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The Synthesis of Single Enantiomers of α-Mycolic Acids of *Mycobacterium tuberculosis* and Related Organisms, with Alternative Cyclopropane Stereochemistries

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**Abstract** We report the synthesis of three stereoisomers of a mycolic acid from *Mycobacterium tuberculosis* containing a di-cis-cyclopropane and of two stereoisomers of a mycolic acid containing a proximal trans-cyclopropane and a distal cis-cyclopropane.

**Key words** mycolic acid, cis-cyclopropane, trans-cyclopropane, stereoisomers

Mycolic acids (MA) from mycobacteria, having a general structure 1 (Figure 1), usually containing 70 to 90 carbons, are present as complex mixtures with varying chain lengths and a number of combinations of distal and proximal substituents X and Y, and different exact compositions depending on the species.1–7

![Figure 1](link)

Figure 1 General structure of mycolic acids

Commonly, group Y is a cis- or α-methyl substituted trans-cyclopropane, a cis-alkene or a trans-alkene with an adjacent methyl substituent. Group X is a cis-cyclopropane (α- and α’-MA), a -CHMeOCHMe- fragment (methoxy-MA) or a -CHMeCO- fragment (keto-MA). Structural assignment has often been based on mass spectra of mixtures of homologues or fragments from them.8,9 It is often difficult from the early literature to judge the certainty with which a structure has been assigned or, indeed, which actual values of a–d have been determined. However, recent detailed studies have clarified the situation considerably.8,9 The presence and proportion of individual classes of MA, and in particular cyclopropanated MA, is known to be important for the virulence of diseases such as tuberculosis.10–13 The free MA are themselves strongly bioactive and indeed synthetic MA of different classes, matching the structures of components of natural mixtures, are selectively active.14

Among the most abundant of these acids are α-MAs containing two cis-cyclopropanes (2).8,9 The acid 2 (a = 19, b = 14, c = 11, d = 23) was reported by Minnikin and Polgar to be the major MA of *Mycobacterium tuberculosis* var hominis.15 (Figure 2)

![Figure 2](link)

Figure 2 Typical α-mycolic acid chain lengths

Indeed, such α-MA make up around 50% of the MA isolated from Mtb cells.7 Although the hydroxy-acid grouping is known to be of R.R-configuration, the absolute stereochemistries of the cyclopropanes are not always clearly defined. There is evidence that the 1-methyl-2-methoxy unit at the distal position from the hydroxy acid in methoxy-MA is S,S,16,17 while the corresponding carbon bearing a methyl group in ketomycolates is also S.18 Much is now known about the enzymes controlling the biosynthesis of MA,19–23 and it has been proposed, for example, that the cis-cyclopropane unit, the α-methyl-trans-cyclopropane and the α-methyl-β-alkoxy unit are formed from a Z-alkene through a common intermediate (Scheme 1).24,25 A consequence of...
this would be that the three subunits should have a common absolute stereochemistry at the carbon bearing the methyl group and C-1 of the cis-cyclopropane.

The stereochemistry B of the trans-cyclopropane unit is consistent with NMR spectra and optical rotations of this fragment in methoxy-MA, and with that of the distal position in methoxy- and keto-MA, and on this basis the cis-cyclopropane stereochemistry is likely to be A. However, there is little direct evidence that this is the case, and an alternative possibility is that the cis-isomer is produced with an alternative stereochemistry in the enzyme-promoted cyclopropanation, or indeed that a mixture of stereoisomers is produced. Although the isomer with stereochemistry A at both cyclopropanes, compound 2a (Figure 3) was reported some time ago, we now describe the synthesis of the three other stereoisomers of 2 containing two cis-cyclopropanes in order that their biological properties may be compared to those of 2a.

The synthetic method used, a simple extension of the method used to prepare 2a, involved the use of a common precursor for the two chiral cyclopropane units, with C–C bonds being created at the positions shown in Figure 4.

The (1S,2R)-aldehyde 3 was prepared by a method described earlier from (1S,2R)-butyryloxyethyl-2-formylcyclopropane. Reaction of (1R)-butyryloxyethyl-(2S)-formyl-cyclopropane 5 with sulfone 4 in a similar way (Scheme 2) led to the (1R,2S)-aldehyde 6.

The aldehyde 6 was homologated to give 8 by reaction with sulfone 7 and base, again to give a mixture of E- and Z-alkenes, followed by reduction to the corresponding alcohols, hydrogenation of the alkenes using di-imide, and then oxidation to the aldehyde 9, or converted into the sulfone 10 (Scheme 3).

The proximal cyclopropane unit 11 was treated with aldehyde 9 in a modified Julia reaction, to give a 1:1 mixture of E- and Z-alkenes. Reduction of the esters to the corresponding alcohols using lithium aluminium hydride, followed by hydrogenation of the alkene, again using di-imide, gave a single enantiomer of alcohol 12 (Scheme 4).
Sulfone 10 was coupled to aldehyde 13 prepared as before,33,34 followed again by reduction of the derived E/Z-mixture of esters to the corresponding alcohols and then saturation of the alkenes (Scheme 5).

![Scheme 5](image)

The fourth stereoisomer (16) was prepared by a similar method using 15 (prepared in a similar way to 10; see the Supporting Information) and the aldehyde 5 (Scheme 6).

![Scheme 6](image)

Each of the alcohols 12, 14 and 16 was then converted into the corresponding sulfone 17, 18 and 19 (Scheme 7).

![Scheme 7](image)

Coupling of these with the protected hydroxy-acid fragment 20, followed by hydrogenation of the derived alkenes and then deprotection, led to the three stereoisomers, 22, 23 and 24 (Scheme 8).

![Scheme 8](image)

The 1H and 13C NMR spectra of all four acids were essentially identical to those of a sample extracted from M. tuberculosis. The natural mixture was protected as the acetate on the alcohol and a methyl ester ([α]D +3.7),27 as a mixture of homologues in which 2 predominates. The specific rotation of the synthetic material 2a, protected in the same way ([α]D +4.2) was close to that of the natural mixture. The specific rotation of the synthetic free acid 2a was [α]D +2.1; the rotation is dominated by the chirality of the hydroxy acid part of the molecule and not indicative of the chirality of the cis-cyclopropanes, which contribute very little. Thus, the three isomers prepared in this work showed specific rotations of +2.0 (22), +2.5 (23) and +2.5 (24).

