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Supplemental Data

CD1c Presentation of Synthetic Glycolipid Antigens with Foreign Alkyl Branching Motifs

Annemieke de Jong, Eva Casas Arce, Tan-Yun Cheng, Ruben P. van Summeren, Ben L. Feringa, Vadim Dudkin, David Crich, Isamu Matsunaga, Adriaan J. Minnaard, and D. Branch Moody

Figure S1. CD8-1 Is a CD1c Restricted β-MPM Specific Reporter T Cell Line

Mock transfected k562 cells and k562 stably transfected with the genes encoding CD1a, CD1b, CD1c and CD1d were used as antigen presenting cells (APC). The APC were incubated with and without 1 µM of C_{32} β-MPM and CD8-1 T cells were added for 24 hours, after which levels of IL-2 released in the culture supernatant were determined by measuring ^3H-thymidine incorporation by IL-2 dependent HT-2 cell line.
Syntheses of MPM Analogs Including Characterization

General experimental remarks: Reagents were purchased from Aldrich, Acros Chimica, Merck or Fluka and were used as received unless otherwise stated. All solvents were reagent grade and were dried and distilled before use according to standard procedures. Chromatography: silica gel, Merck type 9385 230-400 mesh, TLC: silica gel 60, Merck, 0.25 mm. Components were visualized by staining with KMnO₄. EI-MS spectra were recorded on an AEI MS-902 and ESI-MS measurements were carried out using a Quattro II, triple quadrupole mass spectrometer. ¹H and ¹³C NMR spectra were recorded on a Varian AMX400 (100.59 MHz) spectrometer in CDCl₃, CD₃OD, D₂O or mixtures of those solvents. Chemical shift values are denoted in δ values (ppm) relative to residual solvent peaks (CHCl₃, ¹H δ = 7.26, ¹³C δ = 76.9 or CH₃OH, ¹H δ = 3.31, ¹³C δ = 49.0). Multiplicity was determined by APT ¹³C experiments. The enantiomeric excess of 4 was determined by HPLC analysis on a Shimadzu LC-10ADVP HPLC equipped with a Shimadzu SPD-M10AVP diode array detector. Racemic 4 was prepared by reaction of 3 with MeMgBr at -78 °C in THF in the presence of CuI and TMSCl. Optical rotations were measured on a Schmidt + Haensch polarimeter (Polartronic MH8) with a 10 cm cell (c given in g/100 ml).

4-Benzylxoy-butan-1-ol (1):¹ To a solution of butane-1,4-diol (5.0 ml, 55.5 mmol) in dry THF (36 ml) was added NaH (60%, 2.7 g, 55.5 mmol) at 0 °C. Subsequently, BnBr (6.6 ml, 55.5 mmol) in THF (12 ml) was added to the mixture followed by a small amount of DMF to increase solubility. The resulting suspension was stirred overnight under nitrogen at room temperature, after which the reaction was quenched with aq. NH₄Cl (sat.). The aqueous layer was extracted with Et₂O and the combined organic layers were washed with brine (sat.), dried (MgSO₄) and concentrated. The product was purified by column chromatography (n-pentane-EtOAc 4:1 to 7:3) to give 4-benzylxoy-butan-1-ol (8.1 g, 44.9 mmol, 81%) as a light yellow oil. NMR-data were as reported in literature.¹

3-Benzylloxy-propionaldehyde (2): ¹ To a solution of PCC (5.3 g, 24.0 mmol, 2.2 eq) in DCM (38 ml) was added a solution of 4-benzyloxy-butan-1-ol (2.0 g, 11.0 mmol) in DCM (7 ml). The resulting dark green mixture was stirred under nitrogen overnight and then filtered over a silica plug (DCM). After concentration, 2 (1.2 g, 6.7 mmol, 61%) was obtained as a colorless liquid, which was used without further purification. ¹H-NMR (CDCl₃, 400 MHz) δ = 1.95 (m, 2H), 2.54 (dt, J = 1.6, 6.8, 14.4 Hz, 2H), 3.50 (t, J = 6.4 Hz, 2H), 4.49 (s, 2H), 7.24-7.37 (m, 5H), 9.77 (t, J = 1.6 Hz, 1H) ppm. ¹³C-NMR (CDCl₃, 100.6 MHz) δ = 22.3 (t), 40.7 (t), 68.9 (t), 72.7 (t), 127.4 (d), 128.1 (d), 138.0 (s), 202.0 (d) ppm.

S-Ethyl (2E)-6-(benzyloxy)hex-2-enethioate (3): ² EtSC(O)C=PPPh₃ (1.21 g, 3.33 mmol, 1.1 eq) was added to a solution of 2 (540 mg, 3.03 mmol) in CHCl₃ (35 ml) and the resulting mixture was refluxed under nitrogen for 3 h. The reaction mixture was cooled to room temperature and then stirred overnight in the presence of DMAP (100 mg, 0.82 mmol), after which the solvent was removed in vacuo. Trans-3 (more polar, 697 mg, 2.64 mmol, 87%) and a small amount of cis-3 (less polar) were obtained as colorless oils after purification by column chromatography (n-pentane-EtOAc 95:5 to 9:1).

¹H-NMR trans-3 (CDCl₃, 400 MHz) δ = 1.28 (t, J = 7.6 Hz, 3H), 1.78 (m, 2H), 2.31 (m, 2H), 2.94 (q, J = 7.6 Hz, 2H), 3.49 (t, J = 6.4 Hz, 2H), 4.50 (s, 2H), 6.11 (d, J = 15.2 Hz, 1H), 6.90 (dt, J = 7.2, 15.2 Hz, 1H), 7.26-7.37 (m, 5H) ppm. ¹³C-NMR trans-3 (CDCl₃, 100.6 MHz) δ = 14.6 (q), 22.9 (t), 27.9 (t), 28.7 (t), 69.0 (t), 72.8 (t), 127.42 (d), 127.44 (d), 128.2 (d), 128.8 (d), 138.2 (s), 144.3 (d), 189.9 (s) ppm. MS (EI) for C₁₅H₂₀O₂S-C₂H₅: m/z = 235 [M⁺].

