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Synthesis of α-Glucosyl Diacylglycerides as Potential Adjuvants for *Streptococcus pneumoniae* Vaccines

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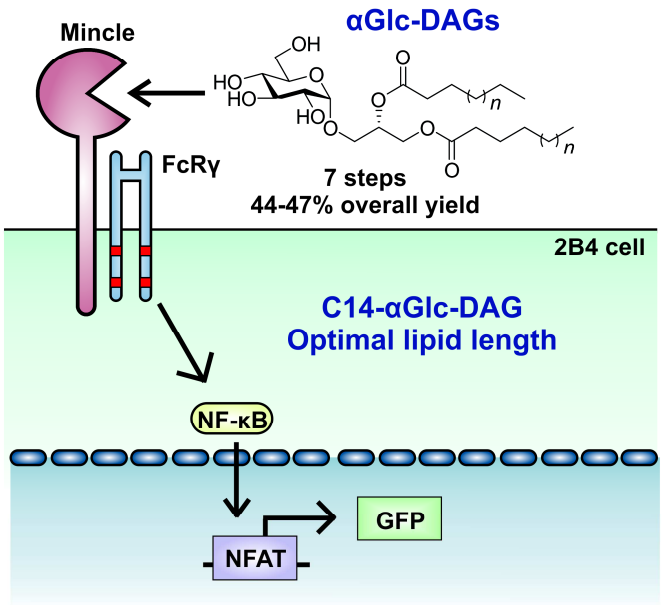
Graphical Abstract



Abstract

α-Glucosyl diacylglycerols (*α*Glc-DAGs) play an important role in providing protective immunity against *Streptococcus pneumoniae* infection through the engagement of the Macrophage inducible C-type lectin (Mincle). Herein, we efficiently synthesised *α*Glc-DAGs containing C12, C14, C16 and C18 acyl chains in 7 steps and 44-47% overall yields, and demonstrated that Mincle signaling was dependent on lipid length using mMincle and hMincle NFAT-GFP reporter cells. The greatest production of GFP in both cell types was elicited by C14 *α*Glc-DAG. Accordingly, C14 *α*Glc-DAG has potential to act as an adjuvant to augment the immune response against *S. pneumoniae* antigens.

Introduction

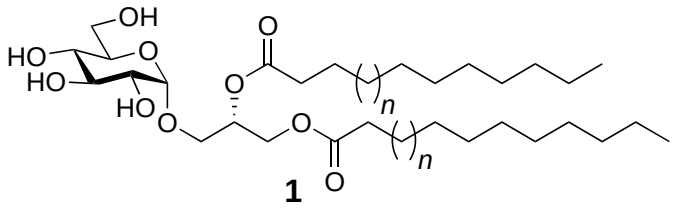
Pneumococcal disease is an infection caused by the gram-positive bacteria *Streptococcus pneumoniae.* People can harbour *S. pneumoniae* in their nose and throat without becoming ill (termed carriage), however infections with the bacteria can lead to sinusitis, otitis media and/or bronchitis, while lower airway infections can lead to pneumococcal pneumonia, which can progress to invasive pneumococcal disease (IPD), septicemia and meningitis.[1,2] Pneumococcal infection is more frequently observed in the young and elderly, with approximately 500,000 infants dying from the disease annually, and with adults greater than 65 years of age being more likely to suffer from significant morbidity and mortality resulting from community-acquired pneumonia or pneumococcal bacteremia and meningitis.[1,2,3,4]

Out of the >90 serotypes of *S. pneumoniae* known so far, approximately 23 are responsible for more than 90% of pneumococcal diseases. The most frequently employed vaccine against *S. pneumoniae* is Pneumovax®, which is composed of capsular polysaccharides purified from the 23 clinically prevalent pneumococcal serotypes, while Prevnar®, a conjugate vaccine containing purified polysaccharides of the clinically most prevalent pneumococcal serotypes coupled to carrier proteins, provides young children (<2 years) with more effective protection against *S. pneumoniae*.[5] Vaccination has certainly decreased the incidence of *S. pneumoniae* infection, although the efficacy of commercially available vaccines and their ability to generate herd immunity varies according to geographic location and age group. This has been attributed to changes in the prevalence and type of capsular polysaccharide antigens found on different *S. pneumoniae* strains, and also to the inability of *S. pneumoniae*-derived

polysaccharides, which behave as T-cell-independent antigens, to induce recall responses following revaccination or infection.[5,6]

Global efforts toward the development of more effective pneumococcal vaccines include increasing the repertoire of *S. pneumoniae*-derived capsular polysaccharides used, or incorporating pneumococcal proteins as antigens.[1,7,8] The use of adjuvants to enhance the efficacy of the immune response to either protein- or carbohydrate-based *S. pneumoniae* antigens has also been deemed a promising strategy,[9] particularly as the engagement of pattern recognition receptors (PRRs) on antigen presenting cells (APCs) may augment antibody boosting and memory responses to polysaccharide pneumococcal antigens. While Toll-like receptor (TLR) agonists do not increase antibody responses when co-administered with polysaccharide antigen (though do if given several days after the antigen),[10,11] Macrophage inducible C-Type Lectin (Mincle) agonists,[12] such as trehalose dimycolate (TDM) and trehalose dibehenate (TDB), show promise in this respect.[9,13]

In 2016, we determined that *α*-glucosyl diacylglycerols (*α*Glc-DAGs, **1**, Figure 1), a class of glycolipid that we structurally elucidated in 2012,[14] played a key role in providing protective immunity against *S. pneumoniae* infection through engagement of Mincle.[15] Here, *α*Glc-DAG containing dipalmitoyl acyl chains was the major Mincle agonist isolated from *S. pneumoniae*. Subsequent studies by Yamasaki and co-workers also confirmed the role of *α*Glc-DAGs in assisting with the suppression of *Streptococcus* infection.[16] Given the potential of Mincle ligands to augment host immunity to *S. pneumoniae*, and the knowledge that lipid length affects the immunostimulatory activity of other classes of Mincle agonist,[17,18,19,20,21] we thus sought to synthesise *α*Glc-DAGs containing C12 (**1a**), C14 (**1b**), C16 (**1c**) and C18 (**1d**) acyl chains so as to investigate their ability to signal through murine and human Mincle. The results from these findings would then inform further studies into the use of *α*Glc-DAGs as adjuvants for *S. pneumonia*e vaccines.



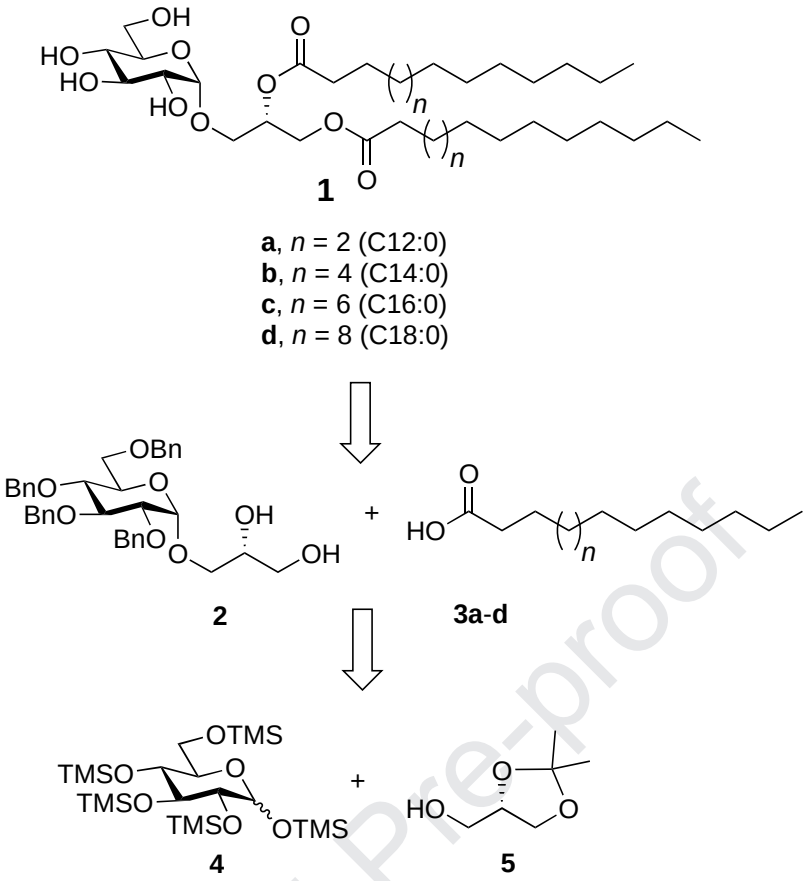
1

a, *n* = 2 (C12:0)
b, *n* = 4 (C14:0)
c, *n* = 6 (C16:0)
d, *n* = 8 (C18:0)

