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Carbohydrate Research

DOI:
10.1016/j.carres.2017.04.023

Published: 07/08/2017

Version created as part of publication process; publisher's layout; not normally made publicly available

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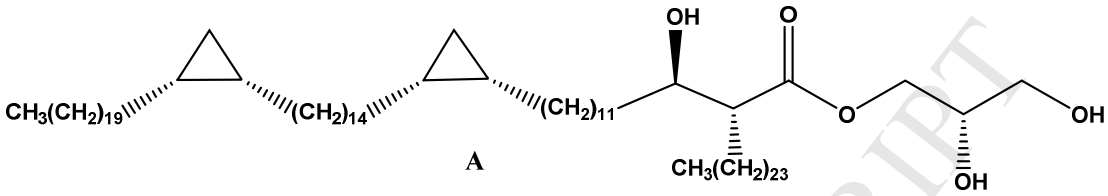
Abstract: R- and S-Glycerol mycolates derived from single synthetic α-, keto- and methoxy-mycolic acids, such as compound A are described.



A

Glycerol mycolates from synthetic mycolic acids

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Abstract: R- and S-Glycerol mycolates derived from single synthetic α-, keto- and methoxy-mycolic acids are described.

Keywords: GroMM, glycerol mycolate, α-mycolic acid, keto-mycolic acid, methoxy-mycolic acid

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1. Introduction

Complex mixtures of mycolic acids are characteristic components of mycobacterial cells, either bound to the wall as arabinose esters or not bound to the wall, as free acids or esterified to sugars such as glucose (GMM), or trehalose (dimycolate, TDM; monomycolate, TMM) [1]. As early as 1956, it was shown that *Mycobacterium tuberculosis* contains mycoloylglycerols (ca. 90 carbons) [2 - 5]. Glycerol monomycolate (GroMM, also known as MMG) is also present in the wax C fraction of BCG [6]. Shorter chain glycerol esters were isolated from *Nocardia rhodochrous*, which contains nocardomycoloylglycerols (40 – 44 carbons) [7], from *Corynebacterium pseudotuberculosis* (30 – 36 carbons) [8], from *Nocardia asteroides* (50 – 56 carbons) [9], and from *Rhodococcus lentifragmentus* (32 – 50 carbons) [10].

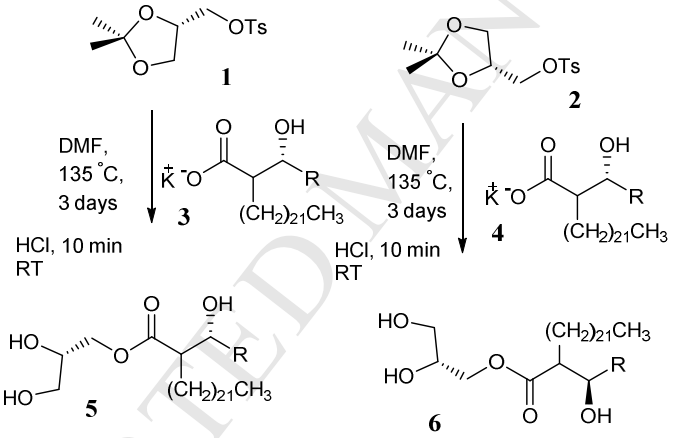
The glycerol is esterified at one of the primary alcohol groups [11]. The antigen isolated from *Mycobacterium bovis BCG* was shown to exist as two stereoisomers at the glycerol by comparison of the NMR spectrum with those of *R*- and *S*-glycerol isomers of GroMM prepared from mycolic acid mixtures isolated from *M.tuberculosis* H37Rv cells [12]. Both semi-synthetic GroMMs stimulate CD-1 restricted T cell clones, the *R*-isomer being more slightly more stimulatory than GroMM from *Mycobacterium bovis BCG*, the *S*-isomer slightly less stimulatory. In this system, *R* and *S*-isomers of GroMM prepared from a 32 carbon corynomycolate almost completely removed the stimulatory effect, in line with earlier results [13]. The secondary alcohol group of the glycerol was also necessary for activity. GroMM was presented by *M. tuberculosis* infected dendritic cells, demonstrating that the antigen is available for presentation during natural infection. *Ex vivo* experiments showed that GroMM stimulated T cells from vaccinated or latently infected healthy donors but not those from patients with active tuberculosis, suggesting that GroMM-specific T cells are primed during infection and their detection correlates with lack of clinically active disease [12]. Hattori *et al.* immunized guinea pigs with bacillus Calmette–Guérin (BCG) expressing high levels of GroMM and then monitored skin reactions at the site of inoculation with GroMM-containing liposome. The host responses to GroMM produced by dormant mycobacteria contribute to their long-term survival in the host [14], again identifying GroMM

as potentially associated with latent infection. GroMM is a ligand for the human, but not mouse macrophage inducible c-type lectin, Mincle [15], the signalling process being dependent on structure [16, 17].

A 32 carbon monomycolyl GroMM analogue demonstrated enhanced immunostimulatory activity in a dioctadecyl ammonium bromide/Ag85B-ESAT-6 formulation. Elevated levels of IFN-gamma and IL-6 were produced in spleen cells from mice immunised with a 32 carbon GroMM analogue comparable activity to the potent Th1 adjuvant, trehalose 6,6'-dibehenate [18 – 22]. Interest in the properties and applications of GroMMs therefore remains high [23 - 26].

Derivatives of GroMMs in which the two other glycerol alcohol groups are acylated have also been reported [27].

Layre *et al*. prepared GroMM using potassium salts of mixtures of mycolates isolated from *M. tuberculosis* H37Rv [12], using (*S*)- and (*R*)-isopropylidene-glycerol *p*-toluenesulfonates **1** and **2** (**Scheme 1**).



Scheme 1: The synthesis of GroMM with natural mycolic acid [13]

Nordly *et al.* synthesised GroMMs from unfunctionalised C_{32} β-hydroxy acids producing a mixture of four stereoisomers, and carried out extensive studies of their bioactivity [21].

In order to determine whether the detailed structure of the individual mycolic acid in the natural mixtures leads to changes in their biological properties, or whether the activity is dominated by the glycerol fragment, we now report the synthesis of GroMMs based on coupling of a set of individual complete synthetic mycolic acids, taken from various Mtb classes, with both glycerol stereoisomers.

