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Synthesis of the *Trypanosoma cruzi* LPPG Heptasaccharyl *myo*-Inositol

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Abstract: Synthesis of the heptasaccharyl *myo*-inositol found in *Trypanosoma cruzi* lipopeptidophosphoglycan was accomplished using a convergent assembly of three building blocks. The target compound is the first complete 2-aminoethyl phosphonic acid substituted glycan related to the glycosylphosphatidylinositol anchor family to be synthesized. The order of assembly enables synthesis of phosphoinositol oligosaccharides related to other glycosylinositolphospholipids in *Tr. cruzi*, the protozoan parasite causing Chagas' disease, which is endemic in South America.

Introduction

Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi*, is a major public health problem in Central and South America, and 18−20 million people are currently infected leading to ∼21 000 deaths each year.[1] At present, there is no cure for this disease, and the available drugs are used selectively due to their cost, significant side effects, and low antiparasitic activity in the prevalent chronic form of the disease.[2] The parasite has a complex life cycle with the mammalian host and insect vector. *Hematophagous reduviid* vectors, infesting the houses of poor people, transmit the parasite to vertebrates by leaving parasite-containing feces after taking a bloodmeal. Disease transmission also occurs through the transfusion of infected blood, and the intake has recently been shown, through ingestion of crushed sugar cane.[3] Thus, elimination of the insect vectors from homes through spraying with insecticide, which has successfully diminished the number of newly infected people, is not sufficient for complete eradication of this disease, and a demand of new treatments and diagnostic tests remains. The recently reported genome sequencing of *T. cruzi* CL Brener clone, a *T. cruzi* II strain, may be a key to determining novel targets for treatments.[4] The organism has a large genome, which plausibly contributes to mechanisms to evade host immune responses.

Herein, we present the synthesis of the heptasaccharyl *myo*-inositol **1** (Figure 1), the inositolphosphoglycan of the *T. cruzi* glycoinositolphospholipid (GIPL) formerly known as the lipopeptidophosphoglycan (LPPG).[5] This glycan, attached to a ceramide lipid anchor, constitutes a major cell membrane constituent in the proliferative epimastigote stage of *T. cruzi* that is found in the insect vector. Characterization of the GIPLs from a number of *T. cruzi* strains has revealed that a number of different glycan structures can be expressed. All contain the same tetramannoside framework linked to a nonacylated glucosamine substituted on O-6 with the uncommon 2-aminoethyl phosphonic acid (2-AEP) motif.[6] The digalactofuranosyl motif appears to be characteristic of *T. cruzi* II strains that predominate in human infections, while other structures contain phosphorylated substituents on the O-6 of the subterminal Man, which is a direct analogue of the glycosylphosphatidylinositol (GPI) anchor that anchors many of the parasitic membrane proteins considered essential for host cell invasion. The *T. cruzi* GIPLs are bifunctional molecules that up- and down-regulate different cell types of the host immune system, with the glycan component influencing B cells.[7]

The development of our synthetic route opens a number of possibilities for future work, including the development of immunogenic glycoconjugates for possible therapy or the production of serum or monoclonal antibodies for diagnostic purposes and the means to probe the immune regulatory properties of different GIPL structures in detail. In addition, smaller synthetic structures can be used in studies of GIPL and GPI biosynthesis, which may reveal novel targets for therapeutic intervention.

Results and Discussion

The inositolphosphoglycan **1** contains structural motifs (Gal*f*, 2-AEP, and *myo*-Ins-1-PO_4), which require a well-planned synthetic strategy. For example, the presence of 2-AEP was a synthetic challenge since protocols to compounds with this motif are restricted because of the low abundance of natural compounds containing 2-AEP. The disconnection sites of target molecule **1** were chosen on the basis of our experience in the successful synthesis of the *Leishmania* LPG core oligosaccha-

(1) WHO, The World Health Report, 2002, www.who.int/tdr/diseases/chagas.
(2) Urbina, J. A.; Docampo, R. *Trends Parasitol.* **2003**, *19*, 495−501.
(3) Health Protection Agency, *CDR Weekly* **2005**, *15*.
(4) El-Sayed et al. *Science* **2005**, *309*, 409−415.
(5) de Lederkremer, R. M.; Lima, C.; Ramirez, M. I.; Ferguson, M. A. J.; Homans, S. W.; Thomas-Oates, J. *J. Biol. Chem.* **1991**, *266*, 23670−23675.
(6) Carreira, J. C.; Jones, C.; Wait, R.; Previato, J. O.; Mendonça-Previato, L. *Glycoconjugate J.* **1996**, *13*, 955−966.
(7) (a) DosReis, G. A.; Peçanha, L. M. T.; Bellio, M.; Previato, J. O.; Mendonça-Previato, L. *Microbes Infect.* **2002**, *4*, 1007−1013. (b) Ropert, C.; Gazzinelli, R. T. *Curr. Opin. Microbiol.* **2000**, *3*, 395−403. (c) Previato, J. O.; Wait, R.; Jones, C.; DosReis, G. A.; Todeschini, A. R.; Heise, N.; Mendonça-Previato, L. *Adv. Parasitol.* **2004**, *56*, 1−41.

