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Preparation of the tri-arabino di-mycolate fragment of mycobacterial arabinogalactan from defined synthetic mycolic acids

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ABSTRACT

An efficient synthetic approach to tri-arabino di-mycolates, using structurally defined synthetic α-, keto and methoxy mycolic acids is described.

1. Introduction

Tuberculosis (TB) is one of the world's deadliest diseases and is caused by *Mycobacterium tuberculosis*, which has a single known host (man) [1,2]. The air-borne nature of this bacterium makes the disease highly infectious, and the World Health Organization (WHO) has reported that one third of the world's population is probably infected with latent TB, and that there is a new case every second [3]. The mycobacterial cell wall has a complex structure made up of lipids, glycolipids, polysaccharides and proteins [4]. There has been growing interest in the construction of D-arabino-oligosaccharides related to mycobacterial cell wall components, arabino-galactan, lipoarabinomannan and arabinomannan from *M. tuberculosis* [5]. The mycolyl-arabinogalactan (mAG) complex is the largest component structure and forms from cross bonding between both D-arabinofuranosyl (Ara*f*) and galactofuranosyl (Gal*f*) with a long-chain α-alkyl-branched β-hydroxylated fatty acid, 'mycolic acid' (MA) [6]. The complex lipids and polysaccharides within the cell wall of *M. tuberculosis* are assumed to be the cause of its characteristic pathogenesis [7].

Anderson and Geiger in 1937 reported the first extraction of arabino-mycolate from the cell wall of *Mycobacterium bovis* using organic solvent [8]. Azuma et al. reported the isolation of arabinose mycolate under acidic conditions [9]. The structure of the mycolyl-arabinogalactan complex of *M. tuberculosis* was reinterpreted using mass spectrometry and NMR spectroscopy [10,11]. Synthetic arabino-furanosyl oligosaccharides, including a branched pentasaccharide were reported in 1998 [12,13]. In 2005, the preparation of a tetra-mycolylpentaarabinose (**1**) (Fig. 1) using a complex natural mixture of MAs was described [14]. However, only in 2010 were structural studies of the composition of the arabinose mycolates of the cell wall of *M. bovis* reported [15]. Ishiwata et al. reported the synthesis of a series of mono- (**3**), di- and tetra-arabinomycolates (**1**) found at the terminal position of the cell wall skeleton of BCG from *M. bovis*, using natural MA mixtures extracted from cells. All of the compounds showed strong TNF-α inducing activity *in vitro*. Such arabinose mycolates have also been reported to show anti-cancer properties [6]. The mechanism of the activity of arabinomycolates is not clear [14].

Although a methoxy trisaccharide of arabinofuranose **2** (Fig. 1) has been prepared and used in an acylation reaction with fatty acids such as behenic acid, palmitic acid and butyric acid [16], there are no examples of the synthesis of arabinofuranose oligosaccharides esterified with structurally defined complete synthetic mycolic acids (MAs) which are the main components of the cell wall of *M. tuberculosis* [6]. MAs **4** are β-hydroxy fatty acids with a long α-alkyl side chain (Fig. 2) [17,18].

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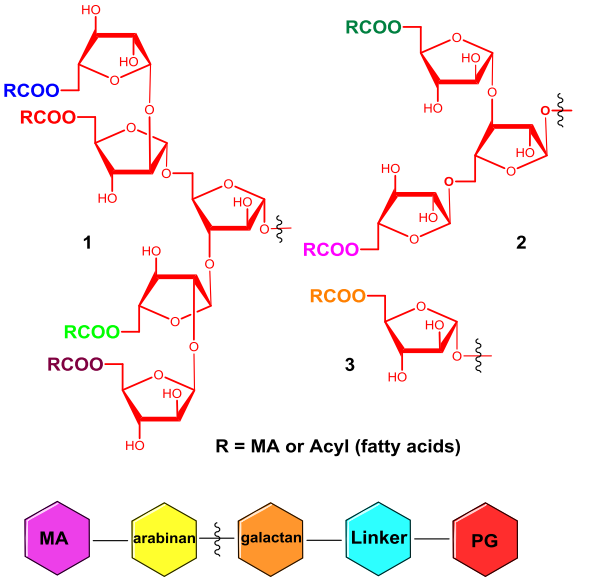


Fig. 1. Structure of targets of mycoloyl-arabinans.

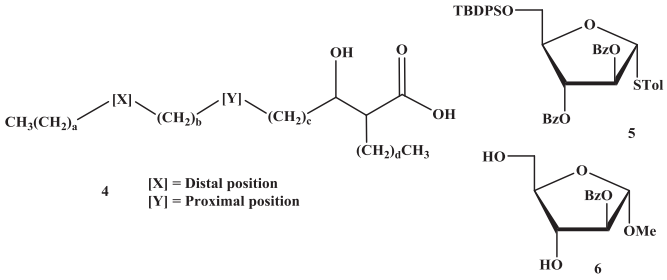


Fig. 2. Generalised MA structure and arabinose building blocks.

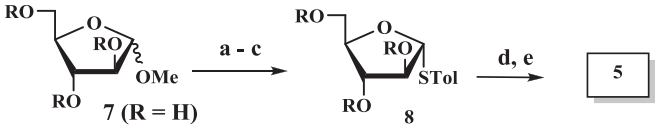
MAs in *M. tuberculosis* have 80–90 carbons, compared to those from corynebacterium (30–36 carbons), *Rhodococcus* (34–38 carbons) and *Nocardia* (46–60 carbons) [19,20]. Natural mixtures extracted from mycobacteria consist of many individual MA containing a range of functional groups, X and Y, including *cis*- and *trans*-cyclopropanes, *cis*- and *trans*-alkenes, methoxy and keto fragments, and different chain lengths [21].

The aim of this study was to synthesize a series of dimycolated triarabinoses (**2**), comprising single defined synthetic mycolic acids, in order that the selectivity of their immune stimulatory activity, e.g. in inducing Interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α), could be evaluated.

2. Results and discussion

2.1. Synthesis of trisaccharides

The target trisaccharide structure **2** has two α-glycosidic linkages, which can be assembled readily from known building blocks, donor **5** [22] and acceptor **6** [23]. The route to the 2,3-*O*-benzoyl-protected thioglycosyl donor **5** was slightly modified compared to the literature method (Scheme 1) [22]. D-(−)-Arabinose was treated with HCl, freshly prepared by addition of acetyl chloride to anhydrous methanol at 0 °C. Work up with pyridine rather than ammonium carbonate [24], gave methyl-α, β-D-Ara*f*, **7** (R = H), with predominant formation of the α-anomer (α/β-D, 3:2) [25,26]. Rather than esterifying with benzoate, the mixture was directly



Scheme 1. Reagents and conditions: (a) Ac_2O, pyridine, 0 °C, **7** (R = Ac) 80%; (b) TolSH (1.2 equiv.), $BF_3.Et_2O$ (5.1 equiv.), CH_2Cl_2, 0 °C/RT, 8 h, **8** (R = Ac), 65%, (c) $NaOCH_3$ (5 equiv.), CH_3OH, RT, 3 h, **8** (R = H), 90%; (d) t-$BuPh_2SiCl$ (1.5 equiv), imidazole, DMF, 0 °C/RT, 16 h, 82%; (e) BzCl (4.6 equiv.), pyridine, 0 °C/RT, 2 h, 83%.

esterified to give triacetate **7** (R = Ac) [22]. Condensation of this with *p*-thiotoluene in the presence of boron trifluoride etherate afforded **8** (R = Ac), which on reaction with $NaOCH_3$ in methanol afforded thioglycoside triol **8** (R = H). Protection of the primary alcohol as a silyl ether followed by benzoylation of the secondary alcohols gave **5**.

