temperature. The bulk of the solvent was removed on a rotary evaporator and the residue dissolved in 5 mL of CH_2Cl_2, dried, and filtered. The volume was reduced to 1.0 mL and the allylic alcohol isolated by preparative TLC (40% ether–*n*-pentane). The middle TLC band contained 1-phenylallyl alcohol (**15**).

Method B. The procedure described above for method A was followed except that 1 mL of dry pyridine was added prior to oxidation. After the oxidation was complete, stirring was continued for 6 h at which time 1.0 mL of water was added and **15** isolated as described above.

Preparation of Enantiomerically Pure (*S*)-(+)-1-Phenylallyl Alcohol (15) via Kinetic Resolution. The Sharpless epoxidation[33] of 2.68 g (20 mmol) of (±)-1-phenylallyl alcohol (**15**) using (+)-DIPT gave 0.82 g (31%) of (*S*)-(+)-**15** and was isolated by column chromatography eluting with 30% ether–*n*-pentane: bp_6 90–95 °C; $[\alpha]^{20}_D$ +12.34 (neat 0.1-mL cell), $[\alpha]^{20}_D$ −8.4 (*c* 5.0, benzene) (lit.[38] $[\alpha]^{20}_D$ +10.0 (neat, in a 2-mL dm cell).

Hydrogenation of (*S*)-(+)-1-Phenylallylic Alcohol (15) to (−)-(*S*)-Phenylethylcarbinol (18). Hydrogenation of (*S*)-**15** was accomplished as previously reported by Duveen and Kenyon[2,38] to afford (*S*)-(−)-phenylethylcarbinol (**18**): bp_6 110 °C; $[\alpha]^{20}_D$ −21.16 (neat, 0.1-mL cell) [lit.[2] $[\alpha]^{20}_D$ −25.91 (1, 1.0 neat)].

Preparation of the Mosher Ester of (*S*)-(+)-1-Phenylallyl Alcohol (15). In a typical procedure a solution of 33 mg (0.25 mmol) of **15** dissolved in 4 mL of dry CCl_4 was added to 0.2 mL of pyridine, a catalytic amount of 4-(*N*,*N*-dimethylamino)pyridine (DMAP), and 1.3 equiv of [(*S*)-(+)-MTACl]. The contents were stirred for 4–5 h or until **15** was shown to be absent by TLC. At this time the solution was diluted with 10 mL of CH_2Cl_2, washed with 5% HCl, water, brine, and dried. Removal of the solvent gave a solid which was purified by preparative TLC developing with 80% *n*-pentane–ether to give 85 mg (95%) of the Mosher ester of (*S*)-**15**: IR (neat) 3150, 3040, 1749, 1455, 1248, 1170, 1080 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.51 (s, 3 H, OMe), 5.25–5.45 (m, 2 H), 5.9–6.15 (m, 1 H), 6.43–6.56 (m, 1 H), 7.28–7.47 (m, 10 H); MS *m/e* 350 (M^+).

Preparation of the Mosher Ester of (±)-1-Phenylallyl Alcohol (15). The procedure described above was followed to give 87 mg (96%) of alcohol **15**: ^1H NMR ($CDCl_3$) δ 3.41 (s, 3 H, OMe), 3.51 (s, 3 H, OMe), 5.25–5.45 (m, 2 H), 5.9–6.15 (m, 1 H), 6.43–6.56 (m, 1 H), 7.28–7.47 (m, 10 H).