Figure 1. *α*-Glucosyl diacylglycerols (*α*Glc-DAGs)

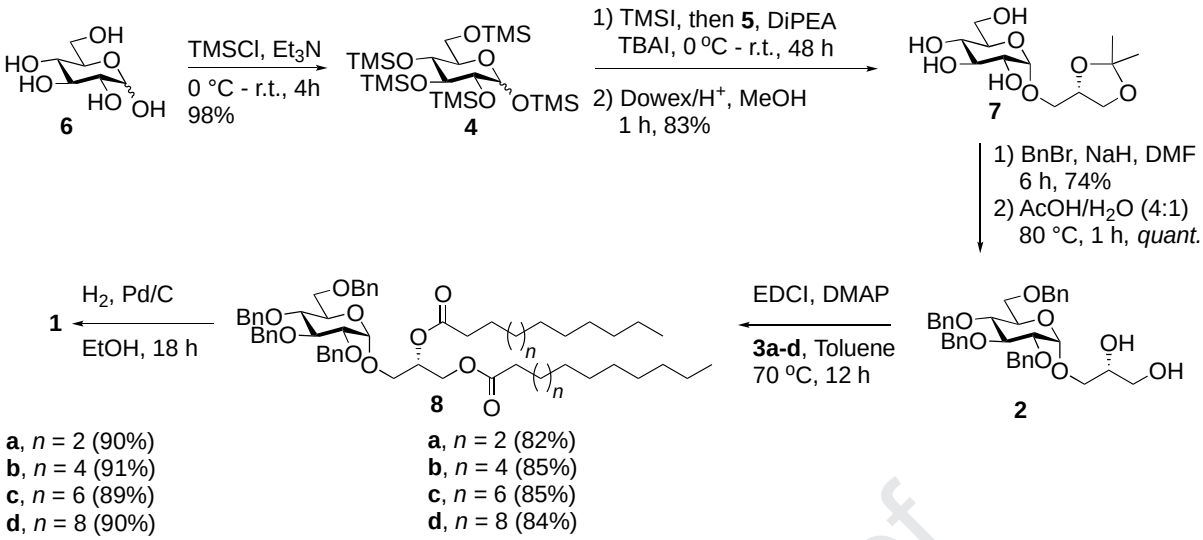
Results and Discussion:

To synthesise *α*Glc-DAGs **1a-d** a retrosynthetic plan was proposed whereby the target glycolipids could be obtained via esterification of benzyl protected 1-*O*-α-glucosyl-*sn*-glycerol **2** with carboxylic acids **3a-d** and subsequent hydrogenolysis of the benzyl groups (Scheme 1). Benzyl-protected glucosylglycerol **2** would in turn be prepared via the glucosylation of TMS-protected glucopyranose **4**,[22,23] which is converted *in situ* to the corresponding glucosyl iodide,[22,23] with commercially available isopropylidene-protected glycerol **5**, followed by protecting group manipulations. Thus, while a change from TMS to benzyl protecting groups is proposed in this retrosynthetic plan to allow for the selective removal of the isopropylidine group under acidic conditions, the glycosyl iodide glycosylation methodology, as developed by Gervay-Hague,[24] provides excellent α-selectivity when using glucose donors, and thus should provide an efficient synthetic route to the target compounds.



Scheme 1. Retrosynthetic plan

With a retrosynthetic strategy in place, the synthesis of TMS-protected glucose was undertaken. Here, per-silylation of D-glucose **6** was achieved under the agency of TMSCl and triethyl amine to produce 1,2,3,4,6-penta-*O*-trimethylsilyl-α-D-glucopyranose **4** in 98% yield (Scheme 2).[23,24] Next, glucopyranose **4** was converted into the glucosyl iodide *in situ*, with the subsequent addition of isopropylidene-protected glycerol **5**, DiPEA and TBAI then providing the TMS-protected glucoside. *In situ* deprotection then yielded 1-*O*-α-glucosyl-*sn*-glycerol **7** in excellent yield (83%) and as only the α-anomer, as determined by ^{1}HNMR analysis whereby the anomeric center was observed at δ = 4.8 ppm with $J_{1\text{-}2}$ = 3.7 Hz. Once determined, the established procedure proved to be very robust with the glucosylation reaction being undertaken on a gram scale without affecting α-selectivity or yield.



Scheme 2. Synthesis of *α*Glc-DAGs (**1a-d**)

In optimising the glucosylation conditions it was observed that reaction time and temperature influenced α:β-selectivity and product yields. As reported by Gervay-Hague,[22,23,24] *in situ* formation of per-*O*-TMS glucosyl iodide from glucopyranose **4** was normally undertaken at room temperature in CH_2Cl_2 with a reaction time of 10 minutes before the addition of the acceptor of choice, stirring for an additional 48 h, and *in situ* removal of the TMS groups. In our hands, when using glycerol **5** as an acceptor, these conditions resulted in a modest (37%) yield of 1-*O*-α-glucosyl-*sn*-glycerol **7** and good α:β-selectivity (10:1). Cooling the reaction to 0 °C and a 20 min reaction time for formation of per-*O*-TMS glucosyl iodide followed by warming to room temperature for the glucosylation reaction led to improved α:β-selectivity (70:1), however, the reaction yield remained modest (48%). A longer (30 min) and warmer (0 °C – r.t.) protocol for formation of the per-*O*-TMS glucosyl iodide prior to the addition of glycerol **5** at 0 °C with warming to room temperature further improved product yield (76%) and α:β-selectivity (96:1), with the optimal product yield (83%) and exclusive α-selectivity (>100:1, α:β) being obtained after formation of per-*O*-TMS glucosyl iodide at 0 °C with warming to room temperature over the course of 30 min, followed by the addition of glycerol **5** at room temperature with stirring for 48 h. In all circumstances, freshly prepared TMS-I,[25] a highly reactive and hygroscopic reagent, led to better product yields.

With 1-*O*-α-glucosyl-*sn*-glycerol **7** in hand, this was then subjected to benzyl protection at the 2-, 3-, 4- and 6-positions via reaction with benzyl bromide in the presence of sodium hydride, followed by isopropylidene deprotection under the agency of acetic acid and water (4:1) to give diol **2** in 74% yield over the two steps. Esterification of **2** with the corresponding commercially available carboxylic acids **3a-d** was then performed in the presence of EDCI and DMAP to give benzyl protected glycolipids **8a-d** in 82-85% yield. The HMBC between the carbonyl carbons (C11 and C11') and the protons of glycerol (H-8 and H-9a+b) confirmed the installation of two lipids at the desired positions. Finally, the benzyl protecting groups on **8a-d** were removed under the agency of Pearlman's catalyst and H_2 to give the target αGlc-DAGs **1a-d** in excellent yields (89-91%). Thus, in sum, the total syntheses of αGlc-DAGs **1a-g** were achieved in 7 steps and 44-47% overall yield. Of compounds **2a-d**, only αGlc-DAG **2d** has previously been synthesized in 5 steps and 36% yield from 1-*O*-*p*-nitrobenzoyl-2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose, with the later prepared in 4 steps from glucose.[26]

With the glycolipids in hand, the ability of these analogues to signal through murine Mincle (mMincle) was assessed using αGlcDAG-coated plates and NFAT-GFP reporter cells.[27,28] Here, Mincle signalling would be monitored by the expression of GFP, as detected by flow cytometry, with TDB as the positive control and FcRγ only as the negative control. Previously, mMincle agonist studies have revealed that increasing lipid length typically leads to better Mincle signalling.[12,21] In our studies, all compounds signaled through mMincle (Figure 2), however, the optimum lipid length for mMincle signalling by αGlc-DAGs centered around glycolipids containing C14 acyl chains. This enhanced Mincle signaling was observed by αGlc-DAG **1b** at both concentrations of glycolipid tested (1 and 4 nmol/well), with a greater level of GFP being produced at the higher concentration of αGlc-DAG **1b**. This concentration dependence was less pronounced for the other glycolipids, and indeed for αGlc-DAG **1d**, a higher concentration of glycolipid resulted in decreased GFP production, which may indicate that the level of coating has reached saturation.[29]

