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A PIM_2 analogue suppresses allergic airway disease

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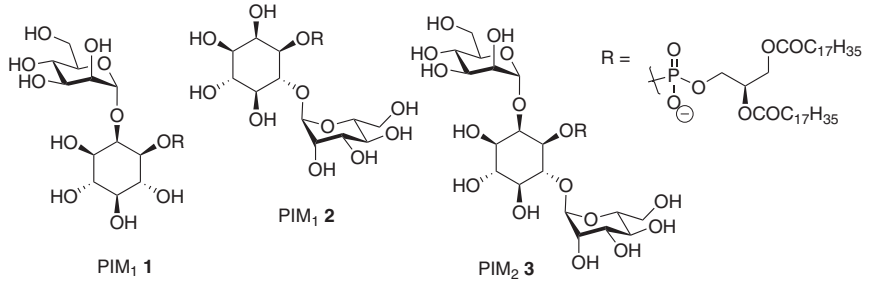
ABSTRACT

Two approaches for the synthesis of a phosphatidylinositol dimannoside (PIM_2) analogue **4** that mimics the suppressive activity of natural PIMs and also synthetic PIM_2 have been developed. This analogue, where the inositol core was replaced by glycerol, was tested for its ability to suppress cellular inflammation in a mouse model of allergic asthma and shown to be effective in suppressing airway eosinophilia. Suppression of all inflammatory cells monitored was observed, indicating a general blockade of cellular activity. These data indicate that the inositol core is not essential for this suppressive activity.

1. Introduction

The increasing prevalence of allergic disease, including asthma, in industrialised nations is commonly linked with decreased childhood exposure to infectious agents including mycobacteria.[1–8] Mycobacterial products and components have been shown to induce a wide range of immune responses including stimulatory and suppressive immune cell function.[9–13] Previously, we have shown that the cell wall components lipoarabinomannan (LAM), and phosphatidylinositol mannanoside (PIM) isolated from *Mycobacterium bovis* exert a suppressive phenotype in a model of allergic airway inflammation.[14] Initial work using native lipoglycans and three discrete synthetic PIM structures: PIM_1 (**1**), PIM_1 (**2**) and PIM_2 (**3**) indicates that suppressive activity requires the presence of fatty acid residues and at least one mannopyranosyl moiety where the mannosylation site can be at either *O*-2 or *O*-6 of the inositol moiety.[14,15]

The heterogeneity of the native PIMs makes their separation technically challenging. As a result a rational approach to progressing structure activity relationship studies on PIMs is to synthesise discrete PIM molecules for testing. However, it is important to note that the *myo*-inositol core complicates the synthetic route to PIMs. Enantiomerically pure *myo*-inositol intermediates for PIM syntheses are available either by low yielding desymmetrization processes[16,17] or via a lengthy multi-step strategy from methyl α-D-glucopyranoside.[18,19] We felt that we could circumvent these problems and evaluate whether the inositol moiety of PIMs was essential for the suppressive activity by elimination of the C-3, -4 and -5 fragment from the central inositol unit to give a



Compounds **1** to **3**

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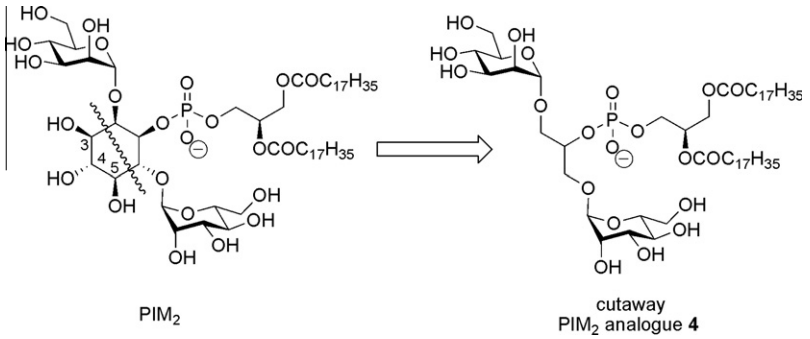


Figure 1. Design of the cutaway PIM analogue.

symmetrical molecule containing a glycerol core (Fig. 1). The advantage of this is that the core structure would contain no asymmetric centres thus simplifying its preparation. Furthermore, the incorporation of two α-D-mannopyrannosyl residues would enhance the water solubility of these PIM-like compounds. We refer here to this simplified PIM_2 structure as a 'cutaway' PIM_2 analogue.

This paper reports our strategy for the synthesis of a cutaway PIM_2 (**4**) analogue that suppresses infiltration of inflammatory cells into the lung in an in vivo allergic airway disease model in detail.[20]

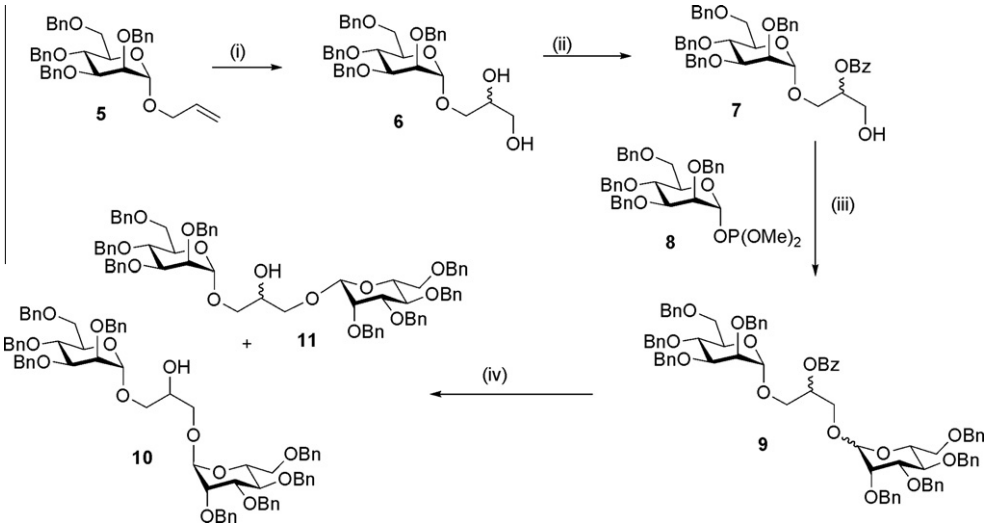
2. Results and discussion

2.1. Chemistry

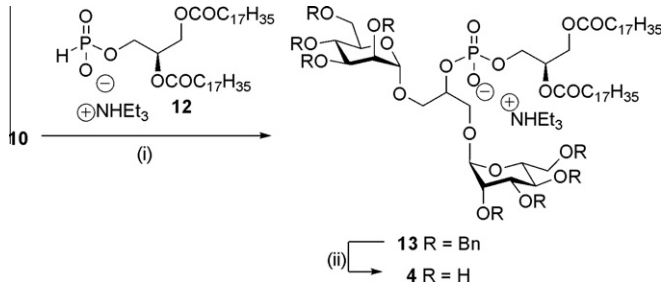
Our initial approach to the synthesis of the cutaway PIM analogue started with the conversion of benzylated allyl mannoside **5**[21] into a 1:1 diastereoisomeric mixture of the glyceryl mannoside **6** by dihydroxylation using the Upjohn process (Scheme 1 and 88% yield). Selective protection of the secondary glyceryl hydroxyl group was achieved by sequential reaction with trityl chloride then benzoyl chloride in pyridine followed by treatment of the crude product with acidified methanol to give the 2-*O*-benzoyl glyceryl glycoside **7** in 77% yield. Mannosylation of **7** with mannosyl phosphite **8**[22,23] gave a 4:1 inseparable mixture of the α,α- and α,β-dimannosylated glycerols **9** in 71% yield. Fortunately, after hydrolysis of the benzoyl group under Zémplen conditions, the glyceryl dimannosides **10** and **11** could be separated in yields of 64 and 5% respectively. The one-bond coupling constants ($^1J_{C-H}$) for each of the anomeric carbon signals of **10** which resonated at δ 98.55 and 98.58 ppm in the ^{13}C NMR spectrum were both 170 Hz confirming the anomeric configuration of each mannose residue as α.[24]

With the dimannoside **10** in hand attention turned to attaching the phosphatidyl group. We initially utilised the *H*-phosphonate method for forming the phosphate ester. Reaction of **10** and *H*-phosphonate **12**[25–27] gave, after oxidation with iodine, the triethylammonium salt of the protected PIM analogue **13** in 76% yield (Scheme 2). The −ve ion mass spectrum showed a peak consistent with the anion of **13** and the ^{31}P NMR spectrum showed a peak at δ 0.15 ppm. The remaining spectral data were also consistent with **13**. The final step in the synthesis was the global hydrogenolysis of the benzyl groups by treatment with hydrogen over 10% Pd on carbon using a 2:1:1:1 mixture of EtOAc, THF, EtOH and H_2O to give the target cutaway PIM_2 analogue **4** as the triethylammonium salt.

