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GLYCOSYL-INOSITOL DERIVATIVES III. SYNTHESIS OF HEXOSAMINE-INOSITOL-PHOSPHATES RELATED TO PUTATIVE INSULIN MEDIATORS*

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Abstract: The disaccharides related to glycosyl phosphatidyl inositol anchors of membrane proteins, 4-O-(2-amino-2-deoxy-α-D-glucopyranosyl)-D-myo-inositol-1-phosphate and 4-O-(2-amino-2-deoxy-α-D-galactopyranosyl)-D-chiro-inositol-1-phosphate, have been prepared from optically resolved myo-inositol derivatives. The chiro-inositol moiety was obtained by epimerization of a selectively blocked myo-inositol-triflate ester. The resolved inositols were subsequently phosphorylated to yield the disaccharide aglycones. The amino-sugar components were prepared by azidonitration of suitably protected glucal and galactal derivatives. The derived pyranosyl chlorides were then condensed with the inositol phosphates using silver triflate as the promoter.

Introduction:

In the past few years, a novel class of glycolipids, commonly known as glycosyl phosphatidyl inositol (GPI) has been identified as the membrane anchor for a variety of cell surface proteins.[1] The structure, biosynthesis and function of GPI are areas of active investigations.[2] Fortuitously, some metabolic fragments of GPI, generated by the combined actions of protease and phospholipase C, have displayed certain biochemical properties previously associated with

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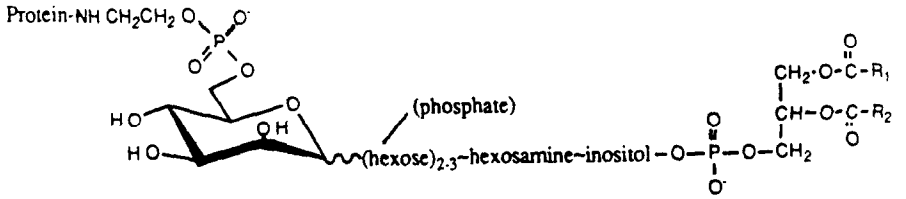
crude preparations of putative-insulin mediators (PIM).[3] These observations not only suggested a structural similarity between GPI and PIM, but also confirmed the heterogeneity of PIM from different cell origins. To facilitate the chemical and biochemical characterizations of multiple forms of GPI and PIM, we have initiated an effort to synthesize their oligosaccharide core structures of the generic type:



The hexosamine-inositol disaccharide moiety in several GPI preparations is D-glucosamine-α-(1--6)-myo-inositol. On the other hand, a hexosamine-(1--4)-inositol linkage was suggested for the anchor of the membrane form of variable surface glycoprotein (VSG) of trypanosomes by periodate oxidation analysis. In a GC-MS experiment, we have found that D-galactosamine and D-chiro-inositol are constituents of a PIM preparation from rat liver.[5] Thus, we elected to synthesize several (1--4) linked hexosamine-inositol phosphates as the first group of structural probes of GPI and PIM.

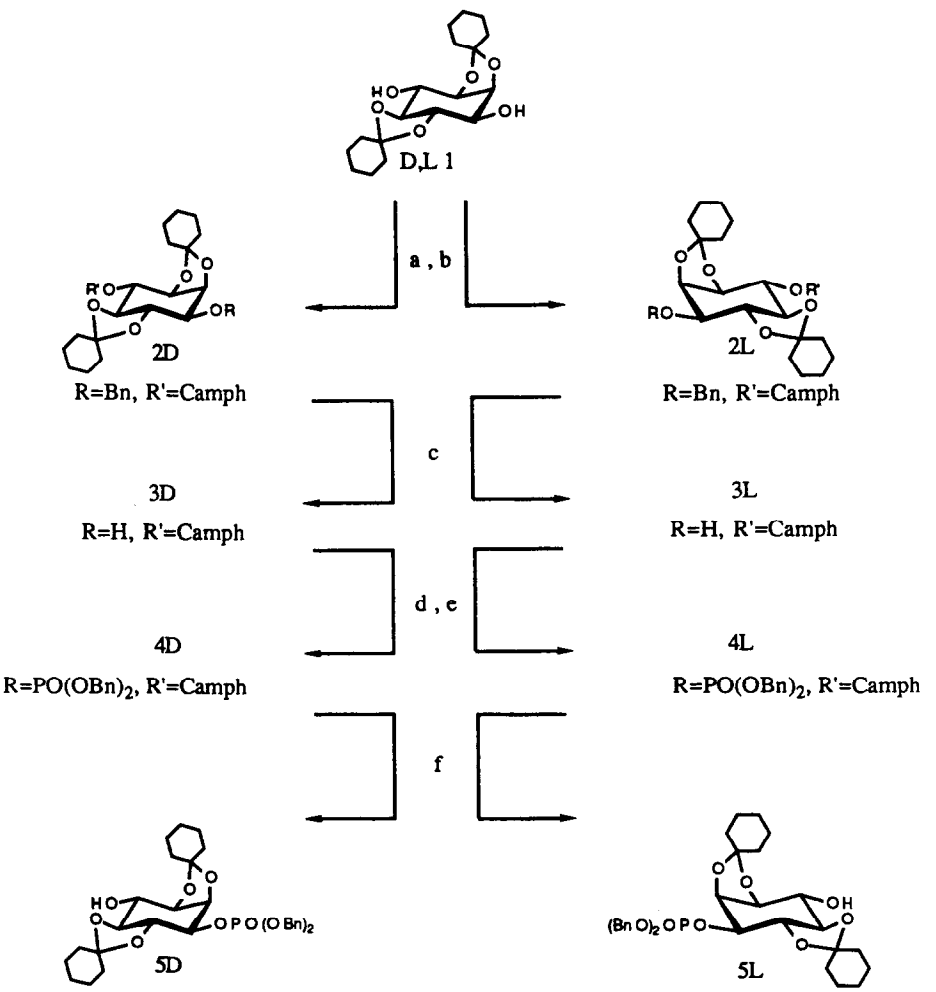
Toward this goal, resolved inositol derivatives bearing suitable functionality to allow for selective phosphorylation at the 1-position and glycosylation at the 4-position were needed. The resolution procedure developed by Vacca et al.[6] to give 3-O-benzyl-6-O-camphanyl-1,2;4,5-di-O-cyclohexylidene-D- and L-myo-inositol appeared the most attractive in terms of the ease of preparation and separation of diastereomers. This strategy also yielded the necessary blocking group requirements in a straightforward manner. As shown in a previous communication,[7] we have extended this approach to blocking groups other than benzyl and have developed a concise synthesis of D and L-chiro-inositol. The synthesis of two hexosamine-inositol phosphates is described in detail below.

RESULTS AND DISCUSSION:

I. Preparation of the inositol-phosphate aglycones (Scheme 1):

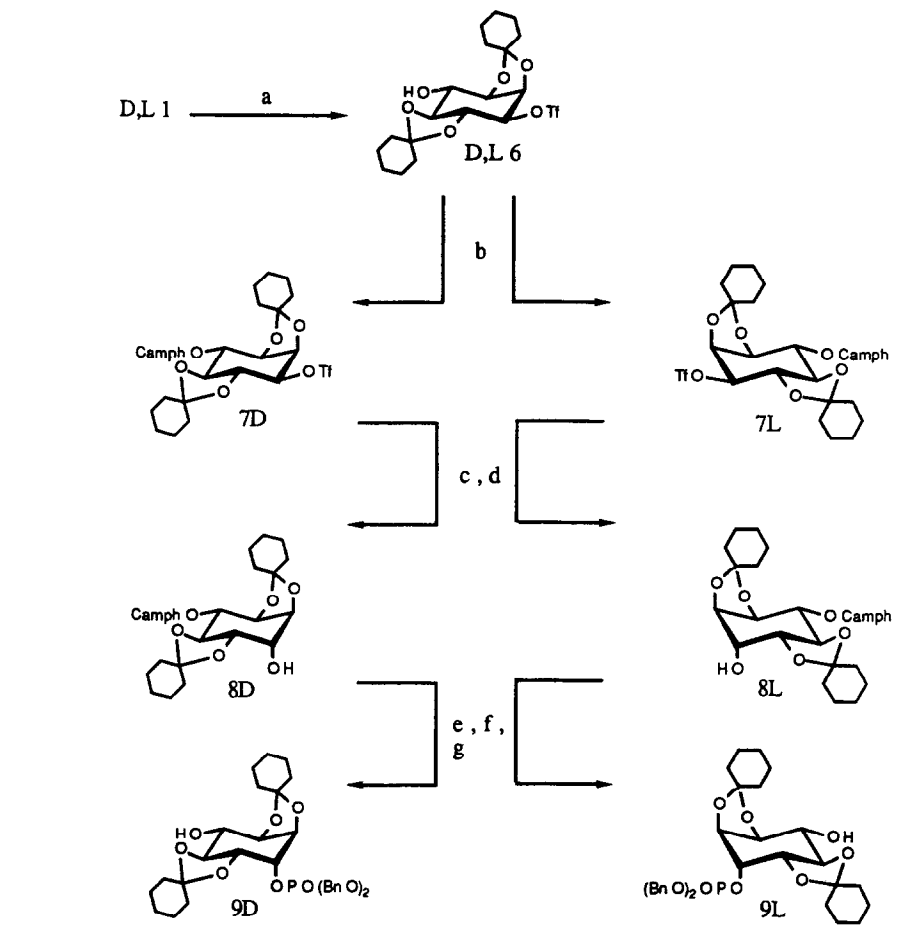
The preparation of the optically resolved inositol derivatives was realized by utilizing the camphanate ester strategy of Vacca et. al.[6] Repetition of the reported resolution gave the 3-benzyl ethers **2D** and **2L** from racemic **1**. The benzyl ether groups were removed by catalytic-transfer hydrogenolysis,[8] using 10% palladium-on-carbon with ammonium formate as the hydrogen source, in methanol at the reflux temperature. The samples **2D** and **2L** were poorly soluble in methanol at

