

Article

Synthesis of a Di-Mycoloyl Tri-Arabinofuranosyl Glycerol Fragment of the Mycobacterial Cell Wall, Based on Synthetic Mycolic Acids

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Academic Editors: Ramón J. Estévez Cabanas and Vito Ferro

Received: 12 August 2019; Accepted: 1 October 2019; Published: 6 October 2019



Abstract: Fragments of mycobacterial cell walls such as arabinoglycerol mycolate and dimycoloyl diarabinoglycerol, comprising complex mixtures of mycolic acids, have immunostimulatory and antigenic properties. A related di-mycoloyl tri-arabinofuranosyl glycerol fragment has been isolated from cell wall hydrolysates. An effective stereoselective synthesis of tri-arabinofuranosyl glycerol, followed by coupling with stereochemically defined mycolic acids of different structural classes, to provide unique di-mycoloyl tri-arabinofuranosyl glycerols is now described.

Keywords: mycobacteria; cell membrane; mycolic acids; triarabinoglycerol

1. Introduction

The cell wall in *Mycobacterium tuberculosis* and in other mycobacteria has an unusual structure, containing a multi-layered and extremely hydrophobic envelope, which is important for the organism to survive in macrophages, and includes characteristic complex mixtures of long-chain (C70–C90), α -alkyl branched β -hydroxylated fatty acids, ‘mycolic acids’ (MA); these include a range of groups (Figure 1), including *cis*- and *trans*-cyclopropanes, and *cis*- and *trans*-alkenes at positions X or Y and methoxy and keto-groups at position X [1,2]. The mycoloyl–arabinogalactan complex (mAG), the largest component structure in the cell wall of mycobacteria, is believed to act as a permeability barrier that prevents the passage of antibiotics. As well as being bound to the wall, largely as penta-arabinose tetramycolates, MA are also present as non-wall-bound sugar esters, such as trehalose dimycolate (TDM) and monomycolate (TMM), and as free acids. Hydrolysis of the cell wall of *Mycobacterium bovis* gave a penta-arabinose tetramycolate, and an arabinose *mono*-mycolate, as well as hexa-arabinose, hepta-arabinose and octa-arabinose tetramycolates [3]. Isolated natural arabinomycolates possess potent adjuvant immunostimulatory activity [4,5]. The preparation of a tetramycoloyl penta-arabinose using a complex natural mixture of MAs has been described [6–10]. Such fragments have also been found to be of value in the treatment of cancer [11]. Smaller fragments, such as glycerol mycolate [12–17] and arabinoglycerol mycolate [18,19], have also been reported and, in the former case, have significant biological activity. In 1992, a new glycolipid, dimycoloyl diarabinoglycerol (DMAG) (2), was isolated from the *Mycobacterium avium*–*Mycobacterium intracellulare* complex (MAC) [20]. High immunoglobulin M (IgM) titres against the glycolipid were observed in enzyme-linked immunosorbent assays (ELISA) of serum from individuals who were culture positive for MAC infection, implying that this serodiagnosis

detects the disease in an active phase [21]. A similar glycolipid mixture was isolated from *M. bovis* Bacille Calmette-Guérin or *Mycobacterium marinum* and from *M. tuberculosis* [22]. The DMAG from *M. marinum* was found to induce the secretion of proinflammatory cytokines (tumour necrosis factor α (TNF- α), and interleukins (IL-8, and IL-1 β)) in human macrophage THP-1 cells, to trigger the expression of the protein ICAM-1 and cluster of differentiation 40 (CD40) cell surface antigens, and to modulate genes related to immune and inflammatory responses, suggesting that DMAG may drive host-pathogen interactions and participate in the immunopathogenesis of mycobacterial infections [23]. There is one report of the isolation of a dimycoloyl triarabinoglycerol (DMTAG, 3) from the degradation of the cell mycoloylarabinogalactan-peptidoglycan-protein complex from *M. tuberculosis* [24].

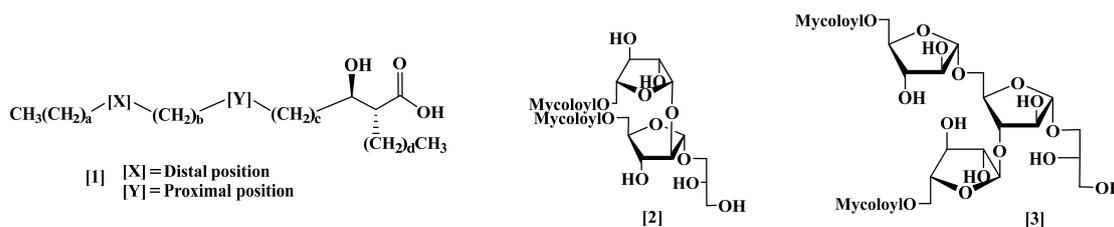
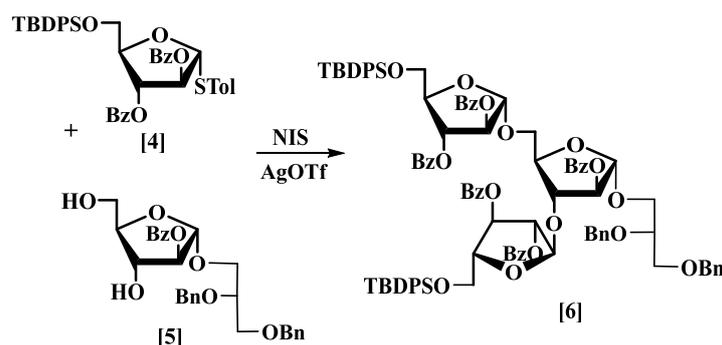


Figure 1. Generalised structure of mycolic acids (1), dimycoloyl diarabinoglycerol (DMAG) (2) and dimycoloyl triarabinoglycerol (DMTAG) (3).

We have already reported the synthesis of triarabinose dimycolates [25], of arabinoglycerol mycolates [19], and of DMAGs containing unique synthetic mycolic acids [26]. Although, so far, it has no reported bioactivity, we now report the extension of the methods used in those synthetic approaches to the synthesis of a single stereoisomer of tri-arabinofuranosyl glycerol (TAG) and of a series of stereo-defined DMTAG glycolipids (Figure 1), through esterification of the glycan with structurally defined synthetic MAs of α - and keto-classes [1,2].

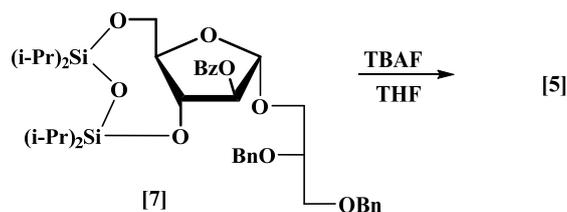
2. Results and Discussion

The target tri-saccharide structure (6) has three α -glycosidic linkages and can be assembled readily from a donor (4) [18,19] and a diol acceptor (5) (Scheme 1), a similar approach to the synthesis of the methoxy tri-saccharide of D-arabinofuranoside [25].



Scheme 1. Synthesis of fully protected glycan tri-arabino glycerol (TAG) (6).

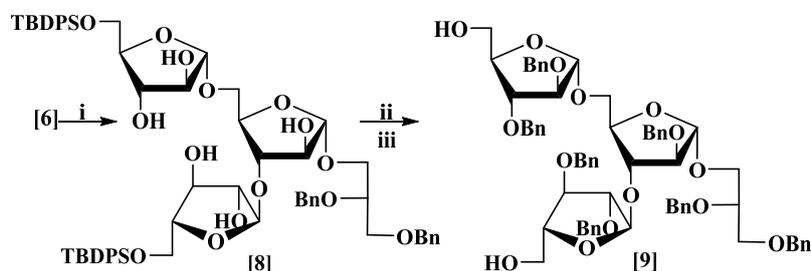
The donor (4) was prepared before [18,19]. The target acceptor (5) was obtained in 95% yield by desilylation of the protected arabinofuranosyl glycerol (7), prepared from D-arabinose [20], using tetrabutylammonium fluoride in dry THF to give two free hydroxyl groups at the C-3 and C-5 positions, respectively (Scheme 2).



Scheme 2. Synthesis of the acceptor (5).

Following the method adopted by Liu et al. [6], the thioglycoside donor (4) was coupled with the acceptor (5) in the presence of N-iodosuccinimide (NIS)/silver trifluoromethane sulfonate (AgOTf) in dry CH_2Cl_2 to give (6) in 91% yield. The $^1\text{H-NMR}$ spectrum of (6) included three broad singlets at δ 5.61, 5.31 and 5.22, corresponding to the protons at the anomeric centre of each ring. The $^{13}\text{C-NMR}$ spectrum (Figure S1, supporting information) established the presence of the α -glycosidic linkages in the tri-arabinofuranosyl glycerol (6), with the signals at δ 106.1 and 105.2 ppm belonging to the three carbons at the anomeric centres. The HSQC-NMR of (6) (Figure S2), showed the three peaks corresponding to the acetal protons, at δ 5.61, 5.31 and 5.22, correlated to their carbons, in agreement with the assignments made by Liu et al. [6,25].