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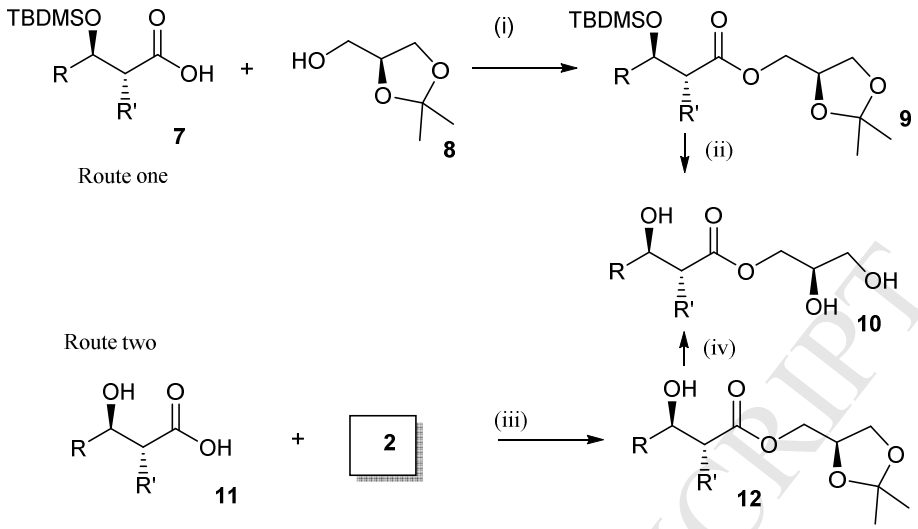
2. Results and discussion

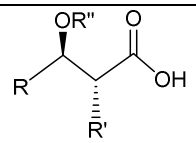
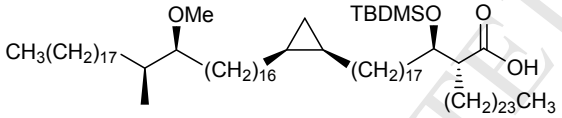
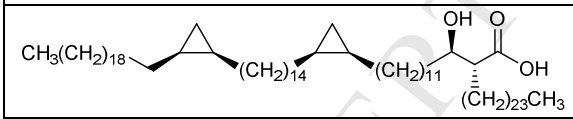
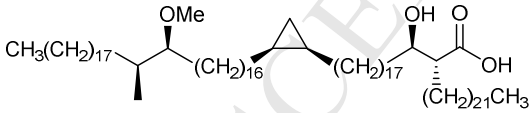
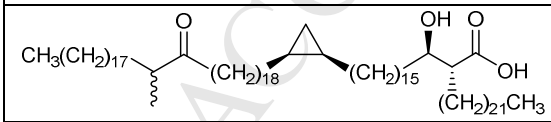
The synthetic mycolic esters of *R*-glycerol **10** were prepared by two methods. In the first, the TBDMS protected MA was coupled with acetonide **8** promoted by DCC and DMAP. In the

second, the unprotected acid was coupled with tosylate **2** in the presence of cesium hydrogen carbonate. (**Scheme 2**)

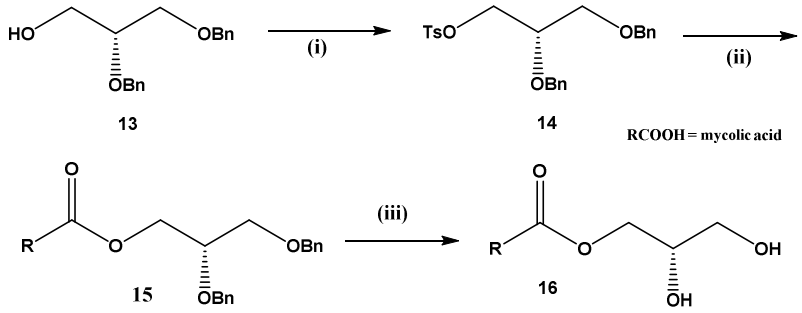


Scheme 2: (i) DMAP, DCC; (ii) TFA, CH_2Cl_2; (iii) $CsHCO_3$; (iv) HCl, THF, H_2O

Table 1: Synthetic mycolates of *R*-glycerol

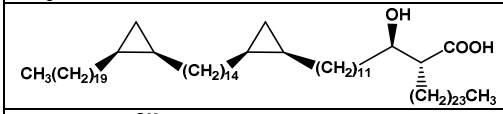
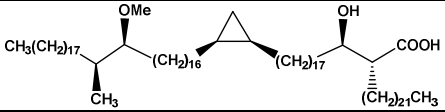
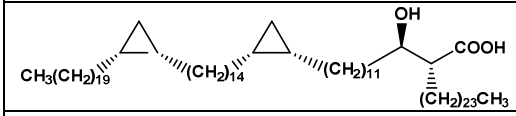
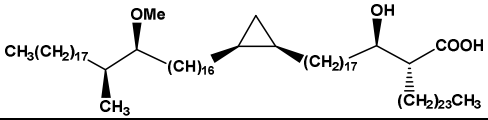
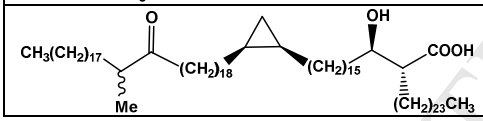
Mycolic acid 	R''	Number	Method	Yield **9/12** (%)	Yield **10** (%)
	**TBDMS**	**7a**	1	**9a** (72)	**10a** (86)
	**H**	**11b**	2	**12b** (85)	**10b** (86)
	**H**	**11c**	2	**12c** (50)	**10c** (88)
	**H**	**11d**	2	**12d** (85)	**10d** (87)

The *S*-glycerol mycolate esters were prepared by a modified method in which the glycerol was protected by benzyl groups, which, after coupling, were removed by hydrogenolysis:



Scheme 3: (i) TsCl, py; (ii) RCOOH, $CsHCO_3$; (iii) $Pd(OH)_2$, H_2, CH_2Cl_2, MeOH

Table 3. Synthetic mycolates of *S*-glycerol

Mycolic acid **RCOOH**	Number	Yield **15**	Yield **16**
	**11b**	**15b** (71)	**16b** (92)
	**11c**	**15c** (62)	**16c** (85)
	**11e**	**15e** (58)	**16e** (74)
	**11f**	**15f (72)**	**16f** (92)
	**11g**	**15g** (59)	**16g** (87)

1. Conclusion

A set of nine R- and S-glycerol mycolates derived from single synthetic α-, keto- and methoxy-mycolic acids are described. The GroMMs **10a** – **10d** did not show any significant effects in the stimulation of cytokines in bone marrow dendritic cells (BMDCs) [28], unlike related TDMs and TMMs, GMMs and arabinose mycolates which activate BMDCs in terms of production of pro-inflammatory cytokines (IL-6 and TNF-α) and reactive oxygen species, upregulation of costimulatory molecules and activation of NLRP3 inflammasome by a mechanism dependent on Mincle [29]. However, they do show strong and selective effects in the stimulation of CD1b-restricted GEM T cell responses, mirroring the effects of the corresponding free mycolic acids [30].