SCHEME 1



a. NaH, BnBr, toluene, reflux; b. Camphanic chloride, Et_3N, DMAP, CH_2Cl_2, 25° C; c. Pd/C, H_4NHCO_2, MeOH, reflux; d. $iPr_2NP(OBn)_2$, tetrazole, CH_2Cl_2, 25° C; e. MCPBA, CH_2Cl_2, 0° C; f. NH_3, MeOH, 25° C.

SCHEME 1
(cont.)



a. Tf_2O, pyr., DMAP, CH_2Cl_2, 25° C; b. Camphanic chloride, Et_3N, DMAP, CH_2Cl_2, 25° C; c. Bu_4NONO_2, DMF, 100° C; d. H_2, Pd/C, MeOH, 25° C; e. $iPr_2NP(OBn)_2$, tetrazole, CH_2Cl_2, 25° C; f. MCPBA, CH_2Cl_2, 0° C; g. NH_3, MeOH, 25° C.

25° C, making pressure hydrogenation difficult. The 3-unprotected myo-inositol derivatives **3D** and **3L** obtained were phosphorylated with the Ku--Fraser-Reid reagent,[9] N,N-diisopropylamino dibenzyl phosphoramidate, followed by oxidation with MCPBA, to give **4D** and **4L** in high yield. Cleavage of the camphanate esters with ammonia--methanol gave the crystalline myo-inositol-phosphate synthons **5D** and **5L** (70% from **3D**).

This procedure proved to be quite general for the resolution of 3-substituted-1,2;4,5-dicyclohexylidene-myo-inositol derivatives. We found that the 3-tosyl, 3-tert-butyldimethylsilyl, and the 3-trifluoromethanesulfonyl derivatives could also be resolved readily as their 6-camphanate esters. For instance, the 3-triflate **6**, prepared by kinetic triflation of **1**, was treated with s-(-)-camphanic acid chloride which yielded the resolved triflates **7D** and **7L** in about 35% yield each. The recently reported tetrabutylammonium nitrate inversion of triflates[10] was applied to **7D** and **7L**. The inversion was clean and, after hydrogenolysis of the nitrate ester, the crystalline chiro-inositol derivatives **8D** and **8L** were isolated in about 80% yield. However, when the triflate **6** was treated directly under these conditions, the yield of the nitrate ester was very low, and the reaction gave many decomposition products.

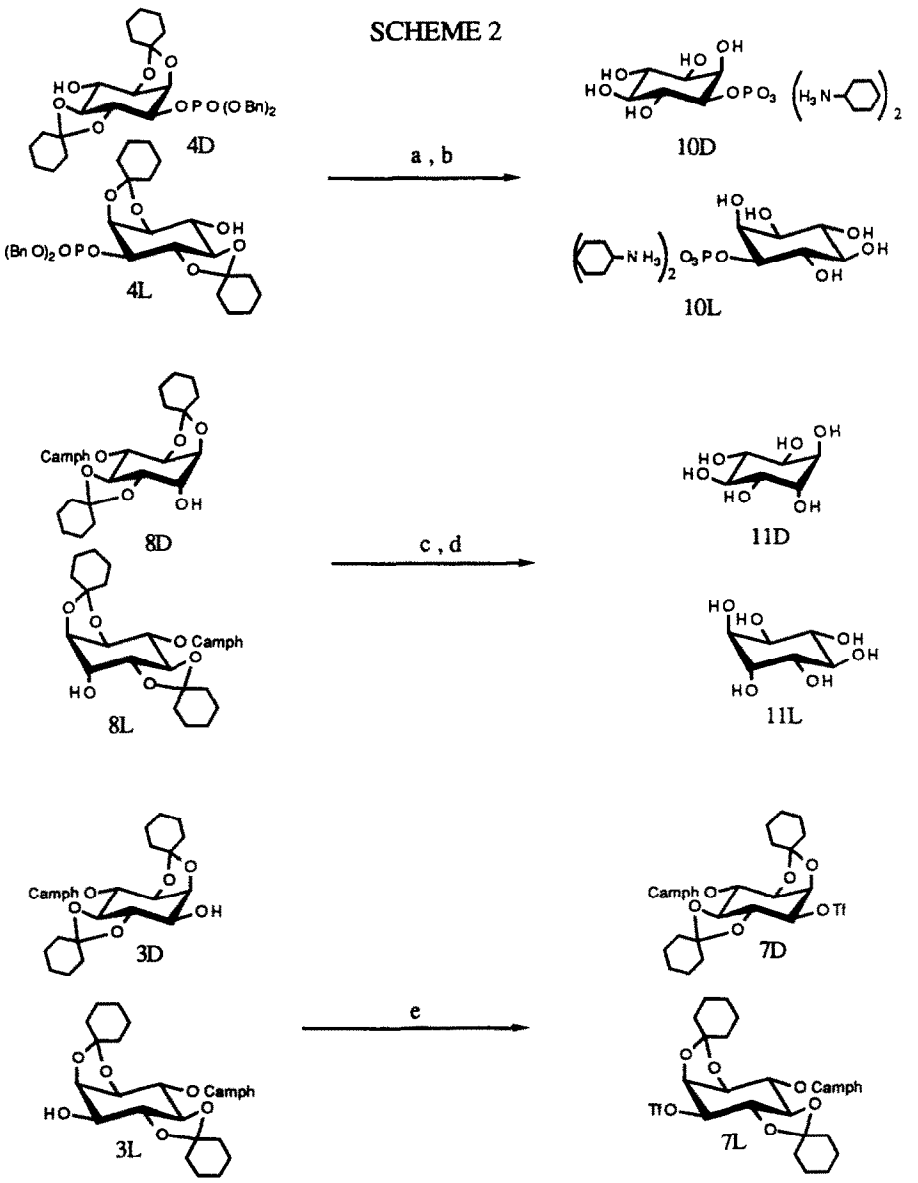
The phosphorylation of compounds **8** with the Ku--Fraser-Reid reagent was not as good as in the myo-inositol derivatives, and the intermediate camphanate-phosphates could not be purified by chromatography. Removal of the ester group by ammonia--methanol gave the chiro-inositol-phosphate synthons **9D** and **9L**, which could be crystallized after purification by column chromatography in about 60% yield.

II. Proof of the absolute configurations of the inositol derivatives (Sheme 2):

The absolute configurations of the resolved pairs of the inositol derivatives was demonstrated by their transformation into derivatives of known absolute configuration. The benzyl groups of the myo-inositol-phosphates **5D** and **5L** were hydrogenolized in methanol and water, which also resulted in the hydrolysis of the bisacetals (vide infera). The known myo-inositol-1-phosphates were isolated by crystallization as their biscyclohexylammonium salts. The melting points and optical rotations of the products were in accord with literature values[11a,11b] (see experimental). The chiro-inositol derivatives **8D** and **8L** were deblocked to give D and L-chiro-inositol (see Scheme 2). The physical constants of the isolated chiro-inositols were in accord with the accepted values[12] (see experimental).

