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Synthesis and investigation of the possible insulin-like activity of 1D-4-*O*- and 1D-6-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-*myo*-inositol 1-phosphate and 1D-6-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-*myo*-inositol 1,2-(cyclic phosphate)

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Abstract

The synthesis of the glycosyl-*myo*-inositol 1-phosphates **1** and **2** and of the glycosyl-*myo*-inositol 1,2-(cyclic phosphate) **3**, starting from previously synthesized intermediates, is reported. Compound **3** was found to display proliferative effects on the early developing inner ear of chick embryo.

Keywords: Glycosyl-inositol phosphate; Insulin mimics; Proliferative effects; Inner ear developing

1. Introduction

Present evidence indicates that insulin promotes the hydrolysis of a glycosyl phosphatidylinositol (GPI) with release of an inositol-containing phospho-oligosaccharide (IPG) which shows insulin-like effects in intact adipocytes and hepatocytes

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[1]. More recently, it has also been shown that insulin-like growth factor-I [2], nerve growth factor [3,4], interleukin-2 [5,6], and thyroid-stimulating hormone [7] stimulate GPI hydrolysis in target cells. The complete structure of these molecules (GPI and IPG) is presently unknown although some data [8–10] suggest structural similarities with the GPIs which anchor protein, polysaccharide, and small oligosaccharides to the outer face of cellular membranes through a covalent linkage [11–17]. On the basis of these data [8–10] and more recent evidence [18], we have reported [19,20] effective routes for the preparation of building blocks suitable for the synthesis of the IPG involved in insulin action. Following this work [19], we now describe the preparation and the investigation of the possible insulin-like activity of 1D-4-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-*myo*-inositol 1-phosphate (**1**), 1D-6-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-*myo*-inositol 1-phosphate (**2**), and 1D-6-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-*myo*-inositol 1,2-(cyclic phosphate) (**3**). Syntheses of fragments [21,22] and of the complete GPI anchor [23] of the variant surface glycoprotein of *Tripanosoma brucei* [24] and other related oligosaccharide fragments have been described [25–29]. It has been shown recently [29] that compound **3**, synthesized using a different procedure, exhibits a dose-dependent stimulation of lipogenesis in contrast with the non-cyclic phosphate **2**.

2. Results and discussion

1D-4-*O*-(6-*O*-Acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-2,3:5,6-di-*O*-isopropylidene-1-*O*-menthoxycarbonyl-*myo*-inositol (**4**) [42] was *O*-deacetylated using catalytic sodium methoxide in methanol [30] and silylated [31,32] to give the 6′-*O*-*tert*-butylsilyl derivative (**5**, 77%). Treatment of **5** with excess of sodium methoxide followed by reaction with dibenzyloxy(diisopropylamino)phosphine [33] and in situ oxidation [34,35] yielded 1D-4-*O*-(2-azido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-*tert*-butyldimethylsilyl-α-D-glucopyranosyl)-1-*O*-dibenzyloxyphosphoryl-2,3:5,6-di-*O*-isopropylidene-*myo*-inositol (**6**, 91%). The ^{1}H NMR spectrum of **6** showed the signal asigned to H-1 as a multiplet at δ 4.65 with $J_{H\text{-}1,P}$ of 8.1 Hz; similarly, the signal for C-1 in the ^{13}C NMR spectrum appeared as a doublet ($J_{C\text{-}1,P}$ 4.8 Hz) at 74.39 ppm. Desilylation of **6**, using tetrabutylammonium fluoride [36], and then hydrogenolysis followed by acid hydrolysis gave **1** (82%); $[\alpha]_D$ +74°; the ^{1}H NMR spectrum of which showed the doublet for H-1′ at 5.50 ppm ($J_{1',2'}$ 3.7 Hz) and the multiplet for H-1 at 3.95 ppm (J 2.7, 9.7 Hz); the ^{13}C NMR spectrum showed the signal for C-1 as a doublet at 76.28 ppm ($J_{C\text{-}1,P}$ 4.5 Hz).

A similar sequence starting from 1D-6-*O*-(6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-2,3:4,5-di-*O*-isopropylidene-1-*O*-menthoxycarbonyl-*myo*-inositol (**7**) [42] led to compound **2** through the corresponding 6′-*O*-*tert*-butyldimethylsilyl derivative (**8**) and the 1-*O*-dibenzyloxyphosphoryl compound (**9**). Compound **2** ($[\alpha]_D$ +80°) showed ^{1}H and ^{13}C NMR spectra in agreement with the proposed structure; the ^{1}H NMR spectrum showed the signal for H-1′ as a doublet ($J_{1',2'}$ 4.0 Hz) at 5.62 ppm and that for H-1 (pH 5) as a multiplet (J 2.7, 9.4 Hz) at

4.13; the ^{13}C NMR spectrum showed the signal for C-1 at 77.04 ($J_{C-1,P}$ 5.0 Hz) and that for C-2 at 78.56 ($J_{C-2,P}$ 2.4 Hz).

The 1,2-(cyclic phosphate) **3** was prepared by treatment of **2** with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride.