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Registry No. (+)-**3a**, 104322-63-6; (−)-**3a**, 104372-31-8; (−)-**4**, 122270-28-4; **5a**, 4346-64-9; **5b**, 88141-36-0; **5c**, 139706-16-4; **5d**, 139706-17-5; (*S*)-**6a**, 88198-16-7; (*R*)-**6b**, 88198-17-8; (*S*)-**6b**, 88198-18-9; (*S*)-**6c**, 139706-18-6; (*S*)-**6d**, 139706-19-7; (*S*)-**9a**, 5056-07-5; (*S*)-**9b**, 88198-18-9; **11a**, 69562-10-3; **11b**, 105882-08-4; **12a**, 139706-20-0; **12b**, 139706-21-1; (*S*)-(*E*)-**13a**, 139706-23-3; (*S*)-(*Z*)-**13b**, 139706-25-5; (*S*)-(*E*)-**14a**, 139706-24-4; (*S*)-(*Z*)-**14b**, 139706-26-6; (*S*)-**15**, 104713-12-4; (*R*)-**15**, 39623-35-3; (*S*)-**15** (Mosher ester), 139706-22-2; (±)-**15** (Mosher exter), 139706-27-7; (*S*)-(−)-**18**, 613-87-6; (*S*)-(+)-MTACl, 20445-33-4; PhSeSePh, 1666-13-3; (*E*)-cinnamyl chloride, 21087-29-6; (*Z*)-cinnamyl chloride, 39199-93-4; (*Z*)-cinnamyl alcohol, 4510-34-3; phenyl acetylene, 536-74-3; 3-phenyl-2-propyn-1-ol, 1504-58-1; bis-(2,4,6-triisopropylphenyl) diselenide, 71518-96-2.

Supplementary Material Available: ^{13}C NMR spectra of **5d**, **6c**, and **6d** (3 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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Synthesis and Characterization of an Insulin-Mimetic Disaccharide

Robert Plourde,[†] Marc d'Alarcao,*[,†] and Alan R. Saltiel[‡]

Department of Chemistry, Tufts University, Medford, Massachusetts 02155, and the Signal Transduction Research Division, Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, Ann Arbor, Michigan 48106

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A glucosaminyl-inositol-1,2-cyclic phosphate, **1**, has been synthesized and evaluated for selected insulin-mimetic properties. The compound was designed to resemble structurally the recently reported inositol glycans which are believed to be insulin second messengers. The synthesis utilized a Koenigs–Knorr glycosylation of optically pure 1-camphanyl-2,3:4,5-di-*O*-cyclohexylidene-D-*myo*-inositol with 2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranosyl bromide followed by phosphitylation by the phosphoramidite method, oxidation, and carbodiimide cyclization. Compound **1** was found to stimulate lipogenesis in rat adipocytes in a dose-dependent manner in the micromolar range up to a level 30–40% of that maximally induced by insulin.

Introduction

Diabetes mellitus affects an estimated 15 million people in the United States alone.[1] Of these, 80% are afflicted with non-insulin-dependent diabetes mellitus (NIDDM), a multifactorial disease often characterized by a relative resistance to insulin. These patients are frequently both hyperglycemic and hyperinsulinemic,[2] reflecting both a decreased responsiveness and sensitivity to insulin at the cellular level. Since reductions in insulin binding to target tissue receptors are usually not sufficient to account for the diminished response, it is likely that the biochemical flaw in patients with NIDDM reflects one or more post-receptor defects probably involving transduction of the insulin signal from the target cell surface receptors to the intracellular metabolic machinery. Therefore, an understanding of insulin signal transduction may lead to new strategies for the treatment of NIDDM.

The mechanism by which insulin elicits its anabolic effect on target tissues is still poorly understood. It is generally accepted that the activation of the insulin receptor involves the insulin-dependent stimulation of its protein tyrosine kinase activity resulting in auto-

[†] Tufts University.
[‡] Warner Lambert Company.

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phosphorylation.[3] Moreover, it is also clear that the activities of several key metabolic enzymes (i.e., glycogen synthetase and pyruvate dehydrogenase) are modulated by dephosphorylation in response to insulin.[4] Although the precise chemical link(s) between the activated cell surface receptor and changes in intracellular protein phosphorylation remain unknown, it is likely that one or more small second messenger molecules are involved.[4] Accordingly, such "mediators" of insulin signal transduction are of great interest with regard to the development of new treatment modalities for NIDDM.

Although evidence for low molecular weight "mediators" of insulin action appeared as early as the 1970's[4] the first data regarding chemical structure emerged in 1986 when Saltiel and Cuatrecasas discovered that insulin stimulates the release of two inositol-containing substances from bovine liver particulate fractions.[5] These substances, which are generated by the phospholipase C-catalyzed hydrolysis of a glycosylphosphatidylinositol in the plasma membrane, were found to modulate the low Km cAMP phosphodiesterase from adipocytes. Labeling studies established that these compounds incorporated *myo*-inositol, glucosamine, and one or more phosphate groups and could be released not only by insulin but also by the phosphatidylinositol-specific phospholipase C from *Staphylococcus aureus*.[6]