The two sets of derivatives were correlated by triflation of the myo-inositol-camphanate esters **3D** and **3L** to give **7D** and **7L**, respectively. The L-isomer of both pairs of the camphanate esters (**2** and **7**) were the chromatographically slower components. These data corroborate the

SCHEME 2



a. H_2, Pd/C, MeOH, H_2O, 25° C; b. $C_6H_{11}NH_2$, 25° C; c, [NaOMe], MeOH, 25° C; d. HOAc, H_2O, 50° C; e. Tf_2O, pyr., CH_2Cl_2, 0° C.

assignment of the absolute configurations of the inositol derivatives described above. In the report by Vacca et al[1], compound **2D** was assigned to the more polar fraction, and **2L** to the less polar fraction. To clarify this descrepancy, compound **3D** was camphorylated to give the D-biscamphanate ester with $[\alpha]_D$ -31°, and **3L** was treated to give the L-biscamphanate ester with a rotation of 10.5°. Decamphanylation of the bis ester derived from **3D** gave 1,2;3,4-di-O-cyclohexylidene-D-myo-inositol, with $[\alpha]_D$ 19.2°, and similar treatment of the L-enantiomer gave the diol with $[\alpha]_D$ -16.1°, which are the correct values reported for the D and L diols. (Personal communication with the original authors has confirmed that the data and the assignments of absolute configurations given in this report are correct).

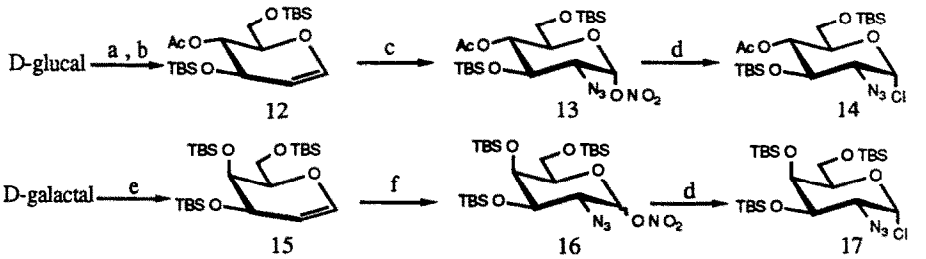
III. Preparation of the glycosyl donors (Scheme 3):

In our synthesis, the 2-azido sugars were selected as the glycosyl donors for the reaction scheme; the azido group was expected to be reduced to an amine concomitant with hydrogenolysis of the phosphate-benzyl-protecting groups of the inositol portion. Schmidt[13] has shown that glucal derivatives will form gluco-azido-nitrates upon azidonitration.[14] However, in our experiment, 2,3,6-tri-O-tert-butyldimethylsilyl-D-glucal gave an inseparable mixture of gluco and manno isomers upon azidonitration. As an alternative, 3,6-di-O-tert-butyldimethylsilyl-D-glucal[17] was prepared in 77% yield by chromatography, followed by acetylation to give **12** in 76% overall yield. Azidonitration of the monoacetate **12** gave a product mixture from which the α-gluco-isomer **13** could be isolated in 30% yield by chromatography. Tetraethylammonium chloride was then used to convert the pyranosyl nitrate into the desired α-pyranosyl chloride **14**.

The galacto derivative was approached first from the 3,4,6-tri-O-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl bromide. This bromide failed to give appreciable amounts of glycoside when treated with silver triflate in the presence of the chiro-inositol-phosphate **9D**. Thus, the trisilyl-derivative **15** was prepared from galactal (in 84% yield) by the standard methods. Azidonitration of **15** under modified conditions gave the D-galacto-azido-nitrate **16** as an α--β mixture (1:1) in good (67%) yield. For the azidonitration reaction, the trisilyl derivative proved to be insoluble in acetonitrile, and tetrahydrofuran was added to aid in mixing. The pyranosyl-chloride **17** was prepared from **16** with tetraethylammonium chloride.

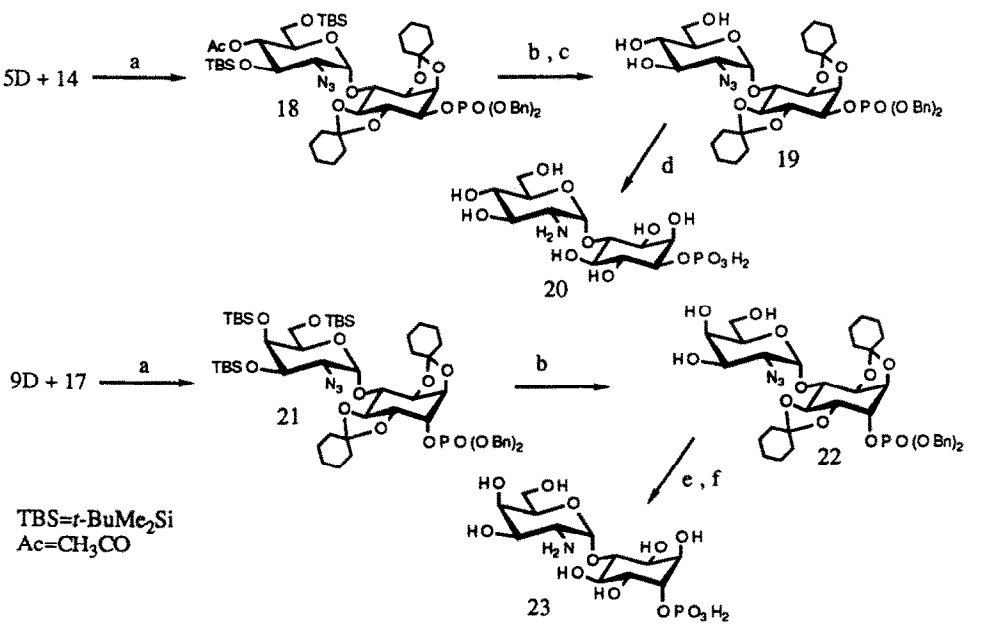
It is interesting to note that the galactal derivatives undergo the azidonitration reaction about 6-times faster than the corresponding glucals. Thus, we prepared 4-O-acetyl-3,6-di-O-tert-butyldimethylsilyl-galactal (data not shown), and this compound undergoes azidonitration about 6-times faster than the corresponding gluco-isomer **12**. This striking difference in rate has been reported by Schmidt[13,16] in separate accounts on the azidonitration of tribenzyl-glucal and galactal.

SCHEME 3



a. TBSCl, imidazole, DMF, 0° C; b. Ac_2O, pyr., 25° C; c. CAN, NaN_3, CH_3CN, -10° C; d. Et_4NCl, CH_3CN, 25° C; e. TBSCl, imidazole, DMAP, DMF, 60° C, f. CAN, NaN_3, CH_3CN, THF, 0° C.

SCHEME 4



a. AgOTf, tetramethylurea, CH_2Cl_2, 4 Å mol. sieves, 25° C; b. Bu_4NF, THF, 0° C; c. NH_3, MeOH, 25° C; d. H_2, Pd/C, MeOH, 25° C; e. H_2, Pd/C, MeOH, 25° C; f. HOAc, H_2O, 70° C.

We also tried tris-trimethylsilyl-galactal (data not shown), but this compound was destroyed under the azidonitration conditions.

IV. Preparation of the disaccharides (Scheme 4):

The glycosidic couplings were carried out with silver triflate as the promoter. A two-fold excess of the glycosyl chlorides was used and the reported yields of the coupling reactions (47% for **18**, and 57% for **21**) are based on the glycosyl acceptors. Both condensations gave a small proportion (10%) of the β-glycoside which was separable by chromatography. The glycosyl bromides gave larger proportions of the β-anomer, and the yields of the coupled products were not increased. The gluco-myo-disaccharide **18** was deblocked first by desilylation using tetrabutylammonium fluoride, with subsequent deacetylation, to give the triol **19** (60% yield). The hydrogenolysis of the phosphate-benzyl-protecting groups and the azide group went smoothly with palladium-on-carbon and hydrogen. As shown by Vacca et. al[8], when methanol--water was used as the hydrogenolysis solvent, the liberated phosphate group of the sample proved acidic enough to hydrolize the cyclohexylidene acetals, thus providing a convenient one-step hydrogenolysis--hydrolysis method. The fully deblocked amino-phosphate **20** was crystallized from water--acetone. The ^{1}H NMR showed only the expected signals, and, the sample appeared quite pure.