Although the chemistry approach above provided the target PIM analogue **4**, problems with synthetic efficiency needed to be addressed. The use of armed donor **8** resulted in an inseparable mixture of bis-mannosides **9**; the separation of debenzoylated glycosides **10** and **11** was inefficient; and hydrogenolysis of benzyl groups of the phosphodiester **13** was unpredictable due to the triethylammonium phosphate moiety. A bis-mannosylation of 2-*O*-*p*-methoxybenzylglycerol **14** with a donor possessing a participating group at *O*-2 (i.e., a disarmed donor) and phosphorylation utilising



Scheme 1. Reagents and conditions: (i) 1% aq OsO_4, 9:1 acetone–H_2O (88%); (ii) (a) TrCl, py, 100 °C, (b) BzCl, py, (c) pTSA, 7:3 CH_2Cl_2–MeOH (77%); (iii) NIS, Et_2O (71%); (iv) 1 M NaOMe, MeOH (64% for **10**, 5% for **11**).

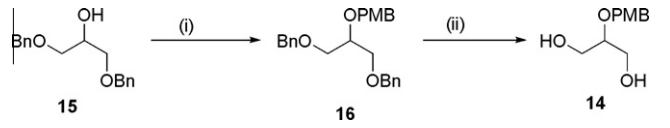


Scheme 2. Reagents and conditions: (i) PivCl, py then I_2 in 9:1 Py–H_2O (76%); (ii) H_2, 10% Pd/C, 2:1:1:1 EtOAc–THF–EtOH–H_2O (69%).

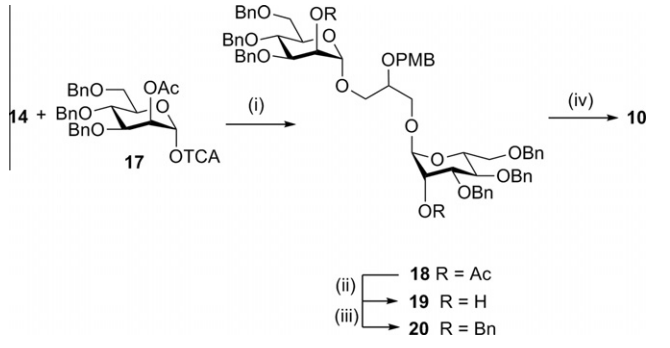
phosphoramidite coupling methodology was used to overcome these issues. Known glycerol derivative **14** was synthesised (Scheme 3) using a modification of the strategy reported by Chong et al.[28]

Commercially available 1,3-di-*O*-benzylglycerol (**15**) was alkylated with *p*-methoxybenzyl chloride under standard conditions to give **16** in 79% yield. Hydrogenolytic removal of the benzyl groups using palladium on carbon as described[28] proved unreliable. Instead, selective hydrogenolysis of the benzyl groups was effected using Raney nickel[29] providing the target glycerol **14** in 77% yield.

Bis-glycosylation of **14** with disarmed trichloroacetimidate (TCA) donor **17**[30] promoted by TMSOTf in a mixture of toluene and CH_2Cl_2 was investigated[31] (Scheme 4). Although the reaction using 20 mol % of the promoter provided the desired α,α-bismannoside **18** in 60% yield, a significant amount of material lacking the *p*-methoxybenzyl group was also recovered. Optimal reaction conditions were obtained by lowering the amount of promoter to 5 mol % to give **18** in 86% yield. This sequence converged with that shown in Scheme 1 by conversion of **18** into the corresponding benzylated bis-mannoside **10** via a sequence of deacetylation to **19**, benzylation to **20** and then oxidative removal of the *p*-methoxybenzyl group with DDQ. Compound **10** produced by this strategy was identical to that synthesised using the route shown in Scheme 1.



Scheme 3. Reagents and conditions: (i) PMBCl, NaH, DMF (79%); (ii) Raney Ni, H_2, EtOH (77%).



Scheme 4. Reagents and condition: (i) 5 mol % TMSOTf, 2:5 CH_2Cl_2–toluene, 0 °C (86%); (ii) NaOMe, CH_2Cl_2–MeOH (90%); (iii) BnBr, NaH, DMF (88%); (iv) DDQ, CH_2Cl_2–H_2O (84%).

An alternative approach to the target PIM analogue **4** was developed from **14** (Scheme 5). Reaction of **14** with disarmed trichloroacetimidate donor **21** possessing a 2-*O*-methoxyacetyl group was attempted under the conditions optimised for donor **17**. In this case the use of 5 mol % TMSOTf to promote this reaction resulted in the formation of orthoester products. Fortunately, increasing the amount of promoter to 20 mol % gave the desired bis-mannoside **22** in 79% yield. The reaction time was kept as short as possible (15 min) to minimise uncontrolled in situ loss of the PMB group. Removal of the PMB protecting group with DDQ to **23** (81%) and subsequent 4,5-dicyanoimidazole-promoted phosphitylation with phosphoramidite **24**[32,33] and subsequent *m*CPBA oxidation resulted in the fully protected PIM_2 analogue **25**. Hydrogenolysis of the benzyl groups gave **26** in 89% yield and followed by selective hydrolysis of the methoxyacetate groups effected by treatment with 0.33 mM sodium methoxide in methanol–CH_2Cl_2 gave the target cutaway PIM_2 analogue **4** isolated as its sodium salt in 84% yield (96% pure by HPLC analysis). Modifying the work-up of this process gave **4** as its triethylamine salt. The spectroscopic data of the triethylamine salt of **4** produced by this route were identical to those of **4** produced by the strategy shown in Scheme 2.

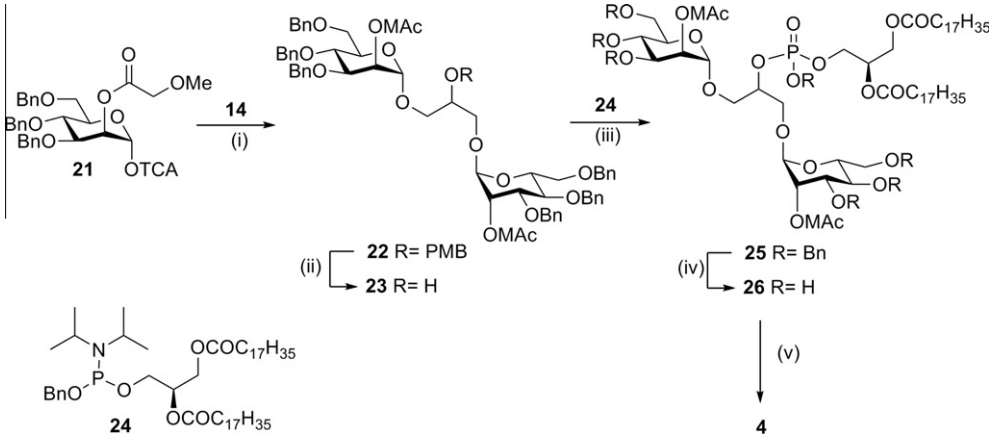
2.2. Suppression of airway eosinophilia

As in earlier studies,[14,15] the cutaway PIM_2 analogue **4** was also assayed in a mouse model of ovalbumin (OVA)-induced allergic airway inflammation using infiltration of eosinophils as a key readout of allergic airways inflammation. As shown in Figure 2A, molecule **4** suppressed the recruitment of eosinophils into the lung of OVA challenged mice. A similar decrease in both macrophage and lymphocyte cell numbers was also observed. As shown in previous studies, the percentage of eosinophils, macrophages and lymphocytes did not change significantly (Fig. 2B). Therefore, like the PIM extracts and synthetic PIMs, the suppression of lung eosinophilia by the cutaway PIM mimic appeared to result from overall suppression of cell infiltration rather than a blockade of a specific cell type.

Previous work had shown that deacylation of native LAM and PIM from *M. bovis*, and the absence of mannose caps in LAM from *M. smegmatis*, results in loss of lipoglycan-dependent suppression of OVA-induced airway eosinophilia.[14,15] The current research now indicates that the inositol core of PIMs is not necessary for suppressive activity in vivo. Similar structure activity requirements have also been observed in relation to the suppressive activity of a closely related compound in the context of LPS-induced macrophage activation in vitro and neutrophil infiltration in vivo.[34] Together with our data, it appears that PIM molecules have the ability to suppress aspects of both adaptive and innate inflammation, and that these immunosuppressive activities require the presence of at least one α-D-mannopyranosyl residue, acyl chains, and the phosphodiester moiety. However, the inositol core of PIMs appears to act solely as a scaffold for the pharmacophore and is not essential for the anti-inflammatory activity.

3. Conclusion

In conclusion, we have developed two approaches for the synthesis of cutaway PIM_2 analogues that mimic the suppressive activity of natural PIMs and also synthetic PIM_2 and added to our understanding of the structural requirements for suppression of allergic airway inflammation by PIMs. As this molecule serves as a lead compound for the development of an immunotherapeutic agent the synthesis of further PIM analogues and investigation of their immunosuppressant activity is underway.



Scheme 5. Reagents and conditions: (i) 20 mol % TMSOTf, 2:5 CH_2Cl_2–toluene, 0 °C (79%); (ii) DDQ, CH_2Cl_2–H_2O (81%); (iii) 4,5-dicyanoimidazole, CH_2Cl_2, then *m*CPBA (82%); (iv) H_2, 20% $Pd(OH)_2$/C, THF–MeOH (89%); (v) NaOMe, CH_2Cl_2–MeOH (84%).