¹H-NMR cis-3 (CDCl₃, 400 MHz) δ = 1.28 (t, J = 7.6 Hz, 3H), 1.78 (m, 2H), 2.74 (m, 2H), 2.92 (q, J = 7.6 Hz, 2H), 3.50 (t, J = 6.4 Hz, 2H), 4.50 (s, 2H), 5.98-6.08 (m, 2H), 7.26-7.36 (m, 5H) ppm. ¹³C-NMR cis-3 (CDCl₃, 100.6 MHz) δ = 14.6 (q), 23.1 (t), 26.7 (t), 29.1 (t), 69.6 (t), 72.8 (t),

126.5 (d), 127.4 (d), 127.5 (d), 128.2 (d), 138.3 (s), 146.0 (d), 189.7 (s) ppm. MS (EI) for C_{15}H_{20}O_{2}S-C_{2}H_{5}S: m/z = 203 [M^+].

S-Ethyl (3R)-6-(benzyloxy)-3-methylhexanethioate ((3R)-4):^2 (R,S)-Josiphos (L*, 67 mg, 0.11 mmol, 6 mol%) and CuBr·SMe\textsubscript{2} (19 mg, 0.09 mmol, 5 mol%) were dissolved in t-BuOMe (17 ml) and stirred for 30 min under nitrogen at room temperature. The mixture was cooled at -78 °C and MeMgBr (3.0 M in Et\textsubscript{2}O, 0.78 ml, 2.3 mmol, 1.3 eq) was added dropwise over 2 min. After stirring for 10 min, a solution of trans-3 (495 mg, 1.87 mmol) in t-BuOMe (2 ml) was added over 5 h by syringe pump and the resulting mixture was stirred overnight at -78 °C. The reaction was quenched with MeOH at -78 °C, removed from the cold bath and diluted with H\textsubscript{2}O, aq. NH\textsubscript{4}Cl (sat.) and Et\textsubscript{2}O. The product was extracted with Et\textsubscript{2}O, the combined organic layers were washed with brine (sat.), dried (MgSO\textsubscript{4}) and concentrated. (3R)-4 (485 mg, 1.73 mmol, 92%, 93% ee) was isolated as a colorless liquid after purification by column chromatography (n-pentane-EtOAc 95:5). [\alpha]_D^{22} = +5.1 ° (c = 1.88, CHCl\textsubscript{3}). \textsuperscript{1}H-NMR (CDCl\textsubscript{3}, 400 MHz) \delta = 0.94 (d, J = 6.8 Hz, 3H), 1.24 (t, J = 7.6 Hz, 3H), 1.22-1.31 (m, 1H), 1.41 (m, 1H), 1.63 (m, 2H), 1.94 (m, 1H), 2.36 (dd, J = 8.4, 14.4 Hz, 1H), 2.54 (dd, J = 5.6, 14.4 Hz, 1H), 2.87 (q, J = 7.2 Hz, 2H), 3.45 (t, J = 6.4 Hz, 2H), 4.50 (s, 2H), 7.26-7.37 (m, 5H) ppm. \textsuperscript{13}C-NMR (CDCl\textsubscript{3}, 100.6 MHz) \delta = 14.7 (q), 19.3 (q), 23.2 (t), 27.0 (t), 30.8 (d), 32.9 (t), 51.2 (t), 70.3 (t), 72.8 (t), 127.4 (d), 127.5 (d), 128.2 (d), 138.4 (s), 199.1 (s) ppm. MS(EI) for C\textsubscript{16}H\textsubscript{24}O\textsubscript{2}S: m/z = 280 [M^+], HRMS calcd for C\textsubscript{16}H\textsubscript{24}O\textsubscript{2}S: 280.150, found: 280.149. Ee determination by chiral HPLC analysis, chiralcel OD-H column, n-heptane-\textit{i}-PrOH 99.5:0.5, retention times: 20.77 (R) / 22.19 (S) min.
S-Ethyl (3S)-6-(benzylxylo)-3-methylhexanethioate ((3S)-4): (S,R)-Josiphos (L*, 34 mg, 0.06 mmol, 6 mol%) and CuBr-SMe2 (10 mg, 0.05 mmol, 5 mol%) were dissolved in t-BuOMe (9 ml) and stirred for 30 min under nitrogen at room temperature. The mixture was cooled at -78 °C and MeMgBr (3.0 M in Et2O, 0.40 ml, 1.18 mmol, 1.3 eq) was added dropwise over 2 min. After stirring for 10 min, a solution of trans-3 (250 mg, 0.94 mmol) in t-BuOMe (1 ml) was added over 5 h by syringe pump and the resulting mixture was stirred overnight at -78 °C. The reaction was quenched with MeOH at -78 °C, removed from the cold bath and diluted with H2O, aq. NH4Cl (sat.) and Et2O. The product was extracted with Et2O, the combined organic layers were washed with brine (sat.), dried (MgSO4) and concentrated. (3S)-4 (225 mg, 0.80 mmol, 85%, 95% ee) was isolated as a colorless liquid after purification by column chromatography (n-pentane-EtOAc 95:5). [α]D22 = -4.5 ° (c = 1.20, CHCl3). Ee determination by chiral HPLC analysis, chiralcel OD-H column, n-heptane-iPrOH 99.5:0.5, retention times: 20.77 (R) / 22.19 (S) min.

