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# Thiol modified mycolic acids

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ABSTRACT

Patient serum antibodies to mycolic acids have the potential to be surrogate markers of active tuberculosis (TB) when they can be distinguished from the ubiquitously present cross-reactive antibodies to cholesterol. Mycolic acids are known to interact more strongly with antibodies present in the serum of patients with active TB than in patients with latent TB or no TB. Examples of single stereoisomers of mycolic acids with chain lengths corresponding to major homologues of those present in *Mycobacterium tuberculosis* have now been synthesised with a sulfur substituent on the terminal position of the α-chain; initial studies have established that one of these binds to a gold electrode surface, offering the potential to develop second generation sensors for diagnostic patient antibody detection.



## 1. Introduction

Mycolic acids such as (**1**)–(**4**) (Scheme 1) are characteristic components of the cell walls of mycobacteria and a number of other species such as *Norcardia* and *Rhodococcus* (Minnikin, 1982; Barry et al., 1998; Verschoor et al., 2012). These long chain β-hydroxy acids consist of a hydrophilic mycolic motif, linked to a long hydrophobic merochain that contains two functional groups. The mycolic motif contains a simple aliphatic hydrocarbon chain in the α position. Based on the two functional groups in the mero-chain the mycolic acids of *Mycobacterium tuberculosis* are classified into three major types, namely α-MA (**1**), methoxy-MA (**2**) and keto-MA (**3**). The cyclopropanes can have a *cis*- or *trans*-stereochemistry. When a *trans*-cyclopropane is present, it has a methyl substituent on the adjacent carbon distal from the hydroxy acid. Mycolic acids are present in cells as complex mixtures of different homologues. They are solvent extractable either as free mycolic acids, or as sugar esters such as trehalose esters, but are mainly covalently bound into the bacterial cell wall by esterification to arabinogalactan. They can be saponified and isolated from cells as complex mixtures of different classes and different chain lengths (Watanabe et al., 2001, 2002). The make-up of these mixtures is characteristic of each particular *Mycobacterium* species. Detailed mass spectral finger-prints of such mixtures can be used directly to identify the *Mycobacterium* species present in a sample and hence the type of mycobacterial infectious disease such as tuberculosis (Uenishi et al., 2008; Salvado-Viader et al., 2007; Yassin, 2011; Shui et al., 2012; Kowalski et al., 2012; Song et al., 2009; Laval et al., 2001). The presence of particular patterns of mycolic acids has also been used to characterise a new genus of bacteria, *Segniliparus* (Hong et al., 2012; Lanéelle et al., 2013).

Mixtures of trehalose esters of mycolic acids have been used in ELISA assays to detect the presence of antibodies in the serum of infected patients and to distinguish this from infection by *Mycobacterium avium* (Maekura et al., 1993; Sakai et al., 2001; Fujiwara et al., 1999; Pan et al., 1999). Despite very positive results, this assay and a large number of related serodiagnostic assays have been found not to reach the required levels of specificity and sensitivity (Steingart et al., 2007, 2009; Morris, 2011; Ireton et al., 2010). Mixtures of free mycolic acids (MAs) isolated from the cells of *M. tuberculosis* have also been used in serodiagnostic assays for TB. In the simplest form they are used to detect interactions with antibodies present in the serum of infected patients using an ELISA assay. One singular advantage of this method is that the signals are retained in HIV co-infected patients (Schleicher et al., 2002). Although this is a very rapid approach to the diagnosis of TB, the sensitivity and specificity are not high enough; i.e., there are too many false negatives and too many false positives (Beukes et al., 2011; Verschoor, 2010). Part of the problem in this case may be that some of the antibodies present are cross-reactive with mycolic acids and cholesterol (Benadie et al., 2008). Improved sensitivity and specificity was obtained by a method based on the use of mycolic acid containing liposomes in an antibody binding inhibition assay using surface plasmon resonance (Verschoor, 2010;

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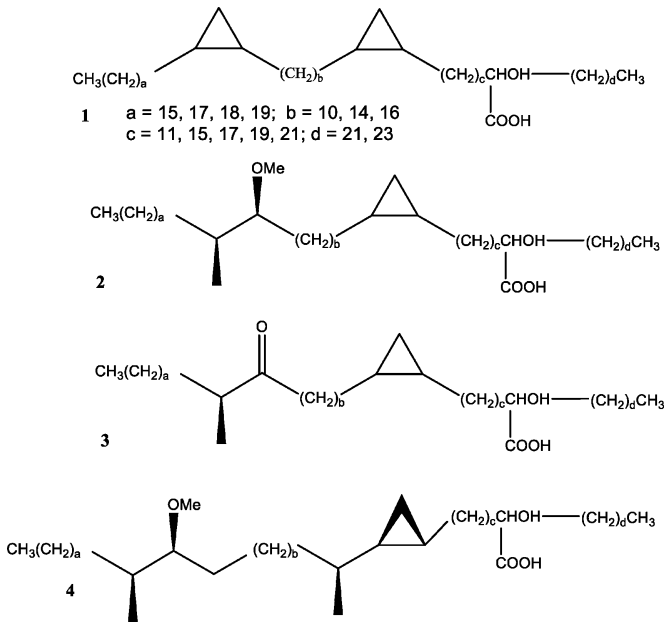



**Scheme 1.** Examples of common classes of mycolic acids in mycobacteria. For (**1**), *a* = 15, 17, 19; *b* = 10, 14, 16; *c* = 11, 13, 15, 17, 19, 21; *d* = 21, 23.

Thanyani et al., 2008; Lemmer et al., 2009). Although this significantly elevated the quality of the test, it remained impractical due to high cost and slow sample throughput.

In order to explore electrochemical methods for immunosensing, Ozoemena et al. coated a polished gold electrode surface with a thiolated fatty acid and adsorbed natural MA antigens onto the hydrophobic fatty acid layer. Using electrochemical impedance spectroscopy (EIS) for the specific antibody detection, they showed that it could distinguish TB+ from TB− sera (Ozoemena et al., 2010; Mathebula et al., 2009). In order to optimise the use of mycolic acids in such diagnostic devices for TB, we wished to bind single synthetic mycolic acid isomers to gold via a thiol group. Since methylation of the carboxylic acid of a natural mixture of MA totally destroys its antigenicity (Lemmer et al., 2009), and subtle stereochemical changes in the functional groups in the merochain of synthetic MAs had a significant effect on antigenicity (Beukes et al., 2011), we argued that suitably thiolated MA would only be accessible via total synthesis. Moreover, we reasoned that the terminus of the invariant alpha-alkyl chain of the mycolic motif is the best place to introduce the thiol moiety without disrupting biological activity.

We have already reported the synthesis of MA with the same structures and chain lengths as the major homologues present in natural mixtures. In each case the molecule was prepared as a single enantiomer (Al Dulayymi et al., 2003, 2005, 2006a,b, 2007). We now report the preparation of four such molecules, in each case modified by the introduction of a thiol substituent at the end of the α-carbon chain of the mycolic motif. This position for thiolation was chosen in order to minimise conformational changes around the β-hydroxy acid and its interaction with the long functionalised mero-chain.

## 2. Experimental

Chemicals used were obtained from commercial suppliers (Sigma–Aldrich, and Alfa Aeser) or prepared from them by the methods described. Solvents which were required to be dry, e.g. ether, tetrahydrofuran were dried over sodium wire and benzophenone under nitrogen, while dichloromethane and HMPA were dried over calcium hydride. All reagents and solvents used were of reagent grade unless otherwise stated. Silica gel (Merck 7736) and silica gel plates used for column chromatography and thin layer chromatography were obtained from Aldrich; separated components were detected using variously UV light, $I_2$ and phosphomolybdic acid solution in IMS followed by charring. Anhydrous magnesium sulfate was used to dry organic solutions. Infra-red (IR) spectra were carried out on a Perkin-Elmer 1600 F.T.I.R. spectrometer as liquid films or KBr disc (solid). Melting points were measured using a Gallenkamp melting point apparatus. NMR spectra were carried out on a Bruker AC250 or Advance 400 spectrometer. $[\alpha]_D$ values were recorded in $CHCl_3$ on a POLAAR 2001 optical activity polarimeter. Mass spectra were recorded on a Bruker matrix-assisted laser desorption/ionisation-time of flight mass spectrometry (MALDI-TOF MS) values are given plus sodium to an accuracy of 2 d.p.

### 2.1. Methyl (2R)-2-[(R)-1-(tert-butyldimethylsilyloxy)-3-hydroxypropyl]-16-(tetrahydro-2H-pyran-yloxy)hexadecanoate (7)

Lithium *bis*(trimethylsilyl)amide (8.63 mL, 9.15 mmol, 1.06 M) was added to a stirred solution of aldehyde (**5**) (2.31 g, 5.86 mmol) (Supplementary Information) and 1-phenyl-5-[12-((tetrahydro-2*H*-pyran-2-yloxy)dodecylsulfonyl]-1*H*-tetrazole (**6**) (3.36 g, 7.03 mmol) (Supplementary Information) in dry THF (150 mL) at −10 °C. The solution turned bright yellow/orange and was allowed to reach room temperature, stirred overnight under nitrogen, then quenched by the addition of a sat.aq. $NH_4Cl$ (150 mL) at −20 °C, extracted with petrol/ethyl acetate (1:2, 3 × 150 mL) and the combined organic layers were dried, filtered and evaporated. Column chromatography (petrol/ethyl acetate, 20:1) gave methyl (2*R*)-2-[(*R*)-3-(benzyloxy)-1-(*tert*-butyldimethylsilyloxy)propyl]-16-(tetrahydro-2*H*-pyran-2-yloxy)-hexadec-4-enoate (2.86 g, 4.42 mmol, 75%) as a colourless oil. Palladium on carbon (10%, 0.5 g) was slowly added under a stream of nitrogen to a stirred solution of the alkene (2.56 g, 3.96 mmol) in IMS (50 mL) and THF (10 mL). The flask purged of air by repeated application of vacuum followed by refilling the system with hydrogen. When the hydrogenation was complete, the mixture was filtered through a pad of Celite®, which was washed with copious ethyl acetate and the solvent was evaporated. Column chromatography (petrol/ethyl acetate, 5:1) gave methyl (2*R*)-2-[(*R*)-1-(*tert*-butyl-dimethylsilyloxy)-3-hydroxypropyl]-16-(tetrahydro-2*H*-pyranyloxy)hexadecanoate (**7**) (1.75 g, 79%) (two diastereoisomers) as a colourless oil, $[\alpha]_D^{23}$ −4.0 (*c* 0.93, $CHCl_3$) {Found $(M+Na)^+$: 581.4215, $C_{31}H_{62}O_6SiNa$ requires: 581.4208}. This showed $\delta_H$: 4.58 (0.8H, dd, *J* 2.9, 4.4), 4.53 (0.6H, ddd, *J* 4.7, 9.6, 11.2 Hz), 4.14 (0.5H, m), 4.28 (1.2H, m), 3.88 (1H, ddd, *J* 3.2, 7.9, 11.4), 3.74 (1.7H, m), 3.68 (1H, s, $OCH_3$), 3.51 (0.7H, m), 3.50 (2H, s, $OCH_3$), 3.39 (1H, dt, *J* 6.7, 9.6), 2.64 (0.4H, ddd, *J* 3.8, 6.9, 10.9, $CHCH(CO)CH_2$), 2.31 (0.6H, ddd, *J* 3.2, 5.4, 8.5, $CHCH(CO)CH_2$), 2.09 (0.6H, m), 1.89–2.02 (1.3H, m), 1.70–1.85 (2.7H, m), 1.58 (10.4H, m), 1.26 (20H, m), 0.89 (9H, s, $C(CH_3)_3$, including smaller s at 0.88), 0.09 (3H, s, $CH_3Si$, including smaller s at 0.11) and 0.08 (3H, s, $CH_3Si$, including smaller s at 0.07); $\delta_C$: 173.1, 99.8, 98.9, 67.7, 65.9, 64.7, 63.1, 62.4, 61.9, 55.0, 47.6, 32.8, 31.7, 30.8, 30.5, 29.8, 29.7, 29.63, 29.60, 29.5, 29.4, 27.2, 26.4, 26.3, 25.74, 25.68, 25.5, 19.7, 19.4, 18.0, −4.3 and −5.0; $\nu_{max}$: 3465, 2926, 2854, 1737 and 1463 $cm^{-1}$.

### 2.2. Methyl (2R)-2-[(R)-1-(tert-butyldimethylsilyloxy)-3-oxopropyl]-16-(tetrahydro-2H-pyran-2-yloxy)hexadecanoate

A solution of alcohol (**7**) (1.73 g, 3.10 mmol) (Al Dulayymi et al., 2007) in dichloro-methane (10 mL) was added to a stirred suspension of PCC (1.67 g, 7.75 mmol) in dichloromethane (100 mL) at room temperature and stirred at room temperature for 3 h. Ethyl acetate (50 mL) was added and the mixture was filtered through a bed of silica and the solvent was evaporated. Column chromatography (petrol/ethyl acetate, 5:2) gave methyl

(2*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilyloxy)-3-oxopropyl]-16-(tetrahydro-2*H*-pyran-2-yloxy)hexadecanoate (1.41 g, 82%) (two diastereoisomers) as a colourless oil, $[\alpha]_D^{21}$ −7.05 (*c* 0.98, $CHCl_3$). This showed $\delta_H$: 9.18 (1H, dd, *J* 1.6, 2.5 Hz), 4.58 (1H, m), 4.43 (1H, m), 3.88 (1H, ddd, *J* 3.3, 7.7, 11.2 Hz), 3.74 (1H, dt, *J* 6.9, 9.6 Hz), 3.69 (3H, s), 3.51 (1H, m), 3.39 (1H, dt, *J* 6.7, 9.6 Hz), 0.07 (3H, s), 2.60 (3H, m), 1.83 (1H, m), 1.72 (1H, m), 1.56 (10H, m), 1.28 (20H, m), 0.86 (9H, s) and 0.08 (3H, s); $\delta_C$: 201.3, 174.1, 98.9, 68.8, 67.7, 62.4, 52.3, 51.6, 48.1, 30.8, 29.8, 29.7, 29.63, 29.56, 29.52, 29.4, 27.8, 27.0, 26.3, 25.6, 25.5, 19.7, 17.9, −4.6 and −4.9; $\nu_{max}$: 2927, 2855, 1735 and 1464 $cm^{-1}$. This aldehyde was used immediately in the next step.

### 2.3. Methyl (2R,3R)-3-(tert-butyldimethylsilyloxy)-2-(14-hydroxytetradecyl) heneicosanoate (**8**)

(i) Lithium *bis*(trimethylsilyl)amide (3.68 mL, 3.90 mmol, 1.06 M) was added to a stirred solution of methyl (2*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilyloxy)-3-oxopropyl]-16-(tetrahydro-2*H*-pyran-2-yloxy)hexadecanoate (1.39 g, 2.50 mmol) and 5-(hexa-decylsulfonyl)-1-phenyl-1*H*-tetrazole (1.30 g, 3.00 mmol) (Al Dulayymi et al., 2007) in dry THF (25 mL) at −10 °C. The solution turned bright yellow/orange and was allowed to reach room temperature, and stirred overnight under nitrogen. It was quenched by addition of a sat.aq. $NH_4Cl$ (100 mL) at −20 °C then extracted with petrol/ethyl acetate (1:2, 3 × 150 mL) and the combined organic layers were dried, filtered and evaporated. Column chromatography (petrol/ethyl acetate, 15:1) gave methyl (2*R*,3*R*)-3-(*tert*-butyldimethylsilyloxy)-2-[14-(tetrahydro-2*H*-pyran-2-yloxy)-tetradecyl]henicos-5-enoate (1.43 g 1.87 mmol, 75%) as a colourless oil.

(ii) Palladium on carbon (10%, 0.3 g) was added to a stirred solution of olefin (1.40 g, 1.83 mmol) in IMS (20 mL) and THF (10 mL) and the mixture was hydrogenated as above then filtered through a pad of Celite®, which was washed with copious ethyl acetate. The solvent was evaporated. Column chromatography (petrol/ethyl acetate, 10:1) gave methyl (2*R*,3*R*)-3-(*tert*-butyldimethylsilyloxy)-2-[14-(tetrahydro-2*H*-pyran-2-yloxy)tetradecyl]henicosanoate (1.30 g, 92%) as a colourless oil, $[\alpha]_D^{21.3}$ −4.15 (*c* 0.92, $CHCl_3$) {Found $(M+Na)^+$: 789.6748, $C_{47}H_{94}O_5SiNa$ requires: 789.6763}. This showed $\delta_H$: 4.58 (1H, dd, *J* 3.0, 4.3 Hz), 3.91 (2H, m), 3.74 (1H, dt, *J* 7.0, 9.6 Hz), 3.66 (3H, s), 3.51 (1H, m), 3.39 (1H, dt, *J* 6.7, 9.6 Hz), 2.53 (1H, ddd, *J* 3.7, 7.2, 11.0 Hz), 1.57–1.83 (16H, m), 1.26 (50H, m), 0.88 (3H, t, *J* 6.9 Hz), 0.87 (9H, s), 0.05 (3H, s) and 0.02 (3H, s); $\delta_C$: 175.2, 98.9, 76.6, 73.2, 67.7, 62.3, 51.6, 51.2, 33.7, 32.8, 31.9, 30.8, 29.83, 29.77, 29.71, 29.66, 29.60, 29.52, 29.46, 29.37, 27.9, 27.5, 26.3, 25.8, 25.5, 23.7, 22.7, 19.7, 18.0, 14.1, −4.4 and −4.9; $\nu_{max}$: 2924, 2853, 1740 and 1464 $cm^{-1}$.

(iii) Pyridinium *p*-toluenesulfonate (40 mg, 1.6 mmol) was added with stirring to methyl (2*R*,3*R*)-3-(*tert*-butyldimethylsilyloxy)-2-[14-(tetrahydro-2*H*-pyran-2-yloxy)-tetra-decyl]-henicosanoate (485 mg, 0.633 mmol) in THF (15 mL), methanol (3 mL) and water (1 mL) at room temperature and stirred overnight. Sat.aq. $NaHCO_3$ (10 mL) and petrol/ethyl acetate (1:1, 10 mL) were added. The mixture was extracted with petrol/ethyl acetate (1:1, 3 × 25 mL) and the combined organic layers were washed with brine (20 mL), dried, filtered and evaporated. Column chromatography (petrol/ethyl acetate, 5:1) gave methyl (2*R*,3*R*)-3-(*tert*-butyldimethylsilyloxy)-2-(14-hydroxy-tetradecyl)heneicosanoate (**8**) (352 mg, 82%) as a colourless oil, $[\alpha]_D^{21.4}$ −3.74 (*c* 0.95, $CHCl_3$) {Found $(M+Na)^+$: 705.6205, $C_{42}H_{86}O_4SiNa$ requires: 705.6188}. This showed $\delta_H$: 3.91 (1H, dt, *J* 4.7, 6.8 Hz), 3.66 (3H, s), 3.65 (2H, t, *J* 6.6 Hz), 2.53 (1H, ddd, *J* 3.8, 7.2, 11.0 Hz), 1.53 (9H, br m), 1.26 (52H, m), 0.88 (3H, t, *J* 7.1), 0.87 (9H, s), 0.05 (3H, s) and 0.02 (3H, s); $\delta_C$: 175.2, 73.2, 63.1, 51.6, 33.7, 32.8, 31.9, 29.8, 29.71, 29.66, 29.63, 29.58, 29.4, 29.3, 27.9, 27.5, 25.8, 25.7, 23.7, 22.7, 18.0, 14.1, −4.3 and −4.9; $\nu_{max}$: 3357, 2924, 2853, 1739 and 1462 $cm^{-1}$.