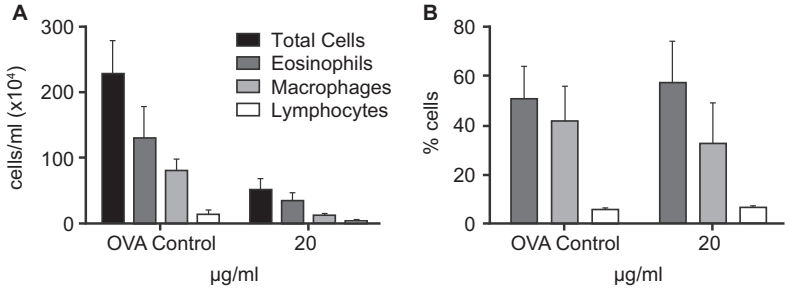


Figure 2. Intra-nasal administration of cutaway PIM_2 mimic **4** suppresses cell infiltration in a mouse model of allergic airway disease (AAD). (A) Cells per mL and (B) cell percentages in the brochoalveolar lavage fluid of OVA challenged mice treated with compound **4**. Graph is representative of two experiments ($n = 5$ per group, ±SEM).

4. Experimental section

4.1. Airway inflammation model

C57BL/6 male mice were bred and housed in a conventional animal facility at the Wellington School of Medicine. All animals used for the experiments were aged between 8 and 12 weeks. Experimental procedures were approved by the animal ethics committee in accordance with Victoria University of Wellington (Wellington, NZ) guidelines for the care of animals.

4.1.1. Immunisation and airway challenge

Mice were injected intraperitoneally (ip) with 2 μg of OVA (Sigma–Aldrich, St. Louis, MO) in 200 μL of alum adjuvant (Serva, Heidelberg, Germany) on day 0 and 14 ($n = 5$ mice/group). On day 28 mice were anaesthetised by an ip injection of a mixture of Ketamine and Xylazine (Sigma–Aldrich) and intranasally (in) challenged with 50 μL of PBS containing 100 μg OVA.

4.1.2. Treatment with PIM analogue 4

One week (day 21) prior to OVA challenge mice were anaesthetised and analogue **4** was administered intranasally (50 μL, 20 μg/mL, in). OVA control mice were given 50 μL of PBS intranasally.

4.1.3. Detection of cell types in the bronchoalveolar lavage fluid

Four days (day 32) post OVA challenge (day 28) the mice were sacrificed and bronchoalveolar lavage (BAL) was performed (three washes with 1 mL PBS). Total BAL cell numbers were counted, cells were fixed onto slides using a cytospin and stained with the Diff-Quick Staining Kit (Dade Behring, Newark, USA). The percentages of different cell types were determined microscopically using standard histological criteria.

4.2. Experimental procedures for synthesis of PIM analogues

Specific optical rotations, given in 10^{-1} deg cm^2 g^{-1}, were measured at ambient temperature using either a Jasco DIP-1000 polarimeter or a Perkin Elmer 241 polarimeter each with a cell of path length 1.0 dm. ^1H NMR spectra were obtained at 500 MHz and referenced to tetramethylsilane (TMS) (0.0 ppm) or the residual solvent peak ($CHCl_3$ 7.26 ppm). Chemical shifts are reported as parts per million (ppm) using the δ scale and coupling constants (J) and separations are reported to the nearest 0.5 Hz. ^{13}C NMR spectra were recorded at 125 MHz and referenced either to TMS (0.0 ppm) or internal solvent ($CDCl_3$ 77.0 ppm). Chemical shifts are reported to 1 decimal place with the chemical shifts for signals that are similar reported to two decimal places. ^{31}P NMR spectra were recorded at 202 MHz and are reported to 1 decimal place with H_3PO_4 (0.0 ppm) as the external reference. Electro-spray ionization (ESI) mass spectra were recorded either on a PerSpective Biosystems Mariner time-of-flight mass spectrometer or a Q-TOF Premier mass spectrometer. Elemental analyses were carried out at the Campbell Microanalytical Laboratory, University of Otago, Dunedin, New Zealand. Thin layer chromatography (TLC) was performed on Merck silica gel DC Alurolle Kieselgel $60F_{254}$ plates and were visualised under an UV lamp and/or with a spray consisting of 5% w/v dodecamolybdophosphoric acid in ethanol with subsequent heating. Flash column chromatography was carried out using Merck Kieselgel 60 (230–400 mesh). All chromatography solvents were reagent grade. Petroleum ether used was bp 60–80° range. Anhydrous solvents were either sourced from Aldrich and used without further treatment or reagent grade solvents distiled fresh prior to use (THF was distiled from sodium-benzophenone ketyl under nitrogen and dichloromethane was distilled from phosphorus pentoxide). Powdered molecular sieves were flame

dried under vacuum immediately prior to use. All compounds were isolated after silica gel column chromatography and fractions collected were one spot by TLC. HPLC analyses were performed on an Agilent 1100 quaternary pump system using an ESA Corona Charged Aerosol Detector (Filter = none). The column used was a Phenomenex Synergi 4 μm Fusion-RP (80 Å, 4.6 × 250 mm). HPLC elution gradient: solvent A H_2O; solvent B 100 mM NH_4OAc; solvent C Methanol; Program 0–10 min 5–0% A, 5% B, 85–95% C, 10–28 min hold (0% A, 5% B, 95% C) and operated at a flow rate of 1.0 mL min^{-1}.

4.2.1. (2*R*∗)-1-*O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl)-glycerol 6

Allyl 2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranoside **5** (1.00 g, 1.72 mmol) and *N*-methylmorpholine-1-oxide (302 mg, 2.58 mmol) were dissolved in a mixture of 9:1 acetone–water (40 mL) and a 1% aqueous osmium tetraoxide solution (1.5 mL) was added. The reaction mixture was stirred overnight at room temperature, then poured into 10% sodium thiosulfate solution (20 mL) and extracted with CH_2Cl_2 (40 mL). The organic layer was washed with water and dried over magnesium sulfate. The solvent was removed and the residue was purified over silica (1:2 hexanes–diethyl ether as eluent) to give the title compound **6** (927 mg, 88%, 1:1 mixture of epimers) as a colourless syrup; R_f 0.4 (1:2 hexanes–EtOAc); (found: C, 71.69; H, 6.92. $C_{37}H_{42}O_8{\cdot}0.5H_2O$ requires C, 71.25; H, 6.95); ν_{max}/cm^{-1} 3439 (OH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.46–3.94 (m, 11H), 4.48–4.64 (m, 5H), 4.71–4.87 (m, 2H), 4.82–4.88 (m, 2H), 7.18–7.21 (m, 2H, Ph-H) and 7.24–7.36 (m, 18H, Ph-H);^{13}C NMR (125 MHz, $CDCl_3$) δ 63.5, 63.6, 69.4, 69.5, 69.9, 70.6, 70.8, 70.9, 72.2, 72.3, 72.37, 72.42, 72.85, 72.9, 73.5, 73.6, 74.9, 75.02, 75.04, 75.10, 75.12, 79.9, 99.1, 127.70, 127.72, 127.76, 127.90, 127.95, 127.99, 128.06, 128.10, 128.40, 128.43, 128.45, 138.0, 138.1, 138.23, 138.24, 138.3, 138.4; LRMS-ESI (+ve) *m*/*z* (%) 638 $[MNa]^+$ (24), 91 (100).

4.2.2. (2*R*∗)-2-(*O*-Benzoyl-3-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)glycerol 7

Glycerol **6** (900 mg, 1.47 mmol) and trityl chloride (490 mg, 1.76 mmol) were dissolved in dry pyridine (60 mL) and heated at 100 °C. The disappearance of **6** was monitored by TLC and after 90 min the reaction was cooled to 0 °C and a solution of benzoyl chloride (1.0 g, 7.0 mmol) in dry CH_2Cl_2 (10 mL) was added. The reaction was warmed to room temperature and stirring was continued for 2 h. The solvent was removed and the residue was dissolved in chloroform (50 mL), washed with 2 M HCl (2 × 20 mL), saturated sodium bicarbonate solution (2 × 20 mL), water (25 mL) and dried over magnesium sulfate. The solvent was removed, the residue was dissolved in a 7:3 CH_2Cl_2–MeOH (50 mL) and *p*-TSA (75 mg) was added. The reaction was stirred at room temperature overnight and solvent was removed. The residue was purified over silica (8:2–7:3 hexanes–diethyl ether as eluent) to afford the title compound **7** (816 mg, 77%) as a pale syrup; R_f 0.4 (2:1 diethyl ether–hexanes); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.62–4.05 (m, 10H); 4.46–4.77 (m, 7H), 4.83 and 4.85 (2 × d, each 1H, *J* 10.5 Hz, PhCH_2), 4.89 and 4.94 (2 × d, each 1H, *J* 2 Hz, H-1), 5.20–5.30(m, 1H, H-2′), 7.10–7.12 and 7.20–7.41 (m, 20 H, Ph-H), 7.51 (t, 1H, *J* 7.5 Hz), 8.05 (br d, 2H, *J* 7.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 57.9, 61.2, 65.25, 65.3, 65.6, 68.9, 69.0, 71.9, 72.0, 72.4, 73.1, 73.2, 73.4, 73.8, 74.6, 74.7, 74.8, 79.6, 79.7, 97.5, 98.0, 127.32, 127.36, 127.38, 127.41, 127.56, 127.59, 127.62, 127.65, 127.74, 127.80, 127.83, 128.07, 128.10, 128.13, 128.2, 128.2, 128.3, 129.5, 129.6, 129.7, 129.8, 132.96, 133.0, 138.05, 138.1, 138.2, 138.3, 166.0, 166.1; ν_{max}/cm^{-1} ($CHCl_3$) 1716; HRMS-ESI (+ve) found: *m*/*z* 719.3220 $[M+H]^+$; $C_{44}H_{47}O_9$ calcd *m*/*z* 719.3220.

