Synthesis of the D-3 Series of Phosphatidylinositol Phosphates

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The 3-mono-, 3,4-bis-, and 3,4,5-trisphosphates of L-α-phosphatidyl-D-*myo*-inositol have been synthesized in their optically active forms from the two enantiomers of 1,2:5,6-di-*O*-cyclohexylidene-*myo*-inositol. These chiral precursors were prepared by a facile biocatalytic resolution, in which the 4-butyryl ester of the racemic compound was subjected to enantioselective hydrolysis by porcine pancreatic lipase in a biphasic system. The overall yield from individual chiral precursors ranged from 32% for the 3,4,5-trisphosphate to 39% for the monophosphate.

Introduction

Among various phospholipids in the plasma membrane, phosphatidylinositol polyphosphates have received much attention because of their pivotal role in signal transduction cascades.[1] Two phosphoinositide-mediated signaling pathways have been demonstrated, both of which originate from phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P_2) (Scheme 1). In the canonical pathway, the activation of phosphatidylinositol-specific phospholipase C (PtdIns-PLC) in response to agonist stimulation leads to a rapid production of D-*myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] and diacylglycerol (DAG) that elicit Ca^{2+} release and protein kinase C (PKC) activation, respectively.[2] The second pathway entails phosphoinositide 3-kinase (PI 3-kinase), of which the activation results in transient accumulations of phosphatidylinositol 3,4,5-trisphosphate [PtdIns(3,4,5)P_3] and phosphatidylinositol 3,4-bisphosphate [PtdIns(3,4)P_2]. This PI 3-kinase pathway has been implicated in diverse physiological responses to growth factors, including mitogenesis, chemotaxis, membrane trafficking, actin reorganization, receptor-down regulation, and cell survival.[3] Evidence is accumulating that PI 3-kinase lipid products are important cellular regulators. For example, PtdIns(3,4,5)P_3 and PtdIns(3,4)P_2, which are undetectable in quiescent cells, appear within seconds after agonist stimulation.[4] In addition, these D-3 phosphoinositides are not substrates for PLC, indicating that they do not serve as precursors to phosphoinositol second messengers.[5] Recent studies have shown that PtdIns(3,4,5)P_3 and PtdIns(3,4)P_2 activate Ca^{2+}-independent protein kinase isoforms δ, ϵ, and η.[6] This stimulatory effect in conjunction with the action of DAG is thought to exert a sustained PKC activation. Another PI 3-kinase product, phosphatidylinositol 3-monophosphate [PtdIns(3)P], has also been implicated in the activation of a protein kinase encoded by the *Akt* proto-oncogene via a pleckstrin homology (PH) domain.[7] Moreover, PtdIns(3,4,5)P_3 has been shown to disrupt the association of PI 3-kinase with tyrosine-phosphorylated proteins by binding to the Src homology 2 (SH2) domains of the p85 subunit.[8]

However, despite recent advances in characterizing the role of PI 3-kinase in diverse signaling pathways, information concerning the physiological targets for its lipid products is lacking, which, in part, is attributed to difficulty in obtaining these novel inositol lipids.[9] As part of our effort to understand the mode of action of PI 3-kinase, we have developed an effective chemoenzymatic synthesis of a series of D-3 phosphoinositides, including PtdIns(3)P, PtdIns(3,4)P_2, and PtdIns(3,4,5)P_3.

Results and Discussion

The syntheses entailed a pair of optically active 1,2:5,6-di-*O*-cyclohexylidene-*myo*-inositols **1** as precursors. These chiral intermediates were prepared by a facile enzymatic method, in which the 4-butyryl ester of racemic **1**, (±)-**2**, was subjected to enantioselective hydrolysis by porcine pancreatic lipase in a biphasic system (Scheme 2).[10]

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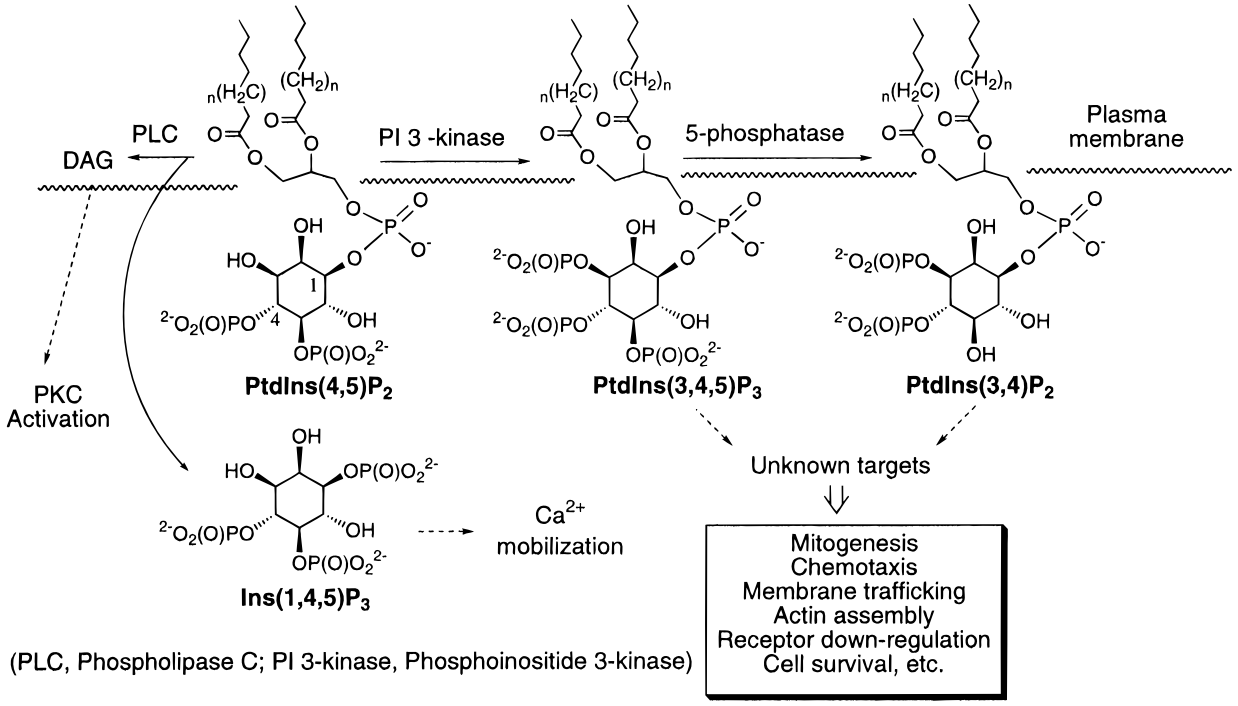
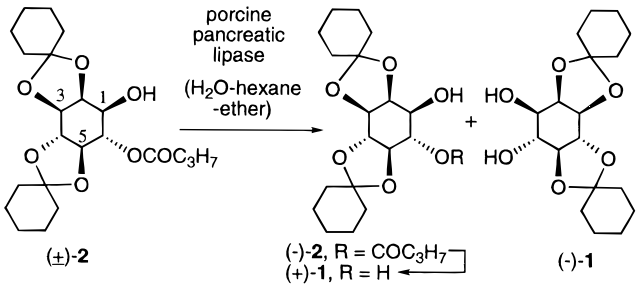
Scheme 1. Phosphoinositide-Mediated Signal Transduction



