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New tetherable derivatives of *myo*-inositol 2,4,5- and 1,3,4-trisphosphates

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ABSTRACT

(±)-*myo*-Inositol 1-(3-aminopropyl hydrogen phosphate) 3,4-bis(disodium phosphate) (**5**) and (±)-*myo*-inositol 2-(3-aminopropyl hydrogen phosphate) 4,5-bis(disodium phosphate) (**11**) have been synthesized by conventional procedures. Each derivative has been immobilized on a polymeric resin in order to give a bioaffinity matrix.

INTRODUCTION

The discovery that 1D-*myo*-inositol 1,4,5-trisphosphate (1,4,5-IP_3) acts as an intracellular second messenger and mediates the release of Ca^{2+} from non-mitochondrial stores[1] has generated a plethora of research on biological studies of this molecule[2]. Several other 1D-*myo*-inositol polyphosphates have also been implicated in the regulation of calcium levels, for instance, the 1,3,4,5-tetrakisphosphate (1,3,4,5-IP_4) which is formed in mammalian cells by phosphorylation of 1,4,5-IP_3 by a 3-kinase[3], and the 1,3,4-trisphosphate (1,3,4-IP_3) formed by the action of a 5-phosphatase on 1,3,4,5-IP_4. In order to elucidate the biological functions of these molecules, we have prepared racemic, P-1 ω-aminoalkyl phosphodiester derivatives of 1,4,5-IP_3 (ref. 4), 1,3,4,5-IP_4 (ref. 5), and IP_6 (ref. 6), and a chiral 1,3,4,5-IP_4 derivative[7]. These "tetherable" ligands have been used to prepare bioaffinity matrices to aid in the purification of receptor proteins[4,8], and radioiodinated photoaffinity analogs in order to allow the identification of specific-binding subunits[9,10].

Increasing attention is being paid to the use of reactive analogs and resin-immobilized derivatives of inositol polyphosphates. Ballou and Tegge[11] have used a

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chiral analog of a P-1 tethered aminopropyl IP_3 derivative[4,12] to prepare an affinity resin and azidosalicylamide photolabel. Jina et al.[13] have synthesized (±)-(1*R*,3*R*,4*R*)-*trans*-*N*-(2-aminoethyl)-3,4-bis(phosphonyloxy)cyclohexane-1-carboxamide and used it to prepare an affinity matrix and a photolabel. Hirata et al.[14] have described the synthesis and biochemical affinities of several 1,4,5-IP_3 photoaffinity labels and two immobilized derivatives for affinity chromatography. A phosphonate analog of *myo*-inositol 1-phosphate was used[15] to prepare an affinity matrix for phospholipase C. Two photolabile arylazido analogs were synthesized from 1D-1-*O*-(2-aminoethylphospho)-*myo*-inositol 4,5-bisphosphate[16], obtained semi-synthetically from 1D-1-*O*-(*sn*-glycer-3-ylphospho)-*myo*-inositol 4,5-bisphosphate[17].

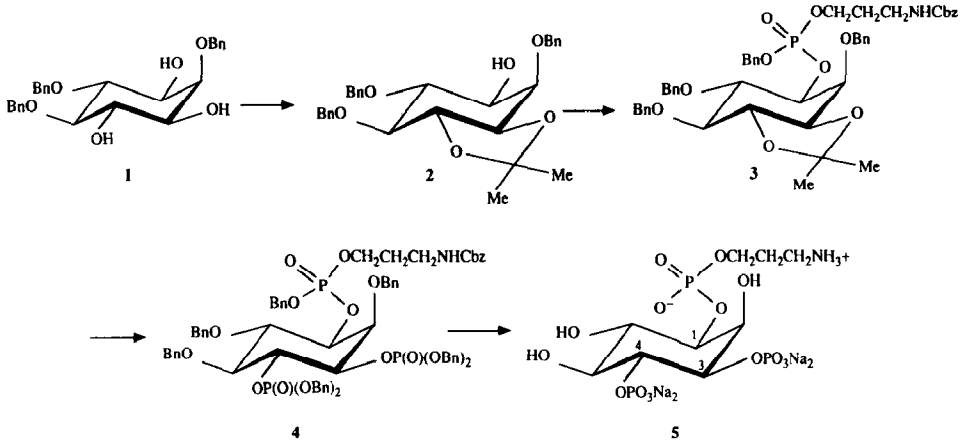
Many researchers are actively investigating how 1,4,5-IP_3 is removed from the cell by the 5-phosphatase and 3-kinase processes[18]. The product of the 3-kinase pathway is 1,3,4,5-IP_4, which is metabolized[19] by a 5-phosphatase to 1,3,4-IP_3. The synthesis of the tetherable 1,3,4-IP_3, derivative **5** would provide access to a series of potential probes for these phosphatase and kinase enzymes. In addition, non-physiological 2,4,5-IP_3 is a good inhibitor of the binding of [^{3}H]-1,4,5-IP_3 to a 1,4,5-IP_3 receptor in isolated olfactory cilia of the catfish[10]. Chiral 2,4,5-IP_3 causes the release of Ca^{2+} from rat basophilic leukemia cells[20]. Therefore, it is desirable to synthesize the tetherable 2,4,5-IP_3 derivative **11** for use in preparing an affinity matrix in an attempt to isolate putative binding proteins.