6-Benzylxylo-(3R)-methyl-hexan-1-ol ((3R)-5): (3R)-4 (555 mg, 1.98 mmol) was dissolved in THF (6 ml) and LiAlH4 (188 mg, 4.95 mmol, 2.5 eq) was added at 0 °C. The resulting suspension was stirred for 45 min at 0 °C under nitrogen and then quenched with water. NaOH (3 M aq.) was added and the mixture was stirred until two clear layers were obtained. The layers were separated and the aqueous layer was extracted with Et2O. The combined organic layers were washed with brine (sat.), dried (MgSO4) and concentrated. Purification by column chromatography (n-pentane-EtOAc 7:3) gave (3R)-5 (421 mg, 1.89 mmol, 96%) as a colorless liquid. [α]D22 = +4.9 ° (c = 1.10, CHCl3). 1H-NMR (CDCl3, 400 MHz) δ = 0.90 (d, J = 6.8 Hz, 3H), 1.22 (m, 1H), 1.40 (m, 2H), 1.54-1.73 (m, 4H), 3.46 (t, J = 6.4 Hz, 2H), 3.67 (m, 2H), 4.50 (s, 2H), 7.26-7.37 (m, 5H) ppm. 13C-NMR (CDCl3, 100.6 MHz) δ = 19.4 (q), 27.0 (t), 29.2 (d), 33.3 (t), 39.7 (t), 61.0 (t), 70.6 (t), 72.8 (t), 127.4 (d), 127.5 (d), 128.2 (d), 138.5 (s) ppm. MS(EI) for C14H22O2: m/z = 222 [M+]. HRMS calcd for C14H22O2: 222.162, found: 222.164.
6-Benzylxy-(3S)-methyl-hexan-1-ol ((3S)-5): (3S)-4 (225 mg, 0.80 mmol) was dissolved in THF (2 ml) and LiAlH₄ (76 mg, 2.00 mmol, 2.5 eq) was added at 0 ºC. The resulting suspension was stirred for 45 min at 0 ºC under nitrogen and then quenched with water. NaOH (3 M aq.) was added and the mixture was stirred until two clear layers were obtained. The layers were separated and the aqueous layer was extracted with Et₂O. The combined organic layers were washed with brine (sat.), dried (MgSO₄) and concentrated. Purification by column chromatography (n-pentane-EtOAc 7:3) gave (3S)-5 (160 mg, 0.72 mmol, 90%) as a colorless liquid. [α]D²² = -5.3 º (c = 1.00, CHCl₃).

6-Benzylxy-(3R)-methyl-hexyl p-toluensulfonate ((3R)-6): (3R)-5 (100 mg, 0.45 mmol) was dissolved in DCM (2 ml) and then pyridine (180 μl, 2.25 mmol, 5.0 eq) and p-TsCl (255 mg, 1.35 mmol, 3.0 eq) were added. The resulting solution was stirred at room temperature under nitrogen overnight and then quenched with NaHCO₃ (sat.). The aqueous layer was extracted with DCM and the combined organic layers were dried (MgSO₄) and concentrated. Purification by column chromatography (n-pentane-EtOAc 95:5) gave (3R)-6 (152 mg, 0.41 mol, 90%) as a colorless oil. [α]D²² = +4.5 º (c = 1.15, CHCl₃). ¹H-NMR (CDCl₃, 400 MHz) δ = 0.82 (d, J = 6.4 Hz, 3H), 1.17 (m, 1H), 1.29 (m, 1H), 1.39-1.75 (m, 5H), 2.43 (s, 3H), 3.41 (t, J = 6.8 Hz, 2H), 4.06 (m, 2H), 4.48 (s, 2H), 7.26-7.38 (m, 7H), 7.77 (s, 1H) ppm. ¹³C-NMR (CDCl₃, 100.6 MHz) δ = 19.0 (q), 21.6 (q), 27.0 (t), 29.1 (d), 33.0 (t), 35.6 (t), 68.9 (t), 70.4 (t), 72.8 (t), 127.4 (d), 127.5 (d), 127.8 (d), 128.3 (d), 129.8 (d), 133.2 (s), 138.5 (s), 144.6 (s) ppm. MS(EI) for C₂₁H₂₈O₄S: m/z = 376 [M⁺], HRMS calcd for C₂₁H₂₈O₄S: 376.171, found: 376.169.

6-Benzylxy-(3S)-methyl-hexyl p-toluensulfonate ((3S)-6): (3S)-5 (150 mg, 0.67 mmol) was dissolved in DCM (3 ml) and then pyridine (270 μl, 3.37 mmol, 5.0 eq) and p-TsCl (383 mg,
2.01 mmol, 3.0 eq) were added. The resulting solution was stirred at room temperature under nitrogen overnight and then quenched with NaHCO₃ (sat.). The aqueous layer was extracted with DCM and the combined organic layers were dried (MgSO₄) and concentrated. Purification by column chromatography (n-pentane-EtOAc 95:5) gave (3R)-6 (222 mg, 0.59 mmol, 88%) as a colorless oil. [α]₀°D²² = -4.9 ° (c = 1.10, CHCl₃).

**1-bromo-heneicosane (7):** To a solution of heneicosanol (900 mg, 2.88 mmol) in DCM (20 ml) was added PPh₃ (910 mg, 3.46 mmol, 1.2 eq) followed by NBS (320 mg, 3.46 mmol, 1.2 eq) at 0 °C. The resulting solution was stirred under nitrogen at 0 °C for 10 min and then warmed to room temperature over 1 h. The reaction mixture was quenched with aq. NaHCO₃ (sat.) and the aqueous layer was extracted with DCM. The combined organic layers were washed with aq. Na₂S₂O₃ (10% v/v) and brine (sat.), dried (MgSO₄), filtered and concentrated. The resulting brown solid was suspended in n-pentane and filtered, after which the residue was washed with n-pentane. The filtrate was concentrated to give 7 (1.06 g, 98%) as a white solid. ¹H-NMR (CDCl₃, 400 MHz) δ = 0.85 (t, J = 6.4 Hz, 3H), 1.00-1.43 (m, 36H), 1.83 (m, 2H), 3.38 (t, J = 6.8 Hz, 2H) ppm. ¹³C-NMR (CDCl₃, 100.6 MHz) δ = 14.1 (q), 22.7 (t), 28.1(t), 28.7 (t), 29.3 (t), 29.4 (t), 29.5 (t), 29.6 (t), 29.7 (t), 31.9 (t), 32.8 (t), 33.8 (t) ppm. MS(EI) for C₂₁H₄₃Br: m/z = 374 [M⁺], HRMS calcd for C₂₁H₄₃Br: 374.255, found: 374.255.

