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Synthesis of an Inositol Phosphoglycan Fragment found in *Leishmania* Parasites

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Abstract—Synthesis of **1** and **2a** is described using a block synthetic strategy. Compound **4** was used as precursor for the two mannose derivatives which, coupled together, forms the dimannoside building block. Thioglycoside **7** was coupled to **8** yielding inositol phosphoglycan **9a**, which was selectively deprotected and reacted with 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranos-1-yl H-phosphonate to form the protected target molecule **12**. Deprotection of **12** by acidic deacetalisation/desilylation and subsequent catalytic hydrogenolysis resulted in cleavage of the anomeric phosphodiester to produce **1**. Debenzylation with sodium in liquid ammonia followed by acidic deacetalisation/desilylation gave the target compound **2a**. © 2000 Published by Elsevier Science Ltd.

Introduction

In the different development stages in the lifecycle of the *Leishmania* parasites, various *myo*-inositol containing oligosaccharides are expressed on the cell surface. During the promastigote stage the cell surface is covered with polydisperse lipophosphoglycans (LPGs) and low molecular-mass glycoinositolphospholipids (GIPLs). In the amastigote stage, the cell surface of the parasite is covered with GIPLs and glycosphingolipids. The GIPLs are the major glycolipids synthesized by *Leishmania* parasites, they coat a significant proportion of the plasma membrane and are immunogenic.[1]

Up to now several GPI-anchors have been synthesized.[2] All characterized GPI-anchors contain the identical ethanolamine-phosphate-6Manα1-2Manα1-6Manα1-4GlcNα1-6*myo*-inositol backbone. The *Leishmania* LPGs, in contrast to the GPI-anchors, all contain a Galα1-6Galα1-3Gal*f*β1-3Manα1-3Manα1-4GlcNα1-core hexasaccharide linked to *lyso*alkylphosphatidylinositol, sharing the common Manα1-4GlcNα1-6Ins-unit with the GPI-anchors.[1] The C6 hydroxyl of the mannose residue most distal to the glucosamine is usually substituted with a glucosyl-α-1-phosphate. The GIPLs of *Leishmania* closely resembles the LPG core oligosaccharide-PI part and some of the GIPLs have been suggested as biosynthetic precursors to LPGs.

These structures are supposedly involved in protection of the parasite against hydrolytic enzymes in the insect midgut (promastigote phase) and against digestion by lysosomal enzymes in the mammalian host macrophages. It has also been suggested that LPGs and GIPLs are involved in mediating host–parasite interactions.[3]

The synthesized lipid free GIPLs, inositolphosphoglycans (IPGs), will be evaluated for cytokine responses in macrophages. In order to understand the biosynthetic pathways of the GIPLs and their biological role, we have synthesized the IPG **1** and **2a** corresponding to parts of the complete LPG. The enzymes that will be studied using these compounds are the putative galactofuranosyltransferase and the alpha 1,3- and alpha 1,6-galactopyranosyltransferases, which attach the remaining three galactose residues to complete the synthesis of the GIPL-core.[4]

Results and Discussion

Compound **4** (Scheme 1) was obtained by benzylation of **3**[5] and was used as a precursor for both mannose residues. Reductive benzylidene opening of **4**, followed by benzylation and removal of the silyl protecting group or monochloroacetylation of position 6 gave derivatives **5** and **6a**, respectively. After transformation of **6a** to **6b**, by bromine treatment, **6b** was coupled to **5** in 80% yield. Previous glycosylations[6] with glycosyl bromide **6b** had shown that the amount of *sym*-collidine is crucial. Absence of *sym*-collidine resulted in deprotection of the silyl group. More than 0.65 mol equiv. of *sym*-collidine, relative to silver triflate, gave lower yields. Disaccharide **7** was activated by dimethyl(methylthio)sulfonium trifluoromethanesulfonate

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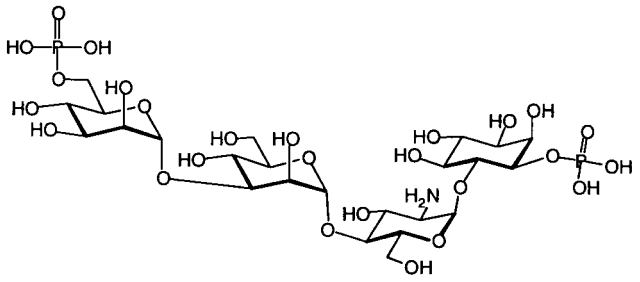