We now report the preparation of **5** and **11** and the corresponding bioaffinity matrices. The biochemical results will be described elsewhere.

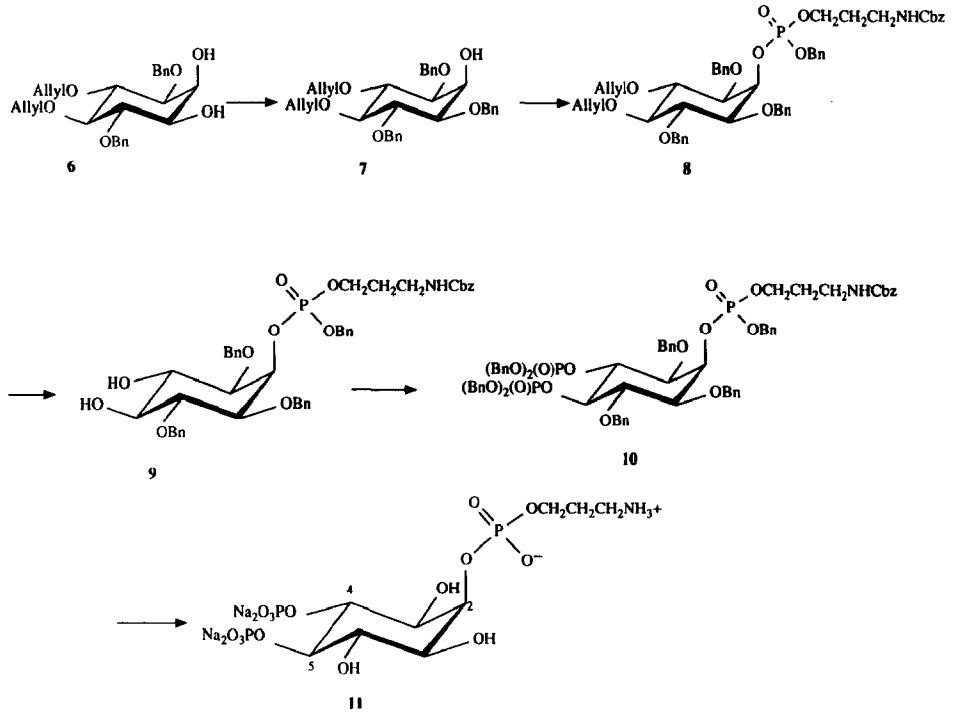
RESULTS AND DISCUSSION

The vicinal 4- and 5-phosphate groups, at least in 1,4,5-IP_3, are required for the calcium-mobilizing effect[21]. The 1-phosphate, or at least a negatively charged group, while not essential, enhances this activity. We elected to append the tether at the isolated P-1 or P-2 phosphate, via a phosphodiester linkage, and leave the vicinal phosphates and the remaining hydroxyl groups unencumbered.

For the synthesis of the P-1 tethered 1,3,4-IP_3 derivative **5**, a convenient starting material was 2,4,5-tri-*O*-benzyl-*myo*-inositol[22] (**1**), which was converted into the known[23] 3,4-*O*-isopropylidene derivative **2** (92%). Treatment of **2** with benzyloxy(3-benzyloxycarbonylaminopropoxy)(diisopropylamino)phosphine[4] followed by oxidation with *m*-chloroperoxybenzoic acid gave the 1-phosphate derivative **3** (84%). The isopropylidene group was removed easily from **3** by mild acid hydrolysis and the hydroxyl groups exposed were phosphorylated by reaction with dibenzyloxy(diisopropylamino)phosphine[24] followed by oxidation with *m*-chloroperoxybenzoic acid to yield the trisphosphate derivative **4**. Catalytic hydrogenolysis (Pd/C) of **4** removed the nine benzyl groups to give the target 1,3,4-IP_3 derivative **5** in quantitative yield.