The biological activity of compounds **1–3** has been investigated. It has been shown [37] that rat liver IPG differentially regulates cell division in the otic vesicle and the associated cochleovestibular ganglion when assayed in chick embryo. The early development of the vertebrate inner ear involves the thickening and invagination of the ectoderm and the formation of the otic vesicle. At developmental stage 18, it consists of a fluid-filled cavity lined by a transporting epithelia [38] and an attached ganglion. Within 48 h, the otocyst goes through a distinct period of cell proliferation evolving towards a more complex structure with signs of growth and morphogenesis. In the otic vesicle, cell division can be arrested in vitro by incubation in serum-free media and then reactivated by the addition of growth factors [39] and also of natural chick embryo-derived IPG [37]. Fig. 1A shows the vesicular growth in the presence and absence of foetal calf serum (10S and control, respectively) and the proliferation induced by 10 nM insulin-like growth factor (IGF-I). The ability of compound **3** to induce proliferation on the otic vesicle epithelium was investigated. Compound **3** was shown to be able to stimulate cell proliferation 2.2-fold by itself (C-3 in Fig. 1A) and was not able to potentiate the effect of bombesin (data not shown). Natural IPG has a small effect when added alone to the incubation medium [37] and, unlike the synthetic compound **3**, potentiates bombesin proliferative action.

The parallel measurement of the incorporation of [^{3}H]thymidine is shown in Fig. 1B. Compound **3** (1 μM) stimulated 2.2-fold the incorporation of [^{3}H]thymidine into the otic vesicle. Whilst compounds **1** and **2** had no significant effects on cell proliferation, DNA synthesis in the presence of 10% foetal calf serum (10S) or 10 nM insulin-like growth factor (IGF-I) was increased 4- and 4.3-fold, respectively.

3. Experimental

General methods.—Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. TLC was performed on Silica Gel GF_{254} (Merck) with detection by charring with H_2SO_4 or phosphomolybdic acid. Column chromatography was performed on Silica Gel (Merck, 70–230 mesh). The ^1H and ^{13}C NMR spectra were recorded with a Varian XL-300 or Bruker AM-200 spectrometer. Optical rotations were measured with a Perkin–Elmer 241 MC polarimeter.

Preparation of explant cultures.—Otic vesicles were aseptically isolated from three-day-old chick embryos as previously described [37]. The standard culture medium consisted of serum-free M-199 medium with Hanks salts and glutamine (Flow Laboratories) supplemented with 25 mM HEPES. Incubations were carried out at 37°C in a water-saturated atmosphere containing 2% CO_2. Otic vesicles were made quiescent by incubation in the absence of serum for 24 h prior to

