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Phosphatidylinositol mannosides: Synthesis and suppression of allergic airway disease

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Abstract—Phosphatidylinositol mannoside (PIM) extracts from mycobacteria have been shown previously to suppress allergic airway inflammation in mice. To help determine the structural requirements for activity, $PIM1_2$ (**1**), $PIM1_6$ (**2**) and PIM2 (**3**) were synthesized and tested for their ability to suppress cellular inflammation in a mouse model of allergic asthma. The synthetic PIMs were all effective in suppressing airway eosinophilia in the asthma model, with $PIM1_6$ being the most effective. Suppression of all inflammatory cells monitored was observed, indicating a general blockade of cellular inflammation. Non-mannosylated phosphatidylinositol (PI) had no suppressive effect, indicating that at least one α-D-mannopyranosyl residue is necessary for activity. The suppressive effect of the three PIM compounds indicates that other members of this set may be of value in treatment of a range of diseases driven by infiltration of inflammatory cells.

1. Introduction

Allergic asthma is increasing in prevalence and severity worldwide.[1] The specific reasons for these increases are unclear, however a variety of contributing factors have been proposed, including a lower incidence of exposure to infectious agents in industrialised nations.[2] It has been postulated that exposure to various bacteria early in childhood moderates the body's response to allergens and lowers the likelihood of the development of allergies later in life.[3–5] Mycobacteria have been identified as organisms that act in this way[6–8] by inducing complex immune responses. These responses have been attributed to the diverse chemical features of the organism that include unmethylated copies of DNA[9] that are released, component proteins such as tuberculin purified protein derivative (PPD)[10] and cell wall constituents.[11] Many studies have been undertaken to determine the potential for exploiting mycobacteria and mycobacterial products in the treatment of immune-mediated diseases ranging from allergies to tuberculosis and cancer.[12–14]

Members of our team have demonstrated that lipoarabinomannan (LAM) and phosphatidylinositol mannan (PIM), glycolipids isolated from the cell wall of *Mycobacterium bovis*, can suppress the infiltration of inflammatory cells into the lung in a mouse model of allergic airway disease.[15] The suppression by the PIM-containing extracts was shown to be associated with the production of the regulatory cytokine IL-10, indicating that the extracts trigger an 'anti-inflammatory' response in vivo. The suppressive effect was shown to be dependent on the presence of the acyl chains and, in the case of LAM, mannose capping on the glycolipids, identifying these moieties as suitable targets for structure–activity relationship studies.

The compositions of the LAM and PIM components of the cell walls vary between different species of Mycobacteria,[16–20] the variability being observed in both the structures of the fatty acid chains and the number of mannose substituents in the PIM compounds. Separation of the different PIM compounds present in cell wall extracts can be technically challenging, however their relatively small molecular sizes

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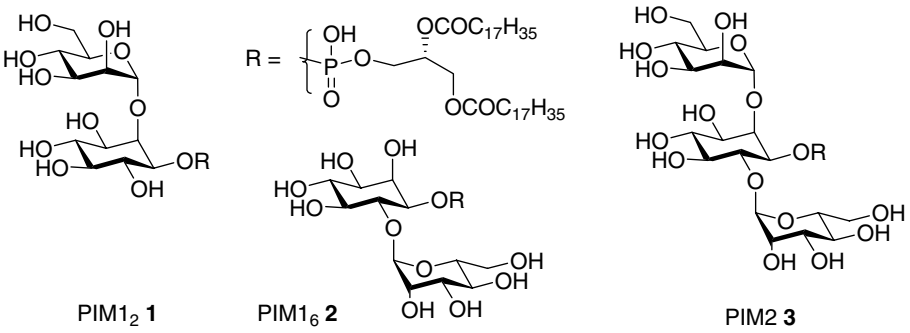


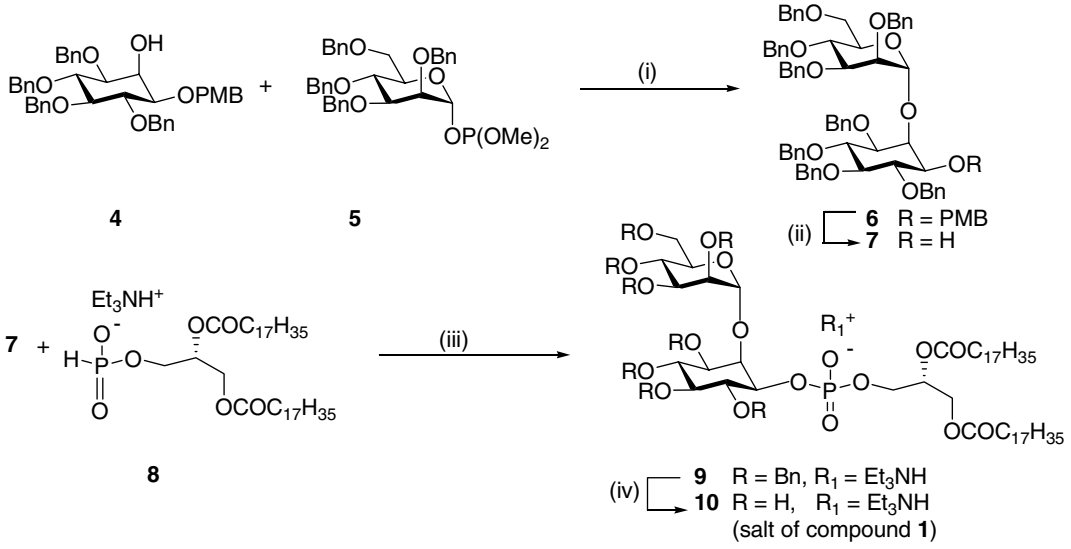
make them amenable to syntheses. To help determine the structural requirements of PIM compounds for anti-inflammatory responses in vivo, we have undertaken the chemical syntheses of PIM compounds PIM1$_2$ (**1**), PIM1$_6$ (**2**) and PIM2 (**3**) and tested them for inhibitory properties in a mouse model of allergic airway inflammation.

The key difference between previously synthesized PIM1$_2$ derivatives and the PIM1$_2$ now described lies in the composition of the acyl group of the phosphatidyl unit. Previous PIM1$_2$ derivatives have incorporated di-*O*-palmitoyl[21] or myristoyl[22–24] acyl groups, whereas we have synthesized a stearoyl PIM1$_2$ derivative, as the stearoyl acyl group has been identified in active PIM extracts.[15] We also describe a versatile synthesis of PIM1$_6$ and PIM2 via a chemoselective mannosylation and dimannosylation of inositol acceptor **11**, respectively. Although various acylated-PIM2 compounds, mannosylated at O-2 and O-6, have been prepared by the groups of Van Boom,[25] Watanabe[26,27] and Fraser-Reid,[28] to our knowledge this is the first report on the synthesis of a PIM1$_6$ entity.

2. Results and discussion

2.1. Synthesis of PIM1$_2$ (1)

The known 4-methoxybenzyl ether **4** was made by selective monoetherification of the previously described tetrabenzyldiol by the procedures of Martin and Wagman.[29] Mannosylation of the C-2 hydroxyl group of product **4** was achieved by reaction with mannosyl phosphite **5**,[26,27] promoted by trimethylsilyl trifluoromethanesulfonate (TMSOTf), to give an inseparable mixture of α-mannoside (**6**) and its β-anomer in 83% combined yield. After removal of the *p*-methoxybenzyl-protecting group with ceric ammonium nitrate (CAN), 2-*O*-mannosyl-*myo*-inositols α-**7** and its β-anomer were isolated in 51% and 15% yields, respectively, and a mixed fraction containing these two glycosides (21%, in similar proportions) was also obtained (Scheme 1). The specific optical rotation of product α-**7** {$[\alpha]_D^{18}$ +15.3 (*c* 1.5, CH_2Cl_2)} compared well with that reported by Elie et al. {$[\alpha]_D$ +16 (*c* 1, $CHCl_3$)},[22] and ^{1}H and ^{13}C NMR data were consistent with those reported in the literature.[22]