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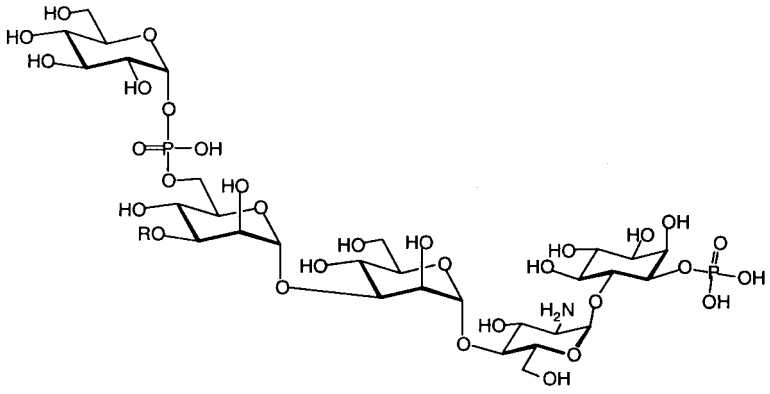
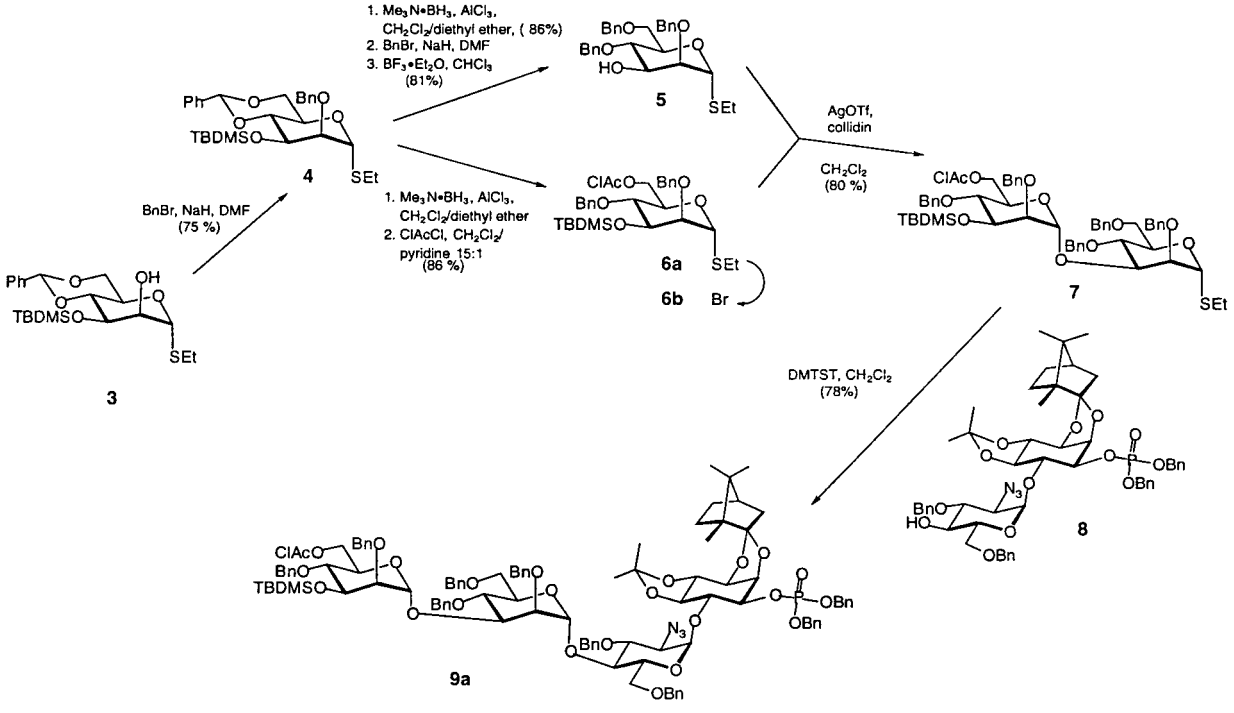
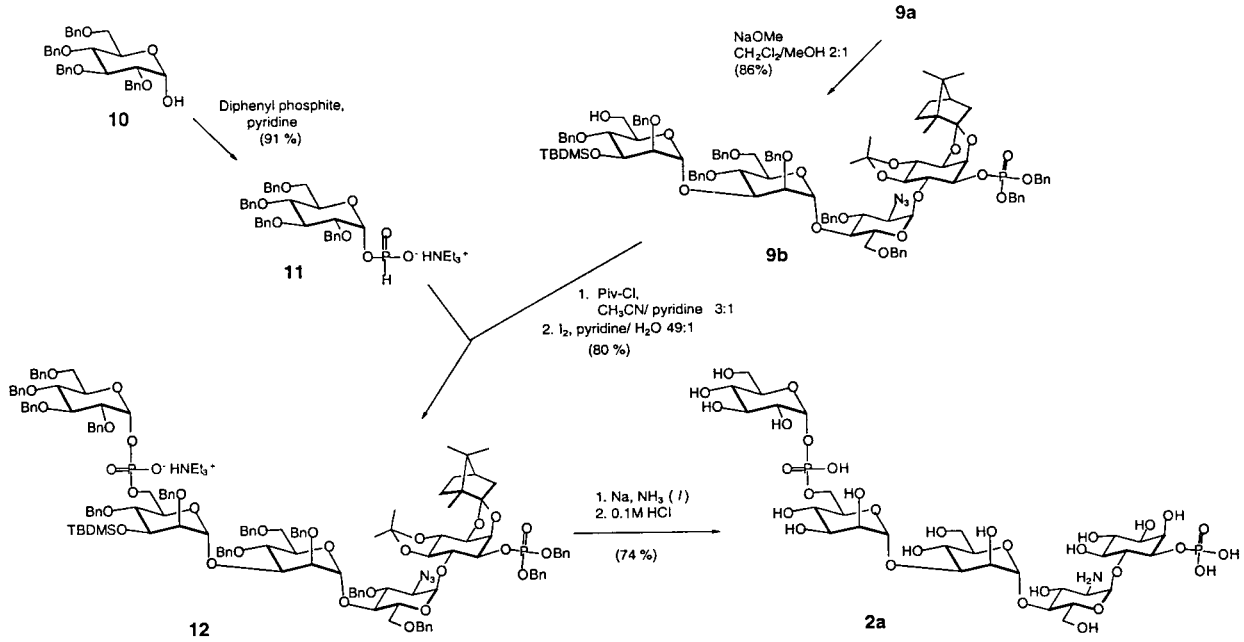


2a R = H
2b R = Gal_p-Gal_p-Gal_f

Figure 1.