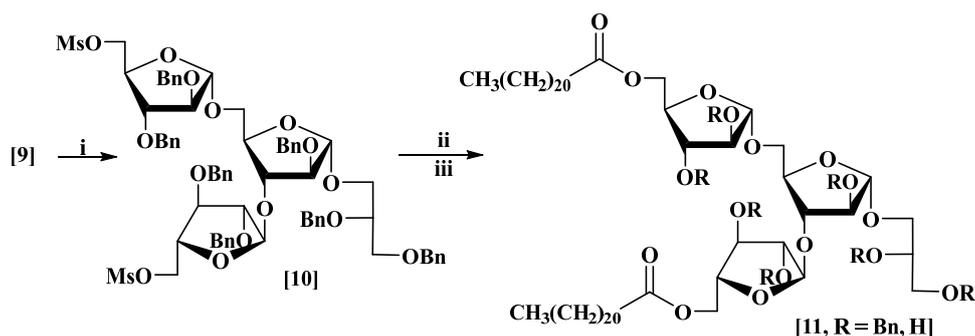
The tri-saccharide (6) was debenzoylated with sodium methoxide to give (8) as a thick oil in 83% yield; in the $^1\text{H-NMR}$ of (8), all the signals corresponding to the protons on the carbon adjacent to the benzoyl ester (6) were shifted up-field. The $^{13}\text{C-NMR}$ spectrum showed the disappearance of the carbonyl signals. Compound (8) was benzylated to protect the five secondary hydroxyl groups using benzyl bromide (BnBr) and sodium hydride in dry dimethylformamide (DMF) in 65% yield, followed by de-protection of the two primary hydroxyl groups using tetrabutylammonium fluoride (TBAF) to afford (9) in 87% yield (Scheme 3).



Scheme 3. Synthesis of compound (9). Reagents and conditions: (i) MeONa (1 M), then ethanoic acid (AcOH), 83%; (ii) benzyl bromide (BnBr), NaH, dimethylformamide (DMF), 65%; (iii) tetrabutylammonium fluoride (TBAF), tetrahydrofuran (THF), 87%.

Compound (9) was then esterified, either by an alkylative coupling using cesium hydrogen carbonate after mesylation of the primary hydroxyl groups in the glycan or by direct coupling with a fatty acid using 1-ethyl-3-(3-dimethylaminopropyl)carbodi-imide (EDCI).

In the first method, the two primary hydroxyl groups in (9) were activated using methanesulfonyl chloride (MsCl) in dry pyridine in the presence of catalytic 4-dimethylamino-pyridine (DMAP) in dry CH_2Cl_2 at 0°C to afford the corresponding mesylate (10) in 87% yield (Scheme 4). The $^1\text{H-NMR}$ spectrum of (10) (Figure S4) showed the expected signals, including two singlets at δ 2.94 and 2.89 for the methyl groups of the mesylates. The $^{13}\text{C-NMR}$ spectrum (Figure S5) showed two signals at δ 37.6 and 37.5 for the carbons of the mesylates. The assignments of the signals were made by comparison with literature values reported for the methoxy tri-arabinose compound, which is identical to (10) except for the absence of the glycerol moiety [16,17].



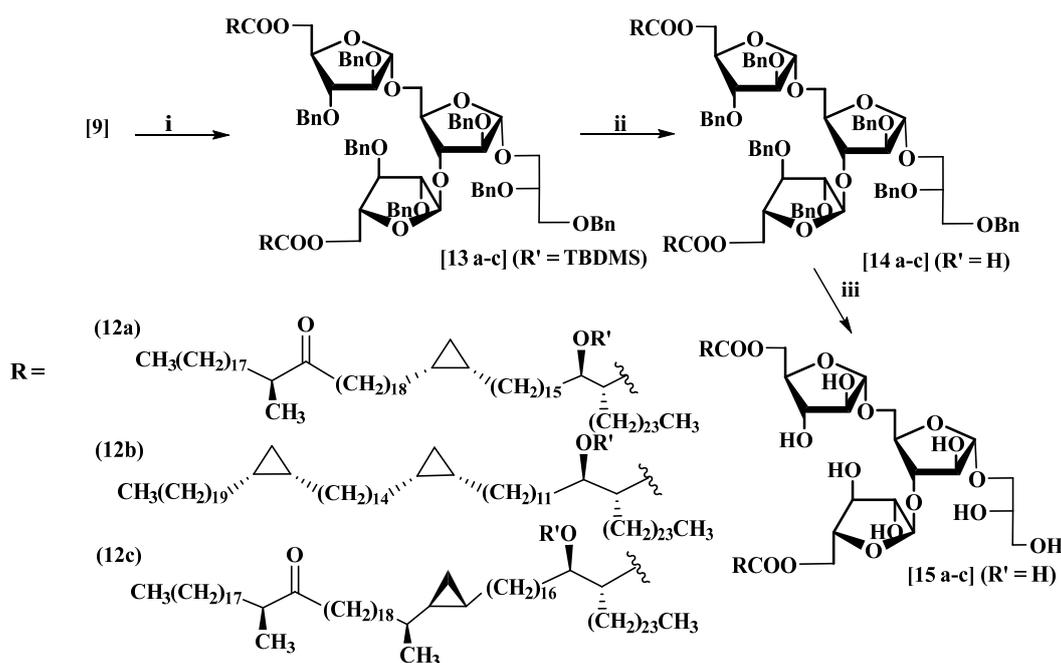
Scheme 4. Synthesis of a model di-behenoyl tri-arabinoglycerol (**11**, R = H). Reagents and conditions: (i) methanesulfonyl chloride (MsCl), 4-dimethylaminopyridine (DMAP), pyridine, 87%; (ii) CsHCO₃, behenic acid, DMF/THF, 70 °C, (**11**, R = Bn, 80%); (iii) Pd(OH)₂/C (20%), H₂, 82%.

The protected di-behenoyl-triarabinoglycerol (**11**, R = Bn) was prepared in 80% yield by coupling the mesylate (**10**) with behenic acid using cesium hydrogen carbonate in dry THF: DMF at 70 °C for 3 days (Scheme 4).

The ¹H-NMR spectrum showed three broad one-proton singlets at δ 5.09, 5.06 and 4.97, corresponding to the three protons at the anomeric centres on the glycan rings. The CH₂ groups adjacent to the carbonyls gave a triplet at δ 2.17 (*J* 7.6 Hz) integrating to four protons. The terminal methyl group showed a triplet signal at δ 0.81 (*J* 6.8 Hz) integrating to six protons. The ¹³C-NMR spectrum showed two signals at δ 173.6 and 173.5 for the carbonyl groups. Signals corresponding to the carbon at the anomeric centre for the three rings appeared at δ 106.5, 106.2 and 105.5. The carbon of the CH₂ group adjacent to the carbonyl in the acid appeared at δ 34.1. The methylene chain ranged from δ 32–22 and the terminal methyl group at δ 14.1.

Compound (**11**, R = Bn) was debenzylated by stirring vigorously in a suspension of Pd(OH)₂/C in dry CH₂Cl₂:MeOH:THF (1:1:1.5) under an atmosphere of hydrogen for 36 h to give the target DMTAG analogue (**11**, R = H) in 82% yield (Scheme 4). The ¹H-NMR spectrum (Figure S7) of compound (**11**, R = H) showed three broad singlets at δ 5.01, 4.97 and 4.90 ppm for the three α-protons, with the remaining 20 protons of the sugar and glycerol moieties appearing between δ 4.30 and 3.50 ppm. The four protons next to the two carbonyls gave a triplet at δ 2.30 ppm (*J* 7.6 Hz), while the terminal methyl groups appeared at δ 0.83 ppm as a triplet (*J* 6.5 Hz). The ¹³C-NMR spectrum obtained for the glycolipid analogue (**11**, R = H) gave signals (Figure S8), which were essentially identical to those for an analogue in the literature [25], bearing a methoxy substituent at C-1 rather than the glycerol substituent in compound (**11**, R = H), and are assigned on that basis (See Supplementary Information, Table S1).

The effects of both free mycolic acids and of their sugar esters on immune responses, and on their recognition by disease antibodies, are known to depend on the specific classes they comprise, and on the detail of their stereochemistry [2,27–29]. The glycan (**9**) was therefore then coupled with three common classes of structurally defined synthetic MAs, *cis*-cyclopropane containing keto-MA (**12a**), α-MA (**12b**) and *trans*-cyclopropane containing keto-MA (**12c**) [30–32], followed by deprotection, as in Scheme 5, to provide examples of three classes for the evaluation of their effects in these areas. In these cases, the coupling was achieved using EDCI-DMAP, which gave somewhat better yields based on the protected synthetic mycolic acids [19,25].



Scheme 5. Synthesis of dimycoloyl triarabinoglycerol (DMTAG) glycolipids. (i) RCOOH ($R' = \text{TBDMS}$) (12a–c), 1-ethyl-3-(3-dimethylaminopropyl)carbodi-imide (EDCI), DMAP, CH_2Cl_2 (a: 51%; b: 84%; c: 46%); (ii) TBAF, THF (a 76%; b: 51%; c: 66%); (iii) Pd(OH)₂/C (20%), H₂, $\text{CH}_3\text{OH}:\text{CH}_2\text{Cl}_2$: THF (1:1:1.5), rt, 36 h, (a: 86%; b: 88%; c: 82%).

The effects of the synthetic triarabinoglycerol mycolates in cytokine and chemokine stimulation, and their recognition by disease antibodies, are currently under investigation.

3. Methods

3.1. General

The chemicals used were obtained from commercial suppliers (Sigma-Aldrich, Lee on Solent, UK, and Alfa Aesar, Heysham, UK) or prepared from them by the methods described. Ether and tetrahydrofuran were dried over sodium wire and benzophenone under nitrogen, while dichloromethane was dried over calcium hydride. The petroleum spirit (petrol) used had boiling point of 40–60 °C. All reagents and solvents used were of reagent grade unless otherwise stated. Silica gel (Merck 7736) used for column and thin-layer chromatography was obtained from Sigma; separated components were detected variously using UV light, I₂ and phosphomolybdic acid solution in IMS followed by charring. Anhydrous MgSO₄ was used to dry organic solutions. Infrared (IR) spectra were carried out on a Perkin–Elmer 1600 F.T.I.R. spectrometer using liquid films or a KBr disc (solid). NMR spectra were carried out on Bruker Avance 400 or 500 spectrometers. $[\alpha]_D$ values were recorded in CHCl₃ on a POLAAR 2001 optical activity polarimeter. Matrix-assisted laser desorption/ionization (MALDI) mass spectra were provided by the Engineering and Physical Science Research Council Mass Spectrometry Service in Swansea University.

