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Note

Synthesis of a glycolipid for studying mechanisms of mitochondrial uncoupling proteins

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Abstract—Glucose-*O*-ω-palmitic acid is an amphipathic molecule that is useful as a tool for studying the mechanism of mitochondrial uncoupling proteins. The synthesis of this glycolipid is described herein. The study of the reaction of a series of glycosyl donors with appropriate acceptors derived from 16-hydroxyhexadecanoic acid showed that a glycosyl trichloroacetimidate donor was more efficient than thioglycoside, glycosyl halide and glycosyl acetate donors for synthesis of this glycolipid.

Keywords: Mitochondrial uncoupling protein; Glycoconjugates; Glucose-*O*-ω-palmitic acid; Palmitic acid; 16-(β-D-Glucopyranosyloxy)-hexadecanoic acid

Mitochondrial uncoupling protein 1 (UCP 1 also known as UCP and thermogenin) is a 32 kDa protein situated in the inner membrane of mitochondria from brown adipose tissue (BAT) of mammals and human infants.[1] The protein uncouples ATP synthesis from oxygen consumption by catalysing the dissipation of the proton electrochemical gradient across the mitochondrial inner membrane. UCP 1 is the basis of the non-shivering thermogenesis generated by BAT under conditions of cold exposure. Long chain free fatty acids, such as palmitic acid, are known to increase proton leak through UCP 1.[2] However, the mechanism by which free fatty acids are involved in the UCP uncoupling process is a matter of investigation. The two main models for the mechanism of action of the uncoupling proteins are: (i) that UCP 1 acts as a proton conduit across the mitochondrial inner membrane and importantly that fatty acids act as cofactors/activators providing an addition carboxyl group at a key intra membrane site enhancing the rate of proton movement[3] or: (ii) that protonated fatty acids freely flip across the mitochondrial inner membrane and uncouple the mitochondria and that the uncoupling proteins act as 'flippases' translocating the anionic fatty acids back across the bilayer leaflets of the inner membrane.[4] Thus the latter model proposes that UCP 1 facilitates a cycle of uncoupling by free fatty acids. There is evidence for both models but crucial experimental evidence distinguishing both models is lacking. To that end, we describe the synthesis of glucose-*O*-ω-palmitic acid **1** (Chart 1) an amphipathic molecule that cannot 'flip' across the mitochondrial inner membrane but can theoretically provide the carboxyl group for catalysis of proton translocation. This glycolipid has been cited previously in the literature[5] but neither details regarding the synthesis of this molecule nor its analytical data nor criteria of purity have been reported to date.

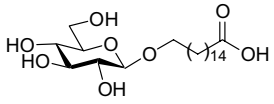

Chart 1. Structure of glycolipid **1**.

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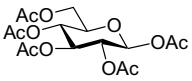
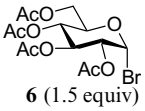
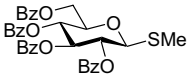
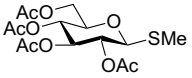
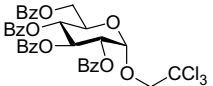
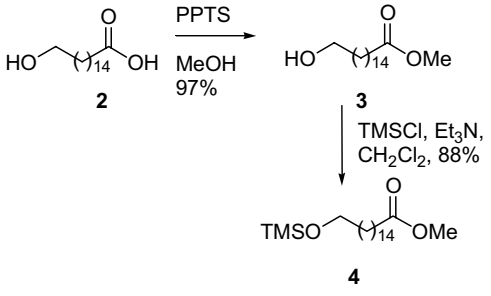
Table 1. Glycosidation reactions[a]