2. Experimental:

2.1 General considerations

All chemicals were purchased from commercial suppliers. THF was distilled over sodium and benzophenone under nitrogen, while dichloromethane was distilled over calcium hydride. Petrol refers to the fraction b.p. 40–60 °C. Organic solutions were dried over anhydrous magnesium sulfate. Reactions carried out under inert conditions were under a slow stream of nitrogen. Column chromatography and thin layer chromatography were carried out using Silica gel (Merck 7736, 40 – 63 micron) and silica gel plates (Merck, 60F254). R_f values for the final GroMMs were all in the range 0.31 – 0.39 using 20:1 chloroform/methanol. Infra-red (IR) spectra were carried out on a Perkin-Elmer 1600 F.T.I.R. spectrometer as films or KBr discs or directly on a Bruker ALPHA FTIR spectrometer. Optical rotations were measured as solutions in chloroform using a Polar 2001 automatic polarimeter. NMR spectra were recorded either on an Advance 400 or 500 spectrometers. Spectra are reproduced in the Supplementary Information. Mass spectra were recorded on a Bruker matrix-assisted laser desorption/ionisation-time of flight mass spectrometry (MALDI-TOF MS) values are given plus sodium to an accuracy of 1 d.p. Accurate mass data were run by Dr Paul Gates (Bristol University) or the EPSRC UK National Mass Spectrometry Facility at Swansea University.

4.2 ((*R*)-2,3-Dihydroxypropyl (*R*)-2-((*R*)-1-hydroxy-18-((1*R*,2*S*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl)hexacosanoate (10a)

(a) (*R*)-2-((*R*)-1-((*tert*-Butyldimethylsilyl)oxy)-18-((1*R*,2*S*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl)hexacosanoic acid (**7a**) [31] (90 mg, 0.06 mmol) and DMAP (10 mg, 0.08 mmol) were added to a stirred solution of (*S*)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanol [32] (10 mg, 0.07 mmol) in CH_2Cl_2 (2 mL). A solution of DCC (20 mg; 0.09 mmol) in CH_2Cl_2 (2 mL) was added dropwise with stirring under nitrogen over 120 min then stirred for 1 h at room temperature. Precipitated dicyclohexyl urea was filtered off and washed with dichloromethane (10 mL). The solvent was evaporated and the crude product was purified by column chromatography eluting with petrol/ethyl acetate (20:1) to give a semi-solid, compound **9a** (70 mg, 72%), $[\alpha]_D^{24} - 6.7$ (*c* 1.4, $CHCl_3$), [Found $(M+Na)^+$: 1505.5, $C_{97}H_{192}NaO_6Si$ requires: 1504.4], which showed δ_H (500 MHz, $CDCl_3$): 4.33 – 4.26 (1H, m), 4.11 (1H, dd, *J* 2.4, 5.4 Hz), 4.06 (1H, dd, *J* 6.4, 8.4 Hz), 3.94 – 3.87 (1H, m), 3.75 (1H, dd, *J* 6.0, 8.4 Hz), 3.34 (3H, s), 3.24 – 3.15 (1H, m), 2.98 – 2.92 (1H, m), 2.55 (1H, ddd, *J* 3.8, 6.9, 10.8 Hz), 2.00 – 1.01 ((153H, m including two singlets at 1.43 (3H, s) and 1.36 (3H, s)), 0.91 – 0.83 (18H, including d for the methyl group (*J* 7 Hz) and a t (*J* 6.8 Hz) for the terminal methyl group and s for the *tert*-butyl group), 0.69 – 0.61 (2H, m), 0.56 (1H, dt, *J* 3.7, 7.6 Hz), 0.04 (3H, s), 0.01 (3H, s), -0.34 (1H, q, *J* 5.2 Hz); δ_C (126 MHz, $CDCl_3$): 174.7, 109.8, 85.6, 73.7, 66.8, 64.4, 57.9, 55.9, 51.6, 35.5, 35.1, 34.0, 32.6, 32.1, 30.7, 30.4, 30.2, 30.1, 29.9, 29.8, 29.6, 29.5, 28.9, 28.0, 27.7, 26.9, 26.3, 26.2, 25.9, 25.6, 25.5, 24.9, 24.1, 22.9, 18.1, 15.9, 15.0, 14.3, 11.1, -4.2, -4.7; ν_{max}/cm^{-1}: 2918, 2849, 1726, 1465, 1256, 1178..

(b)Trifluoroacetic acid in dichloromethane (1:1, 0.4 mL) was added to a stirred solution of ester (**9a**) (50 mg, 0.03 mmol) in CH_2Cl_2 (2 mL) at 0 °C. The solution was stirred at 0 °C for 12 h, when TLC showed no starting material was left and the reaction mixture was worked up by quenching with sat. aq. $NaHCO_3$ (20 mL) and the product was extracted with CH_2Cl_2 (2 ×

10 mL) and the combined organic layers were dried and evaporated. The crude product was purified by column chromatography eluting with chloroform/methanol (15:1) to give a semi-solid, the title compound (**10a**) (40 mg, 86%), $[\alpha]_D^{23} - 8.4$ (*c* 1.1, $CHCl_3$), [MALDI-Found $(M+Na)^+$: 1350.3199, $C_{88}H_{174}NaO_6$ requires: 1350.3203], which showed δ_H (500 MHz, C_6D_6): 4.25 (1H, dd, *J* 6.6, 11.4, Hz), 4.12 (1H, dd, *J* 3.9, 11.4, Hz), 3.81 – 3.71 (2H, m), 3.47 (1H, dd, *J* 4.2, 11.3, Hz), 3.40 (1H, dd, *J* 5.5, 11.4, Hz), 3.27 (3H, s), 3.03 – 2.97 (1H, m), 2.55 (1H, dd, *J* 9.0, 12.5, Hz), 1.9-1.25 (149H, m), 1.00 (3H, d, *J* 6.8 Hz), 0.93 (7H, including t, *J* 6.6 Hz), 0.78 – 0.71 (2H, m), 0.68 (1H, dt, *J* 3.7, 7.6 Hz), -0.01 (1H, q, *J* 5.0 Hz); δ_C (126 MHz, C_6D_6): 175.2, 85.4, 73.2, 70.4, 65.5, 63.6, 57.6, 53.0, 35.9, 35.8, 33.0, 32.4, 30.9, 30.8, 30.6, 30.5, 30.3, 30.2, 30.1, 30.0, 29.9, 29.8, 29.3, 28.2, 28.0, 26.7, 26.0, 23.1, 16.3, 15.3, 14.4, 11.5; ν_{max}/cm^{-1}: 3499 2928, 2854, 1744, 1435, 1267, 1178.