The initial step in the synthetic route to acceptor **6** [23], the separation of the two anomers of **7** (R = H), was carried out using two methods (Supplementary data, Schemes 1 and 2).

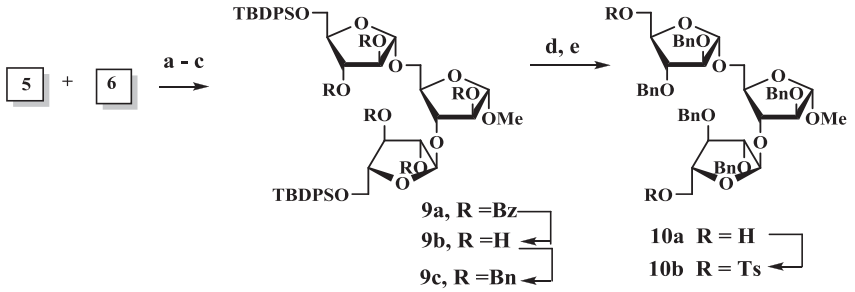
Liu et al. [16] have reported the coupling of the mono-saccharide building blocks to prepare the trisaccharide through the reaction of a 2-*O*-benzylated glycosyl acceptor and thioglycoside **5** using silver trifluoromethane sulfonate and *N*-iodosuccinimide in dry CH_2Cl_2. In the present work, the benzoylated glycosyl acceptor **6** and thioglycoside **5** were reacted under the reported conditions (Scheme 2). Trisaccharide **9a** was obtained as a single stereoisomer in 87% yield, which is similar to that reported for the benzyl compound [16]. The product showed the expected three acetal signals in the proton NMR spectrum, two broad singlets at δ 5.10 and 5.33, for the newly formed α-glycosidic links, and a third singlet at 5.56; these correlated by HSQC with three signals in the carbon spectrum for the glycosidic links at δ 107.0, 106.0 and 105.3 respectively. Compound **9a** was deprotected with sodium methoxide to give **9b** as a thick oil in 81% yield. This was benzylated to protect the five secondary hydroxyl groups using benzyl bromide and sodium hydride in dry DMF to give **9c**. Desilylation of the two primary hydroxyl groups gave **10a**. Direct esterification of the primary hydroxyl group of Ara*f* with a carboxyl group in natural mycolic acid mixtures has been achieved in a low yield (30%) [14], while activating the sugar as a tosylate raised the yield to 79%. Therefore, the two hydroxyl groups in compound **10a** were tosylated using *p*-toluenesulfonyl chloride in dry pyridine in the presence of catalytic 4-dimethylaminopyridine to afford the tosylate **10b** (Scheme 2).

The next step was the coupling of trisaccharide **10b** with the structurally defined synthetic mycolic acids, which had been prepared earlier [27–29]. Firstly, a model tri-arabino-di mycolate was prepared by coupling tosylate **10b** with palmitic acid through the alkylative esterification strategy using cesium hydrogen carbonate in dry DMF: THF at 70 °C for four days [14], to give compound **11a**, which was then debenzylated to give compound **12a** (Scheme 3). The synthesis of this glycolipid has been reported using a different method. Data obtained for **12a** was identical to that reported [16].

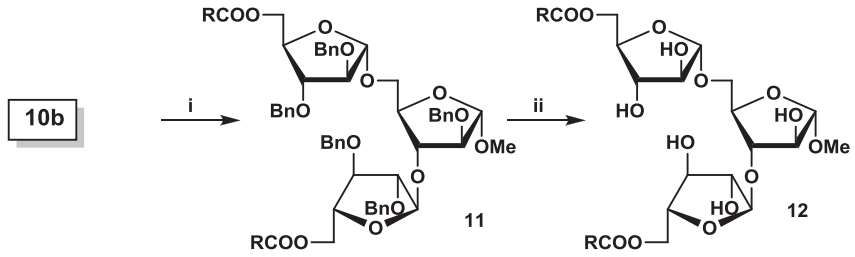
A series of triarabino-dimycolates were then prepared according to the above procedure; the structures of the mycolic acid moieties, including α-, methoxy- and keto-classes are shown in Scheme 3. In each case, the products the proton NMR spectra included the expected three characteristic broad single-hydrogen singlets for the acetal hydrogens in the region δ 4.9–5.2.

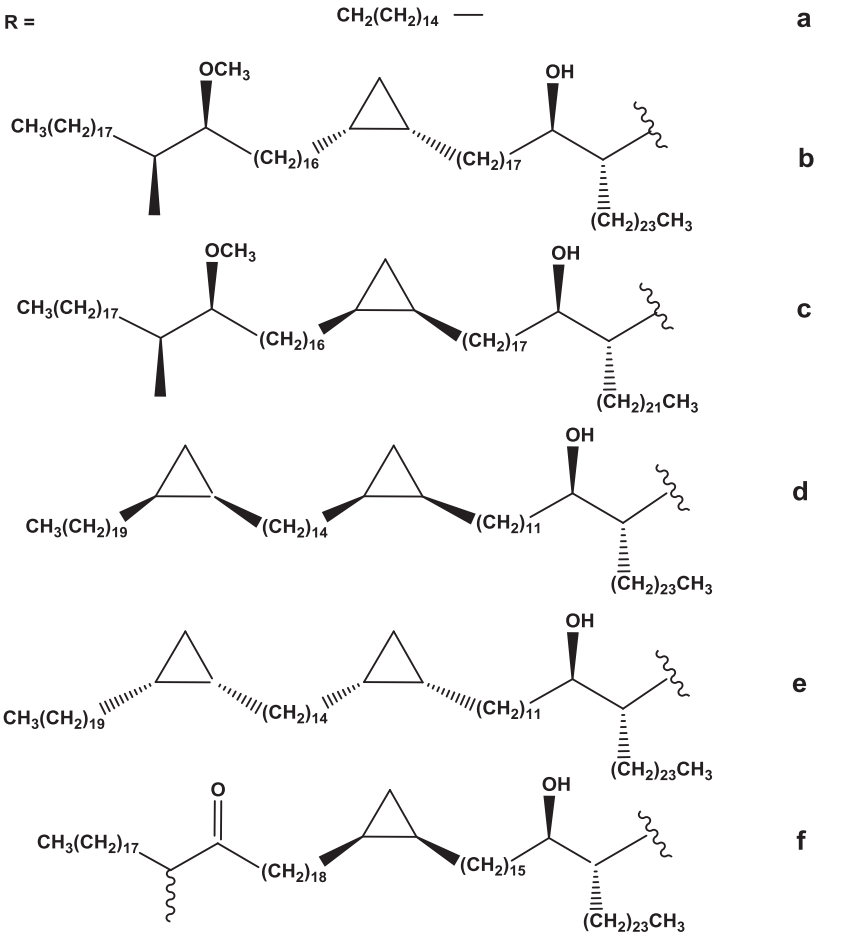
2.2. IL-6 and TNF-α secretion assays with the synthesized compounds

Ishiwata et al. reported the synthesis of a series of mono-, di- and tetra-arabinomycolates found in the terminal position of the



Scheme 2. Reagents and conditions: (a) NIS, AgOTf, (**9a**, 87%); (b) $NaOCH_3$ (cat.), CH_3OH, rt, 2 h, (**9b**, 86%); (C) NaH, BnBr, DMF, 0 °C/rt 16 h, (**9c**, 79%); (d) TBAF, THF, 0 °C/rt, 16 h (**10a** 77%); (e) TsCl, pyridine, DMAP, CH_2Cl_2 at 0 °C (**10b**, 74%).





