.41, 29.35, 29.31, 27.31, 27.21, 25.71, 22.68, 14.10; ν_{max}: 3530, 2915, 2848, 2360, 1684, 1468, 1378, 1208, 965, 717 cm^{-1}.

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