The 2,4,5-IP_3 derivative **11** was prepared in a slightly different manner. 5,6-Di-*O*-allyl-1,4-di-*O*-benzyl-*myo*-inositol (**6**), available from the synthesis of *myo*-inositol 1,4,5-trisphosphate[25], was reacted with 1 equiv of benzyl bromide in the presence of sodium hydride to give 51% of the 1,3,4-tri-*O*-benzyl derivative **7**. The phosphitylation of the 2-hydroxyl group in **7** with benzyloxy(3-benzyloxycarbonylaminopropoxy)(diisopropylamino)phosphine followed by oxidation with *m*-chloro-



peroxybenzoic acid involves two modifications from the usual protocol. First, because the axial HO-2 is hindered, the phosphitylation was allowed to proceed overnight instead of the usual 3–4 h. Second, the use of a low temperature and a short oxidation time allowed the phosphite to be oxidized to the phosphate without affecting the allyl groups. Thus, **8** (80%) was prepared from **7**. The allyl groups were removed by isomerization to the prop-2-enyl ethers, using Wilkinson's catalyst, followed by hydrolysis with dilute acid[26] to give **9**. Phosphorylation of HO-5 and HO-6 in **9** was effected with dibenzyloxy(diisopropylamino)phosphine and oxidation with *m*-chloroperoxybenzoic acid to give **10** (95%). Catalytic hydrogenolysis removed the nine benzyl groups in **10** to yield the target 2,4,5-IP_3 derivative **11**.

Affinity columns were prepared from both **5** and **11** using an *N*-hydroxysuccinimide-activated resin (Affi-Gel 10). Applications of these new tethered IP_3 regioisomers for the purification of catfish olfactory receptors[10] and in the isolation of other IP_n-binding proteins is in progress.

EXPERIMENTAL

General methods.—The ^1H (300 MHz) and ^{13}C NMR (75 MHz) spectra were obtained with a GE QE-300 spectrometer on solutions in $CDCl_3$ (internal Me_4Si). ^{31}P NMR spectra (101.3 MHz) were obtained with a Bruker AC-250 spectrometer (external aq 85% H_3PO_4). Mass spectra were measured on a Kratos MS-80 RFA spectrometer. Combustion analyses were performed by Atlantic Microlab, Norcross, GA (USA). Column chromatography was performed on EM silica gel 60 (230–400 mesh), using HPLC grade solvents, and TLC was performed on silica gel G (Machery Nagel) plates with detection by UV light or with 10% phosphomolybdic acid in EtOH followed by heating. Dichloromethane was distilled from P_2O_5 and stored over 4A molecular sieves. The phosphitylating reagents were prepared by published procedures[4,24]. The 10% Pd/C catalyst was obtained from Johnson Matthey (Catalog No. 11702). Affi-Gel 10 resin was obtained from Bio-Rad Laboratories. Solvents were evaporated at $< 30°$ (40 mmHg) or were removed in vacuo at 20–25°. All the compounds described below are racemic.

*2,5,6-Tri-*O*-benzyl-3,4-*O*-isopropylidene*-myo-*inositol* (**2**).—2,2-Dimethoxypropane (9 mL) and *p*-toluenesulfonic acid monohydrate (90 mg) were added to a solution of 2,4,5-tri-*O*-benzyl-*myo*-inositol (**1**; 0.90 g, 2.0 mmol) in dry acetone (30 mL). The solution was stirred at 25° for 3.5 h, then neutralized with triethylamine (0.9 mL) and $NaHCO_3$ (0.9 g). The solvent was evaporated under reduced pressure and finally in vacuo. The residue was extracted with ether (2×50 mL) and the combined extracts were concentrated. The residue was chromatographed on silica gel (60 g) with diethyl ether–light petroleum–triethylamine (100:100:1) to yield **2** (0.91 g, 92%) as a colorless oil. ^1H NMR data: δ 1.41 (s, 3 H, CH_3), 1.44 (s, 3 H, CH_3), 3.43 (dd, 1 H, J 1.44 and 10.9 Hz, CH of inositol), 3.61 (m, 3 H, 3 CH of inositol), 4.13 (m, 2 H, 2 CH of inositol), 4.61 and 4.94 (ABq, 2 H, J 10.9 Hz,

PhCH_2), 4.69 and 4.88 (ABq, 2 H, J 11.6 Hz, PhCH_2), 4.75 and 4.85 (ABq, 2 H, J 11.4 Hz, PhCH_2), 7.3 (m, 15 H, 3 Ph).