MA containing trans-cyclopropanes at the position in the chain closest to the hydroxy-acid are reported to have a particular effect on the cell wall and therefore on the susceptibility of mycobacterial species to hydrophobic antibiotics.5 A purified trehalose ester of MA lacking trans-cyclopropane rings is five times more potent in stimulating macrophages, and is important as a suppressor of Mtb induced inflammation and virulence.10 The biosynthesis apparently involves conversion of a cis-alkene into an α-methyl-trans-alkene caused by MmaS1 and SAM. This is then cyclopropanated by the CmaA2 gene, again with SAM, to give the α-methyl-trans-cyclopropane.21–25 Inactivation of CmaA2 causes the accumulation of unsaturated derivatives in both methoxy- and keto-MA and the lack of trans-cyclopropanes.26 Although α-MA containing a proximal trans-cyclopropane and a distal cis-cyclopropane do not appear to be present in Mtb, they are present in other Mycobacteria such as Mycobacterium kansasii and Mycobacterium avium complex.8
We therefore describe the synthesis of two stereoisomers of one such compound. The aldehyde 25\textsuperscript{28} was coupled to sulfone 27 (see the Supporting Information) in a modified Julia–Kocienski reaction, followed by saturation of the derived mixture of alkenes with di-imide to give 28, and then oxidative cleavage of the acetal to produce the cis-aldehyde 29. Treatment with base isomerised this to the trans-cyclopropane aldehyde 30. The corresponding aldehyde 31 was prepared by a similar sequence (Scheme 9).

Chain extension of the two aldehydes by standard methods provided the aldehydes 33 and 34 (Scheme 10), which were then coupled to sulfone 35\textsuperscript{15} to provide, after hydrolysis of the protecting groups, the free MA 37 and 38 (Scheme 11).

The effects of the four stereoisomeric MAs 2a and 22–24 in stimulating T-cells have recently been reported. Rather surprisingly, mycolic acid 24 is somewhat more stimulatory than 2a, both having a somewhat stronger effect than 22, with 23 having the least effect.\textsuperscript{36} The trans-cyclopropane 38 is moderately stimulatory, while 37 has a smaller effect.

MA are present both as bound tetramycolyt penta-arabinose clusters and as extractable trehalose 6,6′-dimycoclates (‘cord factor’).\textsuperscript{2–7} The MA 37 and 38 were converted into the corresponding TDM and TMM (see the the Supporting Information) using methods described before.\textsuperscript{37} The effects of the resulting TDMs and TMMs, and those derived from acid 22 in activating bone marrow dendritic cells to produce proinflammatory cytokines (IL-6 and TNF-α, is somewhat more stimulatory

Chemicals used were obtained from commercial suppliers or prepared from them by methods described. Solvents which had to be dry, for example diethyl ether and tetrahydrofuran were dried over sodium wire. Petroleum was of boiling point 40–60 °C. Reactions under inert conditions were carried out under a slow stream of nitrogen. Reactions carried out at low temperatures were cooled using a bath of anhydrous magnesium sulfate. IR spectra were carried out with a Perkin–Elmer 1600 FTIR spectrometer as liquid films. NMR spectroscopy was carried out with Bruker Avance 400 or 500 spectrometers. [α]D values were recorded in CHCl₃ with a POLAR 2001 Optical Activity polarimeter. Mass spectra were recorded with a Bruker-MALDI-TOF MS.
instrument (to an accuracy of 1 d. p.); accurate mass values were obtained in Bangor with a Bruker Microtof LC-MS or by the EPSRC MS service in Swansea or in Bristol University.

(1R,25S)-2-Eicosyclopropanecarbaldehyde (6)

(a) 5-{Nonadecyl-1-sulphonyl}-1-phenyl-1H-tetrazole 4 (15.0 g, 0.032 mol) and butyric acid (1R,25S)-(cis-2-formylcyclopropyl) methyl ester 5 (4.8 g, 0.028 mol) was dissolved in anhydrous THF (250 ml) and cooled to –10 °C. LiHMDS (38.6 mL, 0.041 mol) was carefully added and the mixture was stirred for 1.5 h, then water (100 mL) was added. The aqueous layer was separated and extracted with petrol/ether 2:1 (3 × 150 mL) and the organic layer was washed with brine (2 × 100 mL), dried and evaporated to obtain a residue; column chromatography, eluting with petrol/EtOAc (20:1) gave butyric acid (E/Z)-1R,25S-cis-2-ecicos-1-enylcyclopropyl-methyl ester (9, 85 %) as a colourless oil, as a mixture of isomers in ratio 2.5:1. The derived ester (8.5 g, 0.020 mol) in THF (50 mL) was gradually added to a stirred suspension of lithium aluminium hydride (1.3 g, 0.034 mol) in THF (50 mL) at 0 °C, under nitrogen. The mixture was allowed to reach r.t., and then was added to a stirred suspension of pyridinium chlorochromate (4.66 g, 22.0 mmol, 2.5 mol equiv) in dichloromethane (60 mL) in portions at r.t. The mixture was stirred for 1 h, then the reaction was quenched with sat. aq. ammonium chloride (200 mL), the aqueous layer was separated and extracted with petrol/ether (1:1, 3 × 200 mL) and the combined organic layers were washed with brine (2 × 100 mL), dried and evaporated to obtain a residue; column chromatography, eluting with petrol/EtOAc (20:1) gave 2,2-dimethylpropanoic acid 12-{(1-phenyl-1H-tetrazole-5-yl)-1,13-((1R,25S)-cis-2-ecicosylcyclopropyl)-tridec-12-enyl ester (6.6 g, 98%) as a white solid, as a mixture of isomers in ratio (2.8 : 1). The derived ester (6.0 g, 10 mmol) in THF (20 mL) was gradually added to a stirred suspension of lithium aluminium hydride (0.77 g, 20 mmol) in THF (50 mL) at 0 °C. The mixture was heated at reflux at 100 °C for 2 h, then worked up and purified as before to give: (E/Z)-13-{(1R,25S)-cis-2-ecicosylcyclopropyl}-tridec-12-en-1-ol (5.0 g, 91%) as a white solid. Hydrazine monohydrate (20 mL), acetic acid (2 mL), and sat. aq. copper sulphate (2 mL) were added in succession to the derived hydroxy alcohol (5.0 g, 9.7 mmol) in isopropanol (250 mL) at 80 °C, and then sodium metaperiodate solution (20 g, 97 mmol) in hot water (60 mL) was carefully added dropwise, maintaining the temperature at 80 °C, then worked up as above. The product was recrystallised from chloroform to give the title compound 8.

Yield: 3.8 g (75%); mp 72–74 °C; [α]D26 +3.8 (c 2.03, CHCl3).

1H NMR (500 MHz, CDCl3): δ = 3.65 (dd, J = 7.2, 11.0 Hz, 1 H), 3.58 (dd, J = 7.9, 11.0 Hz, 1 H), 1.67 (br s, 1 H), 1.50–1.38 (m, 4 H), 1.32–1.2 (m, 35 H), 1.15–1.09 (m, 1 H), 0.88 (t, J = 6.9 Hz, 3 H), 0.69 (dt, J = 4.7, 8.2 Hz, 1 H), –0.01 (q, J = 5.2 Hz, 1 H).

13C NMR (126 MHz, CDCl3): δ = 73.4, 73.3, 60.7, 27.8, 26.8, 24.8, 23.0, 22.9, 17.7, 14.8, 14.4, 14.1.

IR: 2991, 2848, 1715, 1469, 1391, 1018, 720 cm–1.

