Pergamon

Tetrahedron, Vol. 53, No. 52, pp. 17727-17734, 1997

0040-4020/97 $17.00 + 0.00

PII: S0040-4020(97)10238-1

Synthesis of Part of a Proposed Insulin Second Messenger Glycosylinositol Phosphate and the Inner Core of Glycosylphosphatidylinositol Anchors

Per J. Garegg, Peter Konradsson*, Stefan Oscarson and Katinka Ruda

Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University,
S-106 91, Stockholm, Sweden

Abstract: Synthesis of 6-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-D-*myo*-inositol 1-phosphate, an inner core structure found in various glycosylphosphatidylinositols, and the corresponding 1,2-cyclic phosphate, proposed as part of an insulin second messenger glycosylinositol phosphate, is described. Chirality in the inositol part of the molecule was achieved by the use of a known D-camphor acetal intermediate. The glycosylation used 4-*O*-allyl-2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl fluoride as glycosyl donor. The allyl group can be chemoselectively removed, opening a route to oligosaccharides bound to the 4-position of the glucosamine unit. The phosphorylation was accomplished by the phosphoramidate procedure.

INTRODUCTION

Glycosylphosphatidylinositol (GPI) anchors occur throughout the eukaryotic kingdom but are much more common in lower organisms, such as protozoa, than in higher organisms, where they make up only a small percentage of the cell surface. Some protozoa, such as in *Leishmania*, have their outer membrane covered with GPIs and glycosylinositolphospholipids (GIPLs).

Although many GPIs are known, they do not appear to vary a great deal structurally. All fully characterized GPIs contain a common trisaccharide core, consisting of 1D-phosphatidylinositol linked to an α-glucosamine, which in turn is linked at position 4 to an α-mannose (Fig. 1).[1] From here on a distinction can be made between the *Leishmania* lipopolysaccharide and the GPI anchors that link proteins to cell surfaces. In *Leishmania* the mannose unit is linked at its 3-position to an α-mannose,[2] in the protein anchors, such as in *Trypanosoma*[1] and rat brain THY-1,[3] the mannose unit is linked at position 6 to a mannose disaccharide. Several syntheses of this pentasaccharide have been completed.[4] Furthermore, in *Trypanosoma* position 3 of the first mannose unit is further α-galactosylated and in the rat brain THY-1 anchor, this mannose unit is galactosylated at position 4 and phosphorylated at position 2.

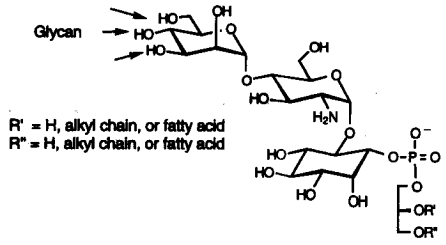


Figure 1. Common structures for GPI

An inositol phosphoglycan (IPG) has been proposed as a second messenger of insulin.[5] It is believed to be structurally related to the glycolipid part of the glycosylphosphatidylinositol anchors. The structure of this insulin sensitive IPG has not been determined, but evidence indicates the prescence of *myo*-inositol glycosidically linked to a non-acetylated glucosamine unit, which itself is coupled to an oligosaccharide (**1a**, Fig. 2). The biologically active IPG is believed to be generated by a specific phospholipase. Biological results have indicated that it is probably the cyclic phosphate (**2a**, Fig. 2) that mediates the action of insulin.[6a] Furthermore, IPG structures are also reported to be generated in response to growth factors.[7]

The structures **1b** and **2b** depicted in Fig. 2 have now been synthesized in a short and efficient way. These structures have also been synthesized by other groups using more laborious routes.[6] Biological results will be published elsewhere.