Since this initial discovery, work from several laboratories has supported the idea that these substances are the elusive second messengers of insulin signal transduction. These molecules have been found to mimic insulin in its ability to modulate several insulin-controlled functions in whole cells including glucose oxidation,[7,8] lipogenesis,[7] lipolysis,[9] and multiple protein phosphorylations.[10] Assays of subcellular fractions have established that these insulin-mimetic properties are due to the ability of these substances to regulate several metabolic enzymes including high affinity cAMP phosphodiesterase,[5,11] pyruvate dehydrogenase,[11] adenylate cyclase,[11] phospholipid methyltransferase,[12] glycerol-3-phosphate acyltransferase,[13] pyruvate dehydrogenase phosphatase,[14] and casein kinase II.[15]

Studies aimed at the elucidation of the structures of these novel insulin-mimetic substances have met with partial success. It is now clear that these substances contain a nonacetylated glucosamine moiety glycosidically linked to a phosphorylated inositol and coupled to an oligosaccharide "tail" (total apparent molecular weight ~1400).[5,6,16] It is equally clear that there is significant structural diversity among these insulin-mimetic phosphooligosaccharides including the presence of *chiro*-inositol in place of *myo*-inositol,[14,16] the presence of mannose vs galactose in the "tail" portion,[16,17] and variable levels of phosphorylation.[18]

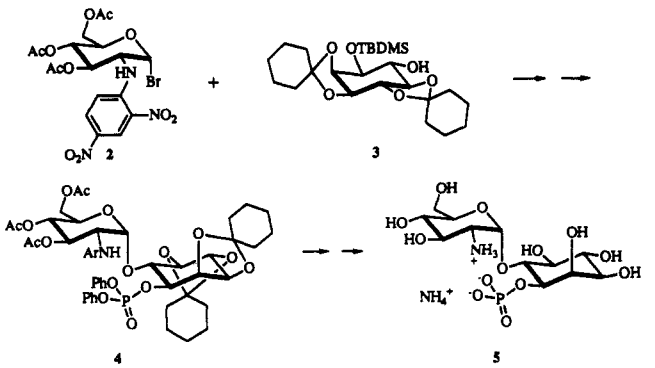

Figure 1. Outline of an earlier[25] synthesis of phosphodisaccharide **5**.

Interestingly, the glycosylphosphoinositides (GPI's) that serve as precursors for the insulin-dependent generation of these proposed second messengers bear structural similarities to the GPI protein anchor by which a diverse group of cell surface proteins is attached to the membrane. Although the hormone-sensitive GPI are not protein-attached and lack the ethanolamine residue found in the protein anchor form, both species of the lipid similarly contain PI glycosidically linked to non-N-acetylated hexosamine, itself attached to a mannose-containing oligosaccharide core.[19-22] Recent studies[23,24] have indicated that inositol phosphate glycan fragments produced from GPI anchors can reproduce the actions of insulin and the insulin-sensitive inositol glycan on fat, muscle, and liver cells. Indeed, a fragment generated by proteolytic digestion of the GPI anchor for the variant surface glycoprotein of *Trypanosoma brucei*[20] specifically mimics the antigluconeogenic action of insulin in rat hepatocytes.[24] Because the exact structure of this GPI anchor is known,[20] we have utilized this core structure to guide the design of insulin mimetic compounds.

In this paper, we report on an efficient synthesis of disaccharide **1** consisting of the terminal substructure of the conserved portion of the membrane anchors and demonstrate its insulin-mimetic activity in the stimulation of lipogenesis. While the syntheses of other compounds related to the inositol glycan have been reported by us[25] and others,[26] this is the first demonstration of insulin-