13-((1R,25S)-cis-2-Eicosyclopropyl)tridecan-1-ol (8)

LIHMDS (18.2 ml, 19.3 mmol) was added to a stirred solution of (1R,25S)-2-eicosyclopropanecarbaldehyde 6 (4.0 g, 11 mmol) and 2,2-dimethylpropanoic acid 12-{(1-phenyl-1H-tetrazole-5-yl)-1,13-((1R,25S)-cis-2-ecicosylcyclopropyl)-tridec-12-enyl ester (6.6 g, 98%) as a white solid, as a mixture of isomers in ratio (2.8 : 1). The derived ester (6.0 g, 10 mmol) in THF (20 mL) was gradually added to a stirred suspension of lithium aluminium hydride (0.77 g, 20 mmol) in THF (50 mL) at 0 °C. The mixture was heated at reflux at 100 °C for 2 h, then worked up and purified as before to give: (E/Z)-13-{(1R,25S)-cis-2-ecicosylcyclopropyl}-tridec-12-en-1-ol (5.0 g, 91%) as a white solid. Hydrazine monohydrate (20 mL), acetic acid (2 mL), and sat. aq. copper sulphate (2 mL) were added in succession to the derived hydroxy alcohol (5.0 g, 9.7 mmol) in isopropanol (250 mL) at 80 °C, and then sodium metaperiodate solution (20 g, 97 mmol) in hot water (60 mL) was carefully added dropwise, maintaining the temperature at 80 °C, then worked up as above. The product was recrystallised from chloroform to give the title compound 8.

Yield: 3.8 g (75%); mp 72–74 °C; [α]D26 +3.8 (c 2.03, CHCl3).

1H NMR (500 MHz, CDCl3): δ = 3.65 (t, J = 6.6 Hz, 2 H), 1.58 (pent, J = 6.65 Hz, 4 H), 1.40–1.22 (m, 57 H), 1.15–1.10 (m, 2 H), 0.89 (t, J = 6.6 Hz, 3 H), 0.65 (m, 2 H), 0.56 (dt, J = 4.0, 7.8 Hz, 1 H), –0.32 (q, J = 5.35 Hz, 1 H).

13C NMR (126 MHz, CDCl3): δ = 63.5, 32.0, 30.5, 29.9, 29.8, 29.5, 28.8, 22.8, 18.5, 16.3, 14.0, 10.0.

IR: 3383, 2917, 2848, 1465, 1064, 723, 449, 426, 417, 412 cm–1.

13-(1R,25S)-2-Eicosyclopropyl)tridecanal (9)

Alcohol 8 (4.5 g, 8.6 mmol) in dichloromethane (80 mL) was added to a stirred suspension of PCC (4.66 g, 22.0 mmol, 2.5 mol equiv) in dichloromethane (60 mL) in portions at r.t. The mixture was stirred for 2 h and then diluted with petrol/ether 2:1 (300 mL), filtered through a pad of Celite on silica, then washed well with warm ether (400 mL) and the filtrate was evaporated to give a residue. This was purified by column chromatography on silica eluting with petrol/ether 2:1 to give the title compound 9.

Yield: 3.6 g (84%); mp 61–64 °C; [α]D26 +1.6 (c 1.2, CHCl3) (enantiomer –1.7 (c 1.2, CHCl3)).

1H NMR (500 MHz, CDCl3): δ = 9.77 (t, J = 7.2, 11.0 Hz, 1 H), 1.89–1.83 (m, 1 H), 1.62–1.55 (m, 2 H), 1.53–1.46 (m, 2 H), 1.43–1.2 (m, 36 H), 1.19 (m, 1 H), 0.88 (t, J = 6.9 Hz, 3 H).

13C NMR (126 MHz, CDCl3): δ = 201.8, 31.9, 30.0, 29.7, 29.64, 29.6, 29.5, 29.4, 29.3, 28.2, 27.8, 26.9, 24.8, 23.0, 22.7, 17.7, 14.8, 14.1, 14.1.

IR: 2921, 2851, 1700, 1465, 1215, 758, 449, 426, 417, 412 cm–1.

13-(1R,25S)-cis-2-Eicosyclopropyl)tridecan-1-ol (8)
(a) $\text{LiHMDS (12.8 mL, 13 mmol) was added dropwise with stirring to aldehyde 9 (3.6 g, 6.9 mmol) and sulphone 11 (3.3 g, 7.5 mmol)}^{21,23}$ in anhydrous THF (30 mL) under nitrogen at –10 to –4 °C. The mixture was allowed to reach r.t., stirred for 5 h, then the reaction was quenched with sat. aq. ammonium chloride (50 mL) and petrol/ether 1:1 (100 mL). 

The organic layer was separated and the aqueous layer was re-extracted with petrol/ether 1:1 (2 × 100 mL). The combined organic layers were washed with water (2 × 200 mL), dried and evaporated. The product was recrystallised from methanol/acetonitrile (1:1), which gave a white solid of the title compound 10.

Yield: 2.1 g (67%); mp 72–74 °C; $[\alpha]_D^{26} +8.3$ (c 0.86, CHCl$_3$).

MS: $m/z [M + H]^+$ calcd for C$_{41}$H$_{83}$O$_3$: 590.0978; found: 590.0980.

$^1$H NMR (500 MHz, CDCl$_3$): $\delta = 7.36$ (m, 5 H), $3.42$ (t, $J = 7.4$ Hz, 2 H), 1.81 (pent, $J = 7.5$ Hz, 2 H), 1.43–1.12 (m, 58 H), 0.91 (t, $J = 6.9$ Hz), 0.87–0.64 (m, 2 H), 0.57 (dt, $J = 4.1$, $3.8$ Hz), $-0.35$ (q, $J = 4.9$ Hz, 1 H).

$^{13}$C NMR (126 MHz, CDCl$_3$): $\delta = 154.8$, 134.2, 133.3, 130.1, 125.9, 33.7, 30.2, 29.7, 29.65, 29.6, 29.5, 29.43, 29.4, 29.1, 29.0, 28.73, 28.7, 22.9, 15.6, 14.4, 11.1.

IR: 3060, 1457, 1378, 1244, 1168, 1015, 693 cm$^{-1}$.

(b) LiAlH$_4$ (0.2 g) was added to stirred THF (100 mL) at 0 °C under nitrogen to ensure THF dryness. Then further LiAlH$_4$ (1.0 g, 26 mmol) was added. A solution of alkenes (3.0 g, 4.5 mmol) in anhydrous THF (20 mL) was added dropwise by using a syringe at 0 °C and the mixture was heated at reflux for 3 h, then cooled to 0 °C and the reaction was quenched with sat. aq. sodium sulphate dehydrate (20 mL), which was added dropwise and stirred at rt until a white precipitate was formed followed by addition of MgSO$_4$ (20 g). THF (40 mL) was added and the mixture was filtered through a pad of silica, dried and the solvent evaporated to give a mixture of alcohols (2.4 g, 89%), which was used for the next step without further purification.