**((4S)-Methyl-heptacosyloxymethyl)-benzene ((4S)-8):** 1-bromoheneicosane (7) (880 mg, 2.34 mmol) in dry THF (5.3 ml) was added dropwise to Mg (80 mg, 3.28 mmol, 1.4 eq) in a nitrogen-purged flask and then stirred for 1.5 h at 40 °C. The resulting solution was titrated with sec-butanol in the presence of 1,10-phenanthroline, showing the concentration of C₂₁H₄₃MgBr to be 0.38 M. The Grignard reagent (3.4 ml, 1.28 mmol, 4.0 eq) was added in a dropwise fashion to a solution of (3R)-6 (120 mg, 0.32 mmol) and CuBr·SMe₂ (16 mg, 0.08 mmol, 25% mol) in dry THF (2.0 ml) at 0 °C under nitrogen. After stirring for 2 h at 0 °C, the solution was allowed to warm to room temperature and stirred overnight. The reaction was quenched with aq. NH₄Cl.
extracted with Et₂O and the combined organic layers were washed with brine (sat.), dried (MgSO₄) and concentrated. (4S)-8 (128 mg, 0.26 mmol, 80%) was isolated as a white solid after purification by column chromatography (n-pentane to n-pentane-EtOAc 95:5). ¹H-NMR (CDCl₃, 400 MHz) δ = 0.87 (d, J = 6.5 Hz, 3H), 0.89 (t, J = 6.5 Hz, 3H), 1.10-1.45 (m, 47H), 1.63 (m, 2H), 3.46 (t, J = 6.7 Hz, 2H), 4.51 (s, 2H), 7.29 (m, 1H), 7.34 (s, 2H), 7.35 (s, 2H) ppm. ¹³C-NMR (CDCl₃, 100.6 MHz) δ = 14.1 (q), 19.6 (q), 22.7 (t), 27.1 (t), 27.3 (t), 29.4 (t), 29.7 (t), 30.0 (t), 31.9 (t), 32.6 (d), 33.3 (t), 37.0 (t), 70.9 (t), 72.9 (t), 127.4 (d), 127.6 (d), 128.3 (d), 138.7 (s) ppm. MS(EI) for C₃₅H₆₄O m/z = 409 [M⁺], HRMS calcd for C₃₅H₆₄O: 500.496, found: 500.498.

BnO

(4R)-Methyl-heptacosyloxymethyl)-benzene ((4R)-8): 1-bromoheneicosane (7) (650 mg, 1.73 mmol) in dry THF (4.0 ml) was added dropwise to Mg (60 mg, 2.45 mmol, 1.4 eq) in a nitrogen-purged flask and then stirred for 1.5 h at 40 °C. The resulting solution was titrated with sec-butanol in the presence of 1,10-phenanthroline, showing the concentration of C₂₁H₄₃MgBr to be 0.38 M. The Grignard reagent (2.7 ml, 1.02 mmol, 4.0 eq) was added in a dropwise fashion to a solution of (3S)-6 (96 mg, 0.25 mmol) and CuBr·SM₂ (13 mg, 0.06 mmol, 25% mol) in dry THF (1.6 ml) at 0 °C under nitrogen. After stirring for 2 h at 0 °C, the solution was allowed to warm to room temperature and stirred overnight. The reaction was quenched with aq. NH₄Cl (sat.), extracted with Et₂O and the combined organic layers were washed with brine (sat.), dried (MgSO₄) and concentrated. (4R)-8 (99 mg, 0.20 mmol, 78%) was isolated as a white solid after purification by column chromatography (n-pentane to n-pentane-EtOAc 95:5). MS(EI) for C₃₅H₆₄O m/z = 409 [M⁺], HRMS calcd for C₃₅H₆₄O: 500.496, found: 500.498.

HO

(4S)-Methyl-heptacosan-1-ol ((4S)-9): (4S)-8 (70 mg, 0.14 mmol) was dissolved in EtOAc (6 ml) and Pd/C (10%, 35 mg) was added. The resulting suspension was degassed with 3 vacuum-nitrogen cycles and then saturated with H₂ by 5 vacuum-H₂ cycles. After stirring for 48 h under an H₂-atmosphere (1 bar), the Pd/C was removed by filtration over celite and the resulting clear
solution was concentrated in vacuo. After purification by column chromatography (n-pentane-EtOAc 95:5) (4S)-9 (53 mg, 0.13, 92%) was isolated as a white solid. $^1$H-NMR (CDCl$_3$, 400 MHz) $\delta = 0.87$ (d, $J = 5.6$ Hz, 3H), 0.88 (t, $J = 6.8$ Hz, 3H), 1.10-1.39 (m, 47H), 1.56 (m, 2H), 3.62 (t, $J = 6.8$ Hz, 2H) ppm. $^{13}$C-NMR (CDCl$_3$, 100.6 MHz) $\delta = 14.1$ (q), 19.6 (q), 22.7 (t), 27.0 (t), 29.4 (t), 29.7 (t), 30.0 (t), 30.3 (t), 31.9 (t), 32.6 (d), 32.9 (t), 37.0 (t), 63.5 (t) ppm. MS(EI) for C$_{28}$H$_{58}$O-H$_2$O m/z = 392 [M$^+$], HRMS calcd for C$_{28}$H$_{58}$O-H$_2$O: 392.438, found: 392.439.

(4R)-Methyl-heptacosan-1-ol ((4R)-9): (4R)-8 (90 mg, 0.18 mmol) was dissolved in EtOAc (7 ml) and Pd/C (10%, 45 mg) was added. The resulting suspension was degassed with 3 vacuum-nitrogen cycles and then saturated with H$_2$ by 5 vacuum-H$_2$ cycles. After stirring for 48 h under an H$_2$-atmosphere (1 bar), the Pd/C was removed by filtration over celite and the resulting clear solution was concentrated in vacuo. After purification by column chromatography (n-pentane-EtOAc 95:5) (4R)-9 (58 mg, 0.14, 78%) was isolated as a white solid. MS(EI) for C$_{28}$H$_{58}$O-H$_2$O m/z = 392 [M$^+$], HRMS calcd for C$_{28}$H$_{58}$O-H$_2$O: 392.438, found: 392.439.