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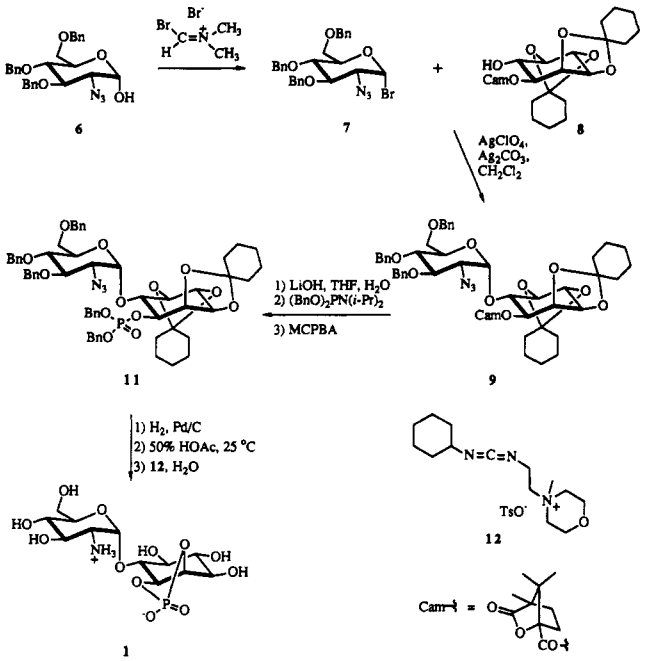

Figure 2. Synthesis of cyclic phosphate 1.

mimetic properties from a synthetic inositol glycan analogue.

Results and Discussion

Synthesis. The principal problem in designing an efficient synthesis of disaccharide 1 was to select glucosamine and inositol precursors which are suitably protected, easily deprotected, and easily preparable in large quantity in optically pure form. In our previous work[25] we utilized dinitrophenyl-protected glucosamine 2[27] (Figure 1) which appeared to meet these criteria and protected inositol 3, available in six steps[25,28] from *myo*-inositol, which marginally qualified, in a synthesis of acyclic phosphate 5. We found, however, that removal of the various protecting groups from disaccharide 4 was accompanied by marginally acceptable yields and difficult purifications. Therefore, our previous synthesis, while succeeding in providing sufficient compound to establish its biological importance, was not adequate for large-scale preparations of 1.

Accordingly, we sought an improved synthesis of 1 based on the coupling of easily preparable precursors protected by groups designed to facilitate purification in the later stages of the synthesis.

As an inositol precursor with the required characteristics we selected camphanic ester 8 (Figure 2). This material is easily preparable[29] in optically pure form in two steps from *myo*-inositol and bears the requisite differential protection at position 1. We have utilized this material in a short synthesis of inositol-4-phosphate[29] and anticipate that 8 will find much use in the synthesis of phosphatidylinositol–glycan derivatives.

As the glucosamine precursor we utilized 2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-glucose (6) which is readily preparable by the $(NH_4)_2Ce(NO_3)_6$-promoted oxidation of 3,4,6-tri-*O*-benzyl-D-glucal in the presence of sodium azide as reported by Kinzy and Schmidt.[30] Conversion of 6 to the corresponding bromide 7 was effected with the Vilsmeier reagent.[31] This method is preferred to the direct conversion of the first formed azidonitrates to the bromide in analogy to the procedure of Lemieux[32] since separation of the desired gluco-configured material from a small amount of manno-configured byproduct is most easily accomplished at the stage of hydroxyl-containing compound 6.

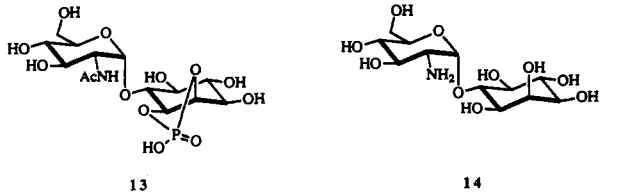

Figure 3. Analogues of 1 in which one of the charged functional groups is missing or blocked.

Koenigs–Knorr glycosylation of inositol 8 with glycosyl bromide 7 provided protected disaccharide 9 together with the β-anomer in 28% yield (α:β = 2.1:1) together with 20% recovered inositol 8 which could be recycled. Hydrolysis of the camphanic ester (LiOH, THF–H_2O) moiety of 9 freed the inositol 1-position producing compound 10 (structure not shown) which was treated with *N*,*N*-diisopropyl dibenzyl phosphoramidite followed by *m*-chloroperbenzoic acid (MCPBA)[33] to produce fully protected phosphate 11 in 75% yield (from 9). Removal of all of the protecting groups was accomplished quantitatively by catalytic hydrogenolysis (Pd/C) followed by treatment with 50% acetic acid at 25 °C. Finally, dehydrative ring closure of the phosphate group to produce the 1,2-cyclic phosphate was effected in aqueous solution with the water-soluble carbodiimide 12.[34] The structure of cyclic phosphate 1 was confirmed by HR-FAB mass spectroscopy as well as by the characteristic downfield shift of hydrogens at C1 and C2 of the inositol ring in the ^1H NMR (δ = 4.34, 4.49 ppm in 1 compared with δ = 3.95, 4.00 ppm in 5, respectively) and the downfield shift of the phosphorus signal in the ^{31}P NMR (δ = 19.9 ppm in 1 compared with δ = 7.0 in 5).