### 2.4. Methyl (2R,3R)-3-(tert-butyldimethylsilyloxy)-2-[14-(tosyloxy)tetradecyl] heneicosanoate

A solution of alcohol (**8**) (256 mg, 0.376 mmol) and triethylamine (1 mL) in dry dichloromethane (10 mL) was cooled to −20 °C under $N_2$ (g) and stirred for 30 min. *p*-Toluenesulfonyl chloride (86 mg, 0.45 mmol) was added in one portion. The solution was kept in the refrigerator overnight and then the solvent was evaporated. Column chromatography (petrol/ethyl acetate, 5:1) gave methyl (2*R*,3*R*)-3-(*tert*-butyldimethylsilyloxy)-2-[14-(tosyloxy)tetradecyl]heneicosanoate (233 mg, 74%) as a colourless oil, $[\alpha]_D^{20.7}$ −0.82 (*c* 0.94, $CHCl_3$) {Found $(M+Na)^+$: 859.33, $C_{49}H_{92}O_6SSiNa$ requires: 859.63}. This showed $\delta_H$: 7.80 (2H, d, *J* 8.5 Hz), 7.36 (2H, d, *J* 7.9 Hz), 4.02 (2H, t, *J* 6.5 Hz), 3.91 (1H, dt, *J* 4.7, 6.9 Hz), 3.66 (3H, s), 2.52 (1H, ddd, *J* 3.7, 7.2, 10.8 Hz), 2.46 (3H, s), 1.63 (8H, m), 1.26 (52H, m), 0.88 (3H, t, *J* 6.9 Hz), 0.87 (9H, s), 0.05 (3H, s) and 0.02 (3H, s) $\delta_C$: 175.1, 144.6, 133.3, 129.8, 127.9, 73.2, 70.7, 60.4, 51.6, 51.2, 33.7, 31.9, 29.8, 29.70, 29.67, 29.64, 29.61, 29.59, 29.51, 29.47, 29.40, 29.36, 28.9, 28.8, 27.9, 27.5, 25.8, 25.3, 23.7, 22.7, 21.6, 21.0, 18.0, 14.2, 14.1, −4.3 and −4.9; $\nu_{max}$: 2925, 2854, 1739, 1598, 1464 $cm^{-1}$.

### 2.5. Methyl (2R,3R)-2-[14-(acetylthio)tetradecyl]-3-(tert-butyldimethylsilyloxy) heneicosanoate (**9**)

Methyl (2*R*,3*R*)-3-(*tert*-butyldimethylsilyloxy)-2-[14-(tosyloxy)tetradecyl]heneicosa-noate (231 mg, 0.276 mmol) and potassium thioacetate (126 mg, 0.111 mmol) in acetone (13 mL) and THF (5 mL) were stirred at room temperature for 4 h, then the solvent was removed. Column chromatography (petrol/ethyl acetate, 5:1) gave methyl (2*R*,3*R*)-2-[14-(acetylthio)tetradecyl]-3-(*tert*-butyldimethylsilyloxy)henicosanoate (**9**) (154 mg, 75%) as a colourless oil, $[\alpha]_D^{24.2}$ −1.7 (*c* 0.47, $CHCl_3$) {Found $(M+Na)^+$: 763.5978, $C_{44}H_{88}O_4SSiNa$ requires: 763.6070}. This showed $\delta_H$: 3.91 (1H, dt, *J* 4.7, 6.9 Hz), 3.66 (3H, s), 2.87 (2H, t, *J* 7.4 Hz), 2.53 (1H, ddd, *J* 3.8, 7.1, 10.9 Hz), 2.32 (3H, s), 1.26–1.55 (60H, m), 0.88 (3H, t, *J* 7 Hz), 0.86 (9H, s), 0.05 (3H, s) and 0.02 (3H, s); $\delta_C$:196.0, 175.1, 73.2, 51.6, 33.7, 31.9, 30.6, 29.8, 29.64 (br), 29.58, 29.50, 29.48, 29.45, 29.37, 29.2, 29.1, 28.8, 27.9, 27.5, 25.8, 23.7, 22.7, 18.0, 14.1, −4.3 and −4.9; $\nu_{max}$: 2926, 2847, 1737, 1695 and 1460 $cm^{-1}$.

### 2.6. Methyl (2R,3R)-2-(14-bromotetradecyl)-3-(tert-butyldimethylsilyloxy)hen-eicosanoate (**10**)

This is described in the Supplementary Information.

### 2.7. Methyl (2R,3R)-2-[14-(acetylthio)tetradecyl]-3-hydroxyheneicosanoate (**11**)

Thioacetate (**9**) (287 mg, 0.388 mmol) was dissolved in dry THF (6 mL) in a dry polyethylene vial under $N_2$ (g) at 0 °C. Pyridine (0.20 mL, 2.5 mmol) and HF.pyridine (1.5 mL, mmol) were added and the mixture stirred at 45 °C overnight, then added slowly to a sat.aq. $NaHCO_3$ (20 mL). The solution was extracted with petrol/ethyl acetate (1:1, 3 × 20 mL) and the combined organic layers were dried, filtered and evaporated. Column chromatography (petrol/ethyl acetate, 10:1) gave methyl (2*R*,3*R*)-2-[14-(acetylthio)tetradecyl]-3-hydroxyhenicosanoate (**11**) (199 mg, 84%) as a white solid, m.p. 66–68 °C, $[\alpha]_D^{24.9}$ +5.9 (*c* 0.59, $CHCl_3$) {Found $(M+Na)^+$: 649.5224, $C_{38}H_{74}O_4SiNa$ requires: 649.5200}. This showed $\delta_H$: 3.71 (3H, s), 3.66 (1H, m), 2.86 (2H, t, *J* 7.4 Hz), 2.44 (1H, dt, *J* 5.3, 9.3, Hz), 2.32 (3H, s), 2.09 (1H, br s, OH), 1.56

(4H, m), 1.45 (4H, m), 1.26 (52H, m) and 0.88 (3H, t, *J* 6.9 Hz); $\delta_C$: 196.0, 176.2, 72.3, 51.5, 51.0, 35.7, 31.9, 30.6, 29.70 (br), 29.65, 29.62, 29.57, 29.50, 29.47, 29.42, 29.3, 29.2, 29.1, 28.8, 27.4, 25.7, 22.7 and 14.1; $\nu_{max}$: 3407, 2921, 2852, 1732, 1688 and 1465 $cm^{-1}$.

### 2.8. (2R,2′R,3R,3′R)-2,2′-Disulfanediylbis(tetradecane-14,1-diyl) bis(3-hydroxy heneicosanoic acid) (**13**)

Lithium hydroxide (4 mol.eq, 10.7 mg, 0.255 mmol) was added to methyl (2*R*,3*R*)-2-[14-(acetylthio)tetradecyl]-3-hydroxyhenicosanoate (**11**) (40 mg, 0.064 mmol) in a mixture of THF (4 mL), water (0.4 mL) and methanol (0.4 mL) and stirred at 45 °C overnight. The reaction was diluted by addition of petrol/ethyl acetate (1:1, 10 mL) and brought to pH 1 by dropwise addition of dil. HCl. The product was extracted with petrol/ethyl acetate (1:1, 10 mL) and the combined organic extracts were dried and evaporated. Column chromatography (petrol/ethyl acetate, 5:2) gave (2*R*,2′*R*,3*R*,3′*R*)-2,2′-disulfanediylbis(tetradecane-14,1-diyl)*bis*(3-hydroxyheneicosanoic acid) (**13**) (9.3 mg, 26%) as a white solid, $[\alpha]_D^{20}$ −0.15 (*c* 0.23, $CHCl_3$) {Found (M-H)$^+$: 1138.3447, $C_{70}H_{137}O_6S_2$ requires 1137.9857}. This showed $\delta_H$: 3.70 (1H, m), 2.70 (2H, t, *J* 7.3 Hz), 2.47 (1H, dt, *J* 4.9, 9.6 Hz), 1.68 (4H, m), 1.52 (3H, m), 1.26 (56H, m) and 0.89 (3H, t, *J* 6.8 Hz); $\delta_C$: 180.7, 72.3, 51.0, 39.5, 35.5, 31.9, 29.70, 29.67, 29.61, 29.56, 29.52, 29.43, 29.36, 29.2, 29.1, 28.9, 28.4, 27.4, 25.7, 22.7, 22.6 and 14.1; $\nu_{max}$($CHCl_3$): 3451, 2916, 2850, 1682 and 1470 $cm^{-1}$.

### 2.9. 2,2-Dimethylpropanoic acid 22-(1-phenyl-1H-tetrazole-5-ylsulfonyl)docosyl ester (**15**)

(i) 1-Phenyl-1*H*-tetrazole-5-thiol (4.40 g, 24.6 mmol), 2,2-dimethylpropanoic acid (22-bromo)docosyl ester (11.0 g, 22.4 mmol) (Supplementary Information) and anhydrous potassium carbonate (6.81 g, 49.3 mmol) were vigorously stirred in acetone (250 mL) for 18 h at room temperature. Water (500 mL) was added and the product was extracted with dichloromethane (1 × 200 mL, 2 × 100 mL). The combined organic layers were washed with brine (2 × 200 mL), dried and evaporated. Column chromatography eluting with petrol/ethyl acetate (10:1) gave a semi-solid 2,2-dimethylpropanoic acid 22-(1-phenyl-1H-tetrazole-5-ylsulfanyl)docosyl ester (13.0 g, 84%) {Found (M + H)$^+$: 587.4344, $C_{34}H_{59}O_2N_4S$ requires: 587.4353}; $\delta_H$ (500 MHz, $CDCl_3$): 7.58–7.51 (5H, m), 4.03 (2H, t, *J* 6.65 Hz), 3.38 (2H, t, *J* 7.55 Hz), 1.84 (2H, pent, *J* 6.5 Hz), 1.60 (2H, pent, *J* 6.3 Hz), 1.44–1.39 (2H, m), 1.33–1.21 (34H, m), 1.18 (9H, s); $\delta_C$: 178.6, 154.4, 133.7, 130.0, 129.7, 123.8, 67.9, 64.4, 53.4, 38.7, 33.3, 30.8, 29.5, 29.48, 29.45, 29.4, 29.2, 29.0, 28.6, 28.55, 27.1, 25.8, 25.6; $\nu_{max}$: 2925, 2853, 1728, 1597, 1500, 1462, 1397, 1283, 1156 $cm^{-1}$.

(ii) Ammonium molybdate (VI) tetrahydrate (13.70 g, 11.09 mmol) in 35% $H_2O_2$ (50 mL), prepared and cooled in an ice bath was added to a stirred solution of the above sulfide (13.01 g, 22.18 mmol) in THF (50 mL) and IMS (100 mL) at 10 °C. The mixture was stirred at room temperature for 2 h. Further ammonium molybdate (VI) tetrahydrate (6.85 g, 5.5 mmol) in 35% $H_2O_2$ (25 mL) was added and the mixture was stirred at room temperature for 18 h, then poured into water (1 L) and extracted with dichloromethane (1 × 250 mL, 3 × 150 mL). The combined organic phases were washed with water (500 mL), dried and the evaporated. The product was purified by column chromatography eluting petrol/ethyl acetate (5:1, then 1:1) to give a white solid, 2,2-dimethylpropanoic acid 22-(1-phenyl-*1H*-tetrazole-5-ylsulfonyl)docosyl ester (**15**) (12.4 g, 90%), mp 41–42 °C {Found (M + Na)$^+$: 641.4071, $C_{34}H_{58}O_2SNa$ requires: 641.4076}; $\delta_H$: (500 MHz, $CDCl_3$); 7.61–7.60 (2H, m), 7.59–7.58 (3 H, m), 4.04 (2H, t, *J* 6.65 Hz), 3.73 (2H, t, *J* 7.4 Hz), 1.95–1.92 (2H, m), 1.61 (2H, pent, *J* 6.95 Hz), 1.50 (2H, pent, *J* 6.65 Hz), 1.37–1.22 (34H, m), 1.19 (9H, s); $\delta_c$: 178.6, 153.5, 133.0, 131.4, 129.7, 125.0, 64.4, 60.3, 56.0, 38.7, 29.7, 29.6, 29.5, 29.47, 29.4, 29.2, 29.15, 28.9, 28.6, 28.1, 27.2, 25.9, 21.9, 21.0; $\nu_{max}$: 2918, 2850, 1725, 1617, 1497, 1473, 1342, 1285, 1155, 824 $cm^{-1}$.

### 2.10. (R)-2-[(R)-1-(tert-Butyldimethylsilanyloxy)-3-hydroxy-propyl]-26-(2,2-dimethylpropionyloxy)-hexacosanoic acid methyl ester (**16**)

(i) Lithium bis(trimethylsilyl)amide (14.6 mL, 15.5 mmol) was added to a stirred solution of (2*R*,3*R*)-5-benzyloxy-3-(*tert*-butyldimethylsilanyloxy)-2-(oxoethyl)penta-noic acid methyl ester (**5**) (3.70 g, 9.38 mmol) and ester (15) (6.39 g, 10.32 mmol) in dry THF (100 mL) under nitrogen at −10 °C. The reaction turned bright yellow and was left to reach r.t. and stirred for 1 h under nitrogen, then quenched with sat.aq. $NH_4Cl$. The product was extracted with petrol/ethyl acetate (20:1,3 × 150 mL), dried, filtered and evaporated. Column chromatography over silica eluting with petrol/ethyl acetate (20:1) gave a colourless oil, methyl (*E*/*Z*)-(*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilanyloxy)-3-benzyloxypropyl]-26-(2,2-dimethylpropionyloxy)-hexacos-3-enoic acid methyl ester (5.0 g, 67%), in ratio (2:1).

(ii) Palladium on carbon 10% (1.0 g) was added to a stirred solution of the alkene in IMS (50 mL) and THF (50 mL) under hydrogen. Hydrogenation was carried out for 2 days. The mixture was filtered over a bed of celite and the solvent was evaporated. Column chromatography eluting with petrol/ethyl acetate (5:1) gave a white solid, (*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilanyloxy)-3-hydroxypropyl]-26-(2,2-dimethyl- propionyloxy)hexacosanoic acid methyl ester (**16**) (3.0 g, 68%), m.p 37–39 °C, $[\alpha]_D^{23}$ −8.89 (*c* 1.54, $CHCl_3$) {Found [M + Na]$^+$: 721.5739. $C_{41}H_{82}O_6SiNa$ requires: 721.5773}; $\delta_H$ (500 MHz, $CDCl_3$): 4.04 (2H, t, *J* 6.5 Hz), 3.81–3.70 (1H, m), 3.78–3.7 (2H, m), 3.67 (3H, s), 2.64 (1H, ddd, *J* 3.75, 6.9, 10.7 Hz), 1.83–1.73 (2H, m), 1.64–1.58 (4H, m), 1.29–1.18 (52H, m, including s at $\delta$ 1.20), 0.88 (9H, s), 0.11 (3H, s), 0.07 (3H, s); $\delta_c$: 178.7, 174.7, 72.1, 64.5, 59.5, 51.6, 51.4, 38.7, 35.2, 29.7, 29.6, 29.55, 29.5, 29.4, 29.2, 28.6, 27.9, 27.2, 25.9, 25.7, 22.6, 22.3, 21.0, 17.9, 14.19, −4.5, −5.0; $\nu_{max}$: 3521, 2925, 2854, 1731, 1463, 1285, 1255, 1163, 1092, 837 $cm^{-1}$.

### 2.11. (R)-2-[(R)-1-(tert-Butyldimethylsilanyloxy)-3-oxopropyl]-26-(2,2-dimethyl-propionyloxy)-hexacosanoic acid methyl ester (**17**)

(*R*)-2-[(*R*)-1-(*tert*-Butyldimethylsilanyloxy)-3-hydroxypropyl]-26-(2,2-dimethyl-propionyloxy)-hexacosanoic acid methyl ester (3.12 g, 4.46 mmol) in dichloromethane (20 mL) was added to a stirred suspension of PCC (2.40 g, 11.2 mmol) in dichloromethane (130 mL) at room temperature. The mixture was stirring vigorously for 2 h, then poured into petrol/ethyl acetate (10:1, 300 mL), filtered through a bed of silica and celite then washed well with petrol/ethyl acetate (10:1) and evaporated. Column chromatography eluting with petrol/ethyl acetate (10:1) gave colourless oil, (*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilanyloxy)-3-oxopropyl]-26-(2,2-dimethylpropionyloxy)hexacosanoic acid methyl ester (**17**) (2.46 g, 82%), $[\alpha]_D^{19}$ −4.2 (*c* 0.96, $CHCl_3$) {Found [M + Na]$^+$: 719.5565, $C_{41}H_{80}O_6SiNa$ requires: 719.5616}, $\delta_H$: (500 MHz, $CDCl_3$): 9.82 (1H, br.s), 4.44–4.41 (1H, m), 4.04 (2H, t, *J* 6.5 Hz), 3.68 (3 H, s), 2.67–2.57 (3H, m), 1.61 (2H, pent, *J* 6.5 Hz), 1.25–1.19 (53 H, m, including s at $\delta$ 1.19), 0.85 (9H, s), 0.074 (3 H, s) 0.072 (3H, s); $\delta_c$: 201.3, 178.6, 174.0, 68.8, 64.5, 52.3, 51.5, 48.1, 38.7, 31.6, 29.7, 29.6, 29.55, 29.5, 29.4, 29.2, 29.0, 28.6, 27.7, 27.2, 27.0, 25.9, 25.6, 22.6, 17.9, 14.1, −4.6, −4.9; $\nu_{max}$: 2927, 2856, 1731, 1463, 1364, 1285, 1162, 1005, 837, 777 $cm^{-1}$.