(c) $\text{Sodium (meta)periodate (20.3 g, 95.0 mmol) in hot water (50 mL) was added over 70 min at 70–80 °C to a stirred solution of above alcohol (2.9 g, 4.8 mmol) in iso propyl alcohol (250 mL), acetic acid (1.5 mmol), sat. aq. copper sulphate (1.5 mL) and hydrazine hydrate (20 mL).}$

The mixture was stirred for 2 h until it reached r.t., then diluted with water (100 mL) and petrol/ether 5:1 (400 mL). Due to the low solubility of the product, the mixture was warmed (40 °C) to allow separation. The aqueous layer was re-extracted with warm petrol/ether 5:1 (3 × 100 mL). The combined organic layers were dried and evaporated to give a solid. Column chromatography on silica gel eluting with petrol/ether 5:1 gave the title compound 12.

Yield: 1.8 g (63%); white solid; mp 72–74 °C; $[\alpha]_D^{26} +8.3$ (c 0.86, CHCl$_3$).

MS: $m/z [M + H]^+$ calcd for C$_{41}$H$_{83}$O$_3$: 590.0978; found: 590.0980.

$^1$H NMR (500 MHz, CDCl$_3$): $\delta = 3.66$ (dd, $J = 7.3, 11$ Hz), 1.50 (dd, $J = 7.9, 11$ Hz), 1.37 (br m, 4 H), 0.89–0.84 (including t at $J = 0.89$ Hz, 4 H), 0.70 (dt, $J = 4.4$, 8.2 Hz, 1 H), 0.68–0.63 (m, 2 H), 0.57 (dt, $J = 4.1$, 8.5 Hz), 1.17 (br m, 4 H), 0.9–0.84 (including t at $J = 0.88$ Hz, 4 H), 0.70 (dt, $J = 4.4$, 8.2 Hz, 1 H), 0.68–0.63 (m, 2 H), 0.57 (dt, $J = 4.1$, 8.5 Hz, 1 H), $-0.03$ (q, $J = 5.35$ Hz, 1 H), $-0.32$ (q, $J = 5.35$ Hz, 1 H).

$^{13}$C NMR (126 MHz, CDCl$_3$): $\delta = 63.4$, 31.9, 30.2, 30.18, 29.7, 29.65, 29.6, 29.4, 28.7, 28.6, 22.7, 18.2, 16.2, 15.8, 14.1, 10.9, 9.5.

IR: 3375, 2852, 1771, 1464, 1370, 1170, 1064, 1037, 964, 932, 823 cm$^{-1}$.

(15SR)-2-[(15RS)-2-Eicosylcyclopropyl]tetradecyl)cyclopropyl Methanol (14)

LiHMDS (3.7 mL, 40 mmol) was added to a stirred solution of butyric acid (15SR)-(2S)-2-formylocyclopropyl)methyl ester 13 (0.5 g, 3.0 mmol) and sulphone 10 (2.1 g, 30 mmol) in anhydrous THF (30 mL) at –10 °C. The mixture was allowed to reach r.t. and stirring was continued for 1.5 h, then worked up and purified as before to give butyric acid (15SR)-2-[(E/Z)-14-((1RS)-2-Eicosylcyclopropyl)tetradec-1-enyl)cyclopropyl)methyl ester (1.2 g, 62%) as a white solid in a mixture of cyclopropylmethanol in ratio 2.5:1. The above ester (1.2 g, 1.8 mmol) in THF (10 mL) was added dropwise to a stirred suspension of lithium aluminium hydride (0.14 g, 3.7 mmol) in THF (10 mL) at –10 °C under nitrogen atmosphere. The reaction was heated at reflux at 100 °C for 2.5 h then worked up as before to give (15SR)-2-[(E/Z)-14-((1RS)-2-Eicosylcyclopropyl)tetradec-1-enyl)cyclopropyl)methyl ester (1.2 g, 62%) as a white solid. Hydrazine monohydrate (7 mL), acetic acid (1 mL), and sat. aq. copper sulphate (1 mL) were added in succession at 70–80 °C to a stirred solution of the above alcohol (0.77 g, 1.3 mmol) in isopropyl alcohol (50 mL). Still maintaining the temperature at 80 °C, a solution of sodium metaperiodate (2.8 g, 13 mmol) in hot water (10 mL) was carefully added dropwise. The mixture was allowed to reach r.t. and stirred for 1 h, then worked up as above to give the title compound 14.

Yield: 0.55 g (71%); white solid; mp 51–53 °C; $[\alpha]_D^{25} +7.3$ (c 1.0, CHCl$_3$).

MS: $m/z [M + Na]^+$ calcd for C$_{41}$H$_{85}$ONa: 611.6; found: 611.5.
**1H NMR (500 MHz, CDCl3):** δ = 7.65–7.5 (m, 5 H), 3.49 (d, J = 7.9 Hz, 2 H), 1.56 (br s, 3 H), 1.49 (m, 1 H), 1.38–1.13 (br m, 63 H), 0.95–0.9 (m, 1 H), 0.88 (t, J = 7.3 Hz, 3 H), 0.83 (dt, J = 5.1, 8.5 Hz, 1 H), 0.67–0.62 (m, 2 H), 0.56 (dt, J = 4.1, 8.2 Hz, 1 H), 0.08 (q, J = 5.4 Hz, 1 H), –0.32 (q, J = 5.4 Hz, 1 H).

**13C NMR (126 MHz, CDCl3):** δ = 154.6, 133.8, 132.1, 130.0, 129.7, 128.5, 128.4, 128.4, 128.3, 35.0, 31.9, 30.2, 30.0, 29.7, 29.65, 29.5, 28.7, 28.5, 22.7, 18.0, 15.8, 14.6, 14.1, 12.5, 10.9.

IR: 3068, 2988, 2916, 2849, 1599, 1502, 1469, 1381, 1234, 1016, 824, 754, 694, 542, 458, 451, 435 cm⁻¹.

(b) Sodium hydrogen carbonate (1.03 g, 12 mmol, 4.5 equiv) was added to a stirred solution of the above tetracazo (2.0 g, 2.7 mmol) in dichloromethane (100 mL), followed by the addition of a mixture of anhydrous 3-chloroperoxybenzoic acid 70% (1.67 g, 9.7 mmol, 2.5 equiv) in CH₂Cl₂ (50 mL). The reaction was stirred at rt. for 48 h to give an off-white precipitate. Aq. sodium hydroxide (58, 80 mL) was added and the aqueous layer was extracted with dichloromethane (3 × 50 mL). The combined organic layers were washed with water (2 × 2 mL) and evaporated. The product was recrystallised from methanol/acetonitrile (1:1) to give the title compound 17.

**Yield:** 1.77 g (85%); [α]D 30 –18 (c 1.5, CHCl₃).

**MS:** m/z [M + H⁺] calc for C₄₈H₈₅N₄O₂S: 782.2976; found: 782.2980.

**1H NMR (500 MHz, CDCl₃):** δ = 7.72–7.68 (m, 2 H), 7.65–7.58 (m, 3 H), 3.98 (dd, J = 5.35, 14.5 Hz, 1 H), 3.57 (dd, J = 9.15, 14.5 Hz, 1 H), 1.5–1.2 (m, 66 H), 1.17–1.1 (m, 1 H), 1.03–0.94 (m, 1 H), 0.90–0.86 (including t at δ 0.89 with J = 6.95 Hz, 4 H), 0.68–0.64 (m, 2 H), 0.56 (dt, J = 4.1, 8.2 Hz, 1 H), 0.25 (q, J = 5.7 Hz, 1 H), –0.32 (q, J = 5.35 Hz, 1 H).