Scheme 2. Preparation of (+)- and (−)-1



This biocatalytic route is amenable to preparing optically active **1** in multigram quantities. Both enantiomers have been used for a systematic synthesis of inositol phosphates,[10] and their utility was extended to the synthesis of novel inositol phospholipids, which is illustrated as shown in Schemes 3 and 4.

(+)-**1** underwent selective 6-*O*-benzylation by reacting with di-*n*-butyltin oxide, followed by benzyl bromide and CsF, to afford (−)-**3**.[11] Subsequent 1-*O*-*p*-methoxybenzylation yielded (−)-**4** in 85% yield. Selective removal of the *trans*-5,6-cyclohexylidene group of **4** was achieved by briefly exposing to CH_3COCl/CH_3OH at room temperature. Since extended exposure of **4** to the acid resulted in the spontaneous hydrolysis of the *cis*-1,2-ketal, the reaction was carefully monitored by TLC and was stopped when all the substrate disappeared. The resulting compound (+)-**5** was subjected to bisbenzylation and then hydrolysis of the *cis*-cyclohexylidene to give (+)-**7**. The subsequent tin-mediated allylation proceeded with extremely high regioselectivity, exclusively at 3-OH, due to the steric hindrance at the axial 2-OH. The product (−)-**8** underwent 2-*O*-benzylation and then deallylation with 10% Pd/C and *p*-toluenesulfonic acid under reflux to yield the desirable alcohol (+)-**10**. Phosphorylation of **10** by reaction with dibenzyl *N*,*N*-diisopropylphosphoramidite and 1*H*-tetrazole, followed by *m*-CPBA, afforded the corresponding 3-(dibenzyl phosphate) (−)-**11**. The 1-*O*-(*p*-methoxybenzyl) function was removed by 10% trifluoroacetic acid (TFA) in CH_2Cl_2 (v/v) to furnish (−)-**12** in order for the subsequent formation of the phosphodiester linkage at the C-1 position. Reaction of **12** with 1,2-di-*O*-palmitoyl-*sn*-glycerol 3-(benzyl *N*,*N*-diisopropylphosphoramidite) (**13**)[12] in the presence of 1*H*-tetrazole, followed by *m*-CPBA, gave the perbenzylated derivative (−)-**14** that underwent hydrogenolysis to afford PtdIns(3)P, with an overall yield of 39% from (+)-**1**.

While (+)-**1** served as a useful precursor to PtdIns(3)P, the efficient use was made of its enantiomer (−)-**1** to prepare PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3 via intermediates (+)-**15** and (−)-**22**, respectively (Scheme 4). These two chiral intermediates have served as key intermediates in our previous synthesis of D-*myo*-inositol 1,3,4-trisphosphate [Ins(1,3,4)P_3] and D-*myo*-inositol 1,3,4,5-tetrakisphosphate [Ins(1,3,4,5)P_4], respectively.[10a]

As shown, regioselective 1-*O*-*p*-methoxybenzylation of (+)-**15** via the aforementioned stannylidene activation followed by 2-*O*-benzylation afforded a fully protected intermediate (+)-**17**. Deallylation of **17** gave the diol (+)-**18** that was subjected to phosphorylation and then removal of the *p*-methoxybenzyl group to yield the key compound (+)-**20**. Coupling of this intermediate with the 1,2-di-*O*-palmitoyl-*sn*-glycerophosphoryl moiety according to the above method gave (−)-**21**. Subsequent hydrogenolysis of the perbenzylated derivative generated PtdIns(3,4)P_2. The overall yield from (+)-**15** was 58%.

The synthetic route for PtdIns(3,4,5)P_3 from (−)-**22** paralleled that converting (+)-**15** to PtdIns(3,4)P_2. Accordingly, it was synthesized with an overall yield of 59%.

