Synthesis of the *Leishmania* LPG Core Heptasaccharyl *myo*-Inositol

Katinka Ruda,[‡,§] Jan Lindberg,[†] Per J. Garegg,[‡] Stefan Oscarson,[‡] and Peter Konradsson*[,†]

Contribution from the Department of Chemistry, Linköping University, Linköping SE-581 83, Sweden, and the Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, Stockholm SE-106 91, Sweden

Received May 1, 2000

Abstract: Total synthesis of the core heptasaccharyl *myo*-inositol, Gal*p*(α1−6)Gal*p*(α1−3)Gal*f*(β1−3)[Glc*p*(α1-PO$_4$-6)Man*p*](α1−3)Man*p*(α1−4)GlcN*p*(α1−6)Ins-1-PO$_4$, and the corresponding hexasaccharyl *myo*-inositol, Gal*p*(α1−6)Gal*p*(α1−3)Gal*f*(β1−3)Man*p*(α1−3)Man*p*(α1−4)GlcN*p*(α1−6)Ins-1-PO$_4$, found in the lipophosphoglycans of *Leishmania* parasites are described. The target molecules contain synthetic challenges such as an unusual internal galactofuranosyl residue and an anomeric phosphodiester. The synthesis was accomplished using a convergent block synthetic strategy. Four building blocks, a trigalactoside, a dimannoside, a glucosyl inositolphosphate, and a glucosyl-α-1-H-phosphonate, all appropriately protected, were used. The trigalactoside was linked to the dimannoside followed by glycosylation with the glucosyl inositolphosphate to produce the fully protected hexasaccharyl *myo*-inositol. Subsequent oxidative coupling of the glucosyl-H-phosphonate formed the anomeric phosphodiester linkage to produce the protected heptasaccharyl *myo*-inositol. Both the assembly order of the subunits and sequence of deprotection were essential for the successful synthesis of these complex molecules. The deprotection was accomplished by deacetylation and clevage of benzyl ethers with sodium in liquid ammonia, followed by acidic deacetalization/desilylation to produce the target molecules.

Introduction

The cell surface on all the various species of *Leishmania* is partly covered with low-molecular mass glycoinositolphospholipids (GIPLs), independent of developmental stage (promastigote or amastigote).[1] In the promastigote stage the surface is also covered with lipophosphoglycans (LPGs) and glycosyl phosphatidylinositol (GPI)-anchored proteins. All, so far characterized, GPI-anchors contain the identical ethanolamine-PO$_4$-6Man*p*(α1−2)Man*p*(α1−6)Man*p*(α1−4)GlcN*p*(α1−6)Ins backbone, and several of these have been synthesized.[2] The *Leishmania* LPGs, in contrast to the GPI-anchors, contain a Gal*p*(α1−6)Gal*p*(α1−3)Gal*f*(β1−3)Man*p*(α1−3)Man*p*(α1−4)-GlcN*p*α1-core hexasaccharide linked to *lyso*alkylphosphatidylinositol, sharing the common Man*p*(α1−4)GlcN*p*(α1−6)Ins-unit with the GPI-anchors.[1] Interestingly, some of the GIPLs contain the same or truncated versions of the carbohydrate core sequence (**1a**) found in the LPGs, suggesting a common biosynthetic pathway. Usually the LPG core hexasaccharide is substituted with a glucosyl-α-1-phosphate (**1b**). The LPGs, compared to GIPLs, are attached into the membrane by one hydrophobic group only, and are therefore liberated more easily from the plasma membrane. The uniqueness of the LPG and GIPLs structures, relative to all other molecules in Nature, makes the strategy of chemotherapeutic intervention appropriate. The findings that a high concentration of LPGs are present only in the promastigote stage, and are easily liberated, make GIPLs most attractive as drug targets (i.e., vaccines and inhibitors).

The target inositol phosphoglycan (IPG), **1b**, corresponding to GIPLs on the cell surface of *Leishmania* parasites, contain carbohydrates (pyranoses and a furanose), a *myo*-inositol unit, and phosphates (a mono-ester and an anomerically linked phosphodiester). Successful total synthesis of a molecule of such diverse complexity requires a high degree of planning such as choice of glycosylation method, orthogonality, order of attachment, protection group pattern, and order of deprotection. A convergent assembly of subunits was planned as depicted in Figure 1. This disconnection is logical, since synthesis of some of the building blocks and couplings between them have previously been accomplished.[3,4]

The benzyl and acetyl groups on the carbohydrate residues and both the acetals on the *myo*-inositol moiety, are persistent protecting groups, to be removed at the final stage of the synthesis.The synthesis of building block **A** has been described.[3] Building block **B**,[4] is a part from the interior of the target molecule and will therefore be used both as a glycosyl donor and acceptor. Thioglycosides have been shown to fulfill the requirements of orthogonality.[5] The monochloroacetyl and *tert*-

* Corresponding author. Telephone: +46-13-281728. Fax: +46-13-281399. E-mail: petko@ifm.liu.se.

† Department of Chemistry, Linköping University, Linköping, Sweden.

‡ Department of Organic Chemistry, Stockholm University, Stockholm, Sweden.

§ Current address: Personal Chemistry, Hamnesplanaden 5, Uppsala, SE-753 19 Sweden.

(1) (a) Turco, S. J.; Descoteaux, A. *Ann. Rev. Microbiol.* **1992**, *46*, 65−94. (b) McConville, M. J.; Ferguson, M. A. J. *Biochem. J.* **1993**, *294*, 305−324 and references therein.

(2) (a) Mootoo, D. R.; Konradsson, P.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1989**, *111*, 8540−8542. (b) Murakata, C.; Ogawa, T. *Carbohydr. Res.* **1992**, *235*, 95−114. (c) Cottaz, S.; Brimacombe J. S.; Ferguson, M. A. *J. Chem. Soc., Perkin Trans. 1* **1993**, 2945−2951. (d) Udodong, U. E.; Madsen, R.; Roberts, C.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1993**, *115*, 7886−7887. (e) Madsen, R.; Udodong, U. E.; Roberts, C.; Mootoo, D. R.; Konradsson, P.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1995**, *117*, 1554−1565. (f) Mayer, T. G.; Schmidt, R. R. *Eur. J. Org. Chem.* **1999**, *1153*, 3−1165.

(3) Garegg, P. J.; Konradsson, P.; Oscarson S.; Ruda, K. *Tetrahedron*, **1997**, *53*, 17727−17734.

(4) Ruda, K., Lindberg, J., Garegg, P. J., Oscarson, S., Konradsson, P. *Tetrahedron* **2000**, *56*, 3969−3975.

(5) Kanie, O.; Ito, Y.; Ogawa, T. *J. Am. Chem. Soc.* **1994**, *116*, 12073−12074.