### 2.12. Methyl (R)-2-[(R)-1-(tert-butyldimethylsilyoxy)-11-hydroxyundecyl]-26-(pivaloyloxy)hexacosanoate (**18**)

(i) Lithium bis(trimethylsilyl)amide (7.3 mL, 8.4 mmol) was added with stirring to ester (**17**) (3.14 g, 4.5 mmol) and 1-phenyl-1*H*-tetrazol-5-[8-(tetrahydro-2*H*-pyran-2-yloxy)octsulfonyl]-1*H*-tetrazole (2.47, 5.8 mmol) in dry THF (80 mL) under nitrogen at −10 °C. The reaction turned bright yellow and was left to reach room temperature and stirred for 1 h under nitrogen, then quenched with sat.aq. $NH_4Cl$ (20 mL). The product was extracted with petrol/ethyl acetate (10:1) (3 × 150 mL). The combined organic layers were dried and evaporated. Column chromatography of the residue eluting with petrol/ethyl acetate (20:1) gave a colourless oil, (*R*)-methyl-2-[(*Z*/*E*)-1-(*tert*-butyldimethylsilyoxy)-11-(tetrahydro-2*H*-pyran-2-yloxy)-undec-3-enyl]-26-(pivaloyloxy)hexacosanoate in ratio (2.5:1) (3.4 g, 85%).

(ii) Pyridinium *p*-toluenesulfonate (0.47 g, 1.90 mmol) was added to a stirred solution of the above alkenes (3.4 g, 3.80 mmol) in MeOH-THF (30 mL: 70 mL) at 50 °C for 3 h. The solvent was evaporated and the residue was treated with sat.aq. $NaHCO_3$ (30 mL) and petrol/ethyl acetate (10:1, 70 mL). The aqueous layer was re-extracted with petrol/ethyl acetate (10:1, 3 × 50 mL). The combined organic layers were dried and evaporated. Chromatography eluting with petrol/ethyl acetate (15:1 then 5:1) gave (*R*)-methyl-2-[(*Z*/*E*)-1-(tert-butyldimethyl-silyoxy)-11-hydroxyundec-3-enyl]-26-(pivaloyloxy)hexacosanoate as a colourless oil (2.7 g, 88%).

(iii) Palladium on carbon 10% (0.7 g) was added to a stirred solution of the above alcohol (2.7 g, 3.33 mmol) in IMS/THF (2:1, 40:20 mL) under hydrogen. Hydrogen-ation was carried out for 1 hr, then the mixture was filtered over a bed of celite and the solvent was evaporated. Column chromatography eluting with petrol/ethyl acetate (5:1) gave methyl (*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilyoxy)-11-hydroxyundecyl]-26-(pivaloyloxy)hexacosanote (**18**) as a semi-solid (2.4 g, 88%), $[\alpha]_D^{22}$ −3.69 (*c* 1.19, $CHCl_3$) {Found $(M+Na)^+$: 833.7025; $C_{49}H_{98}O_6SiNa$ requires: 833.7030}; $\delta_H$ (500 MHz, $CDCl_3$): 4.03 (2H, t, *J* 7.3 Hz), 3.90–3.87 (1H, m), 3.64 (3H, s), 3.62 (2H, t, *J* 6.5 Hz), 2.51 (1H, ddd, *J* 3.75, 7.25, 10.7 Hz), 1.61–1.50 (6H, m), 1.44–1.18 (68H, m, including s at $\delta$ 1.18), 0.85 (9H, s), 0.03 (3H, s), 0.009 (3H, s); $\delta_c$: 178.6, 175.1, 73.2, 64.4, 63.0, 51.5, 51.2, 38.7, 33.6, 32.8, 29.8, 29.7, 29.6, 29.5, 29.48, 29.4, 29.2, 28.6, 27.8, 27.2, 25.9, 25.8, 25.7, 23.7, 21.0, 17.9, 14.2, −4.4, −5.0; $\nu_{max}$: 3344, 2927, 2854, 1732, 1655, 1546, 1463, 1284, 1253, 1157, 1034, 836, 775 $cm^{-1}$.

### 2.13. (R)-2-[(R)-1-tert-Butyldimethylsilanyloxy)-11-oxopropyl]-26-(2,2-dimethyl-propionyloxy)hexacosanoic acid methyl ester (**19**)

The ester **18** (1.20 g, 1.47 mmol) in dichloromethane (20 mL) was added to a stirred suspension of pyridinium chlorochromate (0.79 g, 3.7 mmol) in dichloromethane (50 mL) at room temperature, stirred vigorously for 2 h, then poured into petrol/ethyl acetate (10:1, 150 mL) and filtered through a bed of silica and celite, washed with petrol/ethyl acetate (50 mL) and evaporated. Chromatography eluting with petrol/ethyl acetate (10:1) gave a colourless oil, (*R*)-2-[(*R*)-1-*tert*-butyldimethyl-silanyloxy)-11-oxopropyl]-26-(2,2-dimethylpropionyloxy)hexacosanoic acid methyl ester (**19**) (1.0 g, 84%), $[\alpha]_D^{20}$ −4.0 (*c* 0.94, $CHCl_3$); $\delta_H$ (500 MHz, $CDCl_3$): 9.77 (1H, t, *J* 3.75 Hz), 4.03 (2H, t, *J* 5.05 Hz), 3.92–3.89 (1H, m), 3.66 (3H, s), 2.54–2.50 (1H, m), 2.44–2.40 (2H, m), 1.62 (2H, pent, *J* 6.9 Hz), 1.43–1.39 (1H, m), 1.28–1.14 (68H, m, including s at $\delta$ 1.20), 0.86 (9H, s), 0.04 (3H, s), 0.02 (3H, s); $\delta_c$: 202.8, 178.6, 175.1, 73.2, 64.5, 51.6, 51.2, 43.9, 38.7, 33.7, 29.8, 29.7, 29.6, 29.58, 29.55, 29.5, 29.48, 29.4, 29.3, 29.3, 29.2, 29.2, 28.6, 27.8, 27.5, 27.2, 25.9, 23.8, 22.1, 18.0, −4.4, −4.9; $\nu_{max}$: 2924, 2852, 1734, 1709, 1607, 1494, 1402, 1107, 1050, 824 $cm^{-1}$.

### 2.14. Methyl (R)-2-{(R)-1-(tert-butyldimethylsiloxy)-18-[(1R,2S)-2-(17S,18S)-17-methoxy-18-methylhexatriacontyl) cyclopropropyl]octadecyl}-26-hydroxyhexa-cosanoate (**21**)

(i) Lithium bis(trimethylsilyl)amide (2.17 mL, 2.30 mmol, 1.06 M) was added dropwise to a stirred solution of ester (**19**) (0.970 g, 1.19 mmol) and sulfone (**20**) (1.23 g, 1.37 mmol) in dry THF (50 mL) at −10 °C under nitrogen. The reaction turned bright yellow and was left to reach room temperature and stirred for 1 hr, then quenched with sat.aq. $NH_4Cl$ (30 mL). The product was extracted with petrol/ethyl acetate (10:1) (3 × 150 mL) dried and evaporated. Column chromatography eluting with petrol/ethyl acetate (20:1) gave a colourless oil, methyl (*E*/*Z*)-(*R*)-2-{(*R*)-1-(*tert*-butyldimethyl-siloxy)-18-(1*R*,2*S*)-2-(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)-cyclopropyl-]octadec-11-enyl}-26-(pivaloyloxy)hexacosanoate (1.2 g, 71%) in ratio 2:1.

(ii) Dipotassium azodicarboxylate (2.00 g, 10.3 mmol) was added to a stirred solution of the above alkenes (1.20 g, 0.81 mmol) in THF (30 mL) and methanol (7 mL) at 5 °C. A solution of glacial acetic acid (3 mL) and THF (3 mL) was added dropwise at 5 °C over a period two days. Further dipotassium azodicarboxylate (1.50 g) then glacial acetic acid (2 mL) in THF (2 mL) were added and stirred overnight. This mixture was poured slowly into sat. aq. $NaHCO_3$ (20 mL). The product was extracted with petrol/ethyl acetate (1:1, 3 × 80 mL). The combined organic layers were washed with water (50 mL), dried and evaporated. Column chromatography eluting with petrol/ethyl acetate (15:1) gave methyl (*R*)-2-{(*R*)-1-(*tert*-butyldimethylsiloxy)-18-[(1*R*,2*S*)-2-(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)-cyclopropyl]octadecyl}-26-(pivaloyloxy)hexacosanoate as a thick colourless oil (0.94 g, 78%), $[\alpha]_D^{23}$ −5.6 (*c* 1.07 g, $CHCl_3$) {Found $[M+Na]^+$: 1504.4639; $C_{97}H_{192}O_6NaSi$ requires: 1504.4380}; $\delta_H$ (500 MHz, $CDCl_3$): 4.06 (2H, t, *J* 6.6 Hz), 3.92–3.89 (1H, m), 3.66 (3H, s), 3.34 (3H, s), 2.97–2.94 (1H, m), 2.53 (1H, ddd, *J* 3.8, 7.25, 11.00 Hz), 1.64–1.48 (8H, m), 1.37–1.13 (145H, m, including s at 1.20), 0.90–0.82 (18H, m, including s at $\delta$ 0.86), 0.66–0.64 (2H, br.m), 0.56 (1H, dt, *J* 3.75, 7.9 Hz), 0.04 (3H, s), 0.02 (3H, m), −0.32 (1H, q, *J*, 5.35 Hz); $\delta_c$: 178.3, 175.1, 85.4, 73.2, 64.5, 57.7, 51.6, 51.2, 38.7, 35.3, 33.7, 32.4, 31.9, 30.5, 30.2, 30.0, 29.8, 29.7, 29.6, 29.5, 29.4, 29.35, 29.2, 28.9, 28.6, 27.8, 27.6, 27.2, 26.2, 25.8, 23.7, 22.68, 18.0, 15.8, 14.9, 14.1, 10.9, −4.4, −4.9; $\nu_{max}$: 2923, 2853, 1732, 1464, 1156, 1099, 836 $cm^{-1}$.

(iii) The above product (0.94 g, 0.63 mmol) in THF (3 mL) was added to a stirred solution of potassium hydroxide (0.53 g, 9.5 mmol) in THF (15 mL), methanol (15 mL) and water (1.5 mL). The mixture was heated at 70 °C for 3 h, then quenched with water (10 mL) and extracted with petrol/ethyl acetate (10:1, 3 × 25 mL). The combined organic extracts were dried, filtered and evaporated. Column chromatography eluting with petrol/ethyl acetate (10:1) gave methyl (*R*)-2-{(*R*)-1-(*tert*-butyldimethylsiloxy)-18-[(1*R*,2*S*)-2-(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl]octadecyl}-26-hydroxyhexacosanoate (**21**) (0.62 g, 70%), as a thick colourless oil, $[\alpha]_D^{23}$ −4.6 (*c* 0.67, $CHCl_3$) {Found $[M+Na]^+$: 1420.37; $C_{92}H_{184}O_5NaSi$ requires: 1420.3805}; $\delta_H$ (500 MHz, $CDCl_3$): 3.92–3.89 (1H, m), 3.66 (3H, s), 3.64 (2H, t, *J* 6.95 Hz), 2.97–2.94 (1H, m), 2.55 (1H, ddd, *J* 3.8, 7.25, 11.00 Hz), 1.59–1.54 (8H, m), 1.37–1.14 (140H, m), 0.90–0.84 (18H, m, including s at $\delta$ 0.86), 0.64 (2H, br.m), 0.56 (1H, dt, *J* 4.1, 8.15 Hz), 0.04 (3H, s), 0.02 (3H, s), −0.32 (1H, q, *J*, 5.00 Hz); $\delta_c$: 175.1, 85.4, 73.2, 63.1, 57.7, 51.6, 51.2, 35.3, 33.7, 32.8, 32.4, 31.9, 30.5, 30.2, 30.0, 29.9, 29.8, 29.6, 29.4, 29.35, 28.7, 27.8, 27.6, 27.5, 26.2, 25.8, 23.7, 22.7, 17.9, 15.8, 14.9,

14.1, 10.9, −4.4, −4.9; $\nu_{max}$: 3371, 2921, 2852, 1741, 1466, 1097, 836 $cm^{-1}$.

### 2.15. Methyl (R)-2-{(R)-1-(tert-butyldimethylsiloxy)-18-[(1S,2R)-2-(17R,18R)-17-methoxy-18-methylhexatriacontyl)cyclopropropyl]octadecyl}-26-hydroxy hexacosanoate (**26**)

(i) Lithium bis(trimethylsilyl)amide (2.17 mL, 2.30 mmol, 1.06 M) was added dropwise to a stirred solution of ester (**19**) (1.00 g, 1.33 mmol) and tetrazole (25) (1.37 g, 1.53 mmol) (Al Dulayymi et al., 2007) in dry THF (50 mL) at −10 °C. The reaction turned bright yellow and was left to reach r.t. and stirred for 1 h under nitrogen then quenched with sat.aq. $NH_4Cl$ (25 mL). The product was extracted with petrol/ethyl acetate (10:1, 3 × 150 mL), dried, filtered and evaporated. Column chromatography eluting with petrol/ethyl acetate (20:1) gave a colourless oil, methyl (*E*/*Z*)-(*R*)-2-{(*R*)-1-(*tert*-butyldimethylsiloxy)-18-[(1*S*,2*R*)-2-(17*R*,18*R*)-17-methoxy-18-methylhexatria-contyl)cyclopropyl]octadec-11-enyl}-26-(pivaloyloxy)hexacosanoate (1.60 g, 89%) in ratio (2:1). Dipotassium azodicarboxylate (2.00 g, 10.3 mmol) was added to a stirred solution of the alkene mixture (1.6 g, 1.1 mmol) in THF (30 mL) and methanol (7 mL) at 5 °C. A solution of glacial acetic acid (3 mL) and THF (3 mL) was prepared and added dropwise at 5 °C over two days. Further dipotassium azodicarboxylate (1.5 g) and glacial acetic acid (2 mL) were added and stirred overnight. This mixture was added slowly to sat.aq. $NaHCO_3$, extracted with petrol/ethyl acetate (1:1, 3 × 80 mL,) and the combined organic layers were washed with water (50 mL) and the solvent was evaporated. The product was purified by column chromatography eluting with petrol/ethyl acetate (15:1) to give methyl (*R*)-2-{(*R*)-1-(*tert*-butyldimethylsiloxy)-18-[(1*S*,2*R*)-2-(17*R*,18*R*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl]octadecyl}-26-(pivaloyloxy)hexacosanoate as a thick colourless oil (1.5 g, 93%), $[\alpha]_D^{22}$ +2.8 (*c* 0.87, $CHCl_3$) {Found $[M+Na]^+$: 1504.4309; $C_{97}H_{192}O_6SiNa$ requires: 1504.4580}, $\delta_H$ (500 MHz, $CDCl_3$): 4.05 (2H, t, *J* 6.65 Hz), 3.95–3.89 (1H, m), 3.66 (3H, s), 3.34 (3H, s), 2.96–2.94 (1H, m), 2.53 (1H, ddd, *J* 3.8, 7.25, 11.05 Hz), 1.64–1.59 (4H, m), 1.37–1.22 (143H, m), 1.21 (9H, s), 0.90–0.84 (15H, m, including s at $\delta$ 0.87), 0.66–0.63 (2H, m), 0.56 (1H, br.dt, *J*, 4.1, 8.2 Hz), 0.04 (3H, s), 0.02 (3H, s), −0.32 (1H, q, *J* 4.75 Hz); $\delta_c$: 178.6, 175.1, 85.4, 73.2, 64.5, 57.7, 51.6, 51.2, 38.7, 35.4, 33.7, 32.4, 31.9, 30.5, 30.2, 30.0, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.36, 29.2, 28.7, 28.6, 27.8, 27.6, 27.5, 27.2, 26.2, 25.9, 25.8, 23.7, 22.7, 18.0, 15.8, 14.9,14.1, 10.9, −4.4, −4.9; $\nu_{max}$: 2924, 2853, 1733, 1465, 1157, 1099, 836, 775 $cm^{-1}$.

(ii) The above product (1.50 g, 1.02 mmol) in THF (3 mL) was added to a stirred solution of potassium hydroxide (0.88 g, 15.51 mmol) in a mixture of THF (15 mL), methanol (15 mL) and water (1.5 mL). The mixture was heated to 70 °C. After 3 h, it was quenched with water (10 mL) and extracted with petrol/ethyl acetate (10:1) (3 × 25 mL). The combined organic extracts were dried, filtered and evaporated. Column chromatography eluting with petrol/ethyl acetate (10:1) gave methyl (*R*)-2-{(*R*)-1-(*tert*-butyldimetheylsiloxy)-18-[(1*S*,2*R*)-2-(17*R*,18*R*)-17-methoxy-18-methylhexa-triacontyl)cyclopropyl]octadecyl}-26-hydroxyhexacosanoate (**26**) (0.85 g, 60%), as a thick colourless oil, $[\alpha]_D^{22}$ +4.1 (*c* 0.94, $CHCl_3$) {Found $[M+Na]^+$: 1420.3663; $C_{92}H_{184}O_5SiNa$ requires: 1420.3805}, $\delta_H$ (500 MHz, $CDCl_3$): 3.92–3.89 (1H, m), 3.66 (3H, s), 3.64 (2H, t, *J* 6.9 Hz), 3.34 (3H, s), 2.97–2.94 (1H, m), 2.53 (1H, ddd, *J* 3.45, 6.95, 10.7 Hz), 1.57 (2H, pent, *J* 6.6 Hz), 1.39–1.15 (146H, m), 0.9–0.84 (15H, m including s at $\delta$ 0.86), 0.67–0.64 (2H, m), 0.57 (1H, dt, *J* 4.1, 8.2 Hz), 0.05 (3H, s), 0.02 (3H, s) −0.32 (1H, q, *J* 5.05 Hz); $\delta_c$: 175.1, 85.5, 73.2, 63.1, 57.7, 51.6, 51.2, 35.4, 33.7, 32.8, 32.4, 31.9, 30.5, 30.2, 30.0, 29.9, 29.8, 29.6, 29.4, 29.35, 28.7, 27.8, 27.6, 27.5, 26.2, 25.8, 23.7, 22.7, 18.0, 15.8, 14.8, 14.1, 10.9, −4.4, −4.9; $\nu_{max}$: 3450, 2923, 2853, 1741, 1464, 1361, 1254, 1099, 836, 720 $cm^{-1}$.