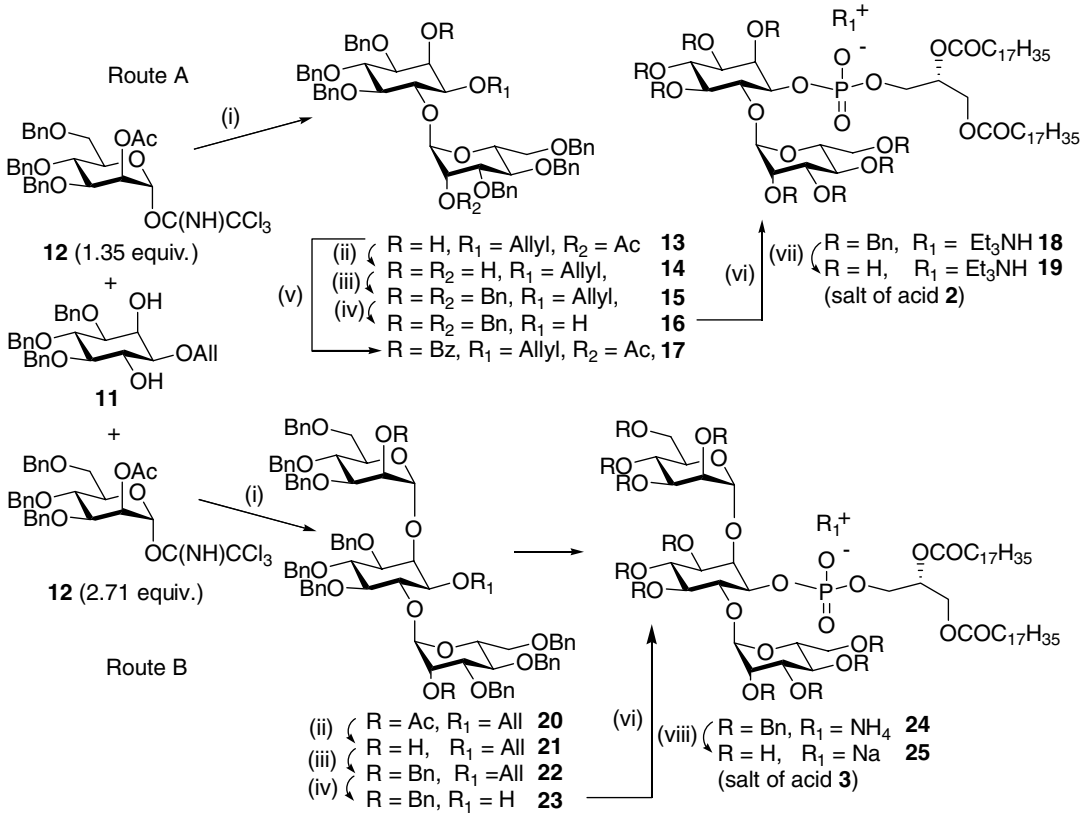
Scheme 1. Reagents: (i) TMSOTf, MS-4A, Et_2O (83%); (ii) CAN, MeCN–H_2O (9:1), (51%); (iii) ^{t}BuCOCl, py, then I_2 in py-H_2O (88%); (iv) H_2, Pd/C, EtOAc–THF–EtOH–H_2O (86%).

The installation of the phospholipid moiety was achieved using the *H*-phosphonate method[30] which involved coupling of alcohol α-**7** with phosphonate **8** in the presence of pivaloyl chloride as an activator. The disubstituted hydrogen phosphonate produced was converted, by in situ oxidation with iodine in wet pyridine, to the corresponding *O*-protected phosphate diester of $PIM1_2$ which was isolated as the triethylammonium salt (**9**) in 88% yield (Scheme 1). Hydrogenolysis of the benzyl-protecting groups over palladium gave the triethylammonium salt (**10**) of $PIM1_2$ (**1**) as a white solid after lyophilisation in 86% yield (Scheme 1). High resolution mass spectrometry established the molecular formula of the anion as $C_{51}H_{96}O_{18}P$. The ^1H NMR spectrum showed all the relevant resonances with the appropriate intensities. In particular, the *sn*-2 proton was evident as a broad multiplet at δ 5.13–5.06 ppm. The anomeric proton signal resonated as a doublet ($J_{1',2'}$ = 1.5 Hz) at δ 4.96 ppm. The ^{13}C NMR spectrum was also consistent with the structure of salt **10**, with the main features being the carbonyl signals at δ 173.7 and 174.0 ppm, the anomeric carbon signal at δ 101.4 and the three oxygenated methylene carbon resonances evident at δ 63.5, 62.8 and 61.3 ppm. A singlet in the ^{31}P NMR spectrum was observed at δ 0.08 ppm.

2.2. Synthesis of $PIM1_6$ (2)

The 6-substituted inositol derivative $PIM1_6$ (**2**) was synthesized using the strategy shown in Scheme 2, Route A. Selective mannosylation of diol **11**, which is readily accessible and has been used previously for the synthesis of PIM derivatives,[28] with the α-directing 2-*O*-acetyl mannosyl donor **12**[31,32] (molar ratio 1:1.35), gave 6-mannoside α-**13** in 50% yield, along with 17% of the dimannoside **20** (see Route B). The positioning of the mannosylation in the major product **13** was assigned by *O*-benzoylation of the free hydroxyl to give compound **17** (65%). The expected downfield shift (5.99 ppm) of the characteristic (br t, J = 2.7 Hz) inositol H-2 proton was evident. Consequently glycosylation of diol **11** occurred preferentially at the equatorial C-6 hydroxyl group. The α-anomeric configuration of glycoside **13** was established from the C-1 heteronuclear one-bond ^{13}C–^1H coupling constant of 175.5 Hz.[33,34]

Compound **13** was then transformed into alcohol **16** by standard deacetylation, O-benzylation and deallylation via compounds **14** and **15**. Introduction of the glycerophosphate moiety and debenzylation, as illustrated in Scheme 1, then led in sequence to the *O*-protected salt **18** and then the triethylammonium salt (**19**) of the required product (**2**). High-resolution mass spectrometry established the molecular formula of the anion as $C_{51}H_{96}O_{18}P$ and the NMR data were consistent with the structure of **19**. The *sn*-2 proton signal was observed as a broad multiplet at δ 5.10–5.02 and the anomeric proton signal resonated as a doublet ($J_{1',2'}$ = 2.0 Hz) at δ 4.91.



Scheme 2. Reagents: (i) TMSOTf, MS-4A, DCM (50% for **13** and 57% for **20**); (ii) NaOMe, MeOH (95% for **14** and 72% for **21**); (iii) BnBr, NaH, DMF (100% for **15** and 79% for **22**); (iv) $(Ph_2MeP)_2(COD)Ir^+ PF_6^-$ (77% for **16** and 76% for **22**); (v) Py, BzCl (65%); (vi) **8**, ^{t}BuCOCl, py, then I_2 in py-H_2O (84% for **18** and 74% for **24**); (vii) H_2, Pd/C, EtOAc–THF–EtOH–H_2O (72%); (viii) H_2, $Pd(OH)_2$/C, ^{t}BuOH (37%).

2.3. Synthesis of PIM2 (3)

The inositol acceptor **11** was also used for the synthesis of PIM2 (**25**) by dimannosylation with excess donor **12** (2.71 mol equiv) (Scheme 2, Route B). By this method the main product from the glycosylation reaction was the dimannoside **20** (57%). The α-anomeric configurations for compound **20** were established from the C-1 one-bond ^{13}C–^{1}H coupling constants. Dimannoside **20** was readily converted to the known[25] alcohol **23**. The diacyl glycerol moiety was installed as before by use of compound **8**, and the final deprotection was effected by hydrogenolysis over $Pd(OH)_2$ on carbon at 150 psi to afford the sodium salt of PIM2 (**25**). High-resolution mass spectrometry established the molecular formula of the anion $C_{57}H_{106}O_{23}P$. The ^{1}H NMR spectrum showed the appropriate intensities with all the expected signals present. In particular, the *sn*-2 proton was evident at δ 5.3 ppm as were the two anomeric protons at δ 5.14 and 5.11 ppm. The ^{13}C NMR was also consistent, with the main observations being: the carbonyl signals at δ 174.8 and 174.5 ppm, the two anomeric signals at δ 101.92 and 101.89 ppm and the four oxygenated methylene carbons evident at δ 63.9, 63.3, 61.7 and 61.5 ppm. The ^{31}P NMR displayed a single peak at δ −3.8 ppm.

2.4. Suppression of airway eosinophilia

In earlier research, members of our group have shown that PIMs isolated from the cell wall of *M. bovis* significantly suppress the recruitment of eosinophils in an asthma mouse model.[15] Eosinophils are a key inflammatory cell associated with inflammatory diseases and tissue damage including airways remodelling in asthmatics. In the current study, intranasal administration of the triethylammonium salts of $PIM1_2$ (**10**) and $PIM1_6$ (**19**) and the sodium salt of PIM2 (**25**) resulted in a decrease in the number of eosinophils per unit volume in the bronchoalveolar lavage (BAL) fluid compared with the phosphate-buffered saline (PBS) controls (Fig. 1A–C), with $PIM1_6$ appearing to cause the greatest suppressive effect.

Interestingly, there was also a general decrease in both macrophage and lymphocyte cell numbers in the BAL fluid. In the case of all three cell types, eosinophils, macrophages and lymphocytes, the percentage of each cell type in the BAL fluid did not change significantly (Fig. 2A–C). It therefore appears that, like the PIM extracts from mycobacteria,[15] the suppression of lung eosinophilia by the PIM compounds results from overall suppression of cell infiltration rather than a blockade of a specific cell type.