10.1021/ja001515t CCC: $19.00
Published on Web 10/27/2000

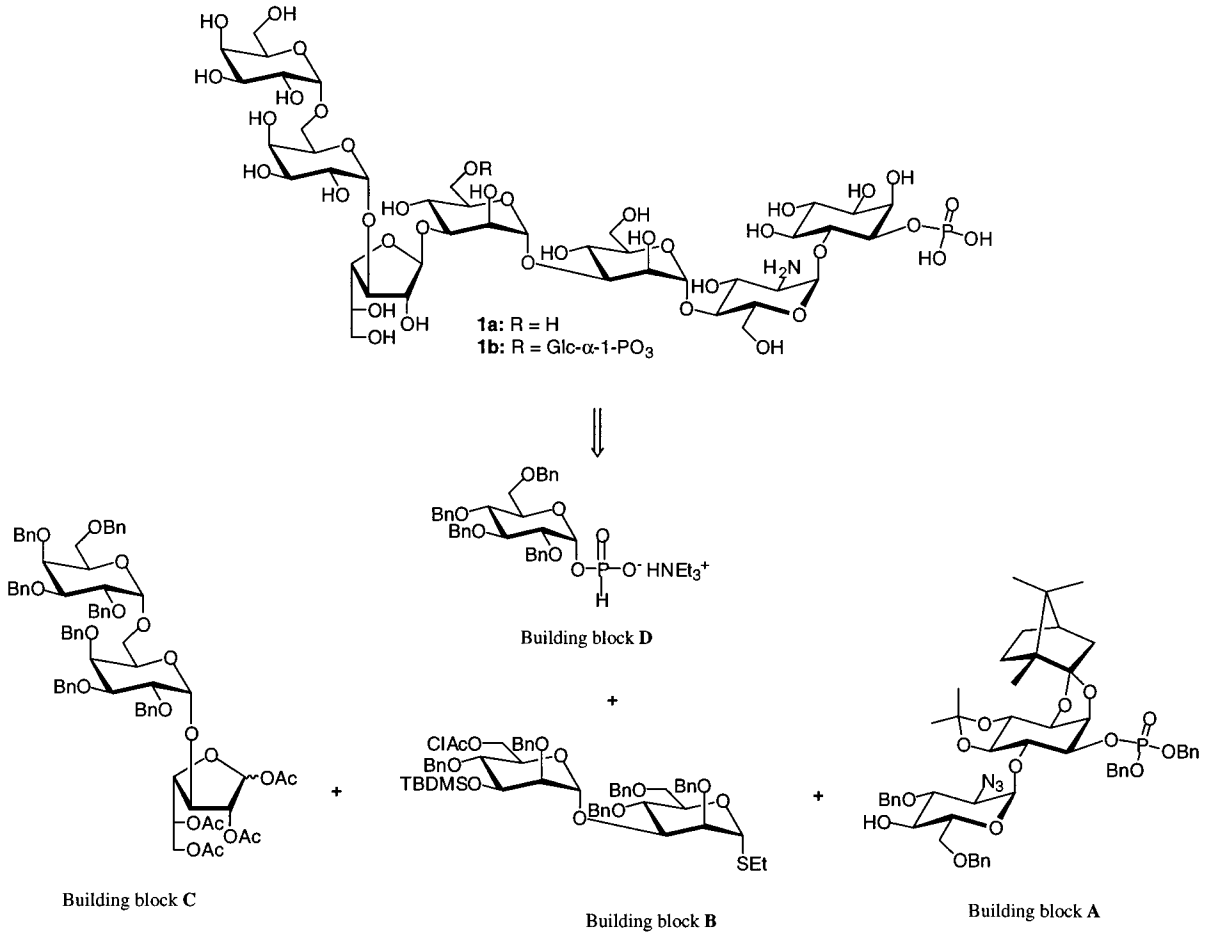


Figure 1. Disconnection of the inositolphosphoglycan (IPG) fragment found on the surface of the *Leishmania* parasite.

butyldimethylsilyl on building block **B** are temporary protecting groups. Each of these groups can selectively be removed in the presence of the other. The trigalactoside, building block **C**, contains an unusual furanoside, which will be anomerically linked to the dimannoside. The high reactivity of furanoside donors[6] (as compared to pyranosides) makes it possible to use an anomeric acetate as a glycosyl donor. Building block **D** with an anomeric H-phosphonate is readily available in anomerically pure form, and it can be coupled to building block **B** and oxidized to form a phosphodiester in high yield.[4,7]

With this synthetic strategy, building block **C** was first connected to building block **B**, and the resulting pentamer was then coupled to building block **A**. The other alternative, connecting building block **A** with **B** and then connecting block **C**, was abandoned because of the use of Lewis acid in the desilylation of building block **B**, which would cause deprotection of the acetals. The hexasaccharyl *myo*-inositol derivative formed was selectively demonochloroacetylated, and the product was phosphorylated with building block **D**. The anomeric phosphodiester had to be introduced in the last step, before complete deprotection, because of the nucleophilicity of the phosphodiester during glycosylations and the instability of anomeric phosphodiesters toward acidic conditions.

Results and Discussion

Ethyl 1-thio-β-D-galactopyranoside was selectively protected in position 6 with a triphenylmethyl group, and then subjected to benzylation, followed by acidic detritylation to yield derivative **2**. Ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside was transformed into the corresponding bromosugar, and compound **2** was glycosylated, using halide-assisted conditions, to give disaccharide **3** in 86% yield. Furanoside derivative **7** was synthesized from **4**, acquired from 1,2:5,6-di-*O*-isopropylidene-α-D-galactofuranose,[8] by benzylation and selective removal of the 5,6-isopropylidene acetal in aqueous acetic acid.[9] To preserve the furanose ring, **4** was acetylated (→**5**) before removal of the 1,2-isopropylidene group and acetylation of the resulting diol (→**6**). After catalytic hydrogenolysis, derivative **7** was ready for coupling with glycosyl donor **3**. The glycosylation promoted by dimethyl(methylthio)sulfonium trifluoromethanesulfonate (DMTST) in diethyl ether gave trisaccharide **8** in 67% yield (Scheme 1).

The protected mannose disaccharide **9a**, described earlier,[4] was desilylated with BF_3 in chloroform to give **9b**. Attempts to remove the TBDMS group with other fluoride reagents resulted in concomitant deprotection of the monochloroacetate. Coupling of **9b** with the galactose trisaccharide derivative **8** in dichloromethane, using trimethylsilyl triflate as a promoter, produced pentasaccharide **10**, in 85% yield. Glycosylation with furanosidic anomeric esters in the presence of a Lewis acid catalyst has also been outlined by others.[6] Thioglycosyl donor **10** was activated by DMTST in diethyl ether and coupled to the *myo*-inositol containing derivative **11**[3] to produce **12a** in 75% yield. A first attempt to deprotect **12a**, starting with acidic deacetalization, followed by deacetylation with sodium methoxide in dichloromethane/methanol and subsequent catalytic hydro-