### 2.16. Methyl (R)-26-(acetylthio)-2-{(R)-1-(tert-butyldimethyl-siloxy)-18-[(1R,2S)-2-(17S,18S)-17-methoxy-18-methyl-hexatriacontyl)cyclopropyl] octadecyl} hexacosanoate (**22**)

(i) Methyl (*R*)-2-{(*R*)-1-(*tert*-butyldimethylsiloxy)-18-[(1*R*,2*S*)-2-(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl] octadecyl}-26-hydroxyhexacosanoate (**21**) (0.61 g, 0.43 mmol) and triethylamine (3 mL) in dry dichloromethane (25 mL) was cooled to −20 °C under nitrogen and stirred for 30 mins, followed by the addition of toluene sulfonylchloride (0.11 g, 0.56 mmol) in one portion. The mixture was kept in a refrigerator for 16 h, then the solvent was evaporated. Column chromatography with petrol/ethyl acetate (10:1) gave methyl (*R*)-2-{(*R*)-1-(*tert*-butyldimethylsiloxy)-18-[(1*R*,2*S*)-2-(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl]octa-decyl}-26-(tosyloxy)hexacosanoate (0.50 g, 75%), as a colourless oil, $[\alpha]_D^{20}$ −7.2 (*c* 0.83, $CHCl_3$) {Found $[M+Na]^+$: 1574.3777; $C_{99}H_{190}O_7NaSiS$ requires: 1574.3894}; $\delta_H$ (500 MHz, $CDCl_3$): 7.79 (2H, d, *J* 8.2 Hz), 7.34 (2H, d, *J* 7.9 Hz), 4.02 (2H, t, *J* 6.3 Hz), 3.92–3.89 (1H, m), 3.65 (3H, s), 3.34 (3H, s), 2.97–2.94 (1H, m), 2.53 (1H, ddd, *J* 3.8, 7.25, 11.05 Hz), 2.45 (3H, s), 1.64–1.60 (2H, m), 1.42–1.11 (142H, m), 0.89–0.82 (18H, m, including s at $\delta$ 0.86), 0.65–0.64 (2H, br.m), 0.56 (1H, dt, *J* 3.4, 8.12 Hz), 0.04 (3H, s), 0.02 (3H, s), −0.32 (1H, q, *J* 5.35 Hz); $\delta_c$: 175.1, 144.5, 133.3, 129.8, 127.9, 85.4, 73.2, 70.7, 57.7, 51.6, 51.2, 35.3, 33.7, 32.4, 31.9, 30.5, 30.2, 30.0, 29.9, 29.8, 29.7, 29.69, 29.6, 29.58, 29.5, 29.4, 29.38, 29.35, 28.9, 28.8, 28.7, 27.8, 27.7, 27.5, 26.1, 25.7, 25.3, 23.7, 22.7, 21.6, 19.4, 17.96, 14.9, 14.2, 10.9, −4.4, −4.9; $\nu_{max}$: 2922, 2853, 1740, 1465, 1369, 1178, 1098, 836 $cm^{-1}$.

(ii) Potassium thioacetate (0.147 g, 1.29 mmol) was added to a stirred solution of the tosylate (0.50 g, 0.32 mmol) in dry THF (5 mL) and acetone (15 mL) at room temperature under nitrogen and stirred for 16 h, then the solvent was evaporated. Column chromatography eluting with petrol/ethyl acetate (20:1) gave methyl (*R*)-26-(acetylthio)-2-{(*R*)-1-(*tert*-butyldimethylsiloxy)-18-[(1*R*,2*S*)-2-(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl]octadecyl}-hexacosanoate **22** (0.35 g, 76%) as a pale yellow thick oil, $[\alpha]_D^{20}$ −5.83 (*c* 1.37, $CHCl_3$) {Found $[M+K]^+$:1478.3724; $C_{94}H_{186}O_4KSSi$ requires: 1478.3473}; $\delta_H$ (500 MHz, $CDCl_3$): 3.92–3.89 (1H, m), 3.66 (3H, s), 3.34 (3H, s), 2.97–2.94 (1H, m), 2.86 (2H, t, *J* 7.25 Hz), 2.53 (1H, ddd, *J* 3.8, 7.25, 10.75 Hz), 2.32 (3H, s), 1.64–1.53 (8H, m), 1.37–1.08 (H, m), 0.90–0.84 (18H, m, including s at $\delta$ 0.86), 0.65–0.64 (2H, br.m), 0.58–0.54 (1H, m), 0.04 (3H,s), 0.02 (3H, s), −0.32 (1H, q, *J*, 5.00 Hz); $\delta_c$: 196.1, 175.1, 85.4, 73.2, 57.7, 51.6, 51.2, 35.3, 33.7, 32.4, 31.9, 30.6, 30.5, 30.2, 30.0, 29.9, 29.8, 29.7, 29.7, 29.6, 29.5, 29.47, 29.4, 29.1, 28.8, 27.8, 27.6, 26.2, 25.8, 23.7, 22.7, 18.0, 15.8, 14.9, 14.1, 10.9, −4.4, −4.9; $\nu_{max}$: 2924, 2853, 1740, 1697, 1465, 1360, 1254, 1099, 836 $cm^{-1}$.

### 2.17. Methyl (R)-26-(acetylthio)-2-{(R)-1-(tert-butyldimethyl-siloxy)-18-[(1S,2R)-2-(17R,18R)-17-methoxy-18-methyl-hexatriacontyl)cyclopropyl]octadecyl} hexacosanoate (**27**)

(i) Toluene sulfonylchloride (0.143 g, 0.754 mmol) was added to a stirred solution of methyl (*R*)-2-{(*R*)-1-(*tert*-butyldimethylsiloxy)-18-[(1*S*,2*R*)-2-(17*R*,18*R*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl]octadecyl}-26-hydroxyhexacosanoate (0.81 g, 0.58 mmol) and triethylamine (3 mL) in dry dichloromethane (25 mL) at −20 °C under nitrogen. The solution was kept in a refrigerator overnight, then evaporated. Column chromatography eluting with petrol/ethyl acetate (10:1) gave methyl

(*R*)-2-{(*R*)-1-(*tert*-butyldimethylsiloxy)-18-[(1*S*,2*R*)-2-(17*R*,18*R*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl]octadecyl}-26-(tosyloxy)-hexacosanoate (0.66 g, 74%), as a colourless oil, $[\alpha]_D^{22}$ +15.17 (*c* 0.87, $CHCl_3$) {Found $[M+Na]^+$: 1574.3817; $C_{99}H_{190}O_7SSiNa$ requires: 1574.3894}, $\delta_H$ (500 MHz, $CDCl_3$): 7.80 (2H, d, *J*, 8.2 Hz), 7.35 (2H, d, *J* 8.00 Hz), 4.02 (2H, t, *J* 6.30 Hz), 3.92–3.89 (1H, m), 3.66 (3H, s), 3.34 (3H, s), 2.96–2.94 (1H, m), 2.53 (1H, ddd, *J* 3.8, 7.25, 10.9 Hz), 2.45 (3H, s), 1.63 (4H, pent, *J* 6.3 Hz), 1.57–1.53 (2H, m), 1.41–1.19 (141H, m), 0.90–0.83 (15H, m, including s at $\delta$ 0.88), 0.68–0.66 (2H, m), 0.56 (1H, dt, *J* 4.1, 8.2 Hz), 0.05 (3H, s), 0.02 (3H, s), −0.32 (1H, q, *J* 5.05 Hz); $\delta_c$: 175.1, 144.6, 133.4, 129.8, 127.9, 85.4, 73.2, 70.7, 57.7, 51.6, 51.2, 41.4, 35.4, 33.7, 32.4, 31.9, 30.5, 30.2, 29.98, 29.9, 29.8, 29.71, 29.7, 29.6, 29.5, 29.45, 29.4, 29.36, 28.9, 28.8, 28.7, 27.8, 27.6, 27.5, 26.2, 25.8, 25.3, 23.7, 22.7, 21.6, 19.4, 18.0, 14.9, 14.1, 10.9, −4.4, −4.9; $\nu_{max}$: 2923, 2852, 1740, 1464, 1253, 1099, 836, 720 $cm^{-1}$.

(ii) Potassium thioacetate (0.2 g, 1.8 mmol) was added to a stirred *solution* of the above tosylate (0.66 g, 0.42 mmol) dissolved in dry THF (5 mL) and acetone (15 mL) at room temperature. After 16 h, the solvent was evaporated. Column chromatography eluting with petrol/ethyl acetate (20:1) gave methyl (*R*)-26-(acetylthio)-2-{(*R*)-1-(*tert*-butyldimethylsiloxy)-18-[(1*S*,2*R*)-2-(17*R*,18*R*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl]octadecyl}hexacosanoate **27** (0.51 g, 82%) as a pale yellow thick oil, $[\alpha]_D^{22}$ +5.3 (*c* 0.87, $CHCl_3$) {Found $[M+Na]^+$: 1478.3565; $C_{94}H_{186}O_5SSiNa$ requires 1478.3682}; $\delta_H$ (500 MHz, $CDCl_3$): 3.91 (1H, br.q, *J* 5.0 Hz), 3.66 (3H, s), 3.34 (3H, s), 2.96–2.94 (1H, m), 2.86 (2H, t, *J* 7.5 Hz), 2.53 (1H, ddd, *J* 3.8, 7.25, 10.70 Hz), 2.32 (3H, s), 1.61–1.51 (6H, m), 1.42–1.14 (141H, m), 0.89–0.83 (15H, m, including s $\delta$ 0.87), 0.67–0.64 (2H, m), 0.56 (1H, dt, *J* 3.75, 8.2 Hz), 0.04 (3H, s), 0.02 (3H, s), −0.32 (1H, br.q, *J*, 5.05 Hz); $\delta_c$: 196.0, 175.1, 85.5, 73.2, 57.71, 51.6, 51.2, 35.4, 33.7, 32.4, 31.9, 30.61, 30.6, 30.5, 30.2, 30.0, 29.95, 29.8, 29.7, 29.71, 29.6, 29.5, 29.49, 29.46, 29.4, 29.2, 29.1, 29.06, 28.8, 27.9, 27.6, 26.2, 25.8, 25.4, 23.8, 22.7, 19.5, 17.8, 14.9, 14.1, 10.9, −4.4, −4.9; $\nu_{max}$: 2923, 2853, 1740, 1465, 1099, 836, 720 $cm^{-1}$.

### *2.18. Methyl (R)-26-(acetylthio)-2-{(R)-1-hydroxy-18-[(1R,2S)-2-(17S,18S)-17-methoxy-18-methylhexatriacontyl) cyclopropyl]octadecyl}hexacosanote (**23**)*

Methyl (*R*)-26-(acetylthio)-2-{(*R*)-1-(*tert*-butyldimethylsiloxy) -18-[(1*R*,2*S*)-2-(17*S*, 18*S*)-17-methoxy-18-methylhexatriacontyl) cyclopropyl]octadecyl}hexacosanoate (**22**) (0.35 g, 0.24 mmol) was dissolved in dry THF (12 mL) in a dry polyethylene vial under $N_2$ at 0 °C. Pyridine (0.1 mL) and hydrogen fluoride-pyridine complex (0.8 mL) were added and the mixture stirred at 45 °C overnight. The mixture was added slowly to sat.aq. $NaHCO_3$ (15 mL), extracted with petrol/ethyl acetate (5:1, 3 × 50 mL) and the combined organic extracts driedand evaporated. Column chromatography (petrol/ethyl acetate, 10:1) gave (*R*)-methyl-26-(acetylthio)-2-{(*R*)-1-hydroxy-18-[(1*R*,2*S*)-2-(17*S*,18*S*)-17-methoxy-18-methyl-hexatriacontyl)cyclopropyl]octadecyl}-hexacosanoate (**23**) (0.20 g, 63%) as a white solid, mp 43–45 °C, $[\alpha]_D^{20}$ −6.0 (*c* 0.88, $CHCl_3$) {Found $[M+Na]^+$: 1364.2836; $C_{88}H_{172}O_5NaS$ requires: 1364.2818}; $\delta_H$ (500 MHz, $CDCl_3$): 3.67 (3H, s), 3.67–3.65 (1H, m), 3.34 (3H, s), 2.97–2.94 (1H, m), 2.87 (2H, t, *J* 7.55 Hz), 2.45–2.40 (1H, m), 2.32 (3H, s), 1.58–1.55 (2H, m), 1.38–1.13 (143H, m), 0.90–0.84 (9H, including t, *J* 6.65 Hz and d, *J* 6.95 Hz), 0.66–0.65 (2H, br.m), 0.56 (1H, dt, *J* 3.82, 7.76 Hz), −0.32 (1H, q, *J* 5.05 Hz); $\delta_c$: 196.2, 176.2, 85.4, 72.3, 57.7, 51.5, 50.9, 35.7, 35.3, 32.4, 31.9, 30.6, 30.5, 30.2, 30.0, 29.9, 29.7, 29.6, 29.57, 29.5, 29.4, 29.35, 29.2, 29.1, 28.8, 28.7, 27.6, 27.4, 26.2, 25.7, 22.7, 15.8, 14.9, 14.1, 10.9; $\nu_{max}$: 3285, 2917, 2850, 1691, 1470, 1167, 1104, 720 $cm^{-1}$.

### *2.19. (R)-27-[((25R,26R)-25-carboxy-26-hydroxy-43-{(1R,2S)-2-[(17S,18S)-17-methoxy-18-methylhexatriacontyl] cyclopropyl}tritetracontyl)disulfanyl]-2-((R)-1-hydroxy-18-{(1R,2S)-2-[(17S,18S)-17-methoxy-18-methylhexatriacontyl] cyclopropyl}octadecyl)heptacosanoic acid (**24**)*

Methyl (*R*)-26-(acetylthio)-2-{(*R*)-1-hydroxy-18-[(1*R*,2*S*)-2-(17*S*,18S)-17-methoxy-18-methylhexatriacontyl)cyclopropyl]octadecyl}hexacosanoate (100 mg, 0.074 mmol) was suspended in a 5% aq. TBAH (20 mL) and heated to 100 °C overnight, then cooled to room temperature and acidified to pH 1 with 1 M HCl and extracted with petrol/ethylacetate (1:1, 3 × 30 mL). The combined organic layers were evaporated. Column chromatography (chloroform/methanol, 10:1) gave a white solid (*R*)-27-[((25*R*,26*R*)-25-carboxy-26-hydroxy-43-{(1*R*,2*S*)-2-[(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl]cyclopropyl}tritetracontyl)disulfanyl]-2-((R)-1-hydroxy-18-{(1*R*,2*S*)-2-[(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl]cyclopropyl}octadecyl) -heptacosanoic acid (**24**) (25 mg, 26%), m.p 60–62 °C, $[\alpha]_D^{23}$ −2.48 (*c* 1.37, $CHCl_3$), $\delta_H$ (500 MHz, $CDCl_3$): 3.73–3.69 (2H, m), 3.35 (6H, s), 2.98–2.95 (2H, m), 2.69 (4H, t, *J* 7.25 Hz), 2.48–2.46 (2H, m), 1.69–1.47 (80H, m), 1.37–1.15 (218H, m), 0.90–0.84 (12H, including t, *J* 6.65 Hz and d, *J* 6.95 Hz), 0.64–0.65 (4H, br.m), 0.58–0.56 (2H, m), −0.32 (2H, q, *J* 4.7 Hz); $\delta_c$: 179.8, 85.6, 72.1, 57.7, 50.9, 39.3, 35.5, 35.3, 32.4, 31.9, 30.5, 30.2, 30.0, 29.9, 29.72, 29.7, 29.6, 29.53, 29.5, 29.4, 29.4, 29.2, 28.7, 28.5, 27.6, 27.3, 26.2, 25.7, 22.7, 15.8, 14.9, 14.1, 10.9; $\nu_{max}$: 3278, 291, 2851, 1708, 1465, 1376, 1098, 719 $cm^{-1}$.

### *2.20. (R)-27-[((25R,26R)-25-carboxy-26-hydroxy-43-{(1S,2R)-2-[(17R,18R)-17-methoxy-18-methylhexatriacontyl]cyclopropyl} tritetracontyl)disulfanyl]-2-((R)-1-hydroxy-18-{(1S,2R)-2-[(17R,18R)-17-methoxy-18-methylhexatriacontyl] cyclopropyl}octadecyl)heptacosanoic acid (**28**)*

(i) The ester (**27**) (0.45 g, 0.30 mmol) was dissolved in dry THF (12 mL) in a polyethylene vial under nitrogen at 0 °C. Pyridine (0.1 mL) and hydrogen fluoride-pyridine complex (0.8 mL) were added and the mixture was stirred at 45 °C overnight. The mixture was added slowly to sat.aq. $NaHCO_3$ (15 mL). The product was extracted with petrol/ethyl acetate (5:1, 3 × 50 mL) and the combined organic extracts were dried and evaporated. The product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give (*R*)-methyl-26-(acetylthio)-2-((*R*)-1-hydroxy-18-{(1*S*,2*R*)-2-[(17*R*,18*R*)-17-methoxy-18-methylhexatriacontyl]cyclopropyl}octa-decyl)hexacosanoate (0.40 g, 97%) as a white solid, mp 40–42 °C, $[\alpha]_D^{23}$ +6.3 (*c* 0.82, $CHCl_3$) {Found $[M+Na]^+$: 1364.2809; $C_{88}H_{172}O_5SNa$ requires: 1364.2818}; $\delta_H$ (500 MHz, $CDCl_3$): 3.71 (3H, s), 3.68–3.64 (1H, m), 3.34 (3H, s), 2.96–2.94 (1H, m), 2.86 (2H, t, *J* 7.6 Hz), 2.46–2.42 (1H, m), 2.32 (3H, s), 1.73–1.69 (2H, m), 1.63–1.53 (4H, m), 1.46–1,14 (142H, m), 0.88 (3H, t, *J* 5.65 Hz), 0.85 (3H, d, *J* 6.9 Hz), 0.66–0.65 (2H, br.m), 0.56 (1H, dt, *J* 4.1, 8.55 Hz), −0.32 (1H, q, *J* 5.00 Hz); $\delta_c$: 196, 176.2, 85.4, 72.3, 57.7, 51.5, 51.0, 35.7, 35.6, 32.4, 31.9, 30.6, 30.5, 30.2, 30.0, 29.97, 29.9, 29.7, 29.6, 29.6, 29.57, 29.5, 29.49, 29.47, 29.4, 29.35, 29.2, 29.1, 28.8, 28.7, 27.6, 27.4, 26.2, 25.7, 22.7, 15.8, 14.9, 14.1, 10.9; $\nu_{max}$: 3518, 2920, 2850, 1709, 1694, 1466, 1165, 1098, 720 $cm^{-1}$.

(ii) The above ester (300 mg, 0.22 mmol) was suspended in 5% aq. tetrabutylammonium hydroxide (20 mL) and heated to 100 °C overnight, then cooled to room temperature and acidified to pH 1 with 1 M HCl and extracted with petrol/ethyl acetate (1:1, 3 × 30 mL). The combined organic layers were dried and evaporated. Column chromatography eluting with chloroform/methanol (10:1) gave (*R*)-27-[((25*R*,26*R*)-25-carboxy-26-hydroxy-43-{(1*S*,2*R*)-2-[(17*R*,18*R*)-17-methoxy-18-methylhexatriacontyl]cyclopropyl}tritetracontyl)disulfanyl]

-2-((*R*)-1-hydroxy-18-{(1*S*,2*R*)-2-[(17*R*,18*R*)-17-methoxy-18-methylhexatriacontyl] cyclopropyl}octadecyl) heptacosanoic acid **28** (150 mg, 54%) as a white solid, $[\alpha]_D^{23} + 3.8$ (*c* 0.85, $CHCl_3$), mp 61–63 °C; $\delta_H$ (500 MHz, $CDCl_3$): 3.61–3.5 (2H, m), 3.29 (6H, s), 2.94–2.91 (2H, m), 2.63 (4H, t, *J* 7.25 Hz), 2.35–2.31 (2H, m), 1.62 (4H, pent, *J* 6.9 Hz), 1.42–1.05 (294H, m), 0.81 (6H, t, *J* 6.65 Hz), 0.79 (6H, d, *J* 6.60 Hz), 0.63–058 (4H, br.m), 0.51 (2H, dt, *J* 4.1, 8.5 Hz), −0.37 (2H, q, *J* 5.05); $\delta_c$: 177.8, 85.6, 72.0, 57.5, 50.9, 39.1, 35.6, 32.3, 31.8, 30.4, 30.1, 29.8, 29.76, 29.6, 29.4, 29.38, 29.3, 29.2, 29.1, 28.6, 28.4, 27.38, 27.3, 26.0, 25.6, 22.5, 15.6, 14.7, 13.9, 10.8; $\nu_{max}$: 3280, 2917, 2849, 1714, 1470, 1377, 1100, 719 $cm^{-1}$.