Entry	Donor	Acceptor	Promoter	Time (h)	Main product (%) α:β
1	 **5** (1.1 equiv)	**4** (1.0 equiv)	$SnCl_4$ (1.0 equiv)	3	**12** (20) <5:95
2	**5** (1.1 equiv)	**4** (1.0 equiv)	$SnCl_4$ (1.0 equiv)	45	**13** (20) >90:10[b]
3	 **6** (1.5 equiv)	**3** (1.0 equiv)	Ag_2CO_3 (3.5 equiv), $AgClO_4$ (0.12 equiv)	2	**12** (31) β
4	 **7** (1.0 equiv)	**3** (1.0 equiv)	AgOTf (0.1 equiv), NIS (1.0 equiv)	2	**10** (33), **11** (58) β
5	 **8** (1.0 equiv)	**3** (1.0 equiv)	AgOTf (0.1 equiv), NIS (1.0 equiv)	2	**14** (51), **15** (29) —[c]
6	 **9** (1.0 equiv)	**3** (1.0 equiv)	TMSOTf (0.1 equiv)	0.33	**10** (55), **11** (5) β

[a] All glycosidations were carried out in CH_2Cl_2 and yields correspond to isolated yield after purification by chromatography.
[b] Higher amounts of α-glycoside **13** were obtained from reactions carried out on >1 g scale after 29 h.
[c] No glycoside product was isolated.

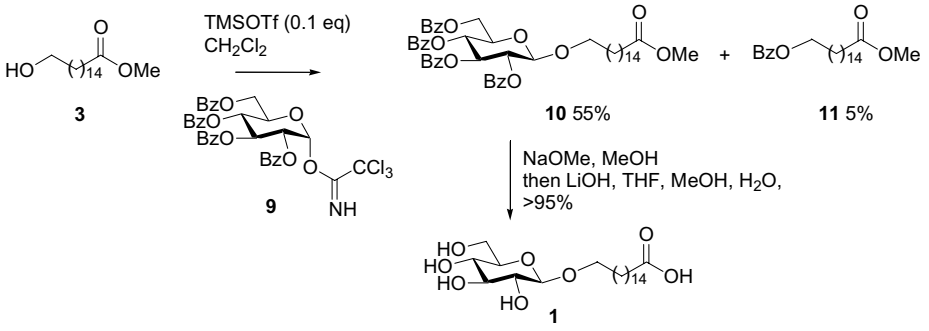
The glycosidation[6] reactions of a series of donors **5**–**9** (Table 1 and Scheme 2)[7–9] in presence of acceptors **3** or **4** were investigated. The acceptors **3** and **4** were obtained from 16-hydroxyhexadecanoic acid **2**; reaction of **2** with MeOH catalysed by PPTS gave ester **3** and subsequent silylation gave **4** (Scheme 1).

The results from glycosidation are summarised in Table 1. The Schmidt–Michel glycosidation from benzoyl protected trichloroacetimidate[10] donor **9** was found to be significantly more efficient than reactions of thioglucosides **7** and **8**, glucosyl bromide (**6**) or β-D-glucose penta-acetate (**5**), giving **10** in 55% yield (Scheme 2) as



Scheme 1. Synthesis of **3** and **4**.

well as a minor amount of benzoate ester **11**. The ester **11** was a major product from reaction of the thioglycoside **7**. A similar by-product, **15**, was isolated, as well as orthoester **14** (Chart 2), from reaction of thioglycoside **8**. A proposal, which would explain the formation of **11** and **15** during glycosidation is shown in Scheme 3. This involves coordination by a Lewis acid reagent to the pyranose 2-O atom followed by formation of the product facilitated by glycosyl oxocarbenium ion formation. The stereoselectivity observed from the $SnCl_4$ catalysed glycosidation of **5** was dependent on the reaction time (Table 1). It was possible to obtain pure β-anomer **12** (Chart 2) after 3 h (Table 1, entry 1), however the α-anomer **13** (>90:10 selectivity) can be obtained if the experiment is conducted over a longer time (45 h, entry 2). The anomerisation[11] reaction of **12** to **13** is catalysed by $SnCl_4$ and can be monitored by TLC and the yield was similar in all experiments (~20%). The Koenigs–Knorr glycosidation of glucosyl bromide **6** gave **12** (31%), with no anomerisation occurring, even after 24 h. The protecting groups were efficiently removed from **10** (or **12**) using first treatment with NaOMe–MeOH and subsequent reaction with LiOH to give the desired glycolipid **1**.