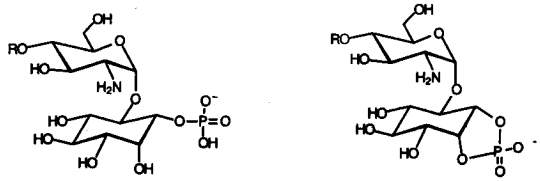
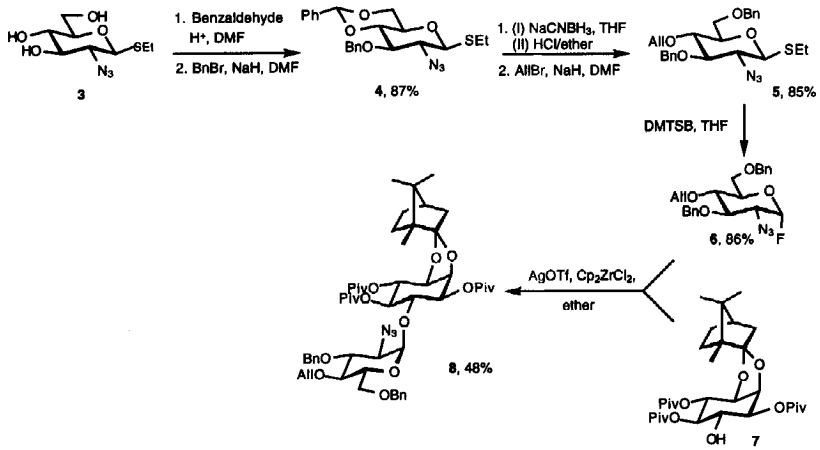


Figure 2. Structures of the inositol phosphoglycans proposed as a "second messenger" of insulin

RESULTS AND DISCUSSION

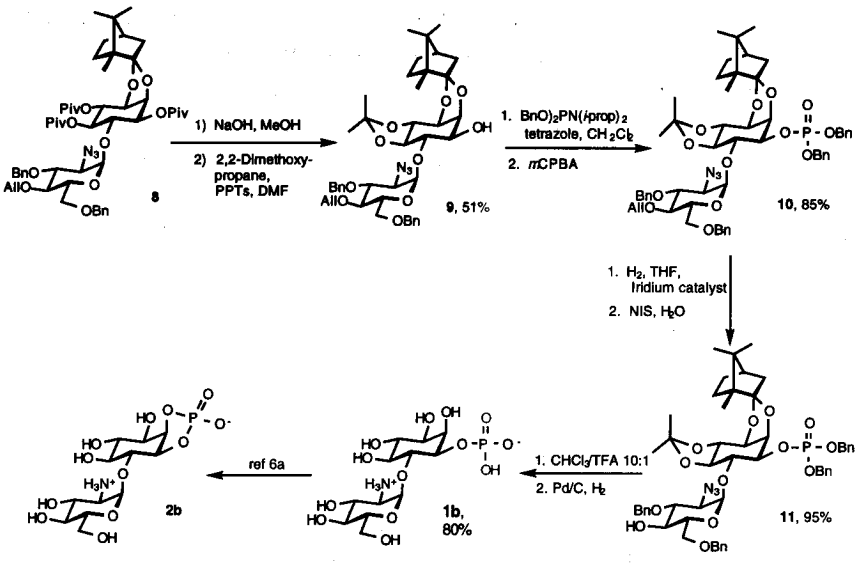
The key compound in the synthesis is the acetal D-2,3-*O*-(D-1,7,7-trimethyl [2.2.1] bicyclohept-2-ylidene)-*myo*-inositol obtained by precipitation in acidic medium from the product mixture formed by reacting the dimethyl ketal of D-camphor with *myo*-inositol as described by Bruzik and Tsai.[8] These authors also described partial pivaloylation of this chiral acetal to produce the monohydroxy compound **7** in only two steps from *myo*-inositol. This easily obtainable chiral inositol derivative has so far not been used as glycosyl acceptor in a synthesis of aminoglycosyl-inositols. A consequence of this strategy is, however, that the remaining OH group in **7** is unreactive with alkylated azido glycosyl donors. Considerable experimentation with various

glycosyl donors, to be described separately, led to the glycosyl donor **6**. Compound **6** was synthesized in five high yielding steps from ethyl 2-azido-2-deoxy-1-thio-β-D-glucopyranoside (**3**)[9] by benzylidenation followed by benzylation to produce **4** (Scheme 1). Regioselective reductive opening of the benzylidene acetal in **4** gave the 4-OH compound, allylation of which produced **5**. Reaction of the thioglycoside with dimethyl(methylthio)sulfonium tetrafluoroborate[10] (DMTSB) in THF produced the glycosyl fluoride donor **6**. Reaction of **6** with the single free OH in **7** in the presence of dicyclopentadienylzirconium dichloride (zirconocene dichloride)[11] gave a reasonable yield (48%) of the glycoside **8**. Only traces of the β-glycoside was obtained.