### 2.21. Methyl (R)-2-((R)-1-acetoxy-18-{(1S,2R)-2-[(17R,18R)-17-methoxy-18-methylhexatriacontyl]cyclopropyl}octadecyl)-26-mercaptohexacosanoate (29)

(i) Excess diazomethane in ether was added to the acid (**28**) (7.0 mg) and stirred for 30 min. The solvent was evaporated to give dimethyl (*R*)-methyl-2-((*R*)-1-hydroxy-18-{(1*S*,2*R*)-2-[(17*R*,18*R*)-17-methoxy-18-methylhexatriacontyl]cyclopropyl} octadecyl)-27-[((25*R*,26*R*)-26-hydroxy-43-{(1*S*,2*R*)-2-[(17*R*,18*R*)-17-methoxy-18-methylhexatriacontyl]cyclopropyl}-25-(methoxycarbonyl)tritetracontyl)disulfanyl] heptacosanoate, which showed $\delta_H$ (400 MHz, $CDCl_3$): 3.70 (6H, s), 3.67–3.64 (2H, m), 3.34 (6H, s), 2.97–2.95 (2H, m), 2.68 (4H, t, *J* 7.28 Hz), 2.46–2.41 (2H, m), 1.72–1.13 (296H, m), 0.88 (6H, t, *J* 6.56 Hz), 0.85 (6H, d, *J* 6.88 Hz), 0.68–0.65 (4H, m), 0.57 (2H, dt, *J* 3.76, 7.92 Hz), −0.32 (2H, br.q, *J* 4.76 Hz); $\delta_c$: 176.3, 85.4, 72.3, 57.7, 51.5, 50.9, 39.2, 35.7, 35.3, 32.4, 31.9, 30.5, 30.2, 30.0, 29.9, 29.7, 29.6, 29.57, 29.5, 29.4, 29.36, 29.3, 29.2, 28.7, 28.5, 27.6, 27.4, 26.2, 25.7, 22.7, 18.4, 15.8, 15.7, 14.9, 14.1, 10.9. The product was used for next step without purification.

(ii) Acetic anhydride (0.3 mL) and pyridine (0.3 mL) were added to a stirred solution of the alcohol in toluene (0.3 mL). The mixture was stirred for 18 h then the solvent was evaporated under reduced pressure to give (*R*)-methyl 2-((*R*)-1-acetoxy-18-{(1*S*,2*R*)-2-[(17*R*,18*R*)-17-methoxy-18-methylhexatriacontyl]cyclopropyl}-octadecyl)-27-[((25*R*,26*R*)-26-acetoxy-43-{(1*S*,2*R*)-2-[(17*R*,18*R*)-17-methoxy-18-methylhexa triacontyl]cyclopropyl}-25-(methoxycarbonyl)tritetracontyl)disulfanyl]-heptacosanoate, which showed $\delta_H$ (400 MHz, $CDCl_3$): 5.10–5.06 (2H, m), 3.68 (6H, s), 3.34 (6H, s), 2.97–2.96 (2H,m), 2.68 (4H, t, *J* 7.28 Hz), 2.62 (2H, ddd, *J* 4.3, 7.0, 10.64 Hz), 2.03 (6H, s), 1.71–1.13 (294H, m), 0.88 (6H, t, *J* 6.40 Hz), 0.85 (6H, d, *J* 6.88 Hz), 0.68–0.66 (4H, m), 0.57 (2H, dt, *J* 3.92, 7.92 Hz), −0.32 (2H, br.q, *J* 4.92 Hz); $\delta_c$: 173.7, 170.4, 85.5, 74.1, 57.7, 51.5, 49.6, 39.2, 35.3, 32.4, 31.9, 31.7, 30.6, 30.1, 30.0, 29.9, 29.7, 29.5, 29.4, 29.2, 29.1, 29.0, 28.7, 28.5, 28.1, 27.6, 27.5, 26.2, 25.0, 22.7, 21.0, 20.6, 15.8, 14.9, 14.1, 10.9. The product was used for next step without purification.

(iii) DL-Dithiothreitol (100 mg) was added with stirring to the above ester in chloroform (1 mL) followed by the addition of one drop of triethylamine under nitrogen. The flask was covered with aluminium foil. The mixture was stirred for 48 h at room temperature. The solvent was evaporated and the product was purified by column chromatography eluting with petrol/ethyl acetate (10:1) to give methyl (*R*)-2-[(*R*)-1-acetoxy-18-((1*S*,2*R*)-2-[(17*R*,18*R*)-17-methoxy-18-methylhexatriacontyl]-cyclopropyl}octadecyl)-26-mercaptohexacosanoate (**29**) (3.5 mg) {MALDI Found $[M+Na]^+$: 1364.1; $C_{88}H_{172}O_5SNa$ requires: 1364.3} which showed $\delta_H$ (400 MHz, $CDCl_3$): 5.13–5.06 (1H, m), 3.68 (3H, s), 3.34 (3H, s), 2.97–2.94 (1H, m), 2.62 (1H, ddd, *J* 4.36, 6.88, 10.88 Hz), 2.53(2H, q, *J* 7.52 Hz), 2.03 (3H, s), 1.17–1.13 (149H, m), 0.83 (3H, t, *J* 6.52 Hz), 0.81 (3H, d, *J* 7.4 Hz), 0.68–0.64 (2H, m), 0.57 (1H, dt, *J* 3.76, 8.04 Hz), −.032 (1H, br.q, *J* 5.16 Hz); $\delta_c$: 173.7, 170.4, 85.4, 74.1, 57.7, 51.6, 49.6, 35.3, 34.1, 32.3, 31.9, 31.7, 30.5, 30.2, 30.0, 29.9, 29.7, 29.6, 29.57, 29.5, 29.47, 29.44, 29.4, 29.36, 29.1, 28.7, 28.4, 28.1, 27.6, 27.5, 26.2, 25.0, 24.7, 22.7, 22.3, 21.0, 15.8, 14.9, 14.1, 10.9; $\nu_{max}$: 2921, 2851, 1746, 1609, 1493, 1452 $cm^{-1}$.

### 2.22. (R)-2-((R)-1-Hydroxy-18-{(1S,2R)-2-[(17R,18R)-17-methoxy-18-methylhexa-triacontyl]cyclopropyl}octadecyl)-26-mercaptohexacosanoic acid (30)

DL-Dithiothreitol (150 mg) was added to a stirred solution of (R)-27-[((25*R*,26*R*)-25-carboxy-26-hydroxy-43-{(1*S*,2*R*)-2-[(17*R*,18*R*)-17-methoxy-18-methylhexatria-contyl]cyclopropyl} tritetracontyl)disulfanyl]-2-((*R*)-1-hydroxy-18-{(1*S*,2*R*)-2-[(17*R*, 18*R*)-17-methoxy-18-methylhexatriacontyl]cyclopropyl}octadecyl)heptacosanoic acid (**28**) (10 mg) in chloroform (1.5 mL) followed by the addition of one drop of triethylamine under nitrogen. The flask was covered with aluminium foil and the mixture was stirred for 48 h at room temperature. The reaction was quenched with 5 drops of dil. HCl (5%) and water (5 mL). The product was extracted with $CHCl_3$ (3 × 10 mL) and the combined organic layers were washed with brine solution, dried and evaporated to give a residue which was purified by column chromatography eluting with chloroform/methanol (10:1) to give (*R*)-2-((*R*)-1-hydroxy-18-{(1*S*,2*R*)-2-[(17*R*,18*R*)-17-methoxy-18-methylhexatriacontyl]cyclopropyl}octadecyl)-26-mercaptohexacosanoic acid (**30**) (6.5 mg) {MALDI Found $[M+Na]^+$: 1308.7; $C_{85}H_{168}O_4SNa$ requires: 1308.3} which showed $\delta_H$ (400 MHz, $CDCl_3$): 3.74–3.69 (1H, m), 3.35 (3H, s), 2.99–2.96 (1H, m), 2.52 (2H, q, *J* 7.52 Hz), 2.49–2.39 (1H, m), 1.74–1.11 (150H, m), 0.88 (3H, t, *J* 6.52 Hz), 0.85 (3H, d, *J* 6.92 Hz), 0.68–0.65 (2H, m), 0.57 (1H, dt, *J* 3.88, 7.76 Hz), −0.32 (1H, br.q, *J* 5.24 Hz); $\delta_c$: 178.0, 85.6, 72.1, 57.7, 50.7, 35.6, 35.3, 34.1, 32.4, 31.9, 30.5, 30.2, 30.0, 29.9, 29.7, 29.6, 29.5, 29.4, 29.36, 29.1, 28.7,28.4, 27.6, 27.3, 26.2, 25.7, 24.7, 22.7, 15.8, 14.9, 14.1, 10.9. When the sample was shaken with $D_2O$, the quartet at $\delta$ 2.52 became a triplet with *J* 7.16 Hz.

### 2.23. Methyl (R)-2-((R)-1-acetoxy-18-{(1R,2S)-2-[(17S,18S)-17-methoxy-18-methylhexatriacontyl]cyclopropyl}octadecyl)-26-mercaptohexacosanoate (31)

Excess diazomethane in ether was added to acid (**24**) (3.5 mg) and stirred for 30 min. The solvent was evaporated to give (*R*)-methyl 2-((*R*)-1-hydroxy-18-{(1*R*,2*R*)-2-[(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl]]cyclopropyl}octadecyl)-27-[((25*R*,26*R*) -26-hydroxy-43-{(1*R*,2*S*)-2-[(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl]cyclopropyl}-25-(methoxycarbonyl)tritetracontyl) disulfanyl]heptacosanoate, which showed an identical spectrum to the product from (**28**) and diazomethane described above. The product was used for next step without purification. Acetic anhydride (0.3 mL) and pyridine (0.3 mL) were added to a stirred solution of the ester in toluene (0.3 mL). After 18 h, the solvent was evaporated under reduced pressure to give dimethyl (*R*)-methyl 2-((*R*)-1-acetoxy-18-{(1*R*,2*R*)-2-[(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl]cyclopropyl}octadecyl)-27-[((25*R*, 26*R*)-26-acetoxy-43-{(1*R*,2*S*)-2-[(17*S*,18*S*)-17-methoxy-18-methylhexa triacontyl]cyclopropyl}-25-(methoxycarbonyl)tritetracontyl)disulfanyl]hepta- cosanoate, which showed an identical spectrum to that above. DL-Dithiothreitol (100 mg) was added to a stirred solution of the ester in chloroform (1 mL) followed by the addition of one drop of triethylamine under nitrogen atmosphere. The flask was covered with aluminium foil. The mixture was stirred for 48 h at room temperature. The solvent was evaporated. Column chromatography eluting with petrol/ethyl acetate (10:1) to give methyl (*R*)-2-((*R*)-1-acetoxy-18-{(1*R*,2*S*)-2-[(17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl]cyclopropyl}octadecyl)-26-mercaptohexacosanoate (**31**) (2 mg) {MALDI Found $[M+Na]^+$:

1364.8; $C_{88}H_{172}O_5SNa$ requires: 1364.3}, which showed essentially identical nmr spectra to those of (**29**) presented above.

### 2.24. Methyl (R)-2-((R)-1-(tert-butyldimethylsilyloxy)-19-{(1S,2R)-2-[(2S,19S,20S)-19-methoxy-20-methyloctatriacontan-2-yl]cyclopropyl}nonadecyl)-26-hydroxy-hexacosanoate (**34**)

(i) Lithium *bis*-(trimethylsilyl)amide (0.96 mL, 1.0 mmol, 1.06 M) was added to a stirred solution of aldehyde (**32**) (Koza et al., 2013) (0.494 g, 0.65 mmol) and methyl and sulfone (**33**) (see Supplementary Information) (0.773 g, 0.781 mmol) in dry THF (15 mL) at 0–5 °C. The solution turned bright yellow/orange and was left to reach room temperature and stirred for 1 h under $N_2$ (g) then quenched with sat.aq. $NH_4Cl$ (10 mL) at −20 °C. The mixture was extracted with petrol/ethyl acetate (1:1, 3 × 15 mL) and the combined organic layers were dried and evaporated. Column chromatography (petrol/ethyl acetate, 20:1) gave methyl (*R*)-2-((*R*)-1-(*tert*-butyldimethylsilyloxy)-19-{(1*S*,2*R*)-2-[(2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatria-contan-2-yl]cyclopropyl}nonadec-10-enyl)-26-(pivaloyloxy)hexacosanoate (0.849 g 86%) as a colourless oil, $[\alpha]_D^{23}$ −8.54 (*c* 1.19, $CHCl_3$).

(ii) Dipotassium azodicarboxylate (2.50 g) was added in excess to a stirred solution of above olefins (0.840 g, 0.552 mmol) in dry THF (10 mL) and methanol (5 mL) at 0 °C under $N_2$ (g). Acetic acid (2 mL) in dry THF (4 mL) was added in small portions throughout the day at 0 °C. Further dipotassium azodicarboxylate followed by more of the solution of acetic acid in THF was added. Again, after stirring overnight, more dipotassium azodicarboxylate was added, followed by more acetic acid in THF. After stirring for a further 24 h the reaction was quenched by adding it in small portions to sat.aq. $NaHCO_3$ (15 mL). The mixture was extracted with petrol/ethyl acetate (5:2, 3 × 25 mL) and the combined organic layers were dried and evaporated. Column chromatography (petrol/ethyl acetate, 20:1) gave methyl (*R*)-2-((*R*)-1-(*tert*-butyl-dimethylsilyloxy)-19-{(1*S*,2*R*)-2-[(2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl]cyclopropyl}nonadecyl)-26-(pivaloyloxy)hexacosanoate (0.727 g, 86%) as a colourless oil, $[\alpha]_D^{21}$ −6.5 (*c* 0.87, $CHCl_3$). This showed $\delta_H$: 4.05 (2H, t, *J* 6.6 Hz), 3.91 (1H, dt, *J* 4.7, 7.0 Hz), 3.66 (3H, s), 3.35 (3H, s), 2.96 (1H, m), 2.53 (1H, ddd, *J* 3.7, 7.2, 10.9 Hz), 1.62 (6H, m), 1.26 (147H, m), 0.89 (23H, m, including a singlet at 0.87), 0.67 (1H, m), 0.42–0.47 (1H, m), 0.10–0.22 (3H, m), 0.05 (3H, s) and 0.02 (3H, s); $\delta_C$: 178.7, 175.2, 143.2, 85.5, 73.2, 64.5, 57.7, 51.6, 51.2, 38.7, 38.1, 37.4, 35.3, 34.5, 32.4, 31.9, 30.5, 30.1, 30.0, 29.9, 29.8, 29.67, 29.63, 29.61, 29.58, 29.54, 29.47, 29.37, 29.2, 29.1, 28.6, 27.6, 27.5, 27.3, 27.2, 26.2, 25.9, 25.7, 22.7, 22.6, 19.7, 18.6, 18.0, 14.9, 14.1, 11.4, 10.5, −4.4, −4.9; $\nu_{max}$: 2924, 2853, 1733, 1464 $cm^{-1}$.

(iii) The above pivalate (0.71 g, 0.47 mmol) was added to a stirred solution of potassium hydroxide (0.39 g, 7.0 mmol) in THF (10 mL), methanol (10 mL) and water (1 mL). The mixture was heated under reflux at 70 °C. After ∼3 h, the reaction was quenched with water (10 mL) and extracted with ethyl acetate (3 × 15 mL). The combined organic extracts were dried and evaporated. Column chromatography (petrol/ethyl acetate, 10:1) gave methyl (*R*)-2-((*R*)-1-(*tert*-butyldimethylsilyloxy)-19-{(1*S*,2*R*)-2-[(2*S*,19*S*,20*S*)-19-methoxy-20-methylocta-triacontan-2-yl]cyclopropyl}-nonadecyl)-26-hydroxyhexacosanoate (**34**) (0.583 g, 86%) as a white solid, m.p. 27–28 °C, $[\alpha]_D^{21}$ −4.5 (*c* 0.69, $CHCl_3$) {MALDI Found $(M+Na)^+$: 1462.45, $C_{95}H_{190}O_5SiNa$ requires: 1462.43}. This showed $\delta_H$: 3.91 (1H, dt, *J* 4.8, 6.6 Hz), 3.66 (3H, s), 3.65 (2H, t, *J* 6.6 Hz), 3.35 (3H, s), 2.96 (1H, m), 2.53 (1H, ddd, *J* 3.6, 7.2, 10.9 Hz), 1.26 (141H, m), 1.58 (9H, br.m), 0.91 (3H, d, *J* 6.6 Hz), 0.89 (3H, t, *J* 7.1 Hz), 0.87 (9H, s), 0.86 (3H, d, *J* 7.0 Hz), 0.66 (1H, m), 0.41–0.48 (1H, m), 0.10–0.21 (3H, m), 0.05 (3H, s) and 0.02 (3H, s); $\delta_C$: 176.3, 175.2, 143.2, 85.5, 73.3, 72.4, 63.1, 57.7, 51.6, 51.2, 38.1, 37.4, 35.3, 34.5, 33.7, 32.8, 32.4, 31.9, 30.5, 30.1, 30.0, 29.9, 29.8, 29.72, 29.64, 29.61, 29.52, 29.45, 29.42, 29.38, 29.1, 28.3, 27.8, 27.6, 27.5, 27.3, 26.2, 26.1, 25.8, 25.5, 23.7, 22.7, 19.7, 18.6, 18.0, 14.9, 14.1, 10.5, −4.4, −4.9; $\nu_{max}$: 3424, 2923, 2853, 1741, 1719, 1463 $cm^{-1}$.