Scheme 3. Reagents and conditions: (i) $CsHCO_3$, THF-DMF, 4 days, 70 °C; (ii) $Pd(OH)_2$, H_2, CH_2Cl_2-MeOH, RT, overnight.

cell-wall skeleton of bacillus calmette Guerin from *M. bovis*, by using natural mycolic acid mixtures extracted from the cell wall and showed they differentially induce TNF- α secretion in a murine macrophage cell line, some at levels similar to trehalose dimycolate

(TDM) [14]. Arabino-mycolates obtained by acid hydrolysis from the originally prepared CWS (SMP-105) of *M. bovis* BCG Tokyo 172, consisting mainly of mono-arabinose mono-mycolate, penta-arabinose tetra-mycolate and hexa-arabinose tetra-mycolate fractions, significantly induced TNF-α production with an intensity comparable to that of cell-wall skeleton, a potent adjuvant, and enhanced delayed type per sensitivity reactions against inactivated tumor cells. The induced TNF-α production was completely dependent on TLR2 and MyD88 pathways [6]. To provide an initial evaluation of the activities of synthesized tri-arabino di-mycolates reported here (**12c** and **12f**) as immune potentiators, secretion inducing assays for IL-6 and TNF-α, cytokines involved in inflammation, were conducted, in comparison with TDM and synthetic mono-arabino mono-mycolates [30]. Surprisingly, neither compound was strongly stimulatory in either assay when compared to synthetic TDMs, trehalose monomycolates or arabinose mycolates [31,32]. Further work to evaluate the biological effects of these molecules is under way.

3. Experimental section

For general experimental detail, see Supplementary Information. Unless otherwise stated, all products were single components by TLC and by proton and carbon NMR.

*3.1. Methyl 2,3-di-O-benzyl-5-O-p-toluenesulfonyl-α-D-arabino-furanosyl-(1 → 3)-[2,3-di-O-benzyl-5-O-p-toluene- sulfonyl-α-D-arabinofuranosyl-(1 → 5)]-2-O-benzyl-α-D-Araf **10b***

(a) Molecular sieves 4 Å (6.6 g) were added to a stirred solution of the acceptor **6** (0.9 g, 3.3 mmol) and the donor **5** (6.0 g, 8.5 mmol) in dry CH_2Cl_2 (30 mL) at r. t. under a nitrogen atmosphere. The reaction mixture was stirred for 30 min then cooled to -60 °C and N-iodosuccinimide (2.09 g, 9.29 mmol) was added followed by the addition of AgOTf (0.36 g, 1.40 mmol). The mixture was stirred at the same temperature until the color became red/dark brown and TLC showed no starting material, then it was quenched by the addition of triethylamine (1 mL) until it turned yellow. The mixture was diluted with CH_2Cl_2 (100 mL) and filtered through celite and the solvent was evaporated under reduced pressure. Column chromatography on silica eluting with hexane/ethyl acetate (5:1) gave compound **9a** as a thick oil (4.2 g, 87%) [Found (MALDI) $(M + Na)^+$: 1447.6, $C_{83}H_{84}NaO_{18}Si_2$, requires: 1447.5], $[\alpha]_D^{20}$ +16 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 8.07 (2H, d, *J* 7.9 Hz), 8.02–7.91 (8H, m), 7.70–7.62 (9H, m), 7.59–7.41 (8H, m), 7.40–7.28 (18H, m), 5.64 (1H, d, *J* 4.5 Hz), 5.61 (1H, d, *J* 4.2 Hz), 5.56 (1H, s), 5.57 (1H, s), 5.53 (1H, br. s), 5.41 (1H, s), 5.32 (1H, br. s), 5.10 (1H, br. s), 4.49–4.35 (4H, m), 4.08 (1H, dd, *J* 11.3, 5.1 Hz), 3.97 (5H, m), 3.44 (3H, s), 1.01 (9H, s), 0.98 (9H, s); δ_C (101 MHz, $CDCl_3$): 165.5, 165.4, 165.2, 165.1, 135.63, 135.6, 135.5, 133.3, 133.2, 133.15, 133.1, 133.0, 129.96, 129.9, 129.8, 129.78, 129.7, 129.6, 129.4, 129.3, 129.2, 129.1, 128.4, 128.31, 128.3, 128.2, 127.6, 107.0, 106.0, 105.3, 84.0, 83.4, 82.5, 82.0, 81.8, 81.6, 80.6, 77.2, 77.1, 66.1, 63.5, 63.3, 54.7, 26.7, 26.6, 19.3, 19.2; ν_{max}: 3069, 3010, 2929, 2859, 1724, 1602, 1451, 1070, 708 cm^{-1}.

(b) A solution of sodium methoxide (2 mL, 1 M, in methanol) was added to a stirred solution of **9a** (0.20 g, 0.14 mmol) in dry MeOH: CH_2Cl_2 (1:1, 5 mL) at r. t. until a pH of 11 was obtained. The mixture was stirred at r. t. for 2 h then the solvent was evaporated under reduced pressure to give an oil. The residue was purified by column chromatography on silica eluting with $CHCl_3$:MeOH (5:2) to give **9b** compound as a thick oil (0.1 g, 83%) [Found (MALDI) $(M + Na)^+$: 927.1, $C_{48}H_{64}NaO_{13}Si_2$, requires: 927.3], $[\alpha]_D^{22}$ +50 (*c* 0.10, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$ + few drops CD_3OD): 7.49–7.34 (20H, m), 5.19 (1H, s), 5.14 (1H, s), 4.86 (1H, s), 4.25–4.20 (2H, m), 4.18 (1H, br. d, *J* 1.8 Hz), 4.12 (2H, br. d, *J* 3.8 Hz), 4.05–4.02 (4H, m), 4.01–3.95 (2H, m), 3.80 (1H, dd, *J* 11.4, 2.3 Hz), 3.77–3.73 (2H, m), 3.70 (2H, br. d, *J* 11.3 Hz), 3.34 (3H, s), 3.01–2.70 (4H, m), 1.10–0.99 (18H, m); δ_C (101 MHz, $CDCl_3$ + few drops CD_3OD): 135.7, 135.6, 135.5, 129.5, 128.3, 128.0, 127.9, 127.8, 109.1, 108.6, 108.4, 88.0, 87.4, 83.8, 82.4, 79.3, 78.9, 78.4, 77.8, 77.7, 66.1, 64.0, 63.85, 54.8, 26.7, 26.6; ν_{max}: 3445, 3010, 2928, 2860, 1494, 1050, 824 cm^{-1}.