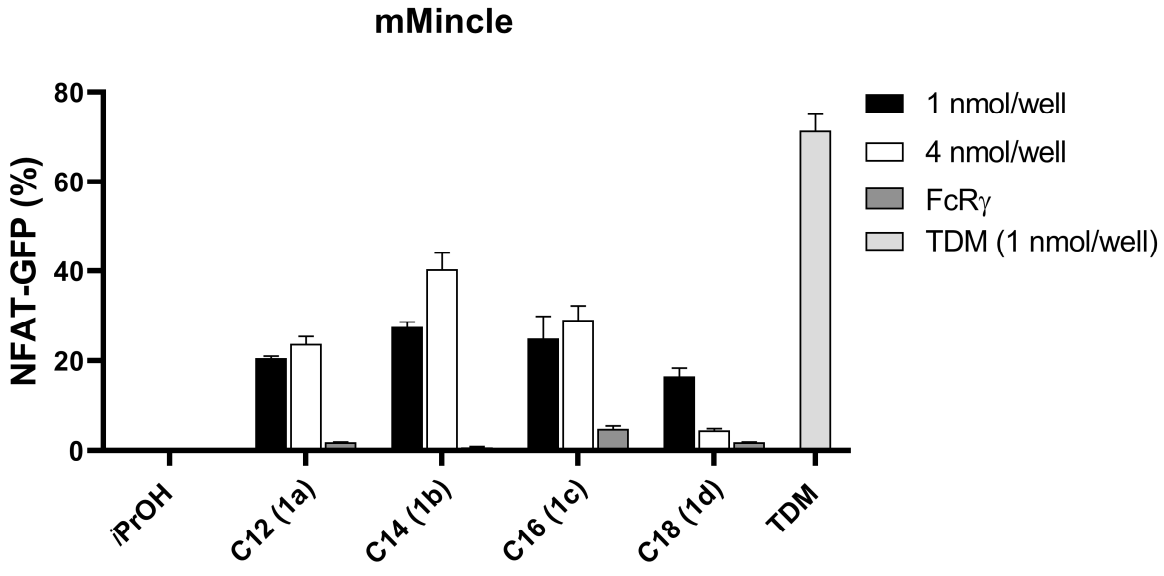


Figure 2. NFAT-GFP 2B4 reporter cells expressing mMincle + FcRγ, or FcRγ-only were stimulated using αGlcDAG-coated plates (1 nmol/well or 4 nmol/well) for 24 h. The cells were then harvested and examined for NFAT-GFP expression. Data reported is representative of three independent experiments performed in duplicate (mean ± SEM).

While there is a high degree of homology between murine and human Mincle,[30] species-specific activity has been observed for select classes of Mincle ligands.[12] Accordingly, we also sought to explore the ability of αGlc-DAGs **1a-d** to signal through hMincle using glycolipid concentrations of 0.1 nmol/well and 1 nmol/well (Figure 3). In agreement with our murine data, all αGlc-DAGs signaled through hMincle, with C14 αGlc-DAG (**1b**) eliciting the most potent response. In general, an increase in lipid length has been associated with increased hMincle signalling,[12] although in a recent study by Evans and co-workers which investigated the effect of the lipid length of α-branched trehalose diesters on mMincle and hMincle agonist activity, those compounds containing chain lengths of 5-14 carbons led to greater cytokine production by HEK cells transfected with hMincle than analogues containing lipid chains of longer or shorter length.[21] However, Evans and co-workers observed a species-specific response, with longer lipids leading to enhanced mMincle agonist activity,[21] while we observed similar relative signalling capacities by both mMincle and hMincle reporter cells following stimulation with the αGlc-DAGs.

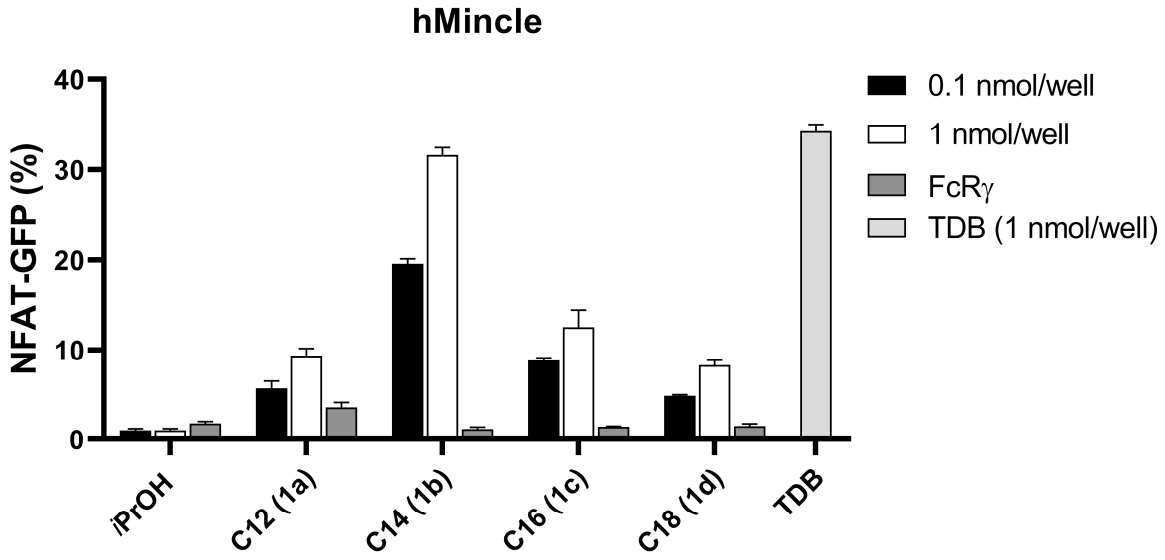


Figure 3. NFAT-GFP 2B4 reporter cells expressing hMincle + FcRγ, or FcRγ-only were stimulated using αGlcDAG-coated plates (0.1 nmol/well or 1 nmol/well) for 18 h. The cells were then harvested and examined for NFAT-GFP expression. Data reported is a representative of two independent experiments performed in duplicate (mean ± SEM).

Thus, in summary, we have demonstrated that C14 αGlc-DAG **1b** contains the optimum glycolipid length for murine and human Mincle signaling and is thus our lead αGlc-DAG agonist. Moreover, the synthetic route is efficient and the glucosylation reaction can be performed on the gram scale without affecting the yield or α:β-selectivity. Further studies into the potential of αGlc-DAG **1b** to augment the immune response against capsular polysaccharide antigens from *S. pneumoniae* will be investigated in due course.

Conclusions

In conclusion we developed an efficient 7-step route for the synthesis of *α*Glc-DAGs and determined that *α*Glc-DAGs **1a-d** signal through both murine and human Mincle. Mincle signaling was dependent on lipid length, with the greatest production of GFP by the NFAT-GFP reporter cells being elicited by *α*Glc-DAG **2b**, which contains C14 acyl chains, rather than those derivatives containing longer or shorter acyl chains. Given the essential role of

*α*Glc-DAGs in providing Mincle-mediated protective immunity against *S. pneumoniae* infection, *α*Glc-DAG **2b** thus shows promise as an adjuvant to enhance immunity against *S. pneumoniae* antigens.

Experimental

Chemistry General. All reactions were performed under an argon atmosphere unless stated otherwise. Prior to use the following solvents were distilled: toluene (ROMIL), acetone (Fisher Scientific), ethyl acetate (Fisher Scientific) and petroleum ether (Merck), DCM (Fisher Chemicals), methanol (Fisher Scientific). Lauric acid (Fisher Scientific), palmitic acid (Fulka), myristic acid (BDH), stearic acid (Fisher Scientific), D-glucose (Applichem), diisopropylethylamine (Sigma Aldich), trimethylsilyl chloride (Sigma Aldrich), hexamethyldisilane (BDH), iodine (Univar), $MgSO_4$ (Pure Science), NaCl (Chem Solute), Et_2O (LabServ), DMAP (Lab Supply), EDCI (Chem Impex), acetic acid (Scharlau), Et_3N (Sigma), $NaHCO_3$ (Pure Science), $KMnO_4$ (AnalR), $CDCl_3$ (Aldrich), CD_3OD (Apollo Scientific), C_5D_5N (Apollo Scientific), and Dowex 50WX8-200, were used as received. Reactions were monitored by TLC analysis by dipping in 10% H_2SO_4 in EtOH followed by charring or dipping in a solution of $KMnO_4$ (0.05 M), K_2CO_3 (0.4 M) and NaOH (0.06%) in water. Column chromatography was performed using Pure Science silica gel (40-63 μm). All solvents were removed by evaporation under reduced pressure. High resolution mass spectra were recorded on an Agilent 6530 Q-TOF mass spectrometer utilising a JetStream^{TM} electro-spray ionisation (ESI) source in positive or negative mode. Optical rotations were recorded on an Autopol II (Rudolph Research Analytical) at 589 nm (sodium D-line). Infrared (IR) spectra were recorded as thin films using a Bruker Platinum-ATR spectrometer and are reported in wave numbers (cm^{-1}). Nuclear magnetic resonance spectra were obtained at 20 °C in $CDCl_3$, CD_3OD or C_5D_5N using a Varian INOVA operating at 500 MHz. Chemical shifts are given in ppm (δ) relative to the solvent residual peak. NMR peak assignments were made using COSY, HSQC, and HMBC 2D experiments.