9-Benzyloxy-nonan-1-ol (10): To a solution of nonane-1,9-diol (200 mg, 1.25 mmol) in dry THF (5 ml) was added NaH (60%, 50 mg, 1.25 mmol, 1.0 eq) at 0 ºC. Subsequently, BnBr (160 µl, 1.38 mmol, 1.1 eq) was added to the mixture and a small amount of DMF to increase solubility. The resulting suspension was stirred overnight under nitrogen at room temperature, after which the reaction was quenched with aq. NH$_4$Cl (sat.). The aqueous layer was extracted with Et$_2$O and the combined organic layers were washed with brine (sat.), dried (MgSO$_4$) and concentrated. The product was purified by column chromatography (n-pentane-EtOAc 7:3) to give 10 (250 mg, 1.00 mmol, 80%) as a light yellow oil. $^1$H-NMR (CDCl$_3$, 400 MHz) $\delta = 1.18$-1.35 (m, 10H), 1.51-1.66 (m, 5H, 2CH$_2$ and OH), 3.46 (t, $J = 6.8$ Hz, 2H), 3.61 (t, $J = 6.4$ Hz, 2H), 4.50 (s, 2H), 7.27 (m, 1H), 7.33 (s, 2H), 7.34 (s, 2H) ppm. $^{13}$C-NMR (CDCl$_3$, 100.6 MHz) $\delta = 25.7$ (t), 26.1 (t), 29.3 (t), 29.4 (t), 29.5 (t), 29.7 (t), 32.7 (t), 62.9 (t), 70.4 (t), 72.8 (t), 127.4 (d), 127.6 (d), 128.3 (d), 138.6
(s) ppm. MS(EI) for \( \text{C}_{16}\text{H}_{26}\text{O}_{2} \): m/z = 250 [M⁺], HRMS calcd for \( \text{C}_{16}\text{H}_{26}\text{O}_{2} \): 250.193, found: 250.194.

\[ \text{BnO} \overset{\text{OTs}}{\longrightarrow} \]

**9-Benzylloxy-nonyl p-toluensulfonate (11):** 10 (200 mg, 0.80 mmol) was dissolved in DCM (4 ml) and then pyridine (320 μl, 4.00 mmol, 5.0 eq) and \( p \)-TsCl (457 mg, 2.40 mmol, 3.0 eq) were added. The resulting solution was stirred at room temperature under nitrogen overnight and then quenched with NaHCO₃ (sat.). The aqueous layer was extracted with DCM and the combined organic layers were dried (MgSO₄) and concentrated. Purification by column chromatography (\( n \)-pentane-EtOAc 95:5) gave 11 (285 mg, 0.70 mmol, 89%) as a colorless oil.

\[ \text{\textsuperscript{1}H-NMR (CDCl₃, 400 MHz)} \quad \delta = 1.17-1.35 \text{ (m, 10H)}, 1.55-1.65 \text{ (m, 4H)}, 2.43 \text{ (s, 3H)}, 3.43 \text{ (t, } J = 6.4 \text{ Hz, 2H)}, 4.01 \text{ (t, } J = 6.4 \text{ Hz, 2H)}, 4.49 \text{ (s, 2H)}, 7.26-7.34 \text{ (m, 7H)}, 7.77 \text{ (s, 2H)}, 7.79 \text{ (s, 2H)} \text{ ppm.} \]

\[ \text{\textsuperscript{13}C-NMR (CDCl₃, 100.6 MHz)} \quad \delta = 21.5 \text{ (q)}, 25.2 \text{ (t, 7H)}, 26.0 \text{ (t, 2H)}, 28.7 \text{ (t, 1H)}, 29.2 \text{ (t, 1H)}, 70.3 \text{ (t, 1H)}, 70.6 \text{ (t, 1H)}, 72.7 \text{ (t, 1H)}, 127.3 \text{ (d, 1H)}, 127.5 \text{ (d, 1H)}, 127.7 \text{ (d, 1H)}, 128.2 \text{ (d, 1H)}, 129.7 \text{ (d, 1H) ppm.} \]

\[ \text{BnO} \overset{\text{Heptacosyloxymethyl-benzene}}{\longrightarrow} \]

**Heptacosyloxymethyl-benzene (12):** Octadecyl bromide (800 mg, 2.40 mmol) in dry THF (5.4 ml) was added dropwise to Mg (82 mg, 3.37 mmol, 1.4 eq) in a nitrogen-purged flask and then stirred for 1.5 h at 40 °C. The resulting solution was titrated with \textit{sec}-butanol in the presence of 1,10-phenanthroline, showing the concentration of \( \text{C}_{21}\text{H}_{43}\text{MgBr} \) to be 0.30 M. The Grignard reagent (5.0 ml, 1.50 mmol, 3.4 eq) was added in a dropwise fashion to a solution of 11 (180 mg, 0.44 mmol) and CuBr-SMe₂ (23 mg, 0.11 mmol, 25% mol) in dry THF (2.7 ml) at 0 °C under nitrogen. After stirring for 2 h at 0 °C, the solution was allowed to warm to room temperature and stirred overnight. The reaction was quenched with aq. NH₄Cl (sat.), extracted with Et₂O and the combined organic layers were washed with brine (sat.), dried (MgSO₄) and concentrated. 12 (183 mg, 0.37 mmol, 84%) was isolated as a white solid after purification by column chromatography (\( n \)-pentane to \( n \)-pentane-EtOAc 95:5). \[ \text{\textsuperscript{1}H-NMR (CDCl₃, 400 MHz)} \quad \delta = 0.89 \text{ (d, } J = 4.8 \text{ Hz, 3H)}, 1.05-1.48 \text{ (m, 48H)}, 1.61 \text{ (m, 2H)}, 3.46 \text{ (t, } J = 8.4 \text{ Hz, 2H)}, 4.51 \text{ (s, 2H)}, 7.28 \text{ (m, 1H)}, 7.34 \text{ (s, 1H),} \]
2H), 7.35 (s, 2H) ppm. $^{13}$C-NMR (CDCl$_3$, 100.6 MHz) $\delta$ = 14.1 (q), 22.7 (t), 26.2 (t), 29.4 (t), 29.5 (t), 29.6 (t), 29.7 (t), 29.8 (t), 31.9 (t), 70.5 (t), 72.8 (t), 127.4 (d), 127.6 (d), 128.3 (d) ppm. MS(EI) for C$_{34}$H$_{62}$O m/z = 486 [M$^+$], HRMS calcd for C$_{34}$H$_{62}$O: 486.480, found: 486.478.