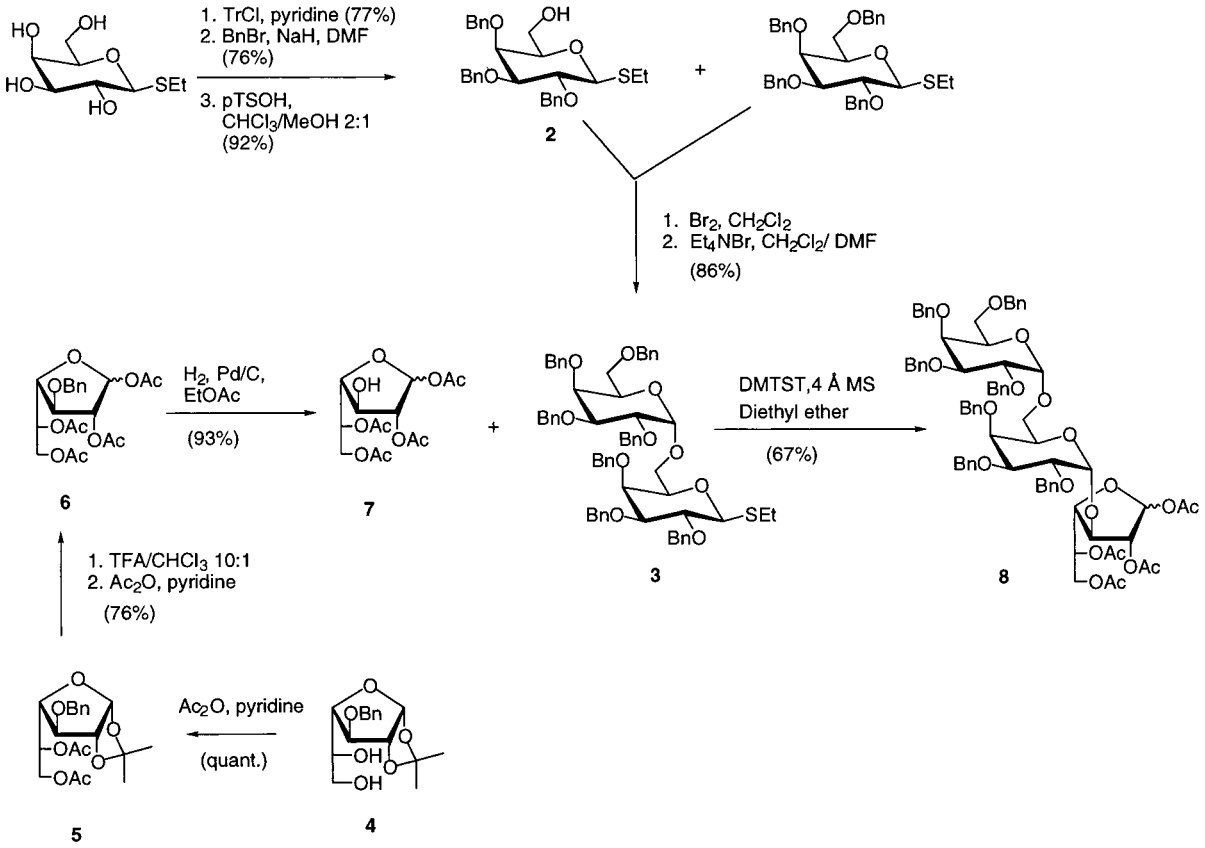
(6) Gallo-Rodriguez, C.; Varela, O.; de Lederkremer, R. M. *J. Org. Chem.* **1996**, *61*, 1886−1889 and references therein.

(7) Hermans, J. P. G.; de Vroom, E.; Elie, C. J. J.; van der Marel, G. A.; van Boom, J. H. *Recl. Trav. Chim. Pays-Bas* **1986**, *105*, 510−511.

(8) Morgenlie, S. *Acta Chem. Scand.* **1973**, *9*, 3609−3610.

(9) Morris, P. E. Jr.; Kiely, D. E. *J. Org. Chem.* **1987**, *52*, 1149−1152.

Scheme 1. Synthesis of Building Block C



genolysis, gave a complex mixture of deprotected isomeric hexasaccharyl *myo*-inositols, identified by a correct HRMS but with distorted integrals of the anomeric protons in the ^{1}H NMR spectrum and missing peaks in the ^{13}C NMR spectrum (Scheme 2).

The suspected cause for the isomerization, base-catalyzed phosphate migration, was investigated by subjecting compound **11** to the same deprotection conditions. The acidic deacetalization proceeded as in previous work[3,4] without phosphate migration. Subsequent treatment with sodium methoxide in dichloromethane/methanol showed by TLC analysis instantaneous disappearance of starting material and formation of several new compounds, not characterized but assumed to be the result of extensive phosphate migration.

These results clearly demonstrate not only the importance of a carefully planned protection group pattern but also that the order of deprotection plays an important role in the synthesis of complex carbohydrates. Debenzylation followed by aqueous acidic deacetalization has previously been shown to proceed without phosphate migration.[4] Deacetylation of **12a** with sodium methoxide in a mixture of dichloromethane and methanol followed by debenzylation with sodium in liquid ammonia and subsequent acidic hydrolysis of the acetals produced **1a** in 78% yield (Scheme 3).

Attempts to selectively remove the chloroacetyl group on compound **12a** with known methods, using hydrazine dithiocarbonate[10] or thiourea,[11] was accompanied with decomposition and low yields. However, solvolysis in dichloromethane/methanol saturated with ammonia accomplished the selective deprotection in 88% yield. Coupling of **12b** and **13**[4,7] with pivaloyl chloride and subsequent in situ oxidation with iodine produced the protected heptasaccharyl *myo*-inositol **14a** in 94% yield. Deprotection of **14a** by the same procedures as for **12a** produced the fully deprotected target molecule **1b** in 72% yield.

Conclusions

The total synthesis of the *Leishmania* LPG core heptasaccharyl *myo*-inositol using a block synthetic strategy was successful. Both the assembly order, choice of deprotection methods, and sequence of deprotection were crucial for the successful synthesis of these complex molecules. Our results strongly support the assignment of the natural compound. The ^{1}H NMR spectrum of **1b** is consistent with the reported spectrum for the characterized IPG of *L. major*,[12] where the difficult assignment of the β-galactofuranoside, was accomplished by comparison with a small synthetic model compound.[13] Our results confirmed that assignment. The unusual upfield shift of the anomeric ^{13}C-peak of the β-galactofuranoside linked to the 3-OH of the α-mannoside is in accordance with that previously reported.[14]

Experimental Section

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 for which 50 °C was used. TLC: 0.25 mm precoated silica gel plates (MERCK silica gel $60F_{254}$); detection by spraying the plates with 8% aqueous H_2SO_4 solution

(10) Van Boeckel, C. A. A.; Beetz, T. *Tetrahedron Lett.* **1983**, *35*, 3775−3778.