Compound data

1,2,3,4,6-Penta-*O*-trimethylsilyl-D-glucose (4). D-Glucose (2.0 g, 0.011 mol) was co-evaporated with DMF (3 × 25 mL) and then suspended in dry DMF (50 mL). Et_3N (8.6 mL,

0.061 mol) was added and the resulting solution was cooled to 0 °C before TMSCl (1.10 g, 3.96 mmol) was added. The mixture was stirred for 4 hours at r.t., after which point hexanes (100 mL) and crushed ice (ca. 50 mL) were added. The aqueous layer was extracted with hexane (3 x 25 mL) and the combined organic layers were washed with water (2 x 50 mL) and brine (3 x 50 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give the titled compound **4** as a colorless oil. The data obtained matched that reported in literature.[31]

3-*O*-(*α*-D-Glucopyranosyl)-1,2-*O*-isopropylidene-*sn*-glycerol (7). TBAI (550 mg, 1.52 mmol), (*S*)-1,2-*O*-isopropylideneglycerol (132 mg, 1.0 mmol) and DIPEA (0.40 mL, 2.02 mmol) were added to an oven-dried flask containing activated 4 Å molecular sieves in dry CH_2Cl_2 (7 mL) and the solution was stirred at r.t. for 30 min. TMS-protected D-glucose **4** (550 mg, 1.01 mmol) was azeotroped with anhydrous toluene (2 × 5 mL) and then dissolved in dry CH_2Cl_2 (2 mL) and cooled to 0 °C. Freshly prepared TMSI (0.14 mL, 1.1 mmol), which was generated by refluxing iodine (2.2 g, 8.7 mmol) over dry distilled hexamethyldisilane (2.0 mL, 9.8 mmol) for 30 minutes followed by distillation, was transferred by syringe into the flask containing TMS-protected glucose. The ice bath was then removed and the solution was stirred at r.t. for 30 min, after which time the *in situ* generated TMS-glucosyl iodide was cannulated into the acceptor flask and the resulting solution stirred for 48 hours at r.t. The reaction mixture was then filtered to remove the molecular sieves, and the flask was rinsed with hexane:EtOAc (1:1, 5 mL). The resulting solution was cooled to 0 °C and TBAI removed by filtration. The solvent was concentrated *in vacuo* and the residue was dissolved in MeOH (5 mL). Dowex/H^+ (300 mgs) and the resulting suspension stirred at r.t. until no TMS protected sugar was observed by TLC (9:1, PE:EtOAc, v/v). The suspension was then filtered to remove the resin, neutralised by the addition of DIPEA (pH = 7) and then concentrated *in vacuo* to give a yellow oil. The product was purified using silica gel flash column chromatography (EtOAc to EtOAc/MeOH, 9:1, v/v) to give the title compound **7** as a colorless oil (247 mg, 0.84 mmol, 83% yield over two steps). R_f = 0.30 (EtOAc/MeOH, 4:1, v/v); $[\alpha]_D^{24.3}$ = +58 (c = 1, CH_2Cl_2); IR (film) = 3385, 2495, 1647, 1450, 1377, 1213, 1154, 1115, 1029, 971, 834, 463 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 4.83 (d, $J_{1,2}$ = 3.7 Hz, 1H, H-1), 4.60 (s, 2H, OH), 4.36 (p, $J_{8,7} = J_{8,9}$ = 5.91, 1H, H-8), 4.10 (dd, $J_{9a,9b}$ = 8.31, $J_{9a,8}$ = 6.53 Hz, 1H, H-9a), 3.81 (dd, $J_{6a,6b}$ = 11.9 Hz, $J_{6a,5}$ = 2.4 Hz, 1H, H-6a), 3.77 (dd, $J_{9b,9a}$ = 6.64 Hz, $J_{9b,8}$ = 2.22 Hz, 1H, H-9b), 3.76–3.74 (m, 1H, H-7b), 3.67 (t, $J_{6a,6b} = J_{6b,5}$ = 5.9 Hz, 1H, H-6b), 3.64 (t, $J_{3,2} = J_{3,4}$ = 8.8 Hz, 1H, H-3), 3.62–3.57 (m, 1H, H-5), 3.51 (dd, $J_{7a,7b}$ = 10.4, $J_{7a,8}$ = 6.2 Hz, 1H, H-7a), 3.39–3.37 (m, 1H, H-2), 3.28 (t, $J_{3,4} = J_{4,5}$ = 9.3 Hz, 1H, H-4), 1.41 (s,

3H, H-1'), 1.35 (s, 3H, H-1'); ^{13}C NMR (125 MHz, CD_3OD) 109.25 (C-2'), 99.07 (C-1), 74.62 (C-8), 73.67 (C-3), 72.44 (C-5), 72.22 (C-2), 70.33 (C-4), 68.59 (C-7), 66.24 (C-9), 61.24 (C-6), 25.64 (C-1'), 24.24 (C-1'); HRMS (ESI) m/z calculated for $[C_{12}H_{22}NaO_8]^+$: 317.1206, found 317.1229.

3-*O*-(2,3,4,6-Tetra-*O*-benzyl-*α*-D-glucopyranosyl)-*sn*-glycerol (2). 3-*O*-(*α*-D-Glucopyranosyl)-1,2-*O*-isopropylidene-*sn*-glycerol **7** (416 mg, 1.41 mmol) was co-evaporated with DMF (3 × 5 mL), suspended in dry DMF (2 mL) and then BnBr (1.0 mL, 8.48 mmol) was added to the reaction mixture. The resulting solution was cooled to 0 °C, NaH [201 mg (60% suspension in mineral oil, 8.48 mmol)] was added, and then the reaction was allowed to warm to r.t. After stirring for 6 hours, the reaction was quenched with methanol (2 mL), concentrated *in vacuo*, re-dissolved in Et_2O (10 mL), washed with water (10 mL) and brine (10 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The resulting residue was purified by silica gel flash column chromatography (PE to PE/EtOAc, 8:1, v/v) to give 1,2-*O*-isopropylidene-3-*O*-(2,3,4,6-tetra-*O*-benzyl-*α*-D-glucopyranosyl)-*sn*-glycerol as a colorless oil (641 mg, 1.05 mmol, 74%). R_f = 0.35 (PE/EtOAc, 4:1, v/v); $[\alpha]_D^{17.7}$ = +32 (c = 1, CH_2Cl_2); IR (film) = 3029, 2984, 1496, 1379, 1209, 1155, 1027, 910, 796, 605, 515, 405 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42–7.24 (m, 18H, CHarom), 7.18-7.11 (d, J = 7.1 Hz, 2H, CHarom), 5.01 (d, $J_{a,b}$ = 11.8 Hz, 1H, CH_a 3-*O*-Bn), 4.90–4.87 (m, 2H, CH_a 4-*O*-Bn, H-1), 4.84 (d, $J_{a,b}$ = 11.8 Hz, 1H, CH_b 3-*O*-Bn), 4.80 (d, $J_{a,b}$ = 11.9 Hz, 1H, CH_a 2-*O*-Bn), 4.68 (d, $J_{a,b}$ = 12.1 Hz, 1H, CH_b 2-*O*-Bn), 4.63 (d, $J_{a,b}$ = 12.2 Hz, 1H, CH_a 6-*O*-Bn), 4.52–4.46 (m, 2H, CH_b 6-*O*-Bn, CH_b 4-*O*-Bn), 4.38 (p, $J_{7,8}$ = $J_{8,9}$ = 6.2 Hz, 1H, H-8), 4.12–4.06 (t, $J_{9,8}$ = 7.6 Hz, 1H, H-9a), 3.99 (t, $J_{3,2}$ = $J_{3,4}$ = 9.3 Hz, 1H, H-3), 3.85-3.74 (m, 3H, H-5, H-6a, H-9b), 3.69–3.62 (m, 3H, H-4, H-7a, H-6b), 3.61–3.56 (m, 2H, H-2, H-7b), 1.45 (s, 3H, H-1'), 1.39 (s, 3H, H-1'); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.89 (C*i*, 3-*O*-Bn), 138.33 (C*i*, 4-*O*-Bn), 138.30 (C*i*, 2-*O*-Bn), 137.95 (C*i*, 6-*O*-Bn), 128.51, 128.45, 128.43, 128.09, 128.01, 127.98, 127.95, 127.76, 127.65 (CHarom), 109.50 (C-2'), 97.56 (C-8), 81.99 (C-3), 80.04 (C-2), 77.65 (C-4), 75.76 (CH_2, 3-*O*-Bn), 75.14 (CH_2, 4-*O*-Bn), 74.65 (C-9), 73.54 (CH_2, 2-*O*-Bn), 73.16 (CH_2, 6-*O*-Bn), 70.41 (C-5), 69.09 (C-7), 68.48 (C-6), 67.10 (C-9), 26.91 (C-1'), 25.53 (C-1'); HRMS (ESI) m/z calculated for $[C_{40}H_{50}NO_8]^+$: 672.3531, found 672.3572. A solution of 1,2-*O*-isopropylidene-3-*O*-(2,3,4,6-tetra-*O*-benzyl-*α*-D-glucopyranosyl)-*sn*-glycerol in AcOH:H_2O (10 mL, 4:1, v/v) was heated to 80 °C. After 1 hour, the reaction mixture was concentrated *in vacuo* and the resulting residue was purified using silica gel flash column chromatography (PE/EtOAc, 10:1-2:1, v/v)