Heptacosanol (13): 12 (163 mg, 0.33 mmol) was dissolved in EtOAc (14 ml) and Pd/C (10%, 82 mg) was added. The resulting suspension was degassed with 3 vacuum-nitrogen cycles and then saturated with H$_2$ by 5 vacuum-H$_2$ cycles. After stirring for 48 h under an H$_2$-atmosphere (1 bar), a suspension was obtained. Toluene was added to get a clear solution and then the Pd/C was removed by filtration over celite. The resulting solution was concentrated in vacuo and after purification by column chromatography (n-pentane-EtOAc 95:5), 13 (110 mg, 0.28, 84%) was isolated as a white solid. $^1$H-NMR (CDCl$_3$, 400 MHz) $\delta$ = 0.88 (t, $J$ = 6.6 Hz, 3H), 1.08-1.38 (m, 48H), 1.56 (m, 2H), 3.64 (t, $J$ = 6.5 Hz, 2H) ppm. $^{13}$C-NMR (CDCl$_3$, 100.6 MHz) $\delta$ = 14.1 (q), 22.7 (t), 25.7 (t), 29.4 (t), 29.6 (t), 29.7 (t), 31.9 (t), 32.8 (t), 63.1 (t) ppm. MS(EI) for C$_{27}$H$_{56}$O-H$_2$O m/z = 378 [M$^+$], HRMS calcd for C$_{27}$H$_{56}$O-H$_2$O: 378.423, found: 378.424.

Diphenyl-(2,3,4,6-tetra-O-acetyl-β-D-mannopyranosyl)-phosphate (β-14): 3 To a solution of 2,3,4,6-tetra-O-acetyl-D-mannopyranose (1.90 g, 5.46 mmol) and DMAP (1.52 g, 12.40 mmol, 2.3 eq) in DCM (32 ml) at room temperature was added a solution of diphenyl chlorophosphate (2.66 ml, 12.80 mmol, 2.4 eq) in DCM (13 ml) by syringe pump over 1 h. After stirring the resulting solution under nitrogen for 2 h, the reaction mixture was diluted with DCM and washed with cold water, cold aq. HCl (0.5 M) and cold aq. NaHCO$_3$ (sat.). The organic layer was dried (MgSO$_4$), filtered and concentrated to give 14 (2.50 g, 4.31 mmol, 79%) as a mixture of anomers (ratio $\alpha:\beta = 1:4$). The pure $\beta$-anomer of 14 (0.98 g, 1.69 mmol, 31%) was obtained after purification by column chromatography (n-pentane-EtOAc 8:3 to 3:2) together with a mixed

fraction of anomers (1.40g, 2.41 mmol, 44%). \( \beta-14 \) was stable in solution at room temperature and for several months at -18 °C when concentrated. \(^1\)H-NMR \( \beta-14 \) (CDCl\(_3\), 400 MHz) \( \delta = 1.94 \) (s, 3H, Ac), 2.00 (s, 6H, Ac), 2.05 (s, 3H, Ac), 3.77 (m, 1H, C5-H), 4.07 (dd, \( J = 2.4, 12.4 \) Hz, 1H, C6-H), 4.23 (dd, \( J = 5.6, 12.4 \) Hz, 1H, C6-H'), 5.07 (dd, \( J = 3.6, 9.6 \) Hz, 1H, C3-H), 5.22 (t, \( J = 9.6 \) Hz, 1H, C4-H), 5.46 (d, \( J = 3.2 \) Hz, 1H, C2-H), 5.61 (d, \( J = 6.8 \) Hz, 1H, C1-H), 7.12-7.32 (m, 10H) ppm. \(^{13}\)C-NMR (CDCl\(_3\), 100.6 MHz) \( \delta = 20.2 \) (q), 20.3 (q), 20.37 (q), 20.38 (q), 61.7 (t), 65.0 (d), 67.8 (d, \( J_{C-P} = 8.5 \) Hz), 69.8 (d), 72.7 (d), 94.5 (d, \( J_{C-P} = 4.6 \) Hz), 120.0 (d, \( J_{C-P} = 4.5 \) Hz), 120.1 (d, \( J_{C-P} = 4.5 \) Hz), 125.4 (d), 125.6 (d), 129.4 (d), 129.6 (d), 149.7 (s, \( J_{C-P} = 7.6 \) Hz), 150.1 (s, \( J_{C-P} = 8.5 \) Hz), 169.3 (s), 169.4 (s), 169.5 (s), 170.3 (s) ppm.