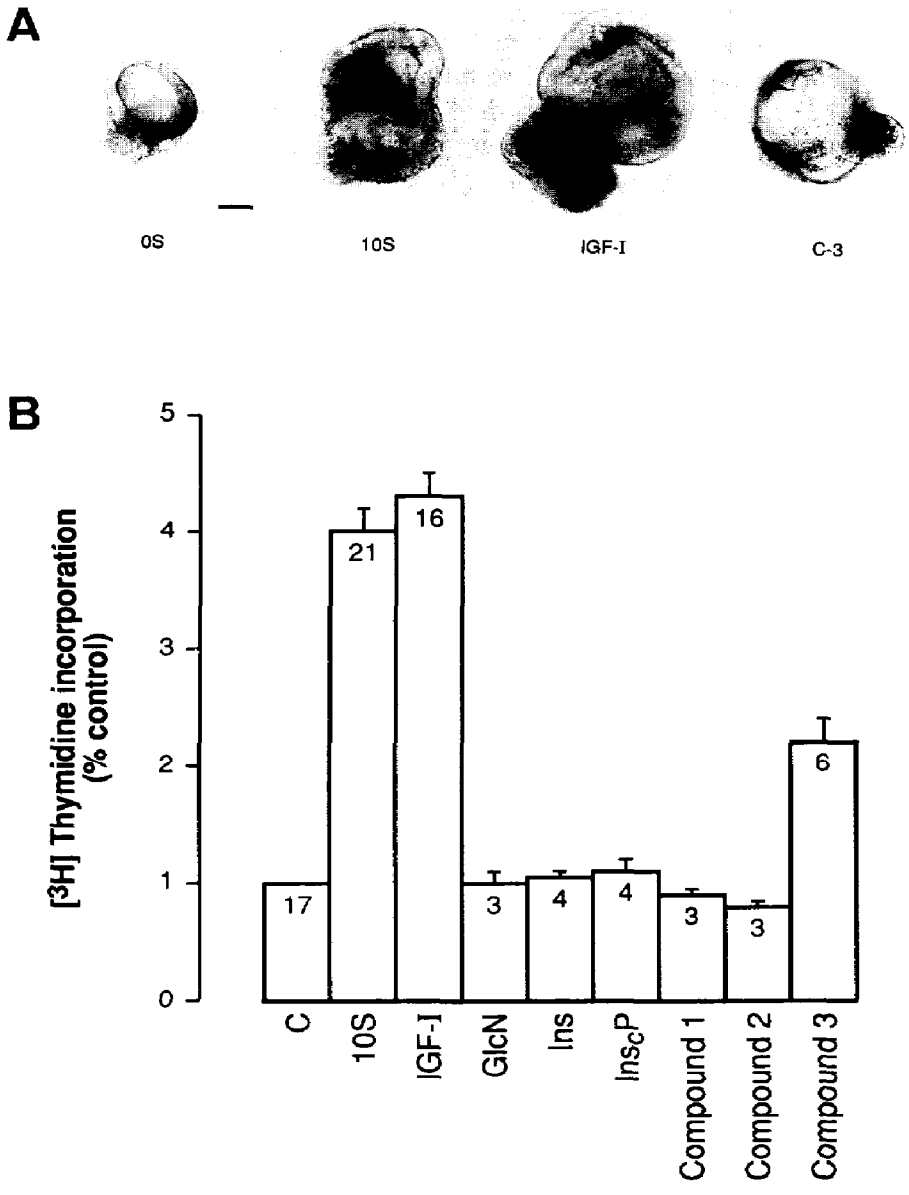
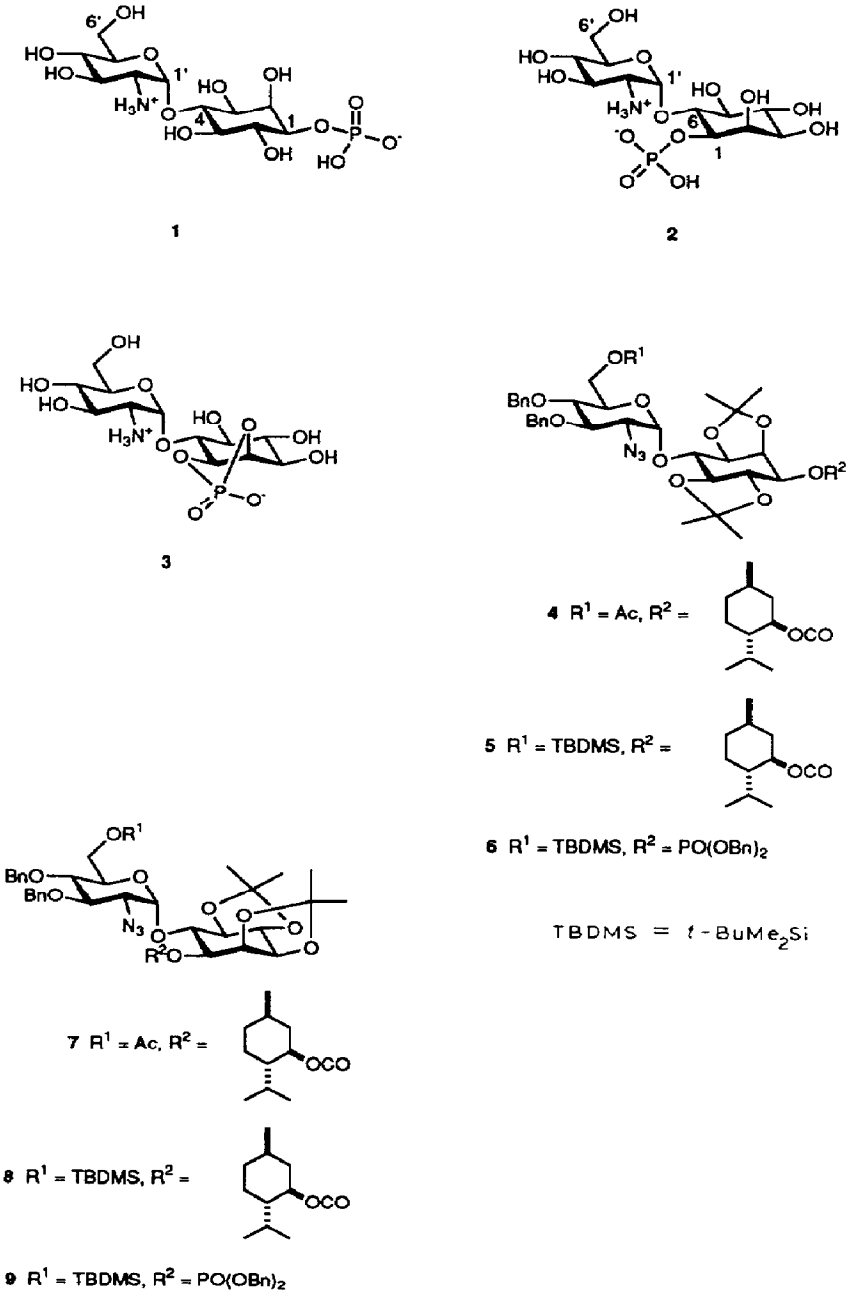


Fig. 1. Effect of compound **3** on cultured otic vesicles. (A) Reactivation of arrested otic vesicles by compound **3**. Quiescent otic vesicles were stimulated for 24 h by addition of 10 nM insulin-like growth factor (IGF-I), 1 μM **3** (C-3), and 10% FCS (10S); or in the absence of additions (OS). Calibration bar: 60 μM. (B) Acid-precipitable [^{3}H]thymidine incorporation by reactivated otocysts. Measurements were done as described in the Experimental section. Values were normalized with respect to those obtained in the absence of serum (8600 $\pm$ 690 cpm, $n = 17$). Vesicles were incubated in the absence of serum (C), or in the presence of 10% serum (10S), 10 nM insulin-like growth factor (IGF-I), 100 μM glucosamine (GlcN), 100 μM *myo*-inositol (Ins), 100 μM *myo*-inositol 1,2-(cyclic monophosphate) (InscP), and 1 μM of either compound **1**, **2**, or **3**. Values are mean $\pm$SE of at least three different experiments with an average of four vesicles per condition.