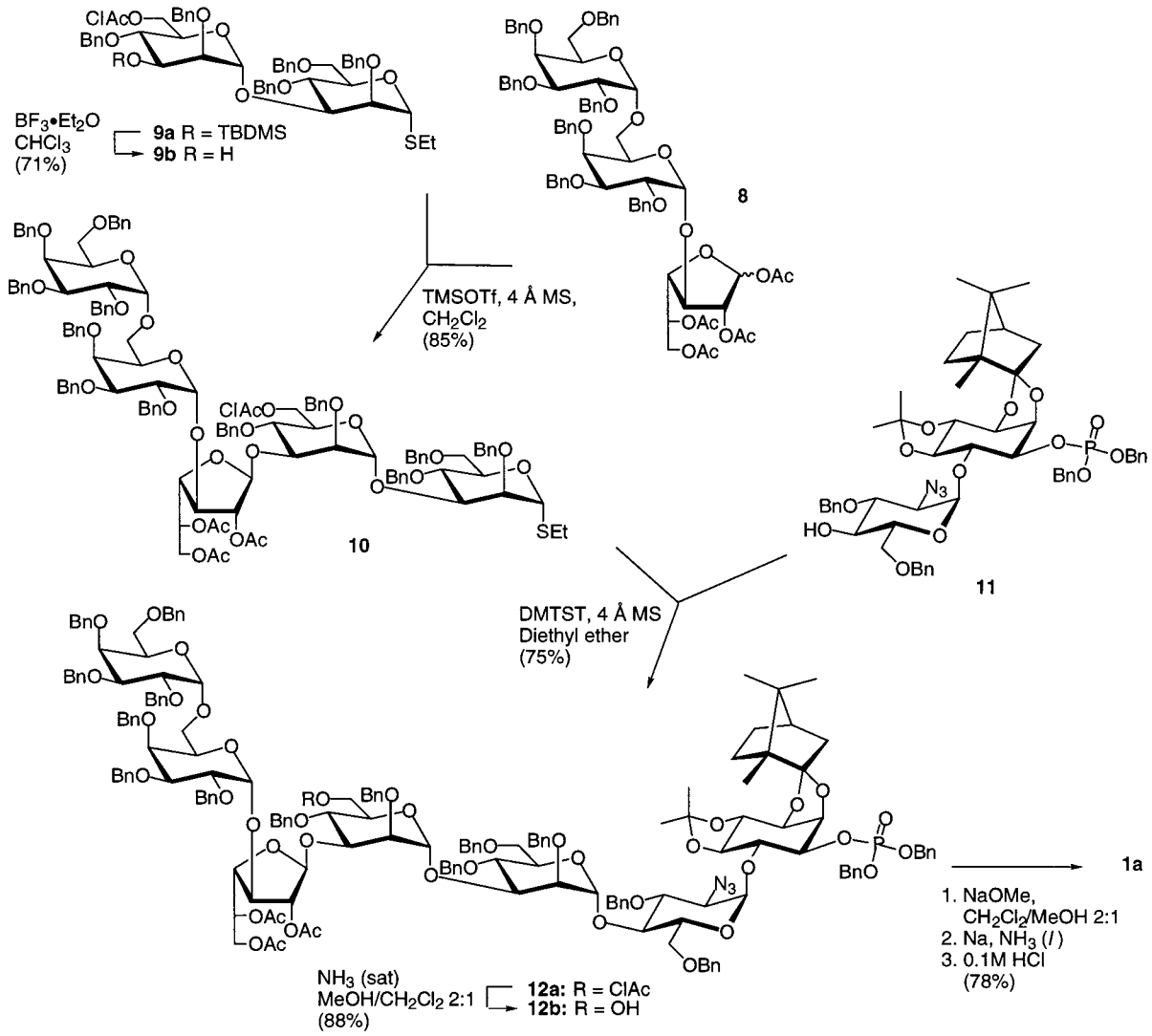
(11) Glaudemans, C. P. J.; Bertolini, M. J. *Methods in Carbohydrate Chemistry*; Academic Press: New York, 1980; Vol. 3, pp 271−275.

(12) McConville, M. J.; Thomas-Oates, J. E.; Ferguson, M. A. J.; Homans, S. W. *J. Biol. Chem.* **1990**, *265*, 19611−19623.

(13) Tsui, D. S.; Gorin, P. A. J. *Carbohydr. Res.* **1986**, *156*, 1−8.

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

Scheme 2. Connecting the Three Building Blocks (A, B, and C) To Form the IPG

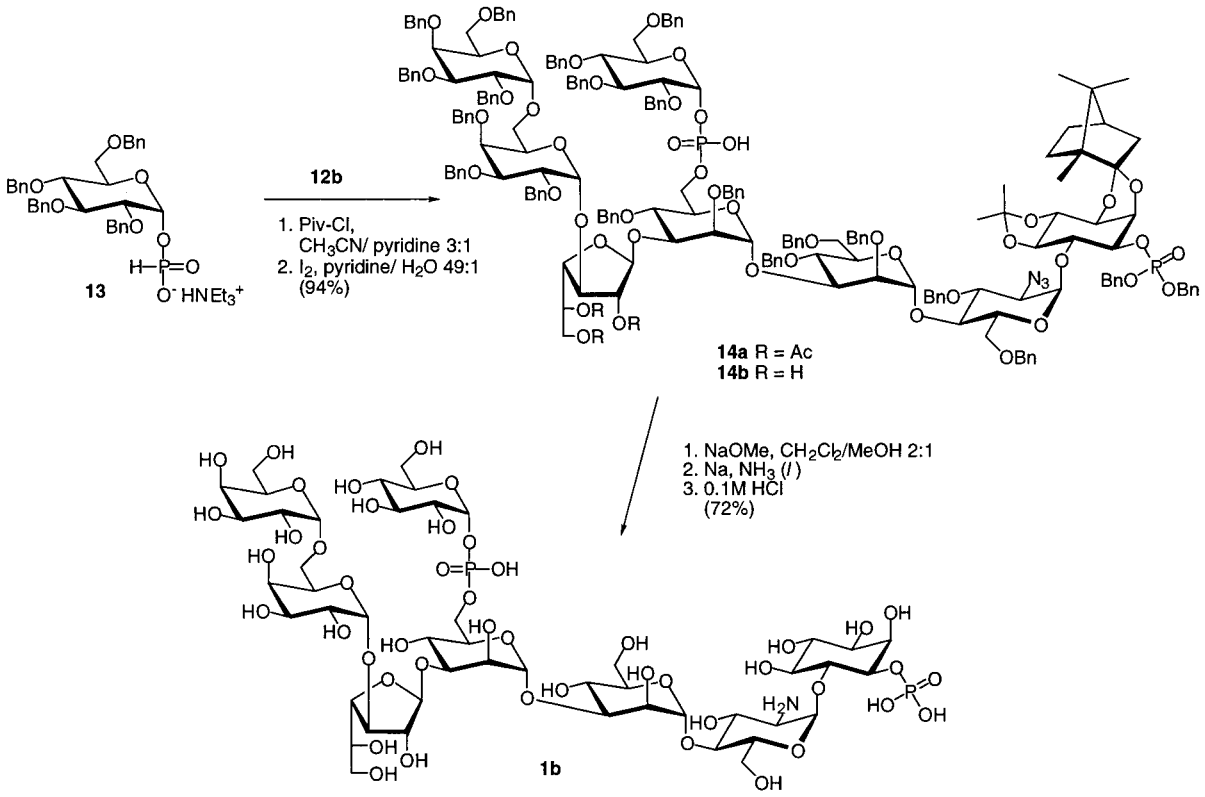


followed by heating at ~250 °C. Optical rotations were recorded at room temperature with a Perkin-Elmer 241 polarimeter. Flash Chromatography (FC): Silica gel MERCK 60 (0.040−0.063 mm). ^{1}H- and ^{13}C NMR spectra were performed on a JEOL JNM-GSX 270, temp 30 °C unless otherwise stated. Chemical shifts are given in ppm relative to TMS in $CDCl_3$ (δ 0.00) or acetone in D_2O (^{13}C: $\delta = 31.00$, ^{1}H: $\delta = 2.22$) as internal standards; ^{31}P, phosphoric acid ($\delta = 0.00$) 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.

