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 (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

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 -CHMeHOME- 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 α–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)

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, and has been shown to have significant biological activity in a number of contexts, 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 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, followed again by reduction of the derived E/Z-mixture of esters to the corresponding alcohols and then saturation of the alkenes (Scheme 5).

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).

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

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).

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), 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 sensitivity of mycobacterial species to hydrophobic antibiotics. 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. 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 cis-cyclopropane and keto-MA and the lack of trans-cyclopropanes. 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 kansasi and Mycobacterium avium complex.
We therefore describe the synthesis of two stereoisomers of one such compound. The aldehyde 25 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 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. 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′-dimycolates ('cord factor'). The MA 37 and 38 were converted into the corresponding TDM and TMM (see the Supporting Information) using methods described before. 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) and reactive oxygen species has recently been reported. Moreover, they are recognised by antibodies in the serum of patients with pulmonary tuberculosis, providing the basis of a diagnostic assay.

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 POLAAR 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,2S)-2-Eicosylcyclopropanecarbaldehyde (6)
(a) 5-(Nonadecyl-1-sulphonyl)-1-phenyl-1H-tetrazole 4 (15.0 g, 0.032 mol) and butyric acid (1R,2S)-cis-2-formylcyclopropyl) methyl ester 5 (4.8 g, 0.028 mol)26 were 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 (1:1, 3 × 150 mL) and saturated aq. ammonium chloride (100 mL). The aqueous layer was added to a stirred suspension of pyridinium chlorochromate (7.6 g, 0.041 mol) in THF (50 mL) at 0 °C. The mixture was stirred for 2 h, then the reaction was quenched with freshly prepared saturated aq. sodium sulfate (200 mL), the aqueous layer was separated and extracted with petrol/ether (1:1, 2 × 100 mL) and evaporated to give a residue. This residue was purified by column chromatography on silica eluting with petrol/ether 2:1 to give the title compound 6 (4.5 g, 91%) 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-(cis-2-eicosyl-1-enylcyclopropyl)methyl ester (6.6 g, 98%) as a white solid, as a mixture of isomers in ratio (2.8 : 1). J 1H NMR (500 MHz, CDCl 3): δ = 3.65 (t, J = 6.6 Hz, 2 H), 1.58 (pent, J = 6.6 Hz, 4 H), 1.40–1.22 (m, 57 H), 1.15–1.10 (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, CDCl 3); δ = 36.5, 32.0, 30.5, 29.9, 29.8, 29.5, 28.6, 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,2S)-cis-2-Eicosylcyclopropyl)tridecan-1-ol (8)
LIHMDS (18.2 mL, 19.3 mmol) was added to a stirred solution of (1R,2S)-2-eicosylcyclopropanecarbaldehyde 6 (4.0 g, 11 mmol) and 2,2-dimethylenopropanoic acid 12-(1-phenyl-1H-tetrazole-5-y1-sulfonyl)iododecyl ester 7 (7.10 g, 14.9 mmol)40 in anhydrous THF (200 mL) at −10 °C under nitrogen. The mixture was allowed to reach r.t., stirred for 2 h, then the reaction was quenched with saturated aq. ammonium chloride (200 mL), the aqueous layer was separated and extracted with petrol/ether (1:1, 3 × 200 mL). The combined organic layers were dried and concentrated and the residue was purified by column chromatography, eluting with petrol/ether (20:1) to give 2,2-dimethylenopropanoic acid (E/Z)-13-(cis-2-eicosyl-1-enylcyclopropyl)tridec-12-yl 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,2S)-cis-2-eicosylcyclopropyl)tridec-12-en-1-ol (5.0 g, 91%) as a white solid. Hydrazine monohydrate (20 mL), acetic acid (2 mL), and saturated aqueous copper sulphate (2 mL) were added in succession to the derived alcohols (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]D 26 +0.33 (c 2.03, CHCl 3).

1H NMR (500 MHz, CDCl 3); δ = 3.65 (t, J = 6.6 Hz, 2 H), 1.58 (pent, J = 6.6 Hz, 4 H), 1.40–1.22 (m, 57 H), 1.15–1.10 (m, 2 H), 0.89 (t, J = 4.7, 8.2 Hz, 1 H), −0.01 (q, J = 5.2 Hz, 1 H)

13C NMR (126 MHz, CDCl 3); δ = 63.5, 32.0, 30.5, 29.9, 29.8, 29.5, 28.6, 22.8, 18.5, 16.3, 14.0, 10.0.