The above synthetic strategy provides an effective access to the D-3 phosphoinositides in optically active form. With these compounds in hand, our current research focuses on examining their modulatory effects on various biomolecules. For example, we have found

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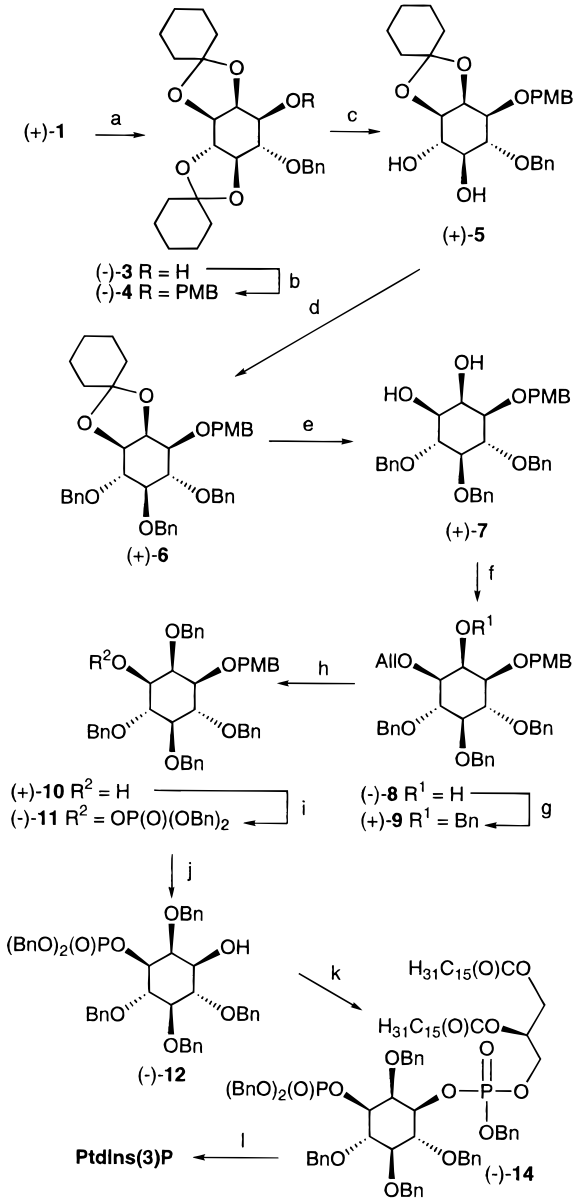
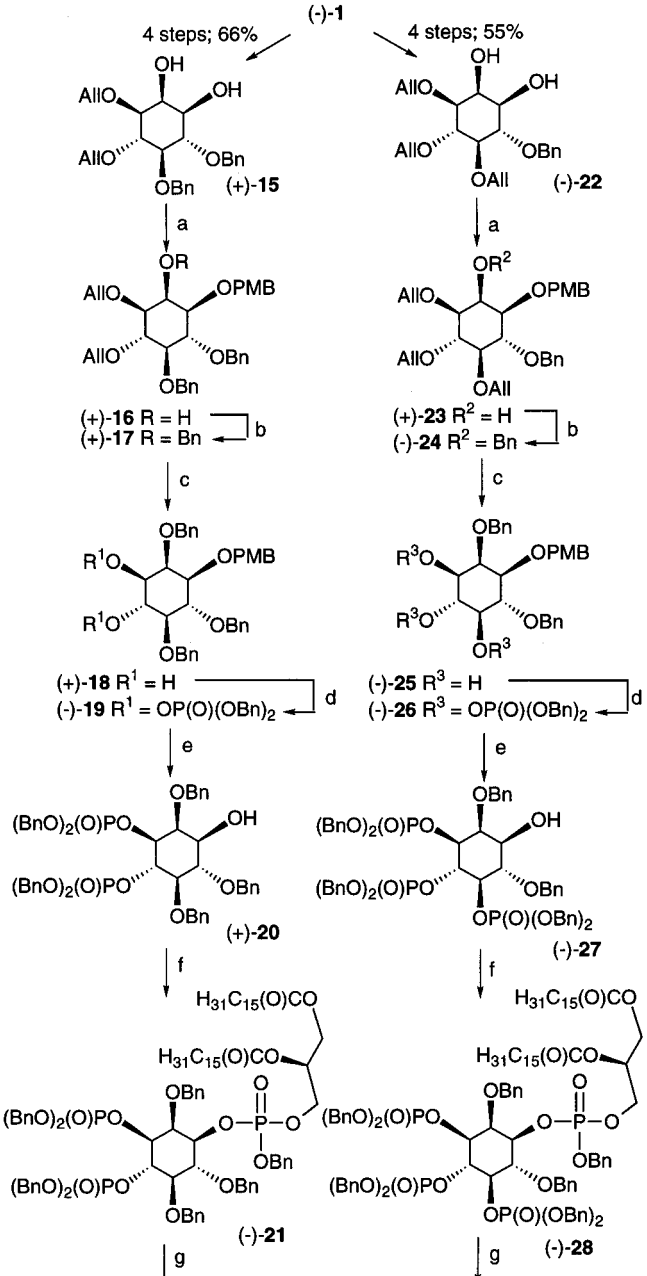
Scheme 3. Synthesis of PtdIns(3)P from (+)-1[a]



[a] Key: (a) *n*-Bu_2SnO, BnBr, CsF; 88%; (b) NaH, PMBCl; 97%; (c) AcCl−CH_3OH; 82%; (d) NaH, BnBr; 97%; (e) AcCl−CH_3OH; 92%; (f) *n*-Bu_2SnO, AllBr, CsF; 95%; (g) NaH, BnBr; 96%; (h) Pd/C, TsOH; 88%; (i) $(BnO)_2PN(i\text{-}Pr)2$, 1*H*-tetrazole, *m*-CPBA; 93%; (j) TFA; 95%; (k) **13**, 1*H*-tetrazole, *m*-CPBA, 89%; (l) palladium black, H_2; 98%.