to give the title compound **2** as a colorless oil (599 mg, 1.046 mmol, *quant.*). R_f = 0.4 (PE/EtOAc, 1:1, v/v); $[\alpha]_D^{21.8}$ = +36 (c = 1, CH_2Cl_2); IR (film) = 3363, 2918, 1453, 1379, 1259, 1155, 1054, 838, 734, 605, 433 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.41–7.22 (m, 15H, CHarom), 7.17–7.10 (m, 2H, CHarom), 4.95 (d, $J_{a,b}$ = 10.9 Hz, 1H, CH_a 3-*O*-Bn), 4.85 (d, $J_{a,b}$ = 11.3 Hz, 1H, CH_b 3-*O*-Bn), 4.84–4.79 (m, 2H, CH_a 4-*O*-Bn, CH_a 2-*O*-Bn), 4.73 (d, $J_{1,2}$ = 3.7 Hz, 1H, H-1), 4.65 (d, $J_{a,b}$ = 11.9 Hz, 1H, CH_b 2-*O*-Bn), 4.60 (d, $J_{a,b}$ = 12.1 Hz, 1H, CH_a 6-*O*-Bn), 4.50–4.45 (m, 2H, CH_b 4-*O*-Bn, CH_b 6-*O*-Bn), 3.97 (t, $J_{2,3}$ = $J_{3,4}$ = 9.3 Hz, 1H, H-3), 3.89–3.82 (m, 2H, H-5, H-8), 3.80 (dd, $J_{7a,b}$ = 10.4 Hz, $J_{7a,8}$ = 3.5 Hz, 1H, 7a), 3.66–3.61 (m, 3H, H-9a,b, H-6a), 3.60–3.56 (m, 2H, H-6b, H-2), 3.46 (dd, $J_{7a,b}$ = 10.5 Hz, $J_{7b,8}$ = 6.8 Hz, 1H, H-7b); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.72 (C*i*, 3-*O*-Bn), 138.16 (C*i*, 4-*O*-Bn), 137.93 (C*i*, 2-*O*-Bn), 137.78 (C*i*, 6-*O*-Bn), 128.70, 128.55, 128.53, 128.52, 128.29, 128.24, 128.18, 128.12, 128.02, 128.00, 127.93, 127.88, 127.79 (CHarom), 98.62 (C-1), 82.18 (C-3), 80.12 (C-2), 77.89 (C-4), 79.95 (CH_2, 3-*O*-Bn), 75.20 (CH_2, 4-*O*-Bn), 73.81 (CH_2, 2-*O*-Bn), 73.64 (CH_2, 6-*O*-Bn), 71.70 (C-7), 70.63 (CH_2, C-8), 70.38 (C-5), 68.53 (C-9), 63.79 (C-6); HRMS (ESI) m/z calculated for $[C_{37}H_{46}NO_8]^+$: 632.3217, found 632.3264.

4.1.2 General esterification procedure: 3-*O*-(2,3,4,6-Tetra-*O*-benzyl-*α*-D-glucopyranosyl)-*sn*-glycerol **2** (1.0 mmol, 1 equiv.) and the appropriate carboxylic acid (4.4 mmol, 4 equiv.) were co-evaporated together with dry toluene, then suspended in dry toluene (5 mL). To the reaction mixture, EDCI (6.6 mmol, 6.6 equiv.) and DMAP (1 mmol, 1 equiv.) were added and the resulting suspension was heated to 70 °C for 12 h. The reaction was cooled to r.t and diluted with Et_2O (5 mL). The organic layer was then washed with water (5 mL), $NaHCO_3$ (5 mL), and brine (5 mL). The combined aqueous phases were re-extracted with Et_2O (5 mL) and the combined organic phases were dried with anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The product was purified using gradient silica-gel flash column chromatography (PE to PE:EtOAc, 9:1, *v/v*).

1,2-di-*O*-Dodecanoyl-3-*O*-(2,3,4,6-tetra-*O*-benzyl-*α*-D-glucopyranosyl)-*sn*-glycerol (8a). By subjecting diol **2** (110 mg, 0.179 mmol), lauric acid **3a** (137 mg, 0.79 mmol), EDCI (193 mg, 1.18 mmol) and DMAP (12.4 mg, 0.18 mmol) to the general esterification procedure, the title compound **8a** was obtained as colorless oil (143 mg, 0.146 mmol, 82%). R_f = 0.55 (PE/EtOAc, 4:1, v/v); $[\alpha]_D^{25.8}$ = +24 (c = 1, CH_2Cl_2); IR (film) = 2922, 2852, 1740, 1697, 1465, 1234, 1028, 907, 733, 563, 435 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.31–7.16 (m, 17H, CHarom), 7.09–7.05 (m, 2H, CHarom), 5.19 (p, $J_{7,8}$ = $J_{8,9}$ = 5.3 Hz, 1H, H-8), 4.90 (d, $J_{a,b}$ = 10.8 Hz, 1H, CH_a 3-*O*-Bn), 4.79–4.67 (m, 4H, H-1, CH_b 3-*O*-Bn, CHa 4-*O*-Bn, CH_a 2-

O-Bn), 4.60–4.51 (m, 2H, CH_a 6-*O*-Bn, CH_b 2-*O*-Bn), 4.41–4.38 (m, 2H, CH_b 6-*O*-Bn, CH_b 4-*O*-Bn), 4.35 (dd, $J_{9a,b}$ = 11.9 Hz, $J_{9a,8}$ = 3.5 Hz, 2H, H-9a), 4.13 (dd, $J_{9a,b}$ = 11.5 Hz, $J_{9a,8}$ = 5.7 Hz, 1H, H-9b), 3.88 (t, $J_{2,3} = J_{3,4}$ = 9.0 Hz, 1H, H-3), 3.71–3.62 (m, 3H, H-7a, H-5, H-6a), 3.61–3.53 (m, 2H, H-4, H-6b), 3.52–3.47 (m, 2H, H-7b, H-2), 2.25–2.18 (m, 4H, H-11, H-11'), 1.56–1.49 (m, 4H, H-12, H-12'), 1.27-1.14 (m, 36H, H-13-20, H-13'-H20'), 0.81 (t, $J_{24,25} = J_{24',25'}$ = 6.8 Hz, 6H, H-21, H-21'); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.53 (C-10'), 173.19 (C-10), 138.92 (C*i*, 3-*O*-Bn), 138.42 (C*i*, 4-*O*-Bn), 138.38 (C*i*, 2-*O*-Bn), 137.99 (C*i*, 6-*O*-Bn), 128.60, 128.52, 128.49, 128.09, 128.00, 127.97, 127.85, 127.81, 127.72 (CHarom), 97.89 (C-1), 81.95 (C-3), 80.17 (C-2), 77.60 (C-4), 75.83 (CH_2, 3-*O*-Bn), 75.20 (CH_2, 4-*O*-Bn), 73.65 (CH_2, 2-*O*-Bn), 73.26 (CH_2, 6-*O*-Bn), 70.69 (C-5), 69.98 (C-8), 68.47 (C-6), 66.54 (C-7), 62.62 (C-9), 34.43 (C-11), 34.26 (C-11'), 32.06 (C-20, C-20'), 29.79, 29.78, 29.74, 29.65, 29.58, 29.50, 29.46, 29.39, 29.27, 29.21 (C-14-19, C14'-19'), 25.06 (C-12), 25.04 (C-12'), 22.83 (C-13, C-13'), 14.279 (C-21), 14.27 (C-21'); HRMS (ESI) *m/z* calculated for $[C_{61}H_{90}NO_{10}]^+$: 996.6559, found 996.6560.