Ethyl 2,3,4-Tri-*O*-benzyl-1-thio-β-D-galactopyranoside (2). Ethyl 1-thio-β-D-galactopyranoside (3.48 g, 15.5 mmol) was dissolved in pyridine (150 mL) and triphenylmethyl chloride (5.19 g, 18.6 mmol), and a catalytic amount of 4-(dimethylamino)pyridine was added. After 48 h the mixture was concentrated, and FC (toluene/EtOAc 1:3) yielded the 6-*O*-tritylated derivative (5.57 g, 0.012 mol, 77%). R_f 0.37 (toluene/EtOAc 1:3). The triol formed (3.02 g, 6.47 mmol), and benzyl bromide (3.46 mL, 29.1 mmol) was dissolved in DMF (50 mL) and added dropwise to a mixture of NaH (60%, 1.17 g, 29 mmol) in DMF (50 mL). After 1.5 h the excess of NaH was destroyed with MeOH, and the solvents were evaporated. The residue was dissolved in toluene and washed with water. Normal workup and FC (light petroleum (45−65)/EtOAc 15:1) afforded the fully protected galactose derivative (3.64 g, 4.94 mmol, 76%). R_f 0.43 (light petroleum (45−65)/EtOAc 9:1). A solution of this derivative (1.84 g, 2.50 mmol) and a catalytic amount of *p*-toluenesulfonic acid in $CHCl_3$/MeOH (2:1, 150 mL) was stirred for 2.5 h, and then washed with $NaHCO_3$(aq) and subjected to normal workup. FC (toluene/EtOAc 6:1) produced **2** (1.14 g, 2.30 mmol, 92%). R_f 0.29 (toluene/EtOAc 6:1); mp 100 °C (from EtOAc/hexane); $[\alpha]_D$ −20 (*c* 1.0, $CHCl_3$); NMR ($CDCl_3$): ^{13}C, δ 15.1, 24.7, 62.0, 72.9, 73.3, 74.2, 75.7, 78.4, 78.7, 84.1, 85.4, 127.6, 127.7, 127.8, 128.2, 128.3, 128.4, 138.3, 138.4; ^{1}H, δ 1.28 (t, 3H, $J = 7.4$ Hz), 2.72 (m, 2H), 3.38−3.53 (m, 3H), 3.73−3.87 (m, 3H), 4.40 (d, 1H, $J = 9.5$ Hz), 4.61−4.96 (m, 6H), 7.32−7.37 (m, 15H); Anal. Calcd for $C_{29}H_{34}O_5S$: C, 70.4; H, 6.9 Found: C, 70.4; H, 7.0.

Ethyl 2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (3). Br_2 (0.122 mL, 2.38 mmol) was added under argon to a solution of ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (1.30 g, 2.22 mmol) in CH_2Cl_2 (10 mL) containing 4 Å molecular sieves. After 5 min, cyclohexene was added until the yellow color disappeared, and then the reaction mixture was concentrated. A solution of **2** (784 mg, 1.58 mmol), Et_4-NBr (466 mg, 2.22 mmol) in CH_2Cl_2 (7.5 mL), and DMF (1 mL) was added to the residue. After 48 h, MeOH (2 mL) was added, and after an additional 15 min the mixture was filtered through Celite, and the filtrate was washed with $NaHCO_3$ (aq). FC (light petroleum (45−65)/EtOAc gradient 9:1−6:1) afforded **3** (1.39 g, 1.36 mmol, 86%). R_f 0.72 (toluene/EtOAc 4:1); mp 117 °C (from EtOAc/hexane); $[\alpha]_D$ +22 (*c* 1.0, $CHCl_3$); NMR ($CDCl_3$):^{13}C, 15.1, 24.5, 67.2, 68.8, 69.4, 72.7, 72.8, 73.5 (2C), 74.0, 74.4, 74.8, 74.9, 75.7, 76.4, 76.8, 78.4, 79.0, 84.0, 85.1, 98.4 ($J_{C,H} = 168.6$ Hz), 127.4, 127.5, 127.6, 127.7, 127.8, 127.9, 128.1, 128.2 (2C), 128.3, 128.4, 138.0, 138.4, 138.5, 138.6, 138.7, 138.8; ^{1}H, 1.24 (t, 3H, $J = 7.1$ Hz), 2.67 (m, 2H), 3.49−3.62 (m, 5H), 3.75−3.97 (m, 6H), 4.02 (dd, 1H, $J = 10.0$, 3.5 Hz), 4.36−4.95 (m, 16H), 7.19−7.41 (m, 35H); Anal. Calcd for $C_{63}H_{68}O_{10}S$: C, 74.4; H, 6.7. Found: C, 74.5; H, 6.8.

Scheme 3. Assembly of the Heptasaccharyl *myo*-Inositol



5,6-Di-*O*-acetyl-3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-galactofuranose (5). 4[8,9] (1.62 g, 5.22 mmol) was treated with pyridine/acetic anhydride (2:1, 60 mL) and 4-(dimethylamino)pyridine (60 mg) for 1 h. Concentration and FC (toluene/EtOAc 9:1) gave **5** (2.06 g, 5.22 mmol, 100%). R_f 0.29 (toluene/EtOAc 18:1); $[\alpha]_D$ −23 (*c* 1.0, $CHCl_3$); NMR ($CDCl_3$): ^{13}C, δ 20.7, 20.8, 26.9, 27.3, 63.0, 70.1, 72.2, 81.4, 82.3, 85.3, 105.0, 114.0, 128.0, 128.1, 128.5, 136.9, 170.1, 170.4; ^{1}H, δ 1.36 (s, 3H), 1.55 (s, 3H), 2.01 (s, 3H), 2.03 (s, 3H), 3.89 (d, 1H, J = 4.6 Hz), 4.07−4.15 (m, 2H), 4.32 (dd, 1H, J = 11.5, 4.0 Hz), 4.50−4.68 (m, 3H), 5.28 (m, 1H), 5.81 (d, 1H, J = 4.0 Hz), 7.33 (s, 5H); Anal. Calcd for $C_{20}H_{26}O_8$: C, 60.9; H, 6.6. Found: C, 60.7; H, 6.8.