Previous work by Sayers et al.[15] showed that the presence of the acyl chains and mannose capping was important for suppressive activity of the larger lipoglycan extract lipoarabinomannan (LAM). LAM isolated from *Mycobacterium smegmatis*, which lacks mannose caps, failed to suppress airway eosinophilia. Because PIM extracted from *M. smegmatis* contains the D-mannopyranosyl residues, we chose to test

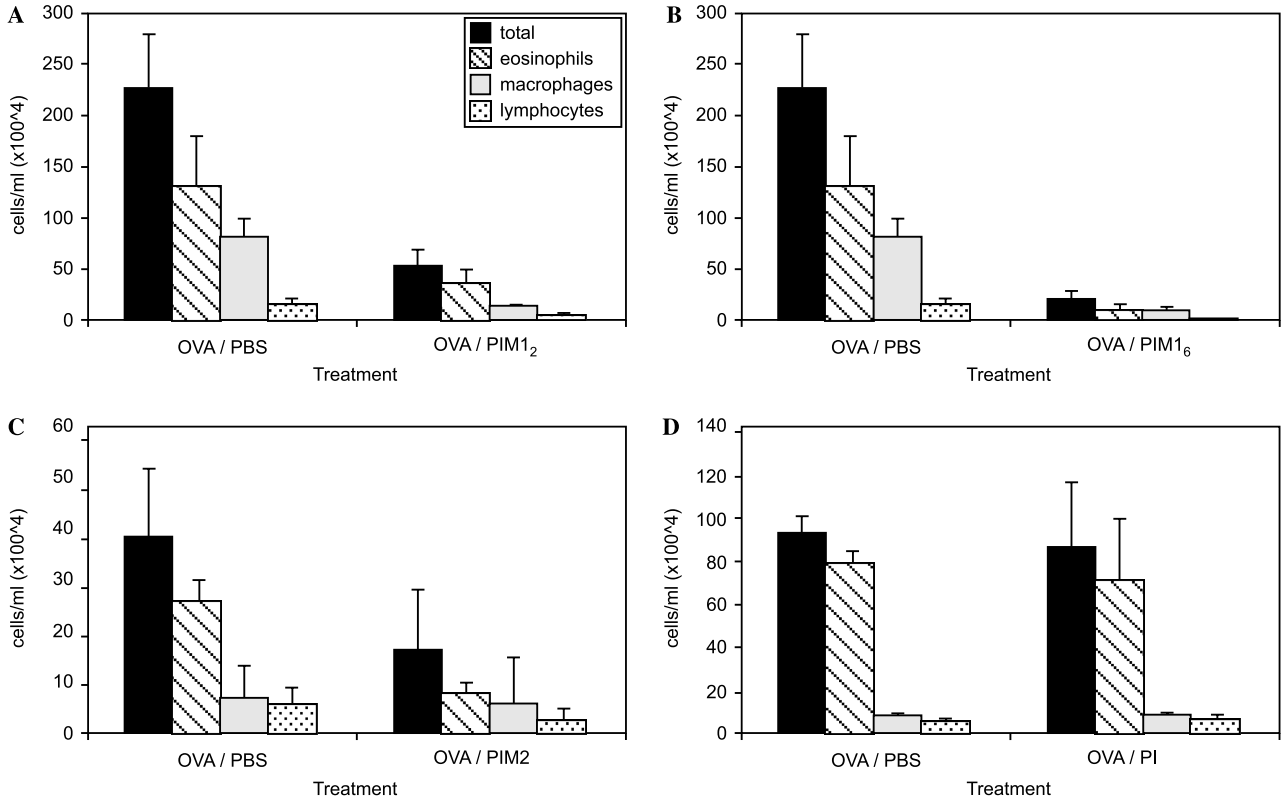


Figure 1. Effects of intranasal administration of PIMs on cell infiltration in a mouse model of allergic airways disease (AAD). Cells per mL in the BAL of ovalbumin-(OVA) challenged mice treated with (A) $PIM1_2$, (B) $PIM1_6$, (C) PIM2 or (D), PI. The bar-graphs each represent results of 2–4 experiments each comprising five mice.

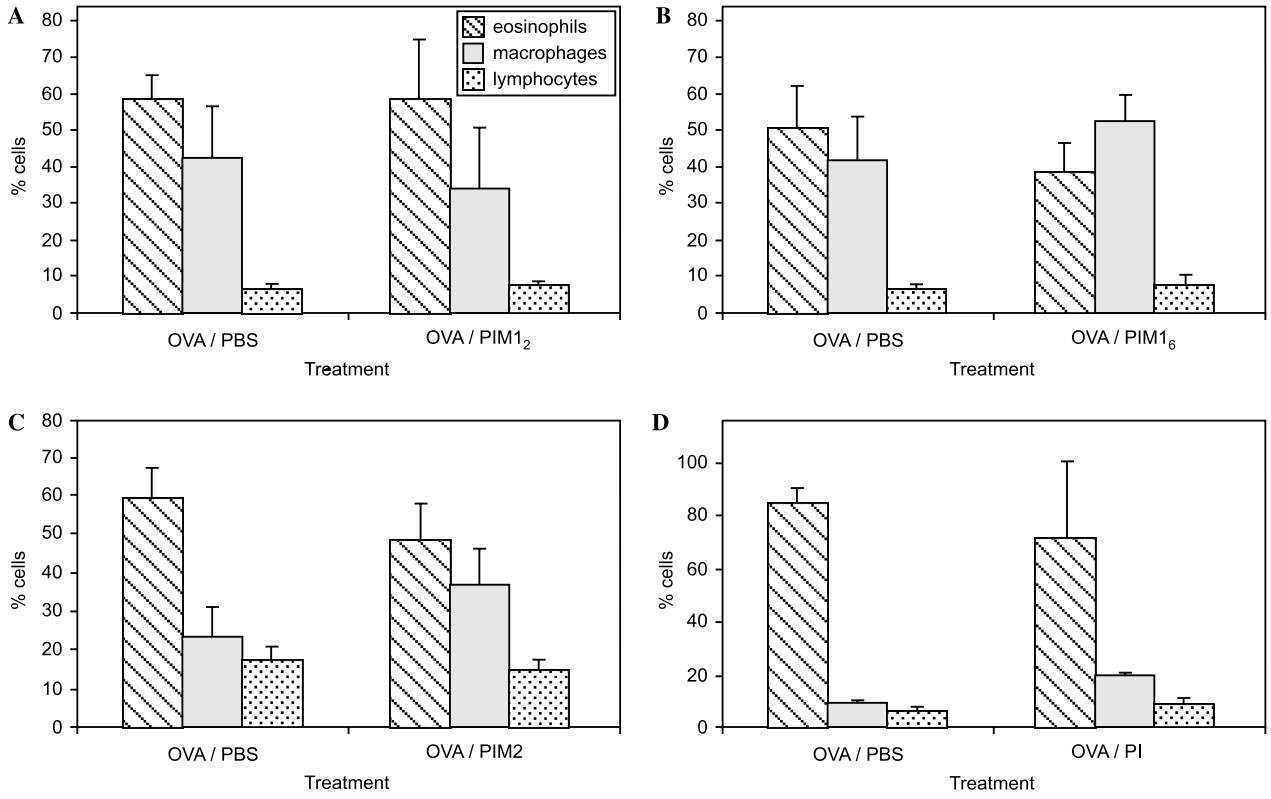


Figure 2. Percentages of different cell types in the BAL fluid of OVA-challenged mice following intranasal administration of (A) $PIM1_2$, (B), $PIM1_6$, (C) PIM2 or (D) PI. Graphs are representative of 2–4 experiments comprising five mice per group.

1,2-diacyl-*sn*-glycero-3-phospho-(1-D-*myo*-inositol) (PI, Aldrich P-5954) in the ovalbumin (OVA) model to determine if the activity of PIMs was dependent on the presence of mannose. As shown in Figures 1D and 2D, PI had no effect on either cell infiltration or cell percentages, respectively, indicating a key role for the mannose sugars in the activity of the synthetic PIM compounds.

3. Conclusions

We have prepared samples of $PIM1_2$ (**10**), $PIM1_6$ (**19**) and PIM2 (**25**), and evaluated their ability to suppress eosinophil recruitment in the lung in an asthma mouse model. The profile of cell type in the BAL fluid suggests that this activity results from an overall suppression of cell infiltration. All three PIMs, **10**, **19** and **25**, possessed this suppressive activity with $PIM1_6$ (**19**) showing the greatest effect. These results suggest that only one α-D-mannopyranosyl residue is required for the observed activity and that the mannosylation site can be at either O-2 or O-6 of the inositol moiety. In addition we have shown that the activity of PIM appears to be dependent on the presence of mannose, however, at this point it is unclear as to whether the type of sugar group is important. We are currently preparing analogues of PIM structures to ascertain the minimal structural requirements for the suppression of recruitment of eosinophils by PIM-based compounds.