*Benzyl (3-benzyloxycarbonylaminopropyl) (2,5,6-tri-*O*-benzyl-3,4-*O*-isopropylidene-*myo*-inosit-1-yl) phosphate* (**3**).—Tetrazole (0.60 g, 8.5 mmol) was added to a solution of **2** (1.15 g, 2.3 mmol) in CH_2Cl_2 (50 mL). The mixture (tetrazole is not completely soluble) was stirred at 20° and a solution of benzyloxy(3-benzyloxycarbonylaminopropoxy)(diisopropylamino)phosphine (1.5 g, 3.4 mmol) in CH_2Cl_2 (2 mL) was added. After stirring at 20° for 5 h, the mixture was cooled to −40° and *m*-chloroperoxybenzoic acid (1.25 g, 7.3 mmol) was added. Stirring was continued at −40° for 5 min, 0° for 30 min, and finally 20° for 30 min. The solution was diluted to 125 mL with CH_2Cl_2, washed with aq 10% Na_2SO_3 (2 × 25 mL), aq 5% $NaHCO_3$ (2 × 25 mL), and water (40 mL), dried ($MgSO_4$), and filtered, and the solvent was evaporated. The residue was chromatographed on silica gel (70 g) with EtOAc–light petroleum–triethylamine (50:50:1) to yield **3** (1.70 g, 84%), isolated as a colorless oil. NMR data: ^{1}H, δ 1.41 (s, 3 H, CH_3), 1.42 (s, 3 H, CH_3), 1.45–1.62 (m, 2 H, $CH_2CH_2CH_2$), 2.90–3.10 (m, 2 H, CH_2CH_2N), 3.35–3.45 (m, 1 H, CH of inositol), 3.58–3.72 (m, 1 H, CH of inositol), 3.75–3.95 [m, 3 H, CH of inositol and P(O)OCH_2], 4.20 (t, 1 H, J 9.6 Hz, CH of inositol), 4.24–4.35 (m, 1 H, CH of inositol), 4.35–4.45 (m, 1 H, CH of inositol), 4.58–5.01 (m, 8 H, 4 × PhCH_2), 5.01 [s, 2 H, PhCH_2OC(O)], 7.15–7.50 (m, 25 H, 5 Ph); ^{31}P (^{1}H decoupled), δ 0.10 and 0.41 (pair of diastereomers).

*Benzyl (3-benzyloxycarbonylaminopropyl) 2,5,6-tri-*O*-benzyl-3,4-di-*O*-(dibenzyloxyphosphoryl)-*myo*-inosit-1-yl] phosphate* (**4**).—A solution of **3** (1.70 g, 2.0 mmol) in MeOH (27 mL) was stirred at 40°, 1 M HCl (3 mL) was added, and the reaction was monitored by TLC (EtOAc–hexane, 1:1). After 10 min, **3** (R_F 0.30) had disappeared and only a single spot at R_F 0.06 was observable. The solution was cooled in an ice bath, then neutralized with $NaHCO_3$ (1 g), the solvent was evaporated in vacuo, and the residue was stirred with CH_2Cl_2 (2 × 50 mL, 5 min each time). The combined extracts were filtered and the solvent was evaporated, leaving a colorless gum (1.54 g). A solution of the gum in *N*,*N*-dimethylformamide (3 mL) was evaporated in vacuo to remove any remaining MeOH or water. The residue was dissolved in CH_2Cl_2 (30 mL), and tetrazole (0.70 g, 10 mmol) and (dibenzyloxy)(diisopropylamino)phosphine (1.90 g, 5.3 mmol) were added. The mixture was stirred at 20° for 5 h (after 30 min all of the tetrazole had dissolved). The solution was cooled to −40° and a solution of *m*-chloroperoxybenzoic acid (2.0 g, 11.6 mmol) in CH_2Cl_2 (10 mL) was added. The solution was stirred at −40° for 5 min, 0° for 30 min, and 20° for 30 min, then diluted to 150 mL with CH_2Cl_2, and worked-up as described for the preparation of **3**. The crude product was chromatographed on silica gel (90 g), using EtOAc–hexane (2:1), to yield **4** (1.86 g, 70%), isolated as a colorless oil. NMR data: ^{1}H, δ 1.60–1.72 (m, 2 H, $CH_2CH_2CH_2$), 3.00–3.12 (m, 2 H, CH_2CH_2NH), 3.32–3.46 (m, 1 H), 3.85–4.00 (m, 2 H), 4.15–4.30 (m, 2 H), 4.60–5.10 (m, 21 H), 5.60–5.70 (m, 1 H, NH),