4.2.3. (2*R*∗)-2-*O*-Benzoyl-1,3-bis-*O*-(2,3,4,6-tetra-*O*-benzyl-D-mannopyranosyl)glycerol 9

Powdered 4 Å molecular sieves (50 mg) were added to a solution of glycerol **7** (730 mg, 1.02 mmol), phosphite **8** (771 mg, 1.22 mmol) and *N*-iodosuccinimide (NIS) (275 mg, 1.22 mmol) in dry diethyl ether (20 mL) under an atmosphere of nitrogen. The reaction was stirred at room temperature for 15 min, trifluoromethanesulfonic acid (110 μL, 1.24 mmol) was added and the stirring was continued for 2 h. The reaction was diluted with more diethyl ether (50 mL) and the organic layer was washed with 10% sodium thiosulfate solution (30 mL), water (2 × 20 mL) and dried over magnesium sulfate. The solvent was removed and the residue was purified over silica (9:1 hexanes–diethyl ether as eluent) to give **9** (900 mg, 71%, 4:1 mixture of αα to αβ diastereoisomers) as a colourless syrup; R_f 0.6 (2:3 hexanes–diethyl ether); ν_{max} cm^{-1}/($CHCl_3$) 1716.7; ^{1}H NMR (500 MHz, $CDCl_3$) δ *inter alia* 3.50–4.13 (m, 16H), 4.42–4.52 (m, 4H), 4.55–4.65 (m, 4H), 4.69 (d, 2H, *J* 12.5 Hz, PhCH_2), 4.72 (d, 2H, *J* 12.5 Hz, PhCH_2), 4.82 (d, 2H, *J* 11 Hz, PhCH_2), 4.86 (d, 2H, *J* 11 Hz, PhCH_2), 4.91 (d, 1H, *J* 2 Hz), 4.96 (d, 1H, *J* 2 Hz), 5.33–5.38 and 5.41–5.53 (2 × m, 1H, H-2′), 7.08–7.38 (m, 42H, Ph-H), 7.55 (t, 1H, *J* 7.7 Hz), 8.00 (d, 2H, *J* 7.7); ^{13}C NMR (125 MHz, $CDCl_3$) δ *inter alia* 65.5, 65.9, 69.1, 69.2, 71.4, 72.2, 72.3, 72.4, 72.65, 72.68, 73.38, 73.42, 74.7, 74.8, 74.9, 75.0, 75.1, 79.9, 80.0, 97.7, 98.4, 127.52, 127.60, 127.63, 127.73, 127.76, 127.78, 127.81, 127.87, 127.92, 128.03, 128.29, 128.33, 128.35, 128.41, 128.5, 129.8, 130.0, 133.2, 138.35 138.42, 138.43, 138.5, 138.5, 138.6, 165.9; HRMS-ESI (+ve) found: *m*/*z* 1241.5595 $[M+H]^+$; $C_{78}H_{81}O_{14}$ calcd *m*/*z* 1241.5626.

4.2.4. 1,3-Bis-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)-glycerol 10

Bis-mannoside **9** (800 mg, 0.65 mmol) was dissolved in 1 M sodium methoxide in methanol (80 mL) and the reaction was stirred overnight at room temperature. The solvent was removed and the residue was dissolved in CH_2Cl_2, washed with 2 M HCl (2 × 50 mL) and water (50 mL), and dried over magnesium sulfate. Removal of the solvent gave a residue (**10** to **11** 4:1, 615 mg) which was purified over silica (75:25–65:35 hexanes–diethyl ether gradient elution) to afford the title compound **10** (470 mg, 64%), a mixture of **10** and **11** (90 mg, 12%), and (2*R*∗)-1-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)-3-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-mannopyranosyl)-glycerol **11** (40 mg, 5%, 1:1 mixture of diastereoisomers).

Data for **10**: R_f 0.55 (2:3 hexanes–diethyl ether); $[\alpha]_D^{20}$ +27 (*c* 0.89, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.45 (dd, 1H, *J* = 7.0, 10.6 Hz), 3.52 (dd, 1H, *J* = 4.1, 10.8 Hz), 3.56 (dd, 1H, *J* = 5.9, 10.8 Hz), 3.61 (dd, 1H, *J* = 4.3, 10.6 Hz), 3.67–3.81 (m, 8H), 3.83–3.92 (m, 3H), 3.94 (d, 1H, *J* = 9.7 Hz), 3.98 (d, 1H, *J* = 9.4 Hz), 4.46–4.52 (m, 4 H), 4.57–4.65 (m, 6H), 4.67–4.76 (m, 4H) 4.82–4.88 (m, 4H), 7.19–7.19 (m, 4H), 7.20–7.38 (m, 36H); ^{13}C NMR 69.26, 69.29, 69.4, 69.6, 70.0, 72.27, 72.29, 72.33, 72.4, 72.8, 73.41, 73.42, 74.9, 75.00, 75.02, 75.05, 75.08, 80.0, 80.1, 98.8 ($^1J_{C-H}$ 170 Hz), 98.9 ($^1J_{C-H}$ 170 Hz), 127.52, 127.55, 127.61, 127.62, 127.64, 127.68, 127.70, 127.8, 128.0, 128.33, 128.34, 128.37, 128.39, 138.3, 138.4, 138.45, 138.47, 138.52; HRMS-ESI (+ve) found: *m*/*z* 1159.5186 $[M+Na]^+$; $C_{71}H_{76}O_{13}Na^+$ calcd *m*/*z* 1159.5184.

Data for **11**: R_f 0.5 (2:3 hexanes–diethyl ether); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.42–.58 (m, 1H), 3.64–4.00 (m, 16H), 4.44–4.76 (m, 15H), 4.83–4.92 (m, 2H), 5.04 (d, 1H, *J* 2 Hz, H-1), 7.16–7.34 (m, 40H, Ph-H), ^{13}C NMR (125 MHz, $CDCl_3$) δ 61.69, 67.03, 69.29, 72.23, 72.36, 72.38, 72.40, 72.71, 72.78, 73.46, 74.88, 74.92, 74.96, 75.11, 75.17, 79.95, 80.14, 98.6 and 97.7 (both C-1), 127.55, 127.59, 127.66, 127.69, 127.76, 127.84, 127.92, 127.97, 128.06, 128.08, 128.14, 128.18, 128.22, 128.37, 128.40, 138.23, 138.26, 138.30, 138.36, 138.42, 138.50, 138.53; LRMS-ESI

(+ve) *m*/*z* 1176 $[M+K]^+$ (100), 1160 $[M+Na]^+$ (89); (found: C, 74.87; H, 6.86; $C_{71}H_{76}O_{13}$ requires C, 74.98; H, 6.74).

4.2.5. Triethylammonium 2-*O*-(1,2-di-*O*-stearoyl-*sn*-glycero-3-phosphoryl)-1,3-bis-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)glycerol 13

A mixture of salt **12**, prepared from (200 mg, 0.32 mmol), salicyl chlorophosphite (107 mg, 0.53 mmol), dimannoside **10** (200 mg, 0.176 mmol)and 1,2-di-*O*-stearoyl-*sn*-glycerol (200 mg, 0.176 mmol) was dried by co-evaporation with pyridine (2 × 20 mL) and was then dissolved in pyridine (8 mL). Pivaloyl chloride (200 μL, 1.62 mmol) was added and the resulting solution was stirred for 1 h at room temperature. After this time a solution of iodine (120 mg, 0.47 mmol) in 9:1 pyridine–water (30 mL) was added and stirring was continued for 45 min. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and stirred for 15 min, washed with 10% sodium thiosulfate solution (20 mL), and with 1 M tetraethylammonium bromide (2 × 20 mL) and water (100 mL). The organic layer was dried over magnesium sulfate and the solvent was removed. The residue was purified over silica (98:1:1 CH_2Cl_2–MeOH–NEt_3 as eluent) to give the title compound **13** (252 mg, 76%) as a clear glass; R_f 0.45 (5:1:0.1 CH_2Cl_2–MeOH–NEt_3); $[\alpha]_D^{22}$ +11.7 (c 1.2, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$) δ 0.88 (t, 6H, *J* 7.5 Hz, 2 × CH_3), 1.15 (t, 9H, *J* 7.5 Hz, 3 × NCH_2CH_3), 1.20–1.32(m, 48H), 1.44–1.58 (br m, 4H, 2 × $COCH_2CH_2$), 2.21 (t, 4H, *J* 7.5 Hz, 2 × $COCH_2$), 2.80–2.90 (br m, 6H, 3 × NCH_2), 3.62–3.90 (m, 14H), 3.92–4.06 (m, 5H), 4.10–4.15 (m, 1H), 4.25–4.74 (m, 15H, $PhCH_2$), 4.80–4.86 (m, 2H, $PhCH_2$), 4.96 (br s, 1H, H-1), 5.03 (d, 1H, *J* 6 Hz, H-1), 5.18–5.22 (m, 1H, H-1″), 7.11–7.38 (m, 40H, Ph-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 8.71, 14.16, 22.73, 24.89, 24.96, 29.20, 29.37, 29.40, 29.57, 29.70, 29.75, 31.96, 34.11, 34.29, 45.43, 62.95, 63.58, 66.97, 69.21, 69.32, 70.52, 72.02, 72.08, 72.54, 72.83, 73.33, 73.37, 74.75, 74.80, 75.04, 75.08, 80.39, 80.47, 98.29 (C-1′), 127.38, 127.45, 127.58, 127.60, 127.65, 127.67, 127.71, 127.74, 127.80, 128.03, 128.04, 128.22, 128.24, 128.27, 128.35, 138.58, 138.66, 138.74, 138.76, 172.99, 173.40; ^{31}P NMR (121 MHz, $CDCl_3$) δ 0.15 ppm; HRMS-ESI (−ve) found: *m*/*z* 1822.0492 $[M-NHEt_3]^-$ $C_{110}H_{150}O_{20}P^-$ calcd *m*/*z* 1822.0458.