4.3 (*R*)-2,3-Dihydroxypropyl (*R*)-2-((*R*)-1-hydroxy-12-((1*R*,2*S*)-2-(14-((1*R*,2*S*)-2-eicosyl-cyclopropyl)tetradecyl)cyclopropyl)dodecyl) hexacosanoate (10b)

(a) Dry cesium hydrogen carbonate (120 mg, 0.33 mmol) was added to a stirred solution of acid (**11b**) [33] (70 mg, 0.06 mmol) in a mixture of THF:DMF (1:1, 4 mL) at room temperature. The mixture was left at room temperature for 1 h then (*R*)-(2,2-dimethyl-1,3-dioxolan-4-yl)methyl 4-methylbenzenesulfonate **2** [34] (40 mg, 0.13 mmol) was added. The mixture was brought to 70 °C and left at this temperature for 18 h, then cooled to room temperature and diluted with ethyl acetate (15 mL). The organic layer washed twice with water, dried and concentrated. Column chromatography eluting with petrol/ethyl acetate (5:1) gave a semi-solid, title compound (**12b**) (66 mg, 85%), $[\alpha]_D^{20} - 4.7$ (*c* 1.2, $CHCl_3$), [Found $(M+Na)^+$: 1274.2, $C_{84}H_{162}O_5Na$ requires: 1274.2]. This showed δ_H (400 MHz, $CDCl_3$): 4.27 – 4.20 (1H, m), 4.10 (1H, dd, *J* 3.3, 9.9 Hz), 4.06 (1H, dd, *J* 4.3, 9.9 Hz), 3.97 (1H, dd, *J* 6.5, 8.5 Hz), 3.65 (1H, dd, *J* 5.9, 8.5 Hz), 3.56 (1H, br s), 2.41 – 2.28 (2H, m), 1.38 – 0.98 ((140H, m including 1.34 (3H, s), 1.26 (3H, s)), 0.78 (6H, t, *J* 6.8), 0.61 – 0.50 (4H, m), 0.50 – 0.41 (2H, m), -0.43 (2H, q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$): 175.3, 109.8, 73.4, 72.3, 66.3, 64.4, 51.3, 35.6, 31.9, 30.2, 29.73, 29.7, 29.6, 29.5, 29.4, 28.7, 27.4, 26.7, 25.7, 25.3, 22.7, 15.7, 14.1, 10.9; ν_{max}/ cm^{-1}: 3430, 3011, 2925, 2886,1744, 1232, 759, 669.

(b) Hydrochloric acid (2 M, 0.04 mL) was added to a stirred solution of ester (**12b**) (55 mg, 0.04 mmol) in THF (25 mL) at room temperature, then stirred at 40 °C for 16 h. The mixture was quenched at room temperature with sat. aq. $NaHCO_3$ (10 mL), the product was extracted with CH_2Cl_2 (25 mL) and the aqueous phase was re-extracted with CH_2Cl_2 (3 × 25). The organic extracts were dried and concentrated. The crude residue was purified by column chromatography eluting with petrol/ethyl acetate (4:1) to give a semi-solid, the title compound (**10b**) (46 mg, 86%), $[\alpha]_D^{22} - 6.5$ (*c* 0.90, $CHCl_3$) [MALDI-Found $(M+Na)^+$: 1234.2005, $C_{81}H_{158}NaO_5$ requires: 1234.2001], which showed δ_H (400 MHz, $CDCl_3$): 4.30 – 4.26 (2H, m), 4.01 – 3.95 (1H, m), 3.76 – 3.61 (3H, m), 2.54 – 2.44 (1H, m), 1.49 – 1.08 (137H, m), 0.90 (6H, t, *J* 6.8 Hz), 0.71 – 0.63 (4H, m), 0.58 (2H, dt, *J* 3.7, 7.6 Hz), -0.31 (2H, q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$): 175.6, 73.1, 70.1, 65.4, 63.4, 52.3, 35.5, 32.1, 30.4, 29.9, 29.8, 29.7, 29.6, 29.5, 29.5, 28.9, 27.6, 25.6, 22.8, 15.9, 14.3, 11.1; ν_{max}/cm^{-1}: 3430, 3011, 2925, 2886,1744, 1232, 759, 669.

4.4 (*R*)-2,3-Dihydroxypropyl (*R*)-2-((*R*)-1-hydroxy-18-((1*R*,2*S*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl)tetracosanoate (10c)

(a) Dry cesium hydrogen carbonate (100 mg, 0.40 mmol) was added to a stirred solution of acid (**11c**) [36] (70 mg, 0.05 mmol) in a mixture of THF:DMF (1:1, 2 mL) at room temperature. The mixture was left at room temperature for 1 h then (*R*)-tosylate (**2**) (80 mg, 0.2 mmol) was added. The mixture was heated at 70 °C for 18 h, then worked up and purified as before to give a semi-solid, compound (**12c**) (70 mg, 50%), $[\alpha]_D^{21} - 6.2$ (*c* 0.90, $CHCl_3$) [Found $(M+Na)^+$: 1362.5, $C_{89}H_{174}NaO_6$ requires: 1362.3]; δ_H (400 MHz, $CDCl_3$): 4.37 – 4.29 (1H, m), 4.20 (1H, dd, *J* 3.3, 9.9 Hz), 4.16 (1H, dd, *J* 2.4, 8.0 Hz), 4.07 (1H, dd, *J* 6.5, 8.5 Hz), 3.75 (1H, dd, *J* 5.9, 8.4 Hz), 3.66 (1H, br s), 3.34 (3H, s), 2.99 – 2.92 (1H, m), 2.52 – 2.37 (2H, m), 1.48 – 1.06 ((149H, m including 1.44 (3H, s), 1.36 (3H, s)), 0.88 (6H, t, *J* 6.8 Hz), 0.85 (3H, d, *J* 6.9 Hz), 0.64 (2H, br s), 0.60 – 0.51 (1H, m), -0.34 (1H, q, *J* 5.3 Hz); δ_C (101 MHz, $CDCl_3$): 175.3, 109.8, 85.5, 73.4, 72.3, 66.3, 64.4, 57.7, 51.3, 35.5, 35.3, 32.4, 31.9, 30.8, 30.2, 30.0, 29.9, 29.7, 29.6, 29.5, 29.4, 29.3, 28.7, 27.6, 27.4, 26.7, 26.1, 25.7, 25.3, 22.7, 15.8, 14.9, 14.1, 10.9; ν_{max}/ cm^{-1}: 3511, 2918, 2849, 1726, 1465, 1256, 1178.