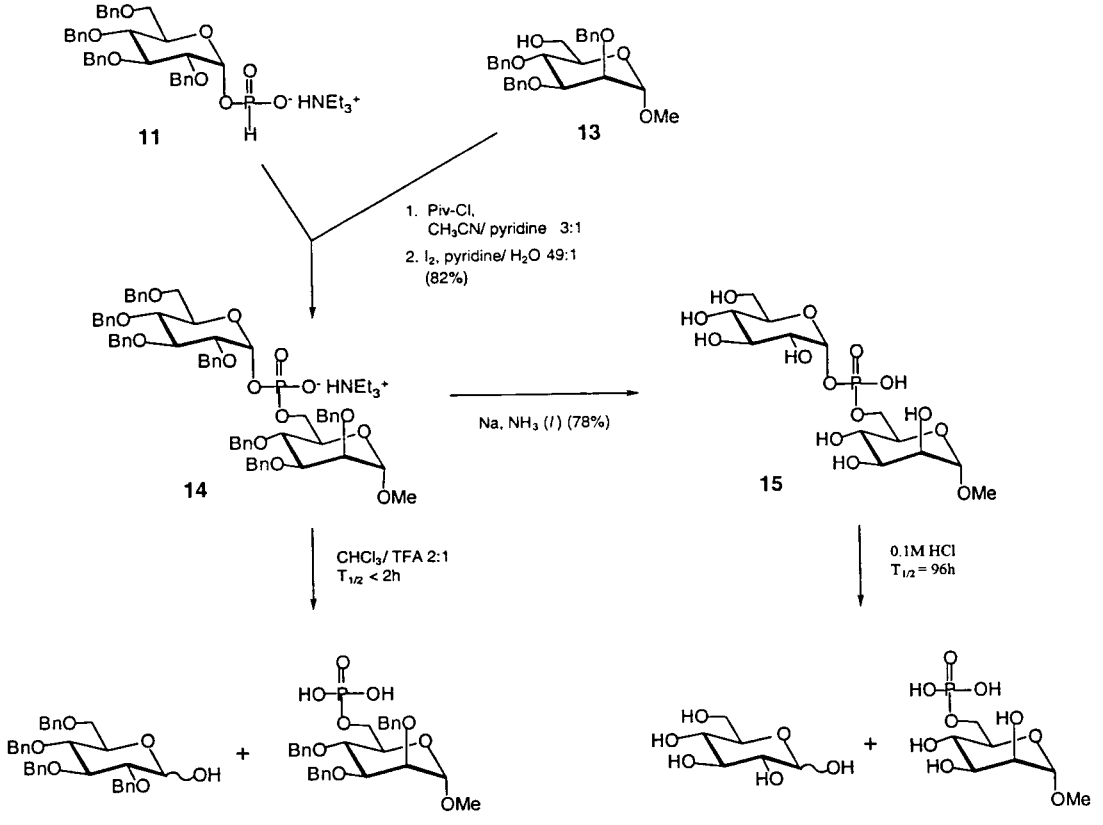
Scheme 1.



Scheme 2.

(DMTST) in dichloromethane and coupled with *myo*-inositol derivative **8**[7] in 78% yield (→**9a**). Building blocks **7** and **8** can be used in the synthesis of other core structures found in *Leishmania*.[1] Inositol phosphoglycan **9a** carries the possibility to introduce the glucose phosphate at the 6 position, as well as the trigalactose unit at the 3 position of the terminal mannose residue (compare structure **2b**, Fig. 1).

The anomerically pure α-D-glucopyranosyl H-phosphonate derivative[8] **11** (Scheme 2) was prepared by phosphonylation[9] of 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose **10**, obtained by recrystallization of commercially available 2,3,4,6-tetra-*O*-benzyl-α/β-D-glucopyranose from ethyl acetate. The base catalyzed anomerization has been shown to be slow enough to permit phosponylation without



Scheme 3.

affecting the anomeric configuration.[10] Coupling of **11** and **9b** followed by in situ oxidation with I_2 in pyridine/water produced compound **12** in 80% yield. The compound **9b** was synthesized from **9a** by removal of the chloroacetyl group with sodium methoxide in a mixture of methanol and dichloromethane. In a first attempt to deprotect **12** it was decided to start the deprotection with the removal of the acetal and silyl protecting groups with trifluoroacetic acid in chloroform followed by catalytic hydrogenolysis with $Pd(OH)_2$/C. However, only **1** could be isolated in 72% yield.

In order to optimize the conditions for the final deprotection, model compounds **14** and **15** were synthesized. Compound **14** (Scheme 3) was dissolved in chloroform/trifluoroacetic acid 10:1, conditions required for the deprotection of the silyl and acetal protecting groups.[7] After 2 h TLC and NMR-analysis showed complete solvolysis of the anomeric phosphate. The rate of hydrolysis for the deprotected compound **15** was determined in 0.1 M HCl. After 96 h 50% of the disaccharide had been hydrolysed. Deprotection of compound **8** with sodium in liquid ammonia followed by acidic hydrolysis of the acetals in 0.1 M HCl, showed after 6 h complete cleavage of the acetals. This pronounced difference in stability of the protected and the deprotected model compounds were also found in compounds **12** and **2a**. Reversal of the deprotection order of compound **12** proceeded as expected without any significant hydrolysis of the anomeric phosphate. Debenzylation with sodium in liquid ammonia followed by acidic hydrolysis of the silyl group and the acetals produced **2a** in 74% yield. The now completed synthesis of fragments **1** and **2a** will be followed up with attempts to synthesize the complete *Leishmania* core octasaccharide **2b**, using a newly constructed galactose trisaccharide unit and **7** and **8** as key building blocks.

Experimental

General methods

Normal workup means drying the organic phase (Na_2SO_4), filtration, and evaporation of the solvent in vacuo at or below 30°C except for *N*,*N*-dimethylformamide when 50°C were used. TLC: 0.25 mm precoated silica-gel plates (MERCK silica-gel $60F_{254}$); detection by spraying the plates with 8% aq. H_2SO_4 solution followed by heating at ca 250°C. Optical rotations were recorded at room temperature with a Perkin–Elmer 241 polarimeter. Flash Chromatography (FC): Silica gel MERCK 60 (0.040–0.063 mm). ^1H and ^{13}C NMR spectra were performed on a JEOL JNM-GSX 270, temperature 30°C unless otherwise stated. Chemical shifts are given in ppm relative to TMS in $CDCl_3$ (δ=0.00) or acetone in D_2O (^{13}C: δ=31.00, ^1H: δ=2.22) as internal standards; ^{31}P, phosphoric acid (δ=0.00) was used as external standard. pH* in D_2O is given as an uncorrected value calibrated against H_2O-buffer solutions. Mass spectra were recorded on a JEOL SX 102 Mass Spectrometer. IR spectra were recorded as films on CaF_2 plates (syrups) or as KBr pellets (solids) on a Perkin–Elmer SPECTRUM 1000 FT-IR Spectrometer.

Ethyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-*t*-butyldimethylsilyl-1-thio-α-D-mannopyranoside (4). A solution of **3**[5] (8.55 g, 20 mmol) and benzyl bromide (4.76 mL, 40 mmol) in DMF (80 mL) was added dropwise to a suspension of NaH (60%, 2.4 mg, 0.06 mmol) in DMF (100 mL). After 15 min the excess NaH was destroyed with MeOH, and the reaction mixture was diluted with toluene and washed with H_2O followed by normal workup. FC (light petroleum (45–65)/EtOAc 19:1) afforded **4** (7.79 g, 15 mmol, 75%) as a colorless syrup. R_f 0.38 (light petroleum (45–65)/EtOAc 6:1); $[\alpha]_D$=+75 (*c* 1.2, $CHCl_3$); IR ν_{max} cm^{-1} 2896, 2953, 2927, 2856, 1455, 1382, 1255, 1212, 1098, 1058, 1014; NMR ($CDCl_3$) ^1H, δ 0.04 (s, 3H), 0.08 (s, 3H), 0.89 (s, 9H), 1.23 (t, 3H, *J*=7.2 Hz), 2.56 (m, 2H), 3.78–3.84 (m, 2H), 4.09–4.20 (m, 4H), 4.69 (benzylic d, 1H, *J*=12.1 Hz), 4.86 (benzylic d, 1H, *J*=11.9 Hz), 5.28 (s, 1H), 5.56 (s, 1H), 7.31–7.47 (m, 10H); ^{13}C, δ −4.8, −4.4, 15.0, 18.4, 25.4, 25.9, 64.9, 68.7, 71.0, 74.0, 79.4, 81.2, 84.1, 101.9, 126.3, 127.8, 127.9, 128.0, 128.4, 128.8, 137.7, 138.3; HRMS Calcd for $C_{28}H_{40}O_5SiS$: $[M+Na]^+$ 539.2263. Found $[M+Na]^+$ 539.2282.

Ethyl 2,4-di-*O*-benzyl-3-*O*-*t*-butyldimethylsilyl-6-*O*-chloroacetyl-1-thio-α-D-mannopyranoside (6a). A mixture of **4** (392 mg, 0.76 mmol), borane trimethylamine complex (1.19 g, 15.2 mmol) and 4 Å MS (2 g) in CH_2Cl_2 (25 mL) and Et_2O (5 mL) was cooled to 0°C. $AlCl_3$ (405 mg, 3.03 mmol) was added and, after 10 min, the reaction mixture was filtered through a pad of Celite. 1 M HCl (30 mL) was added to the filtrate and the mixture was stirred for 30 min. The organic phase was then separated and washed with $NaHCO_3$ (aq). Normal workup and FC (toluene/EtOAc 9:1) yielded ethyl 2,4-di-*O*-benzyl-3-*O*-*t*-butyldimethylsilyl-1-thio-α-D-mannopyranoside (338 mg, 0.65 mmol, 86%). R_f 0.24 (toluene/EtOAc 9:1). To a solution of this compound (3.74 g, 7.20 mmol) in CH_2Cl_2 (45 mL) and pyridine (3 mL) was added chloroacetyl chloride (0.86 mL, 11 mmol). After 5 min, the reaction mixture was washed with 10% $CuSO_4$ (aq) (300 mL) and $NaHCO_3$ (aq). **6a** was quantitatively isolated (4.23 g, 7.20 mmol) as a colorless syrup, after normal workup and FC (light petroleum (45–65)/EtOAc 18:1→6:1). R_f 0.80 (toluene/EtOAc 9:1); $[\alpha]_D$=+103 (*c* 1.2, $CHCl_3$); IR ν_{max} cm^{-1} 2955, 2928, 2884, 2857, 1764, 1738, 1454, 1259, 1101; NMR ($CDCl_3$) ^1H, δ 0.11 (s, 3H), 0.12 (s, 3H), 0.95 (s, 9H), 1.23 (t, 3H, *J*=7.3 Hz), 2.57 (m, 2H), 3.73 (s, 1H), 3.85–3.90 (m, 3H), 4.06–4.20 (m, 2H), 4.29–4.40 (m, 2H), 4.55, (d, 1H, *J*=11.4 Hz), 4.65 (d, 1H, *J*=11.9 Hz), 4.77 (d, 1H, *J*=11.9 Hz), 4.89 (d, 1H, *J*=11.4 Hz), 5.28 (s, 1H), 7.30–7.37 (m, 10H); ^{13}C, δ −4.7, −4.4, 15.0, 18.0, 25.4, 25.9, 40.6, 64.9, 70.4, 72.7, 74.0, 75.0, 75.3, 80.7, 82.4, 127.5, 127.6, 127.7, 127.8, 128.2, 128.3, 138.0, 138.3, 166.8; HRMS Calcd for $C_{30}H_{43}O_6ClSiS$: $[M+Na]^+$ 617.2136. Found $[M+Na]^+$ 617.2149.