4.2.6. Triethylammonium 2-*O*-(1,2-*O*-distearoyl-*sn*-glycero-3-phoshoryl)-1,3-bis-*O*-(α-D-mannopyranosyl)glycerol 4

Phosphate **13** (100 mg, 0.055 mmol) was dissolved in 2:1:1:1 EtOAc–THF–EtOH–H_2O (30 mL). 10% Pd/C (200 mg) was added and the reaction was stirred under the atmosphere of hydrogen for 18 h. The mixture was filtered through Celite, the filter pad was washed with THF (5 mL), methanol (5 mL) and CH_2Cl_2 (2 × 5 mL), and the solvent from the combined filtrates was removed in vacuo. The water was removed by azeotropic distillation with toluene (5 × 4 mL). The residue was purified by silica gel preparative plate chromatography (94:5:1 CH_2Cl_2–MeOH–NEt_3 as eluent). The baseline region of the plate was cut and the silica washed with warm methanol (20 mL) and CH_2Cl_2 (20 mL). The solvent was removed to give a residue which was lypophilised from a methanol and water mixture to give the title compound **4** (42 mg, 69%) as a white solid; R_f 0.2 (2:1:0.17 $CHCl_3$–MeOH–H_2O); $[\alpha]_D^{20}$ +38 (c 0.6, 2:1:0.17 $CHCl_3$–MeOH–H_2O); ^1H NMR (500 MHz, 2:1:0.17 $CDCl_3$–CD_3OD–D_2O) δ 0.85–0.92 (m, 6H), 1.20–1.37 (m, 65H), 1.55–1.67 (m, 4H), 2.28–2.38 (m, 4H), 3.13 (q, 6H, *J* = 7.3 Hz), 3.58–3.69 (m, 6H), 3.71–3.78 (m, 4H), 3.82–3.91 (m, 6H), 36.94–4.02 (m, 2H), 4.17–4.22 (obs m, 1H), 4.35–4.45 (obs m, 2H), 4.82 (d, 1H, *J* = 1.6 Hz), 4.84 (d, 1H, *J* = 1.6 Hz), 5.21–5.27 (m, 1H); ^{13}C NMR (125 MHZ, 2:1:0.17 $CDCl_3$–CD_3OD–D_2O) δ 8.0, 13.4, 22.2, 24.4, 24.5, 28.66, 28.69, 28.8, 28.9, 29.06, 29.09, 29.14, 29.19, 29.22, 31.4, 33.6, 33.8, 46.0, 47.5, 47.7, 47.8, 48.0, 48.2, 48.3, 48.5, 61.00, 61.03, 62.5, 63.0, 66.0, 66.4, 66.88, 66.93, 70.00, 70.04, 70.1, 70.2, 70.7, 72.5, 72.6, 76.7, 77.0, 77.3, 99.8 ($^1J_{C-H}$ = 170), 99.9 ($^1J_{C-H}$ = 169), 173.4, 173.8; ^{31}P NMR (202 MHz, 2:1:0.17 $CDCl_3$–CD_3OD–D_2O) δ −0.9; HRMS-ESI (−ve) found *m*/*z* 1101.6699 $[M-H]^-$; $C_{54}H_{102}O_{20}P^-$ calcd 1101.6702;

4.2.7. 2-*O*-(4-Methoxybenzyl)glycerol 14

A solution of **16**[29] (2.19 g, 5.58 mmol) in ethanol (35 mL) was stirred with Raney®-Ni (50% in water, 4.5 g) under an H_2 atmosphere for 24 h, filtered and concentrated. Flash chromatography on silica gel (deactivated with 8% w/w water) using 4:5–9:10 EtOAc–petroleum ether gave **14** (917 mg, 77%) as a colourless solid. R_f 0.13 (2:1 EtOAc–petroleum ether); ^1H NMR (500 MHz, $CDCl_3$) δ 2.35–2.41 (m, 2H), 3.55 (quintet, 1H, *J* = 4.7 Hz, 2-H), 3.64–3.77 (m, 4H), 3.79 (s, 3H, CH_3), 4.56 (s, 2H, $ArCH_2$), 6.86–6.90 (m, 2H), 7.25–7.29 (m, 2H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 55.3, 62.3, 71.7, 78.9, 114.0, 129.5, 130.2, 159.5; HRMS-ESI (+ve) found: *m*/*z* 235.0947 $[M+Na]^+$; $C_{11}H_{16}O_4Na^+$ calcd 235.0946.

4.2.8. 1,3-Bis-(2-*O*-acetyl-3,4,5-tri-*O*-benzyl-α-D-mannopyranosyl)-2-*O*-(4-methoxybenzyl)glycerol 18

A solution of **14** (48 mg, 0.22 mmol) and donor **17** (357 mg, 0.56 mmol) in CH_2Cl_2 (10 mL) was concentrated and dried under high vacuum for 30 min. The mixture was re-dissolved in dry CH_2Cl_2 (2 mL) and dry toluene (5 mL). To the homogeneous solution was added powdered 4 Å molecular sieves (approx 1 g) and the mixture stirred for 1 h at ambient temperature before being cooled in an ice-water bath. Trimethylsilyl trifluoromethanesulfonate (2 μL, 0.011 mmol) was added and the reaction was monitored for completion by TLC (3:7 EtOAc–petroleum ether). After 1 h at 0 °C NEt_3 (25 μL, 0.18 mmol) was added and the crude reaction mixture was filtered, concentrated and purified by silica gel chromatography (3:7 EtOAc–petroleum ether) to provide **18** (225 mg, 86%). R_f 0.15 (1:2 EtOAc–petroleum ether); $[\alpha]_D^{20}$ +33 (*c* 0.92, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$) δ 2.13 (s, 6H, 2 × $C(O)CH_3$), 3.45–3.55 (m, 2H), 3.63–3.82 (m, 12H), 3.85–3.97 (m, 4H), 4.42–4.54 (m, 8H), 4.62–4.72 (m, 4H), 4.81–4.88 (m, 4H), 5.34–5.38 (m, 2H, 2-H′ and 2-H″), 6.74–6.79 (m, 2H, Ph-H), 7.12–7.35 (m, 32H, Ph-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.1, 55.2, 67.2, 67.4, 68.6, 68.7, 68.8, 71.5, 71.7, 71.8, 71.9, 72.3, 73.37, 73.45, 74.3, 75.1, 76.1, 78.1, 97.8 ($^1J_{C-H}$ = 171 Hz), 98.2 ($^1J_{C-H}$ = 171 Hz), 113.8, 127.5, 127.76, 127.83, 128.1, 128.3, 128.4, 129.6, 130.2, 138.0, 138.3, 138.5, 138.6, 159.3, 170.4; HRMS-ESI (+ve) *m*/*z* found 1183.5016 $[M+Na]^+$; $C_{69}H_{76}O_{16}Na^+$ calcd 1183.5031.

4.2.9. 1,3-Bis-(3,4,5-tri-*O*-benzyl-α-D-mannopyranosyl)-2-*O*-(4-methoxybenzyl)glycerol 19

To a solution of **18** (173 mg, 0.149 mmol) in 2:3 CH_2Cl_2–MeOH (5 mL) was added sodium methoxide (30%, 25 μL) and the reaction mixture was stirred at ambient temperature. The reaction was monitored by TLC (1:1 EtOAc–petroleum ether) and at 4.5 h the reaction was quenched by the addition of DowexWX8-100 (H^+) resin. Flash chromatography on silica gel, using 3:2 EtOAc–petroleum ether gave the title compound **19** (145 mg, 90%) as a colourless foam. R_f 0.22 (3:2 EtOAc–petroleum ether); $[\alpha]_D^{20}$ +55 (*c* 0.88, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$) δ 2.45 (br s, 2H), 3.45–3.55 (m, 2H), 3.61–3.80 (m, 12H), 3.80–3.88 (m, 4H), 3.97–4.01 (m, 2H, 2-H′ and 2-H″), 4.44–4.53 (m, 6H), 4.57–4.70 (m, 6H), 4.79–4.83 (m, 2H), 4.86 (d, 1H, *J* = 1.5 Hz, 1-H′), 4.90 (d, 1H, *J* = 1.5 Hz, 1-H″), 6.75–6.79 (m, 2H), 7.13–7.36 (m, 32H); ^{13}C NMR 125 MHz, $CDCl_3$) δ 55.2, 67.0, 67.4, 68.3, 68.4, 68.90, 68.94, 71.2, 71.3, 72.0, 72.1, 73.4, 73.5, 74.2, 74.3, 75.06, 75.07, 76.1, 80.09, 80.11, 99.4, 99.6, 113.8, 127.52, 127.55, 127.59, 127.62, 127.82, 127.84, 127.86, 127.87, 127.89, 127.91, 127.93, 128.0, 128.30, 128.31, 128.51, 128.52, 129.5, 130.3, 137.92, 137.93, 138.3, 138.4, 138.5, 159.2; HRMS-ESI (+ve) found *m*/*z* 1099.4824 $[M+Na]^+$; $C_{65}H_{72}O_{14}Na^+$ calcd 1099.4820.