Scheme 1.

A protecting group manipulation sequence was now required in order to attach a phosphate group at the 1-position of the inositol. Thus, the pivaloyl groups were removed from **8** and the product formed was converted into the 3,4-*O*-isopropylidene inositol acetal **9** (Scheme 2). The remaining OH group was then phosphorylated with dibenzyl *N,N*-diisopropyl phosphoramidite[12] followed by oxidation with *m*-chloroperbenzoic acid (→**10**). Since the camphor ketal is easily isomerized (giving two isomers), reaction conditions (pH) must be carefully controlled. The presence of a chemoselectively removable allyl group in the glycosylinositol opens the route to further glycosidation at that position in order to make larger oligosaccharylinositol substances. Since GPI anchors do not contain a free reducing end the allyl group can also be used as a spacer precursor by photoinduced thiolation.[13] The best way to perform the deallylation (→**11**) was to use an iridium catalyst followed by a neutral NIS-H_2O hydrolysis.[14] Further deprotection in two steps, consisting of deacetalization and catalytic hydrogenolysis, then gave the first target compound **1b**. Conversion of **1b** into the 1,2-cyclic phosphate **2b** was performed as previously described.[6]



Scheme 2.

In summary the targets **1b** and **2b** were synthesized using Bruzik and Tsai's[8] two-step route to the chiral inositol derivative **7**, combined with the use of the new glycosyldonor **6** in the crucial glycosylation step.

EXPERIMENTAL SECTION

General methods. The compounds, reagents and solvents used were all dried. Normal workup means drying of the organic phase (Na_2SO_4), filtration, and evaporation of the solvent in vacuo at or below 40 °C. TLC: 0.25 mm precoated silica-gel plates (MERCK silica-gel 60F254); detection by spraying the plates with 8% aq. H_2SO_4 soln. followed by heating at ca 250 °C. Optical rotations: recorded at room temperature with a Perkin-Elmer 241 polarimeter. Flash Chromatography (FC) : Silica gel MERCK 60 (0.040-0.063 mm), unless otherwise stated. ^1H- and ^{13}C- NMR spectra were performed on a JEOL JNM-GSX 270, 30 °C. Chemical shifts are given in ppm relative to TMS as internal standard. Mass spectra were obtained on a JEOL SX 102 Mass Spectrometer. Melting points are corrected.

Ethyl 2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-1-thio-β-D-gluco-pyranoside (4). A catalytic amount of *p*-toluenesulfonic acid was added to a solution of ethyl 2-azido-2-deoxy-1-thio-β-D-glucopyranoside **3**[9] (1.71 g, 6.87 mmol) and benzaldehyde dimethyl acetal (1.26 g, 8.25 mmol) in DMF (15 mL). The reaction flask was placed on a rotavapor under reduced pressure at a bath temperature of 30 °C. After 4 h the reaction mixture was neutralized with triethylamine. The solvent was removed, and the residue purified by FC (toluene/ EtOAc 9:1) to give ethyl 2-azido-4,6-*O*-benzylidene-2-deoxy-1-thio-β-D-glucopyranoside in 87% yield (2.02 g, 5.99 mmol). Recrystallization from diethyl ether: n-hexane. R_f 0.56 (toluene/ EtOAc 6:1). A solution of this compound (2.86g, 8.48 mmol) and benzyl bromide (4.35 g, 25 mmol) in DMF (30 mL) was

added dropwise to a suspension of NaH (1.36 g, 34 mmol) in DMF (20 mL). After 10 min excess NaH was destroyed with CH_3OH, the solvent was removed and the residue was partitioned between CH_2Cl_2 and water. Normal workup and FC (light petroleum-EtOAc 9:1) afforded **4** quantitatively as white crystals, (3.65 g, 8.48 mmol). R_f 0.46 (light petroleum bp (40-60)/ EtOAc 6:1). Recrystallization from diethyl ether: n-hexane; mp 87 °C; $[\alpha]_D$ -128° (*c* 0.4, $CHCl_3$); NMR ($CDCl_3$): ^1H, δ 1.30 (t, 3H), 2.72 (m, 2H), 3.40-3.49 (m, 2H), 3.60-3.79 (m, 3H), 4.30-4.37 (m, 2H), 4.87 (dd, 2H, *J* = 26.0, 11.0 Hz), 5.56 (s, 1H), 7.32 (m, 10H); ^{13}C, δ 15.0, 24.9, 65.8, 68.5, 70.4, 75.0, 80.8, 81.5, 84.9, 101.3, 125.9, 127.9, 128.2, 128.3, 124.4, 129.1, 137.1, 137.7; Anal. Calcd for $C_{22}H_{25}N_3O_4S$: C, 61.81; H 5.89; N 9.83. Found: C, 61.90; H, 5.82; N, 9.82.

