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Chemical Synthesis and Immunosuppressive Activity of Dipalmitoyl Phosphatidylinositol Hexamannoside

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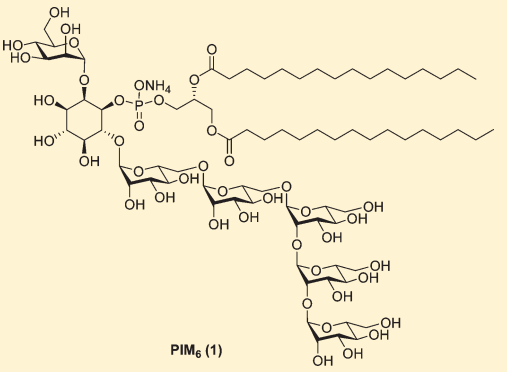
Supporting Information

ABSTRACT: Phosphatidylinositol mannosides (PIMs) isolated from mycobacteria have been identified as an important class of phosphoglycolipids with significant immune-modulating properties. We present here the synthesis of dipalmitoyl phosphatidylinositol hexamannoside (PIM_6) **1** and the first reported functional biology of a synthetic PIM_6. Key steps in the synthetic protocol included the selective glycosylation of an inositol 2,6-diol with a suitably protected mannosyl donor and construction of the glycan core utilizing a [3 + 4] thio-glycosylation strategy. The target **1** was purified by reverse phase chromatography and characterized by standard spectroscopic methods, HPLC, and chemical modification by deacylation to $dPIM_6$. The ^1H NMR spectrum of synthetic $dPIM_6$ obtained from **1** matched that of $dPIM_6$ obtained from nature. PIM_6 (**1**) exhibited dendritic cell-dependent suppression of $CD8^+$ T cell expansion in a human mixed lymphocyte reaction consistent with the well established immunosuppressive activity of whole mycobacteria.



INTRODUCTION

Mycobacteria and their soluble products have strong inherent immunomodulatory properties. The bulk of this activity is associated with the components of the mycobacterial cell wall[1] that include lipoglycans such as lipoarabinomannan and phosphatidylinositol mannosides (PIMs).

PIMs are phosphoglycolipids embedded in the cell walls of mycobacteria that anchor an array of more complex glycolipids to the cell membrane[2] and exhibit both immunosuppressive and adjuvant activities. Previous studies have shown that PIMs can modulate T cell-driven immune responses,[3,4] by altering T cell function and expansion.[5−8] PIMs or their corresponding glycans are reported to have vaccine adjuvant activity in mouse models,[9−11] and there is evidence that PIMs also have the ability to interfere with LPS-induced immune responses.[12]

Native PIM_6 has been reported to expand T cell subsets;[8] however, to our knowledge, other than DC-SIGN binding experiments,[13] currently there is no functional data published on the biological activities of the higher glycosylated synthetic PIMs (i.e., PIM_6). Therefore we decided to embark on the synthesis of a PIM_6, utilizing a thioglycoside as the tetrasaccharide donor in a convergent [3 + 4] glycosylation strategy. A key aspect of the synthesis was the use of the anchimeric directing group 2-(azidomethyl)benzoate (AZMB) as a temporary protecting group orthoganol to benzoate. The target compound was purified by reverse phase chromatography and evaluated for its effect on DC and antibody-mediated human T cell expansion.

RESULTS AND DISCUSSION

In previous work we demonstrated that dipalmitoyl phosphatidylinositol tetramannoside (PIM_4) could be prepared from synthetic protocols that utilized a glycosylation of a suitably protected pseudotrisaccharide similar to **2** with a dimannosyl trichloroacetimidate donor in a [3 + 2] glycosylation strategy. Subsequent introduction of the phosphatidic acid moiety using **3**, followed by global deprotection afforded the desired target PIM_4.[14] We envisaged that dipalmitoyl PIM_6 (**1**) could be prepared in an analogous manner by utilizing the acceptor **2** along with the thio-tetramannosyl donor **4** as the coupling partner to effect a convergent [3 + 4] glycosylation approach (Scheme 1). Subsequent phosphitylation with a phosphoramidite and deprotection would afford PIM_6 (**1**). In related work a similar approach to the synthesis of higher order PIMs has been reported by Seeberger et al. for Ac_1PIM_6[15] and PIM glycans.[10]

Synthesis of Thio-tetramannosyl Donor 4. Tetramannoside donor **4** was prepared from monomannoside building blocks **5**, **6** and **7**. The incorporation of the directing benzoyl and AZMB

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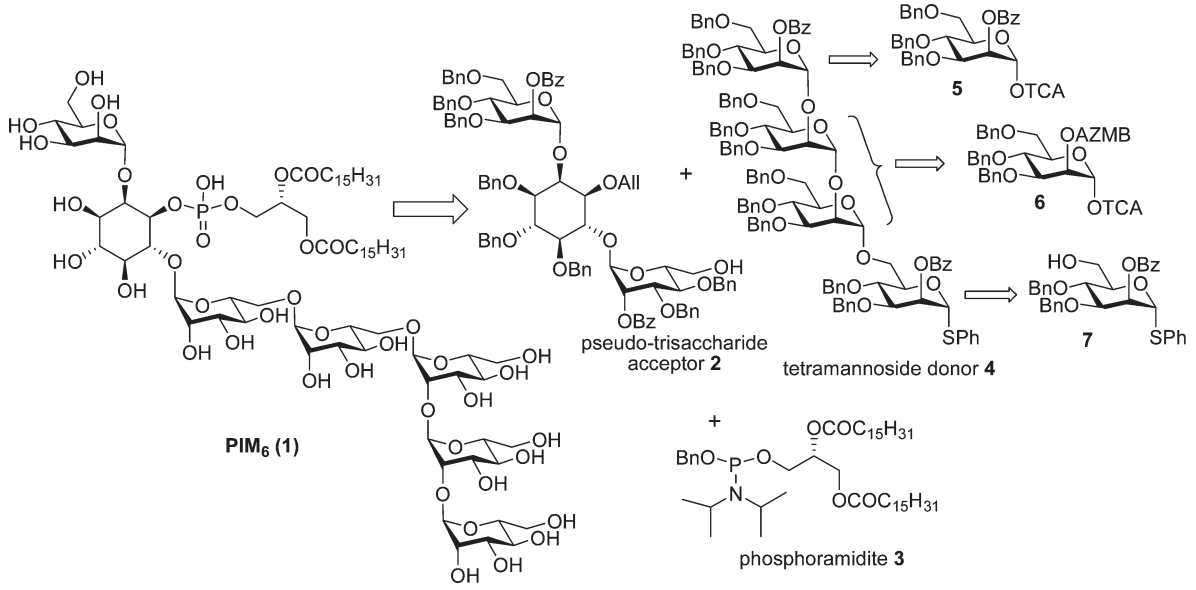
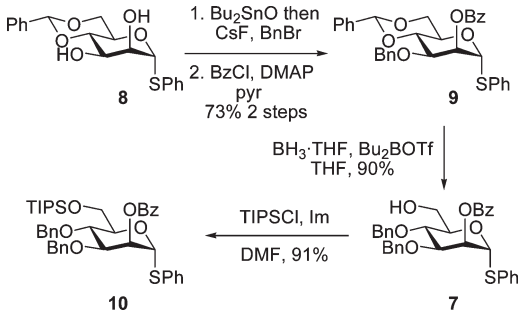
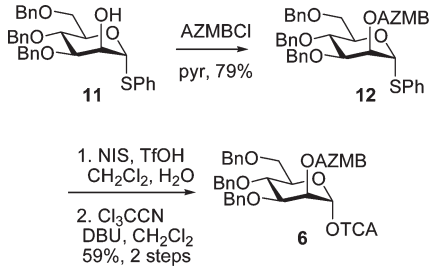
Scheme 1. Retrosynthetic Analysis of PIM_6



Scheme 2. Synthesis of Thiomannosyl Donors 7 and 10



Scheme 3. Syntheis of 2-*O*-AZMB Protected Donor 6



groups[16] at the O-2 positions of these donors would ensure high α-selectivity in manosylation reactions. Furthermore, the AZMB group can be selectively removed in the presence of benzoyl groups and would allow subsequent construction of the two (1→2) mannosyl linkages at the nonreducing end of **4**.

Often 2-*O*-acyl-α-D-mannopyranosyl donors are generated using orthoester chemistry and we used this approach to synthesize mannosyl donor **5** using standard protocols.[17] Due to the accessibility of thiophenyl α-D-mannopyranosides we synthesized donors **6** and **7** using the approach shown in Schemes 2 and 3. The diol **8**[18] was a convenient starting material for the synthesis of **7** and was readily prepared from D-mannose in a modified, four step procedure[19] without the need for column chromatography. The C-3 hydroxyl group of **8** was selectively benzylated via prior stannylene acetal formation and the C-2 hydroxyl was subsequently benzoylated to afford **9** in good yield. Traces of tin residues were removed by chromatography on silica gel impregnated with KF.[20] The benzylidene acetal was regioselectively reduced with borane-THF complex in the presence of dibutylboron triflate or scandium triflate to give primary alcohol **7**, the initial acceptor for the synthesis of tetrasaccharide **4**. Furthermore, thiomannoside **7** was silylated with triisopropylsilyl chloride (TIPSCl) to afford **10**, a glycosyl donor for the synthesis of pseudotrisaccharide **2**.

Trichloroacetimidate (TCA) donor **6** was synthesized from thioglycoside **11**[21] by acylation with 2-(azidomethyl)benzoyl chloride (AZMBCl) to give **12** followed by hydrolysis of the thioglycoside moiety with aqueous *N*-iodosuccinimide and reaction with trichloroacetonitrile (Scheme 3).

Glycosylation of **7** with **6** promoted by TMSOTf afforded disaccharide **13** in 94% yield (Scheme 4). Selective hydrolysis of the AZMB group was achieved using a Staudinger reduction of the azide moiety with tributylphosphine[22] to give the disaccharide acceptor **14** in 83% yield. Reiteration of glycosylation with **6** to give **15** and removal of the AZMB protecting group afforded trisaccharide **16** in 68% yield for the two steps. In the final step, **16** was mannosylated with donor **5** to give the key tetrasaccharide building block **4** in 56% yield.