### 2.25. Methyl (R)-26-(acetylthio)-2-((R)-1-(tert-butyldimethylsilyloxy)-19-{(1S,2R)-2-[(2S,19S,20S)-19-methoxy-20-methyloctatriacontan-2-yl]cyclopropyl}nona-decyl) hexacosanoate (**35**)

(i) Alcohol (**34**) (0.474 g, 0.327 mmol) and triethylamine (2 mL) in dry dichloro-methane (25 mL) was cooled to −20 °C under $N_2$ (g) and stirred for 30 min, followed by the addition of *p*-toluenesulfonyl chloride (0.081 g, 0.43 mmol) in one portion. The solution was kept in a refrigerator overnight, then the solvent was evaporated. Column chromatography (petrol/ethyl acetate, 10:1) gave methyl (*R*)-2-((*R*)-1-(*tert*-butyldimethylsilyl)oxy)-19-{(1*S*,2*R*)-2-[(2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl]cyclopropyl}-nonadecyl)-26-(tosyloxy)hexacosanoate (0.333 g, 65%) as a colourless oil, $[\alpha]_D^{23}$ −5.0 (*c* 0.63, $CHCl_3$) {Found $(M+Na)^+$: 1618.15, $C_{102}H_{196}O_7SSiNa$ requires: 1616.44}. This showed $\delta_H$: 7.80 (2H, d, *J* 8.2 Hz), 7.36 (2H, d, *J* 7.9 Hz), 4.03 (2H, t, *J* 6.5 Hz), 3.91 (1H, dt, *J* 4.8, 6.6 Hz), 3.66 (3H, s, $OCH_3$), 3.35 (3H, s, $OCH_3$), 2.96 (1H, m), 2.53 (1H, ddd, *J* 3.6, 7.2, 10.9 Hz), 2.46 (3H, s), 1.63 (6H, m), 1.26 (143H, m), 0.91 (3H, d, *J* 6.6 Hz), 0.89 (3H, t, *J* 7.2 Hz), 0.87 (9H, s), 0.86 (3H, d, *J* 6.7 Hz), 0.66 (1H, m), 0.42–0.47 (1H, m), 0.09–0.21 (3H, m), 0.05 (3H, s) and 0.02 (3H, s); $\delta_C$: 175.1, 144.5, 133.3, 129.8, 127.9, 85.5, 73.2, 70.7, 60.4, 57.7, 51.6, 51.2, 38.1, 37.4, 35.5, 34.5, 33.7, 32.4, 31.9, 30.5, 30.1, 30.01, 30.00, 29.9, 29.72, 29.68, 29.62, 29.60, 29.52, 29.47, 29.40, 29.3, 28.9, 28.8, 27.8, 27.6, 27.5, 27.3, 26.2, 26.1, 25.8, 25.3, 23.7, 22.7, 21.6, 19.7, 18.6, 18.0, 14.9, 14.1, 10.5, −4.4, −4.9; $\nu_{max}$: 2923, 2853, 1740, 1719, 1464 $cm^{-1}$.

(ii) A solution of tosylate (0.399 g, 0.251 mmol) and potassium thioacetate (0.115 g, 1.003 mmol) in acetone (15 mL) and THF (5 mL) was stirred at room temperature overnight, then the solvent was evaporated. Column chromatography (petrol/ethyl acetate, 20:1) gave methyl (*R*)-26-(acetylthio)-2-((*R*)-1-(*tert*-butyldimethylsilyloxy)-19-{(1*S*,2*R*)-2-[(2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl]cyclo-propyl}nonadecyl)hexacosanoate (**35**) (0.227 g, 61%) as a colourless oil, $[\alpha]_D^{24}$ −4.4 (*c* 0.73, $CHCl_3$) {Found $(M+Na)^+$: 1520.42, $C_{97}H_{192}O_5SSiNa$ requires: 1520.42}. This showed $\delta_H$: 3.90 (1H, dt, *J* 4.7, 7.1 Hz), 3.65 (3H, s), 3.33 (3H, s), 2.96 (1H, m), 2.85 (2H, t, *J* 7.4 Hz), 2.52 (1H, ddd, *J* 3.6, 7.3, 10.9 Hz), 2.31 (3H, s), 1.55 (6H, m), 1.26 (143H, m), 0.90 (3H, d, *J* 6.9 Hz), 0.88 (3H, t, *J* 6.6 Hz), 0.86 (9H, s), 0.85 (3H, d, *J* 7.0 Hz), 0.65 (1H, m), 0.41–0.48 (1H, m), 0.09–0.20 (3H, m), 0.04 (3H, s) and 0.02 (3H, s); $\delta_C$: 195.9, 175.1, 85.4, 73.4, 73.2, 71.0, 57.7, 51.6, 51.2, 38.1, 37.7, 37.4, 35.3, 34.5, 33.7, 32.8, 32.8, 32.6, 31.9, 30.6, 30.5, 30.4, 30.1, 30.00, 29.96, 29.84, 29.73, 29.67, 29.64, 29.62, 29.60, 29.57, 29.53, 29.50, 29.46, 29.39, 29.1, 28.8, 27.8, 27.6, 27.5, 27.3, 26.2, 26.1, 25.7, 23.7, 22.7,19.7, 18.6, 17.9, 14.9, 14.1, 10.5, −4.3, −4.9; $\nu_{max}$: 2921, 2851, 1731,1643, 1463 $cm^{-1}$.

### 2.26. Methyl (R)-26-(acetylthio)-2-((R)-1-hydroxy-19-{(1S,2R)-2-[(2S,19S,20S)-19-methoxy-20-methyloctatriacontan-2-yl]cyclopropyl}nonadecyl)hexacosanoate

Thioacetate (**35**) (50 mg, 0.033 mmol) was dissolved in dry THF (4 mL) in a dry polyethylene vial under $N_2$ (g) at 0 °C. Pyridine (98 mg, 7.8 mmol, 0.1 mL) and HF.pyridine (88 mg) were added and the mixture was stirred at 45 °C overnight then added slowly to sat.aq. $NaHCO_3$ (10 mL). The solution was extracted with petrol/ethyl acetate (1:1, 3 × 15 mL)

and the combined organic extracts were dried, filtered and evaporated. Column chromatography (petrol/ethyl acetate, 10:1) gave methyl (*R*)-26-(acetylthio)-2-((*R*)-1-hydroxy-19-{(1*S*,2*R*)-2-[(2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl]cyclopropyl}nonadecyl)hexacosanoate (41.1 mg, 90%) as a white solid, m.p. 41–43 °C, $[\alpha]_D^{21}$ −2.27 (*c* 2.14, $CHCl_3$) {Found $(M+Na)^+$: 1406.33, $C_{91}H_{178}O_5SNa$ requires: 1406.33}. This showed $\delta_H$: 3.72 (3H, s), 3.66 (1H, m), 3.35 (3H, s, $OCH_3$), 2.96 (1H, m), 2.87 (2H, t, *J* 7.4), 2.45 (1H, dt, *J* 5.4, 9.1), 2.33 (3H, s), 1.58 (8H, m), 1.26 (142H, m), 0.91 (3H, d, *J* 6.6 Hz), 0.89 (3H, t, *J* 6.9 Hz), 0.86 (3H, d, *J* 6.9 Hz), 0.66 (1H, m), 0.41–0.48 (1H, m) and 0.09–0.21 (3H, m); $\delta_C$: 196.1, 176.3, 85.5, 76.6, 72.3, 57.7, 51.5, 50.9, 38.1, 37.4, 35.7, 35.3, 34.5, 32.4, 31.9, 30.6, 30.5, 30.09, 30.05, 30.00, 29.96, 29.72, 29.66, 29.62, 29.59, 29.57, 29.51, 29.44, 29.38, 29.2, 29.1, 28.8, 27.6, 27.4, 27.3, 26.2, 26.1, 25.7, 22.7, 19.7, 18.6, 14.9, 14.1; $\nu_{max}$: 3418, 2922, 2851, 1709, 1687, 1465 $cm^{-1}$.

### 2.27. (R)-26-[((25R,26R)-25-carboxy-26-hydroxy-43-{(1S,2R)-2-[(2S,19S,20S)-19-methoxy-20-methyloctatriacontan-2-yl]cyclopropyl}tritetracontyl)disulfanyl]-2-((R)-1-hydroxy-19-{(1S,2R)-2-[(2S,19S,20S)-19-methoxy-20-methyloctatriacont-an-2-yl]cyclopropyl}nonadecyl)hexacosanoic acid (**36**)

Methyl (*R*)-26-(acetylthio)-2-((*R*)-1-hydroxy-19-{(1*S*,2*R*)-2-[(2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl]cyclopropyl}nonadecyl)hexacosanoate (14 mg, 0.010 mmol) was suspended in 5% aq. TBAH (2 mL) and heated to 100 °C overnight. The solution was cooled to room temperature, acidified to pH 1 with 1 M HCl and extracted with diethyl ether (3 × 15 mL). The combined organic layers were dried, filtered and the solvent evaporated. Column chromatography (chloroform/methanol, 10:1) gave (R)-26-[((25*R*,26*R*)-25-carboxy-26-hydroxy-43-{(1*S*,2*R*)-2-[(2*S*,19*S*,20*S*)-19-methoxy-20-methyloctatriacontan-2-yl]cyclopropyl}tritetracontyl) disulfanyl]-2-((R)-1-hydroxy-19-{(1*S*,2*R*)-2-[(2*S*,19*S*,20*S*)-19-methoxy-20-methyl octatriacontan-2-yl]cyclopropyl}nonadecyl)hexacosanoic acid (36) (7.7 mg, 58%) as a white solid, $[\alpha]_D^{22}$ −2.8 (*c* 0.77, $CHCl_3$). This showed $\delta_H$: 3.91 (1H, m), 3.35 (3H, s), 2.97 (1H, m), 2.69 (2H, t, *J* 7.4 Hz), 2.47 (1H, dt, *J* 5.4, 9.1 Hz), 1.67 (8H, m), 1.26 (144H, m), 0.90 (3H, d, *J* 6.6 Hz), 0.89 (3H, t, *J* 7.0 Hz), 0.86 (3H, d, *J* 6.9 Hz), 0.66 (1H, m), 0.44 (1H, m) and 0.08–0.20 (3H, m); $\delta_C$: 85.5, 57.9, 50.9, 45.3, 39.4, 38.1, 37.4, 35.3, 34.5, 32.4, 31.9, 30.9, 30.5, 30.1, 30.0, 29.9, 29.5, 29.4, 29.2, 28.5, 27.6, 27.3, 26.1, 22.7, 19.77, 18.6, 14.9, 14.1, 10.5, 8.6; $\nu_{max}$: 3423, 2924, 2852, 1718, 1465 $cm^{-1}$.

### 2.28. Methyl (R)-2-[(R)-1-(tert-butyldimethylsilyloxy)-12-((1R,2S)-2-{14-[(1R,2S)-2-eicosylcyclopropyl]-tetradecyl}-cyclopropyl)dodecyl]-26-(pivaloyloxy) hexacosanoate (**38**)

(i) Lithium bis(trimethylsilyl)amide (0.73 mL, 0.78 mmol) was added to a stirred solution of aldehyde (**19**) (0.350 g, 0.430 mmol) and sulfone (**37**) (0.405 g, 0.515 mmol) in dry THF (30 mL) at −10 °C under $N_2$. The reaction turned bright yellow and was left to reach room temperature and stirred for 1 h under $N_2$. The reaction was quenched by adding sat.aq. $NH_4Cl$. The product was extracted with petroleum/ethyl acetate (10:1, 3 × 100 mL) dried and evaporated. Column chromatography eluting with petrol/ethyl acetate (20:1) gave a semi-solid, methyl (*E*/*Z*)-(*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilyloxy)-12-((1*S*,2*S*)-2-{14-[(1*R*,2*S*)-2-eicosylcyclopropyl]tetra-decyl}-cyclopropyl)dodec-10-enyl]-26-(pivaloyloxy)hexacosanoate as a mixture of two isomers in ratio (3:1) (0.35 g, 60%).

(ii) Dipotassium azodicarboxylate (2.00 g, 10.3 mmol) was added to a stirred solution of the alkene above (0.34 g, 0.25 mmol) in THF (20 mL) and methanol (4 mL) at 5 °C. A solution of glacial acetic acid (2.5 mL) and THF (2.5 mL) was prepared and half was added at 5 °C dropwise and the mixture was stirred at r.t. for 2 h. The other half was added at r.t. and the mixture was stirred overnight. Dipotassium azodicarboxylate (2.00 g) and glacial acetic acid (2 mL) were added and stirred overnight. This mixture was slowly added to sat.aq. $NH_4Cl$ and extracted with petroleum/ethyl acetate (1:1, 3 × 100 mL) and the combined organic layers were washed with water (100 mL) and the solvent was evaporated. The procedure was repeated. Column chromatography eluting with petroleum/ethyl acetate (5:1) gave methyl (*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilyloxy)-12-((1*R*,2*S*)-2-{14-[(1*R*,2*S*)-2-eicosylcyclopropyl]tetradecyl}-cyclopropyl)dodecyl]-26-(pivaloyloxy)hexacosanoate (**38**) as a white solid (0.310 g, 91%), mp 40–42 °C, $[\alpha]_D^{21}$ +1.4 (*c* 0.90, $CHCl_3$) {Found $(M+Na)^+$: 1388.3154 $C_{90}H_{176}O_5SiNa$ requires: 1388.3179} which showed $\delta_H$ (500 MHz, $CDCl_3$): 4.05 (2H, t, *J* 6.6 Hz), 3.92–3.89 (1H, m), 3.66 (3H, s), 2.54–2.50 (1H, m), 1.37 (139H, m, including s at 1.20), 0.90–0.82 (16H, m, including s at 0.86), 0.68–0.61 (4H, m), 0.56 (2H, dt, *J* 4.1, 8.2 Hz), 0.05 (3H, s), 0.02 (3H, s), - 0.32 (2H, br.q, *J* 5.0 Hz); $\delta_C$: 178.6, 175.1, 64.4, 51.6, 51.2, 38.7, 33.7, 31.9, 30.2, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 28.7, 28.6, 27.8, 27.5, 27.2, 25.7, 23.7, 22.6, 22.3, 17.9, 15.7, 14.0, 10.9; $\nu_{max}$: 2920, 2852, 1732, 1638, 1464, 1363, 1284, 1253, 1161, 836, 775, 720 $cm^{-1}$.

### 2.29. Methyl (R)-2-[(R)-1-(tert-butyldimethylsilyloxy)-12-((1R,2S)-2-{14-[(1R,2S)-2-eicosylcyclopropyl]tetradecyl}cyclopropyl)dodecyl]-26-(hydroxy)-hexacosanoate (**39**)

(ii) The above ester (0.310 g, 0.224 mmol) in (3 mL) THF was added to a stirred solution of potassium hydroxide (0.19 g, 3.6 mmol) in a mixture of THF (10 mL), methanol (10 mL) and water (1 mL), then heated under reflux at 70 or 80 °C. After 3 h, the reaction was quenched with water (10 mL) and extracted with petroleum/ethyl acetate (10:1, 3 × 25 mL). The combined organic extracts were dried, filtered and evaporated. Column chromatography with petroleum/ethyl acetate (10:1) gave methyl (*R*)-2-[(*R*)-1-(*tert*-butyldimethylsilyloxy)-12-((1*R*,2*S*)-2-{14-[(1*R*,2*S*)-2-eicosylcyclo-propyl]tetradecyl}cyclopropyl)dodecyl]-26-hydroxyhexacosanoate (**39**) as a white solid (0.196 g, 67%), mp 42–43 °C, $[\alpha]^{22}{}_D$ + 1.2 (*c* 1.1, $CHCl_3$) {Found $(M+Na)^+$: 1304.2560. $C_{85}H_{168}O_4SiNa$ requires: 1304.2604} which showed $\delta_H$ (500 MHz, $CDCl_3$): 3.92–3.89 (1H, m), 3.66 (3H, s), 3.65 (2H, t, *J* 6.6 Hz), 2.55–2.51 (1H, m), 1.60–1.14 (125H, br.m), 0.91–0.82 (22H, m, including s at 0.89), 0.66–0.61 (4H, m), 0.57 (2H, dt, *J* 3.75, 7.85 Hz), 0.05 (3H, s), 0.02 (3H, s), −0.32 (2H, br.q, *J* 5.05 Hz); $\delta_C$: 175.1, 63.0, 51.5, 51.1, 34.1, 33.7, 32.8, 31.9, 30.8, 30.2, 29.8, 29.7, 29.6, 29.5, 29.4, 28.7, 27.8, 27.4, 25.7, 23.7, 22.6, 17.9, 15.7, 14.9, 10.9, −4.3, −4.9; $\nu_{max}$: 3414, 2918, 2850, 1739, 1638, 1469, 1384, 1167, 836, 720, 617 $cm^{-1}$.

### 2.30. Methyl (R)-26-(acetylthio)-2-{(R)-1-[(tert-butyldimethylsilyl)oxy]-12-[((1R,2S)-2-{14-[(1R,2S)-2-eicosylcyclopropyl]tetradecyl}cyclopropyl)dodecyl]} hexacosanoate (**40**)

(i) (*R*)-methyl 2-[(*R*)-1-[(tert-butyldimethylsilyl)oxy]-12-[(1*R*,2*S*)-2-(14-[(1*R*,2*S*)-2-eicosylcyclopropyl]tetradecyl)cyclopropyl]dodecyl]-26-hydroxyhexacosanoate (0.196 g, 0.53 mmol) and triethylamine (1.5 mL) in dry dichloromethane (10 mL) was cooled to −20 °C under $N_2$, and stirred for 30 min, followed by the addition of toluenesulfonyl chloride (0.034 g, 0.18 mmol) in one portion. The solution was kept in a refrigerator overnight then the solvent was evaporated. Column chromatography (petroleum/ethyl acetate, 10:1) gave methyl (*R*)-2-[(*R*)-1-[(*tert*-butyldimethylsilyl)-oxy]-12-((1*R*,2*S*)-2-{14-[(1*R*,2*S*)-2-eicosylcyclopropyl]tetradecyl}cyclopropyl)do-decyl]-26-(tosyloxy)hexacosanoate (0.155 g, 71%) as a thick oil which soldified later, $[\alpha]_D^{22}$ +1.1 (*c* 0.10, $CHCl_3$) {Found $(M+Na)^+$:

1458.2668; $C_{92}H_{174}O_6SSiNa$ requires: 1458.2693}, which showed $\delta_H$ (500,MHz,$CDCl_3$): 7.70 (2H, d, *J* 8.2 Hz), 7.34 (2H, d, *J* 7.85 Hz), 4.02 (2H, t, *J* 6.6 Hz), 3.92–3.87 (1H, m), 3.65 (3H, s), 2.55–2.51 (1H, m), 2.45 (3H, s), 1.63 (2H, pent, *J* 6.6 Hz), 1.38–1.20 (129H, m), 0.90–0.82 (15H, m, including s at 0.87), 0.66–0.61 (4H, m), 0.56 (2H, dt, *J* 4.1, 8.15 Hz), 0.05 (3H, s), 0.02 (3H,s), 0.32 (2H, br.q, *J* 5.05 Hz); $\delta_C$: 175.0, 144.5, 133.3, 129.7, 127.8, 73.2, 70.6, 51.5, 51.1, 33.6, 31.9, 30.2, 29.8, 29.6, 29.5, 29.4, 29.3, 28.9, 28.8, 28.7, 27.8, 27.4, 25.7, 25.3, 23.7, 22.6, 21.5, 17.9, 15.7, 14.0, 10.9, −4.3, −4.9; $\nu_{max}$: 2923, 2852, 1738, 1644, 1464, 1366, 1177, 719 $cm^{-1}$.