**13C NMR (126 MHz, CDCl₃):** δ = 153.7, 133.1, 131.4, 129.7, 125.2, 57.1, 31.9, 30.2, 29.64, 29.6, 29.44, 29.34, 29.27, 28.27, 22.7, 15.9, 15.8, 14.1, 11.4, 10.9, 8.0.

IR: 2990, 2916, 2849, 1498, 1470, 1340, 1152, 761, 718, 687 cm⁻¹.

5-[[(15S,2)-2-[(15R,2)-2-Eicosylcyclopropyl]tetradecyl]cyclopropylmethylsulfanyl]-1-phenyl-1H-tetrazole (18)

(a) Alcohol 14 (0.55 g, 0.85 mmol) was dissolved in anhydrous THF (5 mL) and then triphenylphosphine (0.3 g, 1.1 mmol) and 1-phenyl-1H-tetrazole-5-thiol (0.2 g, 1.1 mmol) were added. The mixture was cooled to 0 °C and treated as before with DEAD (0.18 mL, 1.1 mmol) in THF (3 × 200 mL). The combined organic layers were washed with water (2 × 2 mL) and evaporated. The product was recrystallised from methanol/acetonitrile (1:1) to give the title compound 17.

**Yield:** 0.7 g (92 %); mp 43–50 °C; [α]D 30 –21 (c 1.7, CHCl₃).

**MS:** m/z [M + Na⁺] calc for C₄₉H₈₄N₄SNa: 771.6; found: 771.4.

**1H NMR (500 MHz, CDCl₃):** δ = 6.91–6.76 (m, 5 H), 3.49 (d, J = 7.9 Hz, 2 H), 1.60–1.11 (including m at 1.47–1.53 for one cyclopropane proton, 67 H), 0.95–0.9 (m, 1 H), 0.89 (t, J = 6.9 Hz, 3 H), 0.84 (dt, J = 4.7, 1 H, 8.2 Hz), 0.67–0.63 (m, 2 H), 0.57 (dt, J = 3.4, 8.2 Hz, 1 H), 0.08 (q, J = 5.4 Hz, 1 H), –0.32 (q, J = 5.4 Hz, 1 H).

**13C NMR (125 MHz, CDCl₃):** δ = 133.8, 130, 123.8, 35.0, 31.9, 30.9, 29.5, 28.5, 22.7, 21.0, 18.0, 15.8, 14.6, 14.1, 12.

IR: 3059, 2923, 1463, 1340, 1275, 1169, 1088 cm⁻¹.

(b) Ammonium molybdate(VI) tetrahydrate (2.90 g, 2.34 mmol) was dissolved in cold hydrogen peroxide (35% w/w, 10 mL) and was added gradually to a stirred solution of the above tetracazo (0.35 g, 0.47 mmol) in IMS/THF (30:10 mL) at 5–10 °C, then allowed to attain r.t. and stirred for another 2 h after which further ammonium
molybdate(VI) tetrahydrate (1.20 g, 0.94 mmol) in cold hydrogen peroxide (5 ml) was added. The reaction was stirred for 18 h and then poured into water (200 ml) and extracted with dichloromethane (3 × 50 ml). The organic layers were washed with water (2 × 50 ml) and concentrated. Column chromatography eluting with petrol/ether (5:2) gave the title compound 18.

Yield: 0.15 g (41%); mp 68–69 °C; [α]D20 +17 (c 1.2, CHCl3).

MS MALDI: m/z [M + Na]+ calcd for C84H126O11N6SNa: 803.6; found: 803.5.

1H NMR (500 MHz, CDCl3): δ = 7.72–7.68 (m, 2 H), 7.65–7.58 (m, 3 H), 3.98 (dd, J = 5.7, 14.9 Hz, 1 H), 3.57 (dd, J = 9.5, 14.9 Hz, 1 H), 1.38–1.18 (m, 68 H), 1.05–0.97 (m, 1 H), 0.89 (t, J = 6.6 Hz, 3 H), 0.67–0.63 (m, 2 H), 0.57 (dt, J = 4.1, 8.2 Hz, 1 H), 0.08 (q, J = 5.6 Hz, 1 H), –0.33 (q, J = 5.0 Hz, 1 H).

13C NMR (126 MHz, CDCl3): δ = 131.4, 129.7, 125.2, 57.1, 31.9, 30.2, 29.7, 29.5, 29.1, 28.7, 22.7, 15.9, 14.1, 11.4, 10.9, 8.0.

IR: 2922, 2852, 1463, 1377, 1339, 1156 cm–1.


(a) LiHMDS (2.78 ml, 2.9 mmol, 1.3 mol equiv, 1.06 M) was added to a stirred solution of the above alkenes (1.77 g, 2.27 mmol) and aldehyde 20 (1.77 g, 2.5 mmol, 1.1 mol equiv) in anhydrous THF (40 ml) at –10 °C under nitrogen. The solution was allowed to react, stirred for 2 h, then petrol/ether 10:1 (100 ml) and sat. aq. NH4Cl (50 ml) were added. The organic layer was separated and the aqueous layer was reextracted with petrol/ether 10:1 (2 × 100 ml). The combined organic layers were dried and evaporated. Column chromatography eluting with petrol/ether 20:1 gave the product alkenes (2.5 g, 87%) as an E/Z-mixture in ratio 2:1.

(b) Dipotassium azodicarboxylate (3.0 g, 0.015 mol) was added to a stirred solution of the above alkenes (2.4 g, 1.9 mmol) in anhydrous THF (20 ml) and MeOH (25 ml) and then cooled to 0 °C. Then acetic acid (5 ml) in THF (5 ml) was added dropwise at a rate of 1 ml/15 min. The reaction turned bright-yellow and was left stirring for 9 h at 5 °C. The procedure was repeated as above with further dipotassium azodicarboxylate, acetic acid, stirring for a further 9 h, then quenched by adding the mixture in small portions to sat. aq. NaHCO3 (50 ml). After extraction, column chromatography eluting with petrol/ether 20:1 gave the title compound 21.

Yield: 2.07 g (86%); [α]D28 +3.8 (c 1.40, CHCl3).

MALDI MS: m/z [M + Na]+ calcd for C85H168O3SiNa: 1288.3; found: 1288.4.

1H NMR (500 MHz, CDCl3): δ = 3.95–3.88 (m, 3 H), 3.7 (s, 3 H), 2.54 (dd, J = 2.9, 6.4, 9.2 Hz, 1 H), 1.58–1.10 (m, 134 H), 0.87 (1 J = 6.7 Hz, 6 H), 0.86 (s, 9 H), 0.68–0.62 (m, 4 H), 0.58 (dt, J = 3.8, 7.9 Hz, 2 H), 0.05 (s, 3 H), 0.03 (s, 3 H), –0.32 (q, J = 4.75 Hz, 2 H).

