J Chem Biol (2013) 6:121–133
DOI 10.1007/s12154-013-0093-6

ORIGINAL ARTICLE

Lack of activation of UCP1 in isolated brown adipose tissue mitochondria by glucose-*O*-ω-modified saturated fatty acids of various chain lengths

Eamon P. Breen · Wayne Pilgrim · Kieran J. Clarke · Cristy Yssel · Mark Farrell · Jian Zhou · Paul V. Murphy · Richard K. Porter

Received: 4 September 2012 / Accepted: 4 March 2013 / Published online: 27 March 2013

Abstract We previously demonstrated that uncoupling protein 1 activity, as measured in isolated brown adipose tissue mitochondria (and as a native protein reconstituted into liposome membranes), was not activated by the non-flippable modified saturated fatty acid, glucose-*O*-ω-palmitate, whereas activity was stimulated by palmitate alone (40 nM free final concentration). In this study, we investigated whether fatty acid chain length had any bearing on the ability of glucose-*O*-ω-fatty acids to activate uncoupling protein 1. Glucose-O-ω-saturated fatty acids of various chain lengths were synthesized and tested for their potential to activate GDP-inhibited uncoupling protein 1-dependent oxygen consumption in brown adipose tissue mitochondria, and the results were compared with equivalent non-modified fatty acid controls. Here we demonstrate that laurate (12C), palmitate (16C) and stearate (18C) could activate GDP-inhibited uncoupling protein 1-dependent oxygen consumption in brown adipose tissue mitochondria, whereas there was no activation with glucose-*O*-ω-laurate (12C), glucose-*O*-ω-palmitate (16C), glucose-*O*-ω-stearate (18C), glucose-*O*-ω-arachidate (20C) or arachidate alone. We conclude that non-flippable fatty acids cannot activate uncoupling protein 1 irrespective of chain length. Our data further undermine the cofactor activation model of uncoupling protein 1 function but are compatible with the model that uncoupling protein 1 functions by flipping long-chain fatty acid anions.

Keywords Mitochondria · UCP1 · Glucose-*O*-ω-fatty acids · BAT

Abbreviations

BAIB	[bis(acetoxy)iodo]benzene
BAT	Brown adipose tissue
DEPT	Distortionless enhancement by polarization transfer
HMBC	Heteronuclear multiple-bond correlation
HMQC	Heteronuclear multiple-quantum correlation
TEMPO	2,2,6,6-Tetramethylpiperidinyloxyl
THF	Tetrahydrofuran
UCP	Uncoupling protein

Introduction

The molecular basis of non-shivering thermogenesis emanating from brown adipose tissue (BAT) is the dissipation of the proton electrochemical gradient across the mitochondrial inner membrane by uncoupling protein 1 (UCP1; also known as UCP and thermogenin). UCP1 is present in all eutherian mammals [1, 2], and recent evidence points strongly to BAT activity in adult humans [3–5]. UCP1 has also recently been found in thymus tissue of rats and mice [6–8] where it appears to have a role in thymocyte processing and T-cell selection [9].

The mechanism of the uncoupling action by UCP1 is still a contentious issue. The two models that have been proposed for the fatty acid-dependent UCP1 uncoupling function both have evidence to support these models. The

Electronic supplementary material The online version of this article (doi:10.1007/s12154-013-0093-6) contains supplementary material, which is available to authorized users.

E. P. Breen · K. J. Clarke · C. Yssel · R. K. Porter (✉)
School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Pearse Street, Dublin 2, Ireland
e-mail: rkporter@tcd.ie

W. Pilgrim · M. Farrell · J. Zhou · P. V. Murphy
School of Chemistry, National University of Ireland Galway, University Road, Galway, Ireland

flip/flop or "flippase" model proposes a two-step event, whereby the protonated fatty acid freely flips across the mitochondrial inner membrane, independently of UCP1; protons dissociate from the fatty acids into the matrix, and the resulting fatty acid anions are flipped back across (non-proton-dependent charge transfer) the inner membrane by UCP1 [10–12]. The strongest evidence for this flip/flop or "flippase" model comes from the observations that undecanesulfonate, which has a pK ~2 and is thus predominantly an anion at neutral pH, can be flipped by UCP1 reconstituted in liposome membranes [13–15]. In a previous publication, we revisited these experiments and confirmed the observations that UCP1 can transfer charge across the proteoliposome membrane in the presence of undecanesulfonate and that the charge transfer is GDP sensitive [16].

The second model proposes that UCP1 acts as a proton conduit across the mitochondrial inner membrane and, importantly, that fatty acids act as cofactors/activators providing additional carboxyl moieties at key intra-membrane sites, thus enhancing the rate of proton conductivity through the membrane [17–19]. The key piece of evidence for the cofactor/activation model comes from tabulated data cited in two reviews [17, 18], stating that the "unflippable" glycolipid, glucose-*O*-ω-palmitate "activates" native UCP1 reconstituted into liposome membranes. We subsequently re-investigated this latter crucial piece of evidence for the cofactor/activation model by first synthesizing glucose-*O*-ω-palmitate [20] and then investigating the ability of the modified long-chain fatty acid to activate UCP1 [16]. We observed no activation by glucose-*O*-ω-palmitate of UCP1 in mitochondria isolated from brown adipose tissue or in native UCP1 reconstituted into liposome membranes, whereas activity was stimulated by palmitate alone. We concluded that our data were consistent with the "flippase" model of UCP 1 function but not the cofactor/activation model of UCP 1 function. However, the possibility remained that glucose-*O*-ω-palmitate may not have had the optimal chain length to activate UCP1, and thus in this study we synthesized glucose-*O*-ω-modified saturated fatty acids of various chain lengths and tested their potential to activate GDP-inhibited UCP1-dependent oxygen consumption in brown adipose tissue mitochondria.

Experimental

Synthesis of glucose-*O*-ω-fatty acids

The synthesis of compound **1b** (Fig. 1) was carried out as previously described via **3b** and **4b** [20]. By a similar sequence, the glycosidation reaction of alcohol **3a** with trichloroacetimidate **2** gave **4a**. Removal of the benzoate protecting groups followed by saponification using LiOH gave the C-12 derivative **1a**. Compounds **3a** and **3b** were prepared from commercially available acid precursors as described previously [20].

The synthesis of **1c**–**1e** (Fig. 2) was not as routine as for the synthesis of **1a** and **1b** as the corresponding hydroxyalkanoic acid derivatives are not commercially available. After some preliminary investigations using cross-metathesis which was not successful, a strategy based on the Wittig reaction was chosen. The precursors to the Wittig reagents **6c**–**e** were first prepared from **5c**–**e** and the aldehyde **8** was then prepared in two steps (benzylation, Swern oxidation) from the commercially available diol **7**. The Wittig olefination using the Wittig reagent prepared by treatment of **6c**–**e** with LiHMDS with **8** gave alcohols **9c**–**e**. LiHMDS or *n*-BuLi was used for the deprotonation of the salt. Subsequent glycosidation reactions and subsequent removal of the benzyl protecting group using catalytic hydrogenation gave the protected hydroxyalkyl β-D-glucosides **10c**–**e**. Oxidation of the primary alcohol to the acid using TEMPO-BAIB followed by methanolysis of the benzoate esters gave the C18, C20 and C22 derivatives **1c**–**e**. All compounds were purified by chromatography before biological testing. The purity of compounds **1a** (82 %), **1b** (94 %), **1c** (81 %), and **1e** (85 %) was determined by ^{1}H-NMR using methyl α-D-glucopyranoside as an internal standard. This technique involved dissolving a known amount of both the product and standard in DMSO-d_6. The anomeric protons for the product and standard were integrated in the resulting ^{1}H-NMR spectrum, and these data were used to calculate the purity. The purity of **1d** was greater than 85 %.

General experimental conditions Optical rotations were determined with a Perkin-Elmer 343 model polarimeter at the sodium D line at 20 °C. NMR spectra were recorded with Varian Inova 300, VNMRS-500 and VNMRS-600 spectrometers. Chemical shifts are reported relative to internal Me_4Si in $CDCl_3$ (δ 0.0) or HOD for D_2O (δ 4.84) or CD_2HOD (δ 3.31) for ^{1}H and Me_4Si in $CDCl_3$ (δ 0.0) or $CDCl_3$ (δ 77.0) or CD_3OD (δ 49.05) for ^{13}C. ^{1}H NMR signals were assigned with the aid of COSY. ^{13}C NMR signals were assigned with the aid of DEPT, HSQC and/or HMBC. Coupling constants are reported in hertz. The IR spectra were recorded with Mattson Galaxy Series FTIR 3000 using thin film between NaCl plates. Melting points were measured on a Barnstead Electrothermal 9300 melting point apparatus. Low- and high-resolution mass spectra were in positive and/or negative mode as indicated in each case. Thin layer chromatography (TLC) was performed on aluminium sheets precoated with silica gel and spots visualized by UV and charring with H_2SO_4–EtOH (1:20) or cerium molybdate. Flash chromatography was carried out with silica gel 60 (0.040–0.630 mm) and using a stepwise

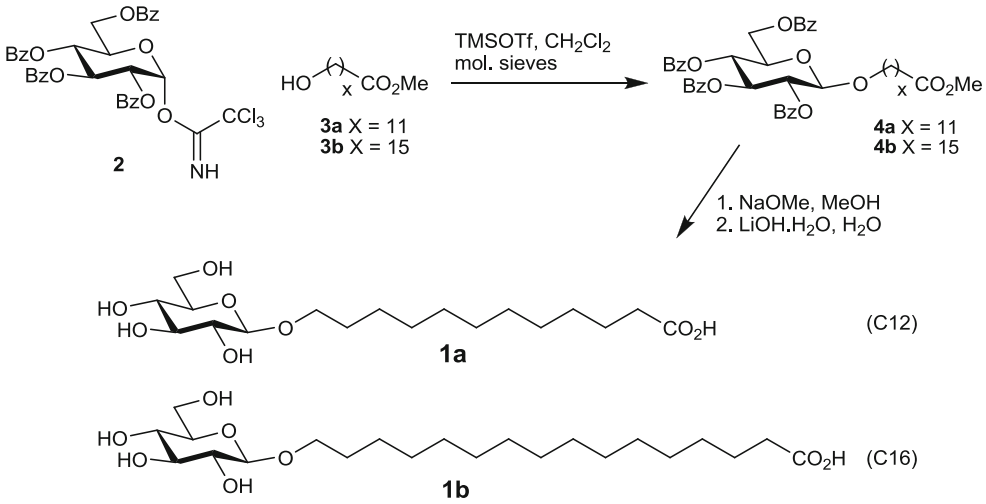
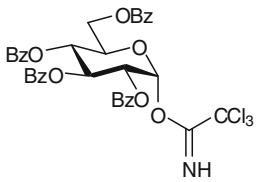


Fig. 1 Synthesis of glucose-*O*-fatty acids (**1a** and **1b**)

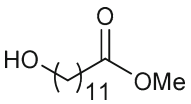
solvent polarity gradient correlated with TLC mobility. Chromatography solvents were used as obtained from suppliers. CH_2Cl_2, MeOH and THF reaction solvents were freshly dried using a Pure Solv™ Solvent Purification System, and acetonitrile, DMF, pyridine and toluene were used as purchased from Sigma-Aldrich.