Synthesis of Pseudotrisaccharide Building Block. With tetrasaccharide **4** in hand it was used in the [3 + 4] glycosylation of pseudotrisaccharide **2**. We have previously reported the synthesis of an analogue of **2**[23] for the synthesis of PIM_4 from an intermediate where the primary hydroxyl group of the mannosyl residue at O-6 of the inositol was protected as a *tert*-butyldiphenylsilyl (TBDPS) ether.[14] However, difficulties in removing TBDPS protecting group in that instance led us to

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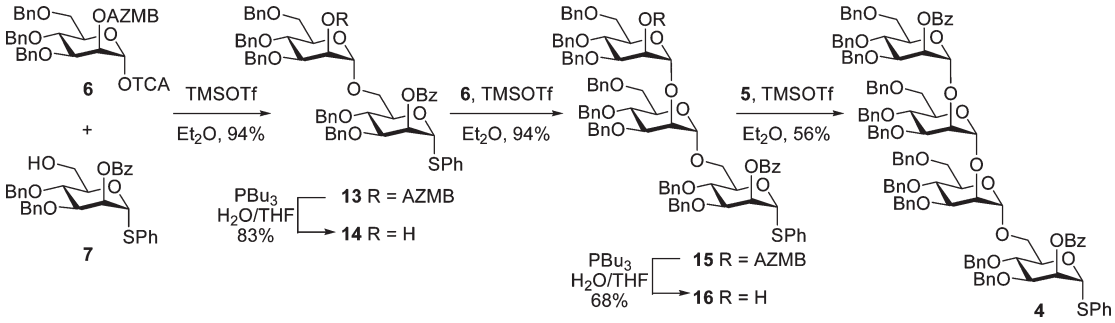
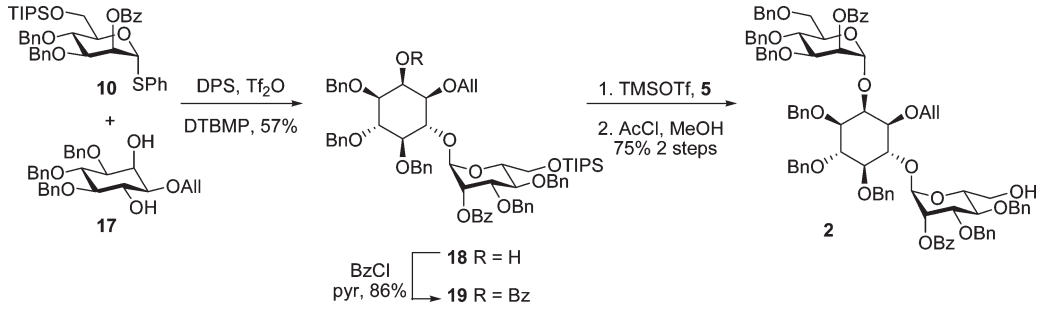
Scheme 4. Synthesis of Tetrasaccharide Donor 4



Scheme 5. Synthesis of Pseudotrisaccharide 2



design the mannosyl donor **10** in which the more acid-labile TIPS group provides the temporary protection required en route to **2** (Scheme 5). Selective O-6 glycosylation of inositol diol **17** was achieved with thiophenyl-mannosyl donor **10** using conditions reported by Codée et al.[24] to give pseudodisaccharide **18** in 57% yield. The corresponding O-6 β-glycoside was not detected under these conditions consistent with previous work in this area.[4,14] The regioselectivity of the glycosylation was evident from NMR data and confirmed by benzoylation that gave the O-2-benzoate **19**. The ^{1}H NMR chemical shift of the characteristic inositol H-2 proton signal of **19** (dd, $J_{1,2}$, $J_{2,3}$ = 2.6 Hz) resonated at 6.00 ppm whereas that for **18** (dd, $J_{1,2}$ = 2.6 Hz, $J_{2,3}$ = 2.7 Hz) was at 4.18 ppm. Glycosylation of **18** with TCA donor **5** afforded an inseparable mixture of α- and β-glycosides. Methanolic HCl-promoted removal of the TIPS protecting group revealed the primary hydroxyl functionality which fortunately facilitated the separation of anomers and the key pseudotrisaccharide building block **2** was obtained in 75% yield from **18** along with the corresponding β-glycoside in 11% yield. The α-stereochemistry of the two glycosidic bonds in **2** were each confirmed as α from the two anomeric $^1J_{C,H}$ coupling constants of 173 and 176 Hz, whereas the α,β-pseudotrisaccharide isomer was observed to have $^1J_{C,H}$ coupling constants of 161 and 176 Hz.

Synthesis of PIM$_6$. The [3 + 4] glycosylation of **2** and thioglycoside **4** was effected by treatment with diphenylsulfoxide (DPS) and triflic anhydride affording the pseudoheptasaccharide **20** in good yield (Scheme 6). All six signals for the anomeric carbons were well resolved in the 125 MHz ^{13}C NMR spectrum. The newly formed glycosidic bond was established confirmed to have an α-configuration as all six of the $^1J_{C,H}$ coupling constants ranged from 170 to 174 Hz. Routine protecting group manipulations, namely debenzoylation to **21**, benzylation to **22** and deallylation afforded alcohol **23**. It was pleasing to note that the analytical data for the intermediate compounds **22** and **23** matched that previously reported.[10] Phosphitylation of **23** with **3** and subsequent *m*CPBA oxidation gave protected PIM$_6$ **24** as a mixture of diastereoisomers in 82% yield. Our experience with this and similarly prepared lipidated late-stage, PIM intermediates is that they are difficult to isolate in high purity from putative phospholipid reaction byproducts. Standard petroleum ether-based silica chromatography is normally complemented well with a second $CHCl_3$-based purification on silica and this was indeed the case here. However, in this instance a reverse-phase purification step ($CHCl_3$/MeOH) was also introduced to remove minor impurities (RP-HPLC). Global deprotection of **24** under hydrogenolytic conditions and reverse-phase chromatography afforded dipalmitoyl PIM$_6$ (**1**) as its ammonium salt in good yield and high purity (96% by HPLC).

The negative ion high resolution mass spectrum of **1** was consistent with the molecular ion $C_{77}H_{138}O_{43}P$. The choice of solvent was critical for obtaining informative NMR spectra and a 30:60:25 mixture of deuterated chloroform, methanol and water provided the best result. In the ^{13}C NMR spectrum four of the six signals for the anomeric carbons were well resolved with the remaining two overlapping at 102.9 ppm.

We have confirmed that the nonlipid core of **1** is identical to that found in nature. To support this **1** was deacylated with hydrazine to afford deacyl PIM$_6$ (dPIM$_6$). The ^{1}H NMR data recorded in d_6-acetone of the synthetic dPIM$_6$ was consistent with that obtained by deacylation of a natural PIM sample as previously reported by Severn et al. (Figure 1).[25]

Further support for the structural relationship of **1** to natural PIM$_6$ is provided by the comparison of the negative ion ESI-MS^2 spectrum of **1** with that reported by Hsu et al.[26] for a

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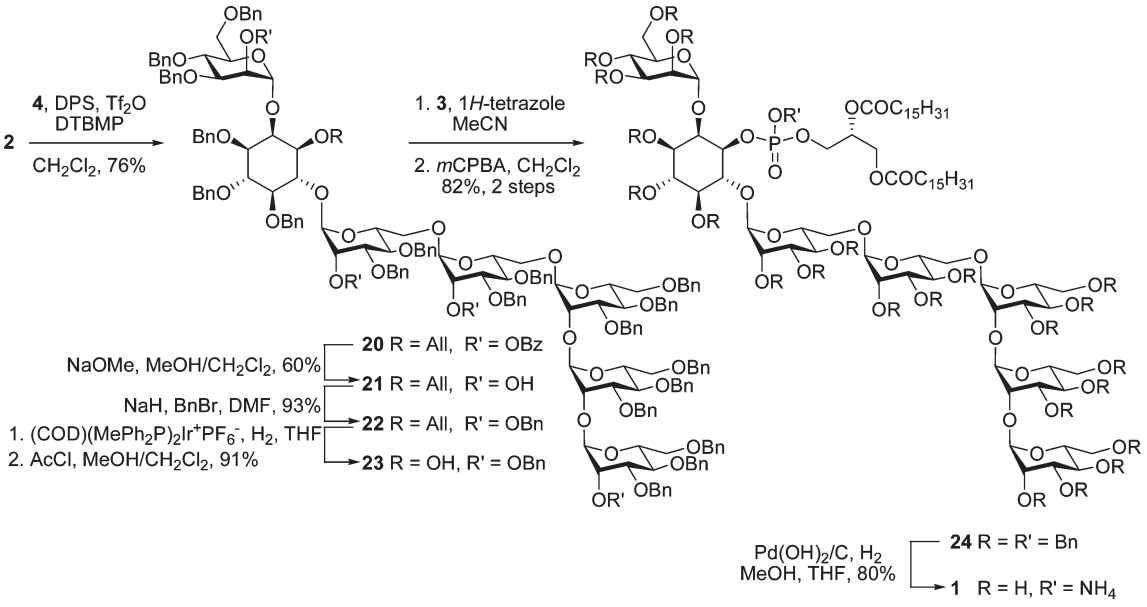
Scheme 6. Synthesis of 1



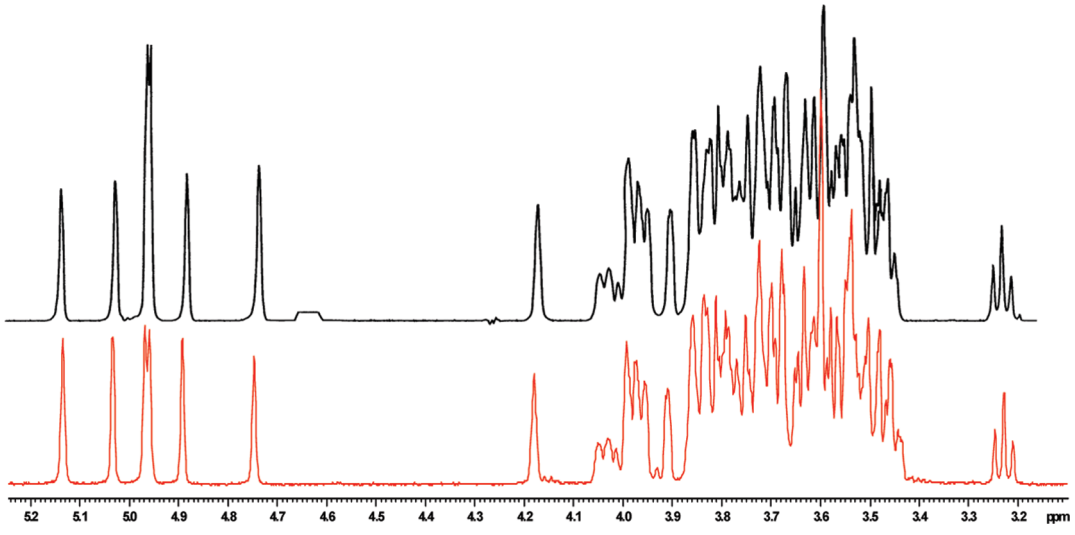


Figure 1. Partial ^{1}H NMR spectra of synthetic dPIM$_6$ (bottom) and dPIM$_6$ from *MTb* (top)[24] recorded in D_2O.

natural PIM$_6$ present in *Mycobacterium bovis*. The MS^2 spectrum of **1** (Supporting Information) selecting for the MS $[M - H]^-$ ion m/z 1781 displays prominent signals at m/z 1525 and 1213 corresponding to loss of the palmitic acid and diacyl glycerol substituents respectively. Taking into account the different acylation state of the natural 19:0/16:0-PIM$_6$ for the spectrum reported by Hsu, a similar fragmentation pattern in the MS^2 spectrum for the MS $[M - H]^-$ ion at m/z = 1823 is observed.