Scheme 2. Synthesis of **1** from trichloroacetimidate donor **9**.

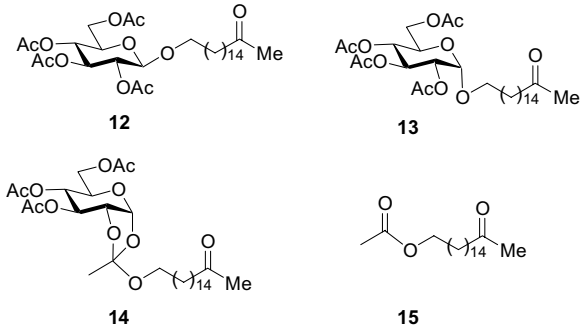
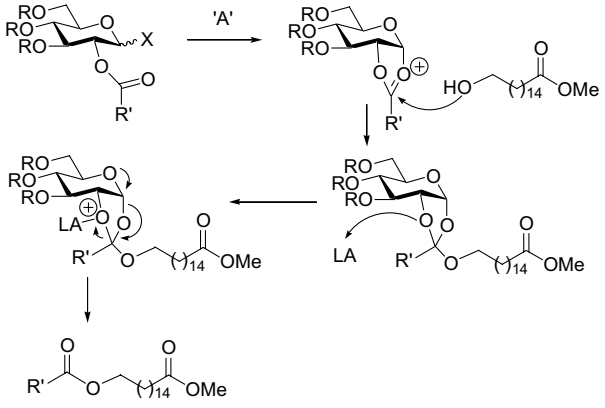


Chart 2. Structure of **12**–**15**.



Scheme 3. A proposal for formation of **11** and **15**.

Biochemists who work in this area are interested in the stability and purity of **1**. We have found that **1** is resistant to acid catalysed hydrolysis and remained unchanged when treated with aq HCl and MeOH, aq HCl and THF or 18% HCl for 24 h at room temp. Hydrolysis or methanolysis of **1** to expected products was only achieved (detected by TLC, MS and ^{1}H NMR), after heating the compound to 60 °C in (i) a 2:1 mixture of aq HCl (9%) and THF for 6 h or (ii) a 1:1 mixture of MeOH and aq HCl (18%) for 3 h. The ^{1}H NMR spectra of **1**, prepared from both **10** and **12**, have been provided in the Supplementary information section. The purity of **1** was determined to be 93% by ^{1}H NMR, using methyl α-D-glucopyranoside as an internal standard. This seems a reasonable assumption given the hygroscopic nature of some carbohydrate compounds. Biochemical experiments with **1** are underway and the insights these provide to the mechanisms, which operate for mitochondrial uncoupling proteins will be reported in due course.

1. Experimental

1.1. General

Optical rotations were determined with a Perkin–Elmer 241 model polarimeter at 23 °C. ^{1}H NMR spectra were recorded with a Varian Inova 300 MHz spectrometer; ^{13}C NMR spectra were recorded on the same instrument at 75 MHz. Chemical shifts are reported relative to internal Me_4Si in $CDCl_3$ (δ 0.0) or HOD for D_2O (δ 4.79) for ^{1}H and (δ 77.16) for ^{13}C. ^{13}C signals were assigned with the aid of DEPT-135. ^{1}H signals were assigned with the aid of COSY. IR spectra were recorded with a Mattson Galaxy Series FTIR 3000 using either thin film between NaCl plates or KBr discs, as specified. High resolution mass spectra were recorded on a Micromass LCT KC420 or Micromass Quattro. TLC was performed on aluminium sheets precoated with Silica Gel 60 (HF254, E. Merck) and spots visualised by UV and charring with 1:20 H_2SO_4–EtOH. Flash column chromatography was carried out with Silica Gel 60 (0.040–0.630 mm, E. Merck) and employed a stepwise solvent polarity gradient correlated with the TLC mobility. Chromatography solvents used were MeOH, CH_2Cl_2 (Riedel-de Haen), EtOAc and cyclohexane (Fluka). Reaction solvents were dried and distilled before use.