2,3,4,6-Tetra-*O*-benzoyl-1-(2,2,2-trichloroethanimidate)-α-D-glucopyranoside **2**



This compound **2** was prepared as described previously by Mbadugha and Menger [21]. Thus, glucose (1.00 g, 5.56 mmol) in pyridine (12 mL) was cooled to 0 °C, and to this benzoyl chloride (4.85 g, 34.5 mmol, 4 mL) was added portion-wise and the reaction mixture was allowed to attain room temperature over 16 h. The reaction mixture was diluted with EtOAc, washed with 1 M HCl, water, and brine, and dried over $MgSO_4$, and the solvent was removed under reduced pressure. Recrystallisation from acetone–water gave α-D-glucopyranose pentabenzoate (2.75 g, 71 %) as a white solid. This pentabenzoate (4 g, 5.71 mmol) was dissolved in CH_2Cl_2 (20 mL) and cooled to 0 °C. To this, HBr (33 % in AcOH, 10 mL) was added and the reaction mixture was stirred at room temperature for 6 h. The mixture was diluted with Et_2O, washed with water, saturated $NaHCO_3$, water, and brine, dried ($MgSO_4$) and concentrated under reduced pressure to give the glycosyl bromide as colourless oil. This was taken up in acetone (100 mL) and water (2 mL); to this, Ag_2CO_3 (788 mg, 2.86 mmol) was added and the reaction mixture was stirred for 16 h at room temperature. The reaction mixture was filtered through celite, which was rinsed with CH_2Cl_2, dried ($MgSO_4$) and concentrated under reduced pressure. Flash chromatography of the residue (EtOAc–cyclohexane, 1:4) gave 2,3,4,6-tetra-*O*-benzoyl-D-glucopyranose as a white solid (2.81 g, 83 %); R_f 0.31 (EtOAc–cyclohexane 1:1). This hemiacetal (2.22 g, 3.73 mmol) was dissolved in CH_2Cl_2 (80 mL) and cooled to 0 °C. To this, Cl_3CCN (3.74 mL, 37.3 mmol) and DBU (five drops) were added. The reaction mixture was stirred for 4 h at 0 °C, then concentrated to 4 mL, and the residue was subjected to chromatography (EtOAc–cyclohexane 1:4) to give **2** (1.87 g, 68 %) as a colourless oil; R_f 0.26 (EtOAc–cyclohexane 1:4); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.56 (1H, s, NH), 7.23–7.96 (20H, aromatic protons) 6.77 (1H, d, J 3.7 Hz, H-1), 6.20 (1H, t, J 10.0 Hz, H-3), 5.74 (1H, t, J 10.0 Hz, H-4), 5.55 (1H, dd, J 10.0 Hz, J 3.7 Hz, H-2), 4.58 (1H, overlapping signals, H-5, H-6a), 4.42 (1H, dd, J 12.5 Hz, J 5.1 Hz, H-6b); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 166.0, 165.6, 165.4, 165.2 (C=O and CCl_3), 160.5 (C=N), 133.5, 133.3, 133.1, 129.9, 129.8, 129.7, 128.4, 128.3 (each Ar–C and CH), 93.1 (C-1), 70.7 (C-2 and C-5), 70.2 (C-3), 68.7 (C-4), 62.5 (C-6). The analytical data we recorded for **2** were in good agreement with that reported previously [20].

Methyl 12-hydroxydodecanoate **3a** [22]



12-Hydroxydodecanoic acid (1.0 g, 4.63 mmol) and *p*-toluenesulfonic acid monohydrate (1.39 mmol, 264 mg) were dried under reduced pressure for 2 h. MeOH (35 mL) was added, and the reaction mixture was stirred at room temperature for 16 h. Solid $NaHCO_3$ (250 mg) was added,

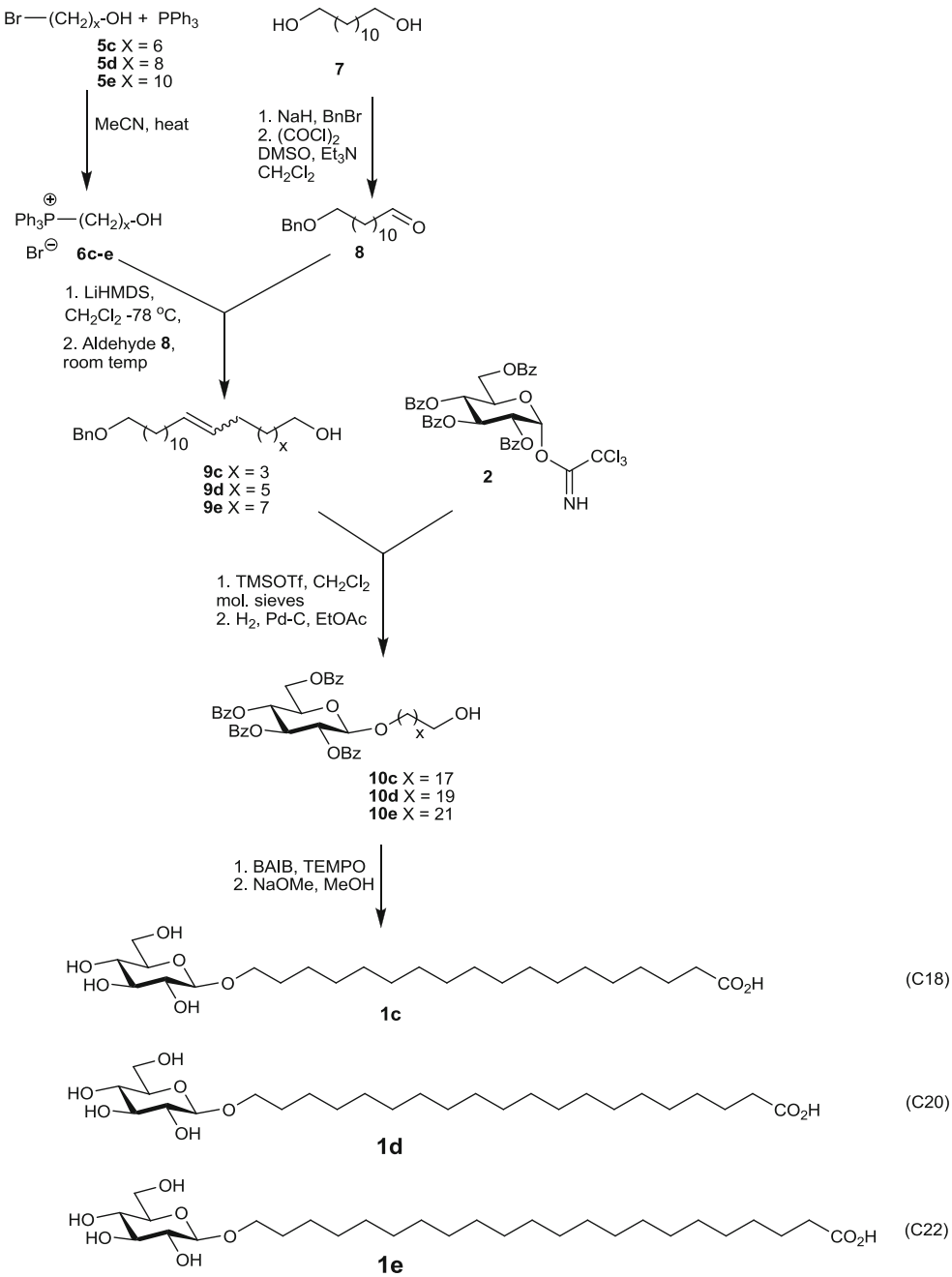


Fig. 2 Synthesis of glucose-*O*-fatty acids (**1c**–**1e**)

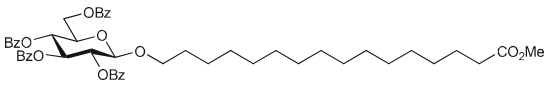
and the mixture was stirred for a further 30 min, then filtered through celite and the solvent removed under reduced pressure. Chromatography of the residue (EtOAc–cyclohexane 1:3) gave **3a** (1.06 g, 100 %) as a white solid; R_f 0.28 (EtOAc–cyclohexane, 1:4); IR (film) cm^{-1}: 3,301 (OH), 2,921, 2,851, 1,743 (C=O), 1,471, 1,369, 1,200, 1,163; ^1H NMR ($CDCl_3$, 500 MHz):"δ 3.66 (3H, s, OCH_3), 3.64 (2H, t, J 6.6 Hz, H-12), 2.30 (2H, t, J 7.6 Hz, H-2), 1.62 (2H, m, H-3), 1.56 (2H, m, H-11), 1.28 (14H, s, 7 CH_2); ^{13}C NMR ($CDCl_3$, 126 MHz): δ 174.30 (CH_2, C-1), 63.05 (CH_2, C-12), 51.39 (OCH_3), 34.10 (CH_2, C-2), 32.79, 29.52, 29.45, 29.37, 29.20, 29.11, 25.71, 24.93 (each CH_2); ES-HRMS calculated for $C_{13}H_{27}O_3$ 231.1960, found m/z 231.1917 $[M+H]^+$.

Methyl 12-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyloxy)-dodecanoate **4a**

A mixture of trichloroacetamide **2** (100 mg, 0.135 mmol), acceptor **3a** (37 mg, 0.162 mmol) and molecular sieves 4 Å (100 mg) was placed under reduced pressure for 1 h. Dichloromethane (3 mL) was added, and the solution was stirred for 40 min at room temperature. The solution was cooled to 0 °C, TMSOTf (0.27 mL of 0.05 M in dichloromethane, 0.