3.2. 2',3'-Di-O-benzyl-L-glycerol-(1'→1)-2-O-benzoyl- α -D-arabinofuranoside (5)

Tetrabutylammonium fluoride (17.8 mL, 0.0616 mol, 1.0 M in THF) was added dropwise with stirring to 2',3'-di-O-benzyl-L-glycerol-(1'→1)-2-O-benzoyl-3,5-O-(tetraisopropylsiloxane-1,3-diyl)- α -D-arabinofuranoside (7) [20] (6.7 g, 0.0089 mol) in anhydrous THF (50 mL) at 0 °C under nitrogen. The mixture was allowed to reach room temperature (rt) and was stirred for 4 h, and it was then diluted with EtOAc (100 mL) and water (50 mL). The aqueous layer was re-extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with sat. aq. NH₄Cl (50 mL), brine (50 mL),

dried and concentrated. Column chromatography eluting with petrol/EtOAc (1:1) gave compound (5) as a colourless thick oil (4.3 g, 95%) [MALDI: Found (M + Na)⁺: 531.2; C₂₉H₃₂NaO₈ requires: 531.2], [α]_D²³ +53 (c 4.0, CHCl₃), which showed δ _H (400 MHz, CDCl₃): 7.98–7.93 (2H, m), 7.53 (1H, t, J 7.4 Hz), 7.39 (2H, t, J 7.7 Hz), 7.33–7.17 (10H, m), 5.20 (1H, br. s), 5.04 (1H, br. s), 4.65 (1H, d, J 12.0 Hz), 4.61 (1H, d, J 12.0 Hz), 4.51 (1H, d, J 12.2 Hz), 4.48 (1H, d, J 12.2 Hz), 4.10 (2H, br. s), 3.85 (1H, dd, J 5.6, 10.3 Hz), 3.80 (1H, m), 3.76 (1H, br. p, J 5.2 Hz), 3.72–3.66 (1H, m), 3.63 (1H, dd, J 4.3, 10.3 Hz), 3.60–3.52 (2H, including br. d, J 4.8 Hz at 3.58), 2.59–2.31 (2H, incl. 2 × OH groups); δ _C (101 MHz, CDCl₃): 166.6, 138.3, 138.0, 133.6, 129.8, 129.0, 128.5, 128.4, 128.3, 127.8, 127.6, 105.3, 85.9, 84.2, 76.6, 76.4, 73.4, 72.2, 69.7, 67.1, 61.9; ν _{max}: 3405, 3065, 3031, 2945, 2868, 1715, 1465, 1105, 884, 712 cm⁻¹.

3.3. 2',3'-Di-O-benzyl-L-glycerol-(1'→1)-2,3-di-O-benzoyl-5-O-tert-butyl-diphenylsilyl- α -D-arabinofuranosyl-(1→3)-[2,3-di-O-benzoyl-5-O-tert-butyl-diphenylsilyl- α -D-arabinofuranosyl-(1→5)]-2-O-benzoyl- α -D-arabinofuranoside (6)

Molecular sieves 4 Å (1.5 g) was added with stirring to furanoside (4) [18] (16.7 g, 28.9 mmol) and furanoside (5) (4.2 g, 8.2 mmol) in dry CH₂Cl₂ (50 mL) at rt under nitrogen. The mixture was stirred for 30 min., then cooled to -36 °C and *N*-iodosucinimide (9.1 g, 0.037 mol) was added followed by silver triflate (2.1 g, 8.2 mmol). The mixture was stirred until it turned dark brown, quenched by the addition of triethylamine (2 mL) until it turned yellow, then diluted with CH₂Cl₂ (100 mL) and filtered through celite. The filtrate was evaporated under reduced pressure; column chromatography eluting with hexane/EtOAc (5:2) gave compound (6) as a colourless thick oil (13.0 g, 91%) [MALDI: Found (M + NH₄)⁺: 1682.6679; C₉₉H₁₀₄NO₂₀Si₂ requires: 1682.6685] (Figure S3), [α]_D¹⁷ -1.4 (c 2.8, CHCl₃); δ _H (400 MHz, CDCl₃): 8.01–7.92 (10H, m), 7.70–7.67 (4H, m), 7.65–7.61 (4H, m), 7.58–7.53 (2H, m), 7.50–7.44 (3H, m), 7.41–7.21 (32H, m), 5.66–5.62 (2H, incl. br. d, J 4.8 Hz at 5.63), 5.61 (1H, br.s), 5.55 (1H, br.d, J 1.3 Hz), 5.51 (1H, br.d, J 0.9 Hz), 5.43 (1H, br.d, J 0.9 Hz), 5.31 (1H, br.s), 5.22 (1H, br.s), 4.68 (2H, br.s), 4.53 (1H, d, J 12.0 Hz), 4.49 (1H, d, J 12.0 Hz), 4.47 (1H, br.s), 4.40 (1H, dd, J 5.2, 9.7 Hz), 4.38–4.32 (2H, incl. br. dd, J 5.8, 10.5 Hz at 4.36), 4.04 (1H, dd, J 4.9, 11.4 Hz), 3.95–3.89 (5H, m), 3.85 (1H, dd, J 2.2, 11.6 Hz), 3.81 (1H, dd, J 5.1, 10.1 Hz), 3.68 (1H, dd, J 4.7, 10.4 Hz), 3.65–3.59 (2H, incl. br. t, J 4.7 Hz at 3.63), 1.00 (9H, s), 0.96 (9H, s); δ _C (101 MHz, CDCl₃): 165.5, 165.4, 165.2, 165.1, 138.6, 138.3, 135.7, 135.6, 135.5, 133.3, 133.2, 133.15, 133.1, 133.0, 130.0, 129.9, 129.8, 129.75, 129.7, 129.6, 129.35, 129.3, 129.25, 129.2, 128.4, 128.35, 128.3, 128.25, 128.2, 127.8, 127.7, 127.6, 127.55, 127.5, 127.4, 126.3, 106.1, 105.2, 83.7, 83.4, 82.3, 82.2, 82.1, 81.8, 80.5, 77.2, 76.6, 73.3, 72.2, 70.1, 67.2, 66.1, 63.3, 26.7, 26.6, 19.3, 19.2; ν _{max}: 3069, 3010, 2932, 2857, 1723, 1602, 1452, 1072, 706 cm⁻¹.

3.4. 2',3'-Di-O-benzyl-L-glycerol-(1'→1)-2,3-di-O-benzyl- α -D-arabinofuranosyl-(1→3)-[2,3-di-O-benzyl- α -D-arabinofuranosyl-(1→5)]-2-O-benzyl- α -D-arabinofuranoside (9)

(a) Sodium methoxide (25 mL, 1M, in methanol) was added to a stirred solution of furanoside (6) (9.1 g, 5.4 mmol) in dry CH₂Cl₂:MeOH (1:1, 50 mL) at rt until a pH of 11 was obtained. The mixture was stirred for 90 min, then neutralized by the addition of acetic acid. The solvent was evaporated under reduced pressure to give an oil; column chromatography eluting with chloroform/methanol (1:1) gave 2',3'-di-O-benzyl-L-glycerol-(1'→1)-5-O-tert-butyl-diphenyl-silyl- α -D-arabino-furanosyl-(1→3)-[5-O-tert-butyl-diphenylsilyl- α -D-arabinofuranosyl-(1→5)]- α -D-arabino-furanoside (8) as a colourless thick oil (5.1 g, 83%) [MALDI: Found (M + Na)⁺: 1167.4913; C₆₄H₈₀NaO₁₅Si₂ requires: 1167.4928], [α]_D¹⁷ +35 (c 6.7, CHCl₃) which showed δ _H (400 MHz, CDCl₃): 7.69–7.62 (7H, m), 7.51–7.18 (23H, m), 5.18 (1H, br.s), 5.12 (1H, br.s), 4.93 (1H, br. s), 4.67 (1H, d, J 12.1 Hz), 4.63 (1H, d, J 12.1 Hz), 4.55 (1H, d, J 12.1 Hz), 4.51 (1H, d, J 12.1 Hz), 4.18 (1H, br. d, J 3.4 Hz), 4.16 (1H, br. dd, J 3.7, 6.1 Hz), 4.12 (1H, br. d, J 3.8 Hz), 4.07 (2H, br. s), 4.03–3.98 (2H, br. m), 3.98–3.93 (2H, incl. br. d, J 2.0 Hz at 3.96), 3.80 (1H, dd, J 3.3, 8.8 Hz), 3.78–3.68 (4H, m), 3.67–3.64 (1H, m), 3.63–3.59 (2H, incl. br. dd, J 3.0, 8.1 Hz at 3.60), 3.57 (1H, dd, J 5.2, 9.9 Hz), 3.02–2.55 (7H, br. m), 1.05 (9H, s), 1.03 (9H, s); δ _C (101 MHz, CDCl₃): 138.4, 138.2, 135.6, 135.5, 131.9, 131.8, 131.7, 131.6, 130.2, 130.1, 130.0, 128.4, 128.35, 128.3, 128.0, 127.9, 127.8, 127.7,

127.6, 127.55, 127.5, 108.6, 108.4, 108.3, 87.8, 87.4, 83.7, 82.3, 79.5, 78.8, 78.5, 77.8, 77.7, 76.7, 76.6, 73.3, 71.9, 69.7, 67.0, 66.0, 64.0, 63.8, 26.7, 26.6, 19.0, 18.9; ν_{\max} : 3418, 3071, 2933, 2858, 1454, 1053, 822 cm^{-1} .