1.2. Methyl 16-hydroxyhexadecanoate (3)

16-Hydroxyhexadecanoic acid **2** (3.0 g, 11 mmol) and *p*-toluenesulfonic acid monohydrate (669 mg, 3.5 mmol) were dried under diminished pressure for 2 h. MeOH (180 mL) was added and the reaction mixture stirred at rt for 16 h. Solid $NaHCO_3$ (750 mg) was then added and the mixture stirred for a further 30 min, then filtered

through Celite and the solvent removed under diminished pressure. Chromatography (1:3 EtOAc–cyclohexane) gave **3** (2.89 g, 92%) as a white powder; mp 53 °C; IR (KBr) cm^{-1}: 3313, 2920, 2850, 1741, 1462; ^1H NMR ($CDCl_3$): δ 3.66 (3H, s, OCH_3), 3.64 (2H, t, J 6.6 Hz, $HOCH_2CH_2$), 2.30 (2H, t, J 7.5 Hz, $CH_2CH_2CO_2Me$), 1.64–1.52 (4H, m, $HOCH_2CH_2$ and $CH_2CH_2CO_2Me$), 1.25 (22H, br s, $11CH_2$); ^{13}C NMR ($CDCl_3$): δ 174.6 (s, CO_2Me), 63.3 (t, CH_2OH), 51.6 (q, OCH_3), 34.4, 33.1, 29.9 (2s) 29.8 (2s), 29.7 (2s), 29.5, 29.4, 26.0, 25.2 (each t, each CH_2); ESI-HRMS calcd for $C_{17}H_{35}O_3$ 287.2586, found m/z 287.2572 $[M+H]^+$. Anal. Calcd for $C_{17}H_{34}O_3$: C, 71.28; H, 11.96. Found: C, 71.30; H, 11.74.

1.3. Methyl 16-trimethylsilyloxyhexadecanoate (4)

Methyl 16-hydroxyhexadecanoate **3** (1.12 g, 3.9 mmol) was dissolved in anhyd CH_2Cl_2 (15 mL) and the soln cooled to 0 °C under N_2. Et_3N (819 μL, 5.9 mmol) and TMSCl (574 μL, 4.7 mmol) were added dropwise and the reaction mixture was stirred at rt for 2 h. The mixture was diluted with CH_2Cl_2 (50 mL) and washed with satd aq $NaHCO_3$ (50 mL). The organic layer was dried ($MgSO_4$), filtered and the solvent was removed under diminished pressure. Chromatography (1:50 Et_3N–cyclohexane) gave **4** as a colourless oil (1.07 g, 88%); IR (film) cm^{-1}: 2926, 2854, 1744, 1463, 1436, 1250, 1098; ^1H NMR ($CDCl_3$): δ 3.65 (3H, s, OCH_3), 3.55 (2H, t, J 6.6 Hz, $TMSOCH_2CH_2$), 2.29 (2H, t, J 7.5 Hz, $CH_2CH_2CO_2Me$), 1.66–1.48 (4H, m, $TMSOCH_2CH_2$ and $CH_2CH_2CO_2Me$), 1.25 (22H, s, CH_2), 0.1 (9H, s, $Si(CH_3)_3$); ^{13}C NMR ($CDCl_3$) δ: 174.5 (s, CO_2Me), 63.0 (t, CH_2OSi), 51.6 (q, OCH_3), 34.4, 33.1, 29.9 (three signals), 29.8, 29.7, 29.5, 29.4, 26.1, 25.2 (each t, each CH_2), −0.2 (q, $Si(CH_3)_3$); Anal. Calcd for $C_{20}H_{42}O_3Si$: C, 66.98; H, 11.80. Found: C, 67.10; H, 11.68.