(b) Hydrochloric acid (2 M, 0.04 mL) was added with stirring to ester (**12c**) (65 mg, 0.05mmol) in THF (34 ml) at room temperature. The mixture was stirred at 40 °C for 16 h, then worked up and purified as before to give a semi-solid, the title compound (**10c**) (56 mg, 88%), $[\alpha]_D^{24} - 9.3$ (*c* 1.2, $CHCl_3$) [MALDI-Found $(M+Na)^+$: 1322.2880, $C_{86}H_{170}NaO_6$ requires: 1322.2890]; δ_H (500 MHz, $CDCl_3$): 4.30 – 4.22 (2H, m), 3.99 – 3.92 (1H, m), 3.74 – 3.67 (2H, m), 3.62 (1H, dd, *J* 5.5, 11.4 Hz), 3.34 (3H, s), 2.98 – 2.92 (1H, m), 2.50 – 2.42 (1H, m), 1.74 – 1.01 (146 H, m), 0.88 (6H, t, *J* 6.9 Hz), 0.84 (3H, d, *J* 6.9 Hz), 0.69 – 0.60 (2H, m), 0.55 (1H, dt, *J* 3.9, 8.4 Hz), -0.34 (1H, q, *J* 5.1 Hz); δ_C (101 MHz, $CDCl_3$): 175.6, 85.6, 73.1, 70.1, 65.4, 63.4, 57.9, 52.3, 35.5, 35.4, 34.1, 32.5, 32.1, 30.6, 30.4, 30.1, 30.0, 29.9, 29.8, 29.7, 29.6, 29.5, 28.9, 27.7, 27.6, 26.3, 25.6, 25.1, 22.8, 15.9, 15.0, 14.3, 11.1; ν_{max}/cm^{-1}: 3511, 2918, 2849, 1726, 1465, 1256, 1178.

4.5 (*R*)-2,3-Dihydroxypropyl (2*R*)-2-((1*R*)-1-hydroxy-16-((1*R*,2*S*)-2-(20-methyl-19-oxo-octatriacontyl)cyclopropyl)hexadecyl)tetracosanoate (10d)

(a) Dry cesium hydrogen carbonate (110 mg, 0.50 mmol) was added to a stirred solution of acid (**11d**) [36] (80 mg, 0.06 mmol) in a mixture of THF:DMF (1:1, 3 mL) at room temperature. The mixture was stirred for 1 h then *R*-tosylate (**2**) (40 mg, 0.13 mmol) was added .The mixture was brought to 70 °C and left at this temperature for 18 h, then worked up and purified as before to give a semi-solid, compound (**12d**) (64 mg, 85%), $[\alpha]_D^{24} - 8.1$ (*c* 1.4, $CHCl_3$) [Found $(M+Na)^+$: 1346.9, $C_{88}H_{170}NaO_6$ requires: 1346.3], which showed δ_H (400 MHz, $CDCl_3$): 4.40 – 4.32 (1H, m), 4.26 (1H, dd, *J* 5.9, 11.4 Hz), 4.20 (1H, dd, *J* 2.2, 5.4 Hz), 4.14 (1H, dd, *J* 4.6, 11.4 Hz), 4.12 – 4.07 (1H, m), 3.78 (1H, ddd, *J* 2.3, 5.7, 8.3 Hz), 3.73 – 3.65 (1H, m), 2.57 – 2.40 (4H, m), 1.66 – 1.11 ((146H, m including 1.46 (3H, s), 1.38 (3H, s)), 1.07 (3H, d, *J* 6.9 Hz), 0.90 (6H, t, *J* 6.8 Hz), 0.71 – 0.63 (2H, m), 0.62 – 0.54 (1H, m), -0.31 (1H, q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$): 215.2, 175.3, 109.8, 73.5, 72.3, 66.2, 64.6, 51.5, 46.3, 41.1, 35.6, 35.5, 33.0, 31.9, 30.2, 29.7, 29.59, 29.5, 29.3, 28.7, 27.3, 26.7, 25.3, 23.7, 22.7, 16.3, 15.7, 14.1, 10.9; ν_{max}/ cm^{-1}: 3455, 2944, 2861, 1722, 1146, 1382, 1214, 1022.

(b) Hydrochloric acid (2 M, 0.04 mL) was added to a stirred solution of ester (**12d**) (60 mg, 0.04 mmol) in THF (30 mL) at room temperature. The mixture was stirred was at 40 °C for 16 h. then worked up and purified as before to give a semi-solid, the title compound (**10d**) (51 mg, 87%), $[\alpha]_D^{22} - 12$ (*c* 1.1, $CHCl_3$) [MALDI-Found $(M+Na)^+$: 1306.2569, $C_{85}H_{166}NaO_6$ requires: 1306.2577], which showed δ_H (400 MHz, $CDCl_3$): 4.30 (1H, dd, *J* 4.1, 11.8 Hz), 4.25 (1H, dd, *J* 6.7, 11.8 Hz), 4.03 – 3.94 (1H, m), 3.77 – 3.70 (2H, m), 3.65 (1H, dd, *J* 4.6, 10.5 Hz), 2.57 – 2.39 (4H, m), 1.76 – 1.10 (143H, m), 1.07 (3H, d, *J* 6.9 Hz), 0.90 (6H, t, *J* 6.8 Hz), 0.66 (2H, br s), 0.58 (1H, dt, *J* 3.7, 7.8 Hz), -0.31 (1H, q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$): 215.3, 175.4, 72.9, 69.7, 65.2, 63.3, 52.2, 46.3, 41.1, 37.9, 35.3, 33.0, 31.9, 30.2, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 28.7, 27.4, 27.3, 25.5, 23.7, 22.7, 16.3, 15.8, 14.1, 10.9; ν_{max}/cm^{-1}: 3455, 2944, 2861, 1722, 1146, 1382, 1214, 1022.

4.6 (*S*)-2,3-Bis(benzyloxy)propyl alkanoate (15): General procedure

Cesium hydrogen carbonate was added to a stirred solution of 1-*O*-*p*-toluene-sulfonyl-(*S*)-2,3-di-*O*-benzylglycerol (**14**) [37], and acid in dry DMF:THF (1:5, 2 mL) at room temperature. The mixture was stirred at 70 ºC for 2 days, then diluted with ethyl acetate (10 mL) and water (10 mL). The organic layer was separated and the aqueous layer was re-extracted with ethyl acetate (3×10 mL). The combined organic layers were washed successively with water (15 mL) and brine (15 mL), dried and evaporated. The residue was purified by column chromatography.

4.7 (*S*)-2,3-Dihydroxypropyl alkanoate (16): General procedure

Palladium hydroxide on activated charcoal (20% Pd, 0.15 fold by weight) was stirred with compounds **(15)** in dry CH_2Cl_2 : MeOH (1:1, 2 mL) at room temperature under hydrogen. The mixture was stirred for 24 h then filtered through celite. The celite was washed with CH_2Cl_2 (10 mL), the filtrate was evaporated, and the residue purified by column chromatography.