4.2.10. 1,3-Bis-(2,3,4,5-tetra-*O*-benzyl-α-D-mannopyranosyl)-2-*O*-(4-methoxybenzyl)glycerol 20

To a solution of **19** (137 mg, 0.127 mmol) and benzyl bromide (76 μL, 0.636 mmol) in DMF (1 mL) stirred in an ice-water bath was added sodium hydride (60%, 20 mg, 0.50 mmol). The cooling bath was removed and after 4 h the reaction was complete (TLC; 1:2 EtOAc–petroleum ether). The reaction mixture was diluted with diethyl ether (50 mL) and washed with water (3 × 30 mL), dried ($MgSO_4$) and concentrated. Flash chromatography on silica gel using 1:3 EtOAc–petroleum ether gave the title compound **20** (140 mg, 88%) as a colourless foam. R_f 0.36 (1:2 EtOAc–petroleum ether); $[\alpha]_D^{20}$ +27 (*c* 1.4, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.41–3.50 (m, 2H), 3.59–3.80 (m, 14H) 3.85–3.90 (m, 2H), 3.97–4.04 (m, 2H), 4.42–4.54 (m, 6H), 4.57–4.74 (m, 10H), 4.84–4.91 (m, 4H), 6.71–6.76 (m, 2H), 7.13–7.38 (m, 42H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 55.1, 66.8, 67.5, 69.1, 69.2, 71.9, 72.0, 72.09, 72.12, 72.2, 72.5, 72.6, 73.2, 73.3, 74.82, 74.84, 74.88, 74.94, 75.0, 76.4, 80.01, 80.02, 98.0, 98.3, 113.7, 127.31, 127.34, 127.40, 127.44, 127.5, 127.56, 127.61, 127.62, 127.64, 127.8, 127.9, 128.16, 128.18, 128.20, 128.22, 128.24, 128.3, 129.3, 130.3, 138.3, 138.36, 138.43, 138.46, 138.48, 138.6, 159.1; HRMS-ESI (+ve) found *m*/*z* 1279.5748 $[M+Na]^+$; $C_{79}H_{84}O_{14}Na^+$ calcd 1279.5759.

4.2.11. 1,3-Bis-(2,3,4,5-tetra-*O*-benzyl-α-D-mannopyranosyl)-glycerol 10

To a rapidly stirred mixture of **20** (117 mg, 93 μmol) in CH_2Cl_2 (3.6 mL) and water (0.4 mL) was added DDQ (25 mg, 0.11 μmol) at ambient T. After 1.5 h trace **20** remained (TLC; 1:2 EtOAc–petroleum ether) and the mixture was diluted with diethyl ether (50 mL) and washed with $NaHCO_3$ (4 × 40 mL) until the aqueous phase was only a pale yellow colour and the combined organic portions were dried ($MgSO_4$) and concentrated. Flash chromatography on silica gel using 7:20 EtOAc–petroleum ether as eluant gave **10** (89 mg, 84%) as a colourless foam. R_f 0.16 (1:2 EtOAc–petroleum ether); Spectroscopic data for **10** prepared in this manner matched well the data for **10** prepared by the alternative route described above.

4.2.12. 3,4,5-Tri-*O*-benzyl-2-*O*-methoxyacetyl-α-D-mannopyranosoyl trichloroacetimidate 21

4.2.12.1. 3,4,5-Tri-*O*-benzyl-1,2-di-*O*-methoxyacetyl-β-D-mannopyranoside. To a solution of 3,4,5-tri-*O*-benzyl-α-D-mannopyranose (3.29 g, 7.29 mmol) in CH_2Cl_2 (11 mL) cooled in an ice/water bath was added pyridine (2.0 mL, 25 mmol) followed by careful addition of methoxyacetyl chloride (1.67 mL, 18 mmol). The reaction was allowed to warm to ambient temperature over 18 h. The mixture was diluted with CH_2Cl_2 (100 mL) and washed with dil. HCl (3 × 50 mL), satd. $NaHCO_3$ (50 mL) and brine (50 mL). The organic phase was dried ($MgSO_4$) and concentrated. Flash chromatography on silica gel using 1:2–2:3 EtOAc–petroleum ether as eluant gave clean samples of each 3,4,5-tri-*O*-benzyl-1,2-di-*O*-methoxyacetyl-α-D-mannopyranoside (2.75 g, 63%) and 3,4,5-tri-*O*-benzyl-1,2-di-*O*-methoxyacetyl-β-D-mannopyranoside (0.39 g, 9%) and a sample as a mixture of anomers (0.93 g, 21%) all as colourless oils. Data for the α-anomer: R_f 0.23 (1:2 EtOAc–petroleum ether); $[\alpha]_D^{20}$ +33 (*c* 1.12, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.41 (s, 3H, CH_3), 3.42 (s, 3H, CH_3), 3.68 (dd, 1H, *J* = 1.6, 10.9 Hz), 3.77–3.85 (m, 2H), 3.91–3.99 (m, 2H), 4.02 (s, 2H, $CH_2(OCH_3)$), 4.13 (s, 2H, $CH_2(OCH_3)$), 4.48–4.59 (m, 3H), 4.65 (d, 1H, *J* = 12.1 Hz), 4.73 (d, 1H, *J* = 11.1 Hz), 4.84 (d, 1H, *J* = 10.6 Hz), 5.45 (dd, 1H, *J* = 2.4, 2.4 Hz, 2-H), 6.22 (d, 1H, *J* = 1.9 Hz, 1-H), 7.15–7.19 (m, 2H), 7.23–7.35 (m, 13 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 59.4, 59.5, 67.9, 68.4, 69.4, 69.5, 72.1, 73.56, 73.60, 74.1, 75.4, 77.4, 91.5 ($^1J_{CH}$ = 177 Hz), 127.7, 127.79, 127.82, 127.95, 127.98, 128.1, 128.36, 128.40, 128.5, 137.5, 138.0, 138.1, 167.9, 169.6; HRMS-ESI (+ve) found *m*/*z* 617.2360 $[M+Na]^+$; $C_{33}H_{38}O_{10}Na^+$ calcd 617.2363. Data for the β-anomer: R_f 0.15 (1:2 EtOAc–petroleum ether); $[\alpha]_D^{20}$ −16 (*c* 0.50, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.44 (s, 6H, 2 × CH_3), 3.59 (ddd, 1H, *J* = 2.1, 4.1, 9.7 Hz, 5-H), 3.72–3.80 (m, 3H), 3.86 (dd, 1H, *J* = 9.5, 9.5 Hz, 4-H), 4.02 (d, 1H, *J* = 16.9 Hz), 4.08 (d, 1H, *J* = 16.9 Hz), 4.15 (d, 1H, *J* = 16.7 Hz), 4.21 (d, 1H, *J* = 16.7 Hz), 4.47–4.55 (m, 3H), 4.64 (d, 1H, *J* = 12.1 Hz), 4.71 (d, 1H, *J* = 11.2 Hz), 4.84 (d, 1H, *J* = 10.8 Hz), 5.66 (dd, 1H, *J* = 0.8, 3.1 Hz, 2-H), 5.85 (d, 1H, *J* = 0.8 Hz, 1-H), 7.13–7.17 (m, 2H), 7.23–7.34 (m, 13H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 59.3, 59.4, 67.9, 68.4, 69.3, 69.5, 71.9, 73.5, 73.6, 75.2, 76.3, 79.6, 91.3 ($^1J_{CH}$ = 162 Hz), 127.7, 127.8, 127.9, 128.0, 128.1, 128.4, 128.5, 137.2, 138.0, 168.3, 170.1; HRMS-ESI (+ve) found *m*/*z* 617.2363 $[M+Na]^+$; $C_{33}H_{38}O_{10}Na^+$ calcd 617.2363.