7.10–7.50 (m, 45 H, 9 Ph); ^{31}P (^1H decoupled), δ −0.49, −0.44, −0.13, 0.26, and 0.73 (mixture of diastereomers).

*(±)-*myo*-Inositol 1-(3-aminopropyl hydrogen phosphate) 3,4-bis(disodium phosphate)* (**5**).—To a solution of **4** (1.38 g, 1.0 mmol) in 95% EtOH (150 mL) was added 10% Pd/C (0.60 g), and hydrogenolysis was allowed to proceed at 50 psi for 7 h. The mixture was filtered through Celite which was then washed with 2:1 EtOH–water (60 mL) and water (20 mL). The combined filtrate and washings were basified with concd ammonia (2 mL) and evaporated to dryness in vacuo at 20°. A solution of the residue in water (5 mL) was applied to a column (8 × 1.4 cm) of Chelex 100 (Na^+) resin, and the product was eluted with water (60 mL). The eluate was concentrated to dryness in vacuo to afford **5** as a white powder in quantitative yield. NMR data (D_2O): ^1H, δ 1.88 (quintet, 2 H, *J* 6.2 Hz, $CH_2CH_2CH_2$), 2.99 (t, 2 H, *J*, 6.9 Hz $CH_2CH_2NH_2$), 3.43 (t, 1 H, *J* 9.0 Hz, CH of inositol), 3.70 (t, 1 H, *J* 9.6 Hz, CH of inositol), 3.75–3.95 [m, 4 H, 2 CH of inositol and $P(O)OCH_2$], 4.06 (q, 1 H, *J* 8.4 Hz, CH of inositol), 4.44 (s, 1 H, H-2 of inositol); ^{31}P (^1H coupled), δ 1.60 (m), 5.11 (d, *J* 7.1 Hz), 6.64 (d, *J* 8.5 Hz).

Anal. Calcd for $C_9H_{18}NNa_4O_{15}P_3 \cdot 4\ H_2O$: C, 16.97; H, 4.11. Found: C, 16.79; H 4.00.

*5,6-Di-O-allyl-1,3,4-tri-O-benzyl-*myo*-inositol* (**7**).—5,6-Di-*O*-allyl-1,4-di-*O*-benzyl-*myo*-inositol (**6**; 2.20 g, 5.0 mmol) was dissolved in benzene (60 mL), and benzyl bromide (0.86 g, 5.0 mmol) was added. The solution was added to NaH (1.20 g, 50 mmol), the mixture was stirred and heated under reflux for 90 min, then cooled to room temperature, and the excess of NaH was destroyed by dropwise addition of water (foaming!). The solution was diluted to 120 mL with benzene, washed with satd aq NaCl (3 × 35 mL), dried ($MgSO_4$), and filtered, and the benzene was evaporated. The residue was chromatographed on silica gel (100 g) with ether–light petroleum (3:2), yielding **7** (1.35 g, 51%). An analytical sample, prepared by crystallization from ether–hexane, had mp 50–51°. NMR data: ^1H, δ 3.15–3.30 (m, 3 H, 3 CH of inositol), 3.74 (t, 1 H, *J* 9.5 Hz, CH of inositol), 3.85 (t, 1 H, *J* 9.5 Hz, CH of inositol), 4.13 (t, 1 H, *J* 2.6 Hz, CH of inositol), 4.22–4.35 (m, 4 H, CH_2=CHCH_2), 4.64 (s, 2 H, PhCH_2), 4.63 and 4.67 (ABq, 2 H, *J* 11.8 Hz, PhCH_2), 4.79 (s, 2 H, PhCH_2), 5.08–5.30 (m, 4 H, 2 CH_2=CH), 5.86–6.03 (m, 2 H, 2 CH_2=C*H*CH_2), 7.20–7.50 (m, 15 H, 3 Ph).

Anal. Calcd for $C_{33}H_{38}O_6$: C, 74.69; H, 7.22. Found: C, 74.70; H, 7.24.