(b) A solution of the above α -D-rabinofuranoside (**8**) (5.0 g, 4.0 mmol) in dry DMF was added dropwise to a stirred suspension of NaH (1.0 g, 43 mmol) (60% *w/w*, dispersion in mineral oil) at rt under nitrogen. The mixture was stirred for 10 min then benzyl bromide (5.2 g, 3.6 mL, 30 mmol) in dry DMF (2 mL) was added, stirred at rt for 6 h, then quenched by the slow addition of methanol (2 mL), and water (10 mL). The aqueous layer was re-extracted with EtOAc (2 \times 50 mL). The combined organic layers were washed with water (25 mL) and brine (25 mL), dried and evaporated under reduced pressure. Column chromatography eluting with petrol/EtOAc (5:1) gave 2',3'-di-O-benzyl-L-glycerol-(1' \rightarrow 1)-2,3-di-O-benzyl-5-O-tert-butylidiphenylsilyl- α -D-arabinofuranosyl-(1 \rightarrow 3)-[2,3-di-O-benzyl-5-O-tert-butylidiphenylsilyl- α -D-arabinofuranosyl-(1 \rightarrow 5)]-2-O-benzyl- α -D-arabinofuranoside as a colourless thick oil (4.5 g, 65%) [MALDI: Found (M+Na)⁺: 1617.7; C₉₉H₁₁₀NaO₁₅Si₂ requires: 1617.7], $[\alpha]_D^{17}$ +43 (*c* 1.9, CHCl₃) which showed δ_H (400 MHz, CDCl₃): 7.71–7.57 (8H, m), 7.48–7.13 (47H, m), 5.18 (2H, br. d, *J* 2.2 Hz), 5.07 (1H, br. s), 4.70 (1H, d, *J* 12.0 Hz), 4.66 (1H, d, *J* 12.0 Hz), 4.58–4.50 (7H, m), 4.47 (2H, d, *J* 11.9 Hz), 4.46 (1H, d, *J* 11.9 Hz), 4.39 (1H, d, *J* 11.9 Hz), 4.38 (1H, d, *J* 11.9 Hz), 4.30 (1H, br.dd, *J* 2.8, 6.8 Hz), 4.18 (2H, br. m), 4.13–4.07 (4H, m), 4.06–3.99 (2H, incl. br. dd, *J* 4.2, 10.6 Hz at 4.03), 3.95 (1H, dd, *J* 4.7, 11.9 Hz), 3.87 (1H, dd, *J* 5.0, 10.5 Hz), 3.84–3.80 (2H, incl. br. dd, *J* 4.0, 11.0 Hz at 3.82), 3.79–3.74 (4H, m), 3.68–3.58 (3H, m), 1.03 (18H, s); δ_C (101 MHz, CDCl₃): 138.6, 138.3, 138.2, 138.0, 137.9, 137.6, 137.5, 135.75, 135.67, 135.65, 135.6, 133.55, 133.5, 133.4, 133.3, 129.6, 129.55, 129.5, 128.4, 128.35, 128.3, 128.25, 128.2, 127.9, 127.85, 127.8, 127.75, 127.7, 127.65, 127.6, 127.55, 127.5, 127.45, 127.4, 106.6, 106.4, 105.4, 88.6, 88.5, 88.0, 83.0, 82.8, 82.3, 81.8, 81.3, 80.3, 73.4, 72.2, 72.0, 71.9, 71.8, 71.7, 71.6, 70.4, 67.0, 66.0, 63.4, 63.2, 26.8, 26.7, 19.3, 19.2; ν_{\max} : 3067, 3031, 2929, 2857, 1495, 1455, 1111, 698 cm^{-1} .

(c) TBAF (14.3 mL, 0.0493 mol, in 1.0 M THF) was added dropwise to a stirred solution of the above furanoside (3.8 g, 2.3 mmol) in anhydrous THF (25 mL) at 0 °C under nitrogen. The mixture was allowed to reach rt and stirred for 8 h then diluted with EtOAc (100 mL) washed with sat. aq. NH₄Cl (50 mL) and brine (50 mL). The organic layer was dried and concentrated; column chromatography eluting with hexane/EtOAc (1:1) gave the title compound (**9**) as a colourless thick oil (2.3 g, 87%) [MALDI-Found (M+Na)⁺: 1141.5, C₆₇H₇₄NaO₁₅ requires: 1141.5], $[\alpha]_D^{17}$ +53 (*c* 4.7, CHCl₃) which showed δ_H (400 MHz, CDCl₃): 7.34–7.16 (35H, m), 5.10 (1H, br. s), 5.07 (1H, br. s), 5.04 (1H, br. d, *J* 0.7 Hz), 4.64 (2H, br. s), 4.55–4.39 (11H, m), 4.31 (1H, d, *J* 11.7 Hz), 4.27 (1H, dd, *J* 3.8, 7.4 Hz), 4.23–4.17 (1H, m), 4.07 (1H, br. d, *J* 2.2 Hz), 4.06 (1H, br. dd, *J* 4.0, 7.6 Hz), 4.02 (1H, br. dd, *J* 2.2, 5.9 Hz), 3.98 (1H, br. dd, *J* 1.3, 3.8 Hz), 3.96 (1H, br. dd, *J* 1.2, 3.6 Hz), 3.88 (1H, br. dd, *J* 4.1, 12.3 Hz), 3.85 (1H, br. dd, *J* 3.2, 6.5 Hz), 3.82 (1H, d, *J* 5.2 Hz), 3.78 (1H, dd, *J* 3.7, 7.4 Hz), 3.76–3.72 (2H, m), 3.71–3.64 (2H, incl. br. dd, *J* 12.3, 2.4 Hz at 3.68), 3.61 (1H, dd, *J* 4.8, 7.3 Hz), 3.59–3.55 (3H, incl. br. d, *J* 5.1 Hz at 3.58), 3.53 (1H, dd, *J* 5.9, 12.3 Hz), 1.5 (2H, br s); δ_C (101 MHz, CDCl₃): 138.5, 138.2, 137.7, 137.6, 137.5, 137.4, 137.2, 128.5, 128.45, 128.4, 128.35, 128.3, 128.0, 127.95, 127.9, 127.85, 127.8, 127.75, 127.7, 127.6, 127.5, 106.1, 106.0, 105.9, 88.7, 88.3, 87.4, 83.0, 82.9, 82.4, 81.9, 80.8, 79.8, 73.4, 72.3, 72.25, 72.2, 72.0, 71.9, 71.8, 70.2, 67.2, 64.8, 62.7; ν_{\max} : 3459, 3064, 3030, 2921, 2860, 1605, 1496, 1115, 820 cm^{-1} .

3.5. 2',3'-Di-O-benzyl-L-glycerol-(1' \rightarrow 1)-2,3-di-O-benzyl-5-O-methanesulfonyl- α -D-arabinofuranosyl-(1 \rightarrow 3)-[2,3-di-O-benzyl-5-O-methanesulfonyl- α -D-arabinofuranosyl-(1 \rightarrow 5)]-2-O-benzyl- α -D-arabinofuranoside (**10**)

Methanesulfonyl chloride (0.57 g, 0.40 mL, 0.0050 mol) and DMAP (0.05g, 0.43 mmol) were added to a stirred solution of furanoside (**9**) (0.56 g, 0.50 mmol) in dry pyridine (5 mL) under nitrogen at rt. After 16 h it was quenched by the addition of water (1 mL); the organic layer was diluted with CH₂Cl₂ (10 mL) then washed with 1M aq. HCl (2 \times 10 mL), sat. aq. NaHCO₃ (2 \times 10 mL), dried and evaporated to give a thick oil; column chromatography eluting with petrol/EtOAc (3:1) gave the title compound (**10**) as a colourless thick oil (0.55 g, 87%) [MALDI: Found (M+Na)⁺: 1297.4; C₆₉H₇₈NaO₁₉S₂ requires: 1297.4], $[\alpha]_D^{17}$ +59 (*c* 0.60, CHCl₃), which showed δ_H (400 MHz, CDCl₃): 7.40–7.15 (35H, m), 5.16 (1H, br.

s), 5.13 (1H, br. s), 5.07 (1H, br. s), 4.68 (2H, br. s), 4.57–4.43 (11H, m), 4.40 (1H, d, J 11.3 Hz), 4.38–4.34 (1H, m), 4.33 (1H, br.dd, J 3.9, 7.3 Hz), 4.30–4.26 (2H, incl. br. dd, J 3.4, 7.3 Hz at 4.29), 4.24 (1H, d, J 5.4 Hz), 4.22–4.12 (2H, m), 4.11–4.07 (2H, m), 4.02–3.97 (2H, m), 3.91–3.84 (4H, m), 3.80 (1H, br. p, J 4.8 Hz), 3.73 (1H, br. dd, J 1.5, 11.5 Hz), 3.67–3.59 (3H, incl. br. dd, J 4.8, 8.7 Hz at 3.64), 2.94 (3H, s), 2.89 (3H, s); δ_C (101 MHz, $CDCl_3$): 138.5, 138.2, 137.4, 137.3, 137.2, 137.0, 128.6, 128.55, 128.5, 128.45, 128.4, 128.3, 128.1, 128.05, 128.0, 127.95, 127.9, 127.7, 127.65, 127.6, 127.55, 106.5, 106.1, 105.8, 88.3, 87.7, 87.5, 82.9, 82.8, 80.3, 80.2, 79.3, 79.2, 77.2, 73.4, 72.35, 72.3, 72.2, 72.1, 72.0, 71.9, 70.2, 68.8, 68.5, 67.2, 65.2, 37.6, 37.5; ν_{max} : 3088, 3065, 3031, 2933, 2871, 1586, 1454, 1177, 745 cm^{-1} .