4.8 (*S*)-2,3-Dihydroxypropyl (*2R*)-2-(1-hydroxy-12-((1*R*,2*S*)-2-(14-((2*S*)-2-eicosylcyclopropyl)tetradecyl)cyclopropyl)dodecyl)hexacosanoate (16b)

(a) Cesium hydrogen carbonate (0.0616 g, 0.317 mmol), tosylate (**14**) (0.0298 g, 0.0698 mmol) and the acid (**11b**) [33] (0.0724 g, 0.0636 mmol) gave a thick colourless oil; column chromatography (hexane/ethyl acetate, 10:1) gave compound (**15b**), (62 mg, 71%); $[\alpha]_D^{22}$ +3.8 (*c* 0.31, $CHCl_3$); δ_H (400 MHz, $CDCl_3$): 7.35 – 7.27 (10H, m), 4.68 (1H, d, *J* 11.8 Hz), 4.65 (1H, d, *J* 11.8 Hz), 4.55 (2H, br.s), 4.43 (1H, dd, *J* 4.1, 11.7 Hz), 4.22 (1H, dd, *J* 5.5, 11.7 Hz), 3.86 – 3.80 (1H, m), 3.67 – 3.55 (3H, including br dd *J* 1.6, 5.4 Hz at δ 3.6), 2.45 (1H, d, *J* 8.0 Hz), 2.43 (1H, br.dd, *J* 3.8, 10.5 Hz), 1.80 – 1.00 (134H, m), 0.89 (6H, t, *J* 6.7 Hz), 0.71 – 0.61 (4H, m), 0.57 (2H, dt, *J* 3.9, 8.4 Hz), -0.32 (2H, br.q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$): 175.4, 138.0, 137.9, 128.4, 128.3, 127.8, 127.7, 127.6, 75.8, 73.5, 72.3, 72.1, 69.6, 63.5, 51.4, 35.5, 31.9, 30.2, 29.7, 29.6, 29.5, 29.4, 29.3, 28.7, 27.5, 25.8, 23.0, 15.8, 14.1, 10.9; ν_{max}: 2991, 2917, 2850, 1732, 1599, 1469 cm^{-1}.

(b) Palladium hydroxide on activated charcoal (0.0077 g) and ester **(15b)** (0.0515 g, 0.0369 mmol) gave a thick colourless oil; column chromatography (chloroform /methanol, 10:1) gave the title compound (**16b**) (40 mg, 92%) [MALDI–Found $(M+Na)^+$: 1234.2001, $C_{81}H_{158}NaO_5$, requires: 1234.2007]; $[\alpha]_D^{22}$ +1.8 (*c* 0.34, $CHCl_3$); δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 4.21 (1H, dd, *J* 4.3, 11.4 Hz), 4.10 (1H, dd, *J* 6.4, 11.5 Hz), 3.89 – 3.80 (1H, m), 3.60 (2H, br dd, *J* 4.1, 11.5 Hz), 3.54 (1H, dd, *J* 5.8, 11.6 Hz), 2.39 (1H, ddd, *J* 4.8, 7.6, 10.0 Hz), 1.86 – 0.94 (137H, m), 0.84 (6H, t, *J* 6.8 Hz), 0.65 – 0.56 (4H, m), 0.52 (2H, dt, *J* 3.9, 8.5 Hz), -0.37 (2H, br.q, *J* 4.9 Hz); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 175.5, 72.6, 69.8, 65.1, 63.0, 52.5, 37.0, 35.0, 31.8, 30.1, 29.6, 29.55, 29.5, 29.45, 29.4, 29.3, 29.2, 28.6, 27.4, 25.3, 22.6, 15.7, 14.0, 10.8; ν_{max}: 3585, 2917, 2849, 1734, 1468 cm^{-1}.

4.9 (*S*)-2,3-Dihydroxypropyl(*R*)-2-((*R*)-1-hydroxy-18-((1*R*,2*S*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl)tetracosanoate (16c)

(a) Cesium hydrogen carbonate (0.0436 g, 0.225 mmol), tosylate (**14**) (0.0211 g, 0.0495 mmol) and acid **11c** (0.0552 g, 0.0450 mmol) [36] gave a thick colourless oil which was purified by column chromatography (hexane/ethyl acetate, 10:1) to afford compound **(15c)**, (41 mg, 62%); $[\alpha]_D^{22}$ +9.0 (*c* 0.31, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.32 – 7.19 (10H, m), 4.62 (1H, d, *J* 11.8 Hz), 4.58 (1H, d, *J* 11.8 Hz), 4.48 (2H, br s), 4.36 (1H, dd, *J* 4.1, 11.7 Hz), 4.15 (1H, dd, *J* 5.5, 11.7 Hz), 3.81 – 3.72 (1H, m), 3.60 – 3.49 (3H, including br dd, *J* 1.6, 5.4 Hz, at 3.53), 3.28 (3H, s), 2.90 (1H, br.p, *J* 4.1 Hz), 2.39 (1H, d, *J* 7.9 Hz), 2.36 (1H, br.dd, *J* 3.5, 7.4 Hz), 1.75 – 0.93 (143H, m), 0.83 (6H, t, *J* 6.8 Hz), 0.79 (3H, d, *J* 6.9 Hz), 0.65 – 0.54 (2H, m), 0.50 (1H, dt, *J* 4.0, 8.4 Hz), -0.39 (1H, br q, *J* 5.2 Hz); δ_C (101MHz, $CDCl_3$): 175.3, 137.9, 137.8, 128.3, 128.2, 127.7, 127.6, 127.5, 85.3, 75.7, 73.4, 72.2, 72.0, 69.5, 63.4, 57.6, 51.3, 38.6, 35.4, 35.2, 32.3, 31.8, 30.4, 30.3, 30.1, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 28.8, 28.6, 27.5, 27.4, 26.0, 25.7, 23.6, 22.9, 22.6, 15.7, 14.8, 14.0, 13.9, 10.9, 10.8; ν_{max}: 2923, 2853, 1737, 1465 cm^{-1}.

(b) Palladium hydroxide on activated charcoal (0.0054 g) and ester **(15c)** (0.0364 g, 0.0245mmol) gave a thick colourless oil; column chromatography (chloroform/methanol, 20:1) gave the title compound **(16c)** (27 mg, 85%); [MALDI–Found $(M+Na)^+$: 1322.2890, $C_{86}H_{170}NaO_6$, requires: 1322.2895]; $[\alpha]_D^{23}$ +1.6 (*c* 2.1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$ + few

drops of CD_3OD): 4.21 (1H, dd, *J* 4.3, 11.4 Hz), 4.11 (1H, dd, *J* 6.4, 11.4 Hz), 3.88 – 3.82 (1H, m), 3.66 – 3.57 (2H, including br dd, *J* 4.3, 11.4 Hz at δ 3.6), 3.54 (1H, dd, *J* 5.8, 11.6 Hz), 3.31 (3H, s), 2.94 (1H, br.p, *J* 4.4 Hz), 2.40 (1H, ddd, *J* 4.8, 7.4, 10.2 Hz), 1.81 – 0.94 (146H, m), 0.84 (6H, t, *J* 7.0 Hz), 0.81 (3H, d, *J* 6.9 Hz), 0.65 – 0.57 (2H, m), 0.52 (1H, dt, *J* 4.0, 8.5 Hz), -0.37 (1H, br.q, *J* 5.1 Hz); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 175.5, 85.5, 72.6, 69.8, 65.1, 63.0, 57.6, 52.4, 35.3, 35.0, 32.3, 31.8, 30.4, 30.1, 29.9, 29.8, 29.7, 29.6, 29.55, 29.5, 29.45, 29.4, 29.3, 28.6, 27.5, 27.4, 26.0, 25.3, 22.6, 15.7, 14.7, 14.0, 10.8; ν_{max}: 3368, 2918, 2850, 1731, 1467 cm^{-1}.