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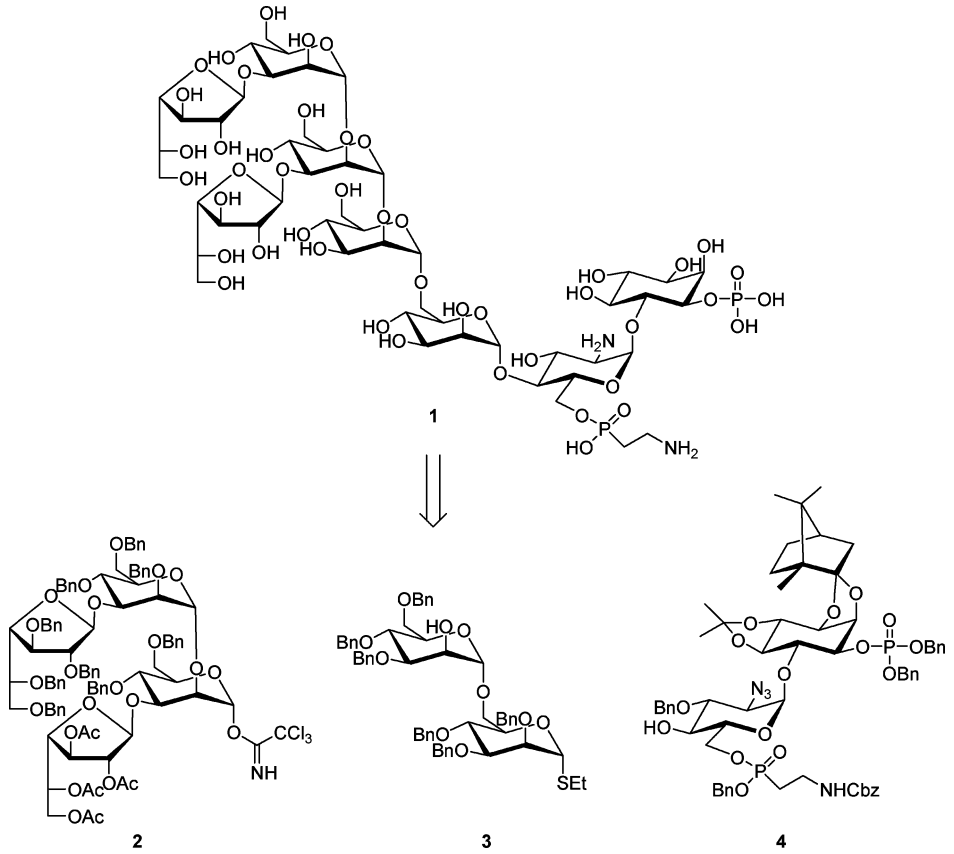


Figure 1. Target molecule **1** and the building blocks **2**−**4**.

ride.[8] In addition, the preparation and utilization of compound **4** has recently been presented.[9] The introduction of this building block was as late as possible in the synthetic route since it exists as a diastereomeric mixture of carbon-phosphonates and requires the largest number of synthetic steps. Synthesis of building block **2** was accomplished by condensation of two Gal*f*-(β1 → 3)-Man*p* disaccharides. A hexasaccharide donor was planned to be formed by condensation of **2** and **3**, followed by glycosylation with derivative **4** to give a fully protected octasaccharide. This order of assembly would open synthetic routes to other relevant glycans found in *T. cruzi*.

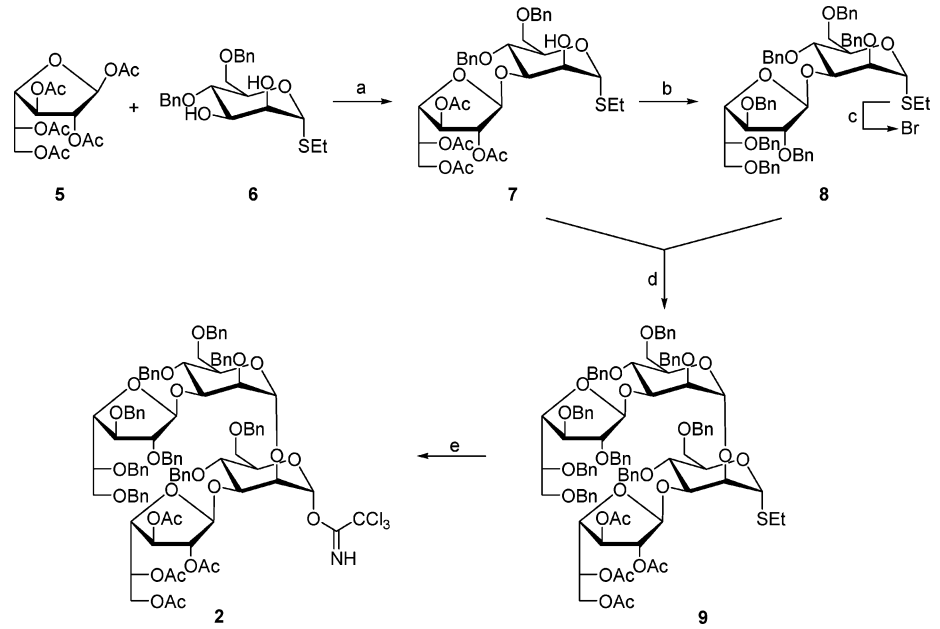
Synthesis of compound **2** started with the regioselective glycosylation of diol **6**[10] with penta-*O*-acetyl-β-D-galactofuranose (**5**),[11] with the 2-*O*-acetyl participating group of the galactofuranoside steering the reaction to the 1,2-trans glycosidic linkage. The Gal*f*-(β1 → 3)-Man*p* disaccharide **7** was obtained in 70% yield using tin tetrachloride activation. The acetates in **7** were exchanged to benzyls to give the fully benzylated compound **8** in 86% yield using first sodium methoxide in CH_2Cl_2 and methanol and then treating the syrup obtained with benzylbromide, silver(I) oxide, and potassium iodide in DMF. Conversion of **8** into a bromoglycoside by treatment with bromine followed by glycosylation with **7** using silver trifluoromethanesulfonate gave the desired tetrasaccharide **9** in 60% yield. Hydrolysis of the thioglycoside was accomplished using tetrabutylammonium periodate, trifluoromethanesulfonic acid, and water in acetonitrile.[12] Treating the obtained reducible tetrasaccharide with DBU and trichloroacetonitrile[13] in CH_2Cl_2 gave α-trichloroacetimidate **2** in 70% yield for the two steps (Scheme 1).