Pyridinium (2,3,4,6-tetra-\( \text{O}-\text{acetyl}\)-\( \beta \)-D-mannopyranosyl)-phosphate (\( \beta-15 \)): \( \beta-14 \) (667 mg, 1.15 mmol) was dissolved in EtOH-EtOAc (14 ml, 1:1 v/v) and PtO\(_2\) (29 mg, 0.13 mmol, 0.11 eq) was added. The resulting suspension was degassed with 3 vacuum-argon cycles and then saturated with H\(_2\) by 5 vacuum-H\(_2\) cycles. After stirring for 60 h under an H\(_2\)-atmosphere (1 bar), the catalyst was removed by filtration over celite and the solution was neutralized with pyridine. After concentration, \( \beta-15 \) (469 mg, 0.92 mmol, 80%) was isolated as a white foam. \(^1\)H-NMR \( \beta-15 \) (D\(_2\)O, 400 MHz) \( \delta = 1.88 \) (s, 3H, Ac), 1.96 (s, 3H, Ac), 1.99 (s, 3H, Ac), 2.10 (s, 3H, Ac), 3.93 (ddd, \( J = 2.0, 3.6, 10.0 \) Hz, 1H, C5-H), 4.07 (dd, \( J = 2.0, 12.8 \) Hz, 1H, C6-H), 4.31 (dd, \( J = 3.6, 12.8 \) Hz, 1H, C6-H'), 5.09 (t, \( J = 10 \) Hz, 1H, C4-H), 5.21 (dd, \( J = 3.2, 10.4 \) Hz, 1H, C3-H), 5.33 (dd, \( J = 0.8, 8.8 \) Hz, 1H, C1-H), 5.40 (d, \( J = 2.8 \) Hz, 1H, C2-H), 7.95 (t, \( J = 7.2 \) Hz, 2H, Py), 8.50 (m, 1H, Py), 8.65 (d, \( J = 5.2 \) Hz, 2H, Py) ppm. \(^{13}\)C-NMR (D\(_2\)O, 100.6 MHz) \( \delta = 18.6 \) (q), 60.3 (t), 64.0 (d), 68.5 (d, \( J_{C-P} = 6.8 \) Hz), 69.6 (d), 70.3 (d), 91.8 (d), 125.9 (d), 171.1 (s), 171.6 (s), 171.7 (s), 172.2 (s) ppm. Positive ion ESI-HRMS calcd for C\(_{14}\)H\(_{21}\)O\(_{13}\)NaP (M-C\(_5\)H\(_6\)N\(^+\) + Na\(^+\) + H\(^+\)\(^+\)): 451.062, found: 451.062.
(4S)-Methylheptacosylphosphoryl-2,3,4,6-tetra-O-acetyl-\(\beta\)-D-mannopyranoside ((4S)-16): \(\beta\)-15 (55 mg, 0.107 mmol, 2.0 eq) was brought into a dry Schlenk tube and co-evaporated with freshly distilled dry toluene (2x; pressure elevated with nitrogen). Subsequently, a solution of (4S)-9 (22 mg, 0.054 mmol) in dry toluene was added and the mixture was co-evaporated with dry toluene (2x). Finally, 2,4,6-triisopropylbenzenesulfonyl chloride (TPSCl) (49 mg, 0.162 mmol, 3.0 eq) was added and after two more additions and evaporations of dry toluene, the residue was dissolved in dry pyridine (1.5 ml) and stirred under nitrogen for 4 days. The reaction was quenched with MeOH (1.5 ml) and stirred for 2 more h before concentration (co-evaporation with toluene). The residue was purified by column chromatography (chloroform-MeOH 95:5) giving (4S)-\(\beta\)-16 (33 mg, 0.040 mmol, 75%) as a white solid. \(^1\)H-NMR (CDCl\(_3\)-CD\(_3\)OD 1:1 v/v, 400 MHz) \(\delta = 0.83\) (d, \(J = 6.4\) Hz, 3H), 0.85 (t, \(J = 6.4\) Hz, 3H), 0.98-1.40 (m, 47H), 1.56 (m, 2H), 1.96 (s, 3H, Ac), 2.04 (s, 3H, Ac), 2.06 (s, 3H, Ac), 2.15 (s, 3H, Ac), 3.81 (m, 3H, C5-H and CH\(_2\)-OP), 4.15 (dd, \(J = 2.4, 12.4\) Hz, 1H, C6-H), 4.26 (dd, \(J = 4.8, 12.4\) Hz, 1H, C6-H'), 5.12 (dd, \(J = 3.2, 10.4\) Hz, 1H), 5.22 (t, \(J = 10.0\) Hz, 1H), 5.35 (d, \(J = 8.0\) Hz, 1H, C1-H), 5.44 (d, \(J = 2.8\) Hz, 1H, C2-H) ppm. \(^{13}\)C-NMR (CDCl\(_3\)-CD\(_3\)OD 1:1 v/v, 100.6 MHz) \(\delta = 14.3\) (q), 19.8 (q), 20.7 (t), 20.8 (t), 23.1 (t), 27.6 (t), 28.6 (t), 28.7 (t), 29.8 (t), 30.1 (t), 30.2 (t), 30.5 (t), 32.4 (t), 33.2 (d), 33.5 (t), 37.5 (t), 62.8 (t), 66.2 (d), 67.1 (t, \(J_{C-P} = 4.3\) Hz), 70.2 (d, \(J_{C-P} = 5.5\) Hz), 71.7 (d), 73.0 (d), 94.2 (d), 170.7 (s), 171.4 (s), 171.7 (s) ppm. \(^{31}\)P-NMR (CDCl\(_3\)-CD\(_3\)OD 1:1 v/v, 161.6 MHz) \(\delta = -3.5\) ppm. Positive ion ESI-HRMS calcd for C\(_{42}\)H\(_{77}\)O\(_{13}\)P (M+Na)\(^+\): 843.499, found: 843.502.

(4R)-Methylheptacosylphosphoryl-2,3,4,6-tetra-O-acetyl-\(\beta\)-D-mannopyranoside ((4R)-16): \(\beta\)-15 (55 mg, 0.107 mmol, 2.0 eq) was brought into a dry Schlenk tube and co-evaporated with freshly distilled dry toluene (2x; pressure elevated with nitrogen). Subsequently a solution of (4R)-9 (22 mg, 0.054 mmol) in dry toluene was added and the mixture was co-evaporated with dry toluene (2x). Finally TPSCl (49 mg, 0.162 mmol, 3.0 eq) was added and after two more
additions and evaporations of dry toluene, the residue was dissolved in dry pyridine (1.5 ml) and stirred under nitrogen for 4 days. The reaction was quenched with MeOH (1.5 ml) and stirred for 2 more h before concentration (co-evaporation with toluene). The residue was purified by column chromatography (chloroform-MeOH 95:5) giving \((4R)-\beta-16\) (30 mg, 0.037 mmol, 68%) as a white solid. Positive ion ESI-HRMS calcd for \(\text{C}_{42}\text{H}_{77}\text{O}_{13}\text{P} (\text{M+Na})^+\): 843.499, found: 843.496.