The galacto-chiro-disaccharide **21** was treated with fluoride to give the triol **22** in 75% yield. Catalytic hydrogenolysis of the benzyl groups and the azide group, followed by acid hydrolysis of the cyclohexylidene acetals gave the fully deblocked disaccharide **23** in 77% yield. The compound has not been crystallized, but is, by ^{1}H NMR, of equal purity to the gluco-myo-disaccharide **20**. Using the phosphate determination method of Ames,[17] a sample of **23** was analized and gave a phosphorus content of 6.85%, with 7.61% as the expected value, using phosphorylethanolamine as the standard.

After the completion of our synthesis of the disaccharide phosphates **20** and **23**, Plourde and d'Alarcao reported the preparation of the D-glucosamine-α-(1--6)-D-myo-inositol-1-phosphate disaccharide, by self-resolution of the Koenigs-Knorr condensation product of 3,4,6-tri-O-acetyl-2-(2,4-dinitrophenylamino)-α-D-glucopyranosyl bromide with 1,2;3,4-di-O-cyclohexylidene-D,L-myo-inositol.[18] These synthetic compounds should serve as valuable research tools and authentic references in the chemical and biochemical characterizations of GPI and PIM.

EXPERIMENTAL:

General Methods: All non-aqueous reactions were carried out under a nitrogen atmosphere. Dichloromethane was distilled from phosphorus pentoxide. Tetrahydrofuran was distilled from the

sodium ketyl of benzophenone.

^{1}H NMR spectra were recorded on a GE QE-300 instrument. Optical rotations were recorded on a Perkin-Elmer 241 spectrometer in 1 dm cells at 25° C. Thin-layer chromatography was carried out on E. Merk 60F-254 plates (glass or aluminum backed). Melting points were determined on a Thomas-Hoover unimelt apparatus, and are uncorrected. Organic solutions were dried over magnesium sulfate, followed by filtration.

Optical resolution of the myo-inositol derivatives: compounds **6-O-camphanoyl-1,2;4,5-di-O-cyclohexylidene-3-O-benzyl-D-myo-inositol** (2D) (less polar) and **6-O-camphanoyl-1,2;4,5-di-O-cyclohexylidene-3-O-benzyl-L-myo-inositol** (2L) (more polar) were prepared from **1,2;4,5-di-O-cyclohexylidene-D,L-myo-inositol** (1) by the method of Vacca, et al.[6] The diastereomers showed the same physical constants as those reported. The triflate derivative **6** was treated with camphanic acid chloride in exactly the same manner to yield 7D and 7L.

6-O-Camphanoyl-1,2;4,5-di-O-cyclohexylidene-D-myo-inositol (3D). By the general method of Bieg and Szeja[5] a mixture of 2D (1.53 g, 2.5 mmol), ammonium formate (0.75 g), and 10% palladium-on-carbon (1.2 g) was heated at reflux in methanol (40 mL) for 2 h. The catalyst was filtered off through Celite, and the filtrate was evaporated to give a white solid. The sample was purified by column chromatography [silica gel, with hexanes--ethyl acetate (1:1) as the eluent] to yield 3D (0.81 g, 61%), which had mp 224-225° C, and $[\alpha]_D$ 12° (c 0.1, chloroform). ^{1}H NMR: δ ($CDCl_3$) 5.36 (dd, 1H, $J_{1,6}$ 6.8 H_z, H-5), 4.48 (t, 1H, $J_{2,3}$ 4.5 Hz, H-2), 4.24 (dd, 1H, $J_{1,2}$ 5.0 Hz, H-1), 4.00 (dd, 1H, $J_{3,4}$ 8.9 Hz, H-3), 3.94 (t, 1H, $J_{4,5}$ 8.9 Hz, H-4) 3.43 (dd, 1H, $J_{5,6}$ 9.1 Hz, H-5), [2.5 (m, 2H), 2.1 (m, 2H), 1.93(m, 2H) 3 camphanate CH_2], 1.4 - 1.8 (m, 21H, cyclohexylidene), [1.12 (s, 3H), 1.05 (s, 3H) 1.00 (s, 3H) 3 camphanate CH_3]. Anal. calc. for $C_{28}H_{40}O_9$: C, 64.60; H, 7.74. Found: C, 64.45; H, 7.72.

6-O-Camphanoyl-1,2;4,5-di-O-cyclohexylidene-L-myo-inositol (3L). By the same method a sample of 2L (0.2 g, 0.33 mmol) gave, after recrystallization from methanol, 3L (0.134 g, 78.5%), which had mp 236-237° C, and $[\alpha]_D$ -25.5° (c 0.11, chloroform). ^{1}H NMR: δ ($CDCl_3$) 5.38 (dd, 1H, $J_{1,6}$ 6.9 Hz, H-6), 4.47 (t, 1H, $J_{2,3}$ 4.4 Hz, H-2), 4.17 (dd, 1H, $J_{1,2}$ 4.9 Hz, H-1), 3.99 (m, 1H, $J_{3,4}$ 9.1 Hz, H-3), 3.94 (t, 1H, $J_{4,5}$ 9.1 Hz, H-4), 3.42 (dd, 1H, $J_{5,6}$ 11.1 Hz, H-5), [2.47 (m, 2H), 2.11 (m, 2H), 1.93 (m, 2H) 3 camphanate CH_2], 1.3-1.8 (m, 21H, cyclohexylidene), [1.12 (s, 3H), 1.07 (s, 3H), 1.01 (s, 3H) camphanate CH_3]. Anal. calc. for $C_{28}H_{40}O_9$: C, 64.60; H, 7.74. Found: C, 64.5; H, 7.73.

6-O-Camphanoyl-1,2;4,5-di-O-cyclohexylidene-3-O-dibenzylphosphoryl-D-myo-inositol (4D). Compound 3D (0.127 g, 0.24 mmol) was treated with N,N-diisopropylamino dibenzyl phosphoramidate according to the general method of Ku and Fraser-Reid.[9] The sample was purified by column chromatography [silica gel, with hexanes--ethyl acetate (1:1) as the eluent] to yield 4D(0.147 g, 78%). The sample had mp 156-157° C, and $[\alpha]_D$ 4.7° (c 0.19, chloroform). ^{1}H NMR: δ ($CDCl_3$) 7.36 (bs, 10H, Ph), 5.38 (dd, 1H, $J_{1,6}$ 6.9 Hz, H-6), 5.1 (m, 4H, CH_2Ph), 4.76 (m, 1H, $J_{3,4}$ 8 Hz, H-3), 4.61 (t, 1H, $J_{2,3}$ 4.8 Hz, H-2), 4.19 (m, 2H, H-1, H-4), 3.45 (dd, 1H, $J_{4,5}$ 9.8 Hz, $J_{5,6}$ 11.1 Hz, H-5), [2.50 (m, 2H), 2.09 (m, 2H), 1.94 (m, 2H), 3 camphanate CH_2], 1.2-1.8 (m, 21 H, cyclohexylidene), [1.12 (s, 3H), 1.05 (s, 3H), 0.998 (s, 3H) 3 camphanate CH_3]. Anal. calc. for $C_{42}H_{53}O_{12}P$*6 H_2O: C, 56.74; H, 6.01. Found: C, 56.58; H, 6.16.

6-O-Camphanoyl-1,2;4,5-di-O-cyclohexylidene-3-O-dibenzylphosphoryl-L-myo-inositol (4L). Treatment of 3L (0.794 g, 1.51 mmol) in the same manner gave 4L (1.06 g, 90% yield), which was isolated by crystallization of the reaction mixture from ethyl acetate--hexanes, mp 138-139° C, and $[\alpha]_D$ 4.17° (c 0.12, chloroform). ^{1}H NMR: δ ($CDCl_3$) 7.35 (bs, 10H, Ph), 5.40 (dd, 1H, $J_{1,6}$ 7.2 Hz, H-6), 5.13 (m, 4H, CH_2Ph), 4.78 (m, 1H, $J_{3,4}$ 10.1 Hz, H-3), 4.60 (t, 1H, $J_{2,3}$ 4.4 Hz, H-2), 4.19 (t, 1H, $J_{4,5}$ 10.1 Hz, H-4), 4.12 (dd, 1H, $J_{1,2}$ 4.2 Hz, H-1), [2.47 (m, 2H), 2.10 (m, 2H), 1.90 (m, 2H), 3 camphanate CH_2], 1.2-1.8 (m, 21H, cyclohexylidene), [1.12 (s, 3H), 1.06 (s, 3H), 1.00 (s, 3H), 3 camphanate CH_3]. Anal. calc. for $C_{42}H_{53}O_{12}P$: C, 64.60; H, 6.84. Found: C, 64.52; H, 6.88.