4.2.12.2. 3,4,5-Tri-*O*-benzyl-2-*O*-methoxyacetyl-D-mannopyranose. To a solution of 3,4,5-tri-*O*-benzyl-1,2-di-*O*-methoxyacetyl-α-D-mannopyranoside (1.22 g, 2.11 mmol) in THF (12 mL) was added methanolic ammonia (7 M, 0.84 mL, 5.9 mmol). The reaction was monitored by TLC (1:1 EtOAc–petroleum ether) and at 80 min was diluted with toluene (20 mL) and concentrated under reduced pressure. Flash chromatography on silica gel using 1:2–2:3 EtOAc–petroleum ether gave 3,4,5-tri-*O*-benzyl-2-*O*-methoxyacetyl-D-mannopyranose (0.967 g, 88%) as a 9:1 mixture of α- and β-anomers as a colourless oil. R_f (α-anomer) 0.35, R_f (β-anomer) 0.24 (1:1 EtOAc–petroleum ether); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.41 (s, 2.7H), 3.42 (s, 0.3H), 3.44–3.49 (m, 0.1H), 3.53 (d, 0.9H, *J* = 3.9 Hz, OH), 3.60 (dd, 0.1H, *J* = 3.1, 9.2 Hz), 3.63–3.74 (m, 3H), 3.96 (d, 0.1H, *J* = 7.8 Hz, OH) 4.01–4.08 (m, 1.8H), 4.09–4.11 (m, 1.8H), 4.13 (d, 0.1H, *J* = 16.6 Hz), 4.20 (d, 0.1H, *J* = 16.6 Hz), 4.44–4.61 (m, 4H), 4.67–4.73 (m, 1H), 4.73–4.76 (m, 0.1H, β-1-H), 4.80–4.86 (m, 1H), 5.21 (dd, 0.9H, *J* = 1.8, 3.8 Hz, α-1-H), 5.44 (dd, 0.9H, *J* = 1.9, 3.2 Hz, α-2-H), 5.52 (dd, 0.1H, *J* = 1.0, 3.1 Hz, β-2-H), 7.13–7.17 (m, 2H), 7.24–7.35 (m, 13H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 59.3, 69.0, 69.3, 69.5, 69.6, 69.7, 71.2, 71.8, 71.9, 73.5, 73.6, 73.9, 74.5, 75.1, 75.2, 77.3, 77.6, 80.2, 92.3, 93.0, 127.69, 127.75, 127.8, 127.91, 127.92, 127.95, 128.00, 128.04, 128.1, 128.2, 128.3, 128.39, 128.41, 128.5, 137.5, 137.86, 137.89, 138.1, 138.2, 169.9, 170.3; HRMS-ESI (+ve) found *m*/*z* 545.2144 $[M+Na]^+$; $C_{30}H_{34}O_8Na^+$ calcd 545.2151.

4.2.12.3. 3,4,5-Tri-*O*-benzyl-2-di-*O*-methoxyacetyl-α-D-mannopyranosyl trichloroacetimidate 21. To a solution of 3,4,5-tri-*O*-benzyl-2-*O*-methoxyacetyl-D-mannopyranose (897 mg, 1.72 mmol) and trichloroacetonitrile (0.86 mL, 8.6 mmol) in CH_2Cl_2 (5 mL) being stirred at 0 °C was added DBU (26 μL, 0.17 mmol). The reaction was monitored for loss of the starting pyranose by TLC (1:2 EtOAc–petroleum ether) and after 15 min the reaction mixture was applied directly to a flash chromatography silica column and eluted with 2:8 EtOAc–petroleum ether to give **21** (1.08 g, 94%) as approx 95:5 mixture of α-/β-anomers as a colourless oil. R_f 0.18 (1:4 EtOAc–petroleum ether); ^{1}H NMR (500 MHz, $CDCl_3$) δ *inter alia* 3.42 (s, 3H), 3.69–3.73 (m, 1H), 3.81–3.85 (m, 1H), 3.96–4.07 (m, 3H), 4.15 (s, 2H), 4.50 (d, 1H, *J* = 12.0 Hz), 4.54 (d, 1H, *J* = 10.7 Hz), 4.59 (d, 1H, *J* = 11.3 Hz), 4.65 (d, 1H, *J* = 12.1 Hz), 4.73 (d, 1H, d, *J* = 11.3 Hz), 4.86 (d, 1H, *J* = 10.7 Hz), 5.58 (dd, 1H, *J* = 2.1, 3.0 Hz, 2-H), 6.31 (d, 1H, *J* = 2.0 Hz, 1-H), 7.17–7.20 (m, 2H), 7.24–7.35 (m, 13H), 8.70 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 59.3, 67.7, 68.3, 69.5, 72.1, 73.4, 73.6, 74.3, 75.4, 90.7, 95.1, 127.6, 127.7, 127.8, 127.9, 128.0, 128.27, 128.32, 128.4, 128.5, 137.3, 138.0, 138.1, 159.9, 169.5; HRMS-ESI (+ve) found *m*/*z* 688.1253 $[M+Na]^+$; $C_{32}H_{34}NO_8Cl_3Na^+$ calcd 688.1248.

4.2.13. 1,3-Bis-(3,4,5-tri-*O*-benzyl-2-*O*-methoxyacetyl-α-D-mannopyranosyl)-2-*O*-(4-methoxybenzyl)glycerol 22

A mixture of glycerol **14** (21 mg, 0.099 mmol), trichloroacetimidate donor **21** (165 mg, 0.247 mmol) and powdered 4 Å molecular sieves (approx 0.5 g) in dry CH_2Cl_2 (1 mL) and dry toluene (2.5 mL)

was stirred for 1 h at ambient temperature before being cooled in an ice-water bath. Trimethylsilyl trifluoromethanesulfonate (3.6 μL, 0.020 mmol) was added and the reaction was monitored for completion by TLC (3:7 EtOAc–petroleum ether). After 15 min at 0 °C NEt_3 (50 μL, 0.36 mmol) was added and the crude reaction mixture was filtered, concentrated and purified by silica gel chromatography (1:3–45:55 EtOAc–petroleum ether) to provide the title compound **22** (95 mg, 79%). R_f 0.26 (1:2 EtOAc–petroleum ether); $[\alpha]_D^{20}$ +31 (*c* 0.91, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.395 and 3.398 (2 × unresolved s, 2 × 3H, 2 × CH_2OCH_3), 3.47–3.56 (m, 2H), 3.62–3.89 (m, 14H), 3.93–3.98 (m, 2H), 4.10 (s, 4H, 2 × C(O)CH_2), 4.41–4.56 (m, 8H, 8 × ArC*H*), 4.61–4.65 (m, 2H, 2 × ArC*H*), 4.68–4.72 (m, 2H, 2 × ArC*H*), 4.81–4.86 (m, 3H, 1-H′ and 2 × ArC*H*), 4.88 (d, 1H, *J* = 1.7 Hz, 1-H″), 5.44–5.47 (m, 2H, 2-H′ and 2-H″), 6.78–6.80 (m, 2H, Ar), 7.12–7.35 (m, 32H, Ar). ^{13}C NMR (125 MHz, $CDCl_3$) δ 55.1, 59.2, 67.1, 67.2, 68.6, 68.8, 68.9, 69.5, 71.4, 71.5, 71.8, 71.9, 72.1, 73.26, 73.33, 74.1, 75.0, 75.9, 77.9, 97.6 ($^1J_{C-H}$ = 171 Hz), 97.9 ($^1J_{C-H}$ = 171 Hz), 113.7, 127.5, 127.6, 127.7, 128.0, 128.2, 128.3, 129.5, 130.0, 137.7, 138.1, 138.26, 138.34, 159.2, 169.7; HRMS-ESI (+ve) found *m*/*z* 1243.5236 $[M+Na]^+$; $C_{71}H_{80}O_{18}Na^+$ calcd 1243.5242.

4.2.14. 1,3-Bis-(3,4,5-tri-*O*-benzyl-2-*O*-methoxycetyl-α-D-mannopyranosyl)glycerol 23

To a stirred mixture of **22** (188 mg, 0.154 mmol) in CH_2Cl_2 (3.6 mL) and water (0.4 mL) was added DDQ (52 mg, 0.231 mmol). After 2.25 h TLC (2:3 EtOAc–petroleum ether) indicated the reaction was complete. The mixture was diluted with diethyl ether (100 mL) and washed $NaHCO_3$ (4 × 20 mL) until the aqueous phase was almost colourless. The organic phase was dried ($MgSO_4$) and concentrated and the residue purified by silica gel chromatography (2:3 to 1:1 EtOAc–petroleum ether) to afford the title compound **23** (138 mg, 81%). R_f 0.26 (1:1 EtOAc–petroleum ether); $[\alpha]_D^{20}$ +27 (*c* 1.4, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.40 (s, 6H, 2 × CH_3), 3.52 (dd, 1H, *J* = 6.6, 10.6 Hz), 3.57 (dd, 1H, *J* = 4.3, 10.7 Hz), 3.63 (dd, 1H *J* = 6.1, 10.7 Hz), 3.65–3.71 (m, 3H), 3.71–3.77 (m, 2H), 3.77–3.85 (m, 4H), 3.95–3.98 (m, 3H), 4.11 (s, 4H, 2 × C(O)CH_2), 4.45–4.50 (m, 4H, 4 × ArC*H*), 4.51–4.56 (m, 2H, 2 × ArC*H*), 4.61–4.65 (m, 2H, 2 × ArC*H*), 4.68–4.72 (m, 2H, 2 × ArC*H*), 4.80–4.85 (m, 2H, 2 × ArC*H*), 4.86–4.89 (m, 2H, 1-H′ and 1-H″), 5.45–5.48 (m, 2H, 2-H′ and 2-H″), 7.12–7.17 (m, 4H, Ar), 7.21–7.34 (m, 26H, Ar); ^{13}C NMR δ 59.3, 68.8, 69.01, 69.04, 69.2, 69.6, 69.7, 69.9, 71.8, 72.03, 72.05, 73.5, 74.2, 75.2, 78.0, 98.2 (1JCH = 171 Hz), 98.3 ($^1J_{CH}$ = 171 Hz), 127.7, 127.8, 127.9, 128.1, 128.3, 128.4, 137.8, 138.1, 138.2, 169.8; HRMS-ESI (+ve) found *m*/*z* 1123.4664 $[M+Na]^+$; $C_{63}H_{72}O_{17}Na^+$ calcd 1123.4667.