IR: 3313, 1463, 1374, 1008, 720 cm⁻1.

13-(1R,2S)-2-Eicosylcyclopropyl)tridecan-1-ol (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 rt. 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]D 26 +1.6 (c 1.2, CHCl 3) (enantiomer −1.7 (c 1.2, CHCl 3)).28

1H NMR (500 MHz, CDCl 3); δ = 9.77 (t, J = 1.9 Hz, 1 H, 2.42 (br td, J = 1.9, 7.3 Hz, 2 H), 1.63 (pent, J = 6.9 Hz, 2 H), 1.38–1.12 (br m, 56 H), 1.15–1.1 (m, 2 H), 0.88 (t, J = 6.9 Hz, 3 H), 0.67–0.61 (m, 2 H), 0.56 (td, J = 3.7, 7.8 Hz, 1 H), −0.32 (br q, J = 5.4 Hz, 1 H).

13C NMR (126 MHz, CDCl 3); δ = 73.0, 31.9, 30.2, 29.7, 29.6, 29.4, 29.3, 29.2, 28.7, 25.7, 22.7, 15.8, 14.1, 10.9.

IR: 2991, 2848, 1715, 1469, 1391, 1018, 720 cm⁻1.
(a) 13-(1R,25)-cis-2-Eicosylcyclopropyl)tridecan-1-ol (0.5 g, 3.0 mmol) was dissolved in anhydrous THF (10 mL) together with triphenylphosphine (2.6 g, 10 mmol) and 1-phenyl-1H-tetrazole-5-thiol (1.8 g, 10 mmol). The mixture was cooled to 0 °C, followed by the addition of DEAD (1.7 mL, 10 mmol) in anhydrous THF (5 mL). The mixture was stirred overnight at r.t., evaporated, then the residue was washed with petrol/ether (10:1) and then filtered. The filtrate was evaporated and the product was purified by column chromatography eluting with chloroform to give 5-[(1R,25)-cis-2-Eicosylcyclopropyl]tridecyl-sulfonyl[phenyl-1H-tetrazole].

Yield: 5.1 g (97%); mp 42–44 °C; [α]D21 +1.2 (c 1.3, CHCl3).

MS: m/z [M + Na]+ calc for C40H72N4O2SNa: 703.5683; found: 703.5658.

1H NMR (500 MHz, CDCl3); δ = 7.62–7.52 (m, 5 H), 3.42 (t, J = 7.4 Hz, 2 H), 1.84 (pent, J = 7.5 Hz, 2 H), 1.47 (pent, J = 7.3 Hz, 2 H), 1.43–1.12 (m, 58 H), 0.91 (t, J = 6.9 Hz, 3 H), 0.71–0.64 (m, 2 H), 0.59 (dt, J = 4, 3.8 Hz, 1 H), –0.35 (q, J = 4.9 Hz, 1 H).

13C NMR (126 MHz, CDCl3); δ = 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: 3375, 2852, 1771, 1464, 1370, 1170, 1064, 1037, 964, 932, 823 cm–1.

(b) LiAlH4 (0.2 g) was added to stirred THF (100 mL) at 0 °C under nitrogen to ensure THF dryness. Then further LiAlH4 (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 decahydrate (20 mL), which was added dropwise and stirred at rt until a white precipitate was formed followed by addition of MgSO4 (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) 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 isopropyl alcohol (250 mL), acetic acid (1.5 mL), sat. aq. copper sulphate (1.5 mL) and hydrazine hydrate (20 mL). The mixture was stirred for 2 h until it reached rt, 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.

Yield: 1.8 g (63%); white solid; mp 72–74 °C; [α]D21 +8.3 (c 0.86, CHCl3).

MS: m/z [M + H]+ calc for C40H72O4Na: 590.0978; found: 590.0980.

1H NMR (500 MHz, CDCl3); δ = 3.66 (dd, J = 7.3, 11 Hz, 1 H), 3.59 (dd, J = 7.9, 11 Hz, 1 H), 1.56 (br m, 4 H), 1.47–1.20 (br m, 60 H), 0.9–0.84 (including t at 0.88 with J = 6.95 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).

13C NMR (126 MHz, CDCl3); δ = 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.