**Heptacosylphosphoryl-2,3,4,6-tetra-O-acetyl-\(\beta\)-D-mannopyranoside (17):** \(\beta-15\) (78 mg, 0.151 mmol, 2.0 eq) was brought into a dry Schlenk tube and co-evaporated with freshly distilled dry toluene (2x; pressure elevated with nitrogen). Subsequently a solution of \(13\) (30 mg, 0.076 mmol) in dry toluene was added and the mixture was co-evaporated with dry toluene (2x). Finally TPSCl (69 mg, 0.228 mmol, 3.0 eq) was added and after two more additions and evaporations of dry toluene, the residue was dissolved in a mixture of dry toluene (1.0 ml) and dry pyridine (1.0 ml). The mixture was stirred under nitrogen for 4 days. The reaction was quenched with MeOH (1.5 ml) and stirred for 2 more h before concentration (co-evaporation with toluene). The residue was purified by column chromatography (chloroform-MeOH 95:5) giving \(\beta-17\) (20 mg, 0.025 mmol, 33%) as a white solid. \(^1\text{H-NMR (CDCl}_3-\text{CD}_3\text{OD} 1:1 \text{v/v}, 400 \text{MHz} ) \delta = 0.87 \text{ (t, } J = 6.8 \text{ Hz, } 3\text{H)}, 1.06-1.38 \text{ (m, } 48\text{H}), 1.58 \text{ (m, } 2\text{H}), 1.96 \text{ (s, } 3\text{H, Ac)}, 2.04 \text{ (s, } 3\text{H, Ac}), 2.07 \text{ (s, } 3\text{H, Ac}), 2.16 \text{ (s, } 3\text{H, Ac}), 3.84 \text{ (m, } 3\text{H, C5-H and CH}_2\text{-OP)}, 4.15 \text{ (dd, } J = 2.0, 12.4 \text{ Hz, } 1\text{H, C6-H)}, 4.27 \text{ (dd, } J = 4.8, 12.4 \text{ Hz, } 1\text{H, C6-H'}), 5.15 \text{ (dd, } J = 2.8, 10.4 \text{ Hz, } 1\text{H)}, 5.22 \text{ (t, } J = 10.0 \text{ Hz, } 1\text{H}), 5.37 \text{ (d, } J = 6.4 \text{ Hz, } 1\text{H, C1-H}), 5.46 \text{ (d, } J = 2.8 \text{ Hz, } 1\text{H, C2-H}) \text{ ppm.} \(^{13}\text{C-NMR (CDCl}_3-\text{CD}_3\text{OD} 1:1 \text{v/v, } 100.6 \text{ MHz} ) \delta = 14.3 \text{ (q), } 20.7 \text{ (t), } 20.8 \text{ (t), } 23.2 \text{ (t), } 26.3 \text{ (t), } 29.9 \text{ (t), } 30.0 \text{ (t), } 30.2 \text{ (t), } 31.1 \text{ (t), } 31.2 \text{ (t), } 32.5 \text{ (t), } 62.9 \text{ (t), } 66.3 \text{ (d), } 66.9 \text{ (t, } J_{\text{C-P}} = 1.9 \text{ Hz)}, 70.4 \text{ (d, } J_{\text{C-P}} = 1.0 \text{ Hz)}, 71.8 \text{ (d), } 73.1 \text{ (d), } 94.3 \text{ (d), } 170.8 \text{ (s), } 171.5 \text{ (s), } 171.8 \text{ (s) ppm.} \(^{31}\text{P-NMR (CDCl}_3-\text{CD}_3\text{OD} 1:1 \text{v/v, } 161.6 \text{ MHz}) \delta = -3.6 \text{ ppm.} \) Positive ion ESI-HRMS calcd for \(\text{C}_{44}\text{H}_{75}\text{O}_{13}\text{P} (\text{M+Na})^+\): 829.484, found: 829.484.
(4S)-Methylheptacosylphosphoryl-β-D-mannopyranoside ((4S)-18): (4S)-β-16 (7.8 mg, 9.50 µmol) was dissolved in DCM-MeOH (0.6 ml, 1:1 v/v) and Et3N was added (20 µl, 0.15 mmol, 14.0 eq). The resulting solution was stirred at room temperature for 2 days, observing the formation of a white solid. The mixture was concentrated and the resulting solid was washed with DCM-MeOH (1:1 v/v) to give (4S)-β-17 (5.1 mg, 7.81 µmol). 1H-NMR (CDCl3-CD3OD-D2O 1.0:1.0:0.1 v/v/v, 400 MHz) δ = 0.85 (d, J = 6.4 Hz, 3H), 0.88 (t, J = 6.8 Hz, 3H), 1.02-1.45 (m, 47H), 1.61 (m, 2H), 3.31 (m, 1H, C5-H), 3.56 (m, 2H, C3-H and C4-H), 3.73 (dd, J = 6.0, 12.4 Hz, 1H, C6-H), 3.85 (m, 3H, C6-H’ and CH2-OP), 3.94 (d, J = 2.4 Hz, 1H, C2-H), 5.08 (d, J = 8.4 Hz, 1H, C1-H) ppm. 13C-NMR (CDCl3-CD3OD-D2O 1.0:1.0:0.1 v/v/v, 100.6 MHz) δ = 14.5 (q), 19.8 (q), 23.3 (t), 27.8 (t), 30.0 (t), 30.3 (t), 30.7 (t), 32.6 (t), 33.4 (d), 33.7 (t), 37.8 (t), 53.1, 61.9, 67.3, 71.9, 72.0, 73.9, 96.2 (s) ppm. 31P-NMR (CDCl3-CD3OD-D2O 1.0:1.0:0.1 v/v/v, 161.6 MHz) δ = -0.6 ppm. Positive ion ESI-HRMS calcd for C34H69O9P (M+Na)^+: 675.457, found: 675.454.

(4R)-Methylheptacosylphosphoryl-β-D-mannopyranoside ((4R)-18): (4R)-β-16 (8.2 mg, 9.99 µmol) was dissolved in DCM-MeOH (0.6 ml, 1:1 v/v) and Et3N was added (20 µl, 0.14 mmol, 13.0 eq). The resulting solution was stirred at room temperature for 2 days, observing the formation of a white solid. The mixture was concentrated and the resulting solid was washed with DCM-MeOH (1:1 v/v) to give (4R)-β-17 (4.9 mg, 7.51 µmol). Positive ion ESI-HRMS calcd for C34H69O9P (M+Na)^+: 675.457, found: 675.461.
Heptacosylphosphoryl-β-D-mannopyranoside (19): β-17 (5.8 mg, 7.19 µmol) was dissolved in DCM-MeOH (0.6 ml, 1:1 v/v) and Et₃N was added (20 µl, 0.14 mmol, 14.0 eq). The resulting solution was stirred at room temperature for 2 days, observing the formation of a white solid. The mixture was concentrated and the resulting solid was washed with DCM-MeOH (1:1 v/v) to give β-19 (3.8 mg, 5.95 µmol). ¹H-NMR (CDCl₃-CD₃OD-D₂O 1.0:1.0:0.1 v/v/v, 400 MHz) δ = 0.87 (t, J = 6.8 Hz, 3H), 1.02-1.38 (m, 48H), 1.60 (m, 2H), 3.31 (m, 1H, C₅-H), 3.55 (m, 2H, C₃-H and C₄-H), 3.72 (dd, J = 5.6, 12.0 Hz, 1H, C₆-H), 3.86 (m, 3H, C₆-H’ and CH₂-OP), 3.93 (m, 1H, C₂-H), 5.07 (d, J = 7.6 Hz, 1H, C₁-H) ppm. ³¹P-NMR (CDCl₃-CD₃OD-D₂O 1.0:1.0:0.1 v/v/v, 161.6 MHz) δ = -0.8 ppm. Positive ion ESI-HRMS calcd for C₃₃H₆₇O₉P (M+Na)⁺: 661.442, found: 661.445.
β-17