Scheme 4. Synthesis of PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3[a]



[a] Key: (a) *n*-Bu_2SnO, PMBCl, CsF; (b) NaH, BnBr; (c) Pd/C, TsOH; (d) $(BnO)_2PN(i\text{-}Pr)_2$, 1*H*-tetrazole, *m*-CPBA; (e) TFA; (f) **13**, 1*H*-tetrazole, *m*-CPBA; (g) palladium black, H_2.

that PtdIns(3,4,5)P_3 and/or PtdIns(3,4)P_2 bind profilin and related actin-regulating proteins with high affinity (Chen, C.-S. Unpublished data), thereby providing a possible link between PI 3-kinase and cytoskeletal rearrangement. Investigations on the interactions of these signaling molecules with other potential targets are underway in this laboratory.

Experimental Section

Materials and Methods. Racemic 1,2:5,6-di-*O*-cyclohexylidene-*myo*-inositol ((±)-**1**) was prepared according to the procedure by Garegg *et al.*[13] Enantiomerically active **1** was prepared with good yield by an enzymatic method in which the 6-butyryl ester of **1**, (±)-**2**, was subjected to enantioselective hydrolysis by porcine pancreatic lipase (Sigma; Type II) in a biphasic system consisting of hexane−ether/water.[10] The optical purity of (+)- and (−)-**1** thus prepared was greater than 98% enantiomeric excess after recrystallization. 1,2-Di-*O*-palmitoyl-*sn*-glycerol 3-(benzyl *N*,*N*-diisopropylphosphoramidite) (**13**) was prepared using a method described by Dreef *et al.*[11] (+)-3,4-Di-*O*-allyl-5,6-di-*O*-benzyl-*myo*-inositol ((+)-**15**) and (−)-3,4,5-tri-*O*-allyl-6-*O*-benzyl-*myo*-inositol ((−)-**22**) were synthesized from (−)-**1** as previously described.

(−)-6-*O*-Benzyl-2,3:4,5-di-*O*-cyclohexylidene-*myo*-inositol (3). A mixture of (+)-**1** (255 mg, 0.75 mmol), Bu_2SnO (205 mg, 0.83 mmol), and toluene (25 mL) was stirred under reflux, with azeotropic removal of water, for 2 h, and then concentrated to dryness. To the residue were added DMF (6 mL), CsF (285 mg, 1.9 mmol), and benzyl bromide at −15 °C. After being stirred at −15 °C for 1 h, the reaction mixture was allowed to warm to room temperature and stirred for 16 h.

(13) Garegg, P. J.; Iversen, T.; Johansson, R.; Lindberg, B. *Carbohydr. Res.* **1984**, *130*, 322.

The solution was then diluted with CH_2Cl_2 (25 mL), washed with water, dried, and concentrated. Column chromatography (hexane−ether, 20:1 → 10:1) of the residue gave (−)-**3** (syrup, 284 mg, 88%): $[\alpha]^{23}_D = -4°$ (*c* 1.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.44−1.75 (m, 20 H), 2.65 (d, J = 1.5 Hz, 1 H), 3.58 (dd, J = 7.6, 10.5 Hz, 1 H), 3.93 (dd, J = 2, 7.8 Hz, 1 H), 4.06−4.07 (m, 1 H), 4.23 (dd, J = 7.6, 10.5 Hz, 1 H), 4.38 (t, J = 7.5 Hz, 1 H), 4.46 (dd, J = 3.6, 7.5 Hz, 1 H), 4.75 (q, J = 11.8, 35 Hz, 2 H), 7.28−7.43 (m, 5 H); MS (EI) *m*/*z* (rel inten) 430 (M^+, 13), 340 (52), 91 (100).

(−)-6-*O*-Benzyl-2,3:4,5-di-*O*-cyclohexylidene-1-*O*-(*p*-methoxybenzyl)-*myo*-inositol (4). A solution of (−)-**3** (268 mg, 0.62 mmol) in DMF (4 mL) was treated with NaH (14 mg, 85%, 0.93 mmol) at 0 °C under argon for 30 min, followed by *p*-methoxybenzyl bromide (140 μL, 0.93 mmol) at 40 °C overnight. Excess NaH was destroyed with CH_3OH, and the solution was diluted with ethyl acetate (50 mL), washed with water, dried, and concentrated. Column chromatography (hexane-ether, 25:1) of the residue afforded (−)-**4** (syrup, 330 mg, 97%): $[\alpha]^{23}_D = -12.1°$ (*c* 0.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.40−1.75 (m, 20 H), 3.47 (dd, J = 7.8, 10.5 Hz, 1 H), 3.73 (t, J = 7.5 Hz, 1 H), 3.79−3.85 (m, 4 H), 4.08 (dd, J = 3.3, 7.3 Hz, 1 H), 4.28−4.37 (m, 2 H), 4.38−4.67 (m, 4 H), 6.84−6.89 (m, 2 H), 7.24−7.34 (m, 7 H); MS (EI) *m*/*z* (rel inten) 550 (M^+, 8), 459 (23), 429 (17), 339 (11), 121 (100).