1,2-Di-*O*-tetradecanoyl-3-*O*-(2,3,4,6-tetra-*O*-benzyl-*α*-D-glucopyranosyl)-*sn*-glycerol (8b). By subjecting diol **2** (110 mg, 0.179 mmol), myristic acid **3b** (180 mg, 0.79 mmol), EDCI (193 mg, 1.18 mmol) and DMAP (12.4 mg, 0.179 mmol) to the general esterification procedure, the title compound **8b** was obtained as a colorless oil (157 mg, 0.152 mmol, 85%). R_f = 0.6 (PE/EtOAc, 4:1, v/v); $[\alpha]_D^{25.9}$ = +26 (c = 1, CH_2Cl_2); IR (film) = 2922, 2852, 1740, 1696, 1419, 1246, 1155, 1070, 733, 557, 414 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37–7.20 (m, 17H, CHarom), 7.15–7.09 (m, 2H, CHarom), 5.24 (p, $J_{7,8} = J_{8,9}$ = 5.6 Hz, 1H, H-8), 4.94 (d, $J_{a,b}$ = 11.2 Hz, 1H, CH_a 3-*O*-Bn), 4.84–4.72 (m, 4H, H-1, CH_b 3-*O*-Bn, CH_a 4-*O*-Bn, CH_a 2-*O*-Bn), 4.63–4.56 (m, 2H, CH_a 6-*O*-Bn, CH_b 2-*O*-Bn), 4.41–4.38 (m, 2H, CH_b 6-*O*-Bn, CH_b 4-*O*-Bn), 4.40 (dd, $J_{9a,b}$ = 11.3 Hz, $J_{9a,8}$ = 3.5 Hz, 2H, H-9a), 4.18 (dd, $J_{9a,b}$ = 11.5 Hz, $J_{9a,8}$ = 3.7 Hz, 1H, H-9b), 3.93 (t, $J_{2,3} = J_{3,4}$ = 9.7 Hz, 1H, H-3), 3.77–3.68 (m, 3H, H-7a, H-5, H-6a), 3.66–3.58 (m, 2H, H-4, H-6b), 3.58–3.52 (m, 2H, H-7b, H-2), 2.33–2.21 (m, 4H, H-11, H-11'), 1.62–1.54 (m, 4H, H-12, H-12'), 1.34–1.10 (m, 48H, H-13-22, H-13'-H22'), 0.87 (t, $J_{24,25} = J_{24',25'}$ = 6.8 Hz, 6H, H-23, H-23'); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.50 (C-10'), 173.16 (C-10), 138.93 (C*i*, 3-*O*-Bn), 138.43 (C*i*, 4-*O*-Bn), 138.39 (C*i*, 2-*O*-Bn), 138.00 (C*i*, 6-*O*-Bn), 128.60, 128.57, 128.51, 128.48, 128.09, 128.02, 127.99, 127.97, 127.85, 127.80, 127.71 (CHarom), 97.89 (C-1), 81.95 (C-3), 80.19 (C-2), 77.60 (C-4), 75.83 (CH_2, 3-*O*-Bn), 75.19 (CH_2, 4-*O*-Bn), 73.65 (CH_2, 2-*O*-Bn), 73.26 (CH_2, 6-*O*-Bn), 70.69 (C-5), 69.98 (C-8), 68.48 (C-6), 66.54 (C-7), 62.62 (C-9), 34.43 (C-11), 34.26 (C-11'), 32.07 (C-22, C-22'),

29.85, 29.83, 29.80, 29.66, 29.51, 29.46, 29.30, 29.27 (C-14-21, C14'-21'), 25.06 (C-12), 25.04 (C-12'), 22.84 (C-13, C-13'), 14.27 (C-23, C-23'); HRMS (ESI) *m/z* calculated for $[C_{65}H_{98}NO_{10}]^+$: 1052.7185, found 1052.7170.

1,2-di-*O*-Hexadecanoyl-3-*O*-(2,3,4,6-tetra-*O*-benzyl-*α*-D-glucopyranosyl)-*sn*-glycerol (8c). By subjecting diol **2** (110 mg, 0.18 mmol), palmitic acid **3c** (202 mg, 0.78 mmol), EDCI (193 mg, 1.18 mmol) and DMAP (12.4 mg, 0.18 mmol) to the general esterification procedure, the title compound **8c** was obtained as a white solid (166 mg, 0.15 mmol, 85%). R_f = 0.6 (PE/EtOAc, 4:1, v/v); $[\alpha]_D^{26.6}$ = +30 (c = 1, CH_2Cl_2); IR (film) = 2922, 2835, 1740, 1735, 1465, 1363, 1241, 1207, 1157, 1089, 1071, 1028, 734, 696 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.33–7.17 (m, 17H, CHarom), 7.09–7.05 (m, 2H, CHarom), 5.19 (p, $J_{7,8} = J_{8,9}$ = 5.4 Hz, 1H, H-8), 4.90 (d, $J_{a,b}$ = 10.8 Hz, 1H, CH_a, 3-*O*-Bn), 4.79–4.67 (m, 4H, H-1, CH_b 3-*O*-Bn, CH_a 4-*O*-Bn, CH_a 2-*O*-Bn), 4.60–4.51 (m, 2H, CH_a 6-*O*-Bn, CH_b 2-*O*-Bn), 4.41–4.38 (m, 2H, CH_b 6-*O*-Bn, CH_b 4-*O*-Bn), 4.35 (dd, $J_{9a,b}$ = 11.3 Hz, $J_{9a,8}$ = 3.5 Hz, 2H, H-9a), 4.13 (dd, $J_{9a,b}$ = 11.5 Hz, $J_{9a,8}$ = 3.7 Hz, 1H, H-9b), 3.88 (t, $J_{2,3} = J_{3,4}$ = 9.3 Hz, 1H, H-3), 3.73–3.63 (m, 3H, H-7a, H-5, H-6a), 3.61–3.53 (m, 2H, H-4, H-6b), 3.52–3.47 (m, 2H, H-7b, H-2), 2.24–2.17 (m, 4H, H-11, H-11'), 1.57–1.49 (m, 4H, H-12, H-12'), 1.28–1.06 (m, 47H, H-13-24, H-13'-H24'), 0.83 (t, $J_{24,25} = J_{24',25'}$ = 6.8 Hz, 6H, H-25, H-25'); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.49 (C-10'), 173.16 (C-10), 138.93 (C*i*, 3-*O*-Bn), 138.43 (C*i*, 4-*O*-Bn), 138.39 (C*i*, 2-*O*-Bn), 138.00 (C*i*, 6-*O*-Bn), 128.60, 128.51, 128.48, 128.08, 128.02, 127.99, 127.97, 127.84, 127.80, 127.71 (CHarom), 97.88 (C-1), 81.95 (C-3), 80.19 (C-2), 77.60 (C-4), 75.82 (CH_2, 3-*O*-Bn), 75.19 (CH_2, 4-*O*-Bn), 73.65 (CH_2, 2-*O*-Bn), 73.26 (CH_2, 6-*O*-Bn), 70.69 (C-5), 69.98 (C-8), 68.49 (C-6), 66.54 (C-7), 62.62 (C-9), 34.43 (C-11), 34.26 (C-11'), 32.07 (C-24, C-24'), 29.85, 29.83, 29.80, 29.66, 29.51, 29.46, 29.30, 29.27 (C-14-23, C14'-23'), 25.06 (C-12), 25.04 (C-12'), 22.84 (C-13, C-13'), 14.27 (C-25, C-25'); HRMS (ESI) *m/z* calculated for $[C_{69}H_{106}NO_{10}]^+$: 1108.7811, found 1108.7819.