(c) Compound **9b** (0.2 g, 0.2 mmol) in dry DMF:THF(1:1) (4 mL) was added dropwise (1 drop each 10 s) to a stirred suspension of NaH (0.1 g, 8.3 mmol) (60% w/w, dispersion in mineral oil, washed with petrol three times) at r. t. under nitrogen. The mixture was stirred for 30 min then benzyl bromide (0.1 mL, 0.8 mmol) in dry DMF (1 mL) was added, stirred at r. t. for 16 h, then quenched by slow addition of methanol (3 mL), and water (10 mL). The organic layer was separated and the aqueous layer was re-extracted with ethyl acetate (2 × 50 mL). The combined organic layers were washed with water (20 mL) and brine (20 mL), dried and the solvent evaporated under reduced pressure. Column chromatography on silica eluting with petrol/ethyl acetate (5:1) gave the title compound as a thick oil (0.23 g, 77%) [Found (MALDI) $(M + Na)^+$: 1377.0, $C_{83}H_{94}NaO_{13}Si_2$, requires: 1377.6], $[\alpha]_D^{22}$ +40 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 7.66–7.51 (7H, m), 7.42–7.17 (38H, m), 5.19 (1H, s), 5.17 (1H, s), 4.94 (1H, s), 4.59–4.37 (10H, m), 4.28 (1H, dd, *J* 6.3, 2.3 Hz), 4.22–4.14 (2H, m), 4.14–4.03 (5H, m), 4.03–3.95 (2H, m), 3.86–3.74 (5H, m), 3.37 (3H, s), 1.03 (18H, s); δ_C (101 MHz, $CDCl_3$): 135.7, 135.65, 135.62, 135.6, 129.6, 129.54, 129.51, 128.5, 128.4, 128.35, 128.34, 128.3, 128.22, 128.2, 127.9, 127.8, 127.79, 127.75, 127.73, 127.66, 127.63, 127.6, 127.56, 127.53, 127.4, 127.35, 127.33, 127.3, 127.28, 107.2, 106.5, 105.4, 88.5, 88.3, 88.0, 83.1, 82.8, 82.3, 81.8, 81.2, 80.4, 72.0, 71.9, 71.73, 71.7, 71.6, 66.2, 63.5, 63.3, 54.7, 26.8, 26.7; ν_{max}: 3466, 3050, 3017, 2958, 2930, 2858, 1493, 1112, 701 cm^{-1}.

(d) Tetrabutylammonium fluoride (0.3 mL, 0.3 mmol, 1 M) was added dropwise to a stirred solution of **9c** (0.2 g, 0.147 mmol) in dry THF (10 mL) at 0 °C under nitrogen atmosphere. The mixture was allowed to reach r. t. and stirred for 16 h then the solvent was evaporated under reduced pressure to give an oil which was purified by column chromatography on silica eluting with hexane/ethyl acetate (1:1) to give compound **10a** as a colourless thick oil (0.1 g, 77%) [Found (MALDI) $(M + Na)^+$: 901.3, $C_{51}H_{58}NaO_{13}$, requires: 901.3], $[\alpha]_D^{20}$ +90 (*c* 0.1, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.38–7.21 (25H, m), 5.16 (1H, s), 5.12 (1H, d, *J* 1.2 Hz), 4.96 (1H, d, *J* 1.2 Hz), 4.61–4.42 (9H,m), 4.38 (1H, d, *J* 12.0 Hz), 4.30 (1H, dd, *J* 7.4, 3.7 Hz), 4.25 (1H, ddd, *J* 6.3, 5.2, 2.9 Hz), 4.16–4.07 (3H, m), 4.03 (1H, dd, *J* 3.7, 1.2 Hz), 4.01 (1H, dd, *J* 3.7, 1.2 Hz), 3.95 (1H, dd, *J* 11.6, 3.8 Hz), 3.91 (1H, dd, *J* 6.6, 2.9 Hz), 3.85–3.71 (4H, m), 3.66 (1H, dd, *J* 12.2, 5.2 Hz), 3.59 (1H, dd, *J* 12.2, 5.8 Hz), 3.40 (3H, s), 2.84 (1H, br. s), 2.48 (1H, br. s); δ_C (101 MHz, $CDCl_3$): 137.7, 137.6, 137.4, 137.3, 137.2, 128.5, 128.44, 128.43, 128.4, 128.38, 127.96, 127.94, 127.9, 127.87, 127.83, 127.8, 127.71, 106.9, 106.2, 105.8, 88.7, 88.2, 87.5, 83.0, 82.9, 82.3, 81.9, 80.9, 79.7, 72.3, 72.2, 72.0, 71.9, 71.8, 64.9, 62.8, 62.7, 54.9; ν_{max}: 3467, 3050, 3017, 2926, 2861, 1609, 1494, 1050, 824 cm^{-1}.

(e) 4-Toluenesulfonyl chloride (2.82 g, 14.7 mmol) was added to a stirred solution of **10a** (1.30 g, 1.47 mmol), pyridine (1.17 g, 1.19 mL, 14.7 mmol) and DMAP (catalytic amount) in dry CH_2Cl_2 (10 mL) at 0 °C under nitrogen. The mixture was allowed to reach r. t. and stirred for 16 h, then diluted with ethyl acetate (100 mL) and water (50 mL), the organic layer was separated and the aqueous layer was re-extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with water (50 mL) and brine (50 mL), dried. The solvent was evaporated under reduced pressure to give an oil; column chromatography on silica eluting with hexane/ethyl acetate (1:1) afforded the title compound as a colorless thick oil **10b** (1.3 g, 74%) [Found (MALDI) $(M + Na)^+$: 1209.2, $C_{65}H_{70}NaO_{17}S_2$, requires:

1209.3], $[\alpha]_D^{22}$ +71 (*c* 0.10, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 7.7 (2H, d, *J* 8.3 Hz), 7.8 (2H, d, *J* 8.4 Hz), 7.37–7.23 (29H, m), 5.09 (1H, br. s), 5.08 (1H, br. s), 4.93 (1H, s), 4.61–4.42 (9H, m), 4.35 (1H, d, *J* 12.0 Hz), 4.30–4.24 (1H, m), 4.22–4.10 (6H, m), 4.10–4.05 (2H, m), 4.0 (1H, dd, *J* 3.3, 1.2 Hz), 3.96 (1H, dd, *J* 3.1, 1.0 Hz), 3.87 (2H, dd, *J* 6.1, 3.1 Hz), 3.84 (1H, dd, *J* 12.0, 4.0 Hz), 3.69 (1H, dd, *J* 12.0, 2.5 Hz), 3.38 (3H, s), 2.38 (3H, s), 2.4 (3H, s); δ_C (101 MHz, $CDCl_3$): 144.8, 144.7, 137.5, 137.4, 137.3, 137.2, 137.1, 132.7, 132.6, 129.8, 129.7, 128.4, 128.38, 128.35, 128.33, 128.3, 128.0, 127.93, 127.9, 127.85, 127.82, 127.8, 127.7, 127.69, 127.67, 106.9, 106.4, 105.5, 88.0, 87.6, 87.4, 82.8, 82.7, 80.5, 80.4, 79.0, 78.7, 72.2, 72.0, 71.8, 71.81, 71.7, 68.7, 68.6, 65.7, 54.8, 21.5; ν_{max}: 3088, 3064, 3031, 2924, 2862, 1598, 1454, 1177, 738 cm^{-1}.