(+)-6-*O*-Benzyl-2,3-*O*-cyclohexylidene-1-*O*-(*p*-methoxybenzyl)-*myo*-inositol (5). A solution of (−)-**4** (304 mg, 0.55 mmol) in $CH_3OH-CH_2Cl_2$ (1:3, 12 mL) was stirred with acetyl chloride (15 μL) at 23 °C for 15 min. Trimethylamine (60 μL) was added, and the solution was concentrated. Column chromatography (hexane−ether, 10:1 → 2:1) of the residue yielded (+)-**5** (syrup, 214 mg, 82%): $[\alpha]^{23}_D = +13.7°$ (*c* 0.68, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.42−1.76 (m, 10 H), 2.74 (br. s, 2H), 3.29 (dd, J = 8.5, 10 Hz, 1 H), 3.65−3.75 (m, 3 H), 3.77 (s, 3 H), 3.91 (dd, J = 7.5, 12.7 Hz, 1 H), 4.27 (q, J = 3.8, 5.1 Hz, 1 H), 4.70 (t, J = 4.9 Hz, 3 H), 4.98 (d, J = 11.2 Hz, 1 H), 6.88−6.98 (m, 2 H), 7.27−7.37 (m, 7H); MS (EI) *m*/*z* (rel inten) 470 (M^+, 9), 469 (12), 379 (5), 349 (20), 121 (100).

(+)-4,5,6-Tri-*O*-benzyl-2,3-*O*-cyclohexylidene-1-*O*-(*p*-methoxybenzyl)-*myo*-inositol (6). Compound (+)-**5** (200 mg, 0.42 mmol) was benzylated, as described for (−)-**4**, to yield (+)-**6** (syrup, 269 mg, 97%): $[\alpha]^{23}_D = +32.3°$ (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.42−1.79 (m, 10 H), 3.40 (dd, J = 8.6, 9.6 Hz, 1 H), 3.67 (dd, J = 3.6, 8.6 Hz, 1 H), 3.77−3.83 (m, 4 H), 3.92 (t, J = 8.4 Hz, 1 H), 4.08 (dd, J = 6.6, 6.8 Hz, 1 H), 4.23 (dd, J = 3.9, 5.4 Hz, 1 H), 4.65−4.92 (m, 8H), 6.84−6.87 (m, 2 H), 7.26−7.36 (m, 17H); MS (EI) *m*/*z* (rel inten) 650 (M^+, 7), 560 (13), 530 (8), 121 (100).

(+)-4,5,6-Tri-*O*-benzyl-1-*O*-(*p*-methoxybenzyl)-*myo*-inositol (7). Compound (+)-**6** (200 mg, 0.31 mmol) was treated with acetyl chloride (25 μL) in $CH_2Cl_2-CH_3OH$ (1:3, 6 mL) at 23 °C for 30 min, and then triethylamine (100 μL) was added, and the solution was concentrated. Column chromatography (hexane-ether, 10:1 → 1:1) of the residue afforded (+)-**7** (amorphous, 161 mg, 92%): $[\alpha]^{23}_D = +16.8°$ (*c* 1.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 2.45 (br s, 2 H), 3.42−3.50 (m, 3 H), 3.80 (s, 3 H), 3.83 (t, J = 9.9 Hz, 1 H), 3.94 (t, J = 9.6 Hz, 1 H), 4.17 (t, J = 3 Hz, 1 H), 4.64 (d, J = 1.5 Hz, 2 H), 4.72−4.97 (m, 6 H), 6.83−6.86 (m, 2 H), 7.24−7.33 (m, 17 H); MS (EI) *m*/*z* (rel inten) 570 (M^+, 9), 480 (17), 450 (9), 121 (100).

Anal. Calcd for $C_{35}H_{38}O_7$: C, 73.70; H, 6.67. Found: C, 73.59, H 6.82.

(−)-3-*O*-Allyl-4,5,6-tri-*O*-benzyl-1-*O*-(*p*-methoxybenzyl)-*myo*-inositol (8). Regioselective allylation of (+)-**7** (140 mg, 0.24 mmol) with Bu_2SnO and CsF, as described for (−)-**3**, yielded (−)-**8** (amorphous, 142 mg, 95%): $[\alpha]^{23}_D = -3.2°$ (*c* 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 2.45 (br s, 1 H), 3.27 (dd, J = 2.7, 9.8 Hz, 1 H), 3.38 (dd, J = 3, 9.6 Hz, 1 H), 3.43 (t, J = 9.6 Hz, 1 H), 3.80 (s, 3 H), 3.91−3.99 (m, 2 H), 4.17−4.20 (m, 3 H), 4.66 (s, 2 H), 4.78−4.91 (m, 6 H), 5.17 (dd, J = 1.2, 10.5 Hz, 1 H), 5.26 (dd, J = 1.5, 17.4 Hz, 1 H), 5.87−6.00 (m, 1 H), 6.84−6.87 (m, 2 H), 7.26−7.31 (m, 17 H); MS (EI) *m*/*z* (rel inten) 610 (M^+, 5), 570 (18), 520 (7), 490 (4), 91 (100).

Anal. Calcd for $C_{38}H_{42}O_7$: C, 74.77; H, 6.88. Found: C, 74.49, H 6.95.