4. Experimental procedures for synthesis of PIM analogues

4.1. General

Mps were measured on a Gallenkamp capillary melting point apparatus and are uncorrected. Specific optical rotations, given in 10^{-1} deg cm^2 g^{-1}, were measured at ambient temperature using either a Jasco DIP-370 or DIP-1000 polarimeter with a cell of path length 1.0 dm. ^{1}H NMR spectra were obtained at either 300 or 500 MHz and referenced to the residual solvent peak. Chemical shifts are reported using the δ scale, and coupling constants (J) and separations are reported in Hz. ^{13}C NMR spectra were recorded at 75 or 125 MHz and chemical shifts are reported using the δ scale. FAB mass spectra were recorded on a Kratos MSORF or a VG70-250S double focusing, magnetic sector mass spectrometer under chemical ionization conditions using isobutene, ammonia or xenon as the ionizing gas, or under high-resolution FAB conditions in a glycerol or nitrobenzyl alcohol matrix. Electro-spray ionization (ESI) mass spectra were recorded on a PerSeptive Biosystems Mariner time-of-flight 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 visualized under UV light and/or with a spray (5% w/v dodecamolybdophosphoric acid in EtOH) with subsequent heating. Flash column

chromatography was carried out using Merck Kieselgel 60 (230–400 mesh). All chromatography solvents were of reagent grade. THF was distilled from sodium-benzophenone ketyl under nitrogen and dichloromethane was distilled from phosphorus pentoxide. All other solvents and reagents were purified using the methods described by Perrin et al.[35] Organic solutions were dried over $MgSO_4$.

4.2. 3,4,5,6-Tetra-*O*-benzyl-2-*O*-(2,3,4,6-tetra-*O*-benzyl-D-mannopyranosyl)-1-*O*-*p*-methoxybenzyl-D-*myo*-inositol (6)

3,4,5,6-Tetra-*O*-benzyl-1-*O*-*p*-methoxybenzyl-D-*myo*-inositol (**4**) (200 mg, 0.30 mmol) and mannosyl phosphite (**5**)[26,27] (200 mg, 0.32 mmol) were dissolved in dry ether (10 mL) and molecular sieves 4 Å (100 mg) were added. The mixture was stirred at room temperature under nitrogen for 15 min, TMSOTf (10 μL, 0.06 mmol) was added and the stirring was continued for a further 30 min. Et_3N (1.0 mL) was added, the mixture was diluted with more ether, washed with water and dried. Removal of the solvent and purification of the residue by chromatography over silica (hexane/ether, 8:2) gave the title compound **6** (296 mg, 83%, undetermined mixture of anomers) as a colourless syrup. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36–7.13 (m, 42H), 6.77 (d, J = 8.5 Hz, 2H), 5.43 (d, J = 1.5 Hz, 1H), 4.92–4.76 (m, 8H), 4.62–4.40 (m, 9H), 4.36 (d, J = 12.5 Hz, 1H), 4.13 (br d, J = 10.0 Hz, 1H), 4.07 (t, J = 9.5 Hz, 1H), 3.84 (dd, J = 10.0, 2.5 Hz, 1H), 3.78 (t, J = 9.5 Hz, 1H), 3.74–3.69 (m, 3H), 3.71 (s, 3H), 3.54 (dd, J = 11.5, 3.5 Hz, 1H), 3.42 (t, J = 9.5 Hz, 1H), 3.35 (t, J = 9.5 Hz, 1H), 3.30 (dd, J = 9.0, 2.5 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.4, 138.9, 138.8, 138.6, 138.5, 138.4, 138.3, 130.0, 129.8, 128.47, 128.45, 128.42, 128.39, 128.35, 128.28, 128.26, 128.16, 128.12, 128.09, 128.04, 127.98, 127.94, 127.68, 127.57, 127.50, 127.44, 127.38, 127.28, 113.9, 98.4, 83.5, 81.5, 81.2, 80.9, 79.2, 78.9, 76.2, 75.8, 75.1, 74.8, 74.4, 73.5, 73.2, 72.1, 72.0, 71.8, 71.7, 68.9, 55.3. MS-ESI $(M+H)^+$ calcd for $C_{76}H_{79}O_{12}$: 1183.5572. Found: 1183.5564.

4.3. 3,4,5,6-Tetra-*O*-benzyl-1-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)-D-*myo*-inositol α-(7) and the anomer *β*-(7)

Ceric ammonium nitrate (200 mg, 0.37 mmol) was added to a solution of compound **6** (200 mg, 0.17 mmol) in MeCN–H_2O (9:1, 5 mL) at 0 °C. The mixture was stirred at 0 °C for 5 min and then at 20 °C for 45 min. $NaHCO_3$ solution (satd 10 mL) was added and the mixture was diluted with CH_2Cl_2. The organic layer was washed with water and dried ($MgSO_4$). Removal of the solvent and purification of the residue by chromatography on silica gel (hexanes/ether 8:2 to 6:4) gave the title compound α-**7** (91 mg, 51%) as a colourless syrup. A mixed fraction of α-**7** and *β*-**7** (37 mg, 21%) and a further fraction containing 3,4,5,6-tetra-*O*-benzyl-2-*O*-(tetra-*O*-benzyl-β-D-mannopyranosyl)-D-*myo*-inositol *β*-(**7**) (27 mg, 15%) were obtained as colourless syrups.

Data for α-**7** $[\alpha]_D^{18}$ +15.3 (*c* 1.5, CH_2Cl_2), lit.[22] $[\alpha]_D^{20}$ +16 (*c* 1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40–7.16 (m, 40H), 5.37 (br s, 1H), 4.95 (d, J = 11.0 Hz, 2H), 4.93 (d, J = 11.0 Hz, 1H), 4.87 (d, J = 11.0 Hz, 1H), 4.79 (d, J = 11.0 Hz, 2H), 4.78 (d, J = 12.5 Hz, 1H), 4.71–4.47 (m, 8H), 4.36 (d, J = 12.0 Hz, 1H), 4.33 (br t, J = 2.0 Hz, 1H), 4.11–4.09 (m, 2H), 3.88–3.85 (m, 1H), 3.79 (t, J = 9.5 Hz, 1H), 3.66–3.64 (m, 1H), 3.57 (dd, J = 11, 2.5 Hz, 1H), 3.53 (t, J = 9.5 Hz, 1H), 3.44–3.34 (m, 4H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.8, 138.68, 138.65, 138.55, 138.52, 138.45, 138.2, 128.8, 128.53, 128.50, 128.4, 128.36, 128.32, 128.25, 128.23, 128.19, 128.16, 128.08, 128.07, 128.05, 128.03, 127.99, 127.96, 127.8, 127.64, 127.60, 127.57, 127.48, 127.42, 127.41, 127.3, 98.9, 83.7, 81.8, 80.9, 79.2, 78.9, 75.9, 75.8, 75.4, 75.2, 73.50, 73.47, 72.37, 72.26, 71.96, 71.90, 71.7, 68.9. HRMS-ESI $(M+H)^+$ calcd for $C_{68}H_{71}O_{11}$: 1063.4996. Found: 1063.5017.

Data for β-**7**: $[\alpha]_D^{19}$ −2.1 (*c* 1.0, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.47–7.18 (m, 40H), 4.94–4.82 (m, 9H), 4.78 (d, J = 11.5 Hz, 1H), 4.67 (d, J = 11.0 Hz, 1H), 4.64–4.51 (m, 5H), 4.45 (d, J = 11.5 Hz, 1H), 4.41 (s, 1H), 4.11 (t, J = 2.5 Hz, 1H), 4.04 (d, J = 3.0 Hz, 1H), 3.91–3.85 (m, 2H), 3.79 (dd, J = 10.5, 2.0 Hz, 1H), 3.78–3.68 (m, 2H), 3.53 (dd, J = 10.0, 2.0 Hz, 1H), 3.50–3.42 (m, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 139.1, 139.0, 138.8, 138.6, 138.3, 138.22, 138.19, 138.12, 128.6, 128.5, 128.45, 128.43, 128.39, 128.29, 128.19, 128.17, 128.07, 128.04, 127.87, 127.71, 127.66, 127.60, 102.6, 83.4, 83.1, 82.4, 81.6, 80.9, 80.1, 75.9, 75.8, 75.7, 75.3, 74.6, 74.3, 74.1, 73.6, 73.2, 72.0, 71.6, 69.4. MS-ESI $(M+H)^+$ calcd for $C_{68}H_{71}O_{11}$: 1063.4996. Found: 1063.4983.