The positive ion MS^2 spectrum of **1** selecting the MS $[M + NH_4]^+$ ion m/z 1800 shows a very clear sequential loss of all six mannose pyranosyl residues after initial loss of ammonia $\{[M\text{-}NH_3\text{-}n(162)]^+\}$ (Supporting Information) and the corresponding signal for the resulting protonated phosphatidyl inositol ion (m/z 811) and the diacylglyceryl ion (m/z 551). This data is consistent with the structure of **1**.

Biological Activity of Synthetic PIM$_6$ in a Human Mixed Lymphocyte Reaction Assay. We have shown previously that natural and synthetic PIM molecules can suppress T cell-driven inflammatory immune responses *in vivo*.[3,4] However, lipid antigen presentation via CD1 molecules is also known to induce expansion of CD1-restricted T cell subsets.[6−8,27,28] Therefore PIM$_6$ has the potential to either suppress or enhance T cell immune responses, or both. Five CD1 molecules, CD1a−e, have been identified in humans,[27] and there is some evidence that both CD1b and CD1d can present PIM molecules.[6,8,7] Furthermore, it appears that efficient presentation of PIM$_6$ to CD1b involves CD1e-dependent intracellular processing.[29−31] As murine systems only express CD1d, we decided to investigate the effect of PIM$_6$ (**1**) on human T cell expansion using a human mixed lymphocyte reaction (MLR), thus ensuring that all relevant CD1 molecules were present for glycolipid processing and

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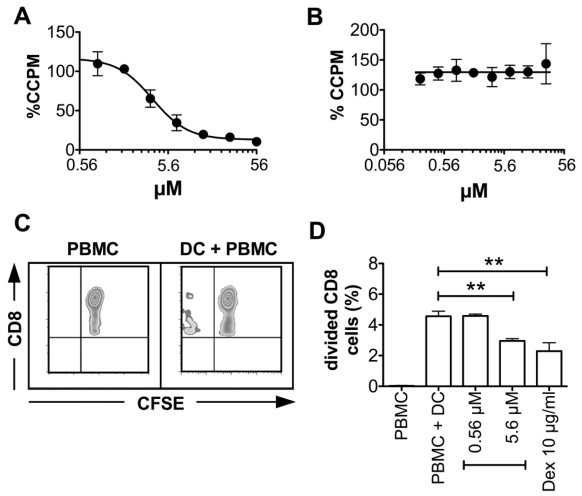

Figure 2. Effect of PIM_6 (**1**) on T cell proliferation in (A) mixed lymphocyte reaction assay and (B) antibody-induced T cell expansion assay. (C) Flow cytometry plot of $CD8^+$ T cell expansion in MLR. (D) PIM_6-induced suppression of $CD8^+$ T cell expansion in MLR (Dex-Dexamethasone).

presentation. This assay system was used to determine whether PIM_6 treatment suppressed or enhanced human T cell expansion *in vitro*.

As shown in Figure 2A, incubation of dendritic cells (DC) and peripheral blood monocytes (PBMC) from different donors induced an allogeneic-driven T cell expansion that was inhibited by PIM_6 (**1**) in a dose-dependent manner (IC_{50} 4.9 μM). Previous work in murine systems showed that direct interaction of PIM molecules with T cells can suppress antibody-induced T cell proliferation *in vitro*.[32] Interestingly, **1** did not directly suppress human T cell expansion induced by anti-CD3/anti-CD28 (Figure 2B) indicating that PIM_6-dependent suppression of T cell expansion in the MLR was DC mediated. Further analysis of carboxyfluorescein succinimidyl ester-labeled T cells by flow cytometry showed that the $CD8^+$ T cells were the predominant population expanding in the MLR at the time of analysis (Figure 2C). PIM_6 (**1**) treatment suppressed $CD8^+$ T cell expansion in a dose-dependent manner and PIM_6-dependent inhibition was comparable to the control anti-inflammatory steroid dexamethasone (Figure 2D). Control $CD4^+$ T cell expansion was not observed (data not shown). It has been shown that in mice the PIM_6 glycan has adjuvant activity when conjugated to the carrier protein KLH.[10] However, our results clearly show the synthetic phosphoglycolipid **1** compound has suppressive activity in the human cell-based assay. These results are potentially representative of the known immunosuppressive effects of *Mtb* when resident in human host cells.

CONCLUSION

In this study, we report the first synthesis of dipalmitoyl PIM_6 **1** utilizing a thio-glycoside donor in the key [3 + 4] glycosylation step. Importantly, common purification difficulties associated with lipoglycans were overcome by employing a combination of reverse phase chromatography and analytical LCMS techniques to deliver target compound **1** in greater than 95% purity. We also report for the first time that a synthetic PIM_6, found in nature,[33] exhibits DC-mediated suppression of human $CD8^+$ T cell proliferation.

EXPERIMENTAL SECTION

General Experimental. Specific optical rotations, given in 10^{-1} deg cm^2 g^{-1}, were measured at ambient temperature using either a Perkin-Elmer 241 or a Rudolph Autopol IV polarimeter with a cell of path length 1.0 dm. ^{1}H NMR spectra were obtained at 300 or 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. Water suppression NMR spectra were collected with the standard Bruker supplied ZGCPPR pulse sequence which employs presaturation with an approximately 7 Hz rf field for a period of 2 s prior to excitation with an on resonance composite excitation pulse (Bax, A. *J. Magn. Reson.* 1985, *65*, 142−145). ^{13}C NMR spectra were recorded at 75 or 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 2 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. Thin layer chromatography (TLC) was performed on Merck silica gel DC Alurolle Kieselgel $60F_{254}$ plates and were visualized 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) unless otherwise stated. All chromatography solvents were AR-grade. Petroleum ether used was bp 60−80° range. Anhydrous solvents were sourced from Aldrich and unless stated were used without further treatment. 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. Analytical HPLC analyses were performed on one of two systems. **HPLC System 1** Column: Phenomenex Luna 5 μm C18 4.6 × 250 mm. Detection: UV at 220 nm. Solvents; A water +0.1% TFA; B 1:1 CH_3CN/THF +0.1% TFA. Gradient Program; 0−20 min 10−0% A, 90−100% B. Column temperature; 40 °C. **HPLC System 2** HPLC: Column: Phenomenex Synergi 4 μm Fusion-RP (80 Å, 3.0 × 250 mm). Detection: ESA Corona charged aerosol detector (Filter = none). Solvents; A H_2O; B Water containing 50 mM NH_4OAc; C Methanol. Gradient Program; 0−10 min 10−0% A, 10% B, 80−90%C, 10−28 min hold (0% A, 10% B, 90% C), 28−30 min return to starting conditions. Column temperature; 40 °C. Chromatograms were corrected by subtraction of a blank injection trace prior to integrating signals after a 3.5 min cutoff.

Phenyl 2-*O*-Benzoyl-3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (9). A mixture of diol **8**[18] (8.40 g, 23.3 mmol) and Bu_2SnO (6.10 g, 24.5 mmol) were refluxed in dry toluene (100 mL) under an atmosphere of argon. After 2 h, the resulting clear solution was allowed to cool to rt and the solvent removed. The residue was dried under high-vacuum then placed under argon. Cesium fluoride (3.72 g, 24.5 mmol) was added, then dry DMF (100 mL) was cannulated onto the mixture. Benzyl bromide (3.42 mL, 25.6 mmol) was added and the reaction stirred at rt overnight. The reaction mixture was diluted with Et_2O (200 mL) and washed with H_2O (2 × 200 mL) and saturated NaCl solution (200 mL), then dried ($MgSO_4$), filtered and concentrated to a residue that was dissolved in CH_2Cl_2 and adsorbed on silica gel. This was purified by chromatography on silica gel containing 10% w/w potassium fluoride[20] (Et_2O/pet. ether =1:9 to 2:8) to afford phenyl 3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (9.43 g, 90%) as a solid. $[\alpha]_D^{20}$ = +206 (c 1.58, $CHCl_3$) [Lit.[16] $[\alpha]_D$ = +228 (c 1.07, $CHCl_3$)]. ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.88 (br s, 1H), 3.86 (dd, J = 10.3, 10.3 Hz, 1H), 3.97 (dd, J = 9.6, 3.3 Hz, 1H), 4.19 (dd, J = 9.5, 9.5 Hz, 1H), 4.21 (dd, J = 1.3, 4.9 Hz, 1H), 4.28−4.30 (m, 1H), 4.31−4.37 (m, 1H), 4.75 (d, J =

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11.8 Hz, 1H), 4.90 (d, J = 11.8 Hz, 1H), 5.60 (br s, 1H), 5.63 (s, 1H), 7.25−7.53 (m, 15H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 64.7, 68.6, 71.5, 73.7, 75.8, 79.1, 87.9, 101.7, 126.1, 127.7, 127.9, 128.1, 128.3, 128.6, 129.0, 129.2, 131.8, 133.4, 137.5, 137.8. HRMS-ESI $[M + Na]^+$ calculated for $C_{26}H_{26}O_5SNa$: 473.1399. Found 473.1418. Benzoyl chloride (7.23 mL, 62.1 mmol) and DMAP (0.253 g, 2.07 mmol) were added to a solution of the intermediate thiomannoside (9.35 g, 20.7 mmol) prepared above in dry pyridine (75 mL) at rt and stirred overnight. H_2O was then added and the reaction stirred for 1 h to ensure that no benzoyl chloride remained. The reaction was diluted into CH_2Cl_2/Et_2O (1:9, 150 mL) and washed with 0.5 M HCl (3 × 200 mL), saturated $NaHCO_3$ (100 mL), saturated NaCl (100 mL) then dried ($MgSO_4$), filtered and the solvent removed. The residue was purified on silica gel (pet. ether to EtOAc/pet. ether = 1:9) to afford the title compound **9** (9.29 g, 81%) as a white solid. $[\alpha]_D^{20}$ = +64 (c 1.94, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.12−8.06 (m, 2H), 7.60−7.21 (m, 18H), 5.83 (dd, J = 3.4, 1.4 Hz, 1H), 5.69 (s, 1H), 5.61 (d, J = 1.4, 1.4 Hz, 1H), 4.77 (d, J = 12.4 Hz, 1H), 4.72 (d, J = 12.4 Hz, 1H), 4.48−4.38 (m, 1H), 4.31−4.23 (m, 2H), 4.12 (dd, J = 9.8, 3.4 HZ, 1H), 3.91 (dd, J = 10.3, 10.3 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.7, 137.8, 137.5, 133.4, 133.4, 132.2, 130.0, 129.7, 129.3, 129.0, 128.5, 128.4, 128.3, 128.1, 127.7, 126.2, 101.8, 87.3, 79.0, 74.3, 72.3, 72.1, 68.6, 65.3. HRMS-ESI $[M + Na]^+$ calculated for $C_{33}H_{30}O_6SNa$: 577.1661. Found 577.1664. Analysis calculated for $C_{33}H_{30}O_6S$: C 71.46, H 5.45. Found C 71.52, H 5.46.