LiHMDS (0.37 mL, 4.0 mmol) was added to a stirred solution of butyric acid (1.5 g, 3.0 mmol) in anhydrous THF (30 mL) at –10 °C. The mixture was allowed to reach rt and stirring was continued for 1.5 h, then worked up and purified as before to give butyric acid (1.2 g, 82% as a white solid in a mixture 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 (15R,2R)-2-[(E,Z)-14-(1R,2S)-2-Eicosylcyclopropyl]tetradecyl-1-enyl)cyclopropylmethyl ester (1.2 g, 62%) as a white solid as a mixture in ratio 2.5:1. The above ether (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 (15R,2R)-2-[(E,Z)-14-(1R,2S)-2-Eicosylcyclopropyl]tetradecyl-1-enyl)cyclopropylmethyl ester.
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\begin{align*}
\text{1H NMR (500 MHz, CDCl}_3\):} & \quad \delta = 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).
\end{align*}
\]

\[
\begin{align*}
\text{1C NMR (126 MHz, CDCl}_3\):} & \quad \delta = 154.6, 133.8, 132.1, 130.0, 129.7, 128.5, 128.4, 128.4, 123.8, 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.
\end{align*}
\]

IR: 3368, 2988, 2981, 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 tetracyle (2.0 g, 2.7 mmol) in dichloromethane (100 mL), followed by the addition of a mixture of anhydrous 3-chlororopropanoxycarbonyl acid 70% (1.67 g, 9.7 mmol, 2.5 equiv) in CH\(_2\)Cl\(_2\) (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 x 100 mL). The combined organic layers were washed with water (2 x 200 mL) and evaporated. The product was recrystallised from methanol/acetic acid (1:1) to give the title compound 17.

Yield: 1.77 g (85%); \(\alpha\) (c 0.5, CHCl\(_3\)).

MS: m/z [M + H\(^+\)] calcd for C\(_{48}\)H\(_{85}\)N\(_4\)O\(_2\)S: 782.2976; found: 782.2980.

\[
\begin{align*}
\text{1H NMR (500 MHz, CDCl}_3\):} & \quad \delta = 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 \(J = 0.89 \text{ Hz}, 4 \text{ H})
\end{align*}
\]

IR: 2990, 2916, 2849, 1498, 1470, 1340, 1152, 761, 718, 687 cm\(^{-1}\).

5-[[1(15R)-2-14-((15R)-2-Eicosyclopropyl)cyclopropyl]tetradecyl]cyclopropylmethanesulfanyl]-1-phenyl-1H-tetrazole (18)

(a) Alcohol 14 (0.55 g, 0.85 mmol) was dissolved in anhydrous THF (5 mL) and then triphenylphosphine (0.30 g, 1.1 mmol) and 1-phenyl-1H-tetrazole-5-thiol (0.2 g, 1.1 mmol) were added. The mixture was cooled to \(0 \text{ °C}\) and treated as above with DEAD (0.18 mL, 1.1 mmol) in THF (20 mL). Work up as before gave (1R,2S)-\([M + H\]^+\) (calcd for C\(_{48}\)H\(_{85}\)N\(_4\)O\(_2\)S: 782.2976; found: 782.2980).

Yield: 1.77 g (85%); \(\alpha\) (c 0.5, CHCl\(_3\)).

MS: m/z [M + H\(^+\)] calcd for C\(_{48}\)H\(_{85}\)N\(_4\)O\(_2\)S: 782.2976; found: 782.2980.
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]+ calcld for C48H84N4SNa: 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: 3059, 2920, 1463, 1339, 1275, 1169, 1088, 1018 cm–1.

R-(2)-(R)-1-(tert-Butyldimethylsilyloxy)-12-((1R,2S)-2-[14-(1R,2S)-2-ecicosylocyclopropyl]tetradecyl)cyclopropyl)hexacosanoic Acid (21)

(a) LiHMDS (2.78 mL, 29.5 mmol, 1.3 mol equiv, 1.06 M) was added to a stirred solution of the above alkene 17 (1.77 g, 2.27 mmol) and aldehydes 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 at r.t., 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 re-extracted 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 alkene (2.5 g, 87%) as an E/Z-mixture in ratio 2.5:1.

(b) Dipotassium azodicarboxylate (3.0 g, 0.015 mol) was added to a stirred solution of the above alkene (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]D20 +3.8 (c 1.40, CHCl3).

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

1H NMR (500 MHz, CDCl3): δ = 3.95–3.88 (m, 1 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 (t, 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).

IR: 2920, 2853, 1742, 1465, 1253 cm–1.