4.4. Triethylammonium 3,4,5,6-tetra-*O*-benzyl-2-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)-1-*O*-(1,2-di-*O*-stearoyl-*sn*-glycero-3-phosphoryl)-D-*myo*-inositol (9)

Mannopyranosyl-inositol α-(**7**) (40 mg, 0.038 mmol) and *H*-phosphonate **8**[30] (56 mg, 0.76 mmol) were mixed, dried by co-evaporation from dry pyridine (2× 10 mL) and dissolved in dry pyridine (4 mL) under nitrogen. Pivaloyl chloride (50 μL, 0.041 mmol) was added and the mixture was stirred for 30 min at 20 °C. A freshly prepared solution of iodine (33 mg) in pyridine–H_2O (9:1, 20 mL) was added and the mixture was stirred for a further 30 min. The solution was diluted with $CHCl_3$ (20 mL), stirred for 15 min and then washed with $Na_2S_2O_3$ solution (10%, 20 mL) and H_2O. The solvents were removed at 50 °C and the residue was dissolved in CH_2Cl_2 (20 mL), washed with triethylammonium bicarbonate buffer (1 M, 2× 20 mL) and with H_2O (100 mL), and dried. The solvent was removed and the residue was purified by chromatography over silica gel (CH_2Cl_2/MeOH/Et_3N, 98:1:1) to give the title compound **9** (61 mg, 88%) as colourless glass. $[\alpha]_D^{18}$ +17.0 (*c* 1.0, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.53 (dd, J = 6.5 Hz, 2H), 7.42 (dd, J = 6.5 Hz, 2H), 7.33–7.17 (m, 36H), 5.74 (d, J = 3.0 Hz, 1H), 5.18–5.08 (m, 1H), 4.99 (dd, J = 11.5, 4.0 Hz, 1H), 4.92–4.79 (m, 10H), 4.73 (d, J = 12.0 Hz, 1H), 4.60–4.52 (m, 4H), 4.39 (dd, J = 12.0, 5.0 Hz, 1H), 4.27 (d, J = 12.0 Hz, 1H), 4.20

(br d, $J = 10.5$ Hz, 1H), 4.15–4.04 (m, 3H), 4.03–3.86 (m, 5H), 3.80 (t, $J = 9.5$ Hz, 1H), 3.75 (t, $J = 9.5$ Hz, 1H), 3.50 (dd, $J = 9.5$, 3.0 Hz, 1H), 3.48–3.44 (m, 2H), 3.16 (dd, $J = 9.5$, 5.5 Hz, 1H), 2.97 and 2.96 ($2 \times$ q, $J = 7.5$ Hz, 6H), 2.20 and 2.17 ($2 \times$ t, $J = 8.0$ Hz, 4H), 1.59–1.47 (m, 4H), 1.32–1.19 (m, 65H), 0.88 (t, $J = 7.0$ Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.3, 172.91, 172.89, 139.12, 139.10, 139.0, 138.8, 138.7, 138.61, 138.57,128.39, 128.36, 128.35, 128.26, 128.25, 128.22, 128.17, 128.11, 128.09, 128.08, 128.01, 127.67, 127.64, 127.59, 127.55, 127.54, 127.52, 127.47, 127.38, 127.34, 127.17, 127.0, 98.0, 83.3, 81.1, 80.9, 80.8, 79.4, 79.3, 76.2, 75.7, 75.5, 75.1, 74.7, 74.0, 73.2, 72.1, 71.5, 71.4, 70.6, 70.5, 70.4, 69.1, 63.8, 63.64, 63.60, 62.81, 62.78, 45.7, 34.32, 34.1, 32.0, 29.8, 29.73, 29.71, 29.59, 29.58, 29.41, 29.38, 29.2, 27.4, 27.3, 24.94, 24.91, 22.7, 14.2, 8.5. ^{31}P NMR (202 MHz, $CDCl_3$) δ 0.0, +0.01. MS-ESI $(M\text{-}NHEt_3)^-$ calcd for $C_{107}H_{144}O_{18}P$: 1748.0090. Found: 1748.0118.

4.5. Triethylammonium 2-*O*-(α-D-mannopyranosyl)-1-*O*-(1,2-di-*O*-stearoyl-*sn*-glycero-3-phosphoryl)-D-*myo*-inositol (10)

A mixture of protected $PIM1_2$ **9** (55 mg, 0.03 mmol) and Pd/C (10%, 100 mg) in a mixture of EtOAc/THF/EtOH/H_2O (2:1:1:1, 20 mL) was stirred at 20 °C under hydrogen for 16 h. The mixture was filtered through a pad of Celite, the pad was washed with MeOH–H_2O (1:1, 10 mL), THF (5 mL) and CH_2Cl_2 (10 mL). The solvent was removed from the combined filtrate and washings, toluene was added and removed. The residue was applied to a preparative silica gel plate (20 cm × 20 cm) and eluted with CH_2Cl_2/MeOH/Et_3N (90:8:2). The silica near the baseline was removed and washed with warm MeOH (30 mL) and then CH_2Cl_2 (30 mL). The solvent was removed and the product was lyophilised from dioxane–water (2:3) to give the title compound **10** (29 mg, 86%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3/CD_3OD/D_2O$, 35:20:3) δ 5.13–5.06 (m, 1H), 4.96 (d, $J = 1.5$ Hz, 1H), 4.29–4.23 (m, 1H), 4.20–3.82 (m, 6H), 4.82–3.78 (m, 1H), 3.72 (br t, $J = 8.5$ Hz, 1H), 3.65 (dd, $J = 10.0$, 2.5 Hz, 1H), 3.61–3.53 (m, 2H), 3.48–3.42 (m, 2H), 3.31 (dd, $J = 10.0$, 2.5 Hz, 1H), 3.10 (t, $J = 9.5$ Hz, 1H), 2.95 (q, $J = 7.0$ Hz, 6H), 2.17 and 2.14 (2t, $J = 8.0$ Hz, 6H), 1.48–1.38 (m, 4H), 1.75–1.07 (m, 65H), 0.73 (t, $J = 8$ Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3/CD_3OD/D_2O$ 35:20:3) δ 174.0 (CO, d, $^6J_{P\text{-}C}$ 2.5 Hz), 173.7 (CO, d, $^5J_{P\text{-}C}$ 5 Hz) 101.4, 78.5, 76.34, 76.29, 74.5, 72.7, 72.3, 71.9, 70.5, 70.44, 70.37, 70.33, 70.1, 67.0, 63.5 (d, $^2J_{C,P} = 0.5$ Hz), 62.8, 61.3, 49.0, 48.8, 48.7, 48.6, 48.4, 48.2, 48.1, 47.9, 46.1, 34.04, 34.02, 33.9, 31.7, 29.5, 29.4, 29.4, 29.3, 29.2, 29.1, 28.94, 28.92, 24.7, 24.61, 22.4, 13.7, 8.3. ^{31}P NMR (202 MHz, $CDCl_3/CD_3OD/D_2O$ 35:20:3) δ 0.08. MS-ESI $(M\text{-}NHEt_3)^-$ calcd for $C_{51}H_{96}O_{18}P$: 1027.6334. Found: 1027.6330.

4.6. 1-*O*-Allyl-6-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,5-tri-*O*-benzyl-6-*O*-D-*myo*-inositol (13)

TMSOTf (25 μL, 0.14 mmol) was added dropwise to a stirred mixture of 1-*O*-allyl-3,4,5-tri-*O*-benzyl-D-*myo*-inositol (**11**) (226 mg, 0.461 mmol), 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl trichloroacetimidate (**12**) (399 mg, 0.626 mmol) and 4 Å molecular sieves (120 mg) in CH_2Cl_2 (15 mL) at −40 °C. The reaction mixture was allowed to warm to 0 °C over 70 min when Et_3N (2.5 mL) was added. The mixture was filtered through Celite and the filter cake washed with further CH_2Cl_2 (2× 100 mL). The solvent was removed and the residue purified by column chromatography on silica gel. Elution with EtOAc/light petroleum (1:9 to 3:7) afforded the dimannosylated inositol derivative **20** (110 mg, 0.076 mmol, 17%) followed by the title compound **13** (222 mg, 0.230 mmol, 50%) as an oil. $[\alpha]_D^{20}$ +11.0 (*c* 5.48, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36–7.01 (m, 30H), 5.97–5.84 (m, 1H), 5.45–5.40 (m, 2H), 5.25–5.15 (m, 2H), 4.90–4.52 (m, 10H), 4.41 (d, $J = 11.0$ Hz, 1H), 4.25 (d, $J = 12.0$ Hz, 1H), 4.18–3.90 (m, 8H), 3.40–3.20 (m, 5H), 2.39 (s, 1H), 2.10 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.8, 139.4, 139.0, 138.9, 138.6, 138.5, 138.3, 134.7, 128.9, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 118.5, 98.6, 81.8, 81.6, 81.0, 80.2, 78.5, 76.3, 76.1, 75.7, 75.3, 74.7, 73.7, 73.1, 72.1, 71.7, 71.6, 69.3, 68.7, 67.1, 21.5. Gated decoupled ^{13}C NMR (75 MHz, $CDCl_3$) selected data, δ 98.6, $^1J_{C1'\text{-}H1'}$ 175.5 Hz. MS-ESI $(M+NH_4)^+$ calcd for $C_{59}H_{68}NO_{12}$: 982.4736. Found: 982.4726. Anal. ($C_{59}H_{64}O_{12}$) C, H.