Ethyl 4-*O*-allyl-2-azido-3,6-di-*O*-benzyl-2-deoxy-1-thio-β-D-gluco-pyranoside (5). Sodium cyanoborohydride (6.23 g, 99.2 mmol) was added at room temperature to a solution of **4** (4.24 g, 9.92 mmol) in THF (150 mL). After stirring for 1 h, the reaction mixture was treated with HCl in diethyl ether, until pH 1, and then filtered through a pad of Celite. The solvent was removed and the residue purified by FC (toluene/EtOAc 9:1) to yield ethyl 2-azido-3,6-di-*O*-benzyl-2-deoxy-1-thio-β-D-glucopyranoside in 85% yield as a colorless syrup (3.65 g, 8.50 mmol) used directly in the next step. R_f 0.43 (toluene-EtOAc 6:1). A solution of the above compound (3.61 g, 8.41 mmol) and allyl bromide (2.03 g, 16.8 mmol) in DMF (15 mL) was added dropwise to a suspension of NaH (1.01 g, 25 mmol) in DMF (10 mL). After 15 min the reaction was quenched with CH_3OH and the solvent was removed. The residue was partitioned between toluene and brine. Normal workup and FC (light petroleum (40-60)/ EtOAc 9:1) afforded **5** quantitatively as white crystals (3.96 g, 8.43 mmol). R_f 0.56 (toluene/ EtOAc 18:1). Recrystallization from n-hexane gave white crystalls with mp 56 °C; $[\alpha]_D$ -44° (*c* 0.5, $CHCl_3$); NMR ($CDCl_3$) ^1H, δ 1.28 (t, 3H), 2.70 (m, 2H), 3.37-3.49 (m, 4H), 3.68-3.71(m, 2H), 4.06 (dd, 1H, *J*=5.5, 12.5 Hz), 4.25 (m, 2H), 4.56 (dd, 2H, *J*=12.1, 22.0), 4.84 (s, 2H), 5.09-5.22 (m, 2H), 5.78-5.88 (m, 1H), 7.30 (m, 10H); ^{13}C, δ 15.1, 24.5, 66.0, 68.8, 73.4, 73.7, 75.6, 77.6, 79.4, 84.1, 84.9, 117.0, 127.5, 127.6, 127.8, 128.1, 128.3, 128.4, 134.5, 137.8, 138.1; Anal. Calcd. for $C_{25}H_{31}N_3O_4S$: C, 63.94; H 6.65; N 8.95. Found: C, 63.99; H, 6.56; N, 8.93.

4-*O*-Allyl-2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl fluoride (6) A mixture of **5** (2.08 g, 4.43 mmol) and freshly activated, powdered 4 Å molecular sieves (4 g) in THF (60 mL) was stirred for 15 min at room temperature DMTSB (1.30 g, 6.65 mmol) was added to the mixture and stirring was continued for 1 h after which pyridine (2 mL) was added. The mixture was filtered through a pad of Celite, which was washed with EtOAc (100 mL). The filtrate was washed with 0.5 M H_2SO_4 (50 mL) and aq. sat. $NaHCO_3$ (50 mL). Normal workup and purification by FC (light petroleum (40-60)/ EtOAc 9:1) yielded 86% of **6** as white crystals (1.63 g, 3.81 mmol). R_f 0.39 (light petroleum (40-60)/ EtOAc 9:1); NMR ($CDCl_3$) ^1H, δ 3.43 (ddd, 1H, *J*=2.8, 10.1, 25.8 Hz), 3.63-3.78 (m, 3H), 3.85-3.92 (m, 2H), 4.04 (dd, 1H, *J*=5.5, 12.3 Hz), 4.26 (dd, 1H, *J*=5.7, 12.3 Hz), 4.57 (dd, 2H, *J*=12.1, 31.1 Hz), 4.86 (dd, 2H, *J*=10.6, 13.0 Hz), 5.11-5.22 (m, 2H), 5.53-5.73 (dd, 1H, *J*=2.8, 52.8 Hz), 5.75-5.87 (m, 1H), 7.25-7.41 (m, 10H); ^{13}C, δ 63.1, 63.5, 67.6, 73.2, 73.3, 73.5, 73.8, 75.6, 77.0, 79.6, 104.4, 107.7, 117.0, 127.8, 128.0, 128.2, 128.4, 128.5, 134.3, 137.6, 137.7; HRMS Calcd for $C_{23}H_{26}O_4N_3FNa$: (M+Na) 450.1805. Found: (M+Na) 450.1792.