(2R)-2-(1R)-1-Hydroxy-12-((1R,2S)-2-[14-(1R,2S)-2-ecicosylocyclopropyl]tetradecyl)cyclopropyl)hexacosanoic Acid (22)

(a) A dry polyethylene vial equipped with an acid-resistant rubber septum was charged with the above TBDMS methyl ester (1.9 g, 1.5 mmol), anhydrous pyridine (2 mL) in anhydrous THF (25 mL) and stirred at r.t. under argon. To it was added hydrogen fluoride-pyridine 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: 1.32 g (76%); [α]D20 +4.3 (c 0.94, CHCl3).

MALDI MS: m/z [M + Na]+ calcld for C79H154O3Na: 1175.0; found: 1174.9.
(R)-2-((R)-1-Hydroxy-12-((1S,2R)-2-[(1R,2S)-2-ecicosylcyclopropyl][tetradecyl][cyclopropyl][dodecyl])hexacosanoic Acid (23)

(a) LHMDS (0.30 mL, 0.24 mmol) was dissolved in THF (15 mL) and MeOH (1.5 mL) and then lithium hydroxide monohydrate (1.10 g, 26.2 mmol, 30 equiv) was added to the stirred mixture at r.t. The mixture was heated at 45 °C for 17 h, then cooled to r.t. and diluted with petrol/THF (7:2, 5 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/THF (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/THF (7:2) to give compound 22.

Yield: 0.17 g (64%); pale yellow solid; mp 51–53 °C; [α]₀²¹ +2.5 (c 1.4, CHCl₃).

MALDI MS: m/z [M + Na]⁺ calcd for C₇₈H₁₅₂O₃Na: 1161.06; found: 1160.59.

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

13C NMR (126 MHz, CDCl₃): δ = 3.75–7.30 (m, 1 H), 1.74–1.71 (m, 136 H), 0.88 (t, J = 7.0 Hz, 6 H), 0.68–0.62 (m, 4 H), 0.57 (dt, J = 4.1, 8.6 Hz, 2 H), –0.32 (q, J = 5.1 Hz, 2 H).

13C NMR (126 MHz, CDCl₃): δ = 182.1, 72.2, 50.8, 35.5, 31.9, 30.2, 29.7, 29.66, 29.55, 29.42, 28.9, 27.3, 25.7, 22.7, 15.8, 14.1, 10.9.

IR: 3281, 2919, 2852, 2367, 1712, 1461, 1377, 721 cm⁻¹.

(R)-2-((R)-1-Hydroxy-12-((1S,2R)-2-[(1R,2S)-2-ecicosylcyclopropyl][tetradecyl][cyclopropyl][dodecyl])hexacosanoic Acid methyl ester (24)

(a) LHMDS (0.32 mL, 0.24 mmol) was dissolved in THF (15 mL) and MeOH (0.5 mL) and then lithium hydroxide monohydrate (0.02 g, 0.48 mmol) was added to the stirred mixture at r.t. The mixture was heated at 45 °C for 18 h, then cooled to r.t. and diluted with petrol/THF (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/THF (7:2, 3 × 10 mL). The combined organic layers were dried and concentrated.

Column chromatography, eluting with petrol/THF (7:2) gave the title compound 23.

Yield: 0.19 g (64%); white solid; mp 51–53 °C; [α]₀²¹ +2.5 (c 1.4, CHCl₃).

MALDI MS: m/z [M + Na]⁺ calcd for C₇₈H₁₅₂O₃Na: 1161.1634; found: 1160.1626.

IR: 3281, 2919, 2852, 1709, 1466, 1377, 721 cm⁻¹.
(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 heated to reflux at 45 °C for 18 h, then cooled to r.t. and worked up purified as before to give the title compound as a white solid; mp 52–53 °C; IR: 3520, 2924, 2855, 2367, 1712, 1461, 1377, 1165, 721 cm⁻¹. MALDI MS: [M + Na]⁺ calcd for C₁₉H₂₆N₄NaO₂S: 397.1674; found: 397.1630.

(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 (3 mL) and then lithium hydride (0.02 g, 0.48 mmol) was added with stirring at r.t. and worked up as above to give (R)-[(R)-1-hydroxy-12-(1R,2S)-[14-(15R,2S)-2-ecicosylcyclopropyl][tetradecyl]cyclopropyl][do-decy]-hexacosanoic acid methyl ester.

Yield: 0.01 (91%); pale-yellow oil; [α]D ²⁰ ̶ 34 (c 1.4, CHCl₃).