1,2-di-*O*-Octadecanoyl-3-*O*-(2,3,4,6-tetra-*O*-benzyl-*α*-D-glucopyranosyl)-*sn*-glycerol (8d). By subjecting diol **2** (110 mg, 0.179 mmol), stearic acid **3d** (224 mg, 0.79 mmol), EDCI (192.6 mg, 1.18 mmol) and DMAP (12.4 mg, 0.18 mmol) to the general esterification procedure, the title compound **8d** was obtained as a white solid (174 mg, 0.150 mmol, 84%). R_f = 0.6 (PE/EtOAc, 4:1, v/v); $[\alpha]_D^{17.7}$ = +32 (c = 1, $CHCl_3$); IR (film) = 3030, 2921, 1740, 1701, 1696, 1559, 1453, 1359, 1207, 1157, 910, 733, 601, 526, 462 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.30–7.17 (m, 17H, CHarom), 7.08 (m, 2H, CHarom), 5.17 (p, $J_{7,8} = J_{8,9}$ = 5.4 Hz, 1H, H-8), 4.88 (d, $J_{a,b}$ = 10.8 Hz, 1H, CH_a 3-*O*-Bn), 4.79 –4.67 (m, 4H, H-1, CH_b 3-

O-Bn, CH_a 4-*O*-Bn, CH_a 2-*O*-Bn), 4.60–4.50 (m, 2H, CH_a 6-*O*-Bn, CH_b 2-*O*-Bn), 4.41–4.38 (m, 2H, CH_b 6-*O*-Bn, CH_b 4-*O*-Bn), 4.35 (dd, $J_{9a,b}$ = 11.3 Hz, $J_{9a,8}$ = 3.5 Hz, 2H, H-9a), 4.13 (dd, $J_{9a,b}$ = 11.5 Hz, $J_{9a,8}$ = 3.7 Hz, 1H, H-9b), 3.88 (t, $J_{2,3}$ = $J_{3,4}$ = 9.3 Hz, 1H, H-3), 3.70–3.62 (m, 3H, H-7a, H-5, H-6a), 3.61–3.53 (m, 2H, H-4, H-6b), 3.50 (m, 2H, H-7b, H-2), 2.23–2.17 (m, 4H, H-11, H-11'), 1.56–1.49 (m, 4H, H-12, H-12'), 1.28-1.06 (m, 56H, H-13-26, H-13'-H26'), 0.83 (t, $J_{24,25}$ = $J_{24',25'}$ = 6.8 Hz, 6H, H-27, H-27'); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.52 (C-10'), 173.19 (C-10), 138.96 (C*i*, 3-*O*-Bn), 138.46 (C*i*, 4-*O*-Bn), 138.42 (C*i*, 2-*O*-Bn), 138.03 (C*i*, 6-*O*-Bn), 128.62, 128.54, 128.51, 128.11, 128.04, 127.02, 127.99, 127.87, 127.83, 127.74 (CHarom), 97.91 (C-1), 81.98 (C-3), 80.22 (C-2), 77.63 (C-4), 75.85 (CH_2, 3-*O*-Bn), 75.22 (CH_2, 4-*O*-Bn), 73.68 (CH_2, 2-*O*-Bn), 73.28 (CH_2, 6-*O*-Bn), 70.72 (C-5), 70.01 (C-8), 68.52 (C-6), 66.57 (C-7), 62.64 (C-9), 34.46 (C-11), 34.29 (C-11'), 32.10 (C-26, C-26'), 29.88, 29.86, 29.83, 29.69, 29.54, 29.49, 29.33, 29.30 (C-14-25, C14'-25'), 25.08 (C-12), 25.07 (C-12'), 22.87 (C-13, C-13'), 14.30 (C-27, C-27'); HRMS (ESI) *m/z* calculated for $[C_{73}H_{114}NO_{10}]^+$: 1164.8437, found 1164.8438.

4.1.3 General debenzylation procedure: To a solution of benzyl-protected glucosyldiacylglycerides **8a-d** dissolved in EtOH:EtOAc (5 mL, 1:1, *v/v*) was added $Pd(OH)_2$/C. H_2 gas was allowed to bubble through the reaction mixture for 18 hours, at which point the suspension was diluted with hot pyridine (5 mL), filtered over celite, and concentrated *in vacuo*. The product was purified using gradient silica gel flash column chromatography (DCM to DCM:MeOH, 9:1, *v/v*).

1,2-Di-*O*-dodecanoyl-3-*O*-*α*-D-glucopyranosyl-*sn*-glycerol (1a). Benzyl protected glucosyldiacylglyceride **8a** (90 mg, 0.091 mmol) and $Pd(OH)_2$/C (70 mg) were subjected to the conditions described in the general procedure for debenzylation to give the title compound **1a** as a white solid (51 mg, 0.082 mmol, 90%). R_f = 0.75 (DCM/MeoH, 1.5:8.5, v/v); $[\alpha]_D^{16.7}$ = +42 (c = 1, C_5H_5N); IR (film) = 3381, 2918, 1648, 1537, 1302, 1230, 1010, 966, 824, 587, 529 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) 5.67 (p, *J* = 5.7 Hz, 1H, H-8), 5.39 (d, $J_{1,2}$ = 3.6 Hz, 1H, H-1), 4.76 (dd, $J_{9a,b}$ = 11.9 Hz, $J_{9a,8}$ = 3.4 Hz, 1H, 9a), 4.62 (t, $J_{2,3}$ = $J_{3,4}$ = 9.2 Hz, 1H, H-3), 4.57–4.50 (m, 2H, H-7a, H-6a), 4.44–4.37 (m, 2H, H-5, H-6b), 4.32–4.23 (m, 2H, H-7a, H-4), 4.18 (dd, $J_{2,3}$ = 9.6 Hz, $J_{1,2}$ = 3.7 Hz, 1H, H-2), 3.95 (dd, $J_{7a,b}$ = 10.7 Hz, $J_{7b,8}$ = 5.5 Hz, 1H, H-7b), 2.46–2.39 (m, 4H, H-11, H11'), 1.73–1.63 (m, 4H, H-12, H-12'), 1.37–1.18 (m, 36H, H-13-22, H-13'-22'), 0.88 (t, $J_{23,22}$ = $J_{23',22'}$ = 6.9 Hz, 6H, H-23, H-23'); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.78 (C10'), 173.60 (C10), 101.43 (C-1), 75.76 (C-3), 75.17 (C-5), 74.21 (C-2), 72.52 (C-4), 71.16 (C-8), 66.75 (C-7), 63.41(C-9), 63.24 (C-6), 34.92

(C11), 34.70 (C-11'), 32.57 (C20, C20'), 30.37, 30.34, 30.24, 30.07, 29.83 (C-14-19, C14'-C-19'), 25.70 (C12, C12'), 23.39 (C-13, C13'), 14.74 (C-21, C-21'); HRMS (ESI) *m/z* calculated for $[C_{33}H_{62}NaO_{10}]^+$: 641.4235, found 641.4230.

1,2-Di-*O*-tetradecanoyl-3-*O*-*α*-D-glucopyranosyl-*sn*-glycerol (1b). Benzyl protected glucosyldiacylglyceride **8b** (150 mg, 0.144 mmol) and $Pd(OH)_2/C$ (86 mg) were subjected to the conditions described in the general procedure for debenzylation to give the title compound as a white solid **1b** (89 mg, 0.131 mmol, 91%). R_f = 0.8 (DCM/MeOH, 1.5:8.5, v/v); $[\alpha]_D^{18.4}$ = +54 (c = 1, C_5H_5N); IR (film) = 3368, 2924, 1648, 1537, 1302, 1230, 1008, 966, 886, 824, 587, 529 cm^1; ^1H NMR (500 MHz, $CDCl_3$) 5.67 (p, J = 5.7 Hz, 1H, H-8), 5.39 (d, $J_{1,2}$ = 4.0 Hz, 1H, H-1), 4.76 (dd, $J_{9a,b}$ = 11.9 Hz, $J_{9a,8}$ = 3.2 Hz, 1H, 9a), 4.62 (t, $J_{2,3}$ = $J_{3,4}$ = 8.9 Hz, 1H, H-3), 4.56–4.50 (m, 2H, H-7a, H-6a), 4.44–4.37 (m, 2H, H-5, H-6b), 4.32–4.22 (m, 2H, H-7a, H-4), 4.18 (dd, $J_{2,3}$ = 9.6 Hz, $J_{1,2}$ = 3.7 Hz, 1H, H-2), 3.95 (dd, $J_{7a,b}$ = 10.7 Hz, $J_{7b,8}$ = 5.5 Hz, 1H, H-7b), 2.46–2.39 (m, 4H, H-11, H11'), 1.73–1.63 (m, 4H, H-12, H-12'), 1.37–1.18 (m, 36H, H-13-22, H-13'-22'), 0.88 (t, $J_{23,22}$ = $J_{23',22'}$ = 6.9 Hz, 6H, H-23, H-23'); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.80 (C10'), 173.63 (C10), 101.43 (C-1), 75.77 (C-3), 75.20 (C-5), 74.22 (C-2), 72.52 (C-4), 71.17 (C-8), 66.74 (C-7), 63.44 (C-9), 63.25 (C-6), 34.95 (C11), 34.73 (C-11'), 32.63 (C22, C22'), 30.51, 30.49, 30.46, 30.44, 30.32, 30.14, 30.13, 29.89, 29.88 (C-13-21, C13'-C-21'), 25.74 (C12, C12'), 23.44 (C-13, C13'), 14.79 (C-23, C-23'); HRMS (ESI) *m/z* calculated for $[C_{37}H_{70}NaO_{10}]^+$: 697.4861, found 697.4858.