6-*O*-(4-*O*-Allyl-2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-1,4,5-tri-*O*-trimethylacetyl-2,3-*O*-(D-1,7,7-trimethyl[2.2.1]bicyclohept-6-ylidene)-D-*myo*-inositol (8). Zirconocene dichloride (2.29 g, 7.84 mmol) and silver trifluoromethane sulfonate (1.73 g, 7.84 mmol) were dissolved in diethyl ether (45 mL) containing 4 Å molecular sieves (3 g). After stirring for 30 min at 0 °C, a solution of **6** (670 mg, 1.57 mmol) and D-1,4,5-tri-*O*-trimethylacetyl-2,3-*O*-(D-1,7,7-trimethyl [2.2.1] bicyclohept-2-ylidene)-*myo*-inositol (**7**, 2.66 g, 4.70 mmol) in diethyl ether (15 mL) was added. After 17 h the reaction mixture was filtered through a pad of Celite, and washed with aq. sat. $NaHCO_3$. Normal workup by purification with FC (using silanized silica-gel 60, acetone/ H_2O, 7:2) gave **8** in 48% yield (735 mg, 0.75 mmol) as a foam. R_f 0.69 (HPTLC, toluene/ EtOAc 9:1); NMR ($CDCl_3$) ^1H, δ 0.86 (s, 3H), 0.94 (s, 3H), 0.97 (s, 3H), 1.05-2.00 (m, 34H), 3.27(dd, 1H, *J*=3.7, 10.3 Hz), 3.55-3.74 (m, 3H), 3.82-3.97 (m, 2H), 3.99-4.19 (m, 2H), 4.21-4.28 (m, 2H), 4.45-4.63 (m, 3H), 4.83 (dd, 2H, *J*=10.6, 19.1 Hz), 5.01 (t, 1H, *J*=4.8 Hz), 5.06-5.20 (m, 2H), 5.21-5.27 (m, 2H), 5.34 (dd, 1H, *J*=4.4, 8.1 Hz), 5.75-5.86 (m, 1H), 7.10-7.40 (m, 10H); ^{13}C, δ 9.4, 20.1, 20.2, 26.9, 27.1 (two peaks), 27.2, 29.6, 38.6, 38.8, 39.0, 43.0, 45.1, 47.8, 51.5, 63.0, 67.8, 69.5, 71.2, 71.4, 71.5, 71.7, 73.5, 73.7, 74.8, 75.3, 75.5, 77.8, 79.4, 98.0, 116.5, 118.0, 127.6, 127.7, 127.8, 127.9, 128.1, 128.3, 128.4, 134.6, 137.8, 138.0, 176.7, 177.2, 177.5; HRMS Calcd for $C_{54}H_{75}O_{13}N_3$: (M^+) 973.5300. Found: (M^+) 973.5306.