Ethyl 2,4,6-tri-*O*-benzyl-1-thio-α-D-mannopyranoside (5). Benzyl bromide (1.2 mL, 0.010 mmol) and ethyl 2,4-di-*O*-benzyl-3-*O*-*t*-butyldimethylsilyl-1-thio-α-D-mannopyranoside (2.63 g, 5.06 mmol) were dissolved in DMF (50 mL). The solution was added dropwise to a slurry of NaH (60%, 405 mg, 0.010 mmol) in DMF (50 mL). MeOH was added after 15 min to destroy the excess of NaH. The mixture was concentrated and the residue was dissolved in toluene and washed with H_2O. The organic phase was concentrated and

the resulting syrup was dissolved in $CHCl_3/BF_3{\cdot}Et_2O$ (50:1, 51 mL). The mixture was heated at 45°C for 1.5 h and then neutralised with $NaHCO_3$ (aq). Normal workup and FC (toluene/EtOAc 20:1) afforded **5** (1.896 g, 3.833 mmol, 76%) as a colorless syrup. R_f 0.44 (toluene/EtOAc 9:1); $[\alpha]_D$=+85 (*c* 0.99, $CHCl_3$); IR ν_{max} cm^{-1} 3546, 3470, 3062, 3029, 2926, 2869, 1496, 1453, 1397, 1351, 1267, 1207, 1097; NMR ($CDCl_3$) ^1H, δ 1.26 (t, 3H, *J*=7.5 Hz), 2.50–2.72 (m, 2H), 3.68–3.85 (m, 4H), 3.95 (dd, 1H, *J*=9.1, 3.6 Hz), 4.13 (ddd, 1H, *J*=9.5, 4.4, 1.8 Hz), 4.48–4.55 (m, 3H), 4.68 (d, 1H, *J*=12.1 Hz), 4.77 (d, 1H, *J*=11.7 Hz), 4.85 (d, 1H, *J*=10.9 Hz), 5.49 (s, 1H), 7.20–7.40 (m, 15H); ^{13}C, δ 14.9, 25.0, 69.1, 71.3, 72.1, 72.3, 73.3, 74.7, 76.8, 79.9, 81.0, 127.4–128.5, 137.6, 138.3, 138.5; HRMS Calcd for $C_{29}H_{34}O_5S$: $[M+Na]^+$ 517.2025. Found $[M+Na]^+$ 517.2037.

Ethyl (2,4-di-*O*-benzyl-3-*O*-*t*-butyldimethylsilyl-6-*O*-chloroacetyl-α-D-mannopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-1-thio-α-D-mannopyranoside (7). Br_2 (20 μL, 0.392 mmol) was added to a solution of **6a** (224 mg, 0.377 mmol) in CH_2Cl_2 (10 mL) containing 4 Å MS (1 g). After 10 min the reaction mixture was concentrated and co-concentrated twice with toluene (Na-dried). This bromosugar **6b**, **5** (149 mg, 0.301 mmol) and collidine (65 μL, 0.489 mmol) were dissolved in CH_2Cl_2 (20 mL) and an additional 0.5 g 4 Å MS was added. The mixture was cooled to −30°C and silver trifluoromethanesulfonate (166 mg, 0.753 mmol) in Na-dried toluene (5 mL) was added dropwise. After 20 min the reaction was quenched by the addition of Et_3N (1 mL). The mixture was filtered through Celite, washed with $NaHCO_3$ (aq), and subjected to normal workup [FC (light petroleum (45–65)/EtOAc 18:1→9:1)] to yield 7 (245 mg, 0.240 mmol, 79%) as a colorless syrup. R_f 0.53 (light petroleum (45–65)/EtOAc 4:1); $[\alpha]_D$=+76 (*c* 1.1, $CHCl_3$); IR ν_{max} cm^{-1} 2954, 2928, 2857, 1763, 1497, 1455, 1253, 1092; NMR ($CDCl_3$) ^1H, δ 0.09 (s, 3H), 0.11 (s, 3H), 0.94 (s, 9H), 1.27 (t, 3H, *J*=7.3 Hz), 2.62 (m, 2H), 3.58–4.30 (m, 16H), 4.45–4.66 (m, 6H), 4.78 (d, 1H, *J*=11.7 Hz), 4.89 (d, 1H, *J*=11.7 Hz), 5.13 (s, 1H), 5.49 (s, 1H), 7.15–7.45 (m, 25H); ^{13}C, δ −4.7, −4.3, 15.0, 18.0, 25.3, 26.0, 40.9, 65.5, 69.1, 70.6, 71.7, 72.2, 73.1, 73.3, 73.4, 74.6, 74.9, 75.4, 75.6, 79.3, 79.5, 79.6, 81.5, 100.4 ($J_{C,H}$=170.5 Hz), 126.9–128.5, 137.9, 138.2, 138.3, 138.4, 138.5, 167.1; HRMS Calcd for $C_{57}H_{71}O_{11}ClSiS$: $[M+Na]^+$ 1049.4073. Found $[M+Na]^+$ 1049.4093.

(2,4-Di-*O*-benzyl-3-*O*-*t*-butyldimethylsilyl-6-*O*-chloroacetyl-α-D-mannopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→4)-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→6)-1-*O*-dibenzyloxyphosphoryl-4,5-*O*-isopropylidene-2,3-*O*-(D-1,7,7-trimethyl[2.2.1]bicyclohept-6-ylidene)-D-*myo*-inositol (9a). To a mixture of 6-*O*-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-1-*O*-dibenzyloxyphosphoryl-4,5-*O*-isopropylidene-2,3-*O*-(D-1,7,7-trimethyl[2.2.1]bicyclohept-6-ylidene)-D-*myo*-inositol **8**[7] (150 mg, 0.153 mmol), **7** (203 mg, 0.198 mmol) and 4 Å MS (1.5 g) in CH_2Cl_2 (15 mL) under an Ar-atmosphere was added DMTST (59 mg, 0.229 mmol). After 1 h, Et_3N (1 mL) was added. The reaction mixture was filtered through a pad of Celite and concentrated. FC (toluene/EtOAc 12:1, 0.25% Et_3N) of the residue yielded 78% (232 mg, 0.119 mmol) of **9a** as a colorless syrup. R_f 0.53 (toluene/EtOAc 4:1); $[\alpha]_D$=+49 (*c* 0.98, $CHCl_3$); IR ν_{max} cm^{-1} 2929, 2107, 1762, 1497, 1454, 1372, 1253, 1111, 1050, 1006; NMR ($CDCl_3$) ^1H, δ 0.05 (s, 3H), 0.06 (s, 3H), 0.83 (s, 3H), 0.89 (s, 3H), 0.91 (s, 9H), 0.98 (s, 3H), 1.10–1.41 (m, 9H), 1.60–2.10 (m, 4H), 3.26 (dd, 1H, *J*=9.3, 3.7 Hz), 3.43–5.20 (m, 45H), 5.49 (s, 1H), 7.10–7.40 (m, 45H); ^{13}C, δ −4.7, −4.3, 10.0, 18.0, 20.3, 20.5, 26.0, 27.0–27.1 (3C), 29.9 40.7, 43.9, 45.2, 48.0, 51.6, 62.9–80.3, 97.3 ($J_{C,H}$=174.1 Hz), 98.6 ($J_{C,H}$=170.5 Hz), 100.5 ($J_{C,H}$=170.5 Hz), 112.5, 119.1, 126.9–129.1, 135.7, 137.8–138.6, 166.9; HRMS Calcd for $C_{108}H_{129}O_{24}ClN_3PSi$: $[M+Cs]^+$ 2078.7216. Found: $[M+Cs]^+$ 2078.7258.