Synthesis of building block **3** was accomplished by treating ethyl 2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1 → 6)-2,3,4-tri-*O*-benzyl-1-α-D-thio-mannopyranoside (**10**)[9] with sodium methoxide in CH_2Cl_2 and methanol (2:1) to give derivative **3**[14] in 90% yield. Logically, attempts were performed to couple thioglycoside **9** with **3** after conversion of the former into the corresponding bromosugar, but without success. Contrary, compounds **2** and **3** were assembled into the desired hexasaccharide **11** in good yield using trimethylsilyl trifluoromethanesulfonate as the promoter in diethyl ether (Scheme 2). This successful glycosylation emphasizes the usefulness of thioglycosides as temporary anomeric protecting groups since they can be transformed into other donors when necessary, not leading to a deadlock in longer synthetic routes.

Dimethyl(methylthio)sulfonium trifluoromethanesulfonate (DMTST) activation of donor **11** in the glycosylation with compound **4**[9] gave the fully protected octasaccharide **12** in 71% yield (Scheme 3). The characterization was complicated by the fact that compound **12** was obtained as a diastereomeric mixture where the diastereomers could not be separated. However, this problem was to be overcome after the final deprotections due to the loss of the chirality of the carbon-phosphorus atom in the target compound.

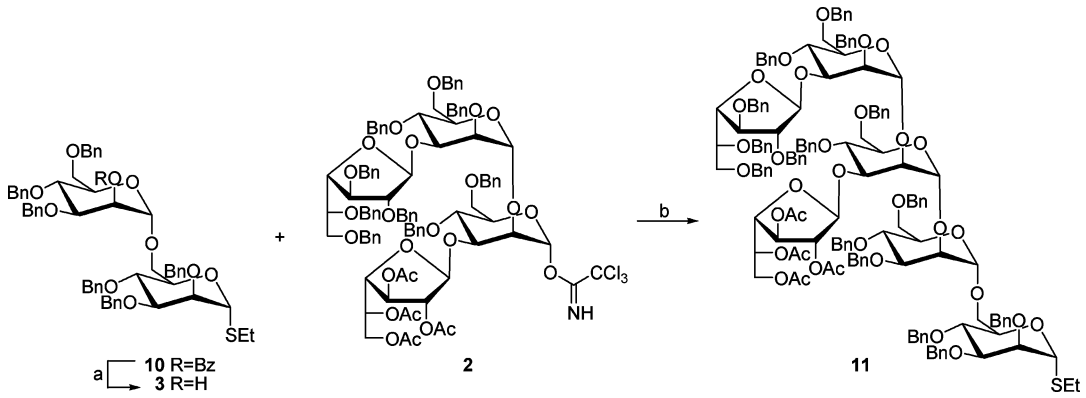
(8) Ruda, K.; Lindberg, J.; Garegg, P. J.; Oscarson, S.; Konradsson, P. *J. Am. Chem. Soc.* **2000**, *122*, 11067−11072.
(9) Hederos, M.; Konradsson, P. *J. Org. Chem.* **2005**, *70*, 7196−7207.
(10) Sarbajna, S.; Misra, A. K.; Roy, N. *Synth. Commun.* **1998**, *28*, 2559−2570.
(11) Lerner, L. M. *Carbohydr. Res.* **1996**, *282*, 189−192.
(12) Hiromi, U.; Wakiyama, Y.; Mukaiyama, T. *Chem. Lett.* **1998**, 567−568.
(13) Schmidt, R. R. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 212−235.
(14) Xue, J.; Shao, N.; Guo, Z. *J. Org. Chem.* **2003**, *68*, 4020−4029.

Scheme 1. Synthesis of Building Block **2**[a]



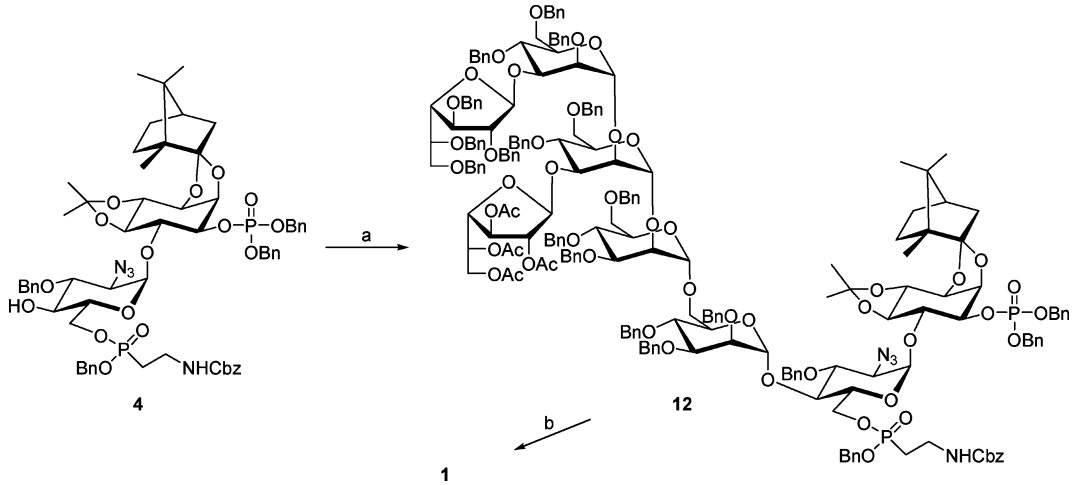
[a] Conditions: (a) $SnCl_4$, 4 Å molecular sieves, −50 °C → rt, CH_2Cl_2, (70%); (b) 1. NaOMe, MeOH/CH_2Cl_2 (1:2); 2. BnBr, KI, Ag_2O, DMF, (86%); (c) Br_2, CH_2Cl_2; (d) AgOTf, 4 Å molecular sieves, CH_2Cl_2, −30 °C, (60%); (e) 1. *n*-Bu_4NIO_4, TfOH, H_2O, acetonitrile; 2. Cl_3CCN, DBU, CH_2Cl_2, (70%).