6-*O*-(4-*O*-Allyl-2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-4,5-*O*-isopropylidene-2,3-*O*-(D-1,7,7-trimethyl[2.2.1]bicyclohept-6-ylidene)-D-*myo*-inositol (9). Compound **8** (1.45 g, 1.49 mmol) and NaOH (2.4 g) were refluxed in MeOH (16 mL) for 15 min. The mixture was neutralized, evaporated and the residue purified by FC (toluene/ EtOAc 3:1) affording 6-*O*-(4-*O*-allyl-2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-2,3-*O*-(D-1,7,7-trimethyl[2.2.1]bicyclohept-6-ylidene)-D-*myo*-inositol in 80% yield as a foam (862 mg, 1.19 mmol). R_f 0.44 (toluene/ EtOAc 2:1). Pyridinium toluene-4-sulfonate (81 mg, 0.32 mmol) was added to a solution of the above compound (1.17 g, 1.62 mmol) and 2,2-dimethoxypropane (3.36 g, 32 mmol) in DMF (12 mL). After 6 h, $NaHCO_3$ (204 mg, 2.43 mmol) was added and the reaction mixture was diluted with toluene and aq. sat. $NaHCO_3$. FC (toluene/ EtOAc 18:1) yielded **9** in 63% as a foam (772 mg, 1.01 mmol). R_f 0.63 (toluene/ EtOAc 6:1). NMR ($CDCl_3$) ^1H δ 0.87 (s, 3H), 0.89 (s, 3H), 1.02 (s, 3H), 1.19 (m, 1H), 1.38-1.48 (m, 8H), 1.71-1.78 (m, 2H), 1.99-2.14 (m, 2H), 3.34 (dd, 1H, *J*=3.7, 10.3 Hz), 3.52-3.67 (m, 3H), 3.76 (dd, 1H, *J*=2.9, 11.0 Hz), 3.87-3.96 (m, 2H), 4.01-4.17 (m, 4H), 4.20-4.28 (m, 1H), 4.40 (m, 1H), 4.58 (dd, 2H, *J*=12.1, 46.2 Hz), 4.85 (s, 2H), 5.09-5.25 (m, 3H), 5.78-5.89 (m, 1H). ^{13}C, δ 9.8, 20.1, 20.5, 26.9, 27.1, 27.2, 29.8, 43.0, 45.1, 48.0, 51.5, 63.2, 68.0, 70.9, 72.7, 73.4, 73.9, 74.9, 75.0, 76.4, 77.7, 78.1, 78.3, 80.0, 96.8, 112.2, 117.1, 118.5, 127.7, 127.8, 128.0, 128.3, 128.4, 134.6, 138.0; HRMS Calcd for $C_{42}H_{55}O_{10}N_3Na$: (M+Na) 784.3785. Found: (M+Na) 784.3801.

6-*O*-(4-*O*-Allyl-2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-4,5-*O*-isopropylidene-2,3-*O*-(D-1,7,7-trimethyl[2.2.1]bicyclohept-6-ylidene)-D-*myo*-inositol 1-Di-*O*-benzyl Phosphate (10). 1*H*-Tetrazole (277 mg, 3.95 mmol) was added to a solution of **9** (860 mg, 1.13 mmol) and dibenzyl diisopropylphosphoramidite (581 mg, 1.69 mmol)[12] in CH_2Cl_2 (40 mL). After 20 min the reaction mixture was cooled to 0 °C and 80% *m*-chloroperbenzoic acid (485 mg, 2.26 mmol) was added. The mixture was stirred for 10 min and then diluted with CH_2Cl_2, washed with aqueous 10% $Na_2S_2O_3$ and aqueous

sat. $NaHCO_3$ and subjected to normal workup. The crude product was purified by silica gel chromatography (toluene/ EtOAc 9:1) to give 85% of **10** (986 mg, 0.96 mmol). R_f 0.23 (toluene/ EtOAc 9:1); NMR ($CDCl_3$): ^1H, δ 0.83 (s, 3H), 0.90 (s, 3H), 0.98 (s, 3H), 1.16-1.96 (m, 13H), 3.22 (dd, 1H, *J*=3.7, 10.3 Hz), 3.44 (t, 1H, *J*=10.3 Hz), 3.56-3.64 (m, 2H), 3.75 (dd, 1H, *J*=11 Hz), 3.88 (t, 2H, *J*=8.8 Hz), 3.97-4.09 (m, 3H), 4.24 (m, 2H), 4.46 (m, 2H), 4.62 (m, 2H), 4.83 (dd, 2H, *J*=11.8, 2.9 Hz), 5.00-5.13 (m, 6H), 5.22 (m, 1H), 5.83 (m, 1H), 7.30 (m, 10H); ^{13}C, δ 10.0, 20.3, 20.5, 26.9, 27.1, 29.8, 43.9, 45.1, 47.9, 51.5, 63.1, 68.0, 69.5 (dd, *J*=5.5 Hz), 70.8, 73.4, 73.7, 73.8, 74.1 (d), 75.3, 76.6, 77.5, 77.8 (d, *J*=6.4 Hz), 78.1, 80.0, 112.2, 117.0, 119.0, 127.6, 127.7, 127.8, 128.0, 128.3, 128.4, 128.5, 129.0, 134.6, 135.6 (two peaks), 138.0, 138.1; ^{31}P, (decoupled) (CH_2Cl_2) δ -1.56; HRMS Calcd for $C_{56}H_{68}O_{13}N_3PCs$: ($M+Cs^+$) 1154.3544. Found: ($M+Cs^+$) 1154.3528.