3.6. L-Glycerol-(1'→1)-5-O-behenate- α -D-arabinofuranosyl-(1→3)-5-O-behenate- α -D-arabinofuranosyl-(1→5)- α -D-arabinofuranoside (**11**, R = H)

(a) $CsHCO_3$ (0.076 g, 0.39 mmol) was added to a stirred solution of dimesylate (**10**) (0.05 g, 0.03 mmol) and behenic acid (0.03 g, 0.09 mmol) in dry DMF:THF (1:5, 1 mL) at rt under nitrogen. The mixture was stirred at 70 °C for 3 days, then diluted with EtOAc (25 mL) and water (5 mL). The aqueous layer was re-extracted with EtOAc (2×10 mL). The combined organic layers were washed with water (5 mL) and brine (5 mL), dried and evaporated under reduced pressure to give a thick oil; column chromatography eluting with petrol/EtOAc (3:1) gave 2',3'-di-O-benzyl-L-glycerol-(1'→1)-2,3-di-O-benzyl-5-O-behenate- α -D-arabinofuranosyl-(1→3)-[2,3-di-O-benzyl-5-O-behenate- α -D-arabinofuranosyl-(1→5)]-2-O-benzyl- α -D-arabinofuranoside (**11**, R = Bn) as a colourless thick oil (55 mg, 80%) [MALDI: Found (M + Na)⁺: 1786.1; $C_{111}H_{158}NaO_{17}$ requires: 1786.1], $[\alpha]_D^{22} +36$ (c 1.0, $CHCl_3$) which showed δ_H (400 MHz, $CDCl_3$): 7.31–7.11 (35H, m), 5.09 (1H, br. s), 5.06 (1H, br. s), 4.97 (1H, br. s), 4.59 (2H, br. s), 4.51–4.35 (10H, m), 4.33 (1H, d, J 11.8 Hz), 4.26 (1H, d, J 11.8 Hz), 4.20 (1H, br. dd, J 3.3, 7.3 Hz), 4.12 (6H, br. m), 4.04 (1H, br. dd, J 2.8, 6.9 Hz), 4.00 (1H, br. d, J 2.5 Hz), 3.95–3.89 (2H, m), 3.84 (1H, dd, J 4.3, 11.8 Hz), 3.81–3.76 (2H, incl. br. dd, J 4.1, 8.5 Hz at 3.78), 3.75 (1H, br. d, J 3.4 Hz), 3.74–3.68 (1H, p, J 5.0 Hz), 3.66 (1H, br. dd, J 2.1, 11.7 Hz), 3.58–3.49 (3H, incl. br. q, J 4.6 Hz at 3.53), 2.17 (4H, t, J 7.6 Hz), 1.56–1.00 (76H, m), 0.81 (6H, t, J 6.8 Hz); δ_C (101 MHz, $CDCl_3$): 173.6, 173.5, 138.6, 138.3, 137.7, 137.6, 137.5, 137.4, 137.3, 128.5, 128.4, 128.35, 128.3, 128.0, 127.95, 127.9, 127.85, 127.8, 127.75, 127.7, 127.65, 127.6, 127.5, 106.5, 106.2, 105.5, 88.3, 88.2, 88.1, 83.4, 83.3, 80.8, 80.3, 79.2, 78.9, 76.9, 73.4, 72.3, 72.2, 72.1, 72.0, 71.9, 71.7, 70.3, 67.1, 65.6, 63.3, 63.2, 34.1, 34.0, 31.9, 29.7, 29.6, 29.5, 29.4, 29.35, 29.3, 29.2, 24.8, 22.7, 14.1; ν_{max} : 3064, 3031, 2923, 2853, 1740, 1718, 1455, 1066, 698 cm^{-1} .

(b) A general procedure for debenzylation was used throughout: Palladium hydroxide on activated charcoal ($Pd(OH)_2-C/20\%$ (1.1 fold by weight) was added to a stirred solution of the benzyl protected compound (0.010 mmol) in CH_2Cl_2 :MeOH:THF (1:1:1.5, 2 mL) at rt under hydrogen. The mixture was stirred for 36 h then filtered through celite and the solvent was evaporated under reduced pressure to give a residue; column chromatography eluting with chloroform/methanol (10:1) afforded the desired compound. In this way, compound (**11**, R = H) was obtained as a colourless thick oil (29 mg, 82%) [MALDI: Found (M + Na)⁺: 1155.8; $C_{62}H_{116}NaO_{17}$ requires: 1155.8], $[\alpha]_D^{18} -21$ (c 1.1, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$ +few drops CD_3OD): 5.01 (1H, br. s), 4.97 (1H, br. s), 4.90 (1H, br. s), 4.28–4.20 (2H, incl. br. dd J 3.2, 11.7 Hz at 4.24), 4.19–4.13 (3H, incl. br. dd J 5.0, 11.8 Hz at 4.17), 4.12 (1H, br. d, J 4.3 Hz), 4.11–4.08 (1H, m), 4.04 (1H, br. q, J 5.5 Hz), 4.01–3.95 (3H, br. m), 3.94 (1H, dd, J 3.6, 11.5 Hz), 3.83–3.76 (3H, m), 3.73 (1H, br. dd, J 4.8, 10.1 Hz), 3.67–3.62 (1H, m), 3.62–3.57 (2H, incl. br. d, J 3.1 Hz at 3.60), 3.56–3.51 (1H, m), 2.30 (4H, t, J 7.6 Hz), 1.64–1.02 (83H, m), 0.83 (6 H, t, J 6.5 Hz); δ_C (101 MHz, $CDCl_3$): 174.05, 174.0, 108.0, 107.7, 83.3, 83.0, 82.4, 81.8, 81.3, 80.7, 79.0, 77.6, 75.8, 69.9, 69.1, 66.4, 63.9, 63.7, 63.5, 34.0, 33.95, 31.8, 29.6, 29.5, 29.45, 29.4, 29.3, 29.25, 29.2, 29.1, 29.0, 24.8, 24.7, 22.6, 13.9.; ν_{max} : 3374, 2920, 2852, 1730, 1723, 1180, 757 cm^{-1} .

3.7. 12L-Glycerol-(1'→1)-5-O-(2R)-2-(1-hydroxy-16-((1S,2R)-2-((S)-20-methyl-19-oxooctatriacontyl)cyclopropyl)hexadecyl)hexacosanoate- α -D-arabinofuranosyl-(1→3)-[5-O-((2R)-2-(1-hydroxy-16-((1S,2R)-2-((S)-20-methyl-19-oxooctatriacontyl)cyclopropyl)hexadecyl)hexacosanoate- α -D-arabinofuranosyl-(1→5)]- α -D-arabinofuranoside (**15a**)

(a) 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) (60 mg, 0.31 mmol), in dry CH₂Cl₂ (1 mL) was added to a stirred solution of α -D-arabinofuranoside (**9**) (35 mg, 0.031 mmol), molecular sieves 4 Å (50 mg), DMAP (38 mg, 0.31) (2R)-2-(1-((*tert*-butyldimethyl-silyl)oxy)-16-((1S,2R)-2-((S)-20-methyl-19-oxooctatriacontyl)cyclopropyl)hexadecyl)hexacosanoic acid (**12a**) (85 mg, 0.062 mmol) [30] in dry CH₂Cl₂ (1 mL) at rt under nitrogen. After 4 days, the precipitate was filtered off and washed with CH₂Cl₂ (10 mL), and the solvent was evaporated; column chromatography eluting with hexane/EtOAc (10:1) gave compound (**13a**) as a colourless thick oil (60 mg, 51%) [MALDI: Found (M + Na)⁺: 3808.2; C₂₄₇H₄₂₆NaO₂₁Si₂ requires: 3808.2], [α]_D²⁰ +18 (c 5.0, CHCl₃), which showed δ _H (400 MHz, CDCl₃): 7.69–6.95 (35H, m), 5.16 (1H, br. s), 5.13 (1H, br. s), 5.04 (1H, br. s), 4.68 (1H, d, *J* 12.1 Hz), 4.65 (1H, d, *J* 12.1 Hz), 4.58–4.45 (9H, m), 4.45 (1H, d, *J* 11.9 Hz), 4.38 (1H, d, *J* 11.9 Hz), 4.33 (1H, d, *J* 11.8 Hz), 4.29–4.22 (5H, incl. br. dd *J* 4.0, 9.1 Hz at 4.25), 4.21–4.16 (2H, m), 4.13 (1H, br. dd, *J* 5.7, 8.2 Hz), 4.06 (1H, br. d, *J* 2.7 Hz), 4.01–3.96 (2H, m), 3.94–3.87 (5H, m), 3.85 (1H, br. dd, *J* 4.8, 10.7 Hz), 3.78 (1H, br. p, *J* 5.1 Hz), 3.76–3.71 (1H, m), 3.65–3.56 (3H, incl. br. dd, *J* 4.8, 8.2 Hz at 3.60), 2.53 (4H, incl. sextet, *J* 5.4 Hz), 2.42 (4H, t, *J* 7.6 Hz), 1.68–1.11 (288H, m), 1.06 (6H, d, *J* 6.9 Hz), 0.89 (12H, t, *J* 6.8 Hz), 0.85 (9H, s), 0.84 (9H, s), 0.71–0.61 (4H, m), 0.57 (2H, dt, *J* 4.0, 8.5 Hz), 0.03 (6H, s), 0.01 (6H, s), –0.32 (2H, br. q, *J* 5.1 Hz); δ _C (101 MHz, CDCl₃): 215.2, 174.3, 174.2, 138.5, 138.2, 137.8, 137.7, 137.6, 137.4, 137.3, 128.5, 128.45, 128.4, 128.35, 128.3, 128.2, 127.9, 127.85, 127.8, 127.75, 127.7, 127.65, 127.6, 127.55, 127.5, 106.6, 106.3, 105.2, 88.3, 88.2, 87.8, 83.7, 83.6, 81.2, 80.2, 79.4, 78.9, 77.2, 73.4, 73.1, 72.3, 72.2, 72.0, 71.9, 71.6, 70.3, 67.0, 65.9, 63.0, 62.7, 51.6, 46.3, 41.1, 33.6, 33.5, 33.0, 31.9, 30.3, 30.2, 29.9, 29.8, 29.75, 29.7, 29.65, 29.6, 29.55, 29.5, 29.45, 29.4, 29.3, 28.7, 27.9, 27.8, 27.3, 27.2, 27.1, 25.8, 24.0, 23.7, 22.7, 22.6, 18.0, 16.4, 15.8, 14.1, 10.9, –4.4, –4.5, –4.7, –4.8; ν _{max}: 3063, 3031, 2920, 2851, 1741, 1714, 1467, 1106, 836, 699 cm^{–1}.