1,2;4,5-Di-O-cyclohexylidene-3-O-dibenzylphosphoryl-D-myo-inositol (5D). A solution of 4D (0.115 g, 0.15 mmol) was stirred in ammonia-saturated methanol (10 mL) for 24 h at 25° C. The solution was evaporated, and the product was isolated by column chromatography [silica gel, with hexanes--ethyl acetate (1:2) as the eluent] to yield 5D (85 mg, 94%). The sample had mp 140-141° C, and $[\alpha]_D$ -13° (c 0.13, chloroform). ^{1}H NMR: δ ($CDCl_3$) 7.35 (bs, 10H, Ph), 5.15 (m, 4H, CH_2Ph), 4.78 (m, 1H, $J_{3,4}$ 9.9 Hz, H-3), 4.56 (t, 1H, $J_{2,3}$ 4.6 Hz, H-2), 4.07 (t, 1H, $J_{4,5}$ 10.6 Hz, H-4), 4.03 (t, 1H, $J_{1,2}$ 5.0 Hz, H-1), 3.88 (dd, 1H, $J_{5,1}$ 5.0 Hz, H-6), 3.36 (dd, 1H, $J_{5,6}$ 9.6 Hz, H-5), 1.3-1.8 (m, 20H, cyclohexylidene). Anal. calc. for $C_{32}H_{41}O_9P$*0.5 H_2O: C, 63.04; H, 6.78. Found: C, 63.07; H, 6.96.

1,2;4,5-Di-O-cyclohexylidene-3-O-dibenzylphosphoryl-L-myo-inositol (5L). Similar treatment of 4L (1.04 g, 1.33 mmol) gave 5L (0.66 g, 84%), and the sample had mp 141-142° C, and $[\alpha]_D$ 10° (c 0.12, chloroform). Anal. calc. for $C_{32}H_{41}O_9P$*0.25 H_2O: C, 63.51; H, 6.91. Found: C, 63.41; H, 6.95.

1,2:4,5-Di-O-cyclohexylidene-3-O-trifluoromethanesulfonyl-myo-inositol (6). To a solution of dicyclohexylidene myo-inositol (**1**) (1.0 g, 2.9 mmol) and pyridine (0.5 mL) in dichloromethane (15 mL) was added a solution of triflic anhydride (0.38 mL, 2.9 mmol) dropwise during 10 min at -40° C. The solution was stirred for 2 h at -40° C. The dichloromethane solution was washed with ice--10% hydrochloric acid aq., ice--sodium bicarbonate sat. aq., and ice--water, dried, and evaporated to a foam. The monotriflate was isolated by column chromatography [20 g silica with hexanes--ethyl acetate 2:1 (100 mL) and then hexanes--ethyl acetate 1:1 as the eluents] to yield **6** (0.96 g, 75%) as a colorless foam. ^{1}H NMR: δ ($CDCl_3$) 5.05 (dd, 1H, $J_{3,4}$ 10.4 Hz, H-3), 4.57 (t, 1H, $J_{2,3}$ 5.0 Hz, H-2), 4.13 (dd, 1H, J1,2 Hz, H-1), 4.11 (t, 1H, $J_{4,5}$ 10.4 Hz, H-4), 3.9 (m, 1H, $J_{6,1}$ 9.2 Hz, H-6), 3.40 (t, 1H, $J_{5,6}$ 10.4 Hz, H-5), 0.8-1.9 (m, 20H, cyclohexylidene).

6-O-Camphanoyl-1,2;4,5-di-O-cyclohexylidene-3-O-trifluoromethanesulfonyl-D-myo-inositol (7D). This less polar compound was isolated by column chromatography [silica gel, with hexanes--ethyl ether (2:1) as the eluent] to yield **7D** (36% yield), which had mp 129-131° C (ethyl ether), and $[\alpha]_D$ 4.46° (c 0.46, chloroform). ^{1}H NMR: δ ($CDCl_3$) 5.38 (dd, 1H, $J_{1,6}$ 7.0 Hz, H-6), 5.06 (dd, 1H, $J_{3,4}$ 9.8 Hz, H-3), 4.56 (t, 1H, $J_{2,3}$ 4.3 Hz, H-2), 4.28 (dd, 1H, $J_{1,2}$ 4.3 Hz, H-1), 4.23 (t, 1H, $J_{4,5}$ 9.6 Hz, H-4), 3.52 (dd, 1H, $J_{5,6}$ 11.2 Hz, H-5), [2.48 (m, 2H), 2.11 (m, 2H), 1.95 (m, 2H), 3 camphanate CH_2], 1.3-1.8 (m, 21 H, cyclohexylidene), [1.12 (s, 3H), 1.04 (s, 3H), 0.98 (s, 3H), 3 camphanate CH_3].

6-O-Camphanoyl-1,2;4,5-di-O-cyclohexylidene-3-O-trifluoromethanesulfonyl-L-myo-inositol (7L). This more polar compound was isolated by direct crystallization of the reaction mixture from ethyl acetate--hexanes. After two recrystallizations **7L** was isolated as fine white needles (31% yield), and had mp 157-159° C, and $[\alpha]_D$ 4.46° (c 1.42, chloroform). ^{1}H NMR: δ ($CDCl_3$) 5.40 (dd, 1H, $J_{1,6}$ 6.8 Hz, H-6), 5.04 (dd, 1H, $J_{3,4}$ 10.2 Hz, H-3), 4.55 (t, 1H, $J_{2,3}$ 4.2 Hz, H-2), 4.22 (t, 1H, $J_{4,5}$ 9.8 Hz, H-4), 4.21 (dd, 1H, $J_{1,2}$ 4.2 Hz, H-1), 3.52 (dd, 1H, $J_{5,6}$ 11.2 Hz, H-5), [2.47 (m, 2H), 2.10 (m, 2H), 1.94 (m, 2H), 3 camphanate CH_2], 1.35-1.82 (m, 21H, cyclohexylidene), [1.13 (s, 3H), 1.06 (s, 3H), 1.00 (s, 3H) 3 camphanate CH_3].

6-O-Camphanoyl-1,2;4,5-di-O-cyclohexylidene-D-chiro-inositol (8D). A sample of the triflate **7D** (0.514 g, 0.82 mmol) and tetrabutylammonium nitrate (0.5 g, 1.64 mmol) were heated in

dimethylformamide (10 mL) for 16 h at 100° C. The solution was evaporated at high vacuum, and the residue was dissolved in dichloromethane, washed twice with water, dried, and evaporated. The crude chiro-inositol ester failed to crystallize, and was treated directly with 10% palladium-on-carbon (50 mg) in methanol (20 mL), on a Parr pressure-reaction-apparatus under hydrogen (55 PSI) for 2.5 h at 25° C. The catalyst was filtered off through Celite, and the solids were washed well with dichloromethane. The filtrate was evaporated and **8D** was obtained by crystallization from methanol. A second crop of crystals was obtained by adding water to the concentrated mother liquors, to yield **8D** (0.33 g, 78%) as fine white needles, mp 185-186° C, $[\alpha]_D$ 31° (c 0.37, chloroform). ^{1}H NMR: δ ($CDCl_3$) 5.34 (dd, 1H, $J_{3,4}$ 10.2 Hz, H-3), 4.54 (t, 1H, $J_{1,6}$ 1.8 Hz, H-6), 4.35 (dd, 1H, $J_{1,2}$ 6.6 Hz, H-1), 4.30 (t, $J_{2,3}$ 6.6 Hz, H-2) 3.98 (t, 1H, $J_{4,5}$ 10.2 Hz, H-4) 3.89 (dd, 1H, $J_{5,6}$ 2.5 Hz, H-5), [2.51 (m, 2H), 2.09 (m, 2H), 1.93 (m, 2H), 3 camphanate CH_2], 1.3-1.8 (m, 21H, cyclohexylidene), [1.11 (s, 3H), 1.06 (s, 3H), 1.01 (s, 3H) 3 camphanate CH_3]. Anal. calc. for $C_{26}H_{40}O_9$: C, 64.62; H, 7.96. Found: C, 64.69; H, 7.67.