Scheme 1.

stimulation. IGF-I, glucosamine, *myo*-inositol, and *myo*-inositol 1,2-(cyclic monophosphate) were purchased from Sigma.

Determination of DNA synthesis.—DNA synthesis was measured as acid-precipitable [^{3}H]thymidine incorporation. Otic vesicles were incubated in standard incubation medium containing 0.3 μM (10 μCi/mL) [^{3}H]thymidine (Amersham, 40 Ci/mmol) for periods of 24 h.

Purification of the inositol phosphoglycan.—Inositol phosphoglycan was prepared by treating purified liver glycosyl phosphatidylinositol with bacterial phosphatidylinositol-specific phospholipase C (PI-PLC) as described [40]. PI-PLC from *Bacillus thuringiensis* was a generous gift of Dr. S. Undenfriend (Roche Institute of Molecular Biology, New Jersey, USA). The biological activity of IPG was assessed in vitro by testing its capacity to inhibit the phosphorylation of histone IIA by the cyclic AMP-dependent protein kinase [41].

*1D-4-O-(2-Azido-3,4-di-O-benzyl-2-deoxy-6-O-*tert*-butyldimethylsilyl-α-D-glucopyranosyl)-2,3:5,6-di-O-isopropylidene-1-O-menthoxycarbonyl-*myo*-inositol* (**5**).—To a solution of **4** [42] (154 mg, 0.181 mmol) in 1:1 CH_2Cl_2–MeOH (2 mL) was added a 2 M solution of NaOMe in MeOH (20 μL, 0.04 mmol). After 3 h at room temperature, the mixture was neutralized with Amberlite IR-120 (H^+) resin, filtered, and concentrated to give a residue (146 mg) which was dissolved in *N*,*N*-dimethylformamide (1 mL). 4-Dimethylaminopyridine (4.4 mg, 0.036 mmol) and imidazole (25 mg, 0.361 mmol) were added and the solution was cooled at 0°C and treated with *tert*-butyldimethylsilyl chloride (41 mg, 0.270 mmol). The mixture was kept for 12 h at room temperature and then diluted with CH_2Cl_2, washed with satd aq $NaHCO_3$ and water, dried, and concentrated. The residue was purified by column chromatography (10:1 hexane–EtOAc) to give **5** (128 mg, 77%) as a syrup; $[\alpha]_D^{20}$ +40° (*c* 0.2, $CHCl_3$); NMR (300 MHz, $CDCl_3$): ^{1}H, δ 7.34–7.19 (m, 10 H, 2 Ph), 5.25 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1'), 4.84 (dd, 1 H, $J_{1,2}$ 4.3, $J_{1,6}$ 10.8 Hz, H-1), 4.81 (ABs, 2 H, CH_2Ph), 4.74 (ABq, 2 H, CH_2Ph), 4.63 (t, 1 H, $J_{1,2} \approx J_{2,3} \approx 4.6$ Hz, H-2), 4.54 (m, 1 H, *J* 4.4, 11.0 Hz, CHOCO), 4.08 (dd, 1 H, $J_{3,4}$ 6.7, $J_{1,3}$ 5.0 Hz, H-3), 3.95 (t, 1 H, $J_{1,6} = J_{5,6} = 10.3$ Hz, H-6), 3.93–3.90 (m, 3 H, H-3', 5', 6'b), 3.86 (dd, 1 H, $J_{3,4}$ 6.7, $J_{4,5}$ 10.7 Hz, H-4), 3.73–3.67 (m, 2 H, H-4', 6'a), 3.48 (dd, 1 H, $J_{4,5}$ 10.7, $J_{5,6}$ 9.6 Hz, H-5), 3.24 (dd, 1 H, $J_{1',2'}$ 3.6, $J_{2',3'}$ 10.3 Hz, H-2'), 1.43, 1.40, 1.22 (3 s, 3 H each, CMe_2), 0.86 (d, 3 H, *J* 6.5 Hz, CH_3CH), 0.85 (s, 9 H, CMe_3), 0.83 (d, 3 H, *J* 7.0 Hz, CH_3CH), 0.73 (d, 3 H, *J* 6.9 Hz, CH_3CH), 0.0 (s, 6 H, 2 MeSi); ^{13}C (50 MHz), δ 153.9, 138.4, 138.0, 128.4, 128.1, 127.8, 127.7, 113.0, 109.0, 96.0, 79.9, 79.4, 78.9, 78.7, 77.8, 77.4, 75.5, 75.0, 74.5, 73.5, 71.4, 63.3, 61.4, 47.2, 40.7, 34.0, 31.4, 27.9, 26.9, 26.7, 26.1, 25.9, 23.3, 21.9, 20.6, 18.3, 20.6, 18.3, 16.3, −5.1 and −5.4 ppm. Anal. Calcd for $C_{49}H_{73}N_3O_{12}Si$: C, 63.68; H, 7.96; N, 4.55. Found: C, 63.97; H, 7.68; N, 4.34.