(ii) The above ester (0.150 g, 0.105 mmol) dissolved in (3 mL) dry THF and (7 mL) acetone. Potassium thioacetate (0.06 g, 0.42 mmol) was added and the solution was stirred at room temperature overnight then the solvent was evaporated. Column chromatography (petroleum/ethyl acetate, 20:1) gave methyl (*R*)-26-(acetylthio)-2-[(*R*)-1-[(*tert*-butyldimethylsilyl)oxy]-12-((1*R*,2*S*)-2-{14-[(1*R*,2*S*)-2-eicosylcyclopropyl]tetradecyl}cyclopropyl)dodecyl]hexacosanoate (**40**) (0.136 g, 94%) as a pale yellow thick oil which soldified later, $[\alpha]_D^{20}$ +1.1 (*c* 0.91 g, $CHCl_3$) {Found $(M+Na)^+$: 1362.2460, $C_{87}H_{170}O_4SSiNa$ requires: 1362.2481} which showed $\delta_H$ (500, MHz,$CDCl_3$): 3.92–3.89 (1H, m), 3.65 (3H, s), 2.86 (2H, t, *J* 7.55 Hz), 2.55–2.50 (1H, m), 2.31 (3H, s), 1.58–1.14 (126H, m), 0.89–0.83 (20H, m, including s at 0.86), 0.65–0.61 (4H, m), 0.56 (2H, dt, *J* 4.1 Hz), 0.04 (3H, s), 0.02 (3H, s), −0.32 (2H, br.q, *J* 4.75 Hz); $\delta_C$: 195.7, 175.0, 73.2, 51.5, 51.1, 41.3, 36.0, 33.6, 31.9, 30.5, 30.2, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.1, 29.0, 28.8, 28.7, 27.8, 27.6, 27.5, 25.8, 25.7, 23.6, 22.7, 22.5, 20.4, 19.4, 18.7, 17.9, 14.2, 11.4, 10.9, −4 .3, −4.9; $\nu_{max}$: 2918, 2849, 1738, 1694, 1465, 1360, 1253, 836, 720 $cm^{-1}$.

### *2.31. Methyl (R)-26-(acetylthio)-2-[(R)-1-hydroxy-12-((1R,2S)-2-{14-[(1R,2S)-2-eicosylcyclopropyl]tetradecyl}cyclopropyl) dodecyl]hexacosanoate*

Methyl (*R*)-26-(acetylthio)-2-[(*R*)-1-[(*tert*-butyldimethylsilyl) oxy]-12-((1*R*,2*S*)-2-{14-[(1*R*,2*S*)-2-eicosylcyclopropyl]tetradecyl} cyclopropyl)dodecyl]hexacosanoate (0.130 g, 0.098 mmol) was dissolved in dry THF (10 mL) in a dry polyethylene vial under $N_2$ at 0 °C. Pyridine (98 mg, 7.8 mmol, 0.1 mL) and hydrogen fluoride-pyridine complex (88 mg, 0.8 mL) were added and the mixture stirred at 45 °C overnight, then, the mixture was added slowly to a sat.aq. $NaHCO_3$ (15 mL). The solution was extracted with petroleum/ethyl acetate (5:1, 3 × 50 mL) and the combined organic extracts were dried, filtered and evaporated. Column chromatography (petroleum/ethyl acetate, 10:1) gave methyl (*R*)-26-(acetylthio)-2-[(*R*)-1-hydroxy-12-((1*R*,2*S*)-2-{14-[(1*R*,2*S*)-2-eicosylcyclopropyl]tetradecyl}cyclopropyl)dodecyl]hexacosanoate (0.065 g, 55%) as a thick oil which soldified later, $[\alpha]_D^{20}$ +1.6 (*c* 1.1, $CHCl_3$) {Found $(M+Na)^+$: 1248.1271 $C_{81}H_{156}O_4SNa$ requires: 1248.1047}, which showed $\delta_H$ (500 MHz,$CDCl_3$); 3.71 (3H, s), 3.67–3.64 (1H, m), 2.86 (2H, t, *J* 7.25 Hz), 2.45–2.42 (1H, m), 2.32 (3H, s), 1.73–1.69 (1H, m), 1.60–1.50 (2H, m), 1.37–1.14 (132H, m), 0.89–0.83 (3H, m), 0.64–0.60 (4H, m), 0.56 (2H, dt, *J* 4.1, 8.2 Hz), −0.32 (2H, br.q, *J* 5.05 Hz); $\delta_C$: 195.9, 176.2, 72.2, 51.4, 50.9, 41.3, 35.6, 31.9, 30.5, 30.2, 29.7, 29.6, 29.5, 29.4, 29.3, 29.1, 29.0, 28.8, 28.7, 27.4, 25.7, 22.6, 22.5, 20.4, 15.7, 14.2, 14.1, 11.4, 10.8; $\nu_{max}$: 2916, 2849, 1695, 1469, 1360, 1166, 836, 720 $cm^{-1}$.

### *2.32. (2R,2′R)-26,26′-disulfanediylbis[2-(R)-1-hydroxy-12-((1R,2S)-2-{14-[(1R,2S) -2-eicosylcyclopropyl] tetradecyl}cyclopropyl)dodecyl]hexacosanoic acid (**41**)*

Methyl (*R*)-26-(acetylthio)-2-[(*R*)-1-hydroxy-12-((1*R*,2*S*)-2-{14-[(1*R*,2*S*)-2-eicosyl- cyclopropyl]tetradecyl}cyclopropyl)dodecyl]hexacosanoate (0.060 g, 0.048 mmol) was suspended in 5% aq. TBAH (10 mL) and heated to 100 °C overnight. The solution was cooled to room temperature and acidified to pH 1 with 1 M HCl and then extracted with ether/petrol (5:2, 3 × 30 mL). The combined organic layers were filtered and the solvent evaporated. Column chromatography (chloroform/methanol, 10:1) gave (*2R,2′R*)-26,26′-disulfanediylbis[2-(*R*)-1-hydroxy-12-((1*R*,2*S*)-2-{14-[(1*R*,2*S*)-2-eicosylcyclopropyl]tetradecyl}cyclopropyl)dodecyl]hexacosanoic acid (**41**) (0.020 g, 33%) as a white solid, mp 54–56 °C, $[\alpha]_D^{22}$ +1.6 (*c* 0.50, $CHCl_3$); $\delta_H$: (500 MHz, $CDCl_3$ + few drops of $CD_3OD$): 3.69–3.64 (2H, m), 2.67 (4H, br.t, *J* 7.55 Hz), 2.43–2.39 (2H, m), 1.71–1.63 (12H, m), 1.52 (24H, br.s), 1.36–1.04 (236H, m), 0.87 (6H, t, *J* 6.6 Hz), 0.64–0.63 (8H, m), 0.55 (4H, dt, *J* 4.1, 8.2 Hz), −0.33 (4H, q, *J* 5.35 Hz), $\delta_C$: 176.2, 72.0, 39.2, 35.5, 31.8, 30.1, 29.6, 29.5, 29.4, 29.3, 29.1, 28.6, 28.4, 27.3, 25.7, 22.6, 15.7, 14.0, 10.8; $\nu_{max}$: 3442, 2917, 2850, 1717, 1469, 1400, 1170, 720 $cm^{-1}$.

### *2.33. Methyl (R)-2-[(R)-1-acetoxy-12-((1R,2S)-2-{14-[(1R,2S)-2-eicosylcyclo- propyl]tetradecyl}cyclopropyl)dodecyl]-26-mercaptohexacosanoate (**42**)*

Excess diazomethane in ether was added to (*2R,2′R*) -26,26′-disulfanediylbis[2-(*R*)-1-hydroxy-12-((1*R*,2*S*)-2-{14-[(1*R*, 2*S*)-2-eicosylcyclopropyl)tetradecyl)cyclopropyl]-dodecyl]hexacosanoic acid and stirred for 30 min. The solvent was evaporated to give dimethyl (2*R*,2′*R*)-26,26′-disulfanediylbis[2-(*R*)-1-hydroxy-12-((1*R*,2*S*)-2-{14-[(1*R*,2*S*)-2-eicosylcyclopropyl]tetradecyl}cyclopropyl)dodecyl]hexacosanoate}. This was used for next step without purification; acetic anhydride (0.3 mL) and pyridine (0.3 mL) were added to a stirred solution of the ester in toluene (0.3 mL). The mixture was stirred for 18 h then the solvent was evaporated to give dimethyl (2*R*,2′R)-26,26′-disulfanediylbis[2-(*R*)-1-acetoxy-12-((1*R*,2*S*)-2-{14-[(1*R*,2*S*)-2-eicosylcyclo propyl]tetradecyl}cyclopropyl)dodecyl]hexacosanoate} which showed $\delta_H$ (400 MHz, $CDCl_3$): 5.11–5.06 (2H, m), 3.68 (6H, s), 2.68 (4H, t, *J* 7.4 Hz), 2.64–2.59 (2H, m), 2.03 (6H, s), 1.69–1.11 (276H, m), 0.88 (6H, t, *J* 6.28 Hz), 0.65 (4H, m), 0.57 (4H, dt, *J* 3.88 Hz), −0.32 (4H, br.q, *J* 4.88 Hz). The product was used for next step without purification. DL-Dithiothreitol (100 mg) was added to a stirred solution of the ester in chloroform (1 mL) followed by the addition of one drop of triethylamine under nitrogen. The flask was covered with aluminium foil. The mixture was stirred for 48 h at room temperature, then the solvent was evaporated. Column chromatography eluting with petrol/ethyl acetate (10:1) gave methyl (*R*)-2-[(*R*)-1-acetoxy-12-((1*R*,2*S*)-2-{14-[(1*R*,2*S*)-2-eicosylcyclopropyl]tetradecyl}cyclopropyl)dodecyl]-26-mercaptohexacosanoate (**42**) {Found (MALDI) $[M+Na]^+$: 1248.92; $C_{81}H_{156}NaO_4S$ requires: 1249.16}, which showed $\delta_H$(400 MHz, $CDCl_3$): 5.11–5.06 (1H, m), 3.68 (3H, s), 2.64–2.59 (1H, m), 2.52 (2H, q, *J* 7.4 Hz), 2.02 (3H, s) 1.63–1.11(137H, m), 0.88 (3H, t, *J* 6.52 Hz), 0.68–0.64 (2H, m), 0.57 (2H, dt, *J* 3.88, Hz), −0.32 (2H, br.q, *J* 5.24 Hz).

### *2.34. Studies using cyclic voltammetry*

#### *2.34.1. Preparation of Solutions*

a. **Phosphate-buffered saline containing sodium azide (AE) and EDTA (PBS/AE, pH 7.4)**: Phosphate buffered saline (PBS) azide EDTA buffer (PBS/AE) with the following: NaCl (8.0 g), KCl (0.2 g), $KH_2PO_4$ (0.2 g) and $Na_2HPO_4$ (1.05 g) dissolved in double distilled deionized (ddd) $H_2O$ (1.0 L) with 1 mM EDTA and sodium azide 0.025% (m/v). The solution was adjusted to pH 7.4.
b. **Ferricyanide/ferrocyanide redox probe solution (1.0 mM)**: In PBS/AE buffer (250 mL) was dissolved $K_3[Fe(CN)_6]$ (82.3 mg, 0.25 mmol) and $K_4[Fe(CN)_6]$ (105.6 mg, 0.25 mmol). The solution was prepared just before use and purged of air by bubbling

nitrogen gas through it for at least 30 min immediately before every electrochemistry experiment.

*2.34.2. Cleaning of Gold Electrode Surface*

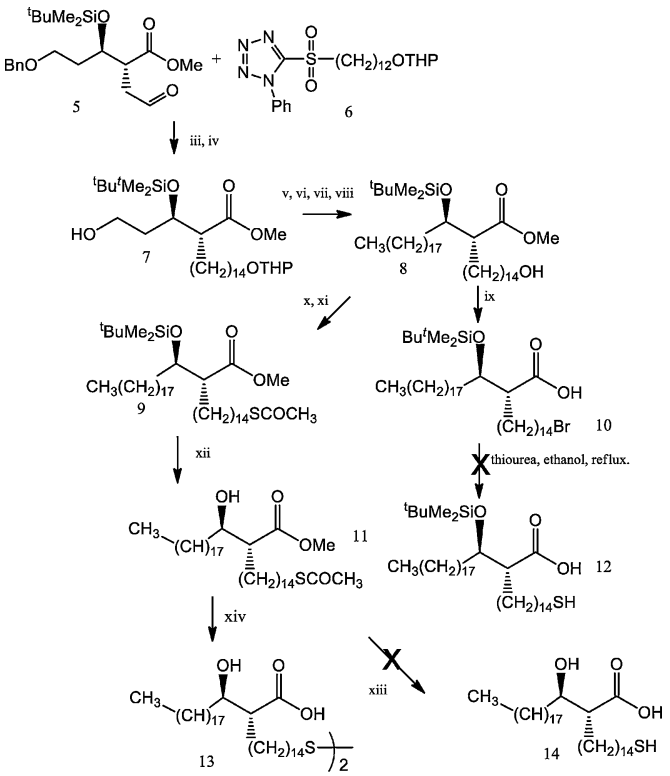
The gold electrode was cleaned according to the following protocol: the electrode was polished on an aqueous slurry of alumina Buehler felt paper. It was rinsed thoroughly with distilled water followed by absolute ethanol and then subjected to ultrasonication in a bath sonicator in absolute ethanol for about 1 h to remove residual alumina particles that might have been trapped on the surface. The electrode was again rinsed with excess distilled water and then absolute ethanol. Next it was incubated in a hot piranha solution for about 2 min followed by a copious rinse with ddd $H_2O$ followed by ethanol. Finally the electrode was cleaned in 0.5 M $H_2SO_4$ at a scan rate of 100 mV/s. A potential window of −0.2 V and −1.2 V measuring 10 scan cycles was used. A reproducible scan characteristic of a clean gold surface was obtained.

*2.34.3. Investigation of Bare Gold Electrode Surface by CV*

CV experiments for the bare gold were run in duplicates in a solution of $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (40 mL, 1 mM) at a scan rate of 25 mV/s and a potential window of −0.2 V to 0.6 V for 20 cycles.

*2.34.4. Fabrication of bare gold electrode with ma disulfide (**24**)*

The cleaned and characterised gold electrode was dipped into a chloroform solution of disulphide (**24**) (1.0 mg/mL) and incubated at room temperature for 34 h. At the end of this period the electrode was removed, rinsed successively in excess chloroform, sonicated in chloroform for 1 min, rinsed again in excess of chloroform, absolute ethanol and finally ddd $H_2O$. This was done to remove any non-covalently bound disulphide (**24**). The gold surface was then blown gently with nitrogen gas. CV characterisation was done immediately as described above for the bare gold.




**Scheme 2.** (i) LiHMDS, THF, 75%; (ii) $H_2$, Pd/C, 79%; (iii) PCC, 82%; (iv) LiHMDS, 5-(hexadecone-1-sulfonyl)-1-phenyl-14-tetrazole, THF, 75%; (v) $H_2$, Pd/C 92%; (vi) pyridinium-*p*-toluene sulphonate, MeOH/THF, 82%; (vii) N-bromosuccinimide, $PPh_3$, $CH_2Cl_2$, 72%; (viii) TsCl, $Et_3N$, 74%; (ix) potassium thioacetate, acetone, 75%; (x) pyridine, HF.pyridine, 84%; (xi) LiOH·$H_2O$ (15.eq), THF/MeOH/$H_2O$; (xii) LiOH.$H_2O$ (4.eq), THF/MeOH/$H_2O$, 26%.

## 3. Results and discussion

In the first series of experiments, a model thiolated β-hydroxy acid (**13**) was prepared as in Scheme 2.

A modified Julia-Kocienski coupling of the aldehyde (**5**) (Koza et al., 2009) with sulfone (**6**), followed by hydrogenation of the derived *E*/*Z*-mixture of alkenes, led to the protected β-hydroxy acid fragment (**7**) (as a pair of diastereoisomers at the OTHP group) with an extended fourteen carbon α-chain. Oxidation of the primary alcohol followed by a second similar chain-extension of the derived aldehyde, then hydrogenation of the alkene and deprotection led to alcohol (**8**). This could be converted into the thioacetate (**9**) using a two-step procedure of tosylation followed by thioacetate substitution (the alternative conversion of (**8**) into (**12**) via the bromide (**10**) was not successful). Deprotection of (**9**) was achieved in two steps. The final deprotection gave the disulphide (**13**) rather than the free thiol (**14**), as indicated by the mass ion in the MALDI MS and by a triplet for the methylene group adjacent to sulphur at $\delta$ 2.70 in the proton NMR spectrum and a signal for the same methylene carbon at ca. $\delta$ 40 in the carbon NMR spectrum. Although this compound was prepared simply as a model, it does represent a thiol substituted form of one of the minor mycolic acid components of *Rhodococcus equi* (Hsu et al., 2011).

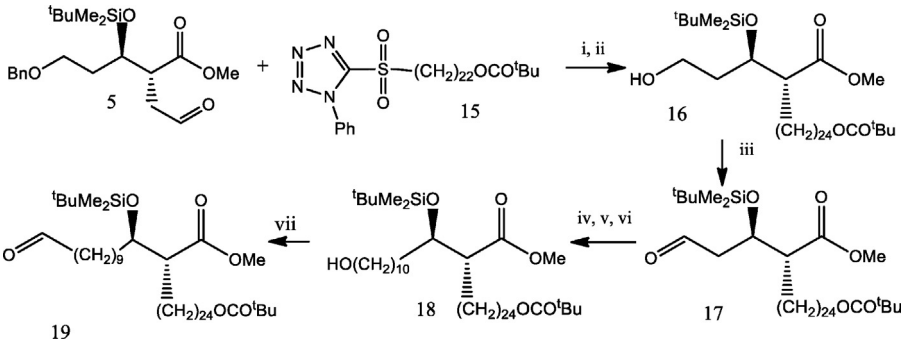
In order to prepare a complete thiolated mycolic acid, the intermediate (**19**) was first prepared as shown in Scheme 3. Coupling of the aldehyde (**5**) (Koza et al., 2009) with the sulfone (**15**) in the presence of base in a modified Julia-Kocienski reaction, followed by hydrogenation of the *E*/*Z*-mixture of alkenes obtained and debenzylation, led to alcohol (**16**). This was chain extended using a similar procedure to give the aldehyde (1**9**) via the aldehyde (**17**) (Scheme 3).

Coupling of this aldehyde (**19**) to the known sulfone (**20**) (Al Dulayymi et al., 2007) in the presence of base followed by hydrogenation led to alcohol (**21**) (Scheme 4). This was converted into the corresponding thioacetate (**22**); the transformation of alcohol to thioacetate was confirmed by the replacement of a triplet in the proton NMR spectrum for the methylene group adjacent to oxygen at $\delta_H$ 3.7 with that adjacent to the thioacetate at $\delta$ 2.86, and the loss of the corresponding carbon signal at $\delta_C$ 63.1. This $^{13}C$ methyl signal in the thioacetate appears in the region 28–34, together with other signals of the MA, but the formation of the thioacetate is confirmed by an additional carbonyl carbon at $\delta_C$ 196.1.