6-O-Camphanoyl-1,2;4,5-di-O-cyclohexylidene-D-chiro-inositol (**8L**). Treatment of **7L** (0.5 g, 0.8 mmol) with tetrabutylammonium nitrate (0.365 g) under the same conditions gave **8L** (0.314 g, 75%), after isolation by column chromatography [silica gel with hexanes--ethyl acetate (2:1) as the eluent]. The sample could be crystallized from ethyl acetate--hexanes, and had mp 211-213° C, and $[\alpha]_D$ -43° (c 1.2, chloroform). ^{1}H NMR: δ ($CDCl_3$) 5.36 (dd, 1H, $J_{3,4}$ 10.0 Hz, H-3), 4.55 (t, 1H, $J_{1,6}$ 2 Hz, H-6), 4.34 (dd, 1H, $J_{1,2}$ 7 Hz, H-1), 4.24 (t, 1H, $J_{2,3}$ 7 Hz, H-2), 3.98 (t, 1H, $J_{4,5}$ 10 Hz, H-4), 3.88 (dd, 1H, $J_{5,6}$ 3 Hz, H-5), [2.48 (m, 2H), 2.12 (m, 2H), 1.94 (m, 2H), 3 camphanate CH_2], 1.3-1.8 (m, 21H, cyclohexylidene), [1.12 (s, 3H), 1.06 (s, 3H), 1.00 (s, 3H), 3 camphanate CH_3].

1,2;4,5-Di-O-cyclohexylidene-3-O-dibenzylphosphoryl-D-chiro-inositol (**9D**). By the general method of Ku and Fraser-Reid[8] a sample of **8D** (0.5 g, 0.96 mmol) was phosphorylated with N,N-diisopropylamino dibenzyl phosphoramidate, with subsequent oxidation with MCPBA. The resulting crude-phosphate triester was treated with ammonia-saturated methanol (10 mL) for 36 h at 25° C. The solvent was evaporated, and the product was isolated by column chromatography [silica gel, with hexanes--ethyl acetate (3:2) as the eluent], to yield **9D** (0.586 g, 61%). After crystallization from ethyl ether--hexanes the sample had mp 136-137° C, $[\alpha]_D$ 38.4° (c 0.13, chloroform). ^{1}H NMR: δ ($CDCl_3$) 7.34 (d, 10H, Ph), 5.1-5.2 (m, 5H, H-1 and CH_2Ph), 4.23 (dd, 1H, $J_{2,3}$ 2.1 Hz, $J_{3,4}$ 5.3 Hz, H-3), 4.0 (t, 1H, $J_{4,5}$ 5.0 Hz, H-4), 3.60-3.70 (m, 3H, H-2, H-5, H-6), 1.3-1.78 (m, 20H, cyclohexylidene). Anal. calc. for $C_{33}H_{41}O_9P$: C 63.04; H, 6.89. Found: C, 63.15; H, 6.89.

1,2;4,5-Di-O-cyclohexylidene-3-O-dibenzylphosphoryl-L-chiro-inositol (9L). A sample of **8L** (0.1 g, 0.19 mmol) was treated as for **8D** to yield **9L** (60 mg, 53%), which had mp 134-135° C (ethyl ether--hexanes), and $[\alpha]_D$ -46.3° (c 0.33, chloroform).

Dicyclohexylammonium D-myo-inositol-1-phosphate (10D). A solution of **5D** (85 mg) was treated in methanol--water (8:2, 30 mL) with 10% palladium-on-carbon (40 mg) on a Parr pressure-reaction-apparatus under an atmosphere of hydrogen (50 PSI) for 16 h at 25° C. The catalyst was removed by filtration through a pad of Celite, and the solids were washed with methanol--water. The filtrate was evaporated, and dissolved in water (4 mL) to which was added cyclohexylamine (2 mL). The solution was stirred for 4 h at 25° C, and the excess amine was then extracted with ethyl ether. Evaporation of the aqueous solution gave a white solid which was recrystallized from water--acetone to give the myo-inositol-phosphate salt **10D** (40 mg, 64%), which had mp 191-192° C, and $[\alpha]_D$ 4.5° (c 1.06, water, pH 9 buffer). Lit.[11c] mp 194-196° C, lit.[11b] $[\alpha]_D$ 3.4° (water).

Dicyclohexylammonium L-myo-inositol-1-phosphate (10L). A sample of **5L** (80 mg) was treated as described for **5D** to yield **10L** (12 mg, 19%), which had mp 190-191° C, $[\alpha]_D$ -2.8° (c 0.39, water, pH 9 buffer). Lit.[11a] mp, 192-193° C, $[\alpha]_D$ -4.9° (water).

L-Chiro-inositol (11L). A sample of the L-chiro-camphanate ester **8L** (0.25 g, 0.4 mmol) was deesterified with methanol containing a catalytic amount of sodium methoxide (20 mL), followed by neutralization with Dry Ice. The sample was poured into dichloromethane and water. The layers were mixed and the separated aqueous layer was extracted with dichloromethane. The combined organic layers were washed with water, dried, and evaporated to a solid. The crude sample was recrystallized from acetone--hexanes (1:2) to give 1,2;4,5-di-O-cyclohexylidene-L-chiro-inositol as white needles (0.12 g, 92%), which had mp 169-171° C. A sample of the bisacetal (50 mg, 0.146 mmol) was hydrolized by heating in 60% aqueous acetic acid (3 mL) for 5 h at 50° C. The solvent was evaporated, and toluene was evaporated from the residue. The sample was dissolved in a minimum of water and diluted with 4 volumes of ethanol to give the L-chiro-inositol as white crystals (18.5 mg, 68%, 62% from **11L**). The sample had mp 226-229° C, and $[\alpha]_D$ -61.6° (c 0.37, water). Lit.[12] mp 240° C, $[\alpha]_D$ -64° (water).

D-Chiro-inositol (11D). A sample of **8D** was converted into D-chiro-inositol in 37% overall yield by the same procedure, and **11D** had mp 235-237° C, and $[\alpha]_D$ 68° (c 0.55, water).

4-O-Acetyl-3,6-di-O-tert-butyldimethylsilyl-D-glucal (12). To a solution of D-glucal (3.1 g, 21.3 mmol) in dimethylformamide (100 mL) was added tert-butyldimethylsilyl chloride (6.74 g, 44.7 mmol) and imidazole (7.25 g, 106.5 mmol), at 0° C, and the mixture was stirred for 6 h at 0° C. The solvent was removed by evaporation and the residue was dissolved in dichloromethane, and washed twice with water, dried, and evaporated. The 3,6-di-O-tert-butyldimethylsilyl-D-glucal was purified by column chromatography [silica gel, with hexanes--ethyl acetate (10:1) as the eluent], to give the diether (6.13 g, 77%). This sample was dissolved in pyridine (15 mL), and cooled to 0° C. To this solution was added a mixture of acetic anhydride (15 mL), and pyridine (15 mL). The solution was stirred for 20 h at 25° C, and then poured into ice--water. The mixture was extracted with dichloromethane, which was washed with 10% hydrochloric acid, sat. sodium bicarbonate, and water, dried, and evaporated. The syrupy product was purified by column chromatography [silica gel, with hexanes--ethyl acetate (5:1) as the eluent], to yield **12** (6.5 g, 99%, 76% from D-glucal), $[\alpha]_D$ -20.9° (c 1.4, chloroform). ^{1}H NMR: δ ($CDCl_3$) 6.35 (d, 1H, $J_{1,2}$ 6.2 Hz, H-1), 4.98 (t, 1H $J_{3,4}$ $J_{4,5}$ 5.2 Hz, H-4), 4.71 (dd, 1H, $J_{2,3}$ 3.6 Hz, H-2), 4.02-4.14 (m, 2H, H-3, H-5), 3.90 (dd, 1H, $J_{5,6a}$ 7.1 Hz, $J_{6a,6b}$ 11.5 Hz, H-6a), 3.73 (dd, 1H, $J_{5,6b}$ 3.1Hz, H-6b), 2.07 (s, 3H, Ac), [0.886 (s, 9H), 0.872 (s, 9H) 2 t-butyl-Si], [0.074 (s, 3H), 0.065 (s, 3H), 0.05 (s, 3H), 0.045 (s, 3H), 4 CH_3-Si]. Anal. calc. for $C_{20}H_{40}O_5Si_2$: C, 57.65; H, 9.68. Found: C, 57.76; H, 9.64.