(+)-3-*O*-Allyl-2,4,5,6-tetra-*O*-benzyl-1-*O*-(*p*-methoxybenzyl)-*myo*-inositol (9). Conventional benzylation of (−)-**8** (120 mg, 0.2 mmol), as described for (−)-**4**, gave (+)-**9** (syrup, 138 mg, 96%): $[\alpha]^{23}_D = +2.1°$ (*c* 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 3.23 (dd, J = 2.1, 9.9 Hz, 1 H), 3.33 (dd, J = 2.4, 9.9 Hz, 1 H), 3.45 (t, J = 9.3 Hz, 1 H), 3.81 (s, 3 H), 4.00−4.10 (m, 5 H), 4.57 (m, 2 H), 4.77−4.93 (m, 8 H), 5.16 (dd, J = 1.2, 10.5 Hz, 1 H), 5.27 (dd, J = 1.5, 17.1 Hz, 1 H), 5.85−5.97 (m, 1 H), 6.83−6.86 (m, 2 H), 7.23−7.44 (m, 22 H); MS (EI) *m*/*z* (rel inten) 700 (M^+, 8), 660 (32), 610 (11), 580 (8), 91 (100).

(+)-2,4,5,6-Tetra-*O*-benzyl-1-*O*-(*p*-methoxybenzyl)-*myo*-inositol (10). A mixture of (+)-**9** (120 mg, 0.17 mmol), Pd/C (20 mg), *p*-toluenesulfonic acid (20 mg, 0.1 mmol), and CH_3OH−water (4:1, 5 mL) was stirred under reflux for 2 h, filtered, and concentrated. Column chromatography (hexane−ether, 20:1 → 10:1) of the residue furnished (+)-**10** (syrup, 100 mg, 88%): $[\alpha]^{23}_D = +7.5°$ (*c* 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 2.19 (d, J = 6.3 Hz, 1 H), 3.42−3.51 (m, 3 H), 3.80 (t, J = 9.3 Hz, 1 H), 3.81 (s, 3 H), 3.99−4.07 (m, 2 H), 4.62−5.01 (m, 10 H), 6.83−6.86 (m, 2 H), 7.24−7.36 (m, 22 H); MS (EI) *m*/*z* (rel inten) 660 (M^+, 4), 659 (9), 569 (15), 539 (10), 121 (100).

(−)-2,4,5,6-Tetra-*O*-benzyl-1-*O*-(*p*-methoxybenzyl)-*myo*-inositol 3-(Dibenzyl phosphate) (11). A solution of 1*H*-tetrazole (33.6 mg, 0.48 mmol) and dibenzyl *N*,*N*-diisopropylphosphoramidite (82.6 mg, 0.24 mmol) in CH_2Cl_2 (2 mL) was stirred at 23 °C under argon for 30 min, and (+)-**10** (80 mg, 0.12 mmol) was added in one portion. The mixture was kept under the same conditions for another 12 h, cooled to −40 °C, and then treated with *m*-chloroperoxybenzoic acid (145 mg, 57% purity, 0.48 mmol). The mixture was stirred at −40 °C for 30 min and then allowed to attain room temperature, diluted with CH_2Cl_2 (25 mL), washed with, in tandem, 10% Na_2SO_3 solution, 10% $NaHCO_3$, and water, dried, and concentrated. Column chromatography (hexane−ether, 25:1 → 0:1) of the residue yielded (−)-**11** (syrup, 104 mg, 93%): $[\alpha]^{23}_D = -6.3°$ (*c* 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 3.34−3.48 (m, 3 H), 3.77−3.83 (m, 4 H), 3.98−4.08 (m, 1 H), 4.23−4.30 (m, 1 H), 4.42−4.56 (m, 2 H), 4.67−5.10 (m, 12 H), 6.80−6.84 (m, 2 H), 7.20−7.32 (m, 32 H); ^{31}P NMR ($CDCl_3$, H_3PO_4 as external standard) δ −1.19; MS (FAB) *m*/*z* (rel inten) 921 (M, 100), 831 (31), 800 (42), 740 (18).

(−)-2,4,5,6-Tetra-*O*-benzyl-*myo*-inositol 3-(Dibenzyl phosphate) (12). Removal of the *p*-methoxybenzyl group was achieved by exposing (−)-**11** (80 mg, 0.087 mmol) to trifluoroacetic acid (TFA) (100 mg, 0.87 mmol) in CH_2Cl_2 (1 mL) at room temperature. After 1 h, triethylamine (100 μL) was added to stop the reaction, and the solution was concentrated. Column chromatography of the residue afforded (−)-**12** (syrup, 66 mg, 95%): $[\alpha]^{23}_D = -5.4°$ (*c* 2.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 2.07 (d, J = 6 Hz, 1 H), 3.43−3.49 (m, 2 H), 3.78 (t, J = 9.6 Hz, 1 H), 4.02 (t, J = 9.6 Hz, 1 H), 4.17 (t, J = 2.4 Hz, 1 H), 4.26−4.33 (m, 1 H), 4.71−4.97 (m, 12 H), 7.22−7.32 (m, 30 H); ^{31}P NMR ($CDCl_3$, H_3PO_4 as external standard) δ −0.664; MS (FAB) *m*/*z* (rel inten) 800 (M, 82), 799 (100), 709 (43), 619 (16).