(2,4-Di-*O*-benzyl-3-*O*-*t*-butyldimethylsilyl-α-D-mannopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→4)-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→6)-1-*O*-dibenzyloxyphosphoryl-4,5-*O*-isopropylidene-2,3-*O*-(D-1,7,7-trimethyl[2.2.1]bicyclohept-6-ylidene)-D-*myo*-inositol (9b). 9a (226 mg, 0.116 mmol) was dissolved in CH_2Cl_2/MeOH (2:1, 30 mL) and methanolic sodium methoxide (1 M, 0.2 mL) was added. The reaction mixture was concentrated after 5 min, whereafter FC (toluene/EtOAc 6:1, 0.25% Et_3N) afforded **9b** (186 mg, 0.100 mmol, 86%) as a colorless syrup. R_f 0.47 (toluene/EtOAc 4:1); $[\alpha]_D$=+40 (*c* 1.1, $CHCl_3$); IR ν_{max} cm^{-1} 2928, 2107, 1497, 1454, 1372, 1256, 1111, 1050, 1006; NMR ($CDCl_3$) ^1H, δ 0.03 (s, 6H), 0.83 (s, 3H), 0.89 (s, 3H), 0.90 (s, 9H), 0.97 (s, 3H), 1.10–1.42 (m, 9H), 1.60–2.18 (m, 4H), 3.24 (dd, 1H, *J*=9.6, 3.5 Hz), 3.43–5.20 (m, 43H), 5.39 (s, 1H), 7.10–7.40 (m, 45H); ^{13}C, δ −4.7, −4.3, 10.0, 18.0, 20.3, 20.5, 26.0, 26.9–27.1 (3C), 29.8, 43.9, 45.1, 48.0, 51.5, 62.5–80.2, 97.2, 99.3, 100.4, 112.5, 119.0, 126.6–128.6, 135.7, 138.0–138.5; HRMS Calcd for $C_{106}H_{128}O_{23}N_3PSi$: $[M+Na]^+$ 1892.8343. Found: $[M+Na]^+$ 1892.8344.

2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranos-1-yl H-phosphonate, triethylammonium salt (11). Diphenyl phosphite (0.50 mL, 2.6 mmol) and 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose **10** (202 mg, 0.374 mmol) were dissolved in pyridine. After 20 min Et_3N/H_2O (1:1, 2 mL) was added and the mixture was stirred for an additional 15 min. The solvents were removed and the residue was dissolved in CH_2Cl_2 and washed twice with 5% $NaHCO_3$ (aq). Normal workup and FC ($CHCl_3$/MeOH 20:1 with 0.5% Et_3N) then yielded **11** (240 mg, 0.340 mmol, 91%) as a colorless syrup. R_f 0.60 ($CHCl_3$/MeOH 5:1); $[\alpha]_D$=+61 (*c* 1.1, $CHCl_3$); IR ν_{max} cm^{-1} 3029, 2914, 2866, 2362, 1496, 1453, 1360, 1224, 1092, 1071, 929; NMR ($CDCl_3$) ^1H, δ 1.18 (t, 9H, *J*=7.2 Hz), 2.89 (m, 6H), 3.66–3.73 (m, 4H), 4.02–4.06 (m, 2H), 4.43–4.77 (m, 4H), 4.82–4.96 (m, 4H), 5.89 (dd, 1H, *J*=8.8, 2.9 Hz), 7.03 (d, 1H, *J*=646 Hz), 7.18–7.37 (m, 20H); ^{13}C, δ 8.4, 45.3, 68.5, 71.5, 72.3, 73.4, 74.8, 75.5, 77.5, 79.8 (d, *J*=5.5 Hz), 81.6, 92.4 (d, *J*=5.5 Hz, J_{C-H}=176 Hz), 127.5–128.3, 138.1, 138.3, 138.6, 138.8; ^{31}P, δ 1.0 (d, *J*=660 Hz); HRMS Calcd for $C_{34}H_{37}O_8P$: $[M+Na]^+$ 627.2124. Found $[M+Na]^+$ 627.2109.