1,2,5,6-Tetra-*O*-acetyl-α,*β*-D-galactofuranose (7). A solution of **5** (391 mg, 0.99 mmol) in $CHCl_3/CF_3COOH$ (10:1, 16.5 mL) was stirred for 8 h, washed with $NaHCO_3$ (aq), and subjected to normal workup. The obtained product was acetylated in pyridine/acetic anhydride (1:1, 20 mL). After 20 min the mixture was concentrated, and the residue was purified by FC (toluene/EtOAc 9:1) to give **6** (329 mg, 0.75 mmol, 76%). R_f 0.21 (toluene/EtOAc 9:1). A solution of **6** (329 mg, 0.75 mmol) in EtOAc (10 mL) containing $Pd(OH)_2$/C (30 mg) was hydrogenolyzed at 1 atm for 24 h, after which filtration of the mixture through Celite, concentration, and FC (toluene/EtOAc 2:1) produced **7** (α/*β* 1:1; 243 mg, 0.70 mmol, 93%). R_f 0.35 (toluene/EtOAc 2:1); NMR ($CDCl_3$):^{13}C, δ 20.5, 20.7, 20.8, 21.0, 62.5, 62.8, 69.9, 71.1, 72.8, 76.4, 77.9, 80.7, 83.3, 84.5, 93.2 ($J_{C,H}$ = 183 Hz), 99.3 ($J_{C,H}$ = 179 Hz), 169.6, 170.7, 170.8, 171.0; ^{1}H, δ 2.05 (s, 3H), 2.06 (s, 3H), 2.10 (s, 3H), 2.12 (s, 3H), 2.13 (s, 9H), 2.14 (s, 3H), 4.00−4.39 (m, 8H), 4.99 (dd, 1H, J = 3.1, 0.9 Hz), 5.06 (dd, 1H, J = 7.9, 4.6 Hz), 5.24−5.33 (m, 2H), 6.18 (s, 1H), 6.29 (d, 1H, J = 4.8 Hz); Anal. Calcd for $C_{14}H_{20}O_{10}$: C, 48.3; H, 5.8. Found: C, 48.4; H, 5.9.

2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl-(1→3)-1,2,5,6-tetra-*O*-acetyl-α,*β*-D-galactofuranose (8). DMTST (252 mg, 0.97 mmol) was added to a mixture of **7** (109 mg, 0.31 mmol), **3** (400 mg, 0.39 mmol), and 4 Å molecular sieves in diethyl ether (30 mL) under an argon atmosphere. After 5 h Et_3N (1 mL) was added, and the mixture was filtered through Celite. The filtrate was concentrated, and FC (toluene/EtOAc 8:1) of the residue yielded **8** (α/*β* 1:1; 273 mg, 0.21 mmol, 67%). R_f 0.48, 0.57 (toluene/EtOAc 4:1); NMR ($CDCl_3$): ^{13}C, δ 20.3, 20.5, 20.7, 20.8, 21.0, 62.4, 62.8, 66.5, 66.7, 68.7, 69.5, 69.6, 70.0, 70.2, 71.6, 72.5, 72.6, 73.4, 73.5, 73.6, 74.6, 74.7, 74.8, 75.9, 76.0, 76.2, 78.6, 79.1, 79.2, 80.7, 81.0, 82.0, 82.9, 93.4, 98.9, 99.0, 99.1, 99.2, 99.5, 127.3−128.3, 138.1, 138.5−138.8, 169.2, 169.3, 169.4 169.7, 170.1, 170.2, 170.4, 170.5; ^{1}H, δ 1.96 (s, 6H), 1.97 (s, 3H), 1.99 (s, 3H), 2.00 (s, 3H), 2.07 (s, 9H), 3.48−5.02 (m, 64H), 5.17 (dd, 1H, J = 8.9, 4.4 Hz), 5.22−5.37 (m, 3H), 6.11 (s, 1H), 6.29 (d, 1H, J = 4.4 Hz), 7.12−7.41 (m, 70H); Anal. Calcd for $C_{75}H_{82}O_{20}$: C, 69.1; H, 6.3. Found: C, 68.9; H, 6.5.

Ethyl 2,4-Di-*O*-benzyl-6-*O*-chloroacetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-1-thio-α-D-mannopyranoside (9b). BF_3·Et_2O (0.4 mL) was added to a solution of **9a**[4] (490 mg, 0.48 mmol) in $CHCl_3$ (15 mL). The mixture was stirred for 26 h, and then neutralized with $NaHCO_3$ (aq). Normal workup and FC light petroleum (45−65)/EtOAc 4:1) afforded **9b** (311 mg, 0.34 mmol, 71%). R_f 0.25 (light petroleum (45−65)/EtOAc 4:1); $[\alpha]_D$ +64 (*c* 0.85, $CHCl_3$); NMR ($CDCl_3$): ^{13}C, δ 15.0, 25.3, 40.9, 65.4, 69.0, 69.5, 71.6, 71.8, 72.3 (2C), 73.4, 74.4, 74.7, 75.5, 76.3, 78.6, 78.7 (2C), 81.5, 98.8, 126.7−128.6, 137.6−138.5, 167.1; ^{1}H, δ 1.27 (t, 3H, J = 7.7 Hz), 2.51−2.76 (m, 2H), 3.47 (t, 1H, J = 9.8 Hz), 3.65−4.80 (m, 22H), 4.91 (d, 1H, J = 11.2 Hz), 5.20 (s, 1H), 5.46 (s, 1H), 7.06−7.40 (m, 25H); HRMS Calcd for $C_{51}H_{57}ClO_{11}S$: $[M + Na]^+$ 935.3208. Found: $[M + Na]^+$ 935.3197.

Ethyl 2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-acetyl-*β*-D-galactofuranosyl-(1→3)-2,4-di-*O*-benzyl-6-*O*-chloroacetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-1-thio-α-D-mannopyranoside (10). TMSOTf (80 μL, 0.44 mmol) was added under argon at 0 °C to a mixture of **8** (150 mg, 0.115 mmol), **9b** (67 mg, 0.073 mmol), and 4 Å molecular sieves in CH_2Cl_2 (10 mL). After 1.5 h, the reaction was quenched by the addition of Et_3N (0.5 mL). The mixture was filtered through a pad of Celite, and the filtrate was concentrated. FC (toluene/EtOAc 9:1) of the residue gave **10** (134 mg, 0.062 mmol, 85%). R_f 0.54 (toluene/EtOAc 4:1); $[\alpha]_D$ +45 (*c* 0.86, $CHCl_3$); NMR ($CDCl_3$): ^{13}C, δ 15.0, 20.5, 20.6, 25.3, 40.9, 62.7, 65.3, 66.0, 68.9, 69.0, 69.5, 69.9, 70.5, 71.5, 72.3, 72.4, 72.6, 72.9, 73.3, 73.5, 73.9, 74.2, 74.6, 74.8, 75.1, 75.5, 76.1, 76.3, 78.4, 78.9, 79.5, 80.3, 81.5, 82.5, 84.1, 99.0 ($J_{C,H}$ = 169 Hz), 100.1 ($J_{C,H}$ = 172 Hz), 100.4 ($J_{C,H}$ = 169 Hz), 101.9 ($J_{C,H}$ = 176 Hz, *β*gal*f*), 127.1−129.0, 137.6−138.9, 167.1, 169.5, 170.1 (2H); ^{1}H, δ 1.25 (t, 3H, J = 7.6

Hz), 1.69 (s, 3H), 1.99 (s, 6H), 2.50−2.70 (m, 2H), 3.45−4.97 (m, 56H), 5.00 (s, 1H), 5.14 (s, 1H), 5.23 (s, 1H), 5.30−5.40 (m, 1H), 5.40 (s, 1H), 7.00−7.40 (m, 60H); HRMS Calcd for $C_{124}H_{135}O_{29}ClS$: $[M + Na]^+$ 2177.8396. Found: $[M + Na]^+$ 2177.8315.