To evaluate the importance of the charged functional groups in 1 to its biological activity, it was desirable to prepare derivatives in which these groups were blocked or absent. Accordingly, the *N*-acetyl derivative 13 (Figure 3) was prepared by treatment of acylic phosphate 5 (Figure 1) with acetic anhydride followed by cyclization as before with 12. The unphosphorylated analogue 14 was generated enzymatically from 5 by treatment with alkaline phosphatase.

Biological Evaluation

The ability of the natural inositol glycan signaling molecules to mimic insulin has been demonstrated in cell-free enzyme assays as well as intact cell systems. Accordingly, we examined the insulin-mimetic activity of our synthetic disaccharides in one system of each type. Compounds 5 (Gln-Ins-1P), 1 (Gln-Ins-1,2-cycP), 13 (*N*-Ac-Gln-Ins-1,2-cycP), and 14 (Gln-Ins) have been evaluated for their ability to modulate cAMP phosphodiesterase

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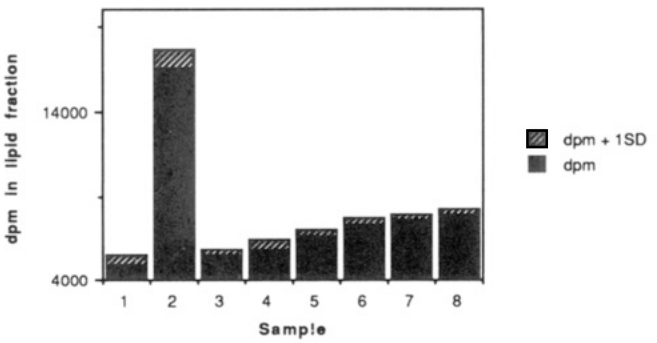
Chart I[a]



[a] Stimulated incorporation of [^{14}C] into rat adipocyte lipids from [U-^{14}C]-glucose. Sample 1 = no additive, 2 = insulin, 3 = 2 μM 1, 4 = 4 μM 1, 5 = 8 μM 1, 6 = 10 μM 1, 7 = 20 μM 1, 8 = 40 μM 1.

(PDE) activity[35] in adipocyte plasma membranes (data not shown). None of the compounds had any effect on the activity of this enzyme in the concentration range tested (1–30 μM). Moreover, these compounds were also without effect in the inhibition of gluconeogenic enzymes in cell-free fractions (not shown). We conclude that none of the disaccharides contain a sufficient substructure of the inositol glycan to elicit these effects at these concentrations.

The same compounds have also been examined for the ability to stimulate lipogenesis in intact rat epididymal adipocytes.[7] In this assay we measured the conversion of [^{14}C]-glucose to lipids using 5 mM [U-^{14}C]-glucose, a concentration at which regulation of metabolic enzymes rather than glucose transport is the rate-limiting step.[7] Under these conditions, basal levels of lipogenesis are somewhat elevated in rat fat cells, reflecting the non-insulin-dependent transport of glucose across the plasma membrane. Compounds **5**, **13**, and **14** are completely inactive (data not shown) while the 1,2-cyclic phosphate **1** exhibits a dose-dependent stimulation of lipogenesis (Chart I). The largest effect (at 40 μM **1**) was about 30–40% of that observed with maximal concentrations of insulin. Interestingly, at higher concentrations the compound was less effective in stimulating lipogenesis (data not shown). This paradoxical dose response is similar to that observed for the anchor-derived inositol glycan although the significance of this finding remains unclear.