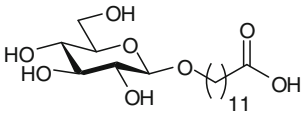
0135 mmol) was added and the mixture was stirred for a further 30 min. Solid $NaHCO_3$ (25 mg) was added and the mixture stirred for 20 min, filtered through celite, which was then rinsed with CH_2Cl_2. The solvent was removed under reduced pressure, and chromatography of the residue (EtOAc–cyclohexane, 1:9) gave **4a** as a colourless syrup (73 mg, 67 %); R_f 0.16 (EtOAc–cyclohexane 1:4); $[\alpha]_D$ + 12.6 (c 3.7,$CHCl_3$); IR (film) cm^{-1}: 3,064, 2,928, 2,854, 1,733, 1,267, 1,108, 1,027, 709; ^1H NMR ($CDCl_3$, 500 MHz): δ 7.25–8.02 (20H, ms, aromatic H), 5.91 (1H, t, J 9.6 Hz, H-3), 5.67 (1H, t, J 9.6 Hz, H-4), 5.52 (1H, dd, J 9.6 Hz, J 7.9 Hz, H-2), 4.84 (1H, d, J 7.9 Hz, H-1), 4.64 (1H, dd, J 12.1 Hz, J 3.4 Hz, H-6a), 4.51 (1H, dd, J 12.1 Hz, J 5.2 Hz, H-6b), 4.16 (1H, ddd, J 9.6 Hz, J 5.2 Hz, J 3.4 Hz, H-5), 3.91 (1H, dt, J 9.7, J 6.3 Hz, CHHO), 3.66 (3H, s, OCH_3), 3.54 (1H, dt, J 9.7 Hz, J 6.7 Hz, CHHO), 2.29 (2H, t, J 7.6 Hz, CH_2CO_2Me), 1.00–1.66 (18H, m, 9×CH_2); ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.24, 166.10, 165.80, 165.17, 165.02 (each C=O) 133.34, 133.14, 133.09, 133.03, 129.78, 129.72, 129.70 (each aromatic CH), 129.65, 129.41, 128.91 128.87 (each aromatic C), 128.34, 128.33, 128.29, 128.27, 128.23 (each aromatic CH), 101.28 (CH, C-1), 72.97 (CH, C-3), 72.16 (CH, C-5), 71.95 (CH, C-2), 70.30 (CH_2O), 69.92 (CH, C-4), 63.26 (CH, C-6), 51.36 (OCH_3), 34.08, 29.38, 29.37, 29.34, 29.18, 29.11, 26.89, 25.74, 24.92 (each CH_2). ES-HRMS calculated for $C_{47}H_{52}O_{12}Na$ 831.3356, found *m/z* 831.3391 $[M+Na]^+$.

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



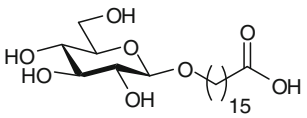
A mixture of trichloroacetamide 2 (200 mg, 0.270 mmol), acceptor 3b (100 mg, 0.350 mmol), and molecular sieves 4 Å (100 mg) were placed under reduced pressure for 1 h. CH_2Cl_2 (5 mL) was added and the solution was stirred for 40 min at room temperature. The solution was cooled to 0 °C and TMSOTf (0.05 M, 0.035 mmol, 0.70 mL) was added and the reaction mixture was stirred for a further 30 min. Solid $NaHCO_3$ (50 mg) was added and the mixture stirred for 20 min, then filtered through celite, which was rinsed with CH_2Cl_2. The solvent was removed under reduced pressure and the residue purified by flash chromatography (EtOAc–cyclohexane 1:9) gave 4b as colourless oil (179 mg, 77 %); R_f 0.39 (EtOAc–cyclohexane 1:4); $[\alpha]_D$ -7 (c 0.1, MeOH); IR (film) cm^{-1}: 2,925, 2,853, 1,733, 1,265, 1,094, 708; ^1H NMR ($CDCl_3$, 500 MHz): δ 7.25–7.8.05 (20H, ms, aromatic H), 5.90 (1H, t, J 9.7 Hz, H-3), 5.66 (1H, t, J 9.7 Hz, H-4), 5.51 (1H, dd, J 7.9 Hz, J 9.7 Hz, H-2), 4.83 (1H, d, J 7.9 Hz, H-1), 4.63 (1H, dd, J 12.1 Hz, J 3.3 Hz, H-6a), 4.51 (1H, dd, J 12.1 Hz, J 5.3 Hz, H-6b), 4.15 (1H, ddd, J 9.7 Hz, J 5.3 Hz, J 3.3 Hz, H-5), 3.91 (1H, dt, J 9.7 Hz, J 6.3 Hz, CHHO), 3.66 (3H, s, OCH_3), 3.53 (1H, dt, J 9.6 Hz, J 6.7 Hz, CHHO), 2.30 (2H, t, J 7.6 Hz, CH_2CO_2), 1.00–1.65 (28H, ms, 14CH_2); ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.31, 166.14, 165.84, 165.21, 165.06 (each C=O), 133.37, 133.17, 133.11, 133.06, 129.88, 129.82, 129.78, 129.76, 129.75, 129.73 (each aromatic CH), 129.66, 129.44, 128.90, 128.88 (each aromatic C), 128.43, 128.38, 128.38, 128.33, 128.30, 128.27 (each aromatic CH), 101.31 (CH, C-1), 72.99 (CH, C-3), 72.18 (CH, C-5), 71.97 (CH, C-5), 70.37 (CH_2O), 69.95 (CH, C-4), 63.29 (CH_2, C-6), 51.40 (OCH_3), 34.13, 29.64, 29.62, 29.59, 29.53, 29.46, 29.42, 29.26, 29.16, 25.79, 24.97 (each CH_2). ES-HRMS calculated for $C_{51}H_{60}O_{12}Na$ 887.3982, found *m/z* 887.3981 $[M+Na]^+$.

12-(β-D-Glucopyranosyloxy)-dodecanoic acid (**1a**)



Protected glycoside 4a (72 mg, 0.0891 mmol) was dissolved in MeOH (5 mL). A catalytic amount of NaOMe (1 M in MeOH) was added, and the resulting solution was stirred for 2 h at room temperature. The reaction was quenched with Amberlite IR-120 (plus) until pH=6.0, the resin was filtered off and washed with THF–MeOH 1:1. The solvent was removed under reduced pressure and dried thoroughly under high vacuum. The white solid was taken up in H_2O (2 mL) and THF was added until the solution became clear. To this, $LiOH \cdot H_2O$ (20 mg) was added, and the reaction mixture was stirred for 1 h. The reaction was quenched using Amberlite IR-120 (plus) until pH=6.0; the resin was filtered off and washed with THF–MeOH 1:1. The solvent was removed under reduced pressure. Chromatography (1:9 MeOH–CH_2Cl_2) gave **1a** (27 mg, 81 %) as a white solid; IR (film) cm^{-1}: 3,362, 2,918, 2,850, 1,553, 1,261, 1,083, 1,037, 839; ^1H NMR (CD_3OD, 500 MHz): δ 4.28 (1H, d, J 7.8 Hz, H-1), 3.92 (1H, dt, J 9.5 Hz, J 6.9 Hz, CHHO), 3.89 (1H, dd, J 11.8 Hz, J 2.0 Hz, H-6a), 3.70 (1H, dd, J 11.8 Hz, J 5.4 Hz, H-6b), 3.57 (1H, dt, J 9.5 Hz, J 6.9 Hz, CHHO), 3.26–3.40 (3H, overlapping signals, H-3, H4 and H-5), 3.20 (1H, dd, J 9.1 Hz, J 7.8 Hz, H-2), 2.29 (2H, t, J 7.4 Hz, CH_2CO_2H), 1.30–1.70 (18H, ms, 9 CH_2); ^{13}C NMR (CD_3OD, 125 MHz): δ 174.62 (C=O, detected indirectly using HMBC), 104.44 (CH, C-1), 78.22 (CH, C-3), 77.98 (CH, C-4), 75.21 (CH, C-2), 71.77 (CH, C-5), 70.96 (CH_2O), 62.87 (CH_2, C-6), 30.86, 30.75, 30.68, 30.64, 30.53, 30.50, 27.15 (each CH_2); ES-HRMS calculated for $C_{18}H_{33}O_8$ 377.2175, found *m/z* 377.2159 $[M-H]^-$.

16-(β-D-Glucopyranosyloxy)-hexadecanoic acid (**1b**)



The protected glycoside **4b** (100 mg, 0.0891 mmol) was dissolved in MeOH (5 mL). A catalytic amount of NaOMe (1 M in MeOH) was added, and the resulting solution was stirred for 2 h at room temperature. The reaction was quenched with Amberlite IR-120 (plus) until pH=6.0; the resin was filtered off and washed with THF–MeOH 1:1. The solvent was removed under reduced pressure, and the residue was dried thoroughly under high vacuum. The white solid was taken up in H_2O (2 mL), and THF was added until the solution became clear. To this, LiOH (20 mg) was added, and the reaction mixture was stirred for 1 h. The reaction was quenched with Amberlite IR-120 (plus) until pH=6.0; the resin was filtered off and washed with 1:1 THF–MeOH. The solvent was then removed under reduced pressure. Chromatography (MeOH–CH_2Cl_2 1:9) gave 1b (32 mg, 64 %) as a white solid; $[\alpha]_D$ -7 (c 0.1, MeOH); ^{1}H NMR (CD_3OD, 500 MHz): δ 4.28 (1H, d, J 7.8 Hz, H-1), 3.92 (1H, dt, J 9.5 Hz, J 6.9 Hz, CHHO), 3.90 (1H, dd, J 11.8 Hz, J 1.8 Hz, H-6a), 3.70 (1H, dd, J 11.8 Hz, J 5.3 Hz, H-6b), 3.58 (1H, dt, J 9.5 Hz, J 6.9 Hz, CHHO), 3.37 (1H, t, J 8.8 Hz, J 8.8 Hz, H-3), 3.31 (1H, t, J 8.8 Hz, J 8.8 Hz, H-4), 3.28 (1H, ddd, J 8.8 Hz, J 5.3 Hz, J 1.8 Hz, H-5), 3.20 (1H, dd, J 8.8 Hz, J 7.8 Hz, H-2), 2.30 (2H, t, J 7.4 Hz, CH_2CO_2), 1.64 (4H, m, 2 CH_2), 1.34 (22H, s, 11 CH_2); ^{13}C NMR (CD_3OD, 125 MHz): δ 178.4 (C=O, detected by HMBC), 104.4 (CH, C-1) 78.08 (CH, C-3), 77.97 (CH, C-4), 75.20 (CH, C-2), 71.77 (CH, C-5), 70.97 (CH_2O), 62.87 (C-6), 30.86, 30.79, 30.78, 30.75, 30.66, 30.48, 30.35, 27.17 (each CH_2); ES-HRMS calculated for $C_{22}H_{47}O_8Na$ 457.2770, found *m*/*z* 457.2762 $[M+Na]^+$.