(b) The protected furanoside (**13a**) (53 mg, 0.014 mmol) was dissolved in dry THF (10 mL) in a dry polyethylene vial equipped with an acid-proof rubber septum, followed by the addition of pyridine (0.1 mL) at rt under nitrogen. The mixture was cooled to 0 °C, and then HF–pyridine complex (70% w, 1.5 mL) was added dropwise. The mixture was stirred at 43 °C for 24 h, then poured slowly into sat. aq. NaHCO₃ and stirred until no more CO₂ was liberated. The aqueous layer was re-extracted with chloroform (3 × 10 mL). The combined organic layers were dried and evaporated. Column chromatography eluting with hexane/EtOAc (10:1) afforded compound (**14a**) as a colourless thick oil (38 mg, 76%) [MALDI: Found (M+Na)⁺: 3579.9; C₂₃₅H₃₉₈NaO₂₁ requires: 3579.9], [α]_D²¹ +19 (c 1.2, CHCl₃); δ _H (400 MHz, CDCl₃): 7.36–7.15 (35H, m), 5.16 (1H, br. s), 5.13 (1H, br. s), 5.05 (1H, br. s), 4.67 (2H, br. s), 4.55 (1H, d, *J* 11.8 Hz), 4.53–4.47 (7H, m), 4.45 (3H, d, *J* 11.8 Hz), 4.40 (1H, d, *J* 11.8 Hz), 4.36–4.22 (7H, m), 4.17 (1H, br. dd, *J* 3.7, 6.9 Hz), 4.11 (1H, br. dd, *J* 3.3, 6.3 Hz), 4.07 (1H, br. d, *J* 2.1 Hz), 4.00–3.96 (2H, m), 3.94–3.88 (2H, m), 3.88–3.82 (2H, incl. br. dd *J* 4.5, 9.8 Hz at 3.86), 3.81–3.75 (2H, m), 3.73 (1H, br. d, *J* 11.7 Hz), 3.67–3.51 (5H, incl. br. d *J* 5.3 Hz at 3.61), 2.51 (2H, sextet, *J* 6.7 Hz), 2.42 (6H, incl. t *J* 7.2 Hz), 2.05–1.10 (288H, m), 1.06 (6H, d, *J* 6.9 Hz), 0.89 (12H, t, *J* 6.7 Hz), 0.69–0.61 (4H, m), 0.57 (2H, dt, *J* 4.1, 8.5 Hz), –0.33 (2H, br. q, *J* 5.0 Hz); δ _C (101 MHz, CDCl₃): 215.2, 175.1, 175.0, 138.6, 138.2, 137.6, 137.5, 137.45, 137.4, 137.2, 128.5, 128.4, 128.35, 128.3, 128.2, 128.1, 128.0, 127.95, 127.9, 127.85, 127.8, 127.75, 127.7, 127.6, 127.5, 106.3, 106.2, 105.5, 88.2, 88.0, 87.9, 83.6, 80.7, 80.3, 79.3, 79.2, 77.2, 73.4, 72.4, 72.2, 72.1, 72.0, 71.7, 70.2, 68.0, 67.1, 65.4, 63.0, 62.9, 51.9, 51.7, 46.3, 41.1, 35.3, 35.2, 33.0, 31.9, 30.3, 30.2, 29.8, 29.7, 29.65, 29.6, 29.55, 29.5, 29.45, 29.4, 29.3, 29.2, 28.7, 27.5, 27.4, 27.3, 25.7, 25.6, 23.7, 22.7, 16.4, 15.8, 14.1, 10.9; ν _{max}: 3511, 3063, 3030, 2918, 2851, 1737, 1714, 1467, 1105, 754, 698 cm^{–1}.

(c) Using the generalised procedure for debenzoylation, compound (**15a**) was obtained as a colourless thick oil (25 mg, 86%) [MALDI: Found (M + Na)⁺: 2949.7; C₁₈₆H₃₅₆NaO₂₁ requires: 2949.7], [α]_D¹⁸ +19 (c 2.2, CHCl₃); δ _H (400 MHz, CDCl₃ + few drops CD₃OD): 5.00 (1H, br. s), 4.96 (1H, br. s), 4.90 (1H, br. s), 4.41 (1H, dd, *J* 4.2, 11.6 Hz), 4.36 (1H, dd, *J* 5.1, 11.9 Hz), 4.26 (1H, dd, *J* 11.9, 5.4 Hz),

4.21 (1H, d, *J* 4.0 Hz), 4.17 (1H, dd, *J* 3.8, 10.3 Hz), 4.12 (1H, br. d, *J* 4.8 Hz), 4.08 (1H, br. q, *J* 6.6 Hz), 4.04 (1H, d, *J* 5.1 Hz), 4.01–3.96 (3H, m), 3.92 (1H, dd, *J* 3.8, 8.0 Hz), 3.90–3.86 (2H, m), 3.77 (1H, dd, *J* 2.7, 8.4 Hz), 3.72 (1H, d, *J* 5.1 Hz), 3.62 (5H, br. m), 3.54 (1H, dd, *J* 2.6, 10.5 Hz), 2.52–2.44 (2H, m), 2.38 (6H, incl. t, *J* 7.5 Hz), 1.65–1.05 (297 H, m), 1.01 (6H, d, *J* 6.9 Hz), 0.84 (12H, t, *J* 6.8 Hz), 0.66–0.57 (4H, m), 0.52 (2H, dt, *J* 4.2, 8.2 Hz), -0.37 (2H, br. q, *J* 4.7 Hz); δ_C (101 MHz, CDCl₃): 216.2, 175.05, 174.9, 109.2, 108.7, 81.95, 81.2, 79.4, 78.0, 77.6, 77.2, 76.5, 72.5, 71.9, 65.0, 63.5, 63.4, 61.5, 52.6, 35.0, 32.8, 31.8, 30.1, 29.6, 29.55, 29.5, 29.4, 29.3, 29.25, 29.2, 28.9, 28.6, 27.3, 27.2, 26.1, 25.3, 23.5, 22.6, 16.1, 15.7, 14.0, 10.8; ν_{\max} : 3511, 3063, 3030, 2918, 2851, 1737, 1714, 1467, 1105, 754, 698 cm⁻¹.

3.8. L-Glycerol-(1'→1)-5-O-(2R)-2-((1R)-1-hydroxy-12-((2R)-2-(14-((2R)-2-eicosylcyclopropyl)tetradecyl)cyclopropyl)dodecyl)hexacosanoate- α -D-arabinofuranosyl-(1→3)-[5-O-(2R)-2-((1R)-1-hydroxy-12-((2R)-2-(14-((2R)-2-icosylcyclopropyl)tetradecyl)cyclopropyl)-dodecyl)hexacosanoate- α -D-arabinofuranosyl-(1→5)]- α -D-arabinofuranoside (**15b**)

(a) A solution of EDCI (48 mg, 0.25 mmol) in dry CH₂Cl₂ (1 mL) was added with stirring to furanoside (**9**) (28 mg, 0.025 mmol), molecular sieves 4 Å (50 mg), DMAP (30 mg, 0.24 mmol) and (2R)-2-((1R)-1-((*tert*-butyldimethylsilyloxy)-12-((2R)-2-(14-((2R)-2-eicosylcyclopropyl)tetradecyl)cyclopropyl)-dodecyl)hexacosanoic acid (**12b**) (62 mg, 0.049 mmol) [31] in dry CH₂Cl₂ (1 mL) at rt under nitrogen. After 4 days, the precipitate was filtered and washed with CH₂Cl₂ (10 mL), the solvent was evaporated and the residue was purified by column chromatography eluting with hexane/EtOAc (10:1) to afford compound (**13b**) as a colourless thick oil (75 mg, 84%) [MALDI: Found (M+Na)⁺: 3607.9; C₂₃₅H₄₀₂NaO₁₉Si₂ requires: 3607.9], $[\alpha]_D^{22} +20$ (c 0.90, CHCl₃), which showed δ_H (400 MHz, CDCl₃): 7.94–6.85 (35H, m), 5.16 (1H, br. s), 5.13 (1H, br. s), 5.04 (1H, br. s), 4.68 (1H, d, *J* 12.1 Hz), 4.65 (1H, d, *J* 12.1 Hz), 4.57–4.45 (9H, m), 4.45 (1H, d, *J* 11.8 Hz), 4.38 (1H, d, *J* 11.8 Hz), 4.32 (1H, d, *J* 11.8 Hz), 4.28–4.20 (5H, m), 4.18 (1H, br. dd, *J* 3.6, 7.4 Hz), 4.15–4.09 (1H, m), 4.06 (1H, br. d, *J* 2.9 Hz), 4.01–3.96 (2H, m), 3.90 (3H, br. m), 3.87–3.81 (2H, incl. br. dd, *J* 4.8, 10.7 Hz at 3.85), 3.78 (1H, br. p, *J* 5.1 Hz), 3.76–3.71 (1H, m), 3.64–3.49 (3H, incl. br. dd, *J* 4.8, 8.3 Hz at 3.60), 2.60–2.47 (2H, m), 1.67–1.06 (270H, m), 0.89 (12H, t, *J* 6.8 Hz), 0.85 (9H, s), 0.84 (9H, s), 0.74–0.62 (8H, m), 0.57 (4H, td, *J* 4.1 Hz), 0.02 (6H, s), 0.00 (6H, s), -0.33 (4H, br. q, *J* 4.9 Hz); δ_C (101 MHz, CDCl₃): 174.3, 174.2, 138.5, 138.3, 137.8, 137.7, 137.6, 137.4, 137.3, 128.5, 128.45, 128.4, 128.35, 128.3, 127.9, 127.85, 127.8, 127.75, 127.7, 127.65, 127.6, 127.55, 127.5, 106.6, 106.3, 105.2, 88.3, 88.2, 87.8, 83.7, 83.6, 81.2, 80.2, 79.4, 78.9, 77.2, 73.4, 73.1, 72.3, 72.2, 71.9, 71.6, 70.3, 67.0, 65.9, 63.0, 62.7, 51.6, 33.6, 33.5, 31.9, 30.3, 30.2, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 28.7, 27.9, 27.8, 27.2, 27.1, 25.9, 25.8, 24.0, 22.7, 18.0, 15.8, 14.1, 11.0, 10.9, -4.4, -4.5, -4.7, -4.8; ν_{\max} : 3063, 2925, 2854, 1737, 1456, 1101, 770 cm⁻¹.