IR: 3317, 2922, 2852, 1681, 1464, 1377, 721 cm⁻¹. 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 (26a) (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; [α]D ²⁰ ̶ 34 (c 1.4, CHCl₃).


IR: 2984, 2831, 1546, 1302, 1298, 1037, 770, 700, 635, 302, 298, 267, 257, 194, 19.2, 8.6. (b) A solution of ammonium molybdate(VI) tetrahydrate (24.7 g, 20.0 mmol) in 35% H₂O₂ (40 mL) was cooled in an ice bath and added to a stirred solution of the above tetrazole (15.0 g, 40.0 mmol) in THF (140 mL) and IMS (300 mL) at 10 °C and stirred for 2 h. A further solution of ammonium molybdate(VI) tetrahydrate (12.4 g, 10.0 mmol) in 35% H₂O₂ (20 mL) was added and the mixture was stirred at r.t. for 18 h. The mixture was poured into water (1.5 L) and extracted with CH₂Cl₂ (1 × 300 mL, 3 × 50 mL). The combined organic layers were washed with water (500 mL), and the solvent was evaporated. Column chromatography eluting with petroleum/EtOAc (5:2 then 1:1) gave the title compound.

Yield: 14 g (87%); yellow oil; [α]D ²⁰ ̶ 37 (c 1.8, CHCl₃).


IR: 3050, 2921, 1857, 1306, 1377, 1175, 712 cm⁻¹. 2017 Syn Open C. Don Lawson et al. THIEME OPEN ACCESS

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IR: 3317, 2922, 2852, 1681, 1464, 1377, 721 cm⁻¹. (S)-2,2-Dimethyl-4-((1R,2R)-2-((S)-2,2-Dimethyl-1,3-dioxolan-4-yl)cyclopropyl)hexadecane-2-yl(cyclopropyl)-1,3-dioxolane (28) LiHMDS (14.6 mL, 15.0 mmol, 1.06 M) was added dropwise to a stirred solution of aldehyde 25 (4.3 g, 9.0 mmol) and sulfone 27 (42. g, 10.3 mmol) in anhydrous THF (50 mL) under nitrogen at −10 °C. The mixture was allowed to reach r.t. and stirred for 2 h, then worked up as above to give (S)-2,2-dimethyl-4-((1R,2R)-2-((E/Z)-(S)-16-2,2-Dimethyl-4-((1R,2R)-2-((S)-2,2-Dimethyl-1,3-dioxolan-4-yl)cyclopropyl)hexadecane-2-yl(cyclopropyl)-1,3-dioxolane (28) LiHMDS (14.6 mL, 15.0 mmol, 1.06 M) was added dropwise to a stirred solution of aldehyde 25 (4.3 g, 9.0 mmol) and sulfone 27 (42. g, 10.3 mmol) in anhydrous THF (50 mL) under nitrogen at −10 °C. The mixture was allowed to reach r.t. and stirred for 2 h, then worked up as above to give (S)-2,2-dimethyl-4-((1R,2R)-2-((E/Z)-(S)-16-
Yield: 3.9 g (84%); thick oil; \[\alpha\]D 22 +4.04 (c 1.1, CHCl3).

MS: m/z [M + Na]+ calcd for C56H110ONa: 821.8454; found: 821.8462.

1H NMR (500 MHz, CDCl3): \(\delta = 4.05\) (br d, \(J = 6.6\) Hz, 2 H), 1.62–1.1, CHCl3).

(b) The above pivalate (2.78 g, 3.13 mmol) was added to a stirred solution of potassium hydroxide (0.7 g, 12.5 mmol) dissolved in a mixture of THF/MeOH/water (30:20:5 mL). The mixture was stirred at 70 °C for 3 h, then worked up as above to give 16-((15Z,2R)-2-(S)-16-((15Z,2R)-2-Octadecylcyclopropyl)hexadecane-2-yl)cyclopropyl)hexadecan-1-oil.

Yield: 1.5 g (60%); white solid; mp 50–52 °C; \[\alpha\]D 22 +3.8 (c 1.1, CHCl3).


1H NMR (500 MHz, CDCl3): \(\delta = 3.65\) (br t, \(J = 6.6\) Hz, 2 H), 1.62–1.1, CHCl3).

(c) The above alcohol (1.50 g, 1.9 mmol) was dissolved in hot CHCl3 (20 mL) and added to a refluxing stirred suspension of PCC (0.94 g, 4.4 mmol) in CH2Cl2 (40 mL). The mixture was stirred vigorously for 2 h, then worked up as above to give aldehyde 33.