4.7. 1-*O*-Allyl-6-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-2-*O*-benzoyl-3,4,5-tri-*O*-benzyl-D-*myo*-inositol (17)

Benzoyl chloride (100 μL, 0.861 mmol) was added dropwise to a stirred solution of alcohol **13** (37 mg, 0.040 mmol) in pyridine (10 mL). After being stirred for 8 h, the mixture was diluted with H_2O (50 mL) and extracted with ether (2× 50 mL). The combined extracts were washed with HCl (0.5 M, 70 mL) and $NaHCO_3$ solution (satd, 50 mL). After drying and filtration, the solvent was removed and the residue purified by column chromatography on silica gel. Elution with EtOAc/light petroleum (3:7 to 2:3) afforded the title compound **17** as an oil (28 mg, 0.026 mmol, 65%). $[\alpha]_D^{22}$ +34 (*c* 0.48, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.06–8.00 (m, 2H), 7.59–7.52 (m, 1H), 7.48–7.40 (m, 2H), 7.35–7.01 (m, 30H), 5.99 (t, $J = 2.7$ Hz, 1H), 5.97–5.81 (m, 1H), 5.50–5.41 (m, 2H), 5.25–5.13 (m, 2H), 4.95–4.55 (m, 10H), 4.42 (d, $J = 10.9$ Hz, 1H), 4.25 (d, $J = 12.0$ Hz, 1H), 4.19–3.90 (m, 7H), 3.58 (dd, $J = 12.5$, 2.8 Hz, 1H), 3.48–3.31 (m, 4H), 2.07 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.6, 166.3, 139.3, 138.9, 138.5, 138.2, 138.0, 134.6, 133.5, 130.3, 128.8, 128.7, 128.6, 128.4, 128.2, 128.1, 128.0, 127.9(2), 127.8(6), 127.7(5), 127.6(9), 118.4, 98.7, 82.2, 81.5, 79.0, 78.8, 78.4, 76.7, 76.3, 76.2, 75.4, 74.7, 73.6, 72.5, 72.0, 71.7, 71.3, 69.1, 68.8, 67.1, 21.4. MS-ESI $(M+NH_4)^+$ calcd for $C_{66}H_{72}NO_{13}$: 1086.4998. Found: 1086.4952.

4.8. 1-*O*-Allyl-3,4,5-tri-*O*-benzyl-6-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-D-*myo*-inositol (14)

Sodium methoxide in MeOH (30%, ca 0.05 mL) was added dropwise to a stirred solution of acetate **13**

(255 mg, 0.264 mmol) in MeOH (12 mL). After being stirred for 3 h, the reaction mixture was diluted with NH_4Cl solution (satd 50 mL). The aqueous phase was extracted with $CHCl_3$ (3× 40 mL) and the combined organic extracts were washed with H_2O (100 ml). After drying and filtration the solvent was removed to afford the title compound **14** as an oil (232 mg, 0.251 mmol, 95%). $[\alpha]_D^{20}$ +30.5 (*c* 4.64, $CHCl_3$). ^1H NMR(300 MHz, $CDCl_3$) δ 7.37–7.04 (m, 30H), 5.96–5.82 (m, 1H), 5.46 (s, 1H), 5.30–5.18 (m, 2H), 4.96–4.61 (m, 9H), 4.55 (d, J = 12.1 Hz, 1H), 4.43 (d, J = 11.1 Hz, 1H), 4.25 (d, J = 12.1 Hz, 1H), 4.19–3.81 (m, 9H), 3.41–3.21 (m, 5H), 2.42 (br s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 139.3, 139.0, 138.9, 138.6, 138.3, 134.5, 128.9, 128.8, 128.7, 128.5, 128.3, 128.2, 128.0, 127.7, 118.4, 100.3, 81.8, 81.7, 81.0, 80.5, 80.2, 76.3, 76.1, 76.0, 75.2, 74.7, 73.7, 73.1, 72.3, 71.4, 69.1, 68.8, 67.1, 21.5. MS-ESI $(M+NH_4)^+$ calcd for $C_{57}H_{66}NO_{11}$: 940.4630. Found: 940.4637.

4.9. 1-*O*-Allyl-2,3,4,5-tetra-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)-D-*myo*-inositol (15)

Sodium hydride (60% dispersion in mineral oil, 40.0 mg, 1.00 mmol) was added to a stirred solution of diol **14** (232 mg, 0.251 mmol) and benzyl bromide (100 μL, 0.840 mmol) in DMF (8 mL) cooled to 0 °C. After 20 min, the ice bath was removed and the reaction mixture stirred at 20 °C for 3 h after which NH_4Cl solution (50 mL, satd) was added. The mixture was extracted with ether (2× 50 mL) and the combined ethereal extracts were washed with H_2O (2 × 50 mL). After drying and filtration, the solvent was removed and the residue purified by column chromatography on silica gel. Elution with EtOAc/light petroleum (1:9 to 1:4) afforded the title compound **15** as an oil (278 mg, 0.252 mmol, 100%). $[\alpha]_D^{22}$ +17.6 (*c* 5.56, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$) δ 7.42–7.03 (m, 40H), 5.82–5.69 (m, 1H), 5.57 (s, 1H), 5.27–5.10 (m, 2H), 4.93–4.57 (m, 14H), 4.45 (d, J = 11.0 Hz, 1H), 4.27–3.77 (m, 10H), 3.41–3.12 (m, 5H). ^{13}C NMR δ 139.6, 139.3, 139.2, 139.1, 138.9, 138.7, 134.9, 128.8, 128.7, 128.6(3), 128.5(9) 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9(2), 127.8(5), 127.7(7), 127.6(7), 127.5(8), 117.5, 98.8, 82.4, 82.3, 81.8, 81.4, 80.5, 76.3, 76.2, 76.0, 75.8, 75.2, 74.4, 73.6, 73.3, 73.2, 72.5, 72.4, 72.2, 71.0, 69.1, 68.8, 67.1, 21.5. MS-ESI $(M+NH_4)^+$ calcd for $C_{71}H_{78}NO_{11}$: 1120.5569. Found: 1120.5628.

4.10. 2,3,4,5-Tetra-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)-D-*myo*-inositol (16)

(1,5-Cyclooctadiene)bis(methyldiphenylphosphine)iridium(I) hexafluorophosphate (40 mg, 0.047 mmol) was added to a stirred solution of allyl ether **15** (259 mg, 0.235 mmol) in THF (10 mL) under argon. This atmosphere was replaced with hydrogen for ca 1 min and the hydrogen was replaced with argon. The mixture was stirred at 20 °C for 70 min, the solvent was removed in vacuo and the residue dissolved with stirring in CH_2Cl_2/MeOH (2:1, 9 mL). Acetyl chloride (0.1 mL, 1.29 mmol) was added to this solution and stirring was continued for a further 90 min when solid $NaHCO_3$ (200 mg, 2.38 mmol) was added. The mixture was stirred for an additional 5 min when H_2O (50 mL) was added. This mixture was extracted with $CHCl_3$ (2× 60 mL) and after drying and filtration the solvent was removed and the residue purified by column chromatography on silica gel. Elution with EtOAc/light petroleum (1:4 to 3:7) afforded the title compound **16** as an oil (192 mg, 0.181 mmol, 77%). $[\alpha]_D^1$ +25.6 (*c* 3.84, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.38–7.06 (m, 40H), 5.56 (d, J = 2.0 Hz, 1H), 5.07 (d, J = 11.5 Hz, 1H), 4.90 (d, J = 11.0 Hz, 1H), 4.85 (d, J = 11.0 Hz, 1H), 4.80 (d, J = 10.5 Hz, 1H), 4.77 (d, J = 10.0 Hz, 1H), 4.74–4.58 (m, 6H), 4.55 (s, 2H), 4.45 (d, J = 11.0 Hz, 1H), 4.30 (d, J = 11.0 Hz, 1H), 4.05–3.80 (m, 7H), 3.49 (dd, J = 11.5, 4.5 Hz, 1H), 3.46–3.42 (m, 3H), 3.27 (t, J = 9.5 Hz, 1H), 2.34 (d, J = 10.1 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 139.0, 138.69, 138.65, 138.53, 138.51, 138.4, 138.3, 138.2, 128.7, 128.6, 128.55, 128.4, 128.30, 128.28, 128.23, 128.20, 128.16, 128.03, 128.00, 127.99, 127.90, 127.85, 127.75, 127.70, 127.6, 127.51, 127.46, 127.39, 127.35, 127.31, 97.3, 81.6, 81.3, 81.2, 79.6, 78.4, 78.1, 75.9, 75.8, 74.90, 74.87, 74.81, 74.6, 73.4, 73.2, 73.1, 72.1, 71.79, 71.76, 68.9. MS-ESI $(M+NH_4)^+$ calcd for $C_{68}H_{74}NO_{11}$: 1080.5256. Found: 1080.5197.