1.4. Methyl 16-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyloxy)-hexadecanoate (10)

A mixture of trichloroacetamide **9**[9] (129 mg, 174 μmol), acceptor **3** (50 mg, 174 μmol) and molecular sieves 4 Å (100 mg) were placed under diminished pressure (<1 mmHg) for 1 h. Anhyd CH_2Cl_2 (2 mL) was added and the sol stirred for 2 h at room temp and TMSOTf (3 μL, 17.4 μmol) then added and stirring continued for a further 20 min at room temp. Solid $NaHCO_3$ (25 mg) was then added and the mixture stirred for 15 min, then filtered through Celite, which was rinsed with CH_2Cl_2 (5 mL). The solvent was removed under diminished pressure and the residue purified by flash chromatography (1:9 EtOAc–cyclohexane) to give the title compound as a colourless syrup (85 mg, 55%) and **11** (3.4 mg, 5%). Analytical data for **10**: $[\alpha]_D$ −7 (c 0.1, MeOH); ^1H NMR ($CDCl_3$, 300 MHz): δ 8.01 (2H, d, J 8.1 Hz, aromatic CH), 7.96 (2H, d, J 8.1 Hz, aromatic CH), 7.90 (2H, d, J 8.1 Hz, aromatic CH), 7.84 (2H, d, J 8.1 Hz, aromatic CH), 7.56–7.26 (12H, m, aromatic CH), 5.90 (1H, t, $J_{2,3}$ 9.6 Hz, $J_{3,4}$ 9.6 Hz, H-3), 5.67 (1H, t, $J_{3,4}$ 9.6 Hz, $J_{4,5}$ 9.9 Hz, H-4), 5.51 (1H, dd, $J_{1,2}$ 7.8 Hz, $J_{3,4}$ 9.6 Hz, H-2), 4.83 (1H, d, $J_{1,2}$ 7.8 Hz, H-1), 4.63 (1H, dd, $J_{6a,6b}$ 12.3 Hz, $J_{5,6a}$ 3.6 Hz, H-6a), 4.50 (1H, dd, $J_{6a,6b}$ 12.3 Hz, $J_{5,6b}$ 5.1 Hz, H-6b), 4.15 (1H, ddd, $J_{4,5}$ 9.9 Hz, $J_{5,6a}$ 3.6 Hz, $J_{5,6b}$ 5.1 Hz, H-5), 3.92 (1H, m, $CHCO_2Me$), 3.66 (3H, s, OCH_3) 3.52 (1H, m, $CHCO_2Me$), 2.30 (2H, t, J 7.5 Hz, OCH_2CH_2), 1.64–1.42 (4H, m, $2CH_2$), 1.27–1.07 (24H, m, $12CH_2$); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 174.5 (s, CO_2Me), 166.2, 166.0, 165.3, 165.2 (each s, each C=O), 133.5, 133.4, 133.3, 133.2 (each s, each aromatic C), 130.0, 129.9, 129.8, 129.5, 129.0, 128.5, 128.4 (each d, each aromatic CH), 101.4 (d, C-1), 73.1, 72.3, 72.0 (each d), 70.5 (t, C-6), 70.0 (d), 63.4 (t, CH_2CH_2OCHO), 51.5 (q, CH_3O), 34.3 (t, $CH_2CH_2CO_2Me$), 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 25.9, 25.1 (each t); ESI-HRMS calcd for $C_{51}H_{60}O_{12}Na$ 887.3982, found m/z 887.3981 $[M+Na]^+$. Analytical data for methyl 16-benzoyloxyhexadecanoate **11**: ^1H NMR ($CDCl_3$, 300 MHz): δ 8.04 (2H, d, J 7.8 Hz, aromatic CH), 7.55 (1H, dd, J 7.8 Hz, J 7.2 Hz, aromatic CH), 7.41 (2H, m, aromatic CH), 4.31 (1H, t, J 6.6 Hz, CH_2O), 3.66 (3H, s, CH_3O-CO), 2.30 (2H, t, J 7.5 Hz, $CH_2CH_2CO_2Me$), 1.77 (2H, m, CH_2CH_2OCOAr), 1.63–1.59 (2H, m, CH_2CH_2CO), 1.25 (22H, m, $11CH_2$); ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 174.5, 166.8 (each s, each C=O), 132.9 (d, aromatic CH), 130.7 (s, aromatic C), 129.7, 128.5 (each d, each aromatic CH), 65.3 (t, CH_2O), 51.6 (q, OCH_3), 34.2 (t, CH_2CO_2), 29.8, 29.7, 29.6, 29.5, 29.3, 28.9, 26.2, 25.9, 25.1 (each t, each CH_2); ESI-HRMS calcd for $C_{24}H_{39}O_4$ 391.2848, found m/z 391.2853 $[M+H]^+$.