13C NMR (126 MHz, CDCl3): δ = 175.1, 73.2, 51.6, 51.2, 41.4, 36.1, 33.7, 33.7, 32.0, 30.3, 29.9, 29.74, 29.74, 29.65, 29.6, 29.5, 29.4, 28.9, 28.8, 27.9, 27.7, 25.5, 25.8, 23.7, 22.7, 22.6, 20.5, 19.4, 18.0, 15.8, 14.1, 10.9, –4.4, –4.9.

IR: 2923, 2853, 1742, 1465, 1253 cm–1.

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Yield: 0.1 g (90%).

(a) A dry polyethylene vial equipped with an acid-resistant rubber septum was charged with the above TBDMS methyl ester (0.10 g, 0.08 mmol) and anhydrous pyridine (0.1 mL) in anhydrous THF (4 mL) and stirred at rt. under nitrogen. To it was added hydrogen fluoride-pyridine complex as ca. 70% hydrogen fluoride (0.7 mL) at 5 °C. The mixture was stirred at 45 °C for 17 h, then worked up and purified as above to give (R)-2-((R)-1-hydroxy-12-((1R,2S)-2-ecicosylcyclopropyl)tetradecyl)cyclopropyl)dodecyl)hexacosanoic acid methyl ester.

Yield: 0.03 g (33%); white solid; mp 55–57 °C; [α]D10 +2.5 (c 1.7, CHCl3).

MALDI MS: m/z [M + Na]+ calcd for C85H168O3SiNa: 1288.3; found: 1288.6.

(b) The above methyl ester (1.0 g, 0.9 mmol) was dissolved in THF (15 mL), MeOH (1 mL) and water (1.5 mL), and then lithium hydroxide monohydrate (1.10 g, 26.2 mmol, 30 equiv) was added to the stirred mixture at rt. The mixture was stirred at 45 °C for 17 h, then cooled to rt. and diluted with petrol/ EtOAc (7: 2, 10 mL) followed by the dropwise addition of sat. aq. potassium hydrogen sulfate (20 mL), which brought the mixture to pH 1. The aqueous layer was separated and re-extracted with warm petrol/ EtOAc (7:2, 3 × 10 mL). The combined organic layers were dried and concentrated to give a crude product, which was purified by column chromatography, eluting with warm petrol/ EtOAc (7:2) to give compound 22.

Yield: 0.92 g (93%); Rf 0.42 (above solvent); [α]D19 +2.0 (c 1.2, CHCl3).

MS: m/z [M + Na]+ calc’d for C95H190O4Na: 1161.06; found: 1160.59.

1H NMR (500 MHz, CDCl3): δ = 3.75–3.70 (m, 1H), 1.85–1.48 (m, 136H), 0.91 (t, J = 6.8 Hz, 6H), 0.73–0.62 (m, 9H), 0.54 (dd, J = 6.8, 4.9 Hz, 2H), –0.35 (q, J = 5.1 Hz, 2H).

13C NMR (126 MHz, CDCl3): δ = 117.4.  IR: 3520, 2924, 2855, 2367, 1712, 1461, 1377, 1165, 721 cm–1.

(c) A dry polyethylene vial equipped with an acid-resistant rubber septum was charged with the above TBDMS methyl ester (0.10 g, 0.08 mmol) and anhydrous pyridine (0.1 mL) in anhydrous THF (4 mL) and stirred at rt. under nitrogen. To it was added hydrogen fluoride-pyridine complex as ca. 70% hydrogen fluoride (0.7 mL) at 5 °C. The mixture was stirred at 45 °C for 17 h, then worked up and purified as above to give (R)-2-((R)-1-hydroxy-12-((1R,2S)-2-ecicosylcyclopropyl)tetradecyl)cyclopropyl)dodecyl)hexacosanoic acid methyl ester.

Yield: 0.03 g (33%); white solid; mp 55–57 °C; [α]D19 +2.5 (c 1.7, CHCl3).

MALDI MS: m/z [M + Na]+ calc’d for C85H168O3SiNa: 1174.2; found: 1174.4.

1H NMR (500 MHz, CDCl3): δ = 3.71 (s, 3H), 3.68–3.62 (m, 1H), 2.46–2.42 (m, 1H), 1.74–1.70 (m, 1H), 1.61–1.57 (m, 2H), 1.48–1.15 (m, 132H), 0.89 (t, J = 6.6 Hz, 6H), 0.68–0.62 (m, 4H), 0.55 (dt, J = 4.1, 8.2 Hz, 2H), –0.33 (q, J = 4.9 Hz, 2H).


IR: 2923, 2853, 1742, 1464, 1254 cm–1.

(d) The above methyl ester (0.030 g, 0.026 mmol) was dissolved in THF (4.0 mL), MeOH (0.5 mL) and water (0.7 mL), and then lithium hydroxide monohydrate (0.02 g, 0.48 mmol) was added to the stirred mixture at rt. The mixture was heated at 45 °C for 18 h, then cooled to rt. and diluted with petrol/ EtOAc (7:2, 5 mL), followed by the dropwise addition of sat. aq. potassium hydrogen sulfate (10 mL) to pH 1. The aqueous layer was extracted with warm petrol/ EtOAc (7:2, 3 × 10 mL). The combined organic layers were dried and concentrated. Column chromatography, eluting with petrol/ EtOAc (7:2) gave the title compound 23.

Yield: 0.019 g (64%); white solid; mp 51–53 °C; [α]D19 +0.42 (above solvent); [α]D21 +2.5 (c 1.4, CHCl3).

MS: m/z [M + Na]+ calc’d for C85H168O4Na: 1160.1634; found: 1160.1626.

1H NMR (500 MHz, CDCl3): δ = 3.73 (td, J = 4.8, 7.9 Hz, 1H), 2.48 (td, J = 5.4, 8.9 Hz, 1H), 1.76–1.71 (m, 1H), 1.63–1.60 (m, 2H), 1.54–1.47 (m, 4H), 1.26 (m, 129H), 0.89 (t, J = 6.9 Hz, 6H), 0.66–0.62 (m, 4H), 0.57 (dt, J = 4.1, 8.2 Hz, 2H), –0.33 (q, J = 4.9 Hz, 2H).

13C NMR (126 MHz, CDCl3): δ = 178.6, 72.1, 50.8, 36.6, 31.9, 30.2, 29.7, 29.6, 29.5, 29.4, 28.7, 27.3, 25.7, 22.7, 15.8, 14.1, 10.9.

IR: 3520, 2925, 2367, 1712, 1461, 1377, 1165, 721 cm–1.