Yield: 1.24 g (85%); white solid; mp 40–42 °C; \[\alpha\]D 24 +5.9 (c 1.0, CHCl3).


1H NMR (500 MHz, CDCl3): \(\delta = 3.65\) (br t, \(J = 6.6\) Hz, 2 H), 1.62–1.1, CHCl3).

(c) The above alcohol (1.50 g, 1.9 mmol) was dissolved in hot CHCl3 (20 mL) and added to a refluxing stirred suspension of PCC (0.94 g, 4.4 mmol) in CH2Cl2 (40 mL). The mixture was stirred vigorously for 2 h, then worked up as above to give aldehyde 33.

Yield: 1.24 g (85%); white solid; mp 40–42 °C; \[\alpha\]D 24 +5.9 (c 1.0, CHCl3).

**Methyl (R)-2-((tert-Butylidemethylsilyloxy)-19-((1S,2R)-2-(S)-16-((1S,2R)-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-((tert-butylidemethylsilyloxy)-3-((1-phenyl-1H-tetrazol-5-yl)thio)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-((tert-butylidemethylsilyloxy)-19-((1S,2R)-2-(S)-16-((1S,2R)-2-octadecylcyclopropyl)hexadecane-2-yl)cyclopropyl)nonadecyl(tertetracosanoate (36).

**Yield:** 0.63 g (70%); white solid; mp 55–56 °C; Rf 0.42 (7:2 petrol/EtOAc; [α]D22 +5.2 (c 0.50, CHCl3).

**MALDI MS:** m/z [M + Na]+ calcd for C37H43O2Na: 530.2612; found: 530.2612.

**IR:** 3411, 2919, 2810, 1689, 1470, 1191, 1016, 720 cm−1.

**1H NMR (500 MHz, CDCl3):** δ = 3.74–3.71 (s, 3 H), 3.66–3.63 (m, 1 H, 1.57) br (s, 8 H), 1.37–1.10 (br m, 132 H, 0.91–0.84 (m, including d integrating 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), –0.27 (br q, J = 5.1 Hz, 1 H).

**13C NMR (126 MHz, CDCl3):** δ = 175.1, 73.2, 51.5, 51.1, 38.1, 37.4, 33.7, 31.9, 30.2, 30.0, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 28.7, 27.5, 27.2, 26.1, 25.7, 23.7, 22.6, 19.6, 18.6, 17.9, 15.7, 14.1, 10.9, 10.4, –4.3, –4.9.

IR: 2923, 2813, 1741, 1465, 1361, 1254, 1166, 836, 775 cm−1.

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Yield: 0.61 g (84%); white solid; mp 54–55 °C; the title acid was isolated. Column chromatography eluting with petroleum/EtOAc (7:2) gave pure product. Organic layers were washed with water (20 mL), dried and evaporated. The combined organic layers were extracted with petroleum/EtOAc (5:1, 3 × 30 mL). The combined layers were washed with water (20 mL), dried and evaporated. Chromatography eluting with petroleum/EtOAc (7:2) gave the title acid.

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

1H NMR (500 MHz, CDCl3): δ = 7.43–7.38 (m, 2 H), 7.33–7.27 (m, 3 H, 2 × C6H5), 6.90–6.83 (m, 1 H), 6.83–6.77 (m, 3 H, terminal 2 × CH3), 0.69–0.61 (m, 3 H, 2 × CH3), 0.57 (dt, δ = 4.1, 8.2 Hz, 6 H, terminal 2 × CH3), 0.46–0.42 (m, 1 H), 0.22–0.09 (m, 3 H), –0.32 (br q, δ = 6.6 Hz, 6 H, terminal 2 × CH3), 0.69–0.61 (m, 3 H, 2 × CH3), 0.57 (dt, δ = 4.1, 8.2 Hz, 6 H, terminal 2 × CH3), 0.46–0.42 (m, 1 H), 0.22–0.09 (m, 3 H), –0.32 (br q, δ = 6.6 Hz, 6 H, terminal 2 × CH3), 0.69–0.61 (m, 3 H, 2 × CH3), 0.57 (dt, δ = 4.1, 8.2 Hz, 6 H, terminal 2 × CH3), 0.46–0.42 (m, 1 H), 0.22–0.09 (m, 3 H), –0.32 (br q, δ = 6.6 Hz, 6 H, terminal 2 × CH3).