Phenyl 2-*O*-Benzoyl-3,4-di-*O*-benzyl-1-thio-α-D-mannopyranoside (7). *Method A.* A solution of BH_3·THF (67.5 mL, 1 M in THF) followed by a solution of Bu_2BOTf (13.5 mL, 1 M in CH_2Cl_2) was added to a stirred solution of benzylidene acetal **9** (7.57 g, 13.5 mmol) in dry THF (50 mL) at 0 °C. After 1 h the reaction was quenched by the careful addition of H_2O. Once the evolution of H_2 ceased, 0.5 M HCl (50 mL) was then added and stirred for a further 15 min. The resultant mixture was extracted into Et_2O and the organic extract washed with 0.5 M HCl (100 mL), saturated $NaHCO_3$ (2 × 100 mL), saturated NaCl (100 mL) then dried ($MgSO_4$), filtered and the solvent removed. The residue was purified on silica gel (EtOAc/pet. ether =1:9 to 1:4) to afford the title compound **7** (6.72 g, 90%). $[\alpha]_D^{20}$ = +82 (c 1.72, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.08−8.04 (m, 2H), 7.61−7.55 (m, 1H), 7.49−7.42 (m, 4H), 7.37−7.23 (m, 13H), 5.84−5.82 (m, 1H), 5.58 (d, J = 1.6 Hz, 1H), 4.94 (d, J = 10.9 Hz, 1H), 4.79 (d, J = 11.5 Hz, 1H), 4.68 (d, J = 10.9 Hz, 1H), 4.62 (d, J = 11.5 Hz, 1H), 4.28−4.19 (m, 1H), 4.11−4.02 (m, 2H), 3.89−3.79 (m, 2H), 1.79 (t, J = 6.8 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.6, 138.2, 137.7, 133.42, 133.37, 132.3, 130.0, 129.8, 129.3, 128.6, 128.52, 128.49, 128.24, 128.17, 128.1, 127.94, 127.89, 86.5, 78.5, 75.4, 74.2, 73.1, 71.8, 70.8, 62.1. HRMS-ESI $[M + Na]^+$ calculated for $C_{33}H_{32}O_6SNa$: 579.1817. Found 579.1842. Analysis calculated for $C_{33}H_{30}O_6S$: C 71.20, H 5.79. Found C 71.41, H 5.86.

Method B. A solution of BH_3·THF (1.00 mL, 1 M in THF) was added to a solution of benzylidene acetal **9** (111 mg, 0.200 mmol) in dry CH_2Cl_2 at rt. After stirring for 5 min, $Sc(OTf)_3$ (15 mg, 0.030 mmol) was added and the reaction stirred overnight. Et_3N was added to quench the reaction, which was then diluted and coevaporated with MeOH (3 × 30 mL). The residue was purified on silica gel (EtOAc/pet. ether = 1:9 to 1:4) to afford the title compound **7** (111 mg, 100%).

Phenyl 2-*O*-benzoyl-3,4-di-*O*-benzyl-6-*O*-triisopropylsilyl-1-thio-α-D-mannopyranoside (10). Triisopropylsilyl chloride (1.68 mL, 7.84 mmol) was added to a solution of alcohol **7** (3.62 g, 6.53 mmol) and imidazole (667 mg, 9.80 mmol) in dry DMF (50 mL) and stirred at rt overnight. H_2O was added and then the reaction was diluted in Et_2O. The mixture was washed with 0.2 M HCl (100 mL), saturated $NaHCO_3$ (2 × 100 mL), saturated NaCl (100 mL) then dried ($MgSO_4$), filtered and the solvent removed. The residue was purified on silica gel (EtOAc/pet. ether =1:9) to afford the title compound **10** (4.25 g, 91%). $[\alpha]_D^{20}$ = +61 (c 1.74, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.12−8.07 (m, 2H), 7.59−7.52 (m, 1H), 7.50−7.38 (m, 4H), 7.35−7.19 (m, 13H), 5.84 (dd, J = 3.1, 1.5 Hz, 1H), 5.61 (d, J = 1.5 Hz, 1H), 4.92 (d, J = 10.7 Hz, 1H), 4.79 (d, J = 11.3 Hz, 1H), 4.74 (d, J = 10.7 Hz, 1H), 4.61 (d, J = 11.3 Hz, 1H), 4.24−4.07 (m, 3H), 4.05 (dd, J = 8.7, 3.1 Hz, 1H), 3.95 (dd, J = 11.1, 1.2 Hz, 1H), 1.12−1.03 (m, 21H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.7, 138.6, 137.9, 134.3, 133.2, 131.5, 130.1, 130.0, 129.0, 128.41, 128.36, 128.2, 128.1, 127.8, 127.7, 127.5, 86.3, 78.8, 75.5, 74.4, 74.1, 71.9, 71.1, 62.6, 18.1, 12.1. HRMS-ESI $[M + Na]^+$ calculated for $C_{42}H_{52}O_6SSiNa$: 735.3152. Found 735.3144.

Phenyl 2-*O*-(2-Azidomethylbenzoyl)-3,4,6-tri-*O*-benzyl-1-thio-α-D-mannopyranoside (12). A solution of alcohol **11** (498 mg, 0.918 mmol) in dry pyridine (5 mL) was canulated onto freshly prepared 2-(azidomethyl)benzoyl chloride[16] (2.98 mmol) and left to stir at rt for 2 h. The reaction was quenched by the addition of H_2O (10 mL), and then extracted into Et_2O (50 mL). The combined organic phases were washed with 0.5 M HCl (2 × 50 mL), saturated $NaHCO_3$ (50 mL), and saturated NaCl (50 mL), then dried ($MgSO_4$), filtered and the solvent removed to give a crude material which was purified on silica gel (EtOAc/pet. ether = 1:4) to yield the title compound **12** (507 mg, 79%). $[\alpha]_D^{20}$ = +63 (c 0.72, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.06−8.02 (m, 1H), 7.57−7.44 (m, 4H), 7.36−7.20 (m, 19H), 5.83 (dd, J = 2.7, 1.9 Hz, 1H), 5.65 (d, J = 1.7 Hz, 1H), 4.90 (d, J = 10.8 Hz, 1H), 4.79 (d, J = 10.8 Hz, 1H), 4.74 (s, 2H), 4.66 (d, J = 11.9 Hz, 1H), 4.65 (d, J = 11.4 Hz, 1H), 4.48 (d, J = 11.9 Hz, 1H), 4.42 (m, 1H), 4.12 (dd, J = 9.3, 9.3 Hz, 1H), 4.06 (dd, J = 9.2, 2.9 Hz, 1H), 3.91 (dd, J = 10.9, 4.8 Hz, 1H), 3.76 (dd, J = 10.9, 1.9 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.8, 138.3, 13737, 137.6, 133.6, 133.0, 132.0, 131.7, 129.6, 129.2, 128.6−127.5, 86.3, 78.6, 75.4, 74.7, 73.5, 72.6, 71.9, 71.2, 69.0, 53.1. HRMS-ESI $[M + Na]^+$ calculated for $C_{41}H_{39}N_3O_6SNa$: 724.2457. Found 724.2477.

2-*O*-(2-Azidomethylbenzoyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl Trichloroacetimidate (6). *N*-Iodosuccinamide (379 mg, 1.68 mmol), then triflic acid (5 μL, 0.056 mmol) were added to a solution of thioglycoside **12** (394 mg, 0.561 mmol) in H_2O/acetone (1:9, 20 mL). After stirring at rt for 2 h the reaction was extracted with CH_2Cl_2 (50 mL) and washed with 10% aqueous $Na_2S_2O_3$, saturated NaCl (50 mL), dried ($MgSO_4$), and the solvent removed. The crude residue was dissolved in dry CH_2Cl_2 (20 mL) and cooled to 0 °C. Trichloroacetonitrile (563 μL, 5.61 mmol) and DBU (8 μL, 0.056 mmol) were added and the reaction stirred for 30 min at this temperature. The solvent was then removed and the crude residue was purified on silica gel (EtOAc/pet. ether = 1:9 to 1:4) to afford the title compound **6** (250 mg, 59%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.71 (s, 1H), 8.08−8.05 (m, 1H), 7.58−7.46 (m, 2H), 7.35−7.20 (m, 17H), 6.43 (d, J = 1.9 Hz, 1H), 5.71 (dd, J = 2.5, 2.5 Hz, 1H), 4.90 (d, J = 10.8 Hz, 1H), 4.80 (d, J = 11.4 Hz, 1H), 4.76 (s, 2H), 4.67 d, J = 11.9 Hz, 1H), 4.66 (d, J = 11.4 Hz, 1H), 4.60 (d, J = 10.8 Hz, 1H), 4.51 (d, J = 11.9 Hz, 1H), 4.22 (dd, J = 9.4, 9.3, Hz, 1H), 4.16 (dd, J = 9.3, 2.9 Hz, 1H), 4.07−4.01 (m, 1H), 3.90 (dd, J = 11.1, 3.4 Hz, 1H), 3.76 (dd, J = 11.1, 1.7 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.5, 160.0, 138.3, 138.1, 137.8, 137.6, 133.1, 131.7, 129.6, 128.6−127.5, 95.3, 90.8, 77.3, 75.6, 74.5, 73.8, 73.5, 72.1, 68.5, 68.1, 53.1. HRMS-ESI $[M + Na]^+$ calculated for $C_{37}H_{35}Cl_3N_4O_7Na$: 775.1469. Found 775.1500.