*Benzyl (3-benzyloxycarbonylaminopropyl) (5,6-di-O-allyl-1,3,4-tri-O-benzyl-*myo*-inosit-2-yl) phosphate* (**8**).—Tetrazole (0.31 g, 4.5 mmol) and benzyloxy(3-benzyloxycarbonylaminopropoxy)(diisopropylamino)phosphine (0.80 g, 1.8 mmol) were added to a solution of **7** (0.64 g, 1.2 mmol) in CH_2Cl_2 (25 mL). The mixture was stirred at 25–30° for 16 h, then cooled to −40°, and *m*-chloroperoxybenzoic acid (0.52 g, 3.0 mmol) was added. The mixture was stirred at −40° for 3 min then at 0° for 12 min. Excess of *m*-chloroperoxybenzoic acid was destroyed by the addition of aq 10% Na_2SO_3 (20 mL), and the mixture was worked-up in the usual way. The crude product was chromatographed on silica gel (60 g) with light petroleum–

EtOAc (2:1) to afford **8** (0.88 g, 80%), isolated as a colorless gum. NMR data: ^{1}H, δ 1.50–1.63 (m, 2 H, $CH_2CH_2CH_2$), 2.92–3.14 (m, 2 H, CH_2CH_2NH), 3.20–3.40 (m, 3 H, 3 CH of inositol), 3.64 (t, 1 H, *J* 9.3 Hz, CH of inositol), 3.72 (dt, 1 H, *J* 2.8 and 9.5 Hz, CH of inositol), 3.86–4.05 [m, 2 H, $P(O)OCH_2$], 4.12–4.35 (m, 3 H, CH of inositol and CH_2=$CHCH_2$), 4.50–5.30 (m, 16 H, CH_2=$CHCH_2$, 5 $PhCH_2$, and 2 CH_2=CH), 5.82–6.03 (m, 2 H, 2 $CHCH_2$ in allyl), 7.20–7.50 (m, 25 H, 5 Ph).

*Benzyl (3-benzyloxycarbonylaminopropyl) (1,3,4-tri-*O*-benzyl-*myo*-inosit-2-yl) phosphate* (**9**).—Diisopropylethylamine (26 mg, 0.2 mmol) and tris(triphenylphosphine)rhodium chloride (93 mg, 0.1 mmol) were added to a solution of **8** (0.88 g, 1.0 mmol) in 95% EtOH (50 mL). The mixture was stirred and heated under reflux under N_2 for 90 min. The EtOH was evaporated in vacuo and the crude residue was chromatographed on silica gel (50 g) with EtOAc–light petroleum–triethylamine (100:100:1), to yield the isomerized intermediate as a light-brown oil (0.9 g). The prop-1-enyl ether groups were removed by heating the oil in MeOH (20 mL) and 1 M HCl (2 mL) at 45° for 45 min. Sodium hydrogen carbonate (1 g) was added, the solvent was evaporated, and the residue was extracted with CH_2Cl_2 (40 mL). The solvent was evaporated and the crude product was chromatographed on silica gel (40 g) with EtOAc–light petroleum (1:1 then 2:1), to give **9** (0.42 g, 52%), isolated as a colorless gum. NMR data: ^{1}H, δ 1.45–1.62 (m, 2 H), 2.97–3.10 (m, 2 H), 3.20–3.30 (m, 1 H), 3.30–3.56 (m, 3 H), 3.60–3.80 (m, 2 H), 3.85–4.00 (m, 2 H), 4.5–5.5 (m, 14 H), 7.1–7.5 (m, 25 H).

*Benzyl (3-benzyloxycarbonylaminopropyl) [1,3,4-tri-*O*-benzyl-5,6-di-*O*-(dibenzyloxyphosphoryl)-*myo*-inosit-2-yl] phosphate* (**10**).—Tetrazole (0.18 g, 2.6 mmol) and dibenzyloxy(diisopropylamino)phosphine (0.45 g, 1.3 mmol) were added to a solution of **9** (0.42 g, 0.5 mmol) in dry CH_2Cl_2 (15 mL). The mixture was stirred at 20° overnight (14 h), then cooled to −40°, and *m*-chloroperoxybenzoic acid (1.0 g, 5.8 mmol) was added. Stirring was continued at −40° for 5 min, 0° for 30 min, and 20° for 30 min. More CH_2Cl_2 (50 mL) was added and the mixture was worked-up in the usual way. The syrupy product was chromatographed on silica gel (40 g) with EtOAc–light petroleum (1.5:1), to yield **10** (0.68 g, 95%), isolated as a colorless syrup. ^{1}H NMR data: δ 1.54–1.78 (m, 2 H), 2.95–3.16 (m, 2 H), 3.32–3.48 (m, 2 H), 3.76–4.11 (m, 2 H), 4.40–5.14 (m, 22 H), 6.92–7.50 (m, 45 H).