*3.2. Methyl5-O-[(2R)-2-(1-hydroxy-18-[(1S,2R)-2-[(17S, 18S) -17-methoxy-18-methylhexatriacontyl)cyclopropyl]octadecyl]-hexacosanoate)]-α-D-arabinofuranosyl-(1 → 3)-5-O-[(2R)-2-(1-hydroxy-18-[(1S,2R)-2-[(17S,18S)-17-methoxy-18-methylhexatriacontyl)-cyclopropyl]octadecyl]hexacosanoate)]-α-D-arabinofuranosyl-(1 → 5)]-α-D-Araf **12b***

(a) Cesium hydrogencarbonate (0.065 g, 0.335 mmol) was added to a stirred solution of **10b** (0.040 g, 0.033 mmol) and (2*R*)-2-{1-hydroxy-18-[(1*S*,2*R*)-2-[(17S,18S)-17-methoxy-18-methylhexatriacontyl]cyclopropyl]octadecyl}hexacosa-noic acid [28] (0.092 g, 0.073 mmol) in dry DMF: THF (1:5, 3 mL) at r. t. under a nitrogen atmosphere. The mixture was stirred at 70 °C for 4 days, then worked up and purified as before to afford **11b** as a colourless thick oil (0.051 g, 45%) [Found (MALDI) $(M + Na)^+$: 3371.9, $C_{221}H_{390}NaO_{19}$, requires: 3371.9], $[\alpha]_D^{23}$ +35 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 7.37–7.23 (25H, m), 5.18 (1H, br. s), 5.13 (1H, br. s), 4.91 (1H, br. s), 4.49–4.37 (9H, m), 4.34 (1H, d, *J* 11.7 Hz), 4.32–4.24 (6H, m), 4.18 (1H, ddd, *J* 9.7, 6.3, 3.0 Hz), 4.11 (1H, dd, *J* 4.4, 2.7 Hz), 4.08 (1H, br. d, *J* 2.9 Hz), 4.00 (1H, dd, *J* 2.9, 0.6 Hz), 3.96 (1H, br. d, *J* 2.3 Hz), 3.93 (1H, dd, *J* 7.8, 3.9 Hz), 3.88–3.78 (2H, m), 3.76 (1H, dd, *J* 11.4, 1.8 Hz), 3.67–3.57 (2H, m), 3.37 (3H, s), 3.35 (6H, s), 2.99–2.93 (2H, m), 2.67 (1H, d, *J* 6.1 Hz), 2.65 (1H, d, *J* 6.5 Hz), 2.41 (2H, dt, *J* 9.0, 5.8 Hz), 1.68–1.03 (294H, m), 0.89 (12H, t, *J* 6.8 Hz), 0.86 (6H, d, *J* 6.9 Hz), 0.71–0.61 (4H, m), 0.57 (2H, dt, *J* 7.6, 4.0 Hz), −0.33 (2H, br. q, *J* 5.1 Hz); δ_C (101 MHz, $CDCl_3$): 175.1, 175.0, 137.7, 137.6, 137.5, 137.4, 137.2, 128.5, 128.41, 128.4, 127.95, 127.9, 127.8, 127.73, 127.7, 107.0, 106.3, 105.5, 88.2, 87.9, 87.8, 85.4, 83.7, 80.6, 80.4, 79.3, 79.1, 72.3, 72.2, 72.1, 71.98, 71.8, 65.6, 63.0, 57.7, 54.8, 51.9, 51.7, 35.3, 32.4, 31.9, 30.5, 30.2, 29.97, 29.9, 29.7, 29.66, 29.5, 29.4, 28.7, 27.6, 26.2, 22.7, 15.8, 14.9, 14.1, 10.9; ν_{max}: 3517, 3063, 3031, 2922, 2852, 1736, 1466, 1101, 757 cm^{-1}.

(b) Palladium (II) hydroxide on activated charcoal (20% $Pd(OH)_2$-C, 12 mg, 0.3 fold by weight) was added to a stirred solution of compound **11b** (0.042 g, 0.014 mmol) in dry CH_2Cl_2: MeOH (1:1, 2 mL) at r. t. under a hydrogen. The mixture was stirred for 16 h, then filtered and the solvent was evaporated under reduced pressure; column chromatography on silica eluting with $CHCl_3$:MeOH (5:1) gave the title compound **12b** as a thick oil (0.03 g, 82%) [Found $(M + Na)^+$: 2921.8, $C_{186}H_{360}NaO_{19}$, requires: 2921.7], $[\alpha]_D^{20}$ +21 (*c* 0.10, $CHCl_3$) which showed δ_H (500 MHz, $CDCl_3$ + few drops CD_3OD): 4.98 (1H, br. s), 4.95 (1H, br. s), 4.77 (1H, br. s), 4.40 (1H, dd, *J* 11.8, 4.3 Hz), 4.33 (1H, dd, *J* 11.6, 4.5 Hz), 4.24 (1H, dd, *J* 11.6, 4.7 Hz), 4.15 (1H, dd, *J* 11.6, 4.3 Hz), 4.13–4.07 (2H, m), 4.07–3.94 (5H, m), 3.94–3.80 (3H, m), 3.67–3.55 (3H, m), 3.36 (3H, s), 3.32 (6H, s), 2.96–2.90 (2H, m), 2.37–2.30 (2H, m), 1.61–0.97 (301H, m), 0.82 (12H, t, *J* 7.0 Hz), 0.79 (6H, d, *J* 6.9 Hz), 0.64–0.55 (4H, m), 0.50 (2H, dt, *J* 8.0, 4.0 Hz), −0.39 (2H, br. q, *J* 5.1 Hz); δ_C (126 MHz, $CDCl_3$ + few drops CD_3OD): 175.1, 175.0, 109.1, 107.9, 107.2, 85.5, 83.0, 82.4, 81.8, 81.0, 79.8, 77.9, 72.5, 72.45, 65.9, 63.4, 63.1, 57.5, 54.8, 52.8, 50.0, 35.2, 34.8, 34.7, 32.2, 31.8, 30.3, 30.1, 29.82, 29.8, 29.62, 29.6, 29.56, 29.5, 29.4, 29.3, 29.2, 29.1, 28.6, 27.4, 27.3, 25.96, 25.2, 25.2, 22.5, 15.6, 14.7, 13.9, 10.7; ν_{max}: 3401, 2919, 2851, 1733, 1467, 1099, 720 cm^{-1}.