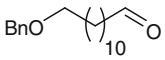
Preparation of alkyltriphenylphosphonium bromides (**6c-e**) [23]

6-Bromohexan-1-ol 5c (687 mg, 3.79 mmol) and triphenylphosphine (994 mg, 3.79 mmol) were dissolved in CH_3CN (10 mL) and the mixture was heated at reflux for 4 days. The solvent was removed to give **6c** as a translucent hygroscopic solid, which was used in the next step without further purification; ^{31}P NMR ($CDCl_3$, 161 MHz): δ 24.5; ES-HRMS calculated for $C_{24}H_{28}OP$ 363.1872, found *m*/*z* 363.1876 $[M-Br^-]^+$. The ^{1}H-NMR and ^{13}C data were in good agreement with that reported previously [24].

(8-Hydroxyoctcyl)triphenylphosphonium bromide (**6d**) and (10-hydroxydecyl)triphenylphosphonium bromide (**6e**) [25]

The salts **6d** and **6e** were prepared by reactions of the appropriate bromoalkyl alcohol (6.57 mmol) and triphenylphosphine (1.74 g, 6.64 mmol) and as described for **6c** and gave **6d** and **6e** as translucent solids, which were used in the next reaction without further purification. Analytical data for **6d**: IR (film) cm^{-1}: 3,385, 3,059, 2,933, 2,857, 1,641, 1,431, 1,113, 748; ES-HRMS calculated for $C_{28}H_{36}OP$ 391.2191, found *m*/*z* 391.2187 $[M-Br^-]^+$. Analytical data for **6e**: IR (film) cm^{-1}: 3,379, 3,055, 2,927, 2,854, 1,621, 1,438, 1,113, 748; ES-HRMS calculated for $C_{28}H_{36}OP$ 419.2498, found *m*/*z* 419.2487 $[M-Br^-]^+$.

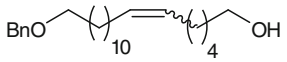
12-(Benzyloxy)dodecan-1-al **8**



Sodium hydride (10.9 mmol, 436 mg) was added to a solution of 1,12-dodecandiol **7** (2.0 g, 9.90 mmol) in THF (70 mL) at 40 °C, and the reaction mixture was stirred for 1 h. Benzyl bromide (1.69 g, 9.90 mmol, 1.18 mL) was added to the mixture, and the reaction mixture was heated to 80 °C for 48 h. The reaction mixture was diluted with Et_2O, washed with water and brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. Chromatography (EtOAc–cyclohexane 1:9) gave an intermediate mono-alcohol (1.42 g, 49 %) as a white solid; mp 35.6–35.9 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.33 (4H, d, *J* 4.4 Hz), 7.27 (1H, m), 4.50 (2H, s, BnCH_2), 3.62 (2H, t, *J* 6.8 Hz, CH_2OH), 3.46 (2H, t, *J* 6.8 Hz, OCH_2Ph), 1.58 (4H, m, 2 CH_2), 1.27 (16H, s, 8 CH_2); ^{13}C NMR (126 MHz, $CDCl_3$) δ 138.70, 128.29, 127.57, 127.41 (aromatic C and CH) 72.82, 70.52, 63.02, 32.79, 29.74, 29.56, 29.55, 29.54, 29.53, 29.45, 29.40, 26.90, 26.17, 25.72 (each CH_2). ES-HRMS calculated for $C_{19}H_{33}O_2$ 293.2481, found *m*/*z* 293.2474 $[M+H]^+$. DMSO (3.70 mmol, 0.26 mL) was added to CH_2Cl_2 (10 mL), and the mixture was cooled to −78 °C. To this, $(COCl)_2$ (1.85 mmol, 0.16 mL) was added, and the solution was stirred for 15 min. The alcohol intermediate (270 mg, 0.924 mmol) in CH_2Cl_2 (3 mL) was added, and the reaction mixture was stirred for a further 1 h, followed by the addition of Et_3N (0.77 mL, 5.55 mmol). The mixture was stirred for a further 30 min and allowed to warm to room temperature, diluted with Et_2O and washed with water and brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure to give **8** (260 mg, 97 %) as a yellow oil which was used without further purification; R_f 0.18 (EtOAc–cyclohexane 1:1); IR (film) cm^{-1}: 2,927, 2,854,

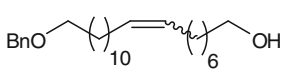
1,726, 1,454, 1,102; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 9.75 (1H, t, J 1.9 Hz, H-C=O), 7.33 (4H, d, J 4.4 Hz, Ar-H), 4.50 (2H, s, benzyl CH_2), 3.46 (2H, t, J 6.7 Hz, CH_2OH), 2.40 (2H, td, J 7.4 Hz, J 1.8 Hz, CH_2CHO), 1.62 (4H, m, 2 CH_2), 1.27 (14H, s, 7 CH_2); ^{13}C-NMR (126 MHz, $CDCl_3$) δ 202. 80 (C=O), 138.72 (Ar–C), 128.28, 127.55, 127.40 (Ar CH) 72.82, 70.49 (each OCH_2), 43.87, 29.74, 29.50, 29.46, 29. 42, 29.35, 29.30, 29.13, 26.16, 22.06 (each CH_2).

18-(Benzyloxy)octadec-6-en-1-ol **9c**



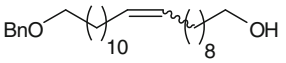
The salt **6c** (130 mg, 0.293 mmol) was dissolved in CH_2Cl_2 (5 mL) and cooled to −78 °C; to this, LiHMDS (1 M, 0.646 mmol, 0.646 mL) was added and the reaction mixture was stirred for 10 min. To this, aldehyde **8** (65 mg, 0.225 mmol) in CH_2Cl_2 (2 mL) was added, and the reaction was allowed to warm to room temperature over 1 h. The reaction mixture was diluted with Et_2O, washed with 1 M HCl, water and brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification via flash chromatography (EtOAc–cyclohexane 1:4) gave alcohol **9c** (60 mg, 72 %) as a colourless oil; R_f 0.26 (EtOAc–cyclohexane 1:4); IR (film) cm^{-1}: 3,366, 3,044, 2,925, 2,853, 1,454, 1,362, 1,101; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.22–7.35 (5H, ms, Ar-H), 5.30–5.40 (2H, ms, alkene CH), 4.50 (2H, s, benzyl CH_2), 3.63 (2H, t, J 6.7 Hz, HOCH_2), 3.46 (2H, t, J 6.7 Hz, BnOCH_2), 1.93–2.07 (2H, m, CH_2), 1.22–1.66 (26H, ms, 13 CH_2)^{13}C NMR ($CDCl_3$, 125 MHz, mixture of isomers): δ 138.71 (Ar–C), 130.38, 130.30 (alkene CH), 129.96, 129.49, 128.29, 127.58, 127.42 (Ar CH), 72. 84, 70.53, 62.99, 62.96 (each OCH_2), 32.70, 32.66, 32.57, 32.50, 29.76, 29.74, 29.62, 29.59, 29.58, 29.52, 29.49, 29. 47, 29.39, 29.29, 29.15, 27.21, 27.14, 26.91, 26.19, 25.39, 25.22 (each CH_2). ES-HRMS calculated for $C_{25}H_{43}O_2$ 375. 3263, found m/z 375.3278 $[M+H]^+$.

18-(Benzyloxy)octadec-6-en-1-ol (**9d**)



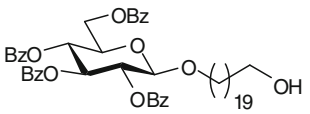
The compound **9d** could be prepared as described for **9c** (30 % yield) or prepared according to the following procedure: the salt **6d** (1.08 g, 2.30 mmol) was dissolved in CH_2Cl_2 (40 mL) and cooled to −78 °C; to this, n-BuLi (1. 16 M, 4.60 mmol, 4.0 mL) was added, and the reaction mixture was stirred for 5 min. To this, aldehyde **8** (667 mg, 2.30 mmol) in CH_2Cl_2 (2 mL) was added, and the reaction mixture was allowed to attain room temperature over 1 h. The reaction mixture was diluted with Et_2O washed with 1 M HCl, water and brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification via flash chromatography (EtOAc–petroleum ether 1:4) gave alcohol **9d** (89 mg, 10 %) as a colourless oil; R_f 0.26 (EtOAc–petroleum ether 1:4); IR (film) cm^{-1}: 3,366, 3,044, 2,928, 2,851, 1,455, 1,365, 1,103; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.25–7.36 (5H, ms, Ar-H), 5.30–5.40 (2H, m, C=CH), 4.51 (2H, s, benzyl CH_2), 3.63 (2H, t, J 6.6 Hz, HOCH_2), 3.47 (2H, t, J 6.7 Hz, BnOCH_2), 1.94–2.06 (4H, m, 2×CH_2), 1.52–1.67 (4H, m, 2 CH_2), 1.22–1.40 (28H, m, 14 CH_2); ^{13}C NMR ($CDCl_3$, 125 MHz, mixture of isomers): δ 138.70 (aromatic C), 130. 66, 130.17 (each alkene CH), 129.96, 129.50, 128.30, 127. 59, 127.43 (each aromatic CH), 72.84, 70.53, 62.97 (each OCH_2), 32.71, 32.87, 32.58, 32.50, 29.77, 29.74, 29.63, 29. 60, 29.58, 29.53, 29.50, 29.48, 29.40, 29.30, 29.16, 27.22, 27.14, 26.92, 26.19, 25.39, 25.23 (each CH_2); ES-HRMS calculatedd for $C_{25}H_{43}O_2$ 403.3576, found m/z 403.3579 $[M+H]^+$.