(b) The protected glycolipid α -D-arabinofuranoside (**13b**) (70 mg, 0.019 mmol) was dissolved in dry THF (10 mL) in a dry polyethylene vial equipped with an acid-proof rubber septum, followed by the addition of pyridine (0.1 mL) at rt under nitrogen. The mixture was cooled to 0 °C, and then HF-pyridine complex (70% w, 1.5 mL) was added dropwise. The mixture was stirred at 43 °C for 24 h, then poured slowly into sat. aq. NaHCO₃ and stirred until no more CO₂ was liberated. The aqueous layer was re-extracted with chloroform (3 × 10 mL). The combined organic layers were dried and evaporated; column chromatography eluting with hexane/EtOAc (10:1) afforded compound (**14b**) as a colourless thick oil (33 mg, 51%) [MALDI: Found (M+Na)⁺: 3379.8; C₂₂₃H₃₇₄NaO₁₉ requires: 3379.8], $[\alpha]_D^{21} +22$ (c 0.90, CHCl₃), which showed δ_H (400 MHz, CDCl₃): 7.37–7.17 (35H, m), 5.16 (1H, br. s), 5.13 (1H, br. s), 5.05 (1H, br. s), 4.67 (2H, br. s), 4.55 (1H, d, *J* 11.9 Hz), 4.53–4.47 (8H, m), 4.46 (2H, d, *J* 11.8 Hz), 4.41 (1H, d, *J* 11.9 Hz), 4.35–4.23 (6H, m), 4.17 (1H, br. dd, *J* 3.7, 6.9 Hz), 4.15–4.08 (3H, incl. br. t, *J* 7.0 Hz at 4.12), 4.07 (1H, br. d, *J* 2.1 Hz), 4.00–3.95 (2H, m), 3.94–3.87 (2H, m), 3.95–3.87 (2H, incl. br. dd, *J* 4.6, 10.3 Hz at 3.86), 3.79 (1H, p, *J* 4.8 Hz), 3.75–3.69 (1H, m), 3.67–3.55 (3H, incl. br. d, *J* 5.3 Hz at 3.61), 2.44–2.37 (2H, m), 1.67–1.03 (270H, m), 0.89 (12H, t, *J* 7.1 Hz), 0.69–0.61 (8H, m), 0.57 (4H, dt, *J* 4.2, 8.5 Hz), -0.32 (4H, br. q, *J* 4.9 Hz); δ_C (101 MHz, CDCl₃): 175.1, 175.0, 138.5, 138.2, 137.6, 137.5, 137.4, 137.4, 137.2, 128.5, 128.4, 128.35, 128.3, 128.0, 127.95, 127.9, 127.85, 127.8, 127.75, 127.7, 127.6, 127.5, 106.3, 106.2, 105.5, 88.2, 88.0, 87.9, 83.6, 80.7, 80.3, 79.3, 79.2, 77.2, 73.4, 72.4, 72.2, 72.0, 71.9, 71.7, 70.3, 67.1, 65.4, 63.1, 63.0, 62.0, 51.9, 51.8, 35.3, 35.2, 34.2, 31.9, 30.3, 30.2, 29.8, 29.75, 29.7, 29.65, 29.6,

29.5, 29.4, 28.7, 27.5, 27.4, 25.8, 25.7, 24.9, 22.7, 22.6, 20.4, 15.8, 14.1, 10.9; ν_{\max} : 3511, 3062, 2921, 2854, 1733, 1725, 1456, 1115, 754 cm^{-1} .

(c) Using the generalised debenzoylation procedure, compound (**15b**) was obtained as a colourless thick oil (22 mg, 88%) [MALDI: Found $(M+Na)^+$: 2749.5; $C_{174}H_{332}NaO_{19}$ requires: 2749.5], $[\alpha]_D^{18} +18$ (c 2.2, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$ +few drops CD_3OD): 5.00 (1H, br. s), 4.96 (1H, br. s), 4.90 (1H, br. s), 4.41 (1H, dd, J 4.6, 11.7 Hz), 4.37 (1H, br. dd, J 5.1, 11.9 Hz), 4.26 (1H, dd, J 5.0, 11.7 Hz), 4.20 (1H, dd, J 4.7, 11.8 Hz), 4.16 (1H, br. q, J 4.8 Hz), 4.12 (1H, br. d, J 6.2 Hz), 4.10–4.06 (2H, incl. br. t, J 7.4 Hz at 4.08), 4.05 (1H, br. d, J 5.6 Hz), 4.02–3.96 (3H, incl. br. d, J 8.5 Hz at 3.99), 3.95–3.86 (3H, m), 3.77 (1H, dd, J 4.2, 7.4 Hz), 3.72 (1H, d, J 5.4 Hz), 3.67–3.57 (4H, incl. br. d, J 10.5 Hz at 3.63), 3.54 (1H, dd, J 2.6, 9.7 Hz), 2.42–2.35 (2H, m), 1.64–1.04 (277H, m), 0.84 (12H, t, J 6.7 Hz), 0.64–0.56 (8H, m), 0.52 (4H, dt, J 4.1, 8.4 Hz), -0.37 (4H, br. q, J 4.9 Hz); δ_C (101 MHz, $CDCl_3$ + few drops CD_3OD): 175.0, 174.9, 108.7, 108.0, 81.7, 81.3, 79.7, 79.4, 77.9, 77.5, 77.2, 76.3, 72.4, 71.9, 69.7, 69.15, 68.9, 67.6, 65.1, 63.4, 62.2, 61.4, 57.8, 52.6, 34.9, 31.8, 30.1, 29.55, 29.5, 29.45, 29.4, 29.3, 29.2, 29.15, 29.1, 28.6, 27.3, 25.3, 22.5, 20.5, 15.6, 13.9, 10.7; ν_{\max} : 3399, 2920, 2851, 1734, 1467, 1043 cm^{-1} .

3.9. L-Glycerol-(1'→1)-5-O-(2R)-2-((1R)-1-hydroxy-17-((1S,2R)-2-((2S)-22-methyl-21-oxotetracontan-2-yl)cyclopropyl)heptadecyl)hexacosanoate- α -D-arabinofuranosyl-(1→3)-[-5-O-(2R)-2-((1R)-1-hydroxy-17-((1S,2R)-2-((2S)-22-methyl-21-oxotetracontan-2-yl)cyclo-propyl)heptadecyl)hexacosanoate- α -D-arabinofuranosyl-(1→5)]- α -D-arabinofuranoside (**15c**)