[(2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranos-1-yl) (2,4-di-*O*-benzyl-3-*O*-*t*-butyldimethylsilyl-α-D-mannopyranos-6-yl triethylammonium phosphate)]-(1→3)-(2,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→4)-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→6)-1-*O*-dibenzyl-

oxyphosphoryl-4,5-*O*-isopropylidene-2,3-*O*-(D-1,7,7-trimethyl[2.2.1]bicyclohept-6-ylidene)-D-*myo*-inositol (12). 11 (58 mg, 0.082 mmol) and **9b** (102 mg, 0.055 mmol) were dissolved in CH_3CN/pyridine (3:1, 16 mL) and pivaloyl chloride (33 μL, 0.269 mmol) was added. The coupling reaction was ready within 5 min according to TLC, when I_2 (28 mg, 0.109 mmol) in pyridine/water (49:1, 10 mL) was added. After an additional 1 h 15 min, the reaction was diluted with $CHCl_3$ and washed with 10% $Na_2S_2O_3$ (aq). Normal workup and FC ($CHCl_3$→$CHCl_3$/MeOH 20:1 with 0.25% Et_3N) gave **12** (114 mg, 0.044 mmol, 81%) as a colorless syrup. R_f 0.27 ($CHCl_3$/MeOH 20:1); $[\alpha]_D$=+37 (*c* 1.2, $CHCl_3$); IR ν_{max} cm^{-1} 2925, 2107, 1497, 1453, 1351, 1252, 1174, 1110, 1009; NMR ($CDCl_3$) ^1H, δ −0.07 (s, 3H,), −0.02 (s, 3H,) 0.83 (s, 3H), 0.85 (s, 9H), 0.91 (s, 3H), 0.98 (s, 3H), 1.02 (t, 9H, *J*=7.3 Hz), 1.10–1.46 (m, 9H), 1.50–1.75 (m, 2H), 1.90–2.08 (m, 2H), 2.62 (m, 6H), 3.22–5.18 (m, 58H), 5.50 (s, 1H), 5.94 (dd, 1H, *J*=7.8, 3.1 Hz), 7.05–7.45 (m, 65H); ^{13}C, δ −4.7, −4.3, 8.4, 10.0, 17.9, 20.2, 20.5, 26.0, 27.0, 27.3 (2C), 29.9, 43.6, 45.2, 47.9, 51.5, 63.6–81.6, 93.1, 97.6, 98.9, 100.7, 112.4, 119.0, 126.9–128.5, 135.6, 135.7, 138.0–139.2; ^{31}P (decoupled), δ −1.1, −0.6; HRMS Calcd for $C_{140}H_{163}O_{31}N_3P_2Si$: $[M-H+2Cs]^+$ 2736.8546. Found $[M-H+2Cs]^+$ 2736.8562.

(α-D-Mannopyranosyl 6-phosphate)-(1→3)-α-D-mannopyranosyl-(1→4)-2-amino-2-deoxy-α-D-glucopyranosyl-(1→6)-D-*myo*-inositol 1-phosphate, pyridinium salt (1). To a stirred solution of **12** (129 mg, 0.050 mmol) in $CHCl_3$ (10 mL) was added CF_3COOH (1 mL). After 4 h the reaction mixture was neutralized with $NaHCO_3$ (aq). The organic phase was concentrated and the residue dissolved in EtOAc/MeOH/H_2O (8:2:1, 11 mL). A catalytic amount of $Pd(OH)_2$ on carbon was added and the mixture was hydrogenolysed at 120 psi for 48 h (after 24 h H_2O (1 mL) was added). The mixture was filtered through Celite and concentrated. Gel filtration of the residue on a Bio-Gel P2 column eluted with a pyridinium acetate buffer (pH 5) afforded **1** (30 mg, 0.036 mmol, 72%) as a white solid. $[\alpha]_D$=+88 (*c* 1.4, H_2O); IR ν_{max} cm^{-1} 3428, 2926, 1635, 1384, 1046; NMR (D_2O) pH^*=5.1 ^1H, δ 3.33 (dd, 1H, *J*=10.6, 3.7 Hz), 3.44 (t, 1H, *J*=9.1 Hz), 3.55–4.28 (m, 21H), 4.35 (s, 1H), 5.06 (s, 1H), 5.28 (s, 1H), 5.65 (d, 1H, *J*=3.6 Hz); ^{13}C, δ 54.8, 60.9, 61.6, 65.5 (d, *J*=3.7 Hz), 66.3, 67.6, 70.5, 70.7, 71.0, 71.1, 71.3, 71.5, 72.4, 73.0, 73.2, 73.3, 74.7, 76.2, 76.7, (d, 1H, *J*=7.4 Hz), 77.5 (d, 1H, *J*=7.4 Hz), 80.1, 95.8, 102.4, 103.7; ^{31}P (decoupled), δ 0.46 (br, 2P); HRMS Calcd for $C_{24}H_{45}O_{26}NP_2$: $[M+H]^+$ 826.1784. Found $[M+H]^+$ 826.1794.

[(α-D-Glucopyranos-1-yl) (α-D-mannopyranos-6-yl) phosphate]-(1→3)-α-D-mannopyranosyl-(1→4)-2-amino-2-deoxy-α-D-glucopyranosyl-(1→6)-D-*myo*-inositol 1-phosphate, ammonium salt (2a). To NH_3 (*l*) (ca. 20 mL) at −33°C was added **12** (39 mg, 0.015 mmol) in THF (2 mL). To the stirred mixture was added a minimum amount of sodium for the mixture to turn deep blue. After 1 min NH_4Cl was added until the color disappeared. The mixture was concentrated and the residue was dissolved in 0.1 M HCl (10 mL). After 6 h the mixture was neutralized with NH_3 (25%, 0.1 mL), washed with ether (10 mL) and concentrated. Gel filtration of the residue on a Pharmacia Sephadex G-15 column eluted with H_2O containing 1% *n*-butanol afforded **2a** (11 mg, 0.011 mmol, 74%) as a white solid. $[\alpha]_D$=+100 (*c* 1.0, H_2O); IR ν_{max} cm^{-1} 3418, 2931, 1635, 1402, 1384, 1224, 1090, 1048; NMR (D_2O) pH^*=5.6 ^1H, δ 3.32 (dd, 1H, *J*=10.6, 4.0 Hz), 3.43 (t, 1H, *J*=9.3 Hz), 3.49 (t, 1H, *J*=9.3 Hz), 3.55–4.27 (m, 26H), 4.29 (t, 1H, *J*=2.2), 5.07 (d, 1H, *J*=0.7 Hz), 5.30 (d, 1H, *J*=1.1 Hz), 5.52 (dd, 1H, *J*=7.3, 3.6 Hz), 5.71 (d, 1H, *J*=4.0 Hz); ^{13}C, δ 54.7, 60.8, 61.1, 61.6, 66.1 (d, *J*=5.5 Hz), 66.3, 67.4, 69.9, 70.6, 70.7, 71.0, 71.3, 71.4, 72.0, 72.1, 72.6, 73.1 (2C), 73.2, 73.3, 73.6, 74.6, 76.0, 76.4 (d, *J*=5.6 Hz), 77.5 (d, *J*=5.6 Hz), 80.0, 95.7, 96.1 (d, *J*=7.4 Hz), 102.3, 103.6; ^{31}P (decoupled), δ −1.11, 1.44; HRMS Calcd for $C_{30}H_{55}NO_{31}P_2$: $[M-H]^-$ 986.2155. Found $[M-H]^-$ 986.2200.