Phenyl (2-*O*-(2-Azidomethylbenzoyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-2-*O*-benzoyl-3,4-di-*O*-benzyl-1-thio-α-D-mannopyranoside (13). TMSOTf (24 μL, 0.131 mmol) was added to a mixture of alcohol **7** (0.713 g, 1.28 mmol), trichloroacetimidate **6** (0.991 g, 1.31 mmol) and 4 Å molecular sieves in dry Et_2O at −30 °C. After stirring for 30 min the reaction was quenched with Et_3N, filtered through Celite and evaporated to dryness. The crude residue was purified on silica gel (EtOAc/pet. ether =1:9 to 1:4 to 3:7) to afford the title compound **13** (1.374 g, 94%) as an oil. $[\alpha]_D^{20}$= +41

(c 1.42, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ 8.11−8.08 (m, 2H), 8.05−8.02 (m, 1H), 7.54−7.10 (m, 36H), 5.88 (dd, J = 2.9, 1.7 Hz, 1H), 5.68 (m, 1H), 5.60 (d, J = 1.6 Hz, 1H), 5.08 (d, J = 1.5 Hz, 1H), 4.90 (d, J = 11.1 Hz, 1H), 4.90 (d, J = 10.9 Hz, 1H), 4.86 (d, J = 10.9 Hz, 1H), 4.82 (d, J = 11.2 Hz, 1H), 4.74−4.67 (m, 3H), 4.62 (d, J = 11.1 Hz, 1H), 4.59 (d, J = 11.1 Hz, 1H), 4.53−4.38 (m, 5H), 4.08−4.00 (m, 4H), 3.94 (dd, J = 9.7, 9.5 Hz, 1H), 3.85−3.72 (m, 3H), 3.63 (dd, J = 10.6, 1.5 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.68, 165.67, 138.5, 138.3, 138.2, 137.8, 137.57, 137.56, 133.8, 133.4, 132.9, 131.7, 131.5, 129.9, 129.5, 129.3, 128.6, 128.5, 128.4−127.7, 127.6, 127.5, 97.9 ($^1J_{C,H}$ = 171.6 Hz), 86.5 ($^1J_{C,H}$ = 167.6 Hz), 78.8, 78.0, 75.29, 75.25, 74.4, 74.3, 73.4, 71.9, 71.8, 71.7, 71.6, 70.7, 69.1, 68.7, 66.6, 53.1. HRMS-ESI $[M + Na]^+$ calculated for $C_{68}H_{65}N_3O_{12}SNa$: 1170.4187. Found 1170.4226.

Phenyl (3,4,6-Tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-2-*O*-benzoyl-3,4-di-*O*-benzyl-1-thio-α-D-mannopyranoside (14). Bu_3P (1.50 mL, 6.0 mmol) was added to a solution of AZMB ester **13** (1.374 g, 1.20 mmol) in H_2O/THF (1:9, 40 mL). After stirring at rt for 1 h the solvent was removed with the aid of toluene to azeotrope all the remaining H_2O. The crude residue was purified on silica gel (EtOAc/pet. ether =1:4 to 3:7) to afford the title compound **14** (0.975 g, 83%) as an oil. $[\alpha]_D^{20}$= +80 (c 1.44, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ 8.07−8.04 (m, 2H), 7.52−7.13 (m, 33H), 5.86 (dd, J = 2.8, 1.7 Hz, 1H), 5.59 (d, J = 1.5 Hz, 1H), 5.06 (d, J = 1.3 Hz, 1H), 4.91 (d, J = 11.0 Hz, 1H), 4.84−4.81 (m 2H), 4.63−4.57 (m, 3H), 4.55 (d, J = 11.1 Hz, 1H), 4.49 (d, J = 10.8 Hz, 1H), 4.45 (d, J = 12.2 Hz, 1H), 4.40−4.36 (m, 1H), 4.09−4.03 (m, 3H), 3.99 (dd, J = 11.2, 5.1 Hz, 1H), 3.94−3.78 (m, 5H), 3.69 (dd, J = 10.8, 4.1 Hz), 3.61 (dd, J = 10.6, 1.5 Hz, 1H), 2.44 (d, J = 2.4 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.6, 138.5, 138.19, 138.17, 137.8, 137.6, 133.9, 133.3, 131.5, 129.88, 129.86,129.2,128.54, 128.52, 128.46, 128.4, 128.33, 128.30, 128.27, 127.94, 127.93, 127.89, 127.81, 127.77, 127.7, 127.61, 127.58, 99.4, 86.4, 80.0, 78.7, 75.3, 75.1, 74.5, 74.2, 73.4, 72.2, 71.9, 71.7, 71.2, 70.7, 68.7, 68.3, 66.2. HRMS-ESI $[M + Na]^+$ calculated for $C_{60}H_{60}O_{11}SNa$: 1011.3754. Found 1011.3795.

Phenyl (2-*O*-(2-Azidomethylbenzoyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-2-*O*-benzoyl-3,4-di-*O*-benzyl-1-thio-α-D-mannopyranoside (15). TMSOTf (19 μL, 0.102 mmol) was added to a mixture of alcohol **14** (0.975 g, 0.986 mmol), trichloroacetimidate **6** (0.772 g, 1.02 mmol) and 4 Å molecular sieves in dry Et_2O at −30 °C. After stirring for 30 min the reaction mixture was quenched with Et_3N, filtered through Celite and evaporated to dryness. The residue was purified on silica gel (EtOAc/pet. ether = 1:4) to afford the title compound **15** (1.317 g, 94%) as a foam. $[\alpha]_D^{20}$= +29 (c 1.30, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 8.06−8.03 (m, 2H), 8.02−7.11 (m, 1H), 7.56−7.07 (m, 51H), 5.83 (dd, J = 2.9, 1.7 Hz, 1H), 5.72 (dd, J = 2.7, 1.8 Hz, 1H), 5.57 (d, J = 1.5 Hz, 1H), 5.16 (d, J = 1.5 Hz, 1H), 4.99 (d, J = 1.6 Hz, 1H), 4.89 (d, J = 11.3 Hz, 1H), 4.88−4.83 (m, 2H), 4.80 (d, J = 11.2 Hz, 1H), 4.72−4.68 (m, 3H), 4.63−4.33 (m, 12H), 4.11−3.96 (m, 6H), 3.94−3.87 (m, 3H), 3.82−3.76 (m, 2H), 3.71−3.58 (m, 4H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.8, 165.6, 138.6, 138.53, 138.47, 138.4, 138.19, 138.16, 138.1, 137.6, 137.5, 134.0, 133.3, 132.8, 131.7, 131.5, 129.8, 129.4, 129.3, 128.7−127.4, 99.4, 99.0, 86.4, 79.6, 78.8, 78.0, 75.23, 75.19 (2 × C), 75.1, 74.6, 74.44, 74.39, 73.4, 73.2, 72.1, 72.04 (2 × C), 72.02, 71.8, 70.7, 69.5, 69.0, 68.9, 66.6, 53.1. Gated decoupled ^{13}C NMR (75 MHz, $CDCl_3$) selected data δ 99.4 $^1J_{C,H}$ = 172.3 Hz, 99.0 $^1J_{C,H}$ = 170.7 Hz, 86.4 $^1J_{C,H}$ = 169.3 Hz. HRMS-ESI $[M + Na]^+$ calculated for $C_{95}H_{93}N_3O_{17}SNa$: 1602.6123. Found 1602.6140.

Phenyl (3,4,6-Tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-manno-pyranosyl)-(1→6)-2-*O*-benzoyl-3,4-di-*O*-benzyl-1-thio-α-D-mannopyranoside (16). Bu_3P (1.04 mL, 4.17 mmol) was added to a solution of AZMB ester **15** (1.317 g, 0.833 mmol) in H_2O/THF (1:9, 40 mL). After stirring at rt for 1 h the solvent was removed with the aid of toluene to azeotrope all the remaining H_2O. The crude residue was purified on silica gel (EtOAc/pet. ether = 1:9 to 1:4 to 3:7) to afford the title compound **16** (0.809 g, 68%) as a foam. $[\alpha]_D^{20}$ = +58 (c 0.64, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ 8.06−8.03 (m, 2H), 7.47−7.07 (m, 48H), 5.82 (dd, J = 2.9, 1.8 Hz, 1H), 5.52 (d, J = 1.4 Hz, 1H), 5.13 (d, J = 1.3 Hz, 1H), 4.98 (d, J = 1.5 Hz, 1H), 4.87−4.77 (m, 4H), 4.61 (d, J = 12.2 Hzm 1H), 4.58−4.44 (m, 9H), 4.42, d, J = 12.2 Hz, 1H), 4.37 (d, J = 12.2 Hz, 1H), 4.32 (m, 1H), 4.12−4.07 (m, 2H), 4.00 (dd, J = 9.1, 3.0 Hz, 1H), 3.97−3.82 (m, 7H), 3.76−3.67 (m, 3H), 3.64−3.55 (m, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.7, 138.7, 138.6, 138.5, 138.27, 138.26, 138.11, 138.10, 137.6, 134.0, 133.4, 131.5, 129.91, 129.88, 129.3, 128.6−127.4, 101.1, 99.2, 88.5, 80.0, 79.6, 78.9, 75.19, 75.16, 75.1, 74.7 (2 × C), 74.5, 74.4, 73.4, 73.2, 72.2, 72.14, 72.11, 72.0, 71.8, 71.6, 70.7, 69.1, 68.9, 68.8, 66.7. HRMS-ESI $[M + Na]^+$ calculated for $C_{87}H_{88}O_{16}SNa$: 1443.5691. Found 1443.5649