Scheme 2. Synthesis of the Hexasaccharide Donor[a]



[a] Conditions: (a) NaOMe, CH_2Cl_2/MeOH (2:1) (90%); (b) TMSOTf, Et_2O, 4 Å molecular sieves, (89%).

Scheme 3. Assembly of the Fully Protected IPG and Subsequent Deprotection to **1**[a]



[a] Conditions: (a) **11**, DMTST, 4 Å molecular sieves, Et_2O, (71%); (b) 1. NaOMe, CH_2Cl_2/MeOH (2:1); 2. Na (s), NH_3 (l); 3. 0.1 M HCl, (83%).

Deprotection of such a complex molecule as **12** is always crucial, and most often there is a limited number of attempts due to the usually small amounts of compounds. Moreover, the order of protecting group removal can be crucial, and the particular deprotection sequence used here was a result from experience in our own laboratory. Deprotection of **12** started

with deacetylation using sodium methoxide in CH_2Cl_2 and MeOH (2:1), followed by debenzylation with sodium in liquid ammonia. Finally, the camphor and isopropylidene acetals were removed using 0.1 M aqueous hydrochloric acid. Changing this particular order has been shown leading to phosphate migration and a complex mixture of products on similar structures.[8] Target compound **1** was obtained in 83% yield after gel filtration. NMR data were in good agreement with reported values from the characterization of the *T. cruzi* GIPL.[6] The anomeric carbons of the two galactofuranoside residues were observed at 106.1 and 105.8 ppm, respectively, which is in accordance with the unusual upfield shift observed from synthetic oligosaccharides constituting the Gal*f*-(β1 → 3)-Man*p* motif (Scheme 3).[15]

Conclusion

The *T. cruzi* LPPG heptasaccharyl *myo*-inositol was synthesized using a convergent building block strategy. The ^{1}H, ^{13}C, and ^{31}P NMR spectra of compound **1** support the assignment of the compound isolated from the insect vector.[6] The order of assembly and earlier synthesis of the LPPG core structure[9] demonstrates the generality of the concept developed with a view to future syntheses of similar phosphoinositol oligosaccharides found in other *T. cruzi* glycoconjugates.

Experimental Procedures

General methods. Organic extracts were dried over $MgSO_4$, filtered, and concentrated in vacuo at 40 °C. NMR spectra were recorded at 25 °C. ^{1}H and ^{13}C chemical shifts are given in ppm relative to TMS (δ = 0.00) and $CDCl_3$ (δ = 77.0) in $CDCl_3$, respectively, and acetone in D_2O (^{13}C: δ = 31.5, ^{1}H: δ = 2.225); ^{31}P, 85% H_3PO_4 (δ = 0.00), was used as an external reference. pH* values in D_2O were calibrated against H_2O-buffer solutions. TLC was performed on Silica Gel F_{254} plates with detection by UV light (254 nm) and/or by charring with AMC [ammonium molybdate 10 g, cerium(IV)sulfate 2 g, dissolved in 10% H_2SO_4 (200 mL)] followed by heating at ∼250 °C. Silica gel (0.040−0.063 mm) was used for flash chromatography (FC). IR spectra were recorded as KBr pellets (solids) or films on CaF_2 crystals (syrups). Gel filtrations were performed using deoxygenated H_2O containing 1% *n*-butanol as the eluent. Mass spectra were recorded at Stockholm University Proteomics Facility, Dept. of Analytical Chemistry, Stockholm University.

Ethyl (2,3,5,6-tetra-*O*-Acetyl-β-D-galactofuranosyl)-(1 → 3)-4,6-di-*O*-benzyl-1-thio-α-D-mannopyranoside (7). Ethyl 4,6-di-O-benzyl-1-thio-α-D-mannopyranoside (**6**)[10] (2.00 g, 4.94 mmol) and penta-*O*-acetyl-β-D-galactofuranose (**5**)[11] (2.31 g, 5.93 mmol) were dissolved in CH_2Cl_2 (40 mL), and 4 Å molecular sieves were added. After stirring for 10 min, the temperature was lowered to −50 °C, and $SnCl_4$ (0.81 mL, 6.92 mmol) was added. The mixture was allowed to attain room temperature and after 10 h was diluted with CH_2Cl_2 and filtered through Celite, washed with saturated aqueous $NaHCO_3$, dried, filtered, and concentrated. FC (toluene/EtOAc 3:1 → 1:1) gave **7** (2.54 g, 3.46 mmol, 70%) as a colorless oil. R_f = 0.39 (toluene/EtOAc 1:1); $[\alpha]_D$ = +60 (*c* 1.0, $CHCl_3$); IR ν_{max} cm^{-1} 3482, 3029, 2926, 2869, 1748, 1638, 1496, 1452, 1382, 1372, 1227, 1092, 966, 739, 699, 602; NMR: ^{1}H (300 MHz, $CDCl_3$), δ 1.28 (t, 3H, *J* = 7.4 Hz), 1.96 (s, 3H), 1.97 (s, 3H), 2.11 (s, 3H), 2.12 (s, 3H), 2.49−2.71 (m, 2H), 3.65 (dd, 1H, *J* = 1.9 10.9 Hz), 3.77 (dd, 1H, *J* = 4.3 10.9 Hz), 3.90 (dd, 1H, *J* = 9.1 9.1 Hz), 4.07 (dd, 1H, *J* = 3.0 9.1 Hz), 4.12−4.17 (m, 4H), 4.28 (dd, 1H, *J* = 3.6 6.0 Hz), 4.48 (d, 1H, *J* = 12.3 Hz), 4.51 (d, 1H, *J* = 11.0 Hz), 4.63 (d, 1H, *J* = 12.3 Hz), 4.82 (d, 1H, *J* = 11.0 Hz), 5.01 (d, 1H, *J* = 2.2 Hz), 5.07 (dd, 1H, *J* = 2.2 6.0 Hz), 5.19 (s, 1H), 5.25−5.30 (m, 1H), 5.38 (d, 1H, *J* = 1.4 Hz), 7.20−7.35 (m, 10H); ^{13}C (75.4 MHz, $CDCl_3$), δ 14.7, 20.4, 20.5, 20.6, 20.7, 24.8, 62.4, 68.4, 68.8, 68.9, 71.4, 73.3 (2C), 74.6, 76.0, 76.3, 80.0, 82.7, 83.5, 101.8 (J_{CH} = 175 Hz), 127.5−128.3, 138.1, 138.3, 169.6, 169.9, 170.3 (2C); HRMS calcd. for $C_{36}H_{46}O_{14}S$: $[M + Na]^+$ 757.2506; Found: 757.2543.