(R)-2-((R)-1-Hydroxy-12-((1R,2S)-2-ecicosylcyclopropyl)tetradecyl)cyclopropyl)dodecyl)hexacosanoic Acid (23)

(R)-2-((R)-1-Hydroxy-12-((1R,2S)-2-ecicosylcyclopropyl)tetradecyl)cyclopropyl)dodecyl)hexacosanoic Acid (24)
(b) Dipotassium azodicarboxylate (2.0 g, 10.3 mmol) was added to a stirred solution of the derived alkenes (0.11 g, 0.09 mmol) in anhydrous THF (3 mL) and MeOH (1.5 mL) and then cooled to 0 °C under nitrogen. Glacial acetic acid (0.5 mL) was dissolved in THF (1.0 mL), and then added dropwise to the mixture, which was stirred overnight at r.t. The process was repeated using the same amount of glacial acetic acid in THF until there was a change in colour from bright yellow to off-white. The reaction was worked up and purified as before to give the title compound (R)-2-(((1S,2S)-2-hexadecyl)cyclopropyl)cyclopropyl-1,3-dioxolane (28). IR: 2984, 2831, 1597, 1500, 1379, 1059, 853, 761 cm\(^{-1}\).

(c) A dry polyethylene vial equipped with an acid-resistant rubber septum was charged with the above ester (0.1 g, 0.08 mmol) and anhydrous pyridine (0.1 mL) in anhydrous THF (4 mL) and stirred at r.t. The reaction was heated to reflux at 45 °C for 18 h, then cooled to 0 °C and worked up purified as before to give (R)-2-((R)-1-hydroxy-12-(1-phenyl-1-propyl)butylthio)-1-phenyl-1H-tetrazole. IR: 3317, 2922, 2852, 1681, 1464, 1377, 721 cm\(^{-1}\).

4. Monoesters

5-(S)-3-((1R,2R)-2-((S)-2,2-Dimethyl-1,3-dioxolan-4-yl)cyclopropyl)butylsulfonyl)-1-phenyl-1H-tetrazole (27)

(a) Diethyl azodicarboxylate (10.6 g, 60.8 mmol) in anhydrous THF (15 mL) was added to a stirred solution of (S)-3-((1R,2R)-2-((S)-2,2-dimethyl-1,3-dioxolan-4-yl)cyclopropyl)butan-1-ol 26\(^\circ\) (10.0 g, 46.7 mmol), triphenylphosphine (17.2 g, 65.5 mmol) and 1-phenyl-1H-tetrazole-5-thiol (11.6 g, 65.0 mmol) in anhydrous THF (100 mL) at 0 °C, allowed to reach r.t. and stirred overnight, then the solvent was evaporated and the residue was heated at reflux with petroleum/EtOAc (5:2, 150 mL) for 1 h and filtered. The filtrate was evaporated; column chromatography eluting with petroleum/EtOAc (5:2) gave 5-((S)-3-((1R,2R)-2-((S)-2,2-dimethyl-1,3-dioxolan-4-yl)cyclopropyl)butylthio)-1-phenyl-1H-tetrazole. Yield: 16 g (91%); pale-yellow oil; \([\alpha]_D^{25} = -34 (c 1.4, CHCl_3)\).

MS: m/z [M + Na\(^+\)] calcd for C\(_{39}\)H\(_{38}\)N\(_2\)O\(_5\)Na: 597.2348; found: 597.2341.
Yield: 3.9 g (84%); thick oil; [α]<sub>D</sub><sup>22</sup> = −8.5 (c 0.6, CHCl<sub>3</sub>).

**MS:** m/z [M + Na]<sup>+</sup> calcd for C₆₆H₁₁₀O₃Na: 905.9030; found: 905.9051.

**IR:** 2920, 2810, 1732, 1470, 1153 cm<sup>−1</sup>.

**1H NMR** (500 MHz, CDCl<sub>3</sub>): δ = 4.13–4.06 (m, 1 H), 3.75–3.65 (m, 2 H), 1.45 (s, 3 H), 1.37–1.13 (br m, including 3 H s at δ 1.36, 67 H), 0.99 (br s, 3 H), 0.88 (t, J = 6.3 Hz, 3 H), 0.83 (dt, J = 4.5, 8.5 Hz, 1 H), 0.71–0.65 (m, 3 H), 0.57 (dt, J = 4.1, 8.5 Hz, 1 H), 0.23 (br q, J = 5.4 Hz, 1 H), −0.33 (br q, J = 5.1 Hz, 1 H).

**13C NMR** (126 MHz, CDCl<sub>3</sub>): δ = 108.2, 77.9, 70.0, 37.4, 33.2, 31.9, 30.0, 29.6, 29.3, 28.7, 27.1, 26.8, 25.7, 23.8, 22.6, 20.0, 19.2, 15.7, 14.1, 10.9, 9.0.

**MS:** m/z [M + Na]<sup>+</sup> calcd for C₅₆H₁₁₀O₂Na: 821.8454; found: 821.8462.

**cis-(1R,2R)-2-(5S,16S)-((1S,2R)-Octadecylcyclopropyl)hexadecane-2-yl)cyclopropanecarbaldehyde (29)**

Periodic acid (3.3 g, 17.1 mmol) was added to stirred solution of the acetate 28 (3.8 g, 5.8 mmol) in anhydrous ether (60 mL) under nitrogen at r.t. The mixture was stirred for 16 h, then worked up as above to give the aldehyde 29.

Yield: 3.1 g (94%); white solid; mp 37–38 °C; [α]<sub>D</sub><sup>22</sup> = 0.015 (c 1.2, CHCl<sub>3</sub>), Cl<sub>3</sub>.

**MS:** m/z [M + Na]<sup>+</sup> calcd for C₃₈H₆₈O₃Na: 681.6556; found: 681.6556.

**1H NMR** (500 MHz, CDCl<sub>3</sub>): δ = 4.13–4.06 (m, 1 H), 3.75–3.65 (m, 2 H), 1.45 (s, 3 H), 1.37–1.13 (br m, including 3 H s at δ 1.36, 67 H), 0.99 (br s, 3 H), 0.88 (t, J = 6.3 Hz, 3 H), 0.83 (dt, J = 4.5, 8.5 Hz, 1 H), 0.71–0.65 (m, 3 H), 0.57 (dt, J = 4.1, 8.5 Hz, 1 H), 0.23 (br q, J = 5.4 Hz, 1 H), −0.33 (br q, J = 5.1 Hz, 1 H).

**13C NMR** (126 MHz, CDCl<sub>3</sub>): δ = 108.2, 77.9, 70.0, 37.4, 33.2, 31.9, 30.0, 29.6, 29.3, 28.7, 27.1, 26.8, 25.7, 23.8, 22.6, 20.0, 19.2, 15.7, 14.1, 10.9, 9.0.

**IR:** 2928, 2848, 1607, 1467, 1369, 1159, 1063, 824, 720 cm<sup>−1</sup>.

**16-(1S,2R)-2-((5S,16S)-((1S,2R)-Octadecylcyclopropyl)hexadecane-2-yl)cyclopropanecarbaldehyde (30)**

Sodium methoxide (0.60 g, 11 mmol) was added to a stirred solution of cis-aldehyde 28 (5.6 g, 0.5 mmol) in MeOH (20 mL) and THF (30 mL) and heated at reflux for 56 h. The mixture was cooled to r.t. and worked up as above to give the trans-aldehyde 30.

Yield: 2.7 g (77%); semi-solid; [α]<sub>D</sub><sup>22</sup> = +8.5 (c 1.0, CHCl<sub>3</sub>), Cl<sub>3</sub>.