Phenyl (2-*O*-Benzoyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-2-*O*-benzoyl-3,4-di-*O*-benzyl-1-thio-α-D-mannopyranoside (4). TMSOTf (12 μL, 0.068 mmol) was added to a mixture of alcohol **16** (0.846 g, 0.595 mmol), trichloroacetimidate **5** (0.467 g, 0.668 mmol) and 4 Å molecular sieves in dry Et_2O at −30 °C. After stirring for 30 min the reaction mixture was quenched with Et_3N, filtered through Celite and evaporated to dryness. The crude residue was purified on silica gel (EtOAc/pet. ether = 1:4) to give a product containing fraction that was further purified on silica gel (Et_2O/toluene =1:49 to 1:24) to afford the title compound **4** (0.656 g, 56%) as a foam. $[\alpha]_D^{20}$ = +26 (c 1.46, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ 8.08−8.02 (m, 4H), 7.57−6.89 (m, 66H), 5.83 (dd, J = 3.1, 1.7 Hz, 1H), 5.75 (m, 1H), 5.51 (d, J = 1.6 Hz, 1H), 5.23 (d, J = 1.9 Hz, 1H), 5.11 (d, J = 1.9 Hz, 1H), 4.97 (d, J = 1.9 Hz, 1H), 4.86−4.78 (m, 5H), 4.73 (d, J = 11.1 Hz, 1H), 4.62−4.29 (m, 17H), 4.13−4.04 (m, 4H), 4.00 (dd, J = 9.0, 2.9 Hz, 1H), 3.97−3.78 (m, 8H), 3.77−3.53 (m, 8H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.6, 165.4, 138.7, 138.64, 138.59, 138.53, 138.49, 138.4, 138.22, 138.16, 138.1, 137.6, 134.0, 133.4, 133.1, 131.5, 130.1, 130.0, 129.9, 129.3, 128.6−127.3, 100.7 ($^1J_{C,H}$ = 173 Hz), 99.5 ($^1J_{C,H}$ = 173 Hz), 99.1 ($^1J_{C,H}$ = 172 Hz), 86.4 ($^1J_{C,H}$ = 169 Hz), 79.6, 79.4, 78.9, 78.2, 75.5, 75.3, 75.17, 75.16, 75.1, 74.8, 74.7, 74.5, 74.3, 73.4, 73.23, 73.20, 72.3, 72.2, 72.1, 72.0, 71.73, 71.65, 70.7, 69.3, 69.1, 69.0, 66.8. HRMS-ESI $[M + Na]^+$ calculated for $C_{121}H_{120}O_{22}SNa$: 1979.7890. Found 1979.7808. Further elution gave the corresponding tetrasaccharide sulfone (0.079 g, 7%). HRMS-ESI $[M+Na]^+$ calculated for $C_{121}H_{120}O_{24}SNa$: 2011.7788. Found 2011.7797. This was followed by the corresponding tetrasaccharide sulfoxide (0.185 g, 16%). HRMS-ESI $[M + Na]^+$ calculated for $C_{121}H_{120}O_{23}SNa$: 1995.7839. Found 1995.7793.

1-*O*-Allyl-6-*O*-(2-*O*-benzoyl-3,4-di-*O*-benzyl-6-*O*-triisopropylsilyl-α-D-mannopyranosyl)-3,4,5-tri-*O*-benzyl-D-*myo*-inositol (18). Tf_2O (144 μL, 0.854 mmol) was added to a solution of thioglycoside donor **10** (435 mg, 0.610 mmol), diphenyl sulfoxide (345 mg, 1.71 mmol), and DTBMP (376 mg, 1.83 mmol) in CH_2Cl_2 (15 mL) at −60 °C. After stirring for 5 min at this temperature, a precooled (−60 °C) solution of diol **17** (360 mg, 0.734 mmol) in CH_2Cl_2 (5 mL) was added. The reaction mixture was stirred for a further 30 min then warmed to rt. Saturated $NaHCO_3$ was added to quench the reaction. The organic layer was separated, washed with saturated NaCl (30 mL), then dried ($MgSO_4$), filtered and the solvent removed. The crude residue was purified on silica gel (EtOAc/pet. ether = 1:9 to 1:4) to afford the title compound **18** (383 mg, 57%) as a white foam. ^1H NMR (300 MHz, $CDCl_3$) δ 8.11−8.05 (m, 2H), 7.57−7.07 (m, 28H), 6.03−5.89 (m, 1H), 5.65 (dd, J = 3.1, 1.9 Hz, 1H), 5.42 (d, J = 1.8 Hz, 1H), 5.25 (dd, J = 17.1, 1.3 Hz, 1H), 5.16 (dd, J = 10.2, 1.3 Hz, 1H), 4.94−4.58 (m, 10H), 4.18 (dd, J = 2.7, 2.6 Hz, 1H), 4.15−3.98 (m, 6H), 3.88−3.82 (m, 1H), 3.54−3.51 (m, 2H), 3.41 (dd, J = 9.7, 2.8 Hz, 1H), 3.35−3.26 (m, 2H), 2.40 (s, 1H), 1.05−1.01 (m, 21H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.8, 139.4, 138.7, 138.5, 138.3, 136.0, 134.4, 133.0, 130.3, 130.1, 128.7−127.2, 118.4,

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98.6, 81.5, 81.3, 80.6, 78.8, 78.4, 76.0, 75.9, 75.6, 75.0, 74.1, 73.8, 72.5, 71.6, 71.4, 69.5, 66.9, 62.1, 18.1, 12.1. HRMS-ESI $[M + Na]^+$ calculated for $C_{66}H_{80}O_{12}SiNa$: 1115.5317. Found 1115.5292.

1-*O*-Allyl-2-*O*-benzoyl-6-*O*-(2-*O*-benzoyl-3,4-di-*O*-benzyl-6-*O*-triisopropylsilyl-α-D-mannopyranosyl)-3,4,5-tri-*O*-benzyl-D-*myo*-inositol (19). Benzoyl chloride (54 μL, 0.470 mmol) was added to a solution of alcohol **18** (51 mg, 0.047 mmol) and DMAP (6 mg, 0.049 mmol) in dry pyridine (10 mL) at rt, and the mixture was stirred for 48 h. H_2O was added and the reaction stirred for a further 30 min then diluted with Et_2O (75 mL). The mixture was washed with 0.5 M HCl (3 × 50 mL), saturated $NaHCO_3$ (100 mL), saturated NaCl (100 mL) then dried ($MgSO_4$), and the solvent removed. The crude residue was purified on silica gel (EtOAc/pet. ether =1:9 to 1:4) to afford the title compound **19** (42 mg, 74%). ^1H NMR (300 MHz, $CDCl_3$) δ 8.09−8.00 (m, 4H), 7.60−7.06 (m, 31H), 6.00 (dd, *J* = 2.6, 2.6 Hz, 1H), 6.00−5.86 (m, 1H), 5.68 (dd, *J* = 3.1, 1.9 Hz, 1H), 5.45 (d, *J* = 1.7 Hz, 1H), 5.23 (dd, *J* = 17.1, 1.5 Hz, 1H), 5.13 (dd, *J* = 10.2, 1.5 Hz, 1H), 4.99−4.77 (m, 6H), 4.69−4.54 (m, 4H), 4.23−3.90 (m, 7H), 3.70−3.56 (m, 3H), 3.49 (dd, *J* = 9.7, 2.7 Hz, 1H), 3.43 (dd, *J* = 9.7, 9.5 Hz, 1H), 1.06−1.02 (m, 21H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.9, 165.7, 139.4, 138.6, 138.4, 138.0, 137.7, 134.3, 133.1, 132.9, 130.3−129.9, 128.5, 127.2, 118.4, 98.6, 81.9, 81.3, 78.7, 78.44, 78.36, 76.3, 76.1, 75.9, 75.1, 74.1, 72.5, 72.2, 71.6, 71.1, 69.3, 66.9, 62.1, 18.2−18.0, 12.1. HRMS-ESI $[M + Na]^+$ calculated for $C_{73}H_{84}O_{13}SiNa$: 1219.5579. Found 1219.5536.

1-*O*-Allyl-2-*O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-6-*O*-(2-*O*-benzoyl-3,4-di-*O*-benzyl-α-D-mannopyranosyl)-3,4,5-tri-*O*-benzyl-D-*myo*-inositol (2). TMSOTf (10 μL, 0.058 mmol) was added to a mixture of alcohol **18** (384 mg, 0.351 mmol), trichloroacetimidate **5** (405 mg, 0.579 mmol) and 4 Å molecular sieves in dry Et_2O at −30 °C. After stirring for 30 min the reaction was quenched with Et_3N, filtered through Celite and evaporated to dryness. The crude residue was purified on silica gel (acetone/toluene =1:49 to 1:24) to afford the pseudotrisaccharide as an inseparable anomeric mixture. This was dissolved in CH_2Cl_2/MeOH (3:7, 20 mL) and cooled to 0 °C. Acetyl chloride (501 μL, 7.02 mmol) was added and the reaction was allowed to warm to rt and stirred overnight after which it was quenched with Et_3N (986 μL, 7.02 mmol) and evaporated to dryness. The crude residue was purified on silica gel (EtOAc/pet. ether =1:9 to 3:7) to give a product containing fraction that was further purified on silica gel (Et_2O/CH_2Cl_2 = 1:49 to 1:24) to afford the title compound **2** (386 mg, 75%). $[\alpha]_D^{20} = -1.3$ (c 1.52, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ 8.09−7.99 (m, 4H), 7.58−7.08 (m, 46H), 5.95−5.81 (m, 1H), 5.72 (dd, *J* = 2.4, 2.3 Hz, 1H), 5.68 (dd, *J* = 3.0, 1.9 Hz, 1H), 5.58 (d, *J* = 1.8 Hz, 1H), 5.26 (d, *J* = 1.9 Hz, 1H), 5.19 (dd, *J* = 17.3, 1.4 Hz, 1H), 5.01 (dd, *J* = 10.4, 1.3 Hz, 1H), 4.95−4.57 (m, 14H), 4.46 (d, *J* = 10.9 Hz, 1H), 4.39−4.33 (m, 2H), 4.26−3.97 (m, 8H), 3.87 (dd, *J* = 9.6, 9.6, Hz, 1H), 3.60 (dd, *J* = 10.7, 3.1 Hz, 1H), 3.55−3.28 (m, 6H), 1.86 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.6, 163.3, 138.8, 138.7, 138.47, 138.47, 138.46, 138.45, 138.2, 138.0, 137.94, 137.93, 133.8, 133.1, 133.0, 130.1−130.0, 128.5−127.4, 118.1, 99.0 ($^1J_{C,H}$ = 173 Hz), 98.3 ($^1J_{C,H}$ = 176 Hz), 81.4, 81.0, 78.8, 78.0, 76.2, 75.8, 75.2, 75.1, 74.14, 74.10, 73.54, 72.3, 72.0, 71.9, 71.7, 71.4, 71.3, 69.0, 68.82, 68.79, 61.5. HRMS-ESI $[M + Na]^+$ calculated for $C_{91}H_{92}O_{18}Na$: 1495.6181. Found 1495.6161. Further elution provided a mostly β-linked mixed fraction (53 mg, 10%) and then the β-linked compound, 1-*O*-allyl-2-*O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl)-6-*O*-(2-*O*-benzoyl-3,4-di-*O*-benzyl-α-D-mannopyranosyl)-3,4,5-tri-*O*-benzyl-D-*myo*-inositol (54 mg, 11%). $[\alpha]_D^{20}$ = −24 (c 1.08, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$) δ 8.08−8.02 (m, 4H), 7.67−6.90 (m, 46H), 5.95−5.81 (m, 2H), 5.47 (dd, *J* = 2.8, 1.9 Hz, 1H), 5.12 (dd, *J* = 17.3, 1.5 Hz, 1H), 5.08 (br s, 1H), 5.04−4.99 (m, 1H), 4.93 (d, *J* = 1.7 Hz, 1H), 4.92−4.47 (m, 16H), 4.15−3.67 (m, 12H), 3.52−3.45 (m, 1H), 3.38−3.31 (m, 3H), 3.23 (dd, *J* = 9.5, 9.4 Hz), 3.15 (dd, *J* = 9.7, 2.4 Hz, 1H), 1.94 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.8, 165.3, 138.9, 138.8, 138.4, 138.3, 138.2, 138.0, 137.8, 135.0, 133.2, 132.4, 130.6, 130.3, 130.0, 128.7−127.2, 117.4, 97.8 ($^1J_{C,H}$ = 161 Hz), 97.6 ($^1J_{C,H}$ = 176 Hz), 81.7, 81.5, 81.4, 80.3, 78.9, 77.8, 75.9, 75.4, 75.2, 75.0, 74.8, 74.4, 74.3, 73.6, 73.3, 71.3, 71.2, 70.1, 69.9, 69.0, 68.7, 61.6. HRMS-ESI $[M + Na]^+$ calculated for $C_{91}H_{92}O_{18}Na$: 1495.6181. Found 1495.6125.