The data have shown that the 1,2-cyclic phosphate **1** but not the noncyclic phosphate **5** is able to mimic partially the activity of insulin as well as the natural inositol glycan to stimulate lipogenesis in intact adipocytes. This suggests that the second messenger may itself be a cyclic phosphate, a notion consistent with studies of the PI-PLC reaction which have demonstrated that PI is hydrolyzed first to inositol 1,2-cyclic phosphate and subsequently to inositol-1-phosphate.[36]

The inability of **1** to affect the activity of cAMP phosphodiesterase (PDE) is presumably due to the absence of a sufficient substructure of the inositol glycan in this molecule, although the absence of this activity may reflect the relative insensitivity of this assay. This together with the lipogenic activity of **1** in intact cells suggests the possibility that the various in vivo targets of the inositol glycan have different structural requirements for modulation of activity. We are currently engaged in the synthesis and evaluation of other structures related to these compounds in an attempt to define these requirements.

Experimental Section

All nonaqueous reactions were carried out under an argon or nitrogen atmosphere. Organic extracts were dried with anhydrous $MgSO_4$. Solvents were removed in vacuo on a Buchi rotary evaporator. Solvents and reagents obtained from commercial sources were used without further purification with the following exceptions.[37] Dichloromethane was distilled from P_2O_5. *N*,*N*-Dimethylformamide (DMF) was distilled at reduced pressure from calcium hydride. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl. Toluene was distilled from P_2O_5. 2,4,6-Trimethylpyridine was distilled from sodium. Molecular sieves were dried at 140 °C for at least 12 h and allowed to cool under vacuum. Silver carbonate and silver perchlorate were dried at reduced pressure (0.1–1.0 mmHg) for 48 h at 100 °C. Carbohydrate intermediates were rendered anhydrous by either (a) maintaining them at reduced pressure (0.1–1.0 mmHg) for at least 12 h at 25 °C or (b) repeated coevaporation with toluene. Reactions were routinely monitored by TLC on Baker glass-backed silica gel plates (0.25-mm thickness) with a 254-nm fluorescent indicator. The chromatograms were visualized by one or more of the following techniques: (a) ultraviolet illumination, (b) dipping in an ethanolic solution of 2.5% *p*-anisaldehyde, 3.5% sulfuric acid, and 1% acetic acid followed by heating, or (c) dipping in 0.1% fluorescamine in acetone. Preparative separations were performed either by flash chromatography on Baker silica gel (40 mm), by gravity chromatography on Baker silica gel (60–200 mm), or by ion-exchange chromatography on Bio-Rad Biogel A (DEAE sephadex). Melting points are uncorrected. Total phosphate was measured colorimetrically by the phosphomolybdate method.[38]

2-Azido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranosyl Bromide (7). To a solution of **6** (225 mg, 0.47 mmol) and collidine (63 μL, 0.58 mmol) in dichloromethane (11 mL) was added (bromomethylene)dimethylammonium bromide[31] (~500 mg, 2.3 mmol). The solution was stirred for 2 h at 0 °C after which another 200–300 mg of brominating reagent was added and the reaction allowed to continue for an additional 2 h at 25 °C. Dichloromethane (30 mL) was added, and the mixture was washed with ice-cold water (2 × 10 mL), 10% sodium bicarbonate (10 mL), and cold water (2 × 10 mL). The organic phase was dried and the solvent removed, affording **7** (231 mg, 91%). The crude product was >95% pure as judged by ^{1}H-NMR. Crude **7** was used immediately or stored as a solution in frozen benzene under argon: ^{1}H-NMR ($CDCl_3$) δ 3.62 (dd, J = 3.8 Hz, 9.8 Hz, 1 H, H2), 3.64 (m, 1 H), 3.83 (m, 1 H), 3.87 (ψt, J = 10 Hz, 1 H), 4.03 (ψt, J = 10 Hz, 1 H), 4.08 (m, 1 H), 4.43–4.92 (m, 6 H), 6.44 (d, J = 3.7 Hz, 1 H, H1), 7.12–7.45 (m, 15 H, aromatic).