4.10 (*S*)-2,3-dihydroxypropyl (*R*)-2-((*R*)-1-hydroxy-12-((1*S*,2*R*)-2-(14-((1*S*,2*R*)-2-eicosylcyclopropyl)tetradecyl)cyclopropyl)dodecyl)hexacosanoate (16e)

(a) Cesium hydrogen carbonate (0.0605 g, 0.312 mmol), tosylate (**14**) (0.0293 g, 0.0687 mmol) and acid **11e** (0.0711 g, 0.0624 mmol) [33] gave a thick colourless oil which was purified by column chromatography (hexane/ethyl acetate, 10:1) to afford compound **(15e)** (51 mg, 58%); $[\alpha]_D^{23}$ +3.6 (*c* 0.88, $CHCl_3$); δ_H (400 MHz, $CDCl_3$): 7.37 – 7.26 (10H, m), 4.68 (1H, d, *J* 11.8 Hz), 4.65 (1H, d, *J* 11.9 Hz), 4.55 (2H, br.s), 4.43 (1H, dd, *J* 4.0, 11.7 Hz), 4.22 (1H, dd, *J* 5.5, 11.7 Hz), 3.86 – 3.80 (1H, m), 3.67 – 3.56 (3H, including br dd *J* 1.6, 5.4 Hz at 3.60), 2.45 (1H, d, *J* 7.9 Hz), 2.43 (1H, br.dd, *J* 3.5, 7.4 Hz) 1.85 – 0.97 (134H, m), 0.89 (6H, t, *J* 7.2 Hz), 0.70 – 0.61 (4H, m), 0.60 – 0.57 (2H, dt, *J* 4.0, 8.5 Hz), -0.32 (2H, br q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$): 175.4, 138.0, 137.8, 128.4, 128.3, 127.7, 127.6, 127.5, 121.9, 75.7, 73.4, 72.3, 72.0, 69.5, 63.4, 51.3, 35.5, 31.9, 30.1, 29.7, 29.6, 29.5, 29.45, 29.4, 29.3, 28.6, 27.4, 25.7, 22.6, 15.7, 14.0, 10.8; ν_{max}: 3435, 2917, 2850, 1732, 1468 cm^{-1}.

(b) Palladium hydroxide on activated charcoal (0.0063g) and ester **(15e)** (0.0425g, 0.0305 mmol) gave a thick oil; column chromatography (chloroform/methanol, 20:1) gave the title compound **(16e)** (27 mg, 74%) [MALDI–Found $(M+Na)^+$: 1234.2001, $C_{81}H_{158}NaO_5$, requires: 1234.2007]; $[\alpha]_D^{23}$ +2.3 (*c* 5.2, $CHCl_3$); δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 4.22 (1H, dd, *J* 4.4, 11.5 Hz), 4.12 (1H, dd, *J* 6.4, 11.5 Hz), 3.89 – 3.83 (1H, m), 3.67 – 3.58 (2H, including br dd, *J* 4.0, 11.1 Hz at 3.61), 3.55 (1H, dd, *J* 5.8, 11.6 Hz), (1H, ddd, *J* 4.4, 7.9, 10.0 Hz), 1.79 – 0.96 (137H, m), 0.85 (6H, t, *J* 6.8 Hz), 0.66 – 0.57 (4H, m), 0.53 (2H, dt, *J* 4.4, 8.4 Hz), -0.36 (2H, br.q, *J* 5.1 Hz); δ_C (126 MHz, $CDCl_3$ + few drops of CD_3OD): 175.4, 72.4, 69.6, 65.0, 62.8, 52.4, 31.7, 30.0, 29.4, 29.1, 29.0, 28.5, 27.2, 25.1, 22.4, 15.5, 13.8, 10.6; ν_{max}: 3400, 3017, 2917, 2850, 1733, 1468 cm^{-1}.

4.11 (*S*)-2,3-dihydroxypropyl (*R*)-2-((*R*)-1-hydroxy-18-((1*R*,2*S*)-2-((17*S*,18*S*)-17-methoxy-18-methylhexatriacontyl)cyclopropyl)octadecyl)hexacosanoate (16f)

(a) Cesium hydrogen carbonate (0.0351 g, 0.181 mmol), tosylate (**14**) (0.0170 g, 0.0399 mmol) and acid **11f** (0.0455 g, 0.0362 mmol) [38]. A thick colourless oil residue which was purified by column chromatography (hexane/ethyl acetate, 10:1) to afford compound **(15f)** (40 mg, 72%); $[\alpha]_D^{23}$ +6.4 (*c* 0.50, $CHCl_3$); δ_H (400 MHz, $CDCl_3$): 7.39 – 7.26 (10H, m), 4.70 (1H, d, *J* 11.8 Hz), 4.66 (1H, d, *J* 11.8 Hz), 4.57 (2H, br.s), 4.44 (1H, dd, *J* 4.1, 11.6 Hz), 4.23 (1H, dd, *J* 5.5, 11.7 Hz), 3.85 (1H, m), 3.69 – 3.57 (3H, including br dd, *J* 1.6, 5.4 Hz at

3.62), 3.37 (3H, s), 2.98 (1H, br.p, *J* 3.8 Hz), 2.47 (1H, d, *J* 7.9 Hz), 2.44 (1H, br.dd, *J* 4.5, 8.5 Hz), 1.88 – 1.03 (147H, m), 0.91 (6H, t, *J* 7.1 Hz), 0.89 (3H, d, *J* 6.8 Hz), 0.71 – 0.63 (2H, m), 0.58 (1H, dt, *J* 4.0, 8.3 Hz), -0.31 (1H, br.q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$): 175.4, 128.4, 128.3, 127.7, 127.6, 127.5, 85.3, 75.7, 73.4, 72.2, 72.0, 69.5, 68.0, 63.4, 57.6, 51.3, 38.6, 35.4, 35.2, 32.3, 31.8, 30.4, 30.3, 30.1, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 28.8, 28.6, 27.5, 27.4, 26.0, 22.6, 15.7, 14.8, 14.0, 10.8; ν_{max}: 3030, 2923, 2853, 1733, 1496 cm^{-1}.