4.2.15. 2-*O*-(Benzyloxy-1,2-di-*O*-stearoyl-*sn*-glycero-3-phosphoryl)-1,3-bis-(3,4,5-tri-*O*-benzyl-2-*O*-methoxycetyl-α-D-mannopyranosyl)glycerol 25

After being dried separately under high vacuum for 2 h alcohol **23** (138 mg, 0.125 mmol) and phosphoramidite **24**[33] (189 mg, s 0.219 mmol) were dissolved together in dry CH_2Cl_2 (6 mL) and cooled in an ice-water bath before adding 4,5-dicyanoimidazole (31 mg, 0.263 mmol). The cooling bath was removed and the initial suspension became homogenous over 15 min. The reaction was monitored for loss of **23** by TLC (2:3 EtOAc–petroleum ether) and after 2 h *m*CPBA (60%, 90 mg as a solution in CH_2Cl_2 and dried over $MgSO_4$, 0.31 mmol) was added over a period of 1 min. After stirring for 30 min the mixture was concentrated under vacuum to half volume, diluted with diethyl ether (150 mL) and washed with 10% sodium thiosulfate solution (100 mL). The aqueous phase was back-extracted with diethyl ether (50 mL) and the combined organic portions were washed with $NaHCO_3$ (3 × 100 mL), brine (1 × 30 mL), dried ($MgSO_4$) and concentrated. Purification using flash silica gel chromatography (35:65–55:45 EtOAc–petroleum ether) afforded **25** (194 mg, 82%). R_f 0.36 (1:3 EtOAc–petroleum ether); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.82–0.82 (m, 6H), 1.09–1.42 (m, 56H), 1.49–1.61 (m, 4H), 2.18–2.27 (m, 4H), 3.37–3.39 (m, 6H), 3.57–3.71 (m, 4H), 3.72–3.90 (m, 8H), 3.90–3.98 (m, 2H), 4.00–4.16 (m, 6H), 4.16–4.27 (m, 2H), 4.39–4.54 (m, 6H), 4.57–4.66 (m, 4H), 4.66–4.71 (m, 1H), 4.77–4.84 (m, 2H), 4.84–4.92 (m, 2H), 5.02–5.09 (m, 2H), 5.09–5.18 (m, 1H), 5.40–5.49 (m, 2H), 7.05–7.17 (m, 4H), 7.18–7.37 (m, 31H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.0, 22.6, 24.7, 29.98, 29.02, 29.20, 29.24, 29.4, 29.6, 31.8, 33.9, 34.0, 59.2, 61.6, 65.5, 66.4, 66.6, 68.6, 68.7, 69.25, 69.32, 69.4, 71.8, 71.9, 73.3, 73.9, 75.1, 75.4, 78.0, 97.8, 98.1, 127.5, 127.6, 127.7, 127.97, 128.01, 128.15, 128.20, 128.5, 135.5, 137.7, 138.1, 138.2, 169.7, 172.6, 173.0; ^{31}P NMR (202 MHz, $CDCl_3$) δ −1.5, −1.4; HRMS-ESI (+ve) found *m*/*z* 1900.0389 $[M+Na]^+$; $C_{109}H_{153}O_{24}PNa^+$ calcd 1900.0387.

4.2.16. 2-*O*-(1,2-Di-*O*-stearoyl-*sn*-glycero-3-phosphoryl)-1,3-bis-(2-*O*-methoxycetyl-α-D-mannopyranosyl)glycerol 26

A mixture of **25** (185 mg, 98 μmol) and $Pd(OH)_2$ (20% on C, 125 mg) in 2:3 THF–MeOH (10 mL) was stirred under an atmosphere of H_2 at ambient temperature for 4 h, filtered and concentrated. Flash chromatography on silica gel, using 1:9–2:3 MeOH–$CHCl_3$ gave **26** as a colourless powder (109 mg, 89%). R_f 0.44 (2:1:0.17 $CHCl_3$–MeOH–H_2O); $[\alpha]_D^{20}$ +24 (*c* 0.6, 2:1 $CHCl_3$–MeOH); ^{1}H NMR (500 MHz, 2:1 $CDCl_3$–CD_3OD) δ 0.86–0.92 (m, 6H), 1.22–1.37 (m, 56H), 1.55–1.67 (m, 4H), 2.28–2.368 (m, 4H), 3.46 (s, 6H, 2 × OCH_3), 3.52–3.60 (m, 2H), 3.62–3.68 (m, 2H), 3.68–3.80 (m, 4H), 3.86–4.05 (m, 8H), 4.08–4.23 (m, 5H), 4.40–4.47 (m, 2H), 4.84 (br s, 1H, 1-H′), 4.86 (d, 1H, *J* = 1.2 Hz, 1-H″), 5.12–5.18 (m, 2H), 5.22–5.29 (m, 1H, 2-H); ^{13}C NMR 125 MHz, 2:1 $CDCl_3$–CD_3OD) δ 13.8, 22.5, 24.76, 24.82, 29.0, 29.2, 29.5, 29.6, 31.8, 34.0, 34.1, 59.0, 61.9, 62.1, 62.7, 63.4, 66.8, 66.9, 68.1, 68.3, 69.3, 70.5, 72.6, 73.0, 73.1, 97.3, 170.3, 170.4, 173.7, 174.0; ^{31}P NMR (202 MHz, 2:1 $CDCl_3$–CD_3OD) δ −0.8; HRMS-ESI (−ve) found *m*/*z* 1245.7139 $[M-H]^-$; $C_{60}H_{110}O_{24}P^-$ calcd 1245.7125.

4.2.17. Sodium salt of 2-*O*-(1,2-di-*O*-stearoyl-*sn*-glycero-3-phosphoryl)-1,3-bis-(α-D-mannopyranosyl)glycerol 4

To a solution of **26** (37 mg, 30.9 μmol) in 1:5 CH_2Cl_2–MeOH (6 mL) was added sodium methoxide (0.5 M, 4 μL, 2 μmol) and the reaction mixture was stirred at ambient T. The reaction was monitored by TLC (2:1:0.17 $CHCl_3$–MeOH–H_2O) and at 2 h a further portion of sodium methoxide was added (0.5 M, 4 μL, 2 μmol). After a further 45 min the reaction was quenched by the addition of DowexWX8-100 (H^+) resin (approx 0.5 g), filtered and subjected to two sequential treatments with DowexWX8-100 (Na^+) resin (2 × approx 0.5 g). Flash chromatography on silica gel, using 2:1:0–2:1:0.05–2:1:0.1 $CHCl_3$–MeOH–H_2O gave **4** as a colourless powder (28 mg, 84%). R_f 0.30 (2:1:0.17 $CHCl_3$–MeOH–H_2O); $[\alpha]_D^{20}$ +37 (*c* 1.4, 2:1:0.17 $CHCl_3$–MeOH–H_2O); ^{1}H NMR (500 MHz, 2:1:0.17 $CDCl_3$–CD_3OD–D_2O) δ 0.83–0.96 (m, 6H), 1.11–1.44 (m, 56H), 1.54–1.68 (m, 4H), 2.26–2.40 (m, 4H), 3.54–3.71 (m, 6H), 3.71–3.80 (m, 4H), 3.80–3.93 (m, 6H), 3.94–4.02 (m, 2H), 4.17–4.23 (obs m, 1H), 4.36–4.45 (obs m, 2H), 4.84 (s, 1H, 1-H′), 4.87 (s, 1H, 1-H″), 5.22–5.28 (m, 1H, 2-H); ^{13}C NMR (125 MHz, 2:1:0.17 $CDCl_3$–CD_3OD–D_2O) δ 14.2, 23.0, 25.2, 25.3, 29.50, 29.54, 29.68, 29.70, 29.8, 29.91, 29.95, 29.98, 30.03, 30.1, 32.3, 34.5, 34.6, 61.6, 61.7, 63.3, 63.8, 63.9, 66.9, 67.2, 67.55, 67.63, 70.76, 70.81, 70.9, 71.0, 71.38, 71.40, 73.3, 73.4, 73.5, 100.57, 100.64, 174.4, 174.7; ^{31}P NMR (202 MHz, 2:1:0.17 $CDCl_3$–CD_3OD–D_2O) δ −0.8; HRMS-ESI (−ve) found *m*/*z* 1101.6700 $[M-H]^-$; $C_{54}H_{102}O_{20}P^-$ calcd 1101.6702; HPLC purity 96.0.

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Supplementary data

Supplementary data (^{1}H and ^{13}C NMR spectra for compounds **6**, **7**, **10**, **13**, **18**, **19**, **20**, **21**, **23** and **25** as well as those of the sodium and triethylammonium salt of the final compound **4** are provided. The HPLC chromatogram of **4** is also provided) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.11.058. These data include MOL files and InChiKeys of the most important compounds described in this article.

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