(−)-1-*O*-(1,2-Di-*O*-palmitoyl-*sn*-glycerol-3-benzyloxyphosphoryl)-2,4,5,6-tetra-*O*-benzyl-*myo*-inositol 3-(Dibenzyl phosphate) (14). A solution of 1,2-di-*O*-palmitoyl-*sn*-glycerol 3-(benzyl *N*,*N*-diisopropylphosphoramidite) (**13**) (221 mg, 0.14 mmol) and 1*H*-tetrazole (43 mg, 0.28 mmol) in CH_2Cl_2 (4 mL) was stirred at 23 °C under argon for 30 min, and (−)-**12** (55 mg, 0.07 mmol) was added. The mixture was kept under the same conditions for another 12 h, cooled to −40 °C, and then treated with *m*-chloroperoxybenzoic acid (85 mg, 57% purity, 0.28 mmol). The mixture was stirred at −40 °C for 30 min, allowed to attain room temperature for 1 h, and concentrated. Column chromatography (hexane−ether, 25:1 → 0:1) of the residue yielded (−)-**14** (syrup, 93 mg, 89%): $[\alpha]^{23}_D = -3.6°$ (*c* 0.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.89 (t, J = 6.3 Hz, 6 H), 1.21−1.28 (m, 48 H), 1.40−1.58 (m, 4 H), 2.13−2.30 (m, 4 H), 3.43 (dd, J = 7.8, 10.5 Hz, 1 H), 3.50−3.62 (m, 1 H), 3.75−3.90 (m, 1 H), 3.92−4.17 (m, 4 H), 4.18−4.36 (m, 1 H), 4.62−5.18 (m, 17 H), 7.14−7.42 (m, 35 H); ^{31}P NMR ($CDCl_3$, H_3PO_4 as external standard) δ −1.13, −1.28; MS (FAB) *m*/*z* (rel intent) 1519.3 (M, 8), 968 (18), 551 (63) 91 (100).

L-α-Phosphatidyl-D-*myo*-inositol 3-Phosphate, Dipalmitoyl [PtdIns(3)P]. A solution of (−)-**14** (90 mg, 0.06 mmol) and palladium black (72 mg) in aqueous 80% EtOH (2 mL) was shaken under H_2 (50 psi) for 24 h, filtered, and concentrated. The residual aqueous solution was lyophilized to furnish PtdIns(3)P (52 mg, 98%): $[\alpha]^{23}_D = -1.2°$ (*c* 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.89 (t, J = 7.2 Hz, 6 H), 1.10−1.32 (m, 48 H), 1.49−1.66 (m, 4 H), 2.28−2.39 (m, 4 H), 3.40−3.80 (m, 6 H), 4.06−4.24 (m, 5 H); ^{31}P NMR ($CDCl_3$, H_3PO_4 as external standard) δ −0.66, 1.65; MS (negative ion FAB) *m*/*z* (rel inten) 889.5 (M − H, 6), 651 (19), 255.2 (18), 183 (100).

(+)-3,4-Di-*O*-allyl-5,6-di-*O*-benzyl-*O*-(*p*-methoxybenzyl)-*myo*-inositol (16). Selective introduction of a *p*-methoxybenzyl function at the C-1 of (+)-**15** (160 mg, 0.36 mmol), as described for (−)-**3**, yielded (+)-**16** (syrup, 195 mg, 96%): $[\alpha]^{23}_D = +14°$ (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 2.67 (d, J = 4.2 Hz, 1 H), 3.51 (dd, J = 3, 10 Hz, 1 H), 3.62−3.81 (m, 2 H), 4.08−4.18 (m, 5 H), 4.20−4.30 (m, 1 H), 4.45−4.58 (m, 2 H), 4.61−4.70 (m, 2 H), 4.90−5.01 (m, 2 H), 5.12−5.23 (m, 4 H), 5.40−5.65 (m, 4 H), 6.21−6.42 (m, 2 H), 6.81−6.87 (m, 2 H), 7.24−7.43 (m, 12 H); MS (EI) *m*/*z* (rel inten) 559.3 (M^+ − H, 18), 469.3 (1.2), 439.2 (2.3), 121 (100).

(+)-3,4-Di-*O*-allyl-2,5,6-tri-*O*-benzyl-1-*O*-(*p*-methoxybenzyl)-*myo*-inositol (17). Benzylation of (+)-**16** (170 mg, 0.3 mmol), as described for (+)-**6**, gave (+)-**17** (syrup, 193 mg, 98%): $[\alpha]^{23}_D = +10°$ (*c* 1.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 3.24 (dd, J = 3, 10 Hz, 1 H), 3.40−3.60 (m, 2 H), 3.80−4.01 (m, 4 H), 4.08−4.30 (m, 4 H), 4.36−4.40 (m, 2 H), 4.61−4.73 (m, 2 H), 4.91−5.12 (m, 6 H), 5.20−5.50 (m, 4 H), 5.97−6.18 (m, 2 H), 6.81−6.87 (m, 2 H), 7.23−7.58 (m, 17 H); MS (EI) *m*/*z* (rel inten) 649.3 (M^+ − H, 2.3), 559.3 (1.6), 529.3 (2.4), 121 (100).

(+)-2,5,6-Tri-*O*-benzyl-1-*O*-(*p*-methoxybenzyl)-*myo*-inositol (18). Deallylation of (+)-**17** (176 mg, 0.27 mmol) with Pd/C and PTSA, as described for (+)-**10**, yielded (+)-**18** (amorphous, 139 mg, 90%): $[\alpha]^{23}_D = +5.4°$ (*c* 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 2.21 (br s, 2 H), 3.42−3.51 (m, 3 H), 3.77−3.83 (m, 4 H), 3.99−4.07 (m, 2 H), 4.62−5.01 (m, 8 H), 6.81−6.87 (m, 2 H), 7.23−7.38 (m, 17 H); MS (FAB) *m*/*z* (rel inten) 593.3 (M + Na, 100), 121 (38).

Anal. Calcd for $C_{35}H_{38}O_7$: C, 73.70; H, 6.67. Found: C, 73.00, H 6.54.