*3.3. Methyl 5-O-(2-[(R)-1-hydroxy-18-[(1R,2S)-2-[(17S, 18S)-17-methoxy-18-methylhexatriacontyl]cyclopropyl]-octadecyl]-tetracosanoate)-α-D-arabinofuranosyl-(1 → 3)-[5-O-(2-[(R)-1-hydroxy-18-[(1R,2S)-2-[(17S,18S)-17-methoxy-18-methyl-hexatriacontyl]cyclopropyl]octadecyl]-tetracosanoate)-α-D-arabinofuranos-yl-(1 → 5)]-α-D-Araf **12c***

(a) Cesium hydrogencarbonate (0.081 g, 0.417 mmol) was added to a stirred solution of **10b** (0.050 g, 0.042 mmol) and (2*R*)-2-{(1*R*)-1-hydroxy-18-[(2*S*)-2-[(17*S*,18*S*)-17-methoxy-18-ethylhexatriacontyl]cyclopropyl]octadecyl}tetracosanoic acid [33] (0.113 g, 0.092 mmol) in dry DMF: THF (1:5, 6 mL) at r. t. under nitrogen. The mixture was stirred at 70 °C for 4 days, then work up and purification as before gave **11c** as a colourless thick oil (50 mg, 36%) [Found $(M + Na)^+$: 3315.8808, $C_{217}H_{382}NaO_{19}$, requires: 3315.8818], $[\alpha]_D^{23}$ +40 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 7.39–7.22 (25H, m), 5.18 (1H, s), 5.13 (1H, s), 4.91 (1H, s), 4.61–4.42 (9H, m), 4.38 (1H, d, *J* 12.0 Hz), 4.30–4.23 (6H, m), 4.22–4.16 (1H, m), 4.14–4.05 (2H, m), 4.03 (1H, dd, *J* 3.2, 1.0 Hz), 3.98 (1H, dd, *J* 3.2, 1.0 Hz), 3.95 (1H, dd, *J* 12.0, 4.6 Hz), 3.86–3.80 (2H, m), 3.78 (1H, dd, *J* 12.0, 2.3 Hz), 3.67–3.57 (2H, m), 3.37 (3H, s), 3.35 (6H, s), 3.01–2.92 (2H, m), 2.67 (2H, br. s), 2.45–2.37 (2H, m), 1.72–1.07 (285 H, m), 0.89 (12H, t, *J* 6.8 Hz), 0.86 (6H, d, *J* 6.9 Hz), 0.71–0.60 (4H, m), 0.61–0.53 (2H, dt, *J* 7.6, 4.0 Hz), −0.32 (2H, br. q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$): 175.1, 174.9, 137.6, 137.51, 137.5, 137.4, 137.2, 128.45, 128.41, 128.4, 128.38, 128.37, 127.9, 127.85, 127.8, 127.74, 127.7, 107.0, 106.3, 105.5, 88.2, 87.9, 87.8, 85.4, 83.7, 83.6, 80.6, 80.4, 79.3, 79.1, 72.3, 72.2, 72.1, 72.0, 71.9, 71.7, 65.5, 63.0, 57.7, 54.8, 51.8, 51.7, 35.3, 32.4, 31.9, 30.5, 30.2, 29.98, 29.9, 29.7, 29.6, 29.5, 29.4, 28.7, 27.6, 27.4, 26.2, 22.7, 15.7, 14.8, 14.1, 10.9; ν_{max}: 3436, 2920, 2851, 1733, 1734, 1174, 734 cm^{-1}.

(b) Palladium (II) hydroxide on activated charcoal (20% $Pd(OH)_2$-C, 0.01 g, 0.30 fold by weight) was added to a stirred solution of compound **11c** (0.037 g, 0.010 mmol) in dry CH_2Cl_2: MeOH (1:1, 2 mL) at r. t. under hydrogen. After 16 h, work up and purification as before gave the title compound **12c** as a white oil (21 mg, 65%) [Found $(M + Na)^+$: 2865.6424, $C_{182}H_{352}NaO_{19}$, requires: 2865.6470], $[\alpha]_D^{16}$ +24 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$ + few drops CD_3OD): 5.02 (1H, d, *J* 1.5 Hz), 4.98 (1H, br. s), 4.80 (1H, br. s), 4.46 (1H, dd, *J* 11.7, 4.3 Hz), 4.37 (1H, dd, *J* 11.6, 4.8 Hz), 4.27 (1H, dd, *J* 11.7, 4.9 Hz), 4.16 (1H, dd, *J* 12.0, 4.8 Hz), 4.14–4.08 (2H, m), 4.08–3.99 (5H, m), 3.95–3.87 (3H, m), 3.62–3.52 (3H, m), 3.35 (3H, s), 3.31 (6H, s), 2.94–2.85 (2H, m), 2.44–2.34 (2H, m), 1.37–1.13 (292H, m), 0.85 (12H, t, *J* 6.8 Hz), 0.82 (6H, d, *J* 6.9 Hz), 0.66–0.57 (4H, m), 0.56–0.49 (2H, dt, *J* 7.6, 4.0 Hz), −0.37 (2H, br. q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$ + few drops CD_3OD): 175.2, 175.0, 109.1, 107.9, 107.2, 85.5, 82.9, 82.5, 82.0, 81.8, 81.0, 80.9, 79.7, 78.0, 72.5, 66.9, 65.9, 63.1, 57.6, 54.8, 52.8, 35.2, 32.3, 31.8, 30.4, 30.1, 29.8, 29.7, 29.66, 29.6, 29.5, 29.3, 29.2, 28.6, 27.4, 26.0, 22.5, 15.7, 14.7, 14.0, 10.8; ν_{max}: 3412, 2919, 2850, 1731, 1466, 1099, 720 cm^{-1}.

*3.4. Methyl 5-O-(2-[(R)-1-hydroxy-12-{(1R,2S)-2-(14-[(1R, 2S)-2-eicosylcyclopropyl]tetradecyl]cyclopropyl}dodecyl)hexacosanoate)-α-D-arabinofuranosyl-(1 → 3)-[5-O-(2-[(R)-1-hydroxy-12-{(1R,2S)-2-(14-[(1R,2S)-2-eicosylcyclopropyl]tetradecyl]cyclopropyl}dodecyl)hexacosanoate)-α-D-arabinofuranosyl-(1 → 5)]-α-D-Araf **12d***

(a) Cesium hydrogencarbonate (0.089 g, 0.458 mmol) was added to a stirred solution of **10b** (0.055 g, 0.046 mmol) and (2*R*)-2-{(1*R*)-

1-hydroxy-12-[(1*R*)-2-[14-[(2*S*)-2-eicosyl-cyclopropyl]tetradecyl] cyclopropyl]dodecyl}hexacosanoic acid [27] (0.115 g, 0.101 mmol) in dry DMF: THF (1:5, 6 mL) at r. t. under hydrogen. The mixture was stirred at 70 °C for 4 days then work up and purification as before afforded **11d** as a colourless oil (56 mg, 18%) [Found (M + Na)$^+$: 3139.7057, $C_{207}H_{358}NaO_{17}$, requires: 3139.7041], $[\alpha]_D^{23}$ +72 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 7.37–7.22 (25H, m), 5.18 (1H, br. s), 5.14 (1H, br. s), 4.92 (1H, br. s), 4.61–4.43 (9H, m), 4.35 (1H, d, *J* 12.0 Hz), 4.32–4.25 (6H, m), 4.22–4.16 (1H, m), 4.09–4.03 (2H, m), 4.01 (1H, dd, *J* 3.0, 1.0 Hz), 3.97 (1H, dd, *J* 3.0, 1.0 Hz), 3.92 (1H, dd, *J* 12.0, 7.5 Hz), 3.88–3.80 (2H, m), 3.77 (1H, dd, *J* 12.0, 2.2 Hz), 3.62–3.55 (2H, m), 3.38 (3H, s), 2.68–2.52 (2H, m), 2.46–2.36 (2H, m), 1.70–1.06 (268H, m), 0.89 (12H, t, *J* 6.8 Hz), 0.71–0.62 (8H, m), 0.60–0.54 (4H, dt, *J* 7.6, 4.0 Hz), −0.32 (4H, br. q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$): 175.1, 174.9, 137.6, 137.51, 137.5, 137.4, 137.2, 128.5, 128.4, 128.38, 128.37, 128.0, 127.9, 127.8, 127.71, 127.7, 107.0, 106.3, 105.5, 88.2, 87.9, 87.8, 83.6, 80.6, 80.4, 79.3, 79.1, 72.3, 72.2, 72.0, 71.9, 71.7, 65.6, 63.0, 54.8, 51.85, 51.7, 35.4, 35.2, 31.9, 30.2, 29.8, 29.75, 29.7, 29.6, 29.5, 29.4, 29.2, 28.7, 27.5, 27.4, 25.7, 22.7, 18.5, 18.3, 15.7, 14.1, 10.9; ν_{max}: 3584, 3064, 3032, 2918, 2850, 1733, 1455, 1018, 732 cm^{-1}.