1,2-Di-*O*-hexadecanoyl-3-*O*-*α*-D-glucopyranosyl-*sn*-glycerol (1c). Benzyl protected glucosyldiacylglyceride **8c** (120 mg, 0.109 mmol) and $Pd(OH)_2/C$ (80 mg) were subjected to the conditions described in the general procedure for debenzylation to give the title compound **1c** as a white solid (71 mg, 0.097 mmol, 89%). R_f = 0.8 (DCM/MeOH, 1.5:8.5, v/v); $[\alpha]_D^{19.8}$ = +48 (c = 1, C_5H_5N); IR (film) = 3376, 2917, 1734, 1648, 1537, 1302, 1230, 1010, 966, 886, 824, 537, 529 cm^1; ^1H NMR (500 MHz, $CDCl_3$) 5.67 (p, J = 5.6 Hz, 1H, H-8), 5.39 (d, $J_{1,2}$ = 3.6 Hz, 1H, H-1), 4.75 (dd, $J_{9a,b}$ = 11.6 Hz, $J_{9a,8}$ = 3.4 Hz, 1H, 9a), 4.62 (t, $J_{2,3}$ = $J_{3,4}$ = 9.2 Hz, 1H, H-3), 4.56–4.48 (m, 2H, H-7a, H-6a), 4.44–4.37 (m, 2H, H-5, H-6b), 4.32–4.22 (m, 2H, H-7a, H-4), 4.17 (dd, $J_{2,3}$ = 9.6 Hz, $J_{1,2}$ = 3.7 Hz, 1H, H-2), 3.94 (dd, $J_{7a,b}$ = 10.7 Hz, $J_{7b,8}$ = 5.5 Hz, 1H, H-7b), 2.48–2.39 (m, 4H, H-11, H11'), 1.74–1.64 (m, 4H, H-12, H-12'), 1.40–1.27 (m, 52H, H-13-22, H-13'-22'), 0.89 (t, $J_{23,22}$ = $J_{23',22'}$ = 6.9 Hz, 6H, H-23, H-23'); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.77 (C10'), 173.60 (C10), 101.41 (C-1), 75.74 (C-3), 75.17 (C-5), 74.19 (C-2), 72.50 (C-4), 71.14 (C-8), 66.72 (C-7), 63.41(C-9), 63.22 (C-6), 34.92 (C11), 34.70 (C-11'), 32.60 (C24, C24'), 30.48, 30.47, 30.43, 30.41, 30.10, 29.86,

29.85 (C-14-23, C14'-C-23'), 25.71 (C12, C12'), 23.42 (C-13, C13'), 14.76 (C-25, C-25'); HRMS (ESI) *m/z* calculated for $[C_{41}H_{78}NaO_{10}]^+$: 753.5487, found 753.5494.

1,2-Di-*O*-octadecanoyl-3-*O*-*α*-D-glucopyranosyl-*sn*-glycerol (1d). Benzyl protected glucosyldiacylglyceride **8d** (125 mg, 0.109 mmol) and $Pd(OH)_2/C$ (90 mg) were subjected to the conditions described in the general procedure for debenzylation to give the title compound **1d** as a white solid (77 mg, 0.098 mmol, 90%). R_f = 0.8 (DCM/MeOH, 1.5:8.5, v/v); $[\alpha]_D^{20.7}$ = +58 (c = 1, C_5H_5N); IR (film) = 3365, 2917, 1648, 1537, 1302, 1230, 1010, 966, 824, 587, 529 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) 5.65 (p, J = 5.7 Hz, 1H, H-8), 5.38 (d, $J_{1,2}$ = 3.6 Hz, 1H, H-1), 4.75 (dd, $J_{9a,b}$ = 11.5 Hz, $J_{9a,8}$ = 3.6 Hz, 1H, 9a), 4.62 (t, $J_{2,3}$ = $J_{3,4}$ = 9.2 Hz, 1H, H-3), 4.57–4.48 (m, 2H, H-7a, H-6a), 4.44–4.36 (m, 2H, H-5, H-6b), 4.30–4.22 (m, 2H, H-7a, H-4), 4.17 (dd, $J_{2,3}$ = 9.6 Hz, $J_{1,2}$ = 3.7 Hz, 1H, H-2), 3.94 (dd, $J_{7a,b}$ = 10.7 Hz, $J_{7b,8}$ = 5.5 Hz, 1H, H-7b), 2.45–2.38 (m, 4H, H-11, H11'), 1.72–1.62 (m, 4H, H-12, H-12'), 1.34–1.20 (m, 36H, H-13-22, H-13'-22'), 0.87 (t, $J_{23,22}$ = $J_{23',22'}$ = 6.9 Hz, 6H, H-23, H-23'); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.79 (C10'), 173.62 (C10), 101.44 (C-1), 75.77 (C-3), 75.20 (C-5), 74.23 (C-2), 72.52 (C-4), 71.16 (C-8), 66.74 (C-7), 63.44 (C-9), 63.25 (C-6), 34.95 (C11), 34.73 (C-11'), 32.62 (C26, C26'), 30.52, 30.50, 30.49, 30.46, 30.43, 30.33, 30.14, 30.12, 29.89, 29.88 (C-14-25, C14'-C-25'), 25.74 (C12, C12'), 23.44 (C-13, C13'), 14.78 (C-27, C-27'); HRMS (ESI) *m/z* calculated for $[C_{45}H_{86}NaO_{10}]^+$: 809.6113, found 809.6121.

Biological Methods

2B4-NFAT-GFP reporter cells assay. Synthesised αGlcDAGs **2a-d** and TDB[18] or TDM (purchased) in chloroform/methanol (2:1, *v*/*v*, 1 mM) were serially diluted with isopropanol and added to the wells of 96-well plates, followed by evaporation of the solvent. The concentration of 2B4-NFAT-GFP reporter cells expressing mMincle + FcRγ, hMincle + FcRγ or FcRγ was adjusted to 4×10^5 cells/mL and 100 µL/well was then added to glycolipid-coated plates (0.1, 1, or 4 nmol/well) for 18 h or 24 h. The reporter cells were harvested, stained with DAPI, and analysed for NFAT-GFP expression using flow cytometry.[27] All synthetic compounds were confirmed to be free of endotoxin at a sensitivity of ≤0.125 EU/mL by Limulus amebocyte lysate (LAL) assay using an endotoxin kit (Pyrotell, Limulus Amebocyte Lysate).

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Synthesis of α-Glucosyl Diacylglycerides as Potential Adjuvants for *Streptococcus pneumoniae* Vaccines

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Highlights

- We efficiently synthesised *α*-glucosyl diacylglycerols (*α*Glc-DAGs) containing C12, C14, C16 and C18 acyl chains in 7 steps and 44-47% overall yields
- Mincle signalling was dependent on lipid length, with C14 *α*Glc-DAG exhibiting the greatest signalling
- C14 *α*Glc-DAG has potential to act as an adjuvant to augment the immune response against *Streptococcus pneumoniae* antigens

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I hereby declare that there are no conflicts of interests to report in relation to the submission of our manuscript:'Synthesis of α-Glucosyl Diacylglycerides as potential adjuvants for *Streptococcus pneumoniae* vaccines.'

Regards,

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