*1D-4-O-(2-Azido-3,4-di-O-benzyl-2-deoxy-6-O-*tert*-butyldimethylsilyl-α-D-glucopyranosyl)-1-O-dibenzyloxyphosphoryl-2,3:5,6-di-O-isopropylidene-*myo*-inositol* (**6**).—To a solution of **5** (120 mg, 0.130 mmol) in 7:3 CH_2Cl_2–MeOH (1 mL) was added a 1 M solution of NaOMe in MeOH (1 mL). After 1 h at room temperature, the mixture was neutralized with solid CO_2, diluted with CH_2Cl_2, and washed with water. The water phase was washed twice with CH_2Cl_2, and the combined organic phases were washed with water and satd aq NaCl, dried, and concentrated. The residue was disolved in 1:1 CH_2Cl_2–MeCN (4 mL) and treated under Ar with dibenzyloxy(diisopropylamino)phosphine (135 mg, 0.39 mmol) and 1*H*-tetrazole (46 mg, 0.65 mmol). The mixture was stirred for 30 min at room temperature and then water (2 mL), $NaIO_4$ (83 mg, 0.39 mmol), and $RuCl_3 \cdot 3H_2O$ (7 mg, 0.003 mmol) were added. After 1 h, CH_2Cl_2 and water were added, and the organic

phase was separated, washed twice with water and then with satd aq NaCl, dried, and concentrated. The residue was purified by column chromatography (3 : 1 → 2 : 1 hexane–EtOAc) to give **6** (118 mg, 91%); $[\alpha]_D^{20}$ +61° (*c* 0.8 $CHCl_3$); NMR ($CDCl_3$): ^1H (300 MHz), δ 7.33–7.22 (m, 20 H, 4 Ph), 5.24 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1'), 5.05 (ABq, 4 H, 2 POCH_2Ph), 4.81 (ABs, 2 H, CH_2Ph), 4.74 (ABq, 2 H, CH_2Ph), 4.65 (ddd, 1 H, $J_{1,2}$ 4.2, $J_{1,6}$ 10.9, $J_{1,P}$ 8.1 Hz, H-1), 4.48 (t, 1 H, $J_{1,2} = J_{2,3} = 4.2$ Hz, H-2), 4.03–3.90 (m, 5 H, H-3,6,3',5',6'b), 3.85 (dd, 1 H, $J_{3,4}$ 6.9, $J_{4,5}$ 10.9 Hz, H-4), 3.72–3.66 (m, 2 H, H-4',6'a), 3.41 (dd, 1 H, $J_{4,5}$ 10.4, $J_{5,6}$ 9.5 Hz, H-5), 3.23 (dd, 1 H, $J_{1',2'}$ 3.7, $J_{2',3'}$ 10.3 Hz, H-2'), 1.43, 1.38, 1.35, 1.18 (4 s, 12 H, 2 CMe_2), 0.84 (s, 9 H, Me_3C), 0.0 (s, 6 H, 2 MeSi); ^{13}C (50 MHz), δ 138.4, 138.1, 135.9, 135.8, 135.7, 135.6, 128.4, 128.1, 127.9, 127.8, 127.7, 127.6, 127.3, 112.9, 110.0, 96.7, 79.8, 79.5, 78.3, 77.8, 77.4, 76.5, 75.5, 75.3, 75.0, 74.4, and 74.3 (C-1, $J_{C\text{-}1,P}$ 4.8 Hz), 71.4, 69.6, 69.5, 69.3, 69.2, 63.3, 61.4, 28.0, 26.9, 26.7, 25.8, 25.7, 18.3, −5.1, and −5.4. Anal. Calcd for $C_{52}H_{68}N_3O_{13}PSi$: C, 62.32; H, 6.84; N, 4.19. Found: C, 62.60; H, 7.07; N, 3.95