(2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranos-1-yl) (methyl 2,3,4-tri-*O*-benzyl-α-D-mannopyranosid-6-yl) triethylammonium phosphate (14). 11 (170 mg, 0.24mmol) and **13**[11] (82 mg, 0.18 mmol) were dissolved in CH_3CN/pyridine (3:1, 12 mL) and pivaloyl chloride (0.15 mL, 1.22 mmol) was added. A 2% solution of I_2 in pyridine/water, 49:1 (7.0 mL, 0.55 mmol) was added to the reaction mixture after 40 min. After an additional 20 min, the reaction was diluted with $CHCl_3$ and washed with 10% $Na_2S_2O_3$ (aq). Normal workup and FC ($CHCl_3$/MeOH 20:1→10:1, 0.25% Et_3N) gave **14** (169 mg, 0.14 mmol, 82%) as a colorless syrup. R_f 0.32 ($CHCl_3$/MeOH 9:1); $[\alpha]_D$=+77 (*c* 0.76, $CHCl_3$); IR ν_{max} cm^{-1} 3030, 2925, 1496, 1453, 1363, 1237, 1098; NMR ($CDCl_3$) ^1H, δ 1.11 (t, 9H, *J*=7.3 Hz), 2.77 (m, 6H), 3.25 (s, 3H), 3.50–92 (m, 8H), 3.95–4.11 (m, 2H), 4.20–4.35 (m, 3H), 4.42–4.94 (m 14H), 5.93 (dd, 1H, *J*=7.7, 2.9 Hz), 7.05–7.41 (m, 35H); ^{13}C, δ 8.4, 45.1, 54.7, 64.6 (d, *J*=4.9 Hz), 68.5, 71.2, 71.3, 71.5, 71.8, 72.2, 73.0, 73.3, 74.7, 74.9, 75.0, 75.3 (2C), 77.4, 80.3, 81.6, 93.0 (d, *J*=5.6 Hz), 98.9, 127.3–128.3, 138.3–139.0; ^{31}P (decoupled), δ −1.01; HRMS Calcd for $C_{62}H_{67}O_{14}P$: $[M+Na]^+$ 1089.4166. Found: $[M+Na]^+$ 1089.4164.

(α-D-Glucopyranos-1-yl) (methyl α-D-mannopyranosid-6-yl) ammonium phosphate (15). To NH_3 (*l*) (ca. 20 mL) at −33°C was added **14** (54 mg, 0.046 mmol) in THF (2 mL). To the stirred mixture was added a minimum amount of sodium for the mixture to turn deep blue. After 1 min NH_4Cl was added until the color disappeared. The mixture was concentrated and gel filtration of the residue on a Pharmacia Sephadex G-15 column eluted with H_2O containing 1% *n*-butanol afforded **15** (16 mg, 0.076 mmol, 78%) as a white solid. $[\alpha]_D$=+36 (*c* 1.4, H_2O); IR ν_{max} cm^{-1} 3404, 2936, 1634, 1401, 1225, 1135, 1093, 1048, 943, 876; NMR (D_2O) pH^*=6.3 ^1H, δ 3.41 (s, 3H), 3.48 (t, 1H, *J*=9.3 Hz), 3.57 (dt, 1H, *J*=9.9, 2.6 Hz), 3.70–3.93 (m, 7H), 4.08–4.21 (m, 2H), 4.76 (d, 1H, *J*=1.8 Hz), 5.52 (dd, 1H, *J*=6.9, 3.6 Hz); ^{13}C, δ 55.6, 61.1, 65.3 (d, *J*=3.7 Hz), 67.0, 69.9, 70.6, 71.2, 72.1, 72.2, 73.3, 73.6, 96.1 (d, *J*=5.5 Hz), 101.7; ^{31}P (decoupled), δ −1.11; HRMS Calcd for $C_{13}H_{25}O_{14}P$: $[M-H]^-$ 435.0904. Found: $[M-H]^-$ 435.0903.

Hydrolytic studies of compounds 14 and 15. To **14** (10 mg) in $CHCl_3$ (3 mL) was added TFA (0.3 mL). After 2 h, when TLC-analysis showed only traces of **14**, pyridine (2 mL) was added and the mixture was concentrated. In the

NMR-spectrum of the residue only traces of the glucose-phosphate anomeric proton could be detected.

15 (4 mg) was dissolved in 0.1 M HCl (10 mL). After 24 h half of the material was neutralized. In order to decrease the salt concentration, Ag_2CO_3 (100 mg) was used for the neutralization to precipitate chloride ions. After stirring for 10 min the mixture was centrifuged and the supernatant was passed through a DOWEX cation exchange resin (NH_4^+-form) to remove traces of Ag^+. After lyophilization the product mixture was quantified by the integrated intensities of the glucose anomeric protons. After 96 h the rest of the material was subjected to the same workup procedures. After 24 h and 96 h 16% and 50% of the anomeric phosphate had been hydrolyzed.

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