(a) A solution of EDCI (35 mg, 0.18 mmol) in dry CH_2Cl_2 (1 mL) was added to a stirred solution of α -D-arabinofuranoside (**9**) (22 mg, 0.019 mmol), molecular sieves 4 Å (25 mg), DMAP (23 mg, 0.18 mmol) and (2R)-2-((1R)-1-((tert-butyl)dimethylsilyloxy)-17-((1S,2R)-2-((2S)-22-methyl-21-oxotetracontan-2-yl)cyclopropyl)-heptadecyl)hexacosanoic acid (**12c**) (50 mg, 0.035 mmol) [32] in dry CH_2Cl_2 (1 mL) at rt under nitrogen. The mixture was stirred for 4 days, then the precipitate was filtered off and washed with CH_2Cl_2 (10 mL), the solvent was evaporated and the residue was purified by column chromatography eluting with hexane/EtOAc (10:1) to afford the title compound (**13c**) as a colourless thick oil (35 mg, 46%) [MALDI: Found $(M + Na)^+$: 3892.2; $C_{253}H_{438}NaO_{21}Si_2$ requires: 3892.2], $[\alpha]_D^{20} +14$ (c 3.0, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.54–7.00 (35H, m), 5.16 (1H, br. s), 5.13 (1H, br. s), 5.04 (1H, br. s), 4.68 (1H, d, J 12.1 Hz), 4.65 (1H, d, J 12.1 Hz), 4.58–4.47 (9H, m), 4.46 (1H, d, J 11.9 Hz), 4.39 (1H, d, J 11.8 Hz), 4.33 (1H, d, J 11.9 Hz), 4.30–4.22 (5H, incl. br. dd, J 3.9, 10.4 Hz at 4.26), 4.22–4.16 (2H, m), 4.13 (1H, br. dd, J 5.6, 8.4 Hz), 4.06 (1H, br. d, J 2.9 Hz), 4.01–3.97 (2H, m), 3.95–3.87 (5H, m), 3.85 (1H, br. dd, J 3.8, 9.4 Hz), 3.78 (1H, br. p, J 4.9 Hz), 3.76–3.72 (1H, m), 3.65–3.55 (3H, incl. br. dd J 4.8, 8.3 Hz at 3.60), 2.53 (4H, incl. sextet, J 5.6 Hz), 2.42 (4H, t, J 7.5 Hz), 1.62–1.14 (292 H, m), 1.06 (6H, d, J 6.9 Hz), 0.89 (12H, t, J 6.8 Hz), 0.88 (6H, d, J 6.8 Hz), 0.85 (9H, s), 0.84 (9H, s), 0.75–0.62 (2H, m), 0.51–0.40 (2H, m), 0.24–0.08 (6H, m), 0.03 (6H, s), 0.01 (6H, s); δ_C (101 MHz, $CDCl_3$): 215.1, 174.2, 174.1, 138.6, 138.3, 137.8, 137.7, 137.6, 137.55, 137.5, 128.45, 128.4, 128.35, 128.3, 128.25, 128.2, 127.9, 127.85, 127.8, 127.75, 127.7, 127.65, 127.6, 127.55, 127.5, 106.6, 106.3, 105.2, 88.3, 88.2, 87.8, 83.8, 83.7, 81.2, 80.2, 79.5, 78.9, 77.2, 73.4, 73.2, 72.3, 72.2, 72.0, 71.9, 71.6, 70.3, 67.0, 65.9, 63.0, 62.7, 51.6, 46.3, 41.1, 38.1, 37.4, 34.5, 33.7, 33.6, 33.0, 31.9, 30.1, 29.9, 29.8, 29.75, 29.7, 29.65, 29.6, 29.55, 29.5, 29.45, 29.4, 29.35, 29.3, 27.8, 27.4, 27.3, 27.2, 27.1, 26.1, 25.9, 25.8, 24.1, 23.7, 22.7, 22.6, 19.7, 18.6, 18.0, 16.4, 14.1, 10.5, -4.4 , -4.5 , -4.7 , -4.8 ; ν_{\max} : 3063, 3032, 2919, 2851, 1739, 1714, 1467, 1105, 836, 698 cm^{-1} .

(b) The protected glycolipid furanoside (**13c**) (31 mg, 0.0080 mmol) was dissolved in dry THF (10 mL) in a dry polyethylene vial equipped with an acid-proof rubber septum, followed by the addition of pyridine (0.1 mL) at rt under nitrogen. The mixture was cooled to 0 °C, and then HF–pyridine complex as (70% w, 1.5 mL) was added dropwise. The mixture was stirred at 43 °C for 24 h, then poured slowly into sat. aq. $NaHCO_3$ and stirred until no more CO_2 was liberated. The aqueous layer was re-extracted with chloroform (3 × 10 mL). The combined organic layers were dried and evaporated; column chromatography eluting with hexane/EtOAc (10:1) afforded compound (**14c**) as a colourless thick oil (19 mg, 66%) [MALDI: Found $(M + Na)^+$: 3664.1; $C_{241}H_{410}NaO_{21}$ requires: 3664.1], $[\alpha]_D^{18} +25$ (c 1.1, $CHCl_3$), which showed δ_H (400 MHz, $CDCl_3$): 7.39–7.18 (35H, m), 5.16 (1H, br. s), 5.13 (1H, br. s), 5.04 (1H, br. s), 4.67 (2H, br. s), 4.55 (1H, d, J 11.9 Hz), 4.53–4.47 (8H, m), 4.45 (2H, d, J 11.8 Hz), 4.40

(1H, d, *J* 11.8 Hz), 4.29 (6H, br. m), 4.17 (1H, br. dd, *J* 3.8, 6.9 Hz), 4.11 (1H, br. dd, *J* 3.4, 6.2 Hz), 4.07 (1H, br. d, *J* 2.5 Hz), 3.99–3.95 (2H, m), 3.94–3.87 (2H, m), 3.87–3.82 (2H, incl. br. dd, *J* 4.6, 10.3 Hz at 3.85), 3.79 (1H, br. p, *J* 4.9 Hz), 3.72 (1H, br. dd, *J* 1.7, 11.6 Hz), 3.64–3.57 (5H, m), 2.51 (2H, sextet, *J* 6.8 Hz), 2.42 (6H, incl. t, *J* 7.5 Hz), 1.87–1.08 (300H, m), 1.05 (6H, d, *J* 6.9 Hz), 0.89 (12H, t, *J* 7.3 Hz), 0.72–0.62 (2H, m), 0.50–0.38 (2H, m), 0.25–0.05 (6H, m); δ_C (101 MHz, CDCl₃): 215.2, 175.1, 175.0, 138.6, 138.3, 137.7, 137.5, 137.45, 137.4, 137.2, 128.5, 128.4, 128.35, 128.3, 128.2, 128.0, 127.95, 127.9, 127.85, 127.8, 127.75, 127.7, 127.6, 106.3, 106.2, 105.4, 88.2, 88.0, 87.9, 83.6, 80.7, 80.3, 79.3, 79.2, 77.2, 73.4, 72.4, 72.2, 72.1, 72.0, 71.9, 71.7, 70.3, 67.1, 65.4, 63.0, 62.9, 51.9, 51.7, 46.3, 41.1, 38.1, 37.4, 35.3, 35.2, 34.5, 33.0, 31.9, 30.1, 29.75, 29.7, 29.65, 29.6, 29.55, 29.5, 29.45, 29.4, 29.3, 27.5, 27.4, 27.35, 27.3, 26.1, 25.7, 23.7, 22.7, 19.7, 18.6, 16.4, 14.1, 10.5; ν_{\max} : 3457, 3063, 3031, 2919, 2852, 1737, 1715, 1464, 1104, 734, 698 cm⁻¹.

(c) Using the generalised debenzoylation procedure, compound (**15c**) was obtained as a colourless oil (9.0 mg, 82%) [MALDI: Found (M+Na)⁺: 3033.8; C₁₉₂H₃₆₈NaO₂₁ requires: 3033.8], [α]_D¹⁸ +26 (c 0.90, CHCl₃); δ_H (400 MHz, CDCl₃+ few drops CD₃OD): 5.01 (1H, br. s), 4.96 (1H, br. s), 4.91 (1H, br. s), 4.42 (1H, dd, *J* 4.9, 12.1 Hz), 4.37 (1H, dd, *J* 4.6, 11.8 Hz), 4.27 (1H, dd, *J* 4.7, 11.1 Hz), 4.22 (1H, br. d, *J* 4.7 Hz), 4.18 (1H, dd, *J* 4.2, 9.0 Hz), 4.13 (1H, br. q, *J* 5.4 Hz), 4.09 (1H, t, *J* 7.0 Hz), 4.06 (1H, d, *J* 5.1 Hz), 4.02–3.97 (3H, br. m), 3.93 (1H, br. dd, *J* 3.3, 7.3 Hz), 3.89 (2H, br. m), 3.80–3.72 (2H, m), 3.67–3.58 (5H, m), 3.55 (1H, dd, *J* 2.4, 9.6 Hz), 2.53–2.44 (2H, sextet, *J* 6.6 Hz), 2.39 (6H, incl. t, *J* 7.4 Hz), 1.57–1.06 (210 H, m), 1.01 (6H, d, *J* 6.9 Hz), 0.85 (12H, t, *J* 7.5 Hz), 0.67–0.57 (2H, m), 0.47–0.34 (2H, m), 0.21–0.02 (6H, m); δ_C (101 MHz, CDCl₃): 215.8, 175.0, 174.75, 109.3, 108.7, 87.1, 82.95, 82.9, 80.9, 79.4, 78.8, 78.2, 77.9, 77.2, 72.6, 71.7, 68.8, 65.0, 64.0, 63.3, 61.8, 52.5, 46.3, 41.1, 38.1, 37.4, 35.1, 34.4, 33.0, 32.9, 31.9, 30.0, 29.7, 29.6, 29.55, 29.5, 29.45, 29.4, 29.35, 29.3, 27.4, 27.35, 27.3, 27.25, 27.2, 26.1, 25.45, 25.4, 23.6, 23.55, 22.6, 19.6, 18.6, 16.3, 16.2, 14.1, 10.4.; ν_{\max} : 3434, 2919, 2852, 1736, 1717, 1467, 1104, 735, 699 cm⁻¹.

Supplementary Materials: Supplementary data related to this article, including the Figures S1–S7 and Table S1 referred to in the text, can be found online at <http://www.mdpi.com/1420-3049/24/19/3596/s1>.

Author Contributions: Conceptualisation, J.R.A.D. and M.S.B.; methodology, O.T.A. and M.O.M.; analysis, O.T.A. and J.R.A.; investigation, O.T.A.; supervision, J.R.A.D. and M.S.B.; resources, M.S.B.; original draft preparation, O.T.A. and J.R.A.D.; writing—review and editing, M.O.M. and M.S.B.; project administration, J.R.A.D. and M.S.B.

Funding: This research received no external funding other than the PhD studentships listed in the Acknowledgements.

Acknowledgments: We thank the Iraqi Ministry of Higher Education and Scientific Research and Mosul University for the award of a PhD studentship to O.T.A. and the Ministry of Higher Education and Scientific Research, Kurdistan Region, Iraq, for the award of a PhD grant to M.O.M. We thank the EPSRC National Mass Spectrometry Service Centre, Swansea, for MALDI measurements.

Conflicts of Interest: There are no conflicts of interest for any of the authors of this paper.

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Sample availability: Samples of the final materials are available from the authors.



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