4.11. Triethylammonium 3,4,5,6-tetra-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)-1-*O*-(1,2-di-*O*-stearoyl-*sn*-glycero-3-phosphoryl)-D-*myo*-inositol (18)

A mixture of protected inositol **16** (30 mg, 0.03 mmol) and phosphonate salt **8** (50 mg, 0.06 mmol) was dried by co-evaporation from dry pyridine (2× 10 mL) and dissolved in the same solvent (4 mL) under nitrogen. Pivolyl chloride (50 μL, 0.04 mmol) was added and the mixture stirred at 20 °C for 30 min. A freshly prepared solution of iodine (30 mg, 0.12 mmol) in pyridine–H_2O (9:1, 20 mL) was added and stirring was continued for 30 min. The solution was diluted with $CHCl_3$ (20 mL) and stirred for 15 min then washed with $Na_2S_2O_3$ solution (10%, 20 mL) and H_2O. After drying, the solvent was removed and the residue was dissolved in CH_2Cl_2 (20 mL), washed with triethylammonium bicarbonate (1 M, 2× 20 mL) and with H_2O (100 mL). The solvent was removed and the residue was purified by chromatography over silica gel (CH_2Cl_2/MeOH/Et_3N, 98:1:1) to give the title phosphate **18** (44 mg, 84%) as a colourless glass. $[\alpha]_D^{20}$ +30.6 (*c* 1, CH_2Cl_2). ^1H NMR (500 MHz, $CDCl_3$) δ 7.50–6.98 (m, 40H), 5.78 (d, J = 2.0 Hz, 1H), 5.28-5.19 (m, 1H), 5.02 (d, J = 11.5 Hz, 1H), 4.91–4.54 (m, 16H), 4.48 (d, J = 11.0 Hz, 1H), 4.54–4.39 (m, 1H), 4.30–4.23 (m, 1H), 4.20–3.94 (m, 8H), 3.55–3.49 (m, 2H), 3.41 (dd, J = 11.5, 1.5 Hz, 1H), 3.38–3.33 (m, 1H), 2.72 (q, J = 7.5 Hz, 6H), 2.25–2.17 (m, 4H), 1.57–1.50 (m, 4H), 1.29–1.20 (m, 56H), 1.09 (t, J = 7.5 Hz, 9H), 0.88 (t, J = 7.5 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.4, 173.0, 139.8, 139.7, 139.58, 139.55, 139.3, 138.9, 138.8, 138.6, 138.3, 128.33, 128.30, 128.23, 128.18, 128.15, 128.12, 128.11, 128.09, 128.07, 127.98, 127.85, 127.7, 127.64, 127.62, 127.60, 127.55, 127.54, 127.45, 127.30, 127.26, 127.18, 127.0, 97.3, 81.8, 81.1, 79.7, 77.3, 77.1, 76.8, 76.1, 75.8, 75.4, 74.9,

74.8, 74.6, 73.0, 72.5, 72.1, 71.5, 71.4, 70.7, 69.1, 63.7, 62.9, 45.6, 34.4, 34.1, 32.0, 29.8, 29.7, 29.6, 29.43, 29.40, 29.2, 27.9, 25.0, 24.9, 22.8, 14.2. ^{31}P NMR (202 MHz, $CDCl_3$) δ −0.6, −0.7. MS-ESI $(M\text{-}NHEt_3)^-$ calcd for $C_{107}H_{144}O_{18}P$: 1748.0090. Found: 1748.0052.

4.12. Triethylammonium 6-*O*-(α-D-mannopyranosyl)-1-*O*-(1,2-di-*O*-stearoyl-*sn*-glycero-3-phosphoryl)-D-*myo*-inositol (19)

A mixture of protected $PIM1_6$ **18** (25 mg, 0.014 mmol) and Pd/C (10%, 50 mg) was stirred at 20 °C under hydrogen for 16 h in EtOAc/THF/EtOH/H_2O (2:1:1:1, 15 mL). The mixture was filtered through a pad of Celite. The filter pad was washed successively with MeOH/H_2O (1:1, 2× 10 mL), THF (5 mL) and CH_2Cl_2 (10 mL). The solvents were removed from the combined filtrate and washings and the residue was dried by co-evaporation from toluene. The residue was purified by preparative thin-layer chromatography on silica gel (CH_2Cl_2/MeOH/Et_3N, 90:8:2 as eluent). The baseline region of silica was cut and washed with warm MeOH (30 mL) and CH_2Cl_2 (30 mL). The solvent was removed and the white residue was lyophilised from dioxane–H_2O water (2:3) to give the deprotected phosphate **19** (11 mg, 72%) as a white fluffy powder. ^{1}H NMR (500 MHz, $CDCl_3$/CD_3OD/D_2O 35:20:3) δ 5.10–5.02 (m, 1H), 4.91 (d, J = 2.0 Hz, 1H), 4.20–3.75 (m, 4H), 3.54 (dd, J = 12.5, 5.5 Hz, 1H), 3.46 (t, J = 9.5 Hz, 1H), 3.22 (dd, J = 10.0, 2.0 Hz, 1H), 3.08 (t, J = 9.5 Hz, 1H), 2.93 (q, J = 7.0 Hz, 6H), 2.16 and 2.12 (t, J = 7.0 Hz, 2H), 1.46–1.35 (m, 4H), 1.13 (t, J = 7.0 Hz, 9H), 0.70 (t, J = 7.5 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.0, 173.7, 101.3, 73.1, 72.6, 70.9, 70.4, 69.9, 67.0, 62.6, 61.3, 48.9, 48.7, 48.5, 48.3, 48.2, 48.0, 47.8, 46.0, 33.9, 33.8, 31.6, 29.40, 29.35, 29.29, 29.25, 29.11, 29.05, 28.9, 28.8, 24.64, 24.56, 22.4, 13.7, 8.2. ^{31}P NMR (202 MHz, $CDCl_3$) δ −0.11, −0.15 ppm. MS-ESI $(M\text{-}NHEt_3)^-$ calcd for $C_{51}H_{96}O_{18}P$: 1027.6334. Found: 1027.6316.

4.13. 1-*O*-Allyl-2,6-di-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,5-tri-*O*-benzyl-D-*myo*-inositol (20)

TMSOTf (100 μL, 0.552 mmol) was added dropwise to a stirred mixture of 1-*O*-allyl-3,4,5-tri-*O*-benzyl-D-*myo*-inositol (**11**) (400 mg, 0.815 mmol), 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl trichloroacetimidate (**12**) (1.41 g, 2.21 mmol) and 4 Å molecular sieves (270 mg) in DCM (50 mL) cooled to −40 °C. The reaction mixture was allowed to warm to 0 °C over 90 min when Et_3N (2.5 mL) was added. The mixture was filtered through Celite and the filter cake washed with further CH_2Cl_2 (2× 100 mL). The solvent was removed and the residue purified by column chromatography on silica gel. Elution with EtOAc/light petroleum (1:9 to 3:7) afforded the diglycoside **20** (665 mg, 0.462 mmol, 57%) as an oil. $[\alpha]_D^{20}$ +27.5 (c 4.75, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38–7.00 (m, 45H), 5.96–5.83 (m, 1H), 5.47–5.43 (m, 3H), 5.23–5.12 (m, 3H), 4.95–4.65 (m, 8H), 4.60–3.79 (m, 21H), 3.55–3.50 (m, 1H), 3.34–3.20 (m, 6H), 2.12 (s, 3H), 2.11 (s, 3H). ^{13}C NMR (75 MHz) δ 170.7, 170.2, 139.4, 139.2, 138.8, 138.6, 138.4, 134.4, 129.0, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 118.0, 99.6, 98.9, 81.8, 81.3, 79.2, 78.6, 77.7, 76.6, 76.4, 76.1, 75.4 (2× CH_2), 74.6, 74.5, 73.8, 73.7, 72.9, 72.6, 72.1, 71.9, 71.7, 69.1, 69.0, 68.9, 68.6, 21.5. Gated decoupled ^{13}C NMR (75 MHz, $CDCl_3$) selected data, δ 99.6, $^1J_{C1'-H1'}$ 173 Hz, δ 98.9, $^1J_{C1''-H1''}$ 174 Hz. MS-ESI $(M+NH_4)^+$ calcd for $C_{88}H_{98}NO_{18}$: 1456.6778. Found: 1456.6777.

4.14. 1-*O*-Allyl-3,4,5-tri-*O*-benzyl-2,6-di-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-D-*myo*-inositol (21)

Sodium methoxide in MeOH (30%, ca 0.05 mL) was added dropwise to a stirred solution of diacetate **20** (603 mg, 0.419 mmol) in MeOH/DCM (2:1, 12 mL). After being stirred for 24 h, the reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with NH_4Cl (satd, 50 mL). The aqueous phase was re-extracted with CH_2Cl_2 (2× 50 mL) and the combined organic extracts were washed with H_2O (100 ml). After drying ($MgSO_4$) and filtration, the solvent was removed and the residue purified by column chromatography on silica gel. Elution with EtOAc/light petroleum (3:7 to 13:7) afforded the diol **21** as an oil (411 mg, 0.303 mmol, 72%). $[\alpha]_D^{20}$ +63 (c 0.82, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40–7.02 (m, 45H), 5.92–5.80 (m, 1H), 5.42 (br s, 1H), 5.29–5.17 (m, 3H), 4.93–3.82 (m, 31H), 3.48 (dd, J = 10.7, 3.6 Hz, 1H), 3.33–3.16 (m, 6H), 2.42 (br s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 139.2, 139.1, 138.9, 138.6, 138.4, 138.3, 134.2, 129.0, 128.8, 128.7, 128.6, 128.5, 128.3, 128.2, 127.9, 127.7, 118.3, 100.8, 100.6, 82.0, 81.9, 81.8, 80.5, 79.8, 79.3, 77.1, 76.3, 76.1, 75.4, 75.3, 74.6, 74.5, 73.8, 73.7, 72.6, 72.3, 72.2, 71.7, 71.5, 69.1, 69.1, 68.7, 68.6. MS-ESI $(M+NH_4)^+$ calcd for $C_{84}H_{94}NO_{16}$: 1372.6573. Found: 1372.6565.