22-(Benzyloxy)docos-10-en-1-ol (**9e**)



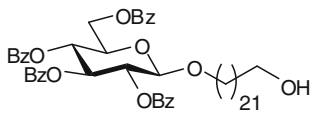
The salt **6e** (430 mg, 0.862 mmol) was dissolved in CH_2Cl_2 (5 mL) and cooled to −78 °C; to this, LiHMDS (1 M, 1.72 mmol, 1.72 mL) was added, and the reaction mixture was stirred for 10 min. Aldehyde **8** (250 mg, 0. 862 mmol) in CH_2Cl_2 (3 mL) was added, and the reaction mixture was allowed to warm to room temperature over 1 h. The reaction mixture was diluted with Et_2O, washed with 1 M HCl, water and brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification via flash chromatography (EtOAc–cyclohexane 1:9) gave alcohol **9e** (114 mg, 33 %) as a colourless oil; R_f 0.26 (EtOAc–Cyclohexane 1:4); IR (film) cm^{-1}: ^{1}H NMR ($CDCl_3$, 500 MHz, mixture of cis and trans isomers): δ 7.22–7.35 (5H, ms, Ar-H), 5.30–5.40 (2H, ms, alkene CH), 4.50 (2H, s, benzyl CH_2), 3.54–3.65 (2H, 2×t, J 6.7 Hz and 6.7 Hz, 2 HOCH_2), 3.36–3.48 (2H, 2 t, J 6.7 Hz and 6.7 Hz, 2× BnOCH_2), 1.20–2.07 (36H, m, 18CH_2); ^{13}C NMR (126 MHz, $CDCl_3$) δ, 130.38, 130.30 (each C), 129.91, 129.89, 129.86, 129.84, 128.30, 128.29, 127.42 (each CH), 72.84, 70.97, 70.95, 70.54, 63.05, 62.75 (each OCH_2), 32.82, 32.74, 32.59, 32.58, 29.78, 29.77, 29.75, 29.65, 29.63, 29.62, 29.60, 29.57, 29.54, 29.53, 29.51, 29.50, 29.48, 29.47, 29.44, 29.42, 29.40, 29.32, 29.31, 29.28, 29.16, 29.12, 27.21, 27.20, 26.20, 26.19, 25.83, 25.74, 25.73 (each CH_2); ES-HRMS calculated for $C_{29}H_{51}O_2$ 431.3889, found m/z 431.3868 $[M+H]^+$.

20-Hydroxyicosyl- 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranoside **10d**



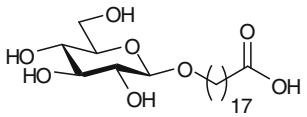
The trichloroacetamide **2** (330 mg, 0.445 mmol) and acceptor **9d** (190 mg, 0.472 mmol) were treated as described for **9c** and gave a benzylated intermediate as a colourless syrup (342 mg, 78 %); ES-HRMS calculated for $C_{61}H_{72}O_{11}Na$ 1,003.4972, found *m/z* 1,003.4968 $[M+Na]^+$. This intermediate (164 mg, 0.167 mmol) was dissolved in EtOAc (20 mL), and the solution was degassed. Ten percent Pd/C (10 %mmol, 35 mg) was added and the atmosphere charged with H_2, and the reaction mixture was stirred vigorously for 16 h. The mixture was filtered through celite which was rinsed with EtOAc and concentrated under reduced pressure to give **10d** (149 mg, 100 %) as a colourless oil; R_f 0.14 (EtOAc–petroleum ether 2:5); IR (film) cm^{-1}: 3,440, 3,067, 2,927, 2,855, 1,731, 1,264, 1,095, 1,068, 708; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.80–8.04 (ms, 8H, aromatic H), 7.26–7.58 (ms, 12H, aromatic H), ^1H NMR (500 MHz, chloroform-*d*): δ 5.90 (t, *J* 9.6 HzHz, 1H, H_3), 5.67 (t, *J* 9.7 Hz, 1H, H_4), 5.52 (dd, *J* 9.8, 7.9 Hz, 1H, H_2), 4.84 (d, *J* 7.9 Hz, 1H, H_1), 4.64 (dd, *J* 12.1, 3.3 Hz, 1H, H_{6a}), 4.51 (dd, *J* 12.1, 5.3 Hz, 1H, H_{6b}), 4.16 (ddd, *J*=9.9, 5.2, 3.3 Hz, H-5), 3.91 (dt, *J* 9.7, 6.3 Hz, 1H, C*H*HO), 3.64 (t, *J* 6.6 Hz, 2H, CH_2CO_2), 3.54 (dt, *J* 9.8, 6.7 Hz, 1H, C*H*HO), (m, 4H, 2×CH_2), 0.7–1.41 (ms, 34H, 17×CH_2);^{13}C NMR ($CDCl_3$, 125 MHz): δ 166.14, 165.83, 165.19, 165.05 (each C=O), 133.38, 133.18, 133.12, 133.07, 129.81, 129.75, 129.72, 129.61, 129.39, 128.84, 128.82, 128.37, 128.32, 128.30, 128.26, (each aromatic C or CH) 101.29 (CH, C-1), 72.94 (CH, C-3), 72.13 (CH, C-5), 71.92 (C-2), 70.38 (CH_2O), 69.89, 63.26 (CH_2, C-6), 63.08, 32.81, 29.69, 29.67, 29.65, 29.63, 29.60, 29.59, 29.53, 29.46, 29.43, 29.40, 29.25, 25.78 (each CH_2); ES-HRMS calculated for $C_{54}H_{68}O_{11}Na$ 915.4659, found *m/z* 915.4663 $[M+Na]^+$.

22-Hydroxydocosanyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranoside **10e**



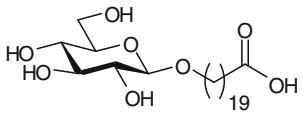
A mixture of trichloroacetamide **2** (252 mg, 0.340 mmol), acceptor **9e** (114 mg, 0.283 mmol) and flame-dried molecular sieves 4 Å (500 mg) were placed under vacuum for 1 h. CH_2Cl_2 (5 mL) was added, and the solution was stirred for 40 min at room temperature. The solution was cooled to 0 °C and TMSOTf (0.05 M, 0.0283 mmol, 0.57 mL) was added, and the reaction mixture was stirred for a further 30 min. Solid $NaHCO_3$ (25 mg) was added, and the mixture stirred for 20 min, filtered through celite, which was rinsed with CH_2Cl_2. The solvent was removed under reduced pressure, and the residue was purified by flash chromatography (EtOAc–cyclohexane 1:9) to give the benzylated intermediate as a colourless syrup (146 mg, 51 %); R_f 0.27 (EtOAc–cyclohexane 1:4); $[\alpha]_D$ +7.4 (c 4.5, $CHCl_3$); IR (film) cm^{-1}: 2,925, 2,853, 1,733, 1,451, 1,265, 1,094, 1,069; ^1H NMR ($CDCl_3$, 500 MHz): δ 7.25–8.05 (25H, ms, aromatic H) 5.90 (1H, t, *J* 9.7 Hz, Hz, H-3), 5.67 (1H, t, *J* 9.7 Hz, H-4), 5.52 (1H, dd, *J* 9.7 Hz, *J* 7.9 Hz, H-2), 5.38 (2H, m), 4.83 (1H, d, *J* 7.9 Hz, H-1), 4.63 (1H, dd, *J* 12.1 Hz, *J* 3.3 Hz, H-6a), 4.51 (1H, dd, *J* 12.1 Hz, *J* 5.3 Hz, H-6b), 4.50 (2H, s, PhCH_2), 4.15 (1H, ddd, *J* 9.7 Hz, *J* 5.3 Hz, *J* 3.3 Hz, H-5), 3.91 (1H, dt, *J* 9.7 Hz, *J* 6.3 Hz, C*H*HO), 3.53 (1H, dt, *J* 9.7 Hz, *J* 6.7 Hz, CH*H*O), 3.46 (2H, t, *J* 6.7 Hz, CH_2COBn), 1.90–2.05 (4H, m, 2 CH_2), 1.64–1.46 (6H, m, 3 CH_2), 1.39–1.01 (30H, m, 15 CH_2);ES-HRMS calculated for $C_{63}H_{76}O_{11}Na$ 1,031.5285, found *m/z* 1,031.5271 $[M+Na]^+$. This intermediate (146 mg, 0.145 mmol) was dissolved in EtOAc (15 mL) and the solution degassed. Ten percent Pd–C (50 mg) was added and the atmosphere was charged with H_2 and the reaction mixture was stirred vigorously for 16 h. The mixture was filtered through celite which was rinsed with EtOAc. Removal of the solvents under reduced pressure gave **10e** (133 mg, 100 %) as a colourless oil; R_f 0.22 (EtOAc–cyclohexane 2:5); $[\alpha]_D$ +8.18 (c 3.5, $CHCl_3$); IR (film) cm^{-1}: 3,539, 3,064, 2,924, 2,853, 1,731, 1,266, 1,094, 1,027; ^1H NMR ($CDCl_3$, 500 MHz): δ 7.25–8.05 (20H, ms, aromatic H) 5.90 (1H, t, *J* 9.7 Hz, H-3), 5.67 (1H, t, *J* 9.7 Hz, H-4), 5.52 (1H, dd, *J* 9.7 Hz, *J* 7.8 Hz, H-2), 4.84 (1H, d, *J* 7.8 Hz, H-1), 4.64 (1H, dd, *J* 12.1 Hz, *J* 3.3 Hz, H-6a), 4.51 (1H, dd, *J* 12.1 Hz, *J* 5.3 Hz, H-6b), 4.16 (1H, ddd, *J* 9.7 Hz, *J* 5.3 Hz, *J* 3.3 Hz, H-5), 3.91 (1H, dt, *J* 9.7 Hz, *J* 6.3 Hz, C*H*HO), 3.63 (2H, t, *J* 6.6 Hz, CH_2OH), 3.54 (1H, dt, *J* 9.7 Hz, *J* 6.7 Hz, CH*H*O), 1.00–1.65 (40H, m, 20 CH_2); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 166.09, 165.80, 165.16, 165.01 (each C=O), 133.32, 133.13, 133.07, 133.01, 129.76, 129.70, 129.68, 129.61, 129.39, 128.84, 128.82, 128.41, 128.32, 128.28, 128.26, 128.21 (each aromatic C or CH), 101.26 (CH, C-1), 72.96 (CH, C-3), 72.13 (CH, C-5), 71.93 (CH, C-2), 70.30 (CH_2O), 69.91 (CH, C-4), 63.25 (CH_2, C-6), 62.98 (CH_2OH), 32.75, 29.74, 29.65, 29.61, 29.59, 29.57, 29.55, 29.52, 29.49, 29.46, 29.42, 29.39, 29.37, 29.21, 25.74, 25.71 (each CH_2); ES-HRMS calculated for $C_{56}H_{72}O_{11}Na$ 943.4972, found *m/z* 943.5019 $[M+Na]^+$.