6-*O*-(2-Azido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-1-*O*-camphanyl-2,3:4,5-di-*O*-cyclohexylidene-D-*myo*-inositol (9). To a mixture of **8**[29] (598 mg, 1.15 mmol), 2,4,6-trimethylpyridine (300 μL, 2.25 mmol), and freshly activated 4-Å molecular sieves (1 g) in dichloromethane (14 mL) were added silver carbonate (700 mg, 2.53 mmol), silver perchlorate (500 mg, 2.39 mmol), and **7** (880 mg, 1.66 mmol, coevaporated with toluene, then dissolved in 9 mL dichloromethane). The mixture was stirred 20 min at 25 °C after which the reaction was quenched with triethylamine (1 mL), diluted with dichloromethane (100 mL), filtered through Celite, concentrated, and chromatographed on 250 g of silica gel with hexane–ether (1:1). This provided **9** (216 mg, 19%), as well as the corresponding β-anomer (102 mg, 9%). Further elution with hexane–ether (1:2) afforded unreacted **8** (118 mg, 20%) which may be recycled. TLC (hexane–ether (1:1)) β-disaccharide, R_f = 0.60; α-disaccharide **9**, R_f = 0.54. For pure **9**: ^{1}H-NMR ($CDCl_3$) δ 1.00, 1.10, 1.11 (3s, 9 H, CH_3), 1.37–1.80 (m, 21 H, 20 cyclohexylidene and 1 camphanyl methylene), 1.93, 2.12, 2.43, (all m, 1 H each, camphanyl methylenes), 3.35 (dd, J = 3.6 Hz, 10.2 Hz, 1 H, H2), 3.44 (ψt, J = 9.6 Hz, 1 H), 3.66 (m, 1 H), 3.82 (m, 2 H), 4.00 (ψt, J = 10.2 Hz, 1 H), 4.14 (m, 2 H),

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4.30 (dd, J = 2.5 Hz, 8.4 Hz, 1 H), 4.56 (m, 5 H), 4.83 (m, 3 H), 5.21, (m, 2 H, H1, H6'), 7.28 (m, 15 H, aromatic); HR FAB MS calcd for $C_{55}H_{67}N_3O_{13}$ 977.4677, found 977.4668.

6-*O*-(2-Azido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-2,3:4,5-di-*O*-cyclohexylidene-D-*myo*-inositol (10). To 43 mg (44 μmol) of **9** in THF (2.2 mL) was added LiOH (1.1 mL of a 1.0 M aqueous solution). The mixture was stirred vigorously for 30 min, diluted with dichloromethane (25 mL), and washed with cold water (4 × 5 mL). Chromatography on a 10-mm flash silica gel column with 3% ether in dichloromethane afforded pure **10** (22 mg, 75%): ^{1}H-NMR ($CDCl_3$) δ 1.28–1.75 (m, 20 H, cyclohexylidenes), 2.80 (d, J = 2.3 Hz, 1 H, OH), 3.43 (m, 2 H), 3.69 (m, 1 H), 3.82 (m, 2 H), 3.98 (m, 2 H), 4.07 (m, 3 H), 4.32 (ψt, J = 7.6 Hz, 1 H), 4.45–4.68 (m, 4 H), 4.83 (m, 3 H), 5.17 (d, J = 3.6 Hz, 1 H, H1), 7.12–7.40 (m, 15 H, aromatic).

6-*O*-(2-Azido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-1-*O*-(dibenzylphosphono)-2,3:4,5-di-*O*-cyclohexylidene-D-*myo*-inositol (11). To a solution of 1*H*-tetrazole (11 mg, 0.15 mmol) and **10** (17 mg, 0.02 mmol) in dichloromethane (1.1 mL) was added dibenzyl diisopropylphosphoramidite[33] (21 mg, 0.06 mmol). After being stirred 20 min at 25 °C the solution was cooled to −40 °C, MCPBA (19 mg, 0.11 mmol in 0.23 mL CH_2Cl_2) was added, and stirring was continued for 30 min. The reaction mixture was diluted with dichloromethane (15 mL), washed with aqueous $Na_2S_2O_5$ (2 × 5 mL), 10% $NaHCO_3$ (2 × 5 mL), water (5 mL), and saturated NaCl (5 mL), dried, and concentrated. The crude product thus obtained was chromatographed on flash silica gel with 3% ether–dichloromethane, providing **11** (23 mg, 100%): ^{1}H-NMR ($CDCl_3$) δ 1.3–1.8 (m), 3.23 (dd, J = 3.5 Hz, 10 Hz, 1 H, H2), 3.48 (dd, J = 7.9 Hz, 10.7 Hz, 1 H), 3.65 (m, 1 H), 3.74–3.83 (m, 2 H), 3.88–4.08 (m, 3 H), 4.20–4.30 (m, 2 H), 4.36 (ψt, J = 7.1 Hz, 1 H), 4.44–4.53 (m, 2 H), 4.58, 4.62, 4.79, 4.82, 4.87 (5d, J = 11 Hz, benzylic H's), 4.98–5.12 (m, 5 H, 4 benzylic H's and H1), 7.15–7.40 (m); ^{31}P NMR ($CDCl_3$) δ −1.8; HR FAB MS calcd for $C_{59}H_{68}N_3O_{13}P$ $[M + H]^+$ 1058.4568, found 1058.4565.