Ethyl (2,3,5,6-tetra-*O*-Benzyl-β-D-galactofuranosyl)-(1 → 3)-2,4,6-tri-*O*-benzyl-1-thio-α-D-mannopyranoside (8). To a solution of compound **7** (500 mg, 0.680 mmol) in CH_2Cl_2/MeOH (2:1, 15 mL) was added NaOMe (4 mg, 0.074 mmol). The solution was stirred for 1 h, and Dowex-H^+ was added, the mixture was filtered, and the filtrate was concentrated. The obtained syrup was dissolved in dry DMF (10 mL), and benzyl bromide (1.62 mL, 13.6 mmol), KI (1.69 g, 10.2 mmol), and Ag_2O (2.36 g, 10.2 mmol) were added. The mixture was stirred for 4 h, diluted with toluene, and washed with water. The organic phase was washed with saturated aqueous $NaHCO_3$, dried, filtered, and concentrated followed by FC (toluene/EtOAc 19:1 → 9:1) to give compound **8** (595 mg, 0.585 mmol, 86%) as a colorless syrup. R_f = 0.42 (toluene/EtOAc 9:1); $[\alpha]_D$ = +11 (*c* 1.2, $CHCl_3$); IR ν_{max} cm^{-1} 3440, 3086, 3062, 3029, 2919, 2867, 1496, 1453, 1384, 1207, 1102, 1028, 737, 697, 619; NMR: ^{1}H (300 MHz, $CDCl_3$), δ 1.26 (t, 3H, *J* = 7.5 Hz), 2.51−2.71 (m, 2H), 3.59 (dd, 1H, *J* = 4.1 9.9 Hz), 3.67−3.83 (m, 4H), 3.91−4.08 (m, 4H), 4.12−4.28 (m, 4H), 4.36−4.52 (m, 8H), 4.57−4.75 (m, 4H), 4.91 (d, 1H, *J* = 11.0 Hz), 5.24 (s, 1H), 5.45 (d, 1H, *J* = 1.9 Hz), 7.13−7.38 (m, 35H); ^{13}C (75.4 MHz, $CDCl_3$), δ 14.8, 25.1, 69.1, 71.1, 71.2, 71.7 (2C), 71.9, 73.0, 73.1, 73.3, 74.0, 74.3, 74.6, 75.4, 76.4, 81.2, 81.7, 83.1, 88.1, 101.8 (J_{CH} = 172 Hz), 127.1−128.3, 137.5−138.6; HRMS calcd. for $C_{63}H_{68}O_{10}S$: $[M + Na]^+$ 1039.4431; Found: 1039.4426.

Ethyl (2,3,5,6-tetra-*O*-Benzyl-β-D-galactofuranosyl)-(1 → 3)-2,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1 → 2)-[(2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranosyl)-(1 → 3)]-4,6-di-*O*-benzyl-1-thio-α-D-mannopyranoside (9). Thioglycoside **8** (775 mg, 0.762 mmol) dissolved in CH_2Cl_2 (15 mL) was treated with Br_2 (42 μL, 0.815 mmol) for 10 min, diluted with dry toluene, and concentrated. The crude bromosugar and compound **7** (560 mg, 0.762 mmol) were dissolved in CH_2Cl_2 (20 mL), and 4 Å molecular sieves were added. The mixture was cooled to −40 °C and stirred for 10 min, and AgOTf (392 mg, 1.524 mmol) was added. The mixture was stirred for 30 min, diluted with CH_2Cl_2, filtered through Celite, washed with saturated aqueous $NaHCO_3$, dried, filtered, and concentrated. FC (toluene/EtOAc 6:1 → 3:1) gave **9** (785 mg, 0.457 mmol, 60%) as a syrup. R_f = 0.29 (toluene/EtOAc 6:1); $[\alpha]_D$ = +6 (*c* 1.0, $CHCl_3$); IR ν_{max} cm^{-1} 3440, 3087, 3060, 3028, 2923, 2865, 1745, 1627, 1494, 1452, 1369, 1224, 1098,1026, 736, 697; NMR: ^{1}H (300 MHz, $CDCl_3$), δ 1.19 (t, 3H, *J* = 7.4 Hz), 1.93 (s, 3H), 1.97 (s, 3H), 1.98 (s, 3H), 2.12 (s, 3H), 2.49 (q, 2H, *J* = 7.4 Hz), 3.50−3.57 (m, 1H), 3.69−3.91 (m, 9H), 4.08−4.64 (m, 24H), 4.69−4.76 (m, 2H), 4.89−4.99 (m, 3H), 5.01 (s, 1H), 5.05−5.07 (d, 1H, *J* = 5.5 Hz), 5.20 (s, 1H), 5.26 (d, 1H, *J* = 1.6 Hz), 5.31−5.37 (m, 1H), 5.45 (s, 1H), 5.65 (s, 1H), 7.17−7.45 (m, 45H); ^{13}C (75.4 MHz, $CDCl_3$), δ 14.9, 20.4 (2C), 20.5, 20.6, 25.5, 62.4, 68.9, 69.1, 69.8, 71.3, 71.9 (2C), 72.2, 72.5, 72.8, 73.1 (2C), 73.2, 73.9, 74.4, 74.5 (3C), 74.7, 74.9, 75.0, 76.2, 76.4, 76.7, 80.2, 81.8, 82.0, 83.0, 83.5, 88.0, 100.7 (J_{CH} = 171 Hz), 101.7 (J_{CH} = 175 Hz), 102.6 (J_{CH} = 173 Hz), 127.0−128.2, 137.7, 137.8, 138.2−138.6, 169.6, 169.8 (2C), 170.2; HRMS calcd. for $C_{97}H_{108}O_{24}S$: $[M + Na]^+$ 1711.6849; Found: 1711.6918.