4.15. 1-*O*-Allyl-3,4,5-tri-*O*-benzyl-2,6-di-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)-D-*myo*-inositol (22)

Sodium hydride (60% dispersion in mineral oil, 50.0 mg, 1.25 mmol) was added to a stirred solution of diol **21** (411 mg, 0.303 mmol) and benzyl bromide (150 μL, 1.26 mmol) in DMF (15 mL) cooled to 0 °C. After 20 min the ice bath was removed and the reaction mixture stirred at 20 °C for 3 h and NH_4Cl (satd, 50 mL) was added. The mixture was extracted with ether (2× 100 mL) and the combined ethereal extracts were washed with H_2O (2× 70 mL). After drying and filtration the solvent was removed and the residue purified by column chromatography on silica gel. Elution with EtOAc/light petroleum (1:4 to 3:7) afforded the title compound **22** as an oil (367 mg, 0.239 mmol, 79%). $[\alpha]_D^{22}$ +31 (c 0.88, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38–7.04 (m, 55H), 5.80–5.67 (m, 1H), 5.52 (br s, 1H), 5.25 (br s, 1H), 5.25–5.07 (m, 2H), 4.92–3.78 (m, 35H), 3.56–3.14 (m, 7H). ^{13}C NMR (75 MHz) δ 139.5, 139.3, 139.2, 139.1, 139.0, 138.9, 138.8, 138.5, 134.4, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.0, 127.8, 127.7, 127.6, 118.2, 99.1, 82.2, 81.9, 81.8, 80.6, 79.4, 79.2, 76.4, 76.4, 76.1, 75.4, 75.3, 75.2, 75.1, 75.0, 73.8, 73.6, 72.8, 72.6, 72.5, 72.4, 72.3, 72.0, 71.5, 71.2, 69.4,

69.1. MS-ESI $(M+NH_4)^+$ calcd for $C_{98}H_{106}NO_{16}$: 1552.7506. Found: 1552.7566.

4.16. 3,4,5-Tri-*O*-benzyl-2,6-di-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)-D-*myo*-inositol[25] (23)

(1,5-Cyclooctadiene)bis(methyldiphenylphosphine)iridium(I) hexafluorophosphate (40 mg, 0.047 mmol) was added to a stirred solution of allyl ether **22** (313 mmol, 0.204 mmol) in THF (12 mL) under argon. The argon was replaced with hydrogen for ca 1 min and the hydrogen atmosphere was, in turn, replaced with argon. After the mixture was stirred at 20 °C for 90 min, the solvent was removed and the residue dissolved with stirring in CH_2Cl_2/MeOH (2:1, 9 mL). Acetyl chloride (0.1 mL) was added to this solution and stirring was continued for 4 h and solid $NaHCO_3$ was added. The mixture was stirred for an additional 5 min when H_2O (50 mL) was added. The mixture was extracted with $CHCl_3$ (2× 80 mL). After drying ($MgSO_4$) and filtration, the solvent was removed in vacuo and the residue purified by column chromatography on silica gel. Elution with EtOAc/light petroleum (1:4 to 3:7) afforded the title compound **23** (234 mg, 0.156 mmol, 76%) as an oil. Spectroscopic data are in agreement with literature[25] values; MS-FAB (+ve) 1626 $[(M+Cs)^+$, 10%], 286 (10), 181 (30), 133 (25), 91 (100). HRMS-FAB $(M+H)^+$ calc for $C_{95}H_{99}O_{16}$: 1495.6933. Found: 1495.6992.

4.17. 3,4,5-Tri-*O*-benzyl-2,6-di-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl-1-*O*-(1,2-di-*O*-stearoyl-*sn*-glycero)-D-*myo*-inositol (24)

A mixture of alcohol **23** (44 mg, 0.029 mmol) and the *H*-phosphonate triethylammonium salt **8** (46 mg, 0.058 mmol) was dried by evaporation with pyridine (2× 5 mL) and re-dissolved in pyridine (5 mL) under argon. Pivaloyl chloride (43.0 μL, 0.348 mmol) was added and the reaction stirred at room temperature for 1 h under argon. TLC analysis (2:3 EtOAc/light petroleum) showed conversion of **23** (R_f 0.35) into a mixture of the diastereomeric H-phosphonates (R_f 0.60 and 0.65). A freshly prepared solution of iodine (30.0 mg, 0.116 mmol) in pyridine/H_2O (95:5, 10 mL) was added and reaction stirred for 30 min. $CHCl_3$ (20 mL) was added and the mixture stirred for a further 15 min. The organic layer was washed with $Na_2S_2O_3$ (10% aq, 20 mL) and aqueous triethylammonium bicarbonate (1 M, 3× 20 mL), dried ($MgSO_4$) and concentrated in vacuo to give the crude product which was purified by semi-preparative reverse phase HPLC (acetonitrile/isopropanol/0.05% ammonia, 40:40:20 to 45:45:10) to afford **24** (48 mg, 74%) as an oil. ^1H NMR (300 MHz, $CDCl_3$) δ 7.40–7.00 (m, 55H), 5.73 (br s, 1H), 5.67 (br s, 1H), 5.12 (br s, 1H), 4.83–3.70 (m, 38H), 3.40–3.02 (m, 6H), 2.16–2.07 (m, 4H), 1.55–1.40 (m, 4H), 1.32–1.10 (m, 56H), 0.89–0.80 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$), selected data, δ 173.8, 173.4, 98.6, 97.9. ^{31}P NMR ($CDCl_3$, 121.5 MHz) δ 0.01. Compound **24** was converted to the sodium salt, see below, HRMS-FAB $(M+H)^+$ calcd for $C_{134}H_{173}O_{23}PNa$: 2204.2003. Found: 2204.1904.

4.18. 2,6-(Di-*O*-α-D-mannopyranosyl)-1-*O*-(1,2-di-*O*-stearoyl-*sn*-glycero-3-phosphoryl)-D-*myo*-inositol (25)

Compound **24** was converted to the sodium salt by ion-exchange chromatography (Dowex 50W X8—100 Na^+ form, eluting with MeOH/$CHCl_3$). A sample (42 mg, 0.019 mmol) was dissolved in ^{t}BuOH (10 mL) and hydrogenated in the presence of $Pd(OH)_2$/C (40 mg, H_2 150 psi, 40 °C) over 72 h during which TLC (5:95 MeOH/CH_2Cl_2) showed disappearance of the starting material (R_f 0.3) to give a baseline product. The mixture was filtered through Celite and purified on silica gel eluting with $CHCl_3$ and $CHCl_3$/MeOH/H_2O (7:4:1) to afford phosphate **3** (8 mg, 37%) as a white powder after lyophilisation from H_2O. $[\alpha]_D^{20}$ +23.0 (*c*, 0.20, $CDCl_3$/CD_3OD/D_2O 70:40:6). ^1H NMR (500 MHZ, $CDCl_3$/CD_3OD/D_2O, 70:40:6), δ 5.30–5.25 (br s, 1H), 5.14 (br s, 1H), 5.11 (br s, 1H), 4.43 (m, 1H), 4.31 (br s, 1H), 4.08–3.95 (m, 7H), 3.85–3.72 (m, 7H), 3.69–3.59 (m, 3H), 3.47 (m, 1H), 3.30 (t, J = 9.1 Hz, 1H), 2.37 (t, J = 7.6 Hz, 2H), 2.33 (t, J = 7.7 Hz, 2H), 1.67–1.55 (m, 4H), 1.35–1.21 (m, 56H), 0.89 (t, J = 6.9 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$/CD_3OD/D_2O 70:40:6), δ 174.8, 174.5, 101.92, 101.89, 78.9, 73.7, 73.3, 73.2, 71.0, 70.9, 70.7, 70.5, 67.5, 67.4, 63.9, 63.3, 61.7, 61.5, 34.6, 34.5, 32.2, 30.1, 30.0, 29.9, 29.8, 29.7, 29.5(3), 29.4(9), 25.3, 25.2, 23.0, 14.2. ^{31}P NMR ($CDCl_3$/CD_3OD/D_2O, 70:40:6, 121.5 MHz) δ −3.8; MS-FAB (−ve) 1190 $[(M-Na)^-$ 20%] 338 (15), 283 (15), 97 (25), 79 (100). HSMS-FAB $(M-Na^+)^-$ calcd for $C_{57}H_{106}O_{23}P$: 1189.6863. Found: 1189.6879.

5. Experimental procedures for the biological tests

5.1. 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 University of Otago (Dunedin, New Zealand) guidelines for the care of animals.

5.2. 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 PBS containing 100 μg OVA.

5.2.1. Treatment with PIM analogues. One week (day 21) prior to OVA challenge mice were anaesthetised and the PIM analogues administered intranasally (50 μl, 20 μg/ml, in). Control mice were given 50 μL PBS.

5.2.2. 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 (3 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.

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