1.5. 16-(β-D-Glucopyranosyloxy)-hexadecanoic acid (1)

Protected glycoside **10** (32 mg, 37 μmmol) was dissolved in dry MeOH (1 mL). A catalytic amount (20 μL) of sodium methanolate (1 M in MeOH) was added, and the resulting soln was stirred for 2 h at rt. The solvent was removed under diminished pressure and water (1 mL) was added. THF was added dropwise into the cloudy solution until it became clear and LiOH (10 mg) then added and the solution was stirred for 1 h at rt. Amberlite IR-120 (plus) was added until pH 6.0. The resin was filtered off and rinsed with THF and then solvent was evaporated under diminished pressure until the soln became cloudy and the remainder of solvent removed by freeze drying. Residual benzoic acid was removed by repeated freeze drying (×3) from water and the title compound further purified by chromatography (MeOH–CH_2Cl_2) to give a white powder (16 mg, 100%); $[\alpha]_D$ −7 (c 0.1, MeOH); mp 85 °C; ^1H NMR (CD_3OD): δ 4.27 (1H, d, $J_{1,2}$ 7.8 Hz, H-1), 3.93–3.68

(2H, m), 3.71–3.65 (1H, m), 3.59–3.51 (1H, m, CH*H*CO_2Me), 3.39–3.27 (3H, m, CH*H*CO_2Me and H-6a,6b), 3.18 (1H, t, *J* 8.4 Hz), 2.30 (2H, t, *J* 7.5 Hz, OC$H_2$$CH_2$), 1.64–1.56 (4H, m, 2C$H_2$), 1.31 (24H, s, 12C$H_2$); ^{13}C NMR ($CD_3OD$): δ 176.1 (s, CO_2H), 104.4 (d, C-1), 78.2, 78.0, 75.2, 71.8 (each d, each CH), 71.0 (t, C-6), 62.8 (t, CH_2*C*H_2OCO), 34.9 (t, *C*H_2CO_2H), 30.9, 30.8, 30.7, 30.6, 30.4, 30.2, 27.2, 26.0 (each t); ESI-HRMS calcd for $C_{22}H_{42}O_8Na$ 457.2770, found *m*/*z* 457.2762 $[M+Na]^+$. To determine the purity, **1** (2.997 mg, 6.90 μmol) and methyl α-D-glucopyranoside as the internal standard (99%, 1.356 mg, 6.99 μmol) were dissolved in Me_2SO-d_6 and the ^{1}H NMR spectrum of the mixture recorded. Integration of the respective anomeric proton signals indicated that the purity of **1** was 93%.