(b) Palladium (II) hydroxide on activated charcoal (20% $Pd(OH)_2$-C, 6.6 mg, 0.30 fold by weight) was added to a stirred solution of compound **11d** (0.030 g, 0.009 mmol) in dry CH_2Cl_2: MeOH (1:1, 2 mL) at r. t. under hydrogen. After16 h, work up and purification as before gave the title compound **12d** as a colourless oil (17 mg, 64%) [Found (M + Na)$^+$: 2689.4696, $C_{172}H_{328}NaO_{17}$, requires: 2689.4694], $[\alpha]_D^{16}$ +36 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$ + few drops CD_3OD): 5.03 (1H, d, *J* 1.5 Hz), 4.99 (1H, br. s), 4.81 (1H, br. s), 4.48 (1H, dd, *J* 11.9, 4.3 Hz), 4.39 (1H, dd, *J* 11.7, 4.7 Hz), 4.28 (1H, dd, *J* 11.5, 4.8 Hz), 4.17 (1H, dd, *J* 11.4, 4.3 Hz), 4.13–3.09 (2H, m), 4.06–3.98 (5H, m), 3.97–3.86 (3H, m), 3.69–3.58 (3H, m), 3.36 (3H, s), 2.51–2.42 (2H, m), 2.42–2.35 (2H, m), 1.70–1.00 (271H, m), 0.86 (12H, t, *J* 6.8 Hz), 0.67–0.58 (8H, m), 0.57–0.50 (4H, dt, *J* 7.6, 4.0 Hz), −0.36 (4H, br. q, *J* 5.1 Hz); δ_C (101 MHz, $CDCl_3$ + few drops CD_3OD): 175.2, 175.0, 109.0, 107.8, 107.3, 83.1, 82.9, 81.5, 81.1, 80.0, 77.97, 72.99, 72.9, 65.7, 63.6, 63.2, 54.9, 52.8, 52.7, 31.9, 30.3, 30.2, 29.8, 29.75, 29.7, 29.6, 29.5, 29.4, 28.7, 27.4, 22.7, 15.8, 14.1, 10.9; ν_{max}: v. br. 3400, 2919, 2851, 1732, 1467, 1099, 760 cm^{-1}.

*3.5. Methyl-5-O-(2-{(R)-1-hydroxy-12-[(1S,2R)-2-(14-[(1S, 2R)-2-eicosylcyclopropyl]tetradecyl)cyclopropyl]dodecyl}-hexacosanoate)-α-D-arabinofuranosyl-(1 → 3)-[5-O-(2-{(R)-1-hydroxy-12-[(1S,2R)-2-(14-[(1S,2R)-2-eicosylcyclopropyl] tetradecyl)-cyclopropyl]dodecyl}hexacosanoate)-α-D-arabinofuranosyl-(1 → 5)]-α-D-Araf **12e***

1. (a) Cesium hydrogencarbonate (0.081 g, 0.417 mmol) was added to a stirred solution **10b** (0.050 g, 0.042 mmol) and 2-{(*R*)-1-hydroxy-12-[(1*S*,2*R*)-2-(14-[(1*S*,2*R*)-2-eicosylcyclo-propyl]tetradecyl)cyclopropyl]dodecyl}hexacosanoic acid [27] (0.105 g, 0.092 mmol) in dry DMF: THF (1:5, 3 mL) at r. t. under hydrogen. The mixture was stirred at 70 °C for 4 days, then work and purification as before gave a colourless oil, compound **11e** (31 mg, 11%) [Found (MALDI) (M + Na)$^+$: 3139.7, $C_{207}H_{358}NaO_{17}$ requires: 3139.7], $[\alpha]_D^{23}$ +80 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 7.35–7.27 (25H, m), 5.18 (1H, br. s), 5.13 (1H, br. s), 4.91 (1H, br. s), 4.61–4.39 (9H, m), 4.40 (1H, d, *J* 12.8 Hz), 4.35 (1H, dd, *J* 9.4, 4.6 Hz), 4.32–4.23 (5H, m), 4.21–4.17 (1H, m), 4.11–4.05 (2H, m), 4.00 (1H, br. d, *J* 2.7 Hz), 3.98–3.95 (1H, m), 3.95–3.91 (1H, m), 3.90–3.85 (2H, m), 3.76 (1H, dd, *J* 11.7, 2.2 Hz), 3.66–3.57 (2H, m), 3.37 (3H, s), 2.69–2.62 (2H, m), 2.45–2.36 (2H, m), 1.64–1.07 (268H, m), 0.89 (12H, t, *J* 6.9 Hz), 0.70–0.61 (8H, m), 0.56 (4H, dt, *J* 7.6, 4.0 Hz), −0.33 (4H, br. q, *J* 5.2 Hz); δ_C (101 MHz, $CDCl_3$): 175.1, 175.0, 137.7, 137.5, 137.4, 137.3, 137.2, 128.5, 128.4, 128.38, 128.37, 127.95, 127.9, 127.8, 127.73, 127.7, 107.0, 106.3, 105.5, 88.2, 87.9, 87.8, 83.6, 80.6, 79.2, 79.1, 72.3, 72.2, 72.1, 72.0, 71.9, 71.7, 65.6, 63.0, 60.4, 54.8, 51.8, 51.7, 31.9, 30.2, 29.72, 29.7, 29.6, 29.5, 29.4, 28.7, 22.6, 15.8, 14.1, 10.9; ν_{max}: 3524, 3035, 2918, 2850, 1735, 1466, 1018, 734 cm^{-1}.