6-*O*-(2-Azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-4,5-*O*-isopropylidene-2,3-*O*-(D-1,7,7-trimethyl[2.2.1]bicyclohept-6-ylidene)-D-*myo*-inositol 1-Di-*O*-benzyl Phosphate (11). A mixture of **10** (110 mg, 0.11 mmol) and [bis(methyldiphenylphosphine)](1,5-cyclooctadiene)iridium(I)PF_6 (9 mg, 0.01 mmol) in freshly distilled THF was degassed and put under an N_2 atmosphere. The catalyst was activated by degassing the mixture and then treating it with H_2 for 5 min. Stirring was continued under N_2 for 15 min after which NIS (121 mg, 0.54 mmol) and water (0.5 g, 28 mmol) were added. After 1 h the solution was diluted with EtOAc and washed with aqueous 10% $Na_2S_2O_3$, aqueous sat. $NaHCO_3$ and subjected to normal workup. FC (toluene/ EtOAc 4:1) provided **11** in 95% as a syrup (100 mg, 0.10 mmol). R_f 0.24 (toluene/ EtOAc 4:1); NMR ($CDCl_3$) ^{13}C, δ 10.0, 20.2, 20.5, 27.0, 27.1, 29.8, 43.9, 45.2, 48.0, 51.5, 62.5, 69.2, 69.3, 69.5, 69.6, 70.0, 72.9, 73.6, 73.7, 74.1, 75.1, 76.8, 76.9, 77.8, 77.9, 79.7, 97.3, 112.3, 119.1, 127.7, 127.8, 127.9, 128.0, 128.1, 128.4, 128.5, 135.7 (two peaks), 137.8, 138.2; HRMS Calcd for $C_{53}H_{64}O_{13}N_3PCs$: ($M+Cs^+$) 1114.3243. Found: ($M+Cs^+$) 1114.3239.

Ammonium 6-*O*-(2-Amino-2-deoxy-α-D-glucopyranosyl)-D-*myo*-inositol 1-Phosphate (1b). Compound **11** (167 mg, 0.17 mmol) was treated with $CHCl_3$-CF_3COOH (10:1, 11 mL) for 2 h. The solvent was removed and the residue purified by FC ($CHCl_3$/ MeOH 18:1) to yield 6-*O*-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-D-*myo*-inositol 1-di-*O*-benzyl phosphate in 85% (117 mg, 0.15 mmol). R_f 0.33 ($CHCl_3$/ MeOH 9:1). NMR ($CDCl_3$) ^{13}C, δ 29.7, 63.0, 69.7, 70.2, 70.8, 71.1, 71.9, 72.7, 73.3, 73.7, 74.9, 77.9, 79.6, 98.2, 127.9, 127.9, 128.0, 128.1, 128.5, 128.6, 128.7, 128.8, 135.2, 137.5, 138.1. 10% Pd/C (15 mg) was added to a solution of of the above compound (47 mg, 0.06 mmol) in a mixture of EtOH-H_2O-AcOH (80:19:1, 2 mL). The mixture was treated with H_2 at 100 psi for 48 h. Filtration through Celite and removal of the solvent afforded the crude product, which was gelfiltered in a pyridinium acetate buffer at pH 5. The counter-ion on the phosphate was exchanged with an 0.3 M ammonium acetate buffer, pH 7, to give **1b** in 94% (24 mg, 0.05 mmol). NMR (D_2O) ^{13}C δ 22.9, 53.4, 59.9, 69.1, 70.0, 70.3, 71.4, 71.6, 72.1, 72.5, 74.8, 77.5, 95.1, 180.2. ^1H and ^{31}P NMR spectra were identical to those previously reported.[6c]

6-*O*-(2-Amino-2-deoxy-α-D-glucopyranosyl)-D-*myo*-inositol 1,2-(Cyclic) phosphate (2b) was made according to Plourde *et al.*[6a]. ^1H NMR ^{31}P NMR spectra were identical to those previously reported[6c].