2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-acetyl-*β*-D-galactofuranosyl-(1→3)-2,4-di-*O*-benzyl-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*-dibenzylphosphoryl-4,5-*O*-isopropylidene-2,3-*O*-(D-1,7,7-trimethyl[2.2.1]bicyclohept-6-ylidene)-D-*myo*-inositol (12a). To a stirred mixture of **10** (167 mg, 0.077 mmol), **11**[3] (83 mg, 0.085 mmol), and 4 Å molecular sieves in diethyl ether (30 mL) under argon was added DMTST (60 mg, 0.233 mmol). After 18 h Et_3N (0.2 mL) was added, and the mixture was filtered through Celite. Evaporation of the solvent and FC (toluene/EtOAc 9:1, 0.25% Et_3N) of the residue yielded **12a** (179 mg, 0.058 mmol, 75%). R_f 0.44 (toluene/EtOAc 4:1); $[\alpha]_D$ +44 (*c* 1.0, $CHCl_3$); NMR ($CDCl_3$): ^{13}C, δ 10.0, 20.3, 20.5, 20.6, 20.7, 26.9, 27.1, 29.7, 40.7, 43.9, 45.2, 48.0, 51.5, 62.5, 62.8 64.8, 66.0, 68.7, 68.9, 69.5, 69.6, 69.7, 69.8, 70.4, 70.5, 71.6, 72.2, 72.4, 72.6, 72.9, 73.0, 73.2, 73.4, 73.5, 73.7, 73.8, 73.9, 74.2, 74.3, 74.4, 74.6, 74.7, 75.2, 75.4, 76.0, 76.3, 76.8, 77.4, 77.6, 78.7, 78.9, 79.2, 79.5, 80.3, 82.6, 83.9, 97.3 ($J_{C,H}$ = 175 Hz), 98.3 ($J_{C,H}$ = 172 Hz), 99.0 ($J_{C,H}$ = 169 Hz), 100.3 ($J_{C,H}$ = 174 Hz), 100.4 ($J_{C,H}$ = 175 Hz), 101.9 ($J_{C,H}$ = 173 Hz), 112.5, 119.1, 127.0−130.0, 135.6, 137.7−138.9, 166.9, 169.5, 170.1, 170.2;^1H, δ 0.83 (s, 3H), 0.89 (s, 3H), 0.98 (s, 3H), 1.10−1.41 (m, 9H), 1.60−2.10 (m, 4H), 1.80 (s, 3H), 1.93 (s, 3H), 1.96 (s, 3H), 3.22 (dd, 1H, *J* = 9.8, 3.4 Hz), 3.40−5.17 (m, 79H), 5.27−5.35 (m, 1H), 5.46 (s, 1H), 7.02−7.38 (m, 80H); HRMS Calcd for $C_{175}H_{193}O_{42}N_3ClP$: $[M + Cs]^+$ 3207.1539. Found: $[M + Cs]^+$ 3207.1584.

(α-D-Galactopyranosyl)−(1→6)−(α-D-galactopyranosyl)−(1→3)−(*β*-D-galactofuranosyl)−(1→3)−(α-D-mannopyranosyl)−(1→3)−(α-D-mannopyranosyl)−(1→4)−(2-amino-2-deoxy-α-D-glucopyranosyl)−(1→6)-D-*myo*-inositol 1-phosphate, ammonium salt (1a). To a stirred solution of **12a** (38 mg, 0.0098 mmol) in CH_2Cl_2/MeOH 2:1 (6 mL) was added NaOMe (11 mg). After 5 h the mixture was diluted with CH_2Cl_2 (25 mL), washed with water, dried, filtered, and concentrated. The residue was dissolved in THF (2 mL) and added to NH_3 (*l*) (∼20 mL) at −33 °C. 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 **1a** (9.4 mg, 0.0076 mmol, 78%). $[\alpha]_D$ +86 (*c* 1.0, H_2O); NMR (D_2O) pH* = 5.8: ^{13}C, δ 54.6, 60.8, 61.5, 62.0 (2C), 63.6, 66.1, 66.7, 67.7, 67.8, 68.9, 69.0, 69.9, 70.0, 70.2 (2C), 70.3, 70.6, 71.3, 71.4 (2C), 71.8 (2C), 72.6, 73.1, 73.6, 74.2, 74.6, 76.1 (2C), 76.3, 78.4 (d, *J* = 6 Hz), 78.6, 80.2, 82.8, 85.4, 96.0, 99.3, 100.3, 102.1, 102.8, 105.6; ^1H, δ 3.33 (dd, 1H, *J* = 10.6, 4.0 Hz), 3.40 (t, 1H, *J* = 9.3 Hz), 3.56 (dd, 1H, *J* = 10.1, 2.7 Hz), 3.62−4.35 (m, 38H), 4.43 (br, 1H) 4.96 (d, 1H, *J* = 3.3 Hz), 5.06 (d, 1H, *J* = 3.3 Hz), 5.16 (d, 1H, *J* = 1.5 Hz), 5.19 (s, 1H), 5.27 (d, 1H, *J* = 1.8 Hz), 5.67 (d, 1H, *J* = 4.0 Hz); ^{31}P (decoupled), δ 1.99; HRMS Calcd for $C_{42}H_{74}O_{38}NP$: $[M - 1]^-$ 1230.3548. Found: $[M - 1]^-$ 1230.3579.

2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-acetyl-*β*-D-galactofuranosyl-(1→3)-2,4-di-*O*-benzyl-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*-dibenzylphosphoryl-4,5-*O*-isopropylidene-2,3-*O*-(D-1,7,7-trimethyl[2.2.1]bicyclohept-6-ylidene)-D-*myo*-inositol (12b). To **12a** (57 mg, 0.019 mmol) in CH_2Cl_2 (3 mL) was added MeOH saturated with NH_3 (6 mL). After 50 min the mixture was concentrated. FC (toluene/EtOAc 6:1, 0.25% Et_3N) of the residue gave **12b** (49 mg, 0.016 mmol, 88%). R_f 0.34 (toluene/EtOAc 4:1); $[\alpha]_D$ +41 (*c* 0.84, $CHCl_3$); NMR ($CDCl_3$):^{13}C, δ 10.0, 20.3, 20.5, 20.5, 20.7 (2C), 26.9 (2C), 27.1, 29.8, 43.9, 45.1, 48.0, 51.5, 62.1, 62.6, 63.1, 66.1, 68.5, 68.8, 69.0, 69.3, 69.5, 69.6, 69.7, 69.8, 70.5, 71.7, 72.3, 72.5, 73.0, 73.1, 73.2, 73.3, 73.4, 73.5, 73.7, 73.8, 74.0, 74.1, 74.5, 74.6, 74.7, 74.8, 75.0, 75.2, 75.9, 76.1, 76.1, 76.7, 77.8, 77.9, 78.0, 78.1, 78.9, 79.4, 80.1, 80.4, 82.0, 84.2, 97.2, 99.1, 99.2, 100.0, 100.2, 102.1, 112.4, 119.0, 126.9−128.6, 135.5, 135.7, 137.8−138.9, 169.5, 170.1, 170.2; ^1H, δ 0.84 (s, 3H), 0.89 (s, 3H), 0.98 (s, 3H), 1.10−1.45 (m, 9H), 1.60−2.10 (m, 4H), 1.83 (s, 3H), 1.91 (s, 3H), 1.98 (s, 3H), 3.22 (dd, 1H, *J* = 9.8, 3.6 Hz), 3.37 (dd, 1H, *J* = 8.8, 5.8 Hz), 3.43−5.17 (m, 76H), 5.27−5.35 (m, 1H), 5.36 (s, 1H), 7.02−7.38 (m, 80H); ^{31}P (decoupled), δ −0.97; HRMS Calcd for $C_{173}H_{192}O_{41}N_3P$: $[M + 1 + Na]^+$ 3022.2701. Found: $[M + 1 + Na]^+$ 3022.2733.