18-(β-D-Glucopyranosyloxy)-octadecanoic acid **1c**



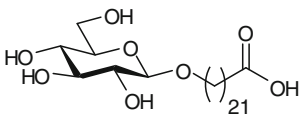
Alcohol **10c** (77 mg, 89.1 μmol) was dissolved in CH_2Cl_2 (2 mL) and H_2O (1 mL); to this, BAIB (310 μmol, 100 mg) was added followed by TEMPO (cat), and the reaction mixture was stirred for 18 h at room temperature. The reaction mixture was diluted with CH_2Cl_2, washed with water, dried over $MgSO_4$ and concentrated under reduced pressure. Chromatography (EtOAc–cyclohexane 1:1) gave the benzoylated protected acid (40 mg, 51 %) as a colourless oil; R_f 0.42 (EtOAc–cyclohexane 1:1); $[\alpha]_D$ +13.3 (c 0.6, $CHCl_3$); IR (film) cm^{-1}: 2,925, 2,853, 1,731 (C=O), 1,266, 1,094, 1,069, 1,027; ^1H NMR ($CDCl_3$, 500 MHz): δ 7.25–8.05 (20H, ms, Ar-H), 5.90 (1H, t, *J* 9.7 Hz, H-3), 5.67 (1H, t, *J* 9.7 Hz, H-4), 5.51 (1H, dd, *J* 9.7 and 8 Hz, H-2), 4.83 (1H, d, *J* 8 Hz, H-1), 4.63 (1H, dd, *J* 12.1 Hz, *J* 3.3 Hz, H-6a), 4.51 (1H, dd, *J* 12.1 Hz, *J* 5.3 Hz, H-6b), 4.15 (1H, ddd, *J* 9.7 Hz, *J* 5.3 Hz, *J* 3.3 Hz, H-5), 3.91 (1H, dt, *J* 9.7 Hz, *J* 6.3 Hz, C*H*HO), 3.53 (1H, dt, *J* 9.7 Hz, *J* 6.7 Hz, CH*H*O), 2.33 (2H, t, *J* 7.5 Hz, CH_2CO_2), 1.00–1.80 (30H, m, 15 CH_2); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 177.96 (*C*O_2H, detected indirectly by HMBC), 166.15, 165.85, 165.20, 165.06 (each C=O), 133.36, 133.17, 133.11, 133.05, 129.82, 129.76, 129.73, 129.65, 129.43, 128.89, 128.87, 128.37, 128.32, 128.30, 128.26 (each aromatic C or CH), 101.30 (CH, C-1), 72.99 (CH, C-3), 72.17 (CH, C-5), 71.96 (CH, C-2), 70.36 (*C*H_2O), 69.94 (CH, C-4), 63.29 (CH, C-6), 34.08 (*C*H_2CO_2), 29.68, 29.66, 29.64, 29.62, 29.61, 29.58, 29.52, 29.46, 29.44, 29.41, 29.26, 29.14, 26.92, 25.78, 24.96 (each *C*H_2); ES-HRMS calculated for $C_{52}H_{61}O_{12}$ 877.41630, found *m*/*z* 877.4130 $[M-H]^-$. This acid (40 mg, 45.6 μmol) was dissolved in MeOH (2 mL); to this, NaOMe/MeOH (1 M, 54 μmol, 54 μL) was added, and the reaction mixture was stirred at room temperature for 16 h. Amberlite IR-120 (plus) was added until pH6.0. The resin was filtered off and rinsed with 1:1 THF–MeOH, and the solvent was removed under reduced pressure. Residual methyl benzoate was removed under high vacuum. Chromatography (CH_2Cl_2–MeOH 9:1) gave **1c** (20 mg, 45 %) as a white solid; $[\alpha]_D$ −2.1 (c 0.33, MeOH); IR cm^{-1}: 3,391 (OH), 2,916, 1,723 (C=O), 1,563, 1,467, 1,408, 1,040; ^1H NMR (CD_3OD, 500 MHz): δ 4.28 (1H, d, *J* 7.8 Hz, H-1), 3.93 (1H, dt, *J* 9.5 Hz, *J* 6.9 Hz, C*H*HO), 3.89 (1H, dd, *J* 11.9 Hz, *J* 2.0 Hz, H-6a), 3.70 (1H, dd, *J* 11.9 Hz, *J* 5.3 Hz, H-6b), 3.57 (1H, dt, *J* 9.5 Hz, *J* 6.8 Hz, C*H*HO), 3.25–3.40 (3H, signals overlapping with CD_2HOD, H-3, H-4, H-5), 3.20 (1H, dd, *J* 9.0 Hz, *J* 7.8 Hz, H-2), 2.24 (2H, t, *J* 7.5 Hz, CH_2CO_2), 1.64 (4H, m, 2 CH_2), 1.42 (2H, m, CH_2), 1.33 (26H, s, 13 CH_2); ^{13}C NMR (CD_3OD, 125 MHz): δ 104.44 (CH, C-1), 78.22 (CH, C-3), 77.98 (CH, C-5), 75.21 (CH, C-2), 71.78 (CH, C-4), 70.98 (*C*H_2O), 62.87 (CH_2, C-6), 30.87, 30.82, 30.81, 30.79, 30.72, 30.67, 30.63, 30.59, 27.17, 27.05 (each *C*H_2); ES-HRMS calculated for $C_{24}H_{45}O_8$ 461.3114, found *m*/*z* 461.3116 $[M-H]^-$.

20-(β-D-Glucopyranosyloxy)-icosanoic acid (**1d**)



Alcohol **10d** (77 mg, 89.1 μmol) was dissolved in CH_2Cl_2 (2 mL) and H_2O (1 mL); to this, BAIB (310 μmol, 100 mg) was added followed by TEMPO (cat), and the reaction mixture was stirred for 18 h at room temperature. The reaction mixture was diluted with CH_2Cl_2, washed with water, dried over $MgSO_4$ and concentrated under reduced pressure. Purification via flash chromatography (EtOAc–cyclohexane 1:1) gave an intermediate acid (40 mg, 51 %) as a colourless oil; R_f 0.42 (EtOAc–cyclohexane 1:1); $[\alpha]_D$ +12.2 (c 0.6, $CHCl_3$); IR (film) cm^{-1}: 2,925, 2,853, 1,730, 1,264, 1,096, 1,069, 1,028; ES-HRMS calculated for $C_{54}H_{65}O_{12}$ 905.4476, found *m*/*z* 905.4481 $[M-H]^-$. This acid (40 mg, 46 μmol) was dissolved in MeOH (2 mL); to this, NaOMe in MeOH (54 μL of 1 M, 54 μmol) was added, and the reaction mixture was stirred at room temperature for 16 h. Amberlite IR-120 (plus) was added until pH6.0. The resin was filtered off and rinsed with THF–MeOH 1:1, and the solvent was removed under reduced pressure. Residual methyl benzoate was removed under high vacuum. Chromatography (CH_2Cl_2–MeOH 6:1) gave **1d** (7.8 mg, 32 %) as a white solid; IR cm^{-1}: 3,394, 2,913, 1,721, 1,566, 1,466, 1,405, 1,041; ^1H NMR (CD_3OD, 500 MHz): δ 4.28 (1H, d, *J* 7.8 Hz, H-1), 3.83–3.92 (2H, m, H-6a and CH(*H*)O), 3.66 (1H, dd, J=11.8, 5.2 Hz, H-6b), 3.53 (1H, dt, *J* 9.5 Hz, *J* 6.8 Hz, C*H*HO), 3.22–3.36 (3H, overlapping with CD_2HOD signal, H-3, H-4, H-5), 3.16 (1H, dd, J=9.5, 8.0 Hz, H-2), 2.26 (2H, t, *J* 7.4 Hz, CH_2CO_2), 1.10–1.80 (34H, m, 17×CH_2); ^{13}C NMR (CD_3OD, 125 MHz): δ 177.1 (C=O, detected using HMBC), 104.43 (CH, C-1), 78.20 (CH, C-3), 77.98 (CH, C-5), 75.19 (CH, C-2), 71.74 (CH, C-4), 70.96 (*C*H_2O), 62.84 (CH_2, C-6), 35.26 (*C*H_2CO_2), 30.87, 30.83, 30.82, 30.80, 30.76, 30.69, 30.67, 30.49, 30.33, 27.18, 26.26 (each *C*H_2); ES-HRMS calculated for $C_{24}H_{45}O_8$ 489.3427, found *m*/*z* 489.3431 $[M-H]^-$.