(−)-2,5,6-Tri-*O*-benzyl-1-*O*-(*p*-methoxybenzyl)-*myo*-inositol 3,4-Bis(dibenzyl phosphate) (19). Phosphorylation of (+)-**18** (150 mg, 0.26 mmol) via the phosphoramidite method, as described for (−)-**11**, provided (−)-**19** (syrup, 250 mg, 88%): $[\alpha]^{23}_D = -7.3°$ (*c* 0.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 3.34−3.48 (m, 3 H), 3.78−3.83 (m, 4 H), 3.98−4.07 (m, 1 H), 4.21−4.30 (m, 1 H), 4.41−4.54 (m, 2 H), 4.69−5.10 (m, 14 H), 6.81−6.86 (m, 2 H), 7.24−7.38 (m, 37 H); ^{31}P NMR ($CDCl_3$, H_3PO_4 as external standard) δ −1.306, −0.856; MS (FAB) *m*/*z* (rel inten) 1090 (M, 100), 121 (34).

(+)-2,5,6-Tri-*O*-benzyl-*myo*-inositol 3,4-Bis(dibenzyl phosphate) (20). Removal of the *p*-methoxybenzyl function of (−)-**19** (230 mg, 0.21 mmol) with TFA, as described for (−)-**12**, gave (+)-**20** (syrup, 190 mg, 93%): $[\alpha]^{23}_D = +1.8°$ (*c* 1.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.80−2.41 (br s, 1 H), 3.38−3.50 (m, 2 H), 3.52−3.70 (m, 1 H), 3.75−3.88 (m, 1 H), 4.20−4.32 (m, 2 H), 4.63−4.74 (m, 2 H), 4.76−4.85 (m, 2 H), 4.86−5.12 (m, 10 H), 7.23−7.42 (m, 35 H); ^{31}P NMR ($CDCl_3$, H_3PO_4 as external standard) δ −1.151, −0.699; MS (FAB) *m*/*z* (rel inten) 971.5 (M, 100), 881.4 (14), 791 (3).

(−)-1-*O*-(1,2-Di-*O*-palmitoyl-*sn*-glycerol-3-benzyloxyphosphoryl)-2,5,6-tri-*O*-benzyl-*myo*-inositol 3,4-Bis(dibenzyl phosphate) (21). Coupling of (+)-**20** (170 mg, 0.175 mmol) with the 1,2-di-*O*-palmitoyl-*sn*-glycerophosphoryl moiety, as described for (−)-**14**, yielded (−)-**21** (syrup, 254 mg, 86%): $[\alpha]^{23}_D = -8.3°$ (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.89 (t, J = 7.2 Hz, 6 H), 1.18−1.40 (m, 48 H), 1.43−1.62 (m, 4 H), 2.26−2.40 (m, 4 H), 3.48−3.55 (m, 2 H), 3.63−3.75 (m, 1 H), 3.80−4.40 (m, 8 H), 4.60−5.15 (m, 16 H), 7.01−7.41 (m, 40 H); ^{31}P NMR ($CDCl_3$, H_3PO_4 as external reference) δ −1.367 (0.25 P), −1.337 (0.5 P), −1.145 (0.25 P), −0.995 (1 P), −0.704 (1 P); MS (negative ion FAB) *m*/*z* (rel inten) 969.3 (M − H, 4), 731.2 (6), 255.2 (100).

L-α-Phosphatidyl-D-*myo*-inositol 3,4-Bisphosphate, Dipalmitoyl [PtdIns(3,4)P_2]. The perbenzylated derivative (−)-**21** (236 mg, 0.14 mmol) was subjected to hydrogenolysis, as described for PtdIns(3)P, to afford PtdIns(3,4)P_2 (lyophilized powder, 133 mg, 98%): $[\alpha]^{23}_D = +1.9°$ (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.89 (t, J = 7.2 Hz, 6 H), 1.13−1.35 (m, 48 H), 1.48−1.62 (m, 4 H), 2.25−2.40 (m, 4 H), 3.42−3.52 (m, 1 H), 3.61−3.70 (m, 2 H), 3.72−3.82 (m, 3 H), 4.02−4.20 (m, 3 H), 4.51 (m, 1 H), 5.32 (m, 1 H); ^{31}P NMR ($CDCl_3$, H_3PO_4 as external reference) δ −1.402, −0.739, −0.327; MS (negative ion FAB) *m*/*z* (rel inten) 969.3 (M − H, 4), 731.2 (6), 255.2 (100).

(+)-3,4,5-Tri-*O*-allyl-6-*O*-benzyl-1-*O*-(*p*-methoxybenzyl)-*myo*-inositol (23). Regioselective protection at the 1-OH of (−)-**22** (140 mg, 0.3 mmol) with a *p*-methoxybenzyl group, as described for (+)-**15**, gave (+)-**23** (syrup, 146 mg, 96%): $[\alpha]^{23}_D = +8.2°$ (*c* 0.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 2.44 (s, 1H), 3.12 (dd, J = 2.3, 9.9 Hz, 1 H), 3.21−3.30 (m, 2 H), 3.78−3.84 (m, 4 H), 3.91−3.97 (m, 2 H), 4.04−4.08 (m, 2 H), 4.25−4.35 (m, 4 H), 4.77−4.87 (m, 4 H), 5.12−5.17 (m, 2 H), 5.23−5.31 (m, 2 H), 5.83−6.02 (m, 2 H), 6.81−6.86 (m, 2 H), 7.21−7.43 (m, 7 H); MS (EI) *m*/*z* (rel inten) 509.4 [(M − H)$^+$, 3], 469.4 (13), 419.4 (21), 389.2 (9), 91 (100).

(−)-3,4,5-Tri-*O*-allyl-2,6-di-*O*-benzyl-1-*O*-(*p*-methoxybenzyl)-*myo*-inositol (24). Conventional benzylation of (+)-**23**, as described for (+)-**6**, yielded (−)-**24** (syrup, 140 mg, 