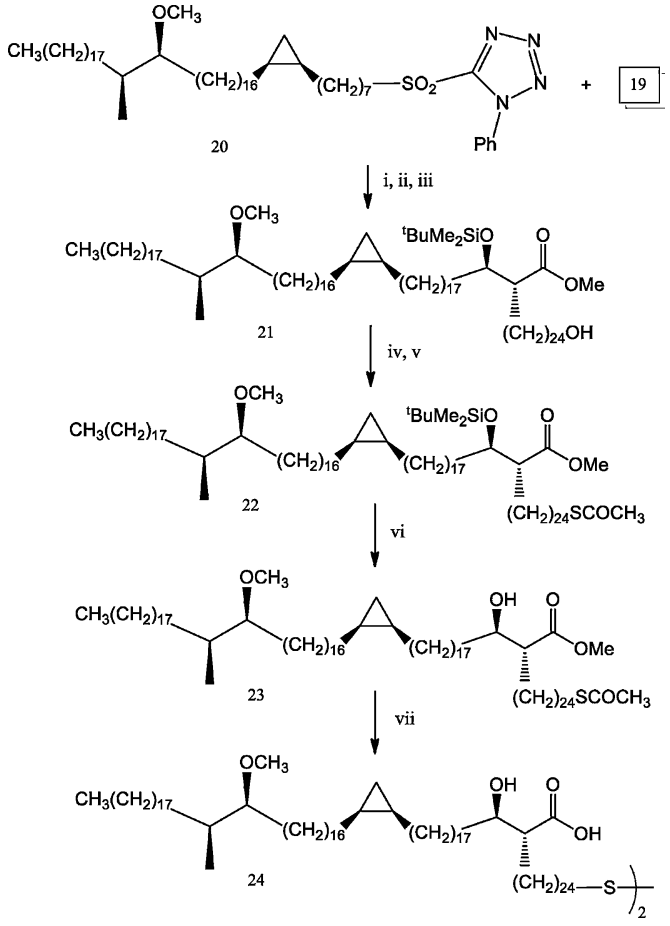
The final deprotection of (**22**) to give the free thio-substituted mycolic acid proved to be very sensitive to reaction conditions. The free thiol was not isolated and the disulfide (**24**) was obtained in only 26% yield. The $^1H$ chemical shift of the methylene group adjacent to sulfur was $\delta$ 2.76 for (**24**) and the corresponding $^{13}C$ resonated at $\delta$ 39.3 ppm. Unfortunately the disulfide did not give the expected molecular ion in MALDI MS. The resolution of this latter problem is described later.

In order to study the effect of the absolute stereochemistry of the mycolic acid on its use in diagnostics, a second diastereoisomer was prepared using the same procedure (Scheme 5).

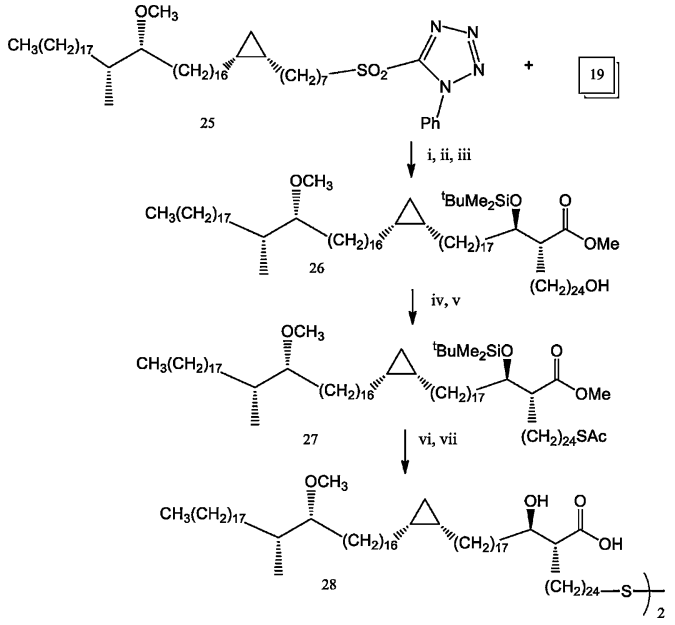
In this case, the final deprotection gave the disulfide (**28**), which again did not provide a molecular ion. As in the case of its diastereomer (**24**), the methylene group adjacent to sulfur appeared at $\delta$ 2.7. The carbons adjacent to sulfur in (**24**) and (**28**) appeared at 39.3 and 39.1 respectively. Compound (**28**) also showed four signals in the carbon NMR spectrum at $\delta$ 85.6, 72.1, 57.7 and 51 characteristic of the CH(OMe), CH(OH), $OCH_3$ and CH(COOH) carbons as seen in the parent methoxymycolic acid with no sulfur substituent on the α-chain (Al Dulayymi et al., 2007). There were also signals at $\delta_C$ 10.9



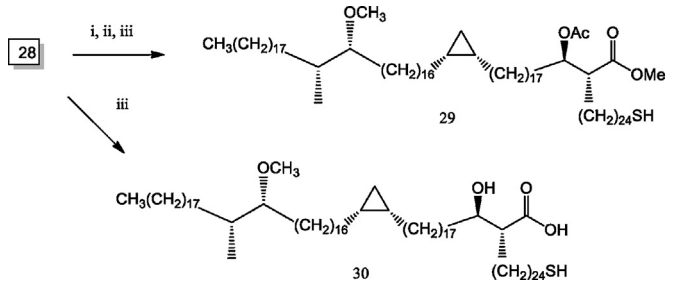
**Scheme 3.** (i) LiHMDS, THF, 67%; (ii) $H_2$, Pd/C 68%; (iii) PCC, 82%; (iv) LiHMDS, 1-phenyl-5-[8-(tetrahydro-2*H*-pyran-2-yl)oxyoctyl]sulfonyl-1*H*-tetrazole, 85%; (v) pyridinium-*p*-toluene sulphonate, MeOH/THF, 88%; (vi) $H_2$, Pd/C 88%; (vii) PCC, 84%.



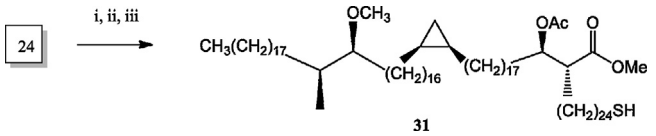
**Scheme 4.** (i) LiHMDS, THF, 71%; (ii) KOOCN=NCOOK, AcOH/MeOH/THF, 78%; (iii) KOH, THF, MeOH, $H_2O$, 70%; (iv) TsCl, $Et_3N$, $CH_2Cl_2$, 75%; (v) potassium thioacetate, THF, acetone, 76%; (vi) HF.pyridine, pyridine, THF, 63%; (vii) tetrabutylammonium hydroxide (5%), 100 °C, 18 h, 26%.



**Scheme 5.** (i) LiHMDS, THF, 89%; (ii) KOOCN=NCOOK, AcOH/MeOH/THF, 93%; (iii) KOH, THF, MeOH, $H_2O$, 60%; (iv) TsCl, $Et_3N$, $CH_2Cl_2$, 74%; (v) potassium thioacetate, THF, acetone, 82%; (vi) HF.pyridine, pyridine, THF, 97%; (vii) tetrabutylammonium hydroxide (5%), 100 °C, 18 h, 54%.



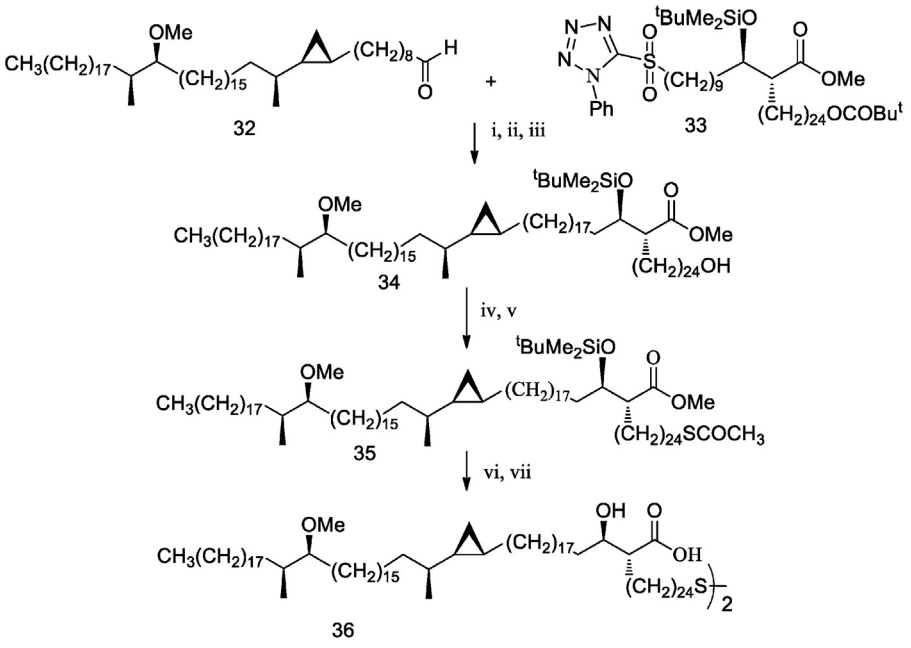
**Scheme 6.** (i) Excess diazomethane; (ii) acetic anhydride, pyridine, toluene; (iii) DL-dithiothreitol.



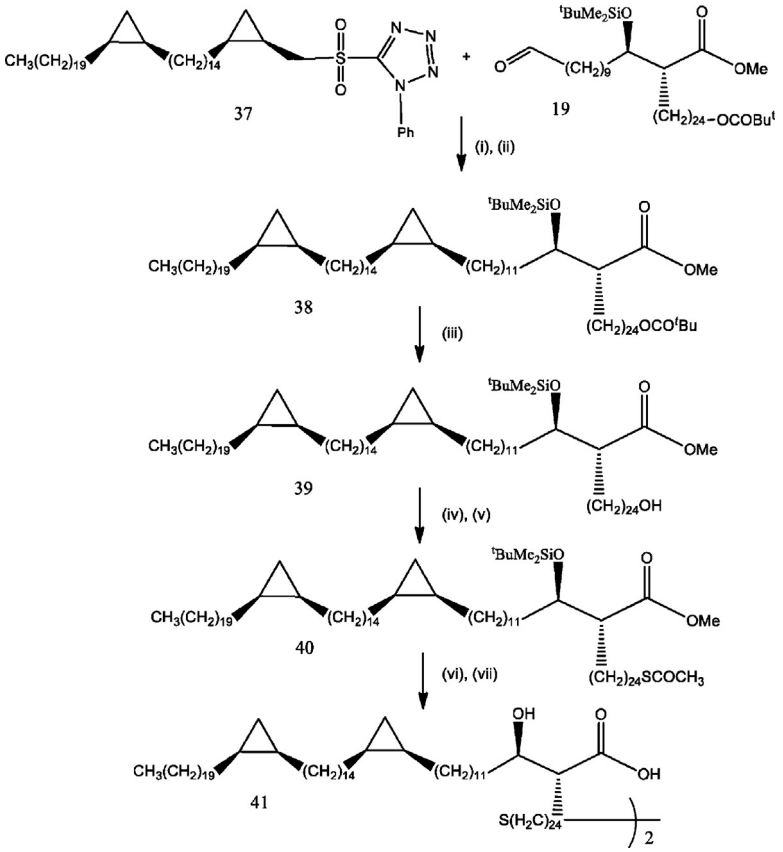
**Scheme 7.** (i) Excess diazomethane; (ii) acetic anhydride, pyridine, toluene; (iii) DL-dithiothreitol.

($CH_2$) and 15.8 (2 × CH) for the cyclopropane carbons, as well as a characteristic signal at $\delta_C$ 22.5 present in all mycolic acids, but no signal at $\delta_C$ 24–26.

In order to fully characterise the disulfides, the acid (**28**) was esterified with diazomethane and the alcohol was reprotected as an acetate. The disulfide was then reductively cleaved to give the thiol (**29**) using DL-dithiothreitol (Scheme 6). This gave the expected molecular ion in MALDI MS and the methylene group adjacent to sulfur appeared as a quartet at 2.53 (*J* 7.52 Hz). In addition, the carbon signal for the methylene group adjacent to sulphur had shifted from around $\delta_C$ 39.5 in the disulphide to 24.7 in the thiol. This is



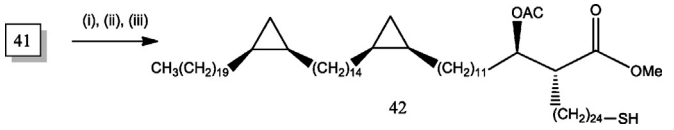
**Scheme 8.** (i) LiHMDS, THF, 86%; (ii) KOOCN=NCOOK, AcOH/MeOH/THF, 86%; (iii) KOH, THF, MeOH, $H_2O$, 86%; (iv) TsCl, $Et_3N$, $CH_2Cl_2$, 65%; (v) potassium thioacetate, THF, acetone, 61%; (vi) HF.pyridine, pyridine, THF, 90%; (vii) tetrabutylammonium hydroxide (5%), 100 °C, 18 h, 58%.



**Scheme 9.** (i) LiHMDS, THF, 60%; (ii) KOOCN=NCOOK, AcOH/MeOH/THF, 91%; (iii) KOH, THF, MeOH, $H_2O$, 67%; (iv) TsCl, $Et_3N$, $CH_2Cl_2$, 71%; (v) potassium thioacetate, THF, acetone, 94%; (vi) HF.pyridine, pyridine, THF, 55%; (vii) tetrabutylammonium hydroxide (5%), 100 °C, 18 h, 33%.

typical for the carbon adjacent to sulfur in sulfides (Anklam and Aced, 1990; Thuo et al., 2011; Angelova et al., 2005).

In the same way, the disulfide (**28**) could be split to free thiol (**30**) without protection of the alcohol and acid groups. The $^1$H NMR spectrum of this again showed a quartet at $\delta$ 2.52 for the methylene group adjacent to sulphur. When the sample was shaken with $D_2O$, this became a triplet with J 7.2 Hz. Once again, the methylene carbon adjacent to sulfur had shifted from $\delta_C$ 39.5 to 24.6. This was confirmed by a proton carbon correlation.




**Scheme 10.** (i) Excess diazomethane; (ii) acetic anhydride, pyridine, toluene; (iii) DL-dithiothreitol.

In the same way, the formation of the diastereoisomer (**24**) could also be confirmed by conversion into the thiol (**31**) (Scheme 7).

In the second part of this work, the same methods were applied to the synthesis of a thiol-substituted methoxymycolic acid containing an α-methyl-*trans*-cyclopropane moiety (Scheme 8).

In this case (Scheme 8), the aldehyde (**32**), containing a stereodefined α-methyl-*trans*-cyclopropane fragment (Koza et al., 2013) was coupled to sulfone (**33**), prepared by a standard route. Saturation of the derived mixture of alkene stereoisomers gave the alcohol (**34**), which was converted into the thioacetate (**35**) using the same method as above. Once again, removal of the protecting groups led to the formation of the disulfide (**36**) rather than the free thiol; seen by the presence of signals at $\delta_H$ 2.69 ($J$ 7.4) and $\delta_C$ 39.5 for the methylene group adjacent to the sulfur.

Finally a thiolated α-mycolic acid was prepared (Scheme 9). The sulfone (**37**) was prepared by the same route as that reported for its enantiomer (Al Dulayymi et al., 2005). Coupling to the aldehyde (**19**) in a modified Julia Kocienski reaction, followed by saturation of the derived alkenes using the same method as before gave (**38**). Removal of the pivaloate protecting group led to (**39**), which was transformed as before into the acetate (**40**). Once again, deprotection led to the corresponding disulfide (**41**).

The formation of the disulfide was seen by the presence of a characteristic carbon signal at $\delta_C$ 39.2 for the methylene group adjacent to sulfur, and the corresponding proton signal at $\delta_H$ 2.67. This was again confirmed by protection of the alcohol and acid groups, followed by the reduction of the disulfide using DL-dithiothreitol to give thiol (**42**) (Scheme 10).

Again, this was confirmed by the shift of the signal for the methylene group adjacent to sulfur for 2.67 to 2.52 in the $^1$H NMR spectrum, the latter appearing as a quartet in chloroform solution. Compound (**42**) represents a thiolated example of an α-mycolic acid with the chain lengths seen for the major homologue of the natural mixture (Al Dulayymi et al., 2003, 2005).

Disulfide (**24**) was immobilised on a gold electrode substrate and the surface investigated by cyclic voltammetry (Fig. 1). The binding of this molecule to gold and its application in the diagnosis of tuberculosis will be reported in full elsewhere. The CV proves the presence of an overlayer on the gold substrate that restricts charge transfer to the electrolyte solution. This is seen as a reduction of the current (the y axis). This CV result importantly indicates

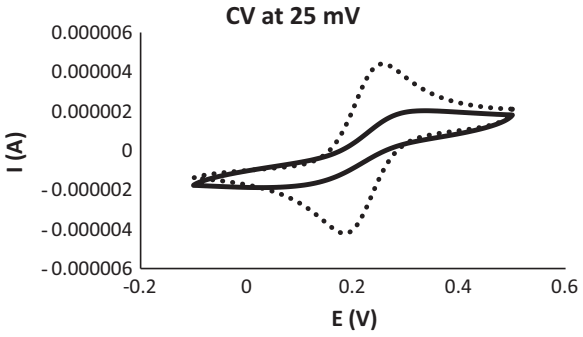



**Fig. 1.** Cyclic voltammograms of gold (-----) and mycolic acid disulfide (**24**) immobilised on gold (——). The gold electrode was incubated with disulfide for 34 h at room temperature.

that the surface-bound thiolated MA is resistant to organic solvent wash and physical agitation by sonication. The development of a stable surface for an immunosensor was a crucial objective in the thiol modification of MAs. It is also significant to note that the compound was stable under 25 mV of electrical perturbations and 20 cycles of ramping. This stability under repeated electrical potential is critical to the application of the thiolated MAs in electrical immunosensors. Ozoemena et al. suggested that different organothiolate self assembled monolayers should be investigated as platforms for electrochemical immunosensors (Mathebula et al., 2009; Ozoemena et al., 2010). The thiolated MAs synthesised here provide this opportunity. The results of studies of their application in devices to distinguish TB+ from TB− serum will be described elsewhere.

## 4. Conclusion

The first synthetic mycolic acids modified by the introduction of a sulfur substituent on the terminal carbon of the alpha-chain have been prepared in order to use them bound to gold surfaces in the diagnosis of tuberculosis. Initial studies show that they do bind to gold.

## Acknowledgements

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemphyslip.2013.03.004.

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