22-(β-D-Glucopyranosyloxy)-docosanoic acid (**1e**)



Alcohol **10e** (63 mg, 68.4 μmol) was dissolved in CH_2Cl_2 (2 mL) and H_2O (1 mL); to this, BIAB

(340 μmol, 109 mg) was added followed by TEMPO (cat), and the reaction mixture was stirred for 18 h at room temperature. The reaction mixture was diluted with CH_2Cl_2, washed with water, dried over $MgSO_4$ and concentrated under reduced pressure. Purification via flash chromatography (EtOAc–cyclohexane 1:2) gave an intermediate acid (30 mg, 47 %) as a colourless oil; R_f 0.35 (EtOAc–cyclohexane 1:1); $[\alpha]_D$ +11.1 (c 1.5, $CHCl_3$); IR (film) cm^{-1}: 2,924, 2,853, 1,732, 1,226, 1,094, 1,069, 709; ^1H NMR ($CDCl_3$, 500 MHz): δ 8.00 (2H, dd, *J* 8.3 Hz, *J* 1.2 Hz, Ar-H), 7.96 (2H, dd, *J* 8.3 Hz, *J* 1.2 Hz, Ar-H), 7.90 (2H, dd, *J* 8.3 Hz, *J* 1.2 Hz, Ar-H), 7.83 (2H, dd, *J* 8.3 Hz, *J* 1.2 Hz, Ar-H), 7.50 (4H, m, Ar-H), 7.39 (4H, m, Ar-H), 7.32 (2H, t, *J* 7.8 Hz, Ar-H), 7.28 (2H, t, *J* 7.8 Hz, Ar-H), 5.90 (1H, t, *J* 9.7 Hz, H-3), 5.67 (1H, t, *J* 9.7 Hz, Hz, H-4), 5.52 (1H, dd, *J* 9.7 Hz, *J* 7.9 Hz, H-2), 4.83 (1H, d, *J* 7.9 Hz, H-1), 4.63 (1H, dd, *J* 12.0 Hz, *J* 3.3 Hz, H-6a), 4.51 (1H, dd, *J* 12.0 Hz, *J* 5.3 Hz, H-6b), 4.15 (1H, ddd, *J* 9.7 Hz, *J* 5.3 Hz, *J* 3.3 Hz, H-5), 3.91 (1H, dt, *J* 9.7 Hz, *J* 6.3 Hz, CH*H*O), 3.53 (1H, dt, *J* 9.7 Hz, *J* 6.7 Hz, C*H*HO), 2.32 (2H, t, *J* 7.5 Hz, CH_2CO_2), 1.63 (2H, m, 2 CH_2), 1.51 (2H, m, CH_2), 1.31–1.02 (36 H, m, 18 CH_2); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 178.78 (CO_2H), 166.09, 165.79, 165.15, 165.00, (each C=O), 133.31, 133.12, 133.06, 133.00, 129.76, 129.70, 129.67, 129.60, 129.37, 128.83, 128.81, 128.32, 128.27, 128.26, 128.25, 128.21 (each aromatic C/CH), 101.25 (C-1), 72.94 (C-3), 72.12 (C-5), 71.91 (C-2), 70.30 (CH_2O), 69.89 (C-4), 63.24 (C-6), 34.35 (CH_2CO_2), 30.83, 29.72 29.64, 29.62, 29.60, 29.59, 29.54, 29.48, 29.41, 29.35, 29.22, 29.20, 29.11, 25.73, 24.98 (each CH_2); ES-HRMS calculated for $C_{56}H_{69}O_{12}$ 933.4789, found *m/z* 933.4752 $[M-H]^-$. This intermediate acid (30 mg, 32.1 μmol) was dissolved in MeOH (2 mL); to this, NaOMe/MeOH (1 M, 39.0 μmol, 39 μL) was added, and the reaction mixture was stirred at room temperature for 16 h. Amberlite IR-120 (plus) was added until pH6.0. The resin was filtered off and rinsed with THF/MeOH 1:1, and the solvent was removed under reduced pressure. Residual methyl benzoate was removed under high vacuum. Flash chromatography (MeOH–CH_2Cl_2 1:9) gave **1e** (10.4 mg, 63 %) as a white solid; R_f 0.29 (MeOH–CH_2Cl_2 1:9); ^1H NMR ($CDCl_3$, 500 MHz): δ 4.28 (1H, d, *J* 7.8 Hz, H-1), 3.93 (1H, dt, *J* 9.5 Hz, *J* 6.9 Hz, C*H*HO), 3.89 (1H, dd, *J* 11.9 Hz, *J* 2.0 Hz, H-6a), 3.70 (1H, dd, *J* 11.9 Hz, *J* 5.3 Hz, H-6b), 3.57 (1H, dt, *J* 9.5 Hz, *J* 6.8 Hz, CH*H*O), 3.36–3.40 (3H, signals overlapping with MeOD, H-3, H-4, H-5), 3.20 (1H, overlapping with CD_3OD sideband, H-2), 2.26 (2H, t, *J* 7.5 Hz, CH_2CO_2), 1.64 (4H, m, 2 CH_2), 0.8-2.0 (2H, ms, CH_2), 1.33 (32H, s, 16 CH_2); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 102.98 (C-1), 76.76 (C-3), 76.51 (C-5), 73.75 (C-2), 70.71 (H-4), 69.5 (CH_2O), 61.40 (C-6), 29.33 and 29.10 (each CH_2) ES-HRMS calculated for $C_{28}H_{53}O_8$ 517.3740, found *m/z* 517.3727 $[M-H]^-$.

Tissue sources and isolation of mitochondria

Wistar rats (*Rattus norvegicus*; 180–200 g) were provided by the BioResources Unit at Trinity College Dublin. All rats were housed at room temperature and fedad libitum. All rats were killed by CO_2 asphyxiation. Ethical consent for use of the rats was granted by the Bio Resources Ethics Committee, Trinity College Dublin, through the Department of Health (Ireland) under guidelines detailed in the Cruelty to Animals Act (1876) as amended by the European Communities regulations 2002 and 2005. Mitochondria from brown adipose tissue were isolated by homogenization, followed by differential centrifugation according to the procedure of Chappell and Hansford [26].

Protein determination

Mitochondrial protein concentrations were determined by the reduction of Folins–Ciocalteau phosphomolybdic–phosphotungstic reagent according to the method of Markwelland Haas [27].

Oxygen consumption by non-phosphorylating brown adipose tissue mitochondria

Oxygen consumption rates were measured using an Oxygraph Respirometer (Oroboros™, Innsbrück, Austria) as previously described [16]. Mitochondria (1 mg/ml) were incubated at 37 °C in medium containing 120 mM KCl, 5 mMHEPES-KOH, pH7.4, 1 mMEGTA, 16 μMfatty acid-free bovine serum albumin (to buffer the fatty acids), 5 μMatractyloside, 5 μMrotenone and 1 μg/ml oligomycin. Non-phosphorylating oxygen consumption rates were measured as the steady-state rates achieved on addition of 7.5 mMsuccinate (succinate–KOH, pH7.4). The sensitivity of this state 4 oxygen consumption rate to GDP (500 μM) was determined, and the sensitivity of the GDP-inhibited oxygen consumption rate to 64 μMfatty acid or modified fatty acid (stock in ethanol) (~40 nM free final concentration [28]) was determined. Finally, the mitochondrial uncoupler FCCP (100pmol/mg) was added to the chamber to determine the maximum oxygen consumption rate attainable. The Oroborus Oxygraph Respirometer was calibrated according to the procedure of Reynafarjeet al. [29], assuming that 406 nmol of oxygen atoms was dissolved in 1 ml of ionic incubation medium at 37 °C. As there were obvious solubility problems with both behenic acid (22C) and glucose-*O*-ω- behenic acid (22C) within the incubation medium, no data were recorded for mitochondrial oxygen consumption in the presence of these compounds.

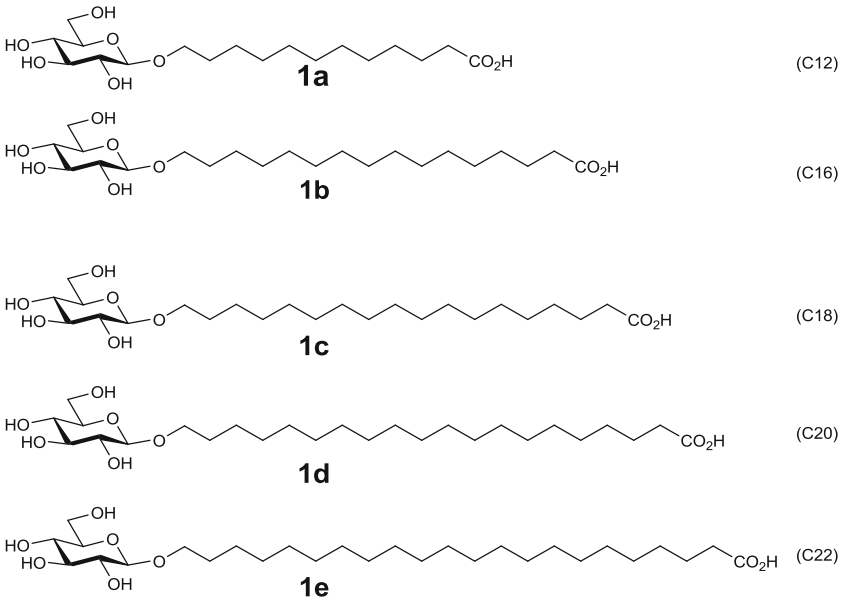


Fig. 3 Schematic representation of the structure of the glucose-*O*-ω-fatty acids used in the study

Results and discussion

The dependence of UCP1 function on the fatty acid chain length of glucose-*O*-ω-modified fatty acids (Fig. 3) and equivalent unmodified fatty acids was investigated. Figure 4 shows significant inhibition of non-phosphorylating oxygen consumption rates (state 4) of BAT mitochondria by GDP (500 μM) for all experiments (Fig. 4a–h). Consequently the GDP-inhibited (UCP1-dependent) component of the oxygen consumption rates were significantly ($p<0.05$) stimulated by