1.6. Methyl 16-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyloxy)-hexadecanoate (12)

Alcohol **3** (50 mg, 0.17 mmol), silver carbonate (164 mg, 0.60 mmol), silver perchlorate (3.6 mg, 0.02 mmol) and molecular sieves were dried under diminished pressure for 3 h. Anhyd CH_2Cl_2 (0.75 mL) was added and the mixture was stirred at rt for 2 h. Bromide **6** (109 mg, 0.26 mmol) in dry CH_2Cl_2 (0.2 mL) was added dropwise and the mixture was stirred under N_2 at rt for 1.5 h. The mixture was diluted with CH_2Cl_2 (20 mL) and washed with water (20 mL). The organic layer was dried ($MgSO_4$), filtered and solvent removed under diminished pressure. Chromatography (1:4–1:3 EtOAc–cyclohexane) gave **12** as a white powder (32 mg, 31%); mp 55 °C; $[\alpha]_D$ −14 (*c* 0.3, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 5.20 (1H, t, $J_{2,3} = J_{3,4} = 9.6$ Hz, H-3), 5.09 (1H, t, $J_{3,4} = J_{4,5} = 9.6$ Hz, H-4), 4.98 (1H, t, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 9.6 Hz, H-2), 4.49 (1H, d, $J_{1,2}$ 7.8 Hz, H-1), 4.27 (1H, dd, $J_{6a,6b}$ 12.3 Hz, $J_{5,6a}$ 4.8 Hz, H-6a), 4.13 (1H, dd, $J_{6a,6b}$ 12.3 Hz, $J_{5,6b}$ 2.4 Hz, H-6b), 3.90–3.83 (1H, m, CH_2CH*H*O), 3.70–3.66 (1H, ddd, $J_{5,6b}$ 2.4 Hz, $J_{5,6a}$ 4.8 Hz, $J_{4,5}$ 9.6 Hz, H-5), 3.66 (3H, s, OCH_3), 3.51–3.43 (1H, m, CH_2C*H*HO), 2.30 (2H, t, *J* 7.5 Hz, CH_2CO_2Me), 2.09, 2.04, 2.02, 2.01 (12H, each s, each CH_3COO), 1.64–1.56 (4H, m, CH_2CH_2O and C$H_2CH_2CO_2$Me), 1.25 (22H, s, CH_2); ^{13}C NMR ($CDCl_3$): δ 174.5 (s, *C*O_2Me), 170.9, 170.5, 169.6, 169.5 (each s, each C=O), 101.1 (d, C-1), 73.2, 72.0, 71.7 (each d), 70.5 (t, C-6), 68.8 (d), 62.3 (t, CH_2*C*H_2OCO), 51.6 (q, CH_3O), 34.3 (t, *C*H_2CO_2Me), 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 26.1, 25.2 (each t), 21.0, 20.9, 20.8 (each q); ESI-HRMS calcd for $C_{31}H_{52}O_{12}Na$ 639.3356, found *m*/*z* 639.3326 $[M+Na]^+$.