(b) Palladium hydroxide on activated charcoal (0.0032g) and ester (**15f**) (0.0215g, 0.0142 mmol) gave a thick colourless oil; column chromatography (chloroform/methanol, 20:1) gave the title compound (**16f**) (18 mg, 92%) [MALDI–Found $(M+Na)^+$: 1350.3203, $C_{88}H_{154}NaO_6$, requires: 1350.3208]; $[\alpha]_D^{23}$ +1.9 (*c* 0.74, $CHCl_3$); δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 4.22 (1H, dd, *J* 4.2, 11.5 Hz), 4.12 (1H, dd, *J* 6.4, 11.5 Hz), 3.89 – 3.83 (1H, m), 3.71 – 3.58 (2H, including br.dd, *J* 4.1, 11.5 Hz at δ 3.61), 3.55 (1H, dd, *J* 5.8, 11.5 Hz), 3.31 (3H, s), 2.94 (1H, br.p, *J* 3.7 Hz), 2.40 (1H, ddd, *J* 4.8, 7.4, 10.4 Hz), 1.68 – 0.94 (150H, m), 0.85 (6H, t, *J* 6.9 Hz), 0.82 (3H, d, *J* 6.9 Hz), 0.65 – 0.58 (2H, m), 0.52 (1H, dt, *J* 4.0, 8.1 Hz), -0.37 (1H, br.q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$ + few drops of CD_3OD): 175.5, 85.5, 72.6, 69.7, 65.1, 63.0, 58.0, 52.5, 35.2, 35.0, 33.0, 31.8, 30.4, 30.1, 29.8, 29.7, 29.6, 29.55, 29.5, 29.4, 29.3, 29.2, 29.1, 28.6, 27.4, 27.3, 26.0, 25.3, 22.6, 15.7, 14.7, 14.0, 10.8; ν_{max}: 3389, 3017, 2919, 2850, 1733, 1467 cm^{-1}.

4.12 (*S*)-2,3-dihydroxypropyl(2*R*)-2-((1*R*)-1-hydroxy-16-((1*R*,2*S*)-2-(20-methyl-19-oxo-octatriacontyl)cyclopropyl)hexadecyl)hexacosanoate (16g)

(a) Cesium hydrogen carbonate (0.0572 g, 0.295 mmol), tosylate (**14**) (0.0277 g, 0.0649 mmol) and acid **11g** (0.0731 g, 0.0590 mmol) [35] gave a thick colourless oil which was purified by column chromatography (hexane/ethyl acetate, 10:1) to afford compound **(15g)**, (52 mg, 59%); $[\alpha]_D^{23}$ +6.5 (*c* 0.55, $CHCl_3$); δ_H (400 MHz, $CDCl_3$): 7.31 – 7.05 (10H, m), 4.60 (1H, d, *J* 11.8 Hz), 4.57 (1H, d, *J* 11.8 Hz), 4.47 (2H, br.s), 4.35 (1H, dd, *J* 4.0, 11.7 Hz), 4.14 (1H, dd, *J* 5.5, 11.7 Hz), 3.80 – 3.71 (1H, m), 3.58 – 3.48 (3H, including br dd, *J* 1.4, 5.4 Hz at 3.52), 2.48 – 2.38 (2H, including OH proton at 2.43), 2.38 – 2.29 (3H, including dt, *J* 5.4, 7.9 Hz, at 2.35), 1.75 – 1.01 (144H, m), 0.98 (3H, d, *J* 6.9 Hz), 0.81 (6H, t, *J* 6.7 Hz), 0.62 – 0.53 (2H, m), 0.49 (1H, dt, *J* 4.0, 8.5 Hz), -0.40 (1H, br.q, *J* 5.1 Hz); δ_C (101 MHz, $CDCl_3$): 215.0, 175.7, 138.0, 128.4, 128.3, 127.7, 127.6, 127.5, 75.7, 73.4, 72.2, 72.0, 69.5, 63.4, 51.3, 46.2, 41.0, 35.4, 33.0, 31.8, 30.1, 29.6, 29.55, 29.5, 29.4, 29.35, 29.3, 29.2, 28.6, 27.4, 27.2, 25.7, 23.7, 23.6, 22.6, 16.3, 15.7, 14.0, 10.8; ν_{max}: 2918, 2850, 1717, 1467 cm^{-1}.

(b) As above, using palladium hydroxide on activated charcoal (0.0075 g) and ester **(15g)** (0.0505 g), eluting with chloroform/methanol (40:1) gave the title compound **(16g)** (38 mg, 87%) [MALDI–Found $(M+Na)^+$: 1334.2890, $C_{87}H_{170}NaO_6$, requires: 1334.2895]; $[\alpha]_D^{23}$ +2.8 (*c* 3.6, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$ + few drops of CD_3OD): 4.22 (1H, dd, *J* 4.2, 11.4 Hz), 4.11 (1H, dd, *J* 6.5, 11.4 Hz), 3.89 – 3.81 (1H, m), 3.68 – 3.57 (2H, including br dd *J* 4.3, 11.5 Hz at δ 3.61), 3.54 (1H, dd, *J* 5.8, 11.6 Hz), 2.53 – 2.44 (1H, m), 2.38 (3H, including br.t, *J* 2.38), 1.68 – 1.05 (147H, m), 1.01 (3H, d, *J* 6.9 Hz), 0.84 (6H, t, *J* 6.7 Hz), 0.65 – 0.57 (2H, m), 0.52 (1H, dt, *J* 4.1, 8.4 Hz), -0.37 (1H, br q, *J* 5.2 Hz); δ_C (126 MHz, $CDCl_3$ + few drops of CD_3OD): 216.0, 175.5, 72.6, 69.7, 65.1, 63.0, 52.5, 46.3, 41.1, 35.0,

32.9, 31.8, 30.1, 29.7, 29.6, 29.55, 29.5, 29.45, 29.4, 29.35, 29.3, 29.25, 29.2, 29.15, 29.1, 28.6, 27.4, 27.2, 25.3, 23.6, 22.6, 16.2, 15.7, 14.0, 10.8; ν_{max}: 3396, 3017, 2922, 2853, 1713, 1467 cm^{-1}.

Acknowledgements

OTA wishes to thank the Iraqi Ministry of Higher Education and Scientific Research and Mosul University for the award of a PhD studentship. We thank the EPSRC UK National Mass Spectrometry Facility at Swansea University and Dr Paul Gates (Bristol University) for carrying out accurate mass measurements.

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Highlights:

Nine examples of R- and S-glycerol mycolates (GroMMs) based on synthetic mycolic acids matching the overall structures of alpha-, keto- and methoxy-mycolic acids of *Mycobacterium tuberculosis* and other mycobacteria.