2. (b) Palladium (II) hydroxide on activated charcoal (20% $Pd(OH)_2$-C, 8.2 mg, 0.30 fold by weight) was added to a stirred solution of compound **11e** (0.027 g, 0.008 mmol) in dry CH_2Cl_2: MeOH (1:1, 2 mL) at r. t. under hydrogen. After 16 h, work up and purification as before gave the title compound **12e** as a colourless oil (23.1 mg, 97%) [Found (MALDI) (M + Na)$^+$: 2689.4, $C_{172}H_{328}NaO_{17}$, requires: 2689.4], $[\alpha]_D^{20}$ +40 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$+ few drops CD_3OD): 4.94 (1H, d, *J* 1.3 Hz), 4.92 (1H, br. s), 4.73 (1H, br. s), 4.35 (1H, dd, *J* 11.8, 4.7 Hz), 4.29 (1H, dd, *J* 11.5, 3.9 Hz), 4.20 (1H, dd, *J* 11.5, 4.8 Hz), 4.13 (1H, dd, *J* 11.7, 4.8 Hz), 4.09–4.02 (2H, m), 4.02–3.92 (5H, m), 3.89–3.77 (3H, m), 3.61–3.49 (3H, m), 3.28 (3H, s), 2.38–2.29 (2H, m), 1.51–0.94 (274H, m), 0.79 (12H, t, *J* 6.8 Hz), 0.61–0.52 (8H, m), 0.47 (4H, dt, *J* 7.6, 4.0 Hz), −0.42 (4H, br. q, *J* 5.1 Hz); δ_C (101 MHz, $CDCl_3$ + few drops CD_3OD): 175.1, 175.0, 109.0, 107.9, 107.3, 82.9, 82.2, 81.8, 81.6, 81.0, 80.9, 79.7, 77.8, 77.5, 72.3, 66.0, 63.4, 63.1, 54.7, 52.7, 49.6, 34.7, 31.7, 30.0, 29.9, 29.6, 29.5, 29.4, 29.2, 29.1, 29.0, 28.5, 27.2, 22.4, 15.5, 13.8, 10.7, 10.6; ν_{max}: 3400, 2918, 2850, 1735, 1467, 1008 cm^{-1}.

*3.6. Methyl5-O-{2-((1R)-1-hydroxy-16-[(1R,2S)-2-(20-methyl-19-oxo-octatriacontyl)cyclopropyl]hexadecyl)hexa-cosanoyl}-α-D-arabinofuranosyl-(1 → 3)-[2,3-di-O-benzyl-5-O-{2-(1R)-1-hydroxy-16-[(1R,2S)-2-(20-methyl-19-oxoocta-triacontyl) cyclopropyl]hexadecyl)hexacosanoyl}-α-D-arabinofuranosyl-(1 → 5)]-α-D-Araf **12f***

(a) Cesium hydrogencarbonate (0.081 g, 0.417 mmol) was added to a stirred solution of **10b** (0.050 g, 0.042 mmol) and (2*R*)-2-{(1*R*)-1-hydroxy-16-[(2*S*)-2-[20-methyl-19-oxo-octa-triacontyl]cyclo-propyl]hexadecyl}hexacosanoic acid [29] (0.114 g, 0.092 mmol) in dry DMF: THF (1:5, 6 mL) at r. t. under nitrogen. The mixture was stirred at 70 °C for 4 days, then work up and purification as before gave compound **11f** as a colourless thick oil (0.05 g, 36%) [Found (MALDI) (M + Na)$^+$: 3339.7, $C_{219}H_{382}NaO_{19}$, requires: 3339.8], $[\alpha]_D^{23}$ +62 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 7.34–7.25 (25H, m), 5.18 (1H, br. s), 5.13 (1H, br. s), 4.91 (1H, br. s), 4.60–4.40 (9H, m), 4.34 (1 H, d, *J* 11.8 Hz), 4.32–4.24 (4H, m), 4.18 (1H, dt, *J* 10.7, 3.5 Hz), 4.11 (1H, dd, *J* 4.1, 2.9 Hz), 4.08 (1H, br. d, *J* 3.0 Hz), 4.04–4.01 (1H, m), 4.00 (1H, br. d, *J* 2.4 Hz), 3.96–3.94 (2H, m), 3.93 (1H, dd, *J* 7.4, 4.4 Hz), 3.88–3.80 (2H, m), 3.76 (1H, dd, *J* 12.0, 2.0 Hz), 3.70–3.55 (3H, m), 3.37 (3H, s), 2.67 (1H, br. s), 2.55–2.34 (8H, m), 1.69–1.10 (288H, m), 1.06 (6H, d, *J* 6.9 Hz), 0.89 (12H, t, *J* 6.8), 0.66–0.62 (4H, m), 0.61–0.51 (2H, dt, *J* 7.6, 4.0 Hz), −0.32 (2H, br. q, *J* 5.1 Hz); δ_C (101 MHz, $CDCl_3$): 215.2, 175.1, 174.96, 137.7, 137.5, 137.4, 137.2, 128.4, 128.41, 128.38, 128.36, 128.0, 127.9, 127.8, 127.73, 127.7, 107.0, 106.3, 105.5, 88.2, 87.9, 87.85, 83.7, 80.6, 80.4, 79.3, 79.1, 72.3, 72.2, 72.1, 71.97, 71.8, 68.9, 65.6, 63.0, 54.8, 51.8, 51.7, 50.9, 46.3, 41.1, 33.0, 32.1, 31.9, 30.2, 29.7, 29.66, 29.6, 29.5, 29.49, 29.4, 29.36, 29.3, 29.2, 28.7, 27.4, 27.3, 25.7, 23.7, 22.8, 22.7, 16.4, 15.7, 14.1, 10.9; ν_{max}: 3522, 3066, 2919, 2851, 1735, 1712, 1467, 1111, 756, 720 cm^{-1}.

3. (b) Palladium (II) hydroxide on activated charcoal (20% $Pd(OH)_2$-C, 13.8 mg, 0.30 fold by weight) was added to a stirred solution of compound **11f** (0.046 g, 0.013 mmol) in dry CH_2Cl_2: MeOH (1:1, 2 mL) at r. t. under hydrogen. After 16 h, work up and chromatography as before gave the title compound **12f** as a colourless oil (26 mg, 65%) [Found (M + Na)$^+$: 2889.6404, $C_{184}H_{352}NaO_{19}$, requires: 2889.6470], $[\alpha]_D^{23}$ +33 (*c* 0.1, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$+ few drops CD_3OD): 5.00 (1H, d, *J* 1.8 Hz), 4.96 (1H, d, *J* 0.6 Hz), 4.79 (1H, br. s), 4.43 (1H, dd, *J* 12.2,

4.6 Hz), 4.35 (1H, dd, *J* 12.2, 4.9 Hz), 4.26 (1H, dd, *J* 11.8, 5.0 Hz), 4.16 (1H, dd, *J* 11.4, 4.2 Hz), 4.13–4.07 (2H, m), 4.04–3.97 (5H, m), 3.94–3.84 (3H, m), 3.67–3.56 (4H, m), 3.34 (3H, s), 3.11 (1H, d, *J* 7.4 Hz), 3.07 (1H, d, *J* 7.3 Hz), 2.47–2.43 (3H, m), 2.41–2.3 (7H, including t at 2.38 with *J* 7.5 Hz), 1.63–1.10 (292H, m), 1.00 (6H, d, *J* 6.9 Hz), 0.84 (12H, t, *J* 6.7 Hz), 0.65–0.56 (4H, m), 0.51 (2H, dt, *J* 7.6, 4.0 Hz), −0.38 (2H, br. q, *J* 5.1 Hz); δ_C (101 MHz, $CDCl_3$ + few drops CD_3OD): 216.3, 175.1, 175.0, 109.1, 107.9, 107.3, 83.0, 82.3, 81.8, 81.7, 81.0, 80.9, 79.7, 77.9, 72.4, 72.3, 65.97, 63.1, 60.4, 54.7, 52.7, 46.2, 41.0, 34.7, 32.9, 31.7, 30.0, 29.5, 29.3, 29.2, 29.1, 28.5, 27.3, 27.1, 25.5, 23.5, 22.5, 16.1, 15.6, 13.8, 10.7; ν_{max}: 3393, 2919, 2850, 1713, 1467, 1100, 720 cm^{-1}.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.carres.2016.11.006.

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