4-O-Acetyl-2-azido-2-deoxy-3,6-di-O-tert-butyldimethylsilyl-α-D-glucopyranosyl nitrate (13). By the general method of Lemieux and Ratcliffe[14] compound **12** (1 g, 2.4 mmol) in acetonitrile (10 mL) was added to a dry mixture of ceric ammonium nitrate (5.23 g, 8.96 mmol) and sodium azide (0.29 g, 4.48 mmol) under a nitrogen atmosphere at -10°C. The suspension was vigorously stirred for 6 h at 0° C. Dichloromethane and ice--water were added, and the organic layer was separated and washed with cold water, dried, and evaporated to a syrup. The product was isolated by column chromatography [silica gel, with hexanes--ethyl acetate (10:1) as the eluent] to give **13** (0.365 g, 30%) as a syrup. The α-gluco isomer eluted separately from the mixed β-gluco and α-manno isomers. ^{1}H NMR: δ ($CDCl_3$) 6.32 (d, 1H, $J_{1,2}$ 1.4 Hz, H-1), 4.95 (t, 1H, $J_{4,5}$ 9.5 Hz, H-4), 3.82 (m, 1H, H-5), 3.88 (t, 1H, $J_{3,4}$ 9.5 Hz, H-3), 3.61 (dd, 1H, $J_{2,3}$ 9.5 Hz, H-2), 3.6 (m, 2H, H-6a, H-6b), 2.08 (s, 3H, Ac), [0.88 (s, 9H), 0.861 (s, 9H) t-butyl-Si], [0.205 (s, 3H), 0.091 (s, 3H), 0.015 (s, 6H) Me-Si].

4-O-Acetyl-2-azido-2-deoxy-3,6-di-O-tert-butyldimethylsilyl-α-D-glucopyranosyl chloride (14). A solution of **13** (0.79 g, 1.5 mmol) in acetonitrile was treated with tetraethylammonium chloride

(0.99 g, 5.95 mmol), and the solution was stirred for 4 h at 25° C. The solution was diluted with dichloromethane and washed with water. The organic layer was dried and evaporated. The product was purified by column chromatography [silica gel, with hexanes--ethyl acetate (10:1) as the eluent] to yield **14** (0.48 g, 65%), as a syrup. ^{1}H NMR: δ ($CDCl_3$) 6.14 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1) 4.99 (t, 1H, $J_{4,5}$ 9.8 Hz, H-4), 4.03 (t, 1H, $J_{3,4}$ 9.6 Hz, H-3), 4.02 (m, 1H, H-5), 3.65 (m, 2H, H6a, H-6b), 3.62 (dd, 1H, $J_{2,3}$ 9.6 Hz, H-2), 2.09 (s, 3H, Ac), 0.881 (bs, 18H, t-butyl-Si), [0.208 (s, 3H), 0.089 (s, 3H), 0.037 (s, 3H), 0.033 (s, 3H) Me-Si].

3,4,6-Tri-O-tert-butyldimethylsilyl-D-galactal (15). A solution of D-galactal[13] (0.5 g, 3.42 mmol), imidazole (1.86 g, 23.4 mmol) and dimethylaminopyridine(0.21 g, 1.71 mmol) was heated in dimethylformamide for 20 h at 60° C, and was stirred for a further 36 h at 25° C. The solution was diluted with dichloromethane and washed with cold water four times, dried, and evaporated. The diester was separated form the triester by column chromatography [silica gel, with hexanes--ethyl acetate (9:1) as the eluent] to yield **8** as a colorless oil (1.4 g, 84%). ^{1}H NMR: δ ($CDCl_3$) 6.21 (d, 1H, $J_{1,2}$ 6.2 Hz, H-1), 4.65 (t, 1H, $J_{2,3}$ 6 Hz, H-2), 3.8-4.15 (m, 5H, H-3, H-4, H-5, H-6a, H-6b), [0.90 (s, 9H), 0.89 (s, 9H), 0.89 (s, 9H) t-butyl-Si], 0.0-0.07 (5 s, 12H, Me-Si).

2-Azido-2-deoxy-3,4,6-tri-O-tert-butyldimethylsilyl-α,β-D-galactopyranosyl nitrate (16). By a modification of the method of Lemieux and Radcliffe,[14] cerric ammonium nitrate (0.6 g, 1.33 mmol) was stirred in dry acetonitrile (2 mL) at 0° C. Sodium azide (40 mg, 0.6 mmol) was added, followed by a solution of **8** (0.2 g, 0.41 mmol) in tetrahydrofuran (1 mL). The mixture was rapidly stirred for 1 h at 0° C, and poured into dichloromethane (20 mL) and ice--water (20 mL). The layers were separated, and the water layer was extracted with dichloromethane. The combined orgainc layers were washed with ice--water, dried, and evaporated. The azido nitrate was purified by column chromatography [silica gel, with hexanes--ethyl acetate (9:1) as the eluent] to yield **9** (0.147 g, 67%). The ^{1}H NMR spectrum of **9** showed an α--β ratio of about 1:1. ^{1}H NMR: δ ($CDCl_3$) 6.32 (d, $J_{1,2}$ 4.3 Hz, H-1α), 5.48 (d, $J_{1,2}$ 8.8 Hz, H-1β), 3.4-4.2 (m, ring protons), 0.82-1.0 (m, t-butyl-Si), 0.0-0.2 (m, Me-Si).

2-Azido-2-deoxy-3,4,6-tri-O-tert-butyldimethylsilyl-α-D-galactopyranosyl chloride (17). The azido nitrate **9** (65 mg, 0.113 mmol) and tetraethylammonium chloride (75 mg, 0.45 mmol) were stirred in acetonitrile (2 mL) for 1 h at 25° C. The reaction mixture was poured into diethyl ether and ice--water, and the layers were mixed. The separated water layer was extracted with more

ether. The combined ether layers were washed with ice--water, dried, and evaporated to yield the crude α-chloride (57 mg, 95%), which was used directly for the preparation of the disaccharide. ^{1}H NMR: δ ($CDCl_3$) 6.17 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 4.13 (dd, 1H, $J_{2,3}$ 10.0 Hz, H-2), 4.07 (d, 1H, $J_{3,4}$ 0 Hz, H-4), 4.06 (dd, 1H, $J_{3,4}$ 1.8 Hz, H-3), 3.92 (dd, 1H, $J_{5,6a}$ 7.2 Hz, $J_{5,6b}$ 6.0 Hz, H-5), 3.71 (dd, 1H, $J_{6a,6b}$ 10.0 Hz, H-6a), 3.62 (dd, 1H, H-6b), [0.92 (s, 9H), 0.90 (s, 9H), 0.89 (s, 9H) t-butyl-Si], [0.20 (s, 3H), 0.17 (s, 3H), 0.11 (s, 3H), 0.05 (s, 6H), 0.05 (s, 3H), Me-Si].

6-O-(4-O-Acetyl-2-azido-2-deoxy-3,6-di-O-tert-butyldimethylsilyl-α-D-glucopyranosyl)-1,2;4,5-di-O-cyclohexylidene-3-O-dibenzylphosphoryl-D-myo-inositol (18). Toluene was evaporated from a mixture of **14** (0.52 g, 1.04 mmol) and **5D** (0.31 g, 0.52 mmol), and the mixture was dried in vacuuo. The mixture was dissolved in dichloromethane (20 mL) and 4 Å molecular sieves powder (0.4 g) was added. The suspension was stirred for 1 h at 25° C. After cooling to 0° C, tetramethylurea (0.12 mL) and silver triflate (0.134 g, 0.52 mmol) were added. The mixture was stirred for 48 h at 25° C. The solids were filtered off through Celite, washed well with dichloromethane, and the filtrate was evaporated. The disaccharide was isolated by column chromatography [silica gel, with hexanes--ethyl acetate (3:1) as the eluent] to give **18** (0.26 g, 47%) as a syrup, which had $[\alpha]_D$ 44° (c 0.8, chloroform). ^{1}H NMR: δ ($CDCl_3$) 7.3-7.45 (m, 10H, Ph), 5.48 (d, 1H, $J_{1,2}$ 3.4 Hz, H'-1), 5.1-5.18 (m, 4H, CH_2Ph), 4.95 (m, 2H, H'-4), 4.76 (m, H-1), 4.59 (t, 1H, $J_{1,2}$ 4.6 Hz, H-2), 3.92-4.24 (m, ring protons), 3.3-3.54 (m, ring protons), 3.14 (dd, 1H, $J_{2,3}$ 10.1 Hz, H'-2), 2.06 (s, 3H, Ac), 1.2-1.8 (m, 20H, cyclohexylidene), [0.87 (s, 9H), 0.88 (s, 9H) t-butyl-Si], [0.06 (s, 3H), 0.04 (s, 3H), 0.04 (s, 3H), 0.02 (s, 3H) Me-Si]. Anal. calc. for $C_{62}H_{90}N_3O_{14}PSi_2$*0.5 H_2O: C, 57.54; H, 7.43; N, 3.87. Found: C, 57.54; H, 7.65; N, 3.73.