*(±)-*myo*-Inositol 2-(3-aminopropyl hydrogen phosphate) 4,5-bis(disodium phosphate)* (**11**).—10% Pd/C (0.25 g) was added to a solution of **10** (0.62 g, 0.46 mmol) in 95% EtOH (60 mL) and hydrogenolysis was allowed to proceed at 50 psi for 7.5 h. The mixture was filtered through Celite which was then washed with EtOH (30 mL), 2:1 EtOH–water (30 mL), and water (30 mL). The combined filtrate and washings were basified with concd ammonia and the solvent was evaporated in vacuo at 20°. The residue was applied to a column (7 × 1.4 cm) of Chelex 100 (Na^+) resin and eluted with water (30 mL). Concentration of the eluate in vacuo gave amorphous **11** in quantitative yield. NMR data (D_2O): ^{1}H, δ 1.91 (quintet, 2 H, *J* 6.3 Hz, $CH_2CH_2CH_2$), 3.04 (t, 2 H, *J* 6.9 Hz, $CH_2CH_2NH_2$), 3.50 (d, 1 H, *J* 7.7 Hz, CH of inositol), 3.63 (d, 1 H, *J* 9.8 Hz, CH of inositol), 3.72–3.82 (m, 2 H,

P(O)OCH_2], 3.90–4.11 (m, 3 H, 3 CH of inositol), 4.41 (d, 1 H, J 8.3 Hz, H-2 of inositol); ^{13}C, δ 27.5, 37.2, 63.7, 70.4, 70.5, 72.5, 76.3, 77.5, 78.3; ^{31}P (^1H coupled), δ 2.50 (m), 6.31 (d, J 5.2 Hz), 6.58 (d, J 7.7 Hz). FABMS: m/z 565.9 $(M + H)^+$.

Preparation of affinity matrices from **5** *and* **11**.—Affi-Gel 10 resin (25 mL) was washed free of the storage solution following the manufacturer's instructions, then added to an aqueous solution of **5** or **11** (120 mg) in ice-cold 0.1 M $NaHCO_3$ (20 mL). The slurry was stirred at 5° overnight, then filtered, and the resin was washed well with ice–water and stored at 5° as a suspension in water. From the combined filtrate and washings ~ 70 mg of unreacted **5** or **11** was recovered after chromatography on DEAE cellulose, indicating the binding of ~ 2 μmol of the ligand/mL of gel.