1-*O*-(2,3,5,6-tetra-*O*-Benzyl-β-D-galactofuranosyl)-(1 → 3)-2,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1 → 2)-[(2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranosyl)-(1 → 3)]-4,6-di-*O*-benzyl-1-thio-α-D-mannopyranosyl trichloroacetimidate (2). To a solution of derivative **9** (590 mg, 0.349 mmol), H_2O (100 μL), and TfOH (6 μL, 0.070 mmol) in acetonitrile (25 mL) at 0 °C was added Et_4NIO_4 (61 mg, 0.140 mmol). The solution was allowed to reach room temperature during 2.5 h, diluted with CH_2Cl_2, and washed sequentially with aqueous saturated $NaHCO_3$ and 10% aqueous $Na_2S_2O_3$. The organic phase was dried, filtered, and concentrated followed by FC (toluene/EtOAc 3:1 → 1:1) to give 2,3,5,6-tetra-*O*-benzyl-β-D-galactofuranosyl)-(1 → 3)-2,4,6-tri-

(15) Gorin, P. A. J.; Barreto-Bergter, E. M.; da Cruz, F. S. *Carbohydr. Res.* **1981**, *88*, 177−188.

O-benzyl-α-D-mannopyranosyl)-(1 → 2)-[(2,3,5,6-tetra-*O*-acetyl-*β*-D-galactofuranosyl)-(1 → 3)]-4,6-di -*O*-benzyl-1-thio-α-D-mannopyranose. [R_f = 0.42 (toluene/EtOAc 2:1:); NMR ^{13}C (75.4 MHz, $CDCl_3$), δ 20.5 (2C), 20.6, 20.7, 62.5, 69.2, 69.3, 69.9, 71.4, 71.7 (2C), 71.9, 72.0, 72.5, 73.0, 73.1 (2C), 73.2, 73.3, 73.9, 74.2, 74.3, 74.6, 74.7 (2C), 75.1, 76.3, 76.5, 80.2, 81.7, 82.0, 83.1, 88.2, 93.1, 100.6, 102.0, 103.1, 127.2−129.0, 137.8−138.7, 169.7, 169.9, 170.0, 170.4.]. To a stirred solution of this residue and trichloroacetonitrile (700 μL, 6.980 mmol) in CH_2Cl_2 (20 mL) at 0 °C, DBU (8 μL, 0.052 mmol) was added. The reaction mixture was stirred overnight, diluted with toluene, and concentrated. FC (toluene/EtOAc 6:1 + 1% Et_3N) gave **2** (444 mg, 0.244 mmol, 70%) as a colorless oil. R_f = 0.32 (toluene/EtOAc 6:1); $[\alpha]_D$ = −4 (*c* 1.0, $CHCl_3$); IR ν_{max} cm^{-1} 3459, 3086, 3063, 3030, 2919, 2863, 1745, 1673, 1495, 1454, 183, 1371, 1225, 1098, 1050, 1026, 969, 796, 737, 697, 645, 602; NMR: ^{1}H (300 MHz, $CDCl_3$), δ 1.89 (s, 3H), 1.91, (s, 3H), 1.95 (s, 3H), 2.10 (s, 3H), 3.47−3.52 (m, 1H), 3.62−3.83 (m, 7H), 3.93−4.59 (m, 25H), 4.64−4.72 (m, 3H), 4.86−4.97 (m, 3H), 5.00−5.04 (m, 2H), 5.21 (s, 1H), 5.27 (d, 1H, *J* = 2.5 Hz), 5.28−5.34 (m, 1H), 5.41 (s, 1H), 6.51 (d, 1H, *J* = 1.4 Hz), 7.13−7.41 (m, 45H), 8.52 (s, 1H); ^{13}C (75.4 MHz, $CDCl_3$), δ 20.4 (2C), 20.5, 20.7, 62.3, 68.5, 69.0, 69.1, 71.4, 72.0, 72.1 (2C), 72.3, 72.5, 73.0, 73.1 (2C), 73.3, 74.0, 74.3, 74.5, 74.6 (2C), 74.7, 75.0, 75.1, 76.3 (2C), 80.1, 81.8, 82.1, 83.1, 88.2, 90.9, 96.6 (J_{CH} = 180 Hz), 100.7 (J_{CH} = 170 Hz), 101.7 (J_{CH} = 172 Hz), 103.1 (J_{CH} = 173 Hz), 127.2−128.3, 137.7−138.6, 159.9, 169.6, 169.8, 169.9, 170.2; HRMS calcd. for $C_{97}H_{104}Cl_3NO_{25}$: $[M + Na]^+$ 1810.5861; Found: 1810.5824.

Ethyl (3,4,6-tri-*O*-Benzyl-*β*-D-mannopyranosyl)-(1 → 6)-2,3,4-tri-*O*-benzyl-1-thio-α-D-mannopyranoside (3). To compound **10**[9] (400 mg, 0.388 mmol) dissolved in CH_2Cl_2/MeOH (2:1, 10 mL), NaOMe (20 mg, 0.370 mmol) was added. The solution was stirred overnight, Dowex-H^+ was added, the solution was filtered, and the filtrate was concentrated. FC (toluene/EtOAc 3:1) gave **3** (324 mg, 0.349 mmol, 90%) as a colorless syrup. R_f = 0.49 (toluene/EtOAc 2:1:); $[\alpha]_D$ = +82 (*c* 1.0, $CHCl_3$); IR ν_{max} cm^{-1} 3467, 3088, 3063, 3029, 2925, 2869, 1496, 1452, 1384, 1363, 1209, 1100, 1050, 1027, 911, 845, 789, 736, 697; NMR spectra were in accordance with those previously published.[14]