Acknowledgements. This work, which was a collaboration with Dr. Peter Strålfors at the Department of Cell Biology, Faculty of Health Sciences, University of Linköping, Sweden, was supported by the Swedish Natural Science Research Council.

REFERENCES

1. Ferguson, M. A. J.; Low, M. G.; Cross, G. A. M. *J. Biol. Chem.* **1985**, *260*, 14547.
2. McConville, M. J.; Ferguson, M. A. J. *Biochem. J.* **199**, *3294*, 305.
3. Ferguson, M. A. J.; Homans, S. W.; Dwek, R. A.; Rademacher, T. W. *Science* **1988**, *239*, 753.
4. a) Mootoo D. R.; Konradsson P.; Fraser- Reid B. *J. Am. Chem. Soc.* **1989**, *111*, 8540, b) Verduyn G. R.; Belien J. J. A.; Dreef-Tromp C. M.; van Marel G. A.; van Boom J. H. *Tetrahedron Lett.* **1991**, *32*, 6637, c) Murakata C.; Ogawa T. *Carbohydr. Res.* **1992**, *235*, 95, d) Boons G.-J.; Price P.; Leslie R.; Ley S. V.; Yeung L. L. *Tetrahedron Lett.* **1993**, *34*, 8523, e) Cottaz S.; Brimacombe J. S.; Ferguson M. A. J. *J. Chem. Soc., Perkin Trans .1* **1993**, 2949, f) Madsen R.; Udodong U. E.; Roberts C.; Mootoo D. R.; Konradsson P.; Fraser-Reid B. *J. Am. Chem Soc.* **1995**, *117*, 1554, g) Campbell S.; Fraser-Reid B. *J. Am. Chem. Soc.*, **1995**, *117*, 10387,. f) Ye J.; Doerrler W. T.; Lehrman M. A.; Falck J. R. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1715.
5. a) Alemany S.; Mato J. M.; Strålfors P. *Nature* **1987**, *330*, 77, b) Strålfors P. *Nature* **1988**, *335*, 554, c) Varela-Nieto I.; Alvarez L.; Mato J. M. *Handbook of Endocrine Research Techniques*, ed Academic Press; San Diego, **1993**, pp 391.
6. a) Plourde R.; d.Aarcao M.; Saltiel A. R. *J. Org. Chem.* **1992**, *57*, 2606, b) Zapata A.; Leon Y.; Mato J. M.; Varela-Nieto I.; Penades S.; Martin-Lomas M. *Carbohydr. Res.* **1994**, *264*, 21, c) Plourde R.; d.Aarcao M. *Tetrahedron Lett.***1990**, *31*, 2693.
7. Gaulton G. N.; Pratt J. C. *Seminars in Immunology* **1994**, *6*, 97.
8. Bruzik K. S.; Tsai M.-D. *J. Am. Chem. Soc.* **1992**, *114*, 6361.
9. Buskas T.; Garegg P. J.; Konradsson P.; Maloisel J.-L. *Tetrahedron Asymm.* **1994**, *5*, 2187.
10. Blomberg L.; Norberg T. *J. Carbohydr. Chem.* **1992**, *11*, 751.
11. Matsumoto T.; Maeta H.; Suzuki K.; Tsuchihashi G. *Tetrahedron Lett.* **1988**, *29*, 3567.
12. Yu K.-L.; Fraser-Reid B. *Tetrahedron Lett.* **1988**, *29*, 979.
13. a) Hall D. N. *J. Org. Chem.* **1967**, *32*, 2082, b) GriesbaumV. K. *Angewandte Chem.* **1970**, *82*, 276.
14. Nukada T.; Lucas H.; Konradsson P.; van Boeckel C. A. A. *Synlett* **1991**, 365.

(*Received in UK* 2 *May* 1997; *revised* 17 *October* 1997; *accepted* 23 *October* 1997)