1D-4-O-(2-Amino-2-deoxy-α-D-glucopyranosyl)-myo-*inositol 1-phosphate* (**1**).—To a solution of **6** (23 mg, 0.023 mmol) in tetrahydrofuran (1.2 mL) was added at 0°C under Ar a 1.1 M solution of tetrabutylammonium fluoride (52 μL, 0.057 mmol). After 2.5 h at room temperature, the mixture was diluted with CH_2Cl_2, washed with aq NaCl, dried, and concentrated. The residue was purified by column chromatography (3 : 2 hexane–EtOAc) and the pure product (21 mg) in 90% EtOH (2 mL) was treated with H_2 in the presence of 10% Pd–C (17 mg) for 36 h. The mixture was filtered on Celite, the solid was washed with 90% EtOH and 5 : 1 MeOH–water, and the solution was concentrated to give a residue (10 mg) which was dissolved in 5 : 1 MeOH–water (1 mL). The solution was diluted with water (1 mL) and treated with Amberlite IR-120 (H^+) resin for 75 min at room temperature. The mixture was filtered on Celite and concentrated, the residue was dissolved in water, and the solution was filtered through a Millipore membrane (0.5 μm) and finally lyophilized to give **1** as a white solid (8 mg, 82%); $[\alpha]_D$ +74.4° (*c* 0.29, ~ H_2O); lit. [27] $[\alpha]_D$ +0.98° (*c* 0.61, H_2O); NMR (D_2O, pH 1): ^1H (300 MHz), δ 5.50 (d, 1 H, $J_{1',2'}$ 3.7 Hz, H-1'), 4.25 (t, 1 H, $J_{1,2} = J_{2,3} = 2.8$ Hz, H-2), 4.07 (dt, 1 H, $J_{4',5'}$ 10, $J_{5',6'a}$ 3.7, $J_{5',6'b}$ 6.4 Hz, H-5'), 3.95 (td, 1 H, $J_{1,2}$ 2.7, $J_{1,6} = J_{H\text{-}1,P}$ = 9.7 Hz, H-1), 3.92 (dd, 1 H, $J_{2',3'}$ 10.6, $J_{3',4'}$ 9.0 Hz, H-3'), 3.87–3.74 (m, 4 H, H-4,6,6'a,6'b) 3.68 (dd, 1 H, $J_{2,3}$ 2.8, $J_{3,4}$ 9.8 Hz, H-3), 3.52 (t, 2 H, *J* 8.9 Hz, H-5,4'), 3.34 (dd, 1 H, $J_{1',2'}$ 3.8, $J_{2',3'}$ 10.7 Hz, H-2'); ^{13}C (50 MHz), δ 97.60 (C-1'), 81.3 (C-4 or C-6), 76.3 and 76.2 (C-1, $J_{1,P}$ 4.5 Hz), 75.7 (C-4' or C-5), 73.41 (C-5'), 73.0 (C-2, and C-4 or C-6), 70.9 (C-4' or C-5), 70.55 (C-3 and C-3'), 61.2 (C-6'), and 55.6 (C-2').

*1D-6-O-(2-Azido-3,4-di-O-benzyl-2-deoxy-6-O-*tert*-butyldimethylsilyl-α-D-glucopyranosyl)-2,3 : 4,5-di-O-isopropylydene-1-O-menthoxycarbonyl*-myo-*inositol* (**8**).—To a solution of **7** [42] (185 mg, 0.217 mmol) in 0.1 : 2 CH_2Cl_2–MeOH (2.1 mL) was added a saturated solution of NaOMe in MeOH (20 μL). After 5 h at room temperature, the mixture was neutralized with Amberlite IR-120 (H^+) resin, filtered, and concentrated. The residue (173 mg) was purified by column chromatography (4 : 1 hexane–EtOAc) to give the 6'-*O*-deacetyl derivative (120 mg,

68%) which was dissolved in *N,N*-dimethylformamide (1 mL). 4-Dimethylaminopyridine (4 mg, 0.03 mmol) and imidazole (20.2 mg, 0.297 mmol) were added and then *tert*-butyldimethylsilyl chloride (33 mg, 0.220 mmol) was added at 0°C. After 12 h at room temperature, the mixture was diluted with CH_2Cl_2, washed with aq $NaHCO_3$, water, and aq NaCl, dried, and concentrated. The residue was purified by column chromatography (5 : 1hexane–EtOAc) to give pure **8** (128 mg, 95%) as a syrup; $[\alpha]_D$ + 20° (*c* 0.3, $CHCl_3$); NMR ($CDCl_3$): ^1H (300 MHz), δ 7.36–7.28 (m, 10 H, 2 Ph), 5.23 (d, 1 H, $J_{1',2'}$ 3.7 Hz, H-1'), 4.98 (t, 1 H, $J_{1,2} = J_{1,6} = 3.6$ Hz, H-1), 4.87 (q, 2 H, CH_2Ph), 4.78 (q, 2 H, CH_2Ph), 4.57 (dd, 1 H, $J_{1,2}$ 4.2, $J_{2,3}$ 6.6 Hz, H-2), 4.53 (m, 1 H, *J* 4.4, 10.9 Hz, CHOCO), 4.36 (bt, 1 H, $J_{2,3} = J_{3,4} = 7.3$ Hz, H-3), 4.03 (dd, 1 H, $J_{1,6}$ 3.2, $J_{5,6}$ 8.4 Hz, H-6), 3.96 (dd, 1 H, $J_{3,4}$ 7.4, $J_{4,5}$ 10.4 Hz, H-4), 3.96–3.74 (m, 4 H, H-3',5',6'a,6'b), 3.71 (t, $J_{3',4'} = J_{4',5'} = 9.4$ Hz, H-4'), 3.51 (dd, 1 H, $J_{4,5}$ 10.7, $J_{5,6}$ 8.4 Hz, H-5), 3.33 (dd, 1 H, $J_{1',2'}$ 3.7, $J_{2',3'}$ 10.2 Hz, H-2'), 1.49, 1.40, 1.33 (3 s, 12 H, 2 CMe_2), 0.91 (d, 3 H, *J* 6.6 Hz, CH_3CH), 0.89 (s, 9 H, CMe_3), 0.86 (d, 3 H, *J* 7.0 Hz, CH_3CH), 0.76 (d, 3 H, *J* 6.7 Hz, CH_3CH), 0.05 (s, 6 H, 2 SiMe); ^{13}C (50 MHz): δ 153.8; 137.9, 137.7, 128.2, 128.1, 127.75, 127.7, 127.5, 112.2, 111.0, 96.5, 79.8, 78.9, 77.8, 77.0, 76.5, 76.4, 76.1, 75.9, 75.2, 74.9, 73.2, 71.6, 63.1, 61.2, 46.7, 40.2, 33.7, 31.1, 26.8, 26.7, 26.2, 25.6, 24.8, 22.9, 21.6, 20.3, 18.0, 15.8, −5.3, and −5.7. Anal. Calcd for $C_{49}H_{73}N_3O_{12}Si$: C, 63.68; H, 7.96; N, 4.55. Found: C, 63.39; H, 7.96; N, 4.76.