Ethyl (2,3,5,6-tetra-*O*-Benzyl-*β*-D-galactofuranosyl)-(1 → 3)-2,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1 → 2)-[(2,3,5,6-tetra-*O*-acetyl-*β*-D-galactofuranosyl)-(1 → 3)]-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1 → 2)-3,4,6-tri-*O*-benzyl-*β*-D-mannopyranosyl)-(1 → 6)-2,3,4-tri-*O*-benzyl-1-thio-α-D-mannopyranoside (11). Compounds **2** (360 mg, 0.198 mmol) and **3** (257 mg, 0.277 mmol) were dissolved in Et_2O (20 mL), and 4 Å molecular sieves were added. The mixture was cooled at 0 °C for 30 min, and TMSOTf (8 μL, 0.040 mmol) was added. After 1 h, solid $NaHCO_3$ was added, and the mixture was filtered through Celite and concentrated. FC (toluene/EtOAc 6:1) gave hexasaccharide **11** (455 mg, 0.176 mmol, 89%) as a syrup. R_f = 0.41 (toluene/EtOAc 6:1); $[\alpha]_D$ = +13 (*c* 0.9, $CHCl_3$); IR ν_{max} cm^{-1} 3463, 3089, 3064, 3031, 2916, 2861, 1747, 1634, 1497, 1454, 1369, 1226, 1099, 1076, 1050, 1028, 738, 699; NMR: ^{1}H (300 MHz, $CDCl_3$), δ 1.14 (t, 3H, *J* = 7.4 Hz), 1.88 (s, 3H), 1.92 (s, 3H), 1.94 (s, 3H), 2.05 (s, 3H), 2.35−2.57 (m, 2H), 3.49−4.75 (m, 57H), 4.80−4.89 (m, 4H), 4.94−4.98 (m, 2H), 5.02−5.05 (m, 3H), 5.21 (s, 2H), 5.26−5.32 (m, 2H), 5.37 (s, 1H), 5.43 (s, 1H), 7.09−7.44 (m, 75H); ^{13}C (75.4 MHz, $CDCl_3$), δ 14.8, 20.3, 20.4, 20.5, 20.6, 25.0, 62.3, 66.5, 69.0, 69.1, 69.2, 69.4, 71.0, 71.2, 71.3, 71.5, 71.8−71.9 (several C), 72.5, 72.7, 72.8, 73.1, 73.2, 73.8, 74.2, 74.5, 74.7−74.8 (several C), 75.0, 75.5, 76.2, 76.3, 76.5, 76.5, 78.9, 80.3, 80.5, 81.4, 81.8, 82.0, 83.1, 88.1, 98.5 (J_{CH} = 172 Hz), 100.4 (J_{CH} = 173 Hz), 100.7 (J_{CH} = 173 Hz), 102.1 (J_{CH} = 176 Hz), 102.6 (J_{CH} = 175 Hz), 127.1−128.9, 137.7−138.8, 169.4, 169.8, 169.9, 170.0; HRMS calcd. for $C_{151}H_{164}O_{34}S$: $[M + Na]^+$ 2576.0722; Found: 2576.0781.

(2,3,5,6-tetra-*O*-Benzyl-*β*-D-galactofuranosyl)-(1 → 3)-2,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1 → 2)-[(2,3,5,6-tetra-*O*-acetyl-*β*-D-galactofuranosyl)-(1 → 3)]-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1 → 2)-3,4,6-tri-*O*-benzyl-*β*-D-mannopyranosyl)-(1 → 6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl-(1 → 4)-2-azido-3-*O*-benzyl-6-*O*-(2-benzyloxycarbonylamino-ethyl)-phosphonic Acid Benzyl Ester-2-deoxy-α-D-glucopyranosyl-(1 → 6)-1-di-*O*-benzyl-phosphoryl-4,5-*O*-isopropylidene-2,3-*O*-(D-1,7,7-tri-methyl[2,2,1]bicyclohept-2-ylidene)-D-*myo*-inositol (12). To a solution of **11** (235 mg, 0.091 mmol), **4**[9] (85 mg, 0.070 mmol), and 4 Å molecular sieves in Et_2O (15 mL) under argon atmosphere, DMTST (50 mg, 0.193 mmol) was added. The mixture was stirred for 4 h when Et_3N (250 μL) was added. The mixture was stirred for 20 min, diluted with toluene, filtered through Celite, and concentrated. FC (toluene/EtOAc 4:1 → 1:1) gave compound **12** (186 mg, 0.050 mmol, 71%) as a diastereomeric mixture. R_f = 0.59 (toluene/EtOAc 2:1); $[\alpha]_D$ = +14 (*c* 1.4, $CHCl_3$); IR ν_{max} cm^{-1} 3430, 3088, 3061, 3030, 2925, 2857, 2105, 1745, 1727, 1498, 1455, 1372, 1228, 1115, 1050, 1026, 738, 698; NMR: ^{1}H (600 MHz, $CDCl_3$, diastereomeric mixture), δ 0.88 (s, 6H), 0.94 (s, 6H), 1.03 (s, 3H), 1.04 (s, 3H), 1.20−1.24 (m, 2H), 1.37−1.47 (m, 16H), 1.70−1.76 (m, 4H), 1.86−2.08 (m, 32H), 3.27−5.13 (m, 182H), 5.18 (s, 1H), 5.20−5.22 (m, 2H), 5.24 (s, 1H), 5.26 (s, 1H), 5.29−5.32 (m, 3H), 5.37 (s, 1H), 5.40 (s, 1H), 5.46 (s, 1H), 5.47 (s, 1H), 5.91 (m, 1H), 6.02 (m, 1H), 7.05−7.47 (m, 200H); ^{13}C (150.9 MHz, $CDCl_3$, diastereomeric mixture), δ 9.9 (2C), 20.1 (2C), 20.3 (5C), 20.4, 20.5 (3C), 20.6, 26.2 (*J* = 139 Hz), 26.4 (*J* = 139 Hz), 26.8 (3C), 26.9, 27.0 (2C), 29.7 (2C), 35.0 (2C), 43.7 (2C), 44.9 (2C), 47.8 (2C), 51.4 (2C), 62.3 (2C), 62.5, 62.6, 63.6, 64.1, 66.1 (3C), 66.2, 66.9 (*J* = 5.9 Hz), 67.1 (*J* = 5.9 Hz), 68.7 (2C), 68.8, 68.9 (2C), 69.2, 69.3 (2C), 69.4−69.5 (several C), 69.6 (2C), 70.7, 70.9, 71.2 (2C), 71.8 (2C), 71.9 (2C), 72.0−72.2 (several C), 72.3, 72.4, 72.7−72.8 (several C), 72.9 (2C), 73.0 (2C), 73.2 (2C), 73.5, 73.6 (2C), 73.8 (2C), 74.0, 74.1, 74.3, 74.4−74.9 (several C), 75.0, 75.2, 75.4, 75.7 (2C), 76.2 (2C), 76.4 (2C), 76.6 (2C), 77.2−77.6 (several C), 78.4, 78.7, 79.7, 79.8, 80.3, 80.4, 81.7, 81.8 (2C), 81.8, 82.9, 83.0, 87.9 (2C), 96.5, 96.6, 98.9, 99.0, 99.7, 99.9, 100.0, 100.3, 100.4, 100.5, 102.1, 102.4, 102.8 (2C), 112.5 (2C), 119.0 (2C), 126.6−128.6, 135.3−136.6, 137.4−138.8, 156.0 (2C), 169.4 (2C), 169.8, 169.9, 170.0 (4C); ^{31}P (decoupled, 121 MHz, $CDCl_3$, diastereomeric mixture), δ −0.32, −0.16, 31.6, 32.3; HRMS calcd. for $C_{212}H_{234}N_4O_{51}P_2$: $[M + 2Na]^{2+}$ 1879.7555; Found: 1879.7485.