6-*O*-(2-Amino-2-deoxy-α-D-glucopyranosyl)-D-*myo*-inositol 1-Phosphate (5). Compound **11** (12 mg, 11 μmol) was dissolved in 1 mL of ethanol–water–acetic acid (80:19:1) and hydrogenolyzed at 48 psig with 10 mg of 10% Pd/C for 24 h. The solvent was removed and, in the same pot, the sample was treated with acetic acid–water (1:1) for 3 h at 25 °C. Solvents were again removed, and the product was redissolved in water (0.5 mL) and filtered through Celite followed by a Waters C18 environmental Sep-Pak. Evaporation of the eluent afforded pure **5** as the free acid (5 mg, 100%): ^{1}H NMR (D_2O) δ 3.20 (dd, J = 3.7, 10 Hz, 1 H, H2), 3.27 (ψt, J = 9 Hz, 1 H), 3.41 (m, 2 H), 3.55 (ψt, J = 9 Hz, 1 H), 3.68–3.82 (m, 4 H), 3.97 (m, 1 H), 4.02 (bs, 1 H), 5.42 (d, J = 3.7 Hz, 1 H, H1). ^{1}H NMR and ^{31}P NMR spectra of the ammonium salt of **5** were identical to those previously reported.[25]

6-*O*-(2-Amino-2-deoxy-α-D-glucopyranosyl)-D-*myo*-inositol 1,2-(Cyclic)phosphate (1). Phosphodisaccharide **5** (3 μmol) was dissolved in 76 μL of distilled water in a 6- × 50-mm culture tube equipped with a magnetic stirring bar. Twenty-five equiv of 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide methyl-*p*-toluenesulfonate (**12**) were added in three portions over 2 h. The reaction was diluted with water (300 μL), passed through two Waters C-18 reversed-phase Sep-Paks and a 3 mL DEAE-cellulose ion exchange column equilibrated with 0.025 M ammonium acetate buffer, pH 7. The column was then eluted with 40 mL of linear gradient from 0.025 to 0.3 M of the same buffer, collecting 1-mL fractions. Lyophilization of the load volume and the first three fractions of the gradient afforded zwitterion **1** (0.6 μmol, 20%). Unreacted **5** (1.2 μmol, 40%) eluted as a monoanion at 0.15 M ammonium acetate and could readily be recycled. For **1**: ^{1}H NMR (D_2O) δ 5.28 (d, J = 3.5 Hz, 1 H, H1), 4.49 (ψt, 1 H, H2'), 4.34 (ddd, J = 4.7, 8.0, 20.1 Hz, 1 H, H1'), 3.90–3.00 (10 H); ^{31}P NMR (D_2O) δ 19.9 (d, $J_{H,P}$ = 20 Hz); HR FAB MS (positive ion mode) calcd for $C_{12}H_{23}NO_{12}P$ $[M + H]^+$ 404.0958, found 404.0954; HR FAB MS (negative ion mode) calcd for $C_{12}H_{21}NO_{12}P$ $[M - H]^-$ 402.0801, found 402.0810.

Assay of Lipogenesis. Lipogenesis was monitored in rat adipocytes prepared by collagenase digestion[39] by assay of [U-^{14}C]-glucose incorporation into lipids.[40] In this assay 25 μL of packed adipocytes were incubated in 0.5 mL of Krebs–Ringer bicarbonate, 5 nM [U-^{14}C]-glucose (1.0 μCi), and 1% bovine serum albumin for 1 h at 37 °C, in the presence of the indicated additives. All results are the means of triplicate determinations which were repeated in several experiments.

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Supplementary Material Available: ^{1}H NMR spectra for compounds **1**, **5**, **7**, and **9**–**11** and ^{31}P spectra (^{1}H coupled and decoupled) for compound **1** (8 pages). Ordering information is given on any current masthead page.

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