2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-acetyl-*β*-D-galactofuranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1-PO_4-6)-2,4-di-*O*-benzyl-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*-dibenzylphosphoryl-4,5-*O*-isopropylidene-2,3-*O*-(D-1,7,7-trimethyl[2.2.1]bicyclohept-6-ylidene)-D-*myo*-inositol triethylammonium salt (14a). 12b (48 mg, 0.016 mmol) and **13**[4,7] (24 mg, 0.034 mmol) were dissolved in CH_3CN/pyridine (3:1, 4 mL), and pivaloyl chloride (20 μL, 0.16 mmol) was added. After 25 min I_2 (2% in pyridine/water 49:1, 1.0 mL, 0.079 mmol) was added. After an additional 30 min, the reaction mixture was diluted with $CHCl_3$ and washed with 10% $Na_2S_2O_3$ (aq). Normal workup and FC ($CHCl_3$/MeOH 100:1→25:1, 0.25% Et_3N) gave **14a** (56 mg, 0.015 mmol, 94%). R_f 0.62 ($CHCl_3$/MeOH 9:1); $[\alpha]_D$ +37 (*c* 1.1, $CHCl_3$); NMR ($CDCl_3$): ^{13}C, δ 8.7, 10.0, 20.2, 20.5 (2C), 20.6, 20.7, 27.0 (2C), 27.3, 29.9, 43.7, 45.2, 48.0, 51.5, 62.8, 63.4, 64.3, 66.0, 68.4, 68.8, 69.0, 69.5, 69.6, 69.7, 70.5, 71.2, 71.7, 71.9, 72.5, 72.5, 72.6, 72.8, 73.1, 73.4, 73.5, 73.8, 73.9, 74.2, 74.5, 74.6, 74.7, 74.9, 75.3, 75.5, 75.7, 76.0, 76.2, 77.3, 77.8, 77.9, 78.0, 78.2, 78.8, 79.0, 79.5, 79.8, 79.9, 80.2, 81.6, 82.9, 83.6, 84.1, 93.1 (d, *J* = 7.4 Hz), 97.4, 98.5, 99.1, 100.4, 100.5, 102.4, 112.4, 119.0, 127.0−128.6, 135.5, 135.7, 137.9−139.0, 169.6, 170.0, 170.3; ^1H, δ 0.83 (s, 3H), 0.90 (s, 3H), 0.97 (s, 3H), 1.00 (t, 9H, *J* = 7.0 Hz), 1.10−1.45 (m, 9H), 1.60−2.10 (m, 13H), 2.61 (m, 6H), 3.22 (dd, 1H, *J* = 9.8, 3.0 Hz), 3.33−5.25 (m, 92H), 5.46 (s, 1H), 5.94 (dd, 1H, *J* = 8.2, 3.1 Hz), 7.02−7.38 (m, 100H); ^{31}P (decoupled), δ −1.06, −0.66; HRMS Calcd for $C_{207}H_{227}O_{49}N_3P_2$: $[M + 2 + Na]^+$ 3625.4804. Found: $[M + 2 + Na]^+$ 3625.4752.

α-D-Galactopyranosyl-(1→6)-α-D-galactopyranosyl-(1→3)-*β*-D-galactofuranosyl-(1→3)-[α-D-glucopyranosyl-(1-PO_4-6)-α-D-mannopyranosyl]-(1→3)-α-D-mannopyranosyl-(1→4)-2-amino-2-deoxy-α-D-glucopyranosyl-(1→6)-D-*myo*-inositol 1-hydrogenphosphate, Ammonium Salt (1b). To a stirred solution of **14a** (54 mg, 0.015 mmol) in CH_2Cl_2/MeOH 2:1 (6 mL) was added NaOMe (25 mg). After 2 h the mixture was diluted with CH_2Cl_2 (50 mL) and washed with 1 M NH_4-Cl. Normal workup and FC ($CHCl_3$/MeOH 25:1 with 0.25% Et_3N) gave **14b** (42 mg, 0.012 mmol, 80%). R_f 0.55 ($CHCl_3$/MeOH 9:1). Deprotection of this derivative by the same procedure as for **12a** gave **1b** (16 mg, 0.011 mmol, 90%); $[\alpha]_D$ +88 (*c* 0.75, H_2O); NMR (D_2O) pH* = 5.6: ^{13}C, δ 54.7, 60.8, 61.1, 61.5, 62.0 (2C), 63.6, 66.0 (d, *J* = 7.4 Hz), 66.3, 67.6, 67.8, 69.0 (2C), 69.9 (2C), 70.0, 70.2 (2C), 70.3, 70.6, 71.3, 71.4, 71.8 (2C), 72.0, 72.1, 72.5, 73.0, 73.1, 73.3, 73.3, 73.6, 74.6, 75.9, 76.0, 76.5 (d, *J* = 5.5 Hz), 77.4 (d, *J* = 3.7 Hz), 80.0, 80.1, 82.8, 85.6, 95.7, 96.1 (d, *J* = 5.5 Hz), 99.3, 100.4, 102.3, 103.4, 105.6; ^1H, δ 3.33 (dd, 1H, *J* = 10.6, 4.0 Hz), 3.44 (t, 1H, *J* = 9.3 Hz), 3.49−4.38 (m, 45H), 4.44 (br, 1H), 4.98 (d, 1H, *J* = 3.3 Hz), 5.06 (d, 1H, *J* = 3.6 Hz), 5.12 (s, 1H), 5.20 (s, 1H), 5.30 (d, 1H, *J* = 1.1 Hz), 5.53 (dd, 1H, *J* = 7.3, 3.3 Hz), 5.69 (d, 1H, *J* = 3.7 Hz); ^{31}P (decoupled), δ −1.15, 1.76; HRMS Calcd for $C_{48}H_{85}NO_{46}P_2$: $[M - H + 2Na]^+$ 1518.3536. Found: $[M - H + 2Na]^+$ 1518.3538.

Acknowledgment. This work, which was part of a collaboration with Dr. Salvatore Turco Department of Biochemistry, University of Kentucky, Lexington, USA and Dr. Hannah Akuffo, Microbiology and Tumor Biology Centre, Karolinska Institute, Stockholm, Sweden, was supported by the Swedish Natural Science Research Council.

JA001515T