(*β*-D-Galactofuranosyl)-(1 → 3)-(α-D-mannopyranosyl)-(1 → 2)-[(*β*-D-galactofuranosyl)-(1 → 3)]-α-D-mannopyranosyl-(1 → 2)-*β*-D-mannopyranosyl)-(1 → 6)-α-D-mannopyranosyl-(1 → 4)-2-amino-6-*O*-(2-amino-ethyl)-phosphonic Acid 2-Deoxy-α-D-glucopyranosyl-(1 → 6)-1-*O*-phosphate-D-*myo*-inositol, Ammonium Salt (1). To a solution of diastereomeric **12** (105 mg, 0.0279 mmol) in CH_2Cl_2/MeOH (6 mL, 2:1) was added NaOMe (2 mg, 0.0370 mmol). The solution was stirred for 1 h, diluted with CH_2Cl_2, and washed with 0.5 M aqueous NH_4Cl. The organic phase was dried, filtered, and concentrated. The obtained residue was dissolved in dry THF (∼2 mL) and added to ∼20 mL of NH_3 (*l*) at −33 °C. A small piece of sodium was added, turning the mixture to dark blue. Stirring was continued for 1 min when NH_4Cl (s) was added until the blue color disappeared. The mixture was concentrated under a stream of N_2 (g), and 10 mL of 0.1 M aqueous HCl was added. The mixture was stirred for 5 h and neutralized with 0.1 mL of NH_3. The aqueous phase was washed with Et_2O (15 mL) and concentrated. The obtained residue was purified on a Sephadex G-15 column using H_2O containing 1% *n*-butanol to give compound **1** (35.2 mg, 0.0231 mmol, 83%) as a solid. $[\alpha]_D$ = +28 (*c* 1.4, H_2O); NMR: ^{1}H (600 MHz, D_2O), δ 1.97−2.14 (m, 2H), 3.18−3.28 (m, 2H), 3.87−3.43 (m, 2H), 3.57 (dd, 1H, *J* = 3.0 10.2 Hz), 3.64−3.86 (m, 21H), 3.91−3.95 (m, 6H), 3.98−4.00 (m, 1H), 4.03−4.16 (m, 13H), 4.19 (m, 1H), 4.25−4.28 (m, 2H), 4.35 (m, 1H), 5.13 (s, 1H), 5.14 (s, 1H), 5.17 (s, 1H), 5.20 (s, 1H), 5.29 (s, 1H), 5.31 (s, 1H), 5.66 (d, 1H, *J* = 3.6 Hz); ^{13}C (150.9 MHz, D_2O), δ 25.6 (*J* = 134 Hz), 36.6, 55.0, 66.2 (2C), 62.3, 64.1 (2C), 64.2 (d, *J* = 5.0 Hz), 66.3, 66.7, 67.0, 67.4, 68.1, 68.2, 71.0 (d, *J* = 6.5 Hz), 71.4 (2C), 71.7 (3C), 71.9, 72.0, 72.9, 73.4, 73.6, 74.0, 74.1, 74.5 (2C), 75.7, 76.3, 76.6, 76.7, 77.0 (d, *J* = 5.4 Hz), 78.0, 78.2, 78.5 (d, *J* = 5.6 Hz), 80.0, 82.6 (2C), 83.9, 84.0,

96.3, 99.5, 101.9, 102.5, 102.8, 105.7, 106.0; ^{31}P (decoupled, 121 MHz, D_2O, $pH^* = 5.1$), δ 22.4, 2.0; HRMS calcd. for $C_{50}H_{90}N_2O_{45}P_2$: $[M + H]^+$ 1501.4370; Found: 1501.4343.

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Supporting Information Available: ^{1}H-, ^{13}C-, and ^{31}P NMR spectra of all new compounds and complete ref 4. This material is available free of charge via the Internet at http://pubs.acs.org.

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