*1*D*-6-*O*-(2-Azido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-*tert*-butyldimethylsilyl-α-*D*-glucopyranosyl)-1-*O*-dibenzyloxyphosphoryl-2,3 : 4,5-di-*O*-isopropylidene*-myo*-inositol* (**9**). —To a solution of **8** (100 mg, 0.108 mmol) in 7 : 2 CH_2Cl_2–MeOH (0.9 mL) was added a 1 M solution of NaOMe in MeOH (1.1 mL). After 7 h at room temperature, the mixture was neutralized with solid CO_2 and diluted with CH_2Cl_2. The aqueous layer was washed twice with CH_2Cl_2, and the combined organic phases were washed with water and aq NaCl, dried, and concentrated. The residue (90 mg) was dissolved in 1 : 1 CH_2Cl_2–MeCN (3.4 mL), and 1*H*-tetrazole (38 mg, 0.54 mmol) and dibenzyloxy(diisopropylamino)phosphine (112 mg, 0.324 mmol) were added under Ar, with stirring. After 1 h at room temperature, water (1.7 mL), $NaIO_4$ (69.3 mg, 0.324 mmol), and $RuCl_3 \cdot 7H_2O$ (0.7 mg, 0.002 mmol) were added. After 70 min, CH_2Cl_2 and water were added, and the organic layer was separated, washed twice with water and once with aq NaCl, dried, and concentrated. The residue was purified by column chromatography (3 : 1 hexane–EtOAc) to give **9** (108 mg, 74%) as a syrup; $[\alpha]_D$ + 34° (*c* 0.4, $CHCl_3$); NMR ($CDCl_3$): ^1H (300 MHz), δ 7.34–7.19 (m, 20 H, 4 Ph), 5.06–5.00 (m, 4 H, 2 CH_2Ph), 4.93 (d, 1 H, $J_{1',2'}$ 3.7 Hz, H-1'), 4.84–4.67 (m, 4 H, CH_2Ph), 4.54 (m, $J_{1,6}$ 2.1, $J_{1,2}$ 3.4 $J_{H\text{-}1,P}$ 8.8 Hz, H-1), 4.45 (m, 1 H, $J_{2,3}$ 6.9 Hz, H-2), 4.31 (t, 1 H, $J_{2,3} = J_{3,4} = 7.3$ Hz, H-3), 4.15 (dd, 1 H, $J_{1,6}$ 1.9, $J_{5,6}$ 8.1 Hz, H-6), 4.01 (dd, 1 H, $J_{3,4}$ 7.6, $J_{4,5}$ 10.7, H-4), 3.89 (dd, 1 H, $J_{5',6'a}$ 2.0, $J_{6'a,6'b}$ 11.5 Hz, H-6'a), 3.85 (t, 1 H, $J_{2',3'} = J_{3',4'} = 10.1$ Hz, H-3'), 3.74–3.63 (m, 3 H, H-4',5',6'b), 3.42 (dd, 1 H, $J_{4,5}$ 10.8 Hz, H-5), 3.10 (dd, 1 H, H-2'), 1.48, 1.34, 1.32, 1.27 (4 s, 12 H, 2 CMe_2), 0.84 (s, 9 H, CMe_3), 0.00 (s, 6 H, 2 SiMe); ^{13}C (50 MHz), δ 138.2, 137.9, 135.7, 135.6, 133.5, 133.4, 128.6, 128.55, 128.5, 128.45, 128.4, 128.3, 128.12, 128.1, 128.05, 128.0, 127.95, 127.9, 127.88, 127.85, 112.54, 112.55, 96.7, 80.1, 78.0, 77.8, 77.3, 77.25, 77.22, 76.5, 76.45, 75.5,

75.1, 74.1, 74.0, 72.0, 69.5, 69.4, 63.3, 61.4, 27.1, 27.0, 26.4, 25.9, 24.9, 18.3, −5.0, and −5.4. Anal. Calcd for $C_{52}H_{68}N_3O_{13}PSi$: C, 62.32; H, 6.84; N, 4.19. Found: C, 62.58; H, 6.92; N, 4.37.