1-*O*-Allyl-2-*O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-6-*O*-[(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-(2-*O*-benzoyl-3,4-di-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-(2-*O*-benzoyl-3,4-di-*O*-benzyl-α-D-mannopyranosyl)]-3,4,5-tri-*O*-benzyl-D-*myo*-inositol (20). Tf_2O (43 μL, 0.258 mmol) was added to a solution of thioglycoside donor **4** (361 mg, 0.184 mmol), diphenyl sulfoxide (104 mg, 0.515 mmol), and DTBMP (113 mg, 0.552 mmol) in dry CH_2Cl_2 (15 mL) at −60 °C. After stirring for 5 min at this temperature, a precooled (−60 °C) solution of alcohol **2** (343 mg, 0.233 mmol) in dry CH_2Cl_2 (5 mL) was added. The reaction was stirred for a further 30 min then allowed to warm to rt. Saturated $NaHCO_3$ was added to quench the reaction. The organic layer was separated, washed with saturated NaCl (30 mL), then dried ($MgSO_4$), filtered and the solvent removed. The crude residue was purified on silica gel (EtOAc/toluene = 1:49 to 1:24) to afford the title compound **20** (466 mg, 76%) as a foam. $[\alpha]_D^{20} = +16$ (c 1.36, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ 8.15−8.13 (m, 2H), 8.09−8.05 (m, 4H), 7.99−7.96 (m, 2H), 7.56−6.79 (m, 111H), 5.92−5.83 (m, 1H), 5.80−5.77 (m, 2H), 5.76 (dd, *J* = 2.8, 1.8 Hz, 1H), 5.68 (dd, *J* = 2.2, 2.2 Hz, 1H), 5.57 (d, *J* = 1.7 Hz, 1H), 5.30 (d, *J* = 1.6 Hz, 1H), 5.25 (d, *J* = 1.8 Hz, 1H), 5.20 (dd, *J* = 17.2, 1.6 Hz, 1H), 5.16 (d, *J* = 1.8 Hz, 1H), 5.06−5.01 (m, 2H), 4.94−3.72 (m, 65H), 3.65−3.54 (m, 4H), 3.48−3.28 (m, 8H), 3.22−3.17 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.7, 165.4, 165.3, 135.1, 138.9, 138.8, 138.7, 138.54, 138.48, 138.47, 138.4, 138.2, 138.12, 138.08, 138.0, 137.9, 137.8, 137.6, 133.7, 133.2, 133.0, 132.8, 130.2−129.8, 128.6−127.3, 127.0, 126.8, 118.1, 100.5 ($^1J_{C,H}$ = 170 Hz), 99.5 ($^1J_{C,H}$ = 173 Hz), 99.2 ($^1J_{C,H}$ = 171 Hz), 99.1 ($^1J_{C,H}$ = 172 Hz), 98.9 ($^1J_{C,H}$ = 174 Hz), 98.4 ($^1J_{C,H}$ = 172 Hz), 81.5, 81.3, 80.7, 79.58, 79.56, 79.4, 78.9, 78.8, 78.4, 78.3, 75.9, 75.87, 75.34, 75.32, 75.11, 75.05, 74.8, 74.63, 74.58, 74.3, 74.14, 74.11, 74.0, 73.43, 73.39, 73.38, 73.31, 73.27, 72.5, 72.4, 72.3, 72.1, 71.9, 71.71, 71.67, 71.5, 71.39, 71.37, 71.1, 70.7, 70.5, 69.1, 69.00, 68.97, 68.84, 68.78, 68.6, 68.3, 66.1, 65.6. HRMS-ESI $[M + Na]^+$ calculated for $C_{206}H_{206}O_{40}Na$: 3342.3983. Found 3342.4109.

1-*O*-Allyl-2-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-6-*O*-[(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-(3,4-di-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-(3,4-di-*O*-benzyl-α-D-mannopyranosyl)]-3,4,5-tri-*O*-benzyl-D-*myo*-inositol (21). Sodium methoxide in MeOH (30% solution) was added dropwise to a stirred solution of tetra-benzoate **20** (559 mg, 0.168 mmol) in CH_2Cl_2/MeOH (1:9, 50 mL) until the pH of the solution was 11. After being stirred for 4 days, the reaction mixture was diluted with saturated NH_4Cl (50 mL). The aqueous phase was extracted with $CHCl_3$ (3 × 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 (EtOAc/pet.ether =1:4 to 1:1) to afford the title compound **21** (294 mg, 60%) as an oil. $[\alpha]_D^{20} = +63$ (c 2.16, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$) δ 7.40−6.98 (m, 95H), 5.94−5.80 (m, 1H), 5.37 (br s, 1H), 5.30−5.16 (m, 4H), 5.14 (br s, 1H), 5.01−4.27 (m, 40H), 4.19−3.62 (m, 30H), 3.57−3.15 (m, 13H), 3.10−3.03 (m, 1H), 2.40−2.26 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.9−137.9, 133.8, 128.7−126.9, 117.9, 101.0, 111.7, 100.2, 100.1, 100.0, 99.1, 81.6, 81.5, 81.3, 80.7, 80.1, 79.9, 79.6, 79.4, 79.0, 78.9, 77.3, 75.9, 75.8, 75.0, 74.8, 74.6, 74.3, 73.8, 73.5, 73.3, 72.4, 72.1, 91.94, 71.89, 71.6,

71.4, 71.3, 71.1, 70.6, 70.5, 69.3, 69.0, 68.8, 68.7, 68.4, 67.6, 66.2, 65.3. HRMS-ESI $[M + Na]^+$ calculated for $C_{178}H_{190}O_{36}Na$: 2926.2935. Found 2926.2996.

1-*O*-Allyl-2-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)-6-*O*-[(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)]-3,4,5-tri-*O*-benzyl-D-*myo*-inositol (22)[10]. NaH (32 mg, 60% in mineral oil, 0.800 mmol) was added to a stirred solution of tetraol **21** (291 mg, 0.100 mmol) in dry DMF (10 mL) at 0 °C. After 10 min BnBr (71 μL, 0.600 mmol) was added and the resultant suspension left to slowly warm to rt and stirred overnight. Saturated NH_4Cl was added to quench the reaction until the evolution of H_2 ceased. The reaction was extracted into CH_2Cl_2/Et_2O (1:4, 50 mL) then washed with H_2O (2 × 500 mL), saturated NaCl (500 mL) then dried ($MgSO_4$), filtered and the solvent removed. The crude residue was purified on silica gel (EtOAc/pet. ether = 1:4 to 3:7) to afford the title compound **22** (303 mg, 93%) as a pale-yellow oil. $[\alpha]_D^{20} = +39$ (c 1.28, $CHCl_3$) [Lit[10] +37.5 (c = 1, $CHCl_3$)]. ^1H NMR (300 MHz, $CDCl_3$) δ 7.37−6.96 (m, 115H), 5.79−5.63 (m, 1H), 5.43 (br s, 1H), 5.23−5.15, (m, 4H), 5.10−5.05 (m, 1H), 5.02−4.73 (m, 12H), 4.68−4−22 (m, 37H), 4.18−3.77 (m, 27H), 3.72−3.06 (m, 16H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 139.2−137.9, 134.0, 128.9−126.7, 117.7, 100.4, 99.4, 99.3, 99.1, 98.8, 98.4, 81.6, 81.5, 81.3, 80.8, 80.03, 79.96, 79.1, 78.9, 78.8, 75.9, 75.8, 75.4, 75.0, 74.9, 74.8, 74.6, 74.3, 73.7, 73.6, 73.4, 73.3, 72.7, 72.6, 72.5, 72.3, 72.2, 72.0, 71.3, 71.2, 71.0, 70.9, 70.5, 69.2, 69.07, 68.95, 68.9, 66.1, 65.9. HRMS-ESI $[M + Na]^+$ calculated for $C_{206}H_{214}O_{36}Na$: 3286.4813. Found 3286.4849.

2-*O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl)-6-*O*-[(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)]-3,4,5-tri-*O*-benzyl-D-*myo*-inositol (23)[10]. (1,5-Cyclooctadiene)bis-(methyldiphenyl-phosphine)iridium(I) hexafluorophosphate (15 mg, 0.018 mmol) was added to a stirred solution of allyl ether **22** (295 mg, 0.090 mmol) in dry THF (10 mL) at rt under argon. The argon was replaced with H_2 for ca. 1 min followed by a gentle stream of argon being passed over the reaction. After 1 h the solvent was removed and the residue dissolved in $MeOH/CH_2Cl_2$ (1:2, 15 mL). Acetyl chloride (150 μL) was added and the reaction was stirred overnight. Solid $NaHCO_3$ was added to quench the reaction then the resulting mixture partitioned between $CHCl_3$ and H_2O. The aqueous layer was extracted with $CHCl_3$ (3 × 30 mL) and the combined organics dried ($MgSO_4$), filtered and the solvent removed. The crude residue was purified on silica gel (EtOAc/pet. ether = 1:4 to 3:7) to afford the title compound **23** (265 mg, 91%) as an oil. $[\alpha]_D^{20} = +34.4$ (*c* 2.3, $CHCl_3$). [Lit[10] +35 (c = 1, $CHCl_3$)]. ^1H NMR (300 MHz, $CDCl_3$) δ 7.36−6.98 (m, 115H), 5.45 (br s, 1H), 5.21−5.17 (m, 3H), 4.97−4.76 (m, 10H), 4.75−4.22 (m, 40H), 4.18−3.74 (m, 26H), 3.70−3.15 (m, 14H), 2.88 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.9−138.0, 128.6−127.2, 100.6, 99.34, 99.31, 98.9, 98.6, 96.2 (br), 81.3, 80.5, 80.0, 79.9, 78.9, 78.7, 75.6, 75.3, 75.01, 74.96, 74.9, 74.8, 74.7, 74.6, 74.4, 74.0, 73.38, 73.35, 73.3, 73.2, 72.8, 72.59, 72.56, 72.4, 72.2, 72.1, 72.04, 72.01, 71.9, 71.8, 71.61, 71.57, 71.3, 71.1, 69.3, 69.1, 69.0, 68.9, 66.7, 66.2. HRMS-ESI $[M + Na]^+$ calculated for $C_{203}H_{210}O_{36}Na$: 3246.4500. Found 3246.4465.