**MS:** m/z [M + Na]<sup>+</sup> calcd for C₆₆H₁₁₀O₃Na: 905.9030; found: 905.9051.

**IR:** 2920, 2810, 1732, 1470, 1158, 1053 cm<sup>−1</sup>.

**1H NMR** (500 MHz, CDCl<sub>3</sub>): δ = 3.65 (br, J = 6.6 Hz, 2 H), 1.62–1.54 (m, including 3 H s at δ 1.36, 67 H), 0.99 (br s, 3 H), 0.88 (t, J = 6.3 Hz, 3 H), 0.83 (dt, J = 4.5, 8.5 Hz, 1 H), 0.71–0.65 (m, 3 H), 0.57 (dt, J = 4.1, 8.5 Hz, 1 H), 0.23 (br q, J = 5.4 Hz, 1 H), −0.33 (br q, J = 5.1 Hz, 1 H).

**13C NMR** (126 MHz, CDCl<sub>3</sub>): δ = 108.2, 77.9, 70.0, 37.4, 33.2, 31.9, 30.0, 29.6, 29.3, 28.7, 27.1, 26.8, 25.7, 23.8, 22.6, 20.0, 19.2, 15.7, 14.1, 10.9, 9.0.

**IR:** 2928, 2848, 1607, 1467, 1369, 1159, 1063, 824, 720 cm<sup>−1</sup>.
Methyl (2R)-2-((1R)-1-(tert-Butyldimethylsilyloxy)-19-(15Z,2R)-2-(5S)-16-(15Z,2S)-2-octadecylcyclopropyl)hexadecane-2-yl)cyclopropyl)nonadecyl)tetracosanoate (36)

LiHMDS (2.4 mL, 2.5 mmol, 1.06 M) was added dropwise to a stirred solution of aldehyde 33 (1.2 g, 1.5 mmol) and methyl (R)-2-((1R)-1-(tert-butyl dimethylsilyloxy)-3-(1-phenyl-1H-tetrazol-5-yl)hydroxy)propyl)tetracosanoate 35 (1.27 g, 1.60 mmol) in anhydrous THF (30 mL) under nitrogen at −10 °C. The reaction was allowed to reach r.t. and stirred for 2 h, then worked up as above to give a colourless oil as a mixture of (E/Z) methyl (R)-2-((1R)-1-(tert-butyl dimethylsilyloxy)-19-(15Z,2R)-2-(5S)-16-(15Z,2S)-2-octadecylcyclopropyl)hexadecane-2-yl)cyclopropyl)nonadec-4-ene/tetracosanoates (1.7 g, 85%).

Dipotassium azodicarboxylate (2.10 g, 10.3 mmol) was added to a stirred solution of the above alkenes (2.5 g, 1.9 mmol) in THF (25 mL) and MeOH (15 mL) at 5 °C. A solution of glacial acetic acid (6.6 mL, 104.4 mmol) and TFA (3 mL) was added for 24 h then additional dipotassium azodicarboxylate (1.5 g, 7.7 mmol) and a solution of glacial acetic acid (2 mL) and THF (2 mL) were added and stirred for further 24 h. The mixture was worked up as above to give methyl (R)-2-((1R)-1-(tert-butyl dimethylsilyloxy)-19-(15Z,2R)-2-(5S)-16-(15Z,2S)-2-octadecylcyclopropyl)hexadecane-2-yl)cyclopropyl)nonadecyl)tetracosanoate 36.

**Yield:** 1.41 g (83%); colourless oil;

**MS:** m/z [M + Na]⁺ calc'd for C₉₀H₁₇₈O₃SiNa: 1358.3443; found: 1358.3443.

**1H NMR** (500 MHz, CDCl₃): δ = 3.92–3.89 (m, 1 H), 3.66 (s, 3 H, 2.53 (ddd, J = 3.7, 7.2, 11.0 Hz, 1 H), 1.57 (br s, 8 H), 1.37–1.10 (br m, 132 H), 0.91–0.84 (m, including integration to 3 H, t integrating to 6 H and s integrating to 9 H, 18 H), 0.70–0.65 (m, 3 H), 0.56 (dt, J = 3.7, 8.1 Hz, 1 H), 0.48–0.42 (m, 1 H), 0.21–0.078 (m, 3 H), 0.049 (s, 3 H), 0.025 (s, 3 H), −0.32 (br q, J = 5.1 Hz, 1 H).


**IR:** 3420, 2917, 2810, 1715, 1618, 1374, 1196, 720 cm⁻¹.

**MALDI MS:** m/z [M + Na]⁺ calc'd for C₃₅H₇₁O₃Na: 1230.25; found: 1230.24.

(15Z)-1-Hydroxy-19-(15Z,2R)-2-(5S)-16-(15Z,2S)-2-octadecylcyclopropyl)hexadecane-2-yl)cyclopropyl)nonadecyl)tetracosanoic Acid (38)

**Yield:** 0.73 g (72%); white solid; mp 47–48 °C; [α]D²⁵ +8.1 (c 0.19, CHCl₃).

**MS:** m/z [M + Na]⁺ calc'd for C₉₀H₁₇₈O₃SiNa: 1244.2578; found: 1244.2612.

**1H NMR** (500 MHz, CDCl₃): δ = 3.71 (s, 3 H), 3.68–3.63 (m, 1 H), 2.49–2.36 (m, 1 H), 2.05 (br s, 1 H, for hydroxyl group), 1.37–1.14 (br m, 140 H), 0.90–0.81 (m, including integration to 3 H and t integrating to 6 H, 9 H), 0.68–0.63 (m, 3 H), 0.57 (dt, J = 4.1, 7.8 Hz, 1 H), 0.48–0.42 (m, 1 H), 0.20–0.10 (m, 3 H), −0.31 (br q, J = 5.3 Hz, 1 H).

ed. Column chromatography eluting with petroleum/EtOAc (7:2) gave
was extracted with petroleum/EtOAc (5:1, 3 × 30 mL). The combined
organic layers were washed with water (20 mL), dried and evaporated.
Column chromatography eluting with petroleum/EtOAc (7:2) gave
the title acid 38.

Yield: 0.61 g (84%); white solid; mp 54–55 °C; Rf 0.42 (above solvent); [α]D24 +5.5 (c 0.60, CHCl3).
MALDI MS: m/z found [M + Na]+: 1230.2; C83H162O3Na requires:

IR: 3419, 2917, 2810, 1712, 1618, 1470, 1375, 1197, 719 cm–1.

13C NMR (126 MHz, CDCl3):

δ = 179.5, 72.1, 50.8, 38.1, 37.4, 35.5, 34.5, 31.9, 30.2, 30.0, 29.7, 29.6, 29.5, 29.4, 29.3, 28.7, 27.3, 27.2, 26.1, 25.7, 22.6, 19.6, 18.6, 14.1, 10.9, 10.4.

IR: 3418, 2918, 2819, 1685, 1470, 1205, 1020, 719 cm–1.

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Supporting Information

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References