1.7. Analytical data for methyl 16-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyloxy)-hexadecanoate (13)

White solid mp 31 °C; $[\alpha]_D$ +87 (*c* 0.3, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 5.48 (1H, t, $J_{2,3}$ 10.0 Hz, $J_{3,4}$ 9.6 Hz, H-3), 5.06 (1H, d, $J_{1,2}$ 3.9 Hz, H-1), 5.06 (1H, t, $J_{3,4}$ 9.6 Hz, $J_{4,5}$ 9.6 Hz, H-4), 4.85 (1H, dd, $J_{1,2}$ 3.9 Hz, $J_{2,3}$ 10.0 Hz, H-2), 4.26 (1H, dd, $J_{6a,6b}$ 12.3 Hz, $J_{5,6a}$ 4.5 Hz, H-6a), 4.08 (1H, dd, $J_{6a,6b}$ 12.3 Hz, $J_{5,6b}$ 2.1 Hz, H-6b), 3.99 (1H, $J_{4,5}$ 10.2 Hz, $J_{5,6a}$ 4.5 Hz, $J_{5,6b}$ 2.1 Hz, ddd, H-5), 3.70–3.66 (4H, m, CH*H*CO_2Me and OCH_3), 3.46–3.40 (1H, m, C*H*HCO_2Me), 2.30 (2H, t, *J* 7.5 Hz, OC$H_2$$CH_2$), 2.09, 2.06, 2.03, 2.01 (12H, each s, each CH_3COO), 1.64–1.56 (4H, m, 2CH_2), 1.25 (24H, se, 12CH_2); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 174.6 (s, *C*O_2Me), 170.9, 170.5, 170.4, 169.9 (each s, each C=O), 95.9 (d, C-1), 71.3, 70.6 (each d), 69.1 (t, C-6), 69.0, 67.5 (each d), 62.3 (t, CH_2*C*H_2OCO), 51.7 (q, OCH_3), 34.4 (t, CH_2*C*H_2CO_2Me), 29.9 (5s), 29.7, 29.6 (2s), 29.5 (2s), 26.3, 25.3 (each t), 21.0, 20.9 (2s) (each q); ESI-HRMS calcd for $C_{31}H_{52}O_{12}Na$ 639.3356, found *m*/*z* 639.3340 $[M+Na]^+$. Anal. Calcd for $C_{31}H_{52}O_{12}$: C, 60.37; H, 8.50. Found: C, 59.97; H, 8.35.

1.8. Analytical data for methyl 16-acetyloxyhexadecanoate (15)

^{1}H NMR ($CDCl_3$, 300 MHz): δ 4.05 (1H, t, *J* 7 Hz, CH_2OCOMe), 3.66 (3H, s, CH_3OCO), 2.31 (2H, t, *J* 7.5 Hz, CH_2CH_2CO_2Me), 2.04 (3H, s, CH_3CO_2), 1.66–1.59 (4H, m, OCH_2CH_2 and CH_2CH_2CO), 1.25 (22H, m, 11CH_2); ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 174.4, 171.3 (s, each C=O), 64.8 (t, *C*H_2O), 51.5 (q, O*C*H_3), 34.2 (q, CH_2CO_2), 29.7, 29.6, 29.5, 28.7, 26.5, 25.0, 21.1 (t, 13*C*H_2).

1.9. Methyl 16-*O*-[2-(3,4,6-tri-*O*-acetyl-α-D-glucopyranose-1,2-di-*O*-yl)ethyl]-hexadecanoate (14)

^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.70 (1H, d, $J_{1,2}$ 5 Hz, H-1), 5.19 (1H, m, H-3), 4.90 (1H, dd, $J_{3,4}$ 10 Hz, $J_{4,5}$ 3 Hz, H-4), 4.31 (1H, m, H-2), 4.20 (2H, m, H-6a,6b), 3.95 (1H, dt, $J_{4,5}$ 3 Hz, $J_{5,6a} = J_{5,6b} = 9$ Hz, H-5), 3.66 (1H, s, CO_2Me), 3.44 (2H, t, *J* 7 Hz, CH_2O), 2.29 (2H, t, CH_2CO_2), 2.11, 2.09, 2.08 (9H, each s, each CH_3CO), 1.71 (3H, s, C$H_3C(O)_3$), 1.63–1.50 (4H, m, CH_2CH_2O and C$H_2CH_2CO_2$Me), 1.25 (22H, m, 11CH_2); ^{13}C NMR ($CDCl_3$, 75 MH_Z): δ (ppm) 174.0, 170.7, 169.7, 169.2 (each s, each C=O), 121.3 (s), 96.9 (s, C-1), 73.1, 70.2, 67.0 (each d), 63.7, 63.1 (each t, CH_2O and C-6), 51.4 (q, *C*H_3O), 34.1 (t, CH_2*C*H_2CO_2Me), 29.6, 29.8, 29.4, 29.2, 26.0, 24.0 (each t, each CH_2), 20.8 (q); ESIMS: *m*/*z* $[M+Na]^+$: calcd 639.3, found 639.3.

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Supplementary data

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