2-*O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl)-6-*O*-[(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)]-3,4,5-tri-*O*-benzyl-1-*O*-(1,2-di-*O*-hexadecanoyl-*sn*-glycero-3-benzylphosphoryl)-D-*myo*-inositol (24). A mixture of alcohol **23** (170 mg, 0.053 mmol) and phosphoramidite **3** (280 mg, 0.347 mmol) was coevaporated from dry acetonitrile (2 × 30 mL) then placed under high vacuum for 30 min. The reagents were dissolved in dry acetonitrile (15 mL) before the addition of 1*H*-tetrazole (24 mg, 0.347 mmol) at rt. After 1 h the reaction mixture was diluted with dry CH_2Cl_2 (10 mL), stirred for 10 min, then cooled to −30 °C and a dried ($MgSO_4$) solution of *m*CPBA (∼60%, 100 mg, 0.347 mmol) in CH_2Cl_2 (3 mL) was added to the reaction. After warming to rt over 1 h the reaction was diluted with CH_2Cl_2 quenched by the addition of solid $NaHCO_3$, water (50 mL) and Na_2SO_3 (10%, 50 mL) and the mixture was extracted with CH_2Cl_2 (2 × 50 mL). The aqueous phase was extracted with EtOAc (50 mL) and the combined organics dried ($MgSO_4$), filtered and the solvent removed. The crude residue was purified on silica gel (EtOAc/pet. ether = 1:9 to 1:4) followed by a further purification on silica gel ($EtOAc/CHCl_3$ = 1:49 to 1:24) to afford a product containing fraction (180 mg) that included an impurity (5% by HPLC system 1). This fraction was further purified on LiChroprep RP-18 ($MeOH/CHCl_3$ = 4:1 to 1:1) to afford to the title compound **24** (170 mg, 0.043 mmol, 82%) (>98% pure by HPLC system 1) as a clear oil. ^1H NMR (500 MHz, $CDCl_3$) mixture of isomers δ 7.37−6.94 (m, 120H), 5.43−5.39 (br s, 1H), 5.31−5.24 (m, 1H), 5.22−5.17 (m, 2H), 5.16−5.06 (m, 1H), 5.05−3.75 (m, 80H), 3.72−3.23 (m, 12H), 3.10−3.03 (m, 2H), 2.24−2.11 (m, 4H), 1.56−1.43 (m, 4H), 1.34−1.14 (m, 48H), 0.91−0.84 (m, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.0, 172.6, 139.1−137.8, 129.1−126.7, 100.4, 99.6, 99.4, 99.2, 99.0 (br), 98.3, 81.0, 80.3, 80.73, 70.70, 80.6, 80.04, 79.97, 79.9, 79.1, 79.0, 78.9, 78.7, 78.5, 77.3, 76.0, 75.81, 75.76, 75.6, 75.5, 75.1, 75.01, 74.95, 74.92, 74.89, 74.8, 74.63, 74.60, 74.5, 74.3, 74.1, 73.7, 73.6, 73.5, 73.41, 73.36, 73.2, 72.8, 72.6, 72.5, 72.4, 72.3, 72.2, 72.0, 71.9, 71.7, 71.3, 71.2, 70.9, 70.2, 70.1, 69.5, 69.4, 69.2, 68.9, 68.8, 66.2, 66.0, 65.8, 61.5, 34.1, 34.0, 33.9, 32.6, 29.8−29.2, 24.8, 22.7, 14.2. ^{31}P NMR (202 MHz, $CDCl_3$) δ −0.11, −0.43. HRMS-ESI $[M + Na]^+$ calculated for $C_{245}H_{283}O_{43}PNa$: 3966.9593. Found 3967.0671.

1-*O*-(1,2-Di-*O*-hexadecanoyl-*sn*-glycero-3-benzylphosphoryl)-2-*O*-(α-D-mannopyranosyl)-6-*O*-[(α-D-mannopyranosyl)-(1→2)-(α-D-mannopyranosyl)-(1→2)-(α-D-mannopyranosyl)-(1→6)-(α-D-mannopyranosyl)-(1→6)-(α-D-mannopyranosyl)]-D-*myo*-inositol (1). $Pd(OH)_2/C$ (20%, 35 mg) was added to a solution of per-benzylated PIM_6 **24** (130 mg, 0.033 mmol) in THF/MeOH (2:3, 10 mL). The mixture was stirred under an H_2 atmosphere for 3 h at rt, then filtered through Celite and the solvent removed. The residue was lyophilized to afford the title compound **1** (58 mg, 0.033 mmol, 99%) as a white powder. A portion (10 mg) was purified on LiChroprep RP-18 (MeOH/water containing 35 mmol ammonium acetate/$CHCl_3$ = 85:15:0 to 45:15:40) to afford to the title compound **1** (8 mg, 96% pure by HPLC system 2) as a white powder. $[\alpha]_D^{20} = +48.9$ (c 0.5, H_2O). ^1H NMR (500 MHz, $CDCl_3/CD_3OD/D_2O$ 30:60:25) δ 5.30 (m, 1H), 5.28 (s, 1H), 5.16 (s, 1H), 5.13 (s, 1H), 5.11 (s, 1H), 5.02 (s, 1H), 4.90 (s, 1H), 4.47 (dd, *J* = 2.5, 12.5 Hz, 1H), 4.31 (s, 1H), 4.27−4.161 (m, 2H), 4.12−3.57 (m, 39H) 3.51 (dd, *J* = 2.5, 10.0 Hz, 1H), 2.39 (ddm, *J* = 7.5, 7.5 Hz, 2H), 2.34 (ddd, *J* = 7.5, 7.5, 1.8 Hz 2H), 1.61 (m, 4H), 1.36−1.26 (m, 48H), 0.91−0.87 (m, 6H). ^{13}C NMR (125 MHz, CD_3OD/D_2O 30:60:25) δ 175.9, 175.7, 103.7, 102.9 (2 × C), 102.3, 100.9, 99.6, 80.4, 79.9, 78.8, 78.2, 74.8, 74.4, 74.3, 72.9, 72.5, 72.2, 72.1, 71.9, 71.8, 71.7, 68.9, 68.6, 68.4, 67.5, 67.1, 64.9, 64.4, 62.9, 62.8, 62.7, 35.6, 35.5, 33.2, 31.0, 30.8, 30.6, 30.5, 30.45, 26.3, 26.2, 23.9, 15.1. ^{31}P NMR (202 MHz, $CDCl_3$) δ −0.7. HRMS-ESI $[M - H]^-$ calculated for $C_{77}H_{138}O_{43}P$: 1781.8349. Found 1781.8326.

BIOLOGICAL ASSAYS

Reagents. Carboxyfluorescein succinimidyl ester (CSFE), RPMI-1640, and Penicillin-streptomycin were from Invitrogen, Auckland, NZ. Human CD3 and CD28 antibodies, and fluorescent antibodies for CD4

and CD8 were from eBiosciences, San Diego, CA, USA. [^{3}H]-thymidine was from Sigma-Aldrich, Auckland, NZ. Lymphoprep was from Medica Pacifica Ltd., Auckland, NZ.

Peripheral Blood Mononuclear Cells (PBMC) Isolation and Generation of Human Dendritic Cells (DC). Whole blood was collected from healthy volunteers into heparinized vacutainer tubes. Blood collection for this study was approved by the Central Regional Ethics Committee (NZ) and all donors provided written, informed consent.

PBMC were isolated from whole blood using a Lymphoprep density gradient according to manufacturer's instructions. Autologous serum was retained for *in vitro* cell cultures. DC were generated by six day culture in RPMI-1640 supplemented with GM-CSF and IL-4 as previously described.[34]

Mixed lymphocyte Reaction (MLR) Assays. *[^{3}H]-Thymidine Assay.* DC from donor one were cultured in 96 well plates (1×10^4/well) with freshly isolated PBMC from donor two (1×10^5/well) in RPMI-1640 (100 μL, 100 U/mL penicillin-streptomyocin, 1% heat inactivated autologous human serum) in the presence of test compounds. Cells were incubated for four days (37 °C, 5% CO_2) then incubated with 0.25 μCi [^{3}H]-thymidine for one day. The cells were then harvested onto filtermats using an automated cell harvester and read on a Topcount Microplate scintillation counter.

Carboxyfluorescein Succinimidyl Ester (CFSE) Assay. Freshly isolated PBMC from donor two were incubated with CFSE (1 μM in RPMI-1640, 8 min) then washed three times with PBS. CFSE-labeled PBMC were cultured with DC from donor one as described above and the cells harvested after 72 h. Cells were fluorescently labeled with antimouse CD8-APC and analyzed on a BD FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA). Proliferation was determined by the percentage of proliferating CFSE-diluted cells compared to the undiluted CFSE-labeled nonproliferating control population. Dexamethasone (10 μg/mL) was included as a positive control.

Antibody-Mediated T-Cell Proliferation Assay. Freshly isolated human PBMC ($(1-2) \times 10^5$ per well in RPMI-1640, 100 U/mL penicillin-streptomyocin, 1% heat inactivated autologous serum) were plated onto 96-well plates coated with antibodies for CD3 (5 mg/mL) and CD28 (0.5 mg/mL). Cells were incubated with the compounds (37 °C, 5% CO_2) for four days. Cells were then incubated in the presence of 0.25 μCi [^{3}H]-thymidine for one day and thymidine uptake measured as above.

■ ASSOCIATED CONTENT

Supporting Information. General experimental details, ^{1}H, ^{13}C, and ^{31}P NMR spectra for all new compounds reported in the manuscript, HPLC trace for compounds **24** and **1** and the ESI-MS^2 spectrum of compound **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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