1-D-6-O-(2-Amino-2-deoxy-α-D-glucopyranosyl)-myo-*inositol 1-phosphate* (**2**).—To a solution of **9** (20 mg, 0.020 mmol) in tetrahydrofuran (1 mL) was added at 0°C under Ar a 1.1 M solution of tetrabutylammonium fluoride (45 μL, 0.050 mmol). After 2.5 h at room temperature, the mixture was diluted with CH_2Cl_2, washed with aq NaCl, dried, and concentrated. The residue was purified by column chromatography (3:2 hexane–EtOAc). The pure product (16 mg) in 90% EtOH (1.5 mL) was treated with H_2 in the presence of 10% Pd–C (13 mg) at room temperature for 18 h. The mixture was filtered on Celite, and the solid washed with 90% EtOH and then with 5:1 MeOH–water. The filtrate and washings were concentrated to give a residue (11 mg) which was dissolved in 5:1 MeOH–water (1 mL). Water (1 mL) was added and the solution was treated with Amberlite IR-120 (H^+) resin for 1.5 h at room temperature. The mixture was filtered on Celite and the filtrate was concentrated. A solution of the residue in water was filtered through a Millipore membrane (0.5 μm) and lyophilized to give **2** as a white solid (7 mg, 83%); $[\alpha]_D^{20}$ + 80° (*c* 0.5, H_2O); NMR (D_2O, pH 1): ^1H (300 MHz), δ 4.22–4.14 (m, 2 H, H-1,2), 4.09 (m, 1 H, $J_{4',5'}$ 10.1, $J_{5',6'a}$ 3.4, $J_{5',6'b}$ 6.4 Hz, H-5'), 3.95–3.89 (m, 2 H, H-6,3'), 3.86–3.80 (m, 2 H, H-6'a,6'b), 3.69 (t, 1 H, $J_{3,4} = J_{4,5}$ = 10.7 Hz, H-4), 3.57–3.50 (m, 2 H, H-3,4'), 3.41 (t, 1 H, $J_{4,5} = J_{5,6}$ = 9.4 Hz, H-5), 3.34 (dd, 1 H, $J_{1',2'}$ 3.9, $J_{2',3'}$ 10.5 Hz, H-2'); (D_2O, pH 5): ^1H (300 MHz), δ 5.62 (d, 1 H, $J_{1',2'}$ 4.0 Hz H-1'), 4.18 (t, 1 H, $J_{1,2} = J_{2,3}$ = 2.7 Hz, H-2), 4.13 (m, 1 H, $J_{1,2}$ 2.7, $J_{1,6} = J_{H\text{-}1,P}$ = 9.4 Hz, H-1), 4.08 (m, 1 H, $J_{4',5'}$ 11.0, $J_{5',6'a}$ 4.0, $J_{5',6'b}$ 7.7 Hz, H-5'), 3.92–3.81 (m, 4 H, H-6,3',6'a,6'b), 3.68 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9.7 Hz, H-4), 3.57–3.50 (m, 2 H, H-3,4'), 3.40 (t, 1 H, $J_{4,5} = J_{5,6}$ = 9.3 Hz, H-5), 3.32 (dd, 1 H, $J_{2',3'}$ 10.7 Hz, H-2'); ^{13}C (50 MHz), δ 96.6, 78.5 (d, $J_{2,P}$ 2.4 Hz, C-2), 77.0 (d, $J_{1,P}$ 5.0 Hz, C-1), 73.9, 73.6, 73.2, 72.9, 72.5, 71.4, 70.4, and 55.0; ^{31}P (D_2O, pD 1, ref H_3PO_3), δ 3.20.

1-D-6-O-(2-Amino-2-deoxy-α-D-glucopyranosyl)-myo-*inositol 1,2-(cyclic phosphate* (**3**).—To a stirred solution of **2** (3 mg, 7.1 mmol) in 177 μL of distilled water was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (75.4 mg, 177 mmol) in three portions over 2 h. The mixture was diluted with water (0.7 mL) and passed through two Waters C-18 reversed phase Sep-Paks and then through a 5-mL DE 32-Cellulose ion-exchange column equilibrated with 0.025 M ammonium acetate buffer, pH 7. The column was then eluted with the buffer (50 mL, linear gradient from 0.025 to 0.3 M), collecting 1-mL fractions. Lyophilization of fractions 5–8 gave **3** (0.6 mg, 20%) as a white solid; NMR (D_2O): ^1H (500 MHz), δ 5.47 (d, 1 H, $J_{1',2'}$ 3.6 Hz, H-1'), 4.59–4.53 (m, 1 H, H-1), 4.05–3.4 (10 H); ^{31}P (D_2O, ref H_3PO_3), δ 17.69.

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