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REFERENCES

1 M.J. Berridge, *Annu. Rev. Biochem.*, 56 (1987) 159–193; M.J. Berridge and R.F. Irvine, *Nature (London)*, 341 (1989) 197–203.
2 B.V.L. Potter, *Nat. Prod. Rep.*, (1990) 1–25; A.B. Reitz (Ed.), *ACS Symp Ser.*, 463 (1991).
3 R.F. Irvine, A.J. Letcher, J.P. Heslop, and M.J. Berridge, *Nature (London)*, 320 (1986) 631–634; C.A. Hansen, S. Mah, and J.R. Williamson, *J. Biol. Chem.*, 261 (1986) 8100–8103; A.J. Morris, K.J. Murray, P.J. England, C.P. Downes, and R.H. Michell, *Biochem. J.*, 251 (1988) 157–163; R.A. Johanson, C.A. Hansen, and J.R. Williamson, *J. Biol. Chem.*, 263 (1988) 7465–7471; R.A.J. Challis and S.R. Nahorski, *J. Neurochem.*, 54 (1990) 2138–2141; S.Y. Lee, S.S. Sim, J.W. Kim, K.H. Moon, J.H. Kim, and S.G. Rhee, *J. Biol. Chem.*, 265 (1990) 9434–9440.
4 G.D. Prestwich, J.F. Marecek, R.J. Mourey, A.B. Theibert, C.D. Ferris, S.K. Danoff, and S.H. Snyder, *J. Am. Chem. Soc.*, 113 (1991) 1822–1825.
5 V.A. Estevez and G.D. Prestwich, *Tetrahedron Lett.*, 32 (1991) 1623–1626.
6 J.F. Marecek and G.D. Prestwich, *Tetrahedron Lett.*, 32 (1991) 1863–1866.
7 V.A. Estevez and G.D. Prestwich, *J. Am. Chem. Soc.*, 113 (1991) 9885–9887; S.L. Bender and R.J. Budhu, *ibid.*, 113 (1991) 9883–9885.
8 A.B. Theibert, V.A. Estevez, C.D. Ferris, S.K. Danoff, R.K. Barrow, G.D. Prestwich, and S.H. Snyder, *Proc. Natl. Acad. Sci. U.S.A.*, 88 (1991) 3165–3169.
9 A.B. Theibert, V.A. Estevez, R.J. Mourey, J.F. Marecek, R.K. Barrow, G.D. Prestwich, and S.H. Snyder, *J. Biol Chem.*, 267 (1992) 9071–9079.
10 D.L. Kalinoski, S.B. Aldinger, A.G. Boyle, T. Huque, J.F. Marecek, G.D. Prestwich, and D. Restrepo, *Biochem. J.*, 281 (1992) 449–456.
11 C.E. Ballou and W. Tegge, *ACS Symp. Ser.*, 463 (1991) 33–42.
12 G.D. Prestwich and J.F. Marecek, *ACS Symp. Ser.*, 463 (1991) 122–131.
13 A.N. Jina, J. Ralph, and C.E. Ballou, *Biochemistry*, 29 (1990) 5203–5209.
14 M. Hirata, Y. Watanabe, T. Ishimatsu, T. Ikebe, Y. Kimura, K. Yamaguchi, S. Ozaki, and T. Koga, *J. Biol. Chem.*, 264 (1989) 20303–20308; M. Hirata, Y. Watanabe, T. Ishimatsu, F. Yanaga, T. Koga,

and S. Ozaki, *Biochem. Biophys. Res. Commun.*, 168 (1990) 379–386; M. Hirata, Y. Kimura, T. Ishimatsu, F. Yanaga, T. Shuto, T. Sasagur, T. Koga, Y. Watanabe, and S. Ozaki, *Biochem. J.*, 276 (1991) 333–336; Y. Watanabe, M. Hirata, T. Ogasawara, T. Koga, and S. Ozaki, *Bioorg. Med. Chem. Lett.*, 1 (1991) 399–402.

15 M.S. Shashidhar, J.F.W. Keana, J.J. Volwerk, and O.H. Griffith, *Chem. Phys. Lipids*, 56 (1990) 159–167.

16 R. Schafer, M. Nehls-Sahabandu, B. Grabowski, M. Dehlinger-Kremer, I. Schulz, and G.W. Mayr, *Biochem. J.*, 272 (1990) 817–825.

17 V. Henne, G.W. Mayr, B. Grabowski, B. Koppitz, and H.-D. Soling, *Eur. J. Biochem.*, 174 (1988) 95–101.

18 S.B. Shears, *Pharmacol. Ther.*, 49 (1991) 79–104.

19 T.M. Connolly, V.S. Bansal, T.E. Bross, R.F. Irivne, and P.W. Majerus, *J. Biol. Chem.*, 262 (1987) 2146–2149.

20 W. Tegge, G.V. Denis, and C.E. Ballou, *Carbohydr. Res.*, 217 (1991) 107–116.

21 G.M. Burgess, R.F. Irvine, M.J. Berridge, J.S. McKinney, and J.W. Putney, *Biochem. J.*, 224 (1984) 741–746; R.F. Irvine, K.D. Brown, and M.J. Berridge, *ibid.*, 222 (1984) 269–272.

22 J. Gigg, R. Gigg, S. Payne, and R. Conant, *J. Chem. Soc., Perkin Trans. 1*, (1987) 1757–1762; *ibid.* (1987) 2411–2414.

23 M.F. Boehm and G.D. Prestwich, *Tetrahedron Lett.*, 29 (1988) 5217–5220.

24 W. Bannwarth and A. Trzeciak, *Helv. Chim. Acta*, 70 (1987) 175–186; K.-L. Yu and B. Fraser-Reid, *Tetrahedron Lett.*, 29 (1988) 979–982.

25 J. Gigg, R. Gigg, S. Payne, and R. Conant, *J. Chem. Soc., Perkin Trans. 1*, (1987) 423–429.

26 E.J. Corey and J.W. Suggs, *J. Org. Chem.*, 38 (1973) 3224–3225.