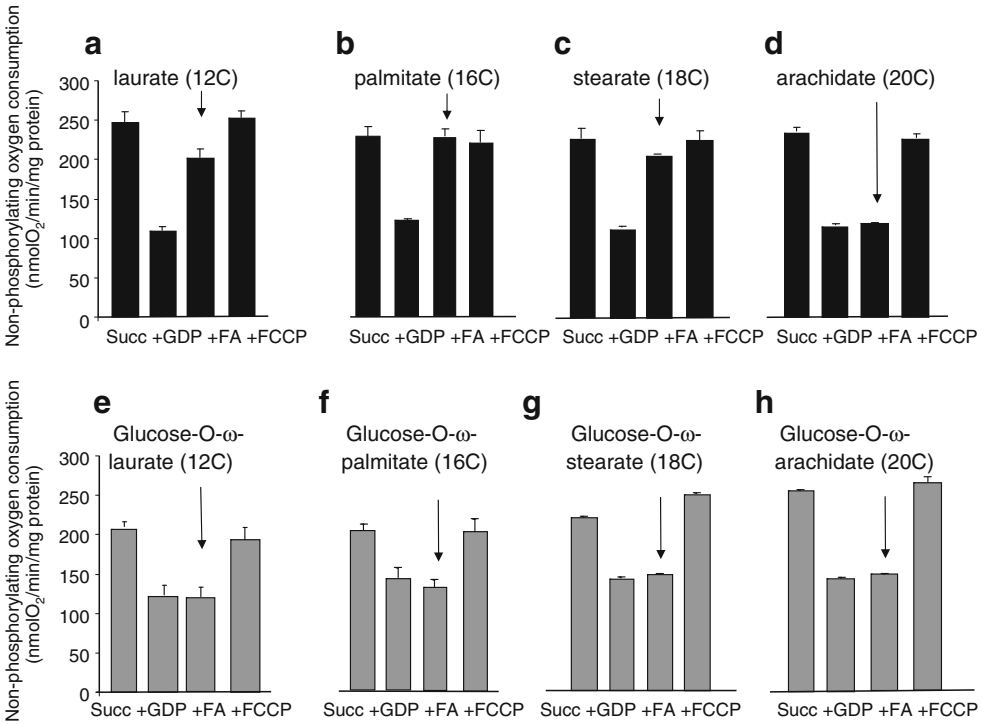


Fig. 4 Measurement of oxygen consumption rates in BAT mitochondria from rat. Mitochondria (0.5 mg/ml) from rat BAT were incubated in the presence of 120 mM KCl, 5 mM HEPES-KOH, pH 7.0, 1 mM EGTA, 7.5 mM succinate (K^+-salt), 16 μM de-fatted bovine serum albumin, 5 μM rotenone, 1 μg/ml oligomycin, and 5 μM atractyloside. Non-phosphorylating steady-state oxygen consumption rates were then measured (*Succ*), followed by addition of 1 mM GDP and subsequent addition of 64 μM fatty acid (*FA*) (~40 nM final free concentration): **a** laurate, **b** palmitate, **c** stearate, **d** arachidate, **e** glucose-*O*-ω-laurate, **f** glucose-*O*-ω-palmitate, **g** glucose-*O*-ω-stearate, or **h** glucose-*O*-ω-arachidate, followed by addition of 100 pmol/mg FCCP. Data are shown as the mean±SEM from at least three independent experiments each performed in triplicate

nanomolar amounts of laurate (12C) (Fig. 4a), palmitate (16C) (Fig. 4b) and stearate (18C) (Fig. 4c), but not arachidate (20C) (Fig. 4d). The aforementioned stimulated rates are close to the maximal uncoupled rate induced by the addition of FCCP. These data allow us to conclude that the functional assay for fatty acid-dependent UCP1 activity in isolated BAT mitochondria is working and that in the case of laurate, palmitate and stearate, at least, we get the expected stimulation of GDP-inhibited UCP1-dependent oxygen consumption as has been observed previously by ourselves [16] and others [1, 2]. Of course, either the cofactor/activation model or the flip/flop model could explain the aforementioned activation of UCP1 dependent oxygen consumption by laurate, palmitate or stearate. The lack of activation of UCP1 in isolated BAT mitochondria by arachidate is probably due to a solubility issue in relation to this long 20-carbon chain fatty.

In theory, if the cofactor activation model is correct, the glucose-*O*-ω-fatty acids should be able to "activate" UCP1, and due to the hydrophilic glucose modification at the omega end of the fatty acid, they are most unlikely to be transported across membranes. Our data demonstrated that the component of the oxygen consumption in BAT mitochondria, due to UCP1, was not stimulated by nanomolar amounts of glucose-*O*-ω-laurate (12C) (Fig. 4e), glucose-*O*-ω-palmitate (16C) (Fig. 4f), glucose-*O*-ω-stearate (18C) (Fig. 4g) or glucose-*O*-ω-arachidate (20C) (Fig. 4h). The lack of apparent activation by the glucose-*O*-ω-modified fatty acids (Fig. 4e–h) we know is not due to the inhibition of the electron transport chain as a maximal uncoupling activity could be induced by the addition of FCCP. In summary, our data show that irrespective of fatty acid chain length, the glucose-*O*-ω-modified saturated fatty acids were unable to "activate" UCP1. These data are consistent with our previous data for UCP1 in isolated mitochondria [16]. The data are not consistent with the "cofactor activation" model of UCP 1 function [17–19] but are comparable with the model that UCP 1 functions by flipping long-chain fatty acid anions [13–15].

Acknowledgments The research in this manuscript was funded by a Science Foundation Ireland Principal Investigator grant to RKP and a Trinity Studentship award to KJC. RKP is a member of the MITOFOOD COST action (FA0602). Higher Education Authority Programme for Research Cycle 3 for funding to PVM.

References

1. Nicholls DG, Locke RM (1984) Thermogenic mechanisms in brown fat. Physiol Rev 64(1):1–64
2. Cannon B, Nedergaard J (2004) Brown adipose tissue: function and physiological significance. Physiol Rev 84(1):277–359
3. Cypess AM, Lehman S, Williams G, Tal I, Rodman D, Goldfine AB, Kuo FC, Palmer EL, Tseng YH, Doria A, Kolodny GM, Kahn CR (2009) Identification and importance ofbrown adipose tissuein adulthumans. N Engl J Med 360(15):1509–1517
4. van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM, Drossaerts JM, Kemerink GJ, Bouvy ND, Schrauwen P, Teule GJ (2009) Cold-activatedbrown adipose tissueinhealthymen. N Engl J Med 360(15):1500–1508
5. Saito M, Okamatsu-Ogura Y, Matsushita M, Watanabe K, Yoneshiro T, Nio-Kobayashi J, Iwanaga T, Miyagawa M, Kameya T, Nakada K, Kawai Y, Tsujisaki M (2009) High incidence of metabolically activebrown adipose tissueinhealthy adulthumans: effects of cold exposure and adiposity. Diabetes 58(7):1526–1531
6. Carroll AM, Haines LR, Pearson TW, Fallon P, Walsh C, Brennan CM, Breen EP, Porter RK (2005) Detection of a functioning UCP 1 in thymus. J Biol Chem 280(16):15534–15543
7. Adams AE, Hanrahan O, Nolan DN, Voorheis HP, Fallon P, Porter RK (2007) Images of mitochondrial UCP 1 in mouse thymocytes using confocal microscopy. Biochim Biophys Acta 1777(2):115–117
8. Adams AE, Carroll AM, Fallon PG, Porter RK (2008) Mitochondrial uncoupling protein 1 expression in thymocytes. Biochim Biophys Acta 1777(7–8):772–776
9. Adams AE, Kelly OM, Porter RK (2010) Absence of mitochondrial uncoupling protein 1 affects apoptosis in thymocytes, thymocyte/T-cell profile and peripheral T-cell number. Biochim Biophys Acta 1797(6–7):807–816
10. Skulachev VP (1991) Fatty acid circuit as a physiological mechanismof uncoupling of oxidative phosphorylation. FEBS Lett 294(3):158–162
11. Garlid KD, Jabůrek M, Ježek P, Vařecha M (2000) How do uncoupling proteins uncouple? Biochim Biophys Acta 1459(2–3):383–389
12. Garlid KD, Jabůrek M, Ježek P (2001) Mechanism of uncoupling protein action. Biochem Soc Trans 29(6):803–806
13. Garlid KD, Orosz DE, Modrianský M, Vassanelli S, Ježek P (1996) On the mechanism of fatty acid-induced proton transport by mitochondrial uncoupling protein. J Biol Chem 271(5):2615–2620
14. Jabůrek M, Vařecha M, Gimeno RE, Demski M, Ježek P, Zhang M, Burn P, Tartaglia LA, Garlid KD (1999) Transport function and regulation of uncoupling proteins 2 and 3. J Biol Chem 274(3):26003–26007
15. Ježek P (1999) Fatty acid interaction with mitochondrial uncoupling proteins. J Bioenerg Biomembr 31(5):457–466
16. Breen EP, Gouin SG, Murphy AF, Haines LR, Jackson AM, Pearson TW, Murphy PV, Porter RK (2006) On the mechanism of mitochondrial uncoupling protein 1 function. J Biol Chem 281(4):2114–2119
17. Klingenberg M, Echtay KS, Bienengraeber M, Winkler E, Huang SG (1999) Structure–function relationship in UCP1. Int J Obes Relat Metab Disord Suppl 6:S24–S29
18. Klingenberg M, Huang SG (1999) Structure and function of uncoupling protein from brown adipose tissue. Biochim Biophys Acta 1415(2):271–296
19. Nicholls DG, Rial E (1999) A history of the first uncoupling protein, UCP1. J Bioenerg Biomembr 31(5):399–406
20. Gouin SG, Pilgrim W, Porter RK, Murphy PV (2005) Synthesis of a glycolipid for studying mechanisms of mitochondrial uncoupling proteins. Carbohydr Res 340(8):1547–1552
21. Mbadugha BNA, Menger FM (2003) Sugar/steroid/sugar conjugates: sensitivity of lipid binding to sugar structure. Org Lett 5(22):4041–4044
22. Qiu X, Ong S, Bernal C, Rhee D, Pidgeon C (1994) A general synthetic route for preparing ether phospholipids suitable for immobilization: a phosphotriester approach. J Org Chem 59(3):537–543
23. Lei H, Atkinson J (2000) Synthesis of phytyl- and chroman-derivatized photoaffinity labels based on alpha-tocopherol. J Org Chem 65(8):2560–2567
24. Mühlhausen U, Schirrmacher R, Piel M, Lecher B, Briegert M, Piee-Staffa A, Kaina B, Rösch F (2006) Synthesis of 131I-labeled

glucose-conjugated inhibitors of O6-methylguanine-DNA methyltransferase (MGMT) and comparison with nonconjugated inhibitors as potential tools for in vivo MGMT imaging. J Med Chem 49(21):263–272
25. Lermer L, Neeland EG, Ounsworth JP, Sims RJ, Tischler SA, Weiler L (1992) The synthesis of beta-keto lactones via cyclization of beta-keto ester dianions or the cyclization of meldrum acid derivatives. Can J Chem 70(5):1427–1445
26. Chappell JB, Hansford RG (1972) In: Birnie GD (ed) Subcellular components: preparation and Fractionation. Butterworths, London, pp. 77–91
27. Markwell MA, Haas SM (1978) A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. Anal Biochem 87(1):206–210
28. González-Barroso MM, Fleury C, Bouillaud F, Nicholls DG, Rial E (1998) The uncoupling protein UCP1 does not increase the proton conductance of the inner mitochondrial membrane by functioning as a fatty acid anion transporter. J Biol Chem 273(25):15528–15532
29. Reynafarje B, Costa LE, Lehninger AL (1985) O_2 solubility in aqueous media determined by a kinetic method. Anal Biochem 145(2):406–418