6-O-(2-Azido-2-deoxy-α-D-glucopyranosyl)-1,2;4,5-di-O-cyclohexylidene-3-O-dibenzylphosphoryl-D-myo-inositol (19). A sample of the silylated disaccharide **18** (0.235 g, 0.22 mmol) was dissolved in tetrahydrofuran (10 mL), and cooled to 0° C. A 1 M solution of tetrabutylammonium fluoride in tetrahydrofuran (1.1 mL) was added and the resulting pale yellow solution was stirred for 1 h at 25° C. The reaction mixture was diluted with dichloromethane, and washed with dil. aq. sodium chloride. The organic layer was dried and evaporated to a syrup. The sample was purified by column chromatography [silica gel, with hexanes--ethyl acetate--methanol (5:5:1) as the eluent] to yield the desilylated disaccharide (0.12 g, 65%). This syrupy monoacetate (0.12 g, mmol) was dissolved in ammonia-saturated methanol (10 mL), and the solution was stirred for 16 h at 25° C. Evaporation of the solvent, and isolation by column

chromatography [silica gel, with hexanes--ethyl acetate--methanol (5:5:1) as the eluent] gave the partially deblocked disaccharide **19** (0.105 g, 92%) as a syrup, which had $[\alpha]_D$ 33.5° (c 0.2, chloroform). ^{1}H NMR: δ ($CDCl_3$) 7.3-7.41 (bs, 10H, Ph), 5.38 (d, 2H, $J_{1,2}$ 3.4 Hz, H'-1), 5.13 (m, 4H, CH_2Ph), 4.78 (m, 1H, H-1), 4.58 (bt, 1H, H-2), 3.4-4.2 (m, ring protons), 3.19 (dd, 1H, $J_{1,2}$ 10.8 Hz, H'-2), 1.2-1.8 (m, 20H, cyclohexylidene). Anal. calc. for $C_{36}H_{50}N_3O_{13}P$*0.5 H_2O: C, 57.28; H, 6.33; N, 5.27. Found: C, 57.08; H, 6.63; N, 5.44.

4-O-(2-Amino-2-deoxy-α-D-glucopyranosyl)-1-O-dihydrogenphosphoryl-D-myo-inositol (20). Compound **19** (0.104 g, 0.133 mmol) was treated with 10% palladium-on-carbon (50 mg) in methanol (10 mL) and water (2 mL) under the hydrogen atmosphere provided by a 20 cm balloon for 24 h at 25° C. The catalyst was filtered off through a pad of Celite, and the solids were washed well with methanol--water. The filtrate was evaporated to give a solid which was recrystallized from water--acetone to give the disaccharide **20** (55 mg, 73%), which had mp 177-178° C (dec.), and $[\alpha]_D$ 0.98° (c 0.61, water). ^{1}H NMR: δ (D_2O) 5.48 (d, 1H, $J_{1,2}$ 3.6 Hz, H'-1), 4.23 (bd, 1H, J 2.3 Hz, H-2), 4.03 (m, 1H, H-1), 3.4-4.0 (m, ring protons), 3.32 (dd, 1H, $J_{2,3}$ 10.8 Hz, H'-2).

3-O-(2-Azido-2-deoxy-3,4,6-tri-O-tert-butyldimethylsilyl-α-D-galactopyranosyl)-1,2;4,5-di-O-cyclohexylidene-1-O-dibenzylphosphoryl-D-chiro-inositol (21). A sample of the azido-chloride **17** (57 mg, 0.107 mmol) and the chiro-inositol-phosphate **9D** (40 mg, 67 μmol) were stirred in dicholormethane (5 mL) with 4 Å molecular sieves powder (0.1 g) for 1 h at 25° C. After cooling to 0° C, tetramethylurea (25 μL) and silver triflate (17 mg, 0.107 mmol) were added. The reaction mixture was allowed to warm, and was stirred for 24 h at 25° C. The solids were filtered off through Celite and were washed well with dichloromethane. The filtrate was evaporated and the disaccharide was isolated by column chromatography [silica gel, with hexanes--ethyl acetate (4:1) as the eluent], to yield **21** (43.2 mg, 57%) as a colorless syrup, $[\alpha]_D$ 67.7° (c 0.22, chloroform). ^{1}H NMR: δ ($CDCl_3$) 7.34, 7.35 (2 s, 10H, Ph), 5.39 (d, 1H, $J_{1,2}$ 3.7 Hz, H'-1), 5.02-5.16 (m, 5H, CH_2Ph, H-1), 4.23 (dd, 1H, $J_{1,2}$ 2.0 Hz, $J_{2,3}$ 5.1 Hz, H-2), 3.74-4.1 (m, ring protons), 3.68 (t, 1H, $J_{5,6a}=J_{6a,6b}$ 9 Hz, H'-6a), 3.58 (dd, 1H, $J_{5,6b}$ 5.5 Hz, H'-6b), 1.4-1.8 (m, 20H, cyclohexylidene), [0.97 (s, 9H), 0.90 (s, 9H), 0.88 (s, 9H) t-butyl-Si], [0.20 (s, 3H), 0.16 (s, 3H), 0.16 (s, 3H), 0.10 (s, 3H), 0.05 (s, 6H), Me-Si]. Anal. calc. for $C_{56}H_{92}N_3O_{13}PSi_3$: C, 59.49; H, 8.20; N, 3.72. Found: C, 59.61; H, 8.39; N, 3.65.

3-O-(2-Azido-2-deoxy-α-D-galactopyranosyl)-1,2;4,5-di-O-cyclohexylidene-1-O-

dibenzylphosphoryl-D-chiro-inositol (22). A sample of the disaccharide **21** (50 mg, 44.2 μmol) in tetrahydrofuran (2 mL) was cooled to 0° C, and a 1 M solution of tetrabutylammonium fluoride (0.256 mL) was added. The resulting pale-yellow solution was stirred for 30 min at 25° C. The reaction mixture was poured into dichloromethane, and washed with cold-dilute aq. sodium chloride solution. The aqueous layer was re-extracted with dichloromethane. The dichloromethane solution was dried, and evaporated. The product was isolated by chromatography [silica gel, with chloroform and then chloroform--methanol (5:1) as the eluents] to yield the desilylated disaccharide **22** (26 mg, 75%) as a pale-yellow syrup, $[\alpha]_D$ 78.1° (*c* 1.2, chloroform). ^{1}H NMR: δ ($CDCl_3$ + D_2O). 7.34, 7.38 (2 s, 10H, Ph), 5.45 (d, 1H, $J_{1',2'}$ 3.8 Hz, H'-1), 5.0-5.15 (m, 5H, CH_2Ph, H-1), 3.75-4.22 (m, 10H, ring protons), 3.57 (dd, $J_{2,3}$ 10.2 Hz, H'-2), 1.2-1.78 (m, 20H, cyclohexylidene).

4-O-2-Amino-2-deoxy-α-D-galactopyranosyl)-1-O-dihydrogenphosphoryl-D-chiro-inositol (23). The disaccharide **22** (20 mg, 25.4 μmol) was treated with 10% palladium-on-carbon (10 mg) in methanol(5 mL) and water (1 mL) under a hydrogen atmosphere provided by a 10 cm diameter balloon for 18 h at 25° C. The catalyst was filtered off through Celite, and was washed well with methanol--water. The filtrates were evaporated to give a white solid (13 mg, 90%). The reduced disaccharide (12 mg, 21 μmol) was heated in acetic acid--water (3:1, 4 mL) at 80° C for 2.5 h. After evaporation of the solvent, toluene was added and evaporated to remove the residual acetic acid to yield the fully-deblocked disaccharide **23** (7.3 mg, 86%, 77% from **22**), as an amporphous white powder, $[\alpha]_D$ 121.3° (*c* 0.52, water). The purity of the sample was established by ^{1}H NMR. ^{1}H NMR: δ (D_2O) 5.54 (d, 1H, $J_{1,2}$ 3.7 Hz, H'-1), 4.38 (m, 1H, $J_{1,2}$ 3.8 Hz, $J_{1,P}$ 8.0 Hz, H-1), 4.31 (t, 1H, $J_{5,6a}=J_{5,6b}$ 6.8 Hz, H'-5), 4.21 (t, 1H, $J_{2,3}$ 3.9 Hz, H-2), 4.11 (dd, 1H, $J_{3,4}$ 3.2 Hz, H'-3), 4.03 (bd, 1H, $J_{4,5}$ 0 Hz, H'-4), 3.90 (dd, 1H, $J_{3,4}$ 9.8 Hz, H-3), 3.7-3.87 (m, 5H, H-4, H-5, H-6, H'-6a, H'-6b), 3.52 (dd, 1H, $J_{2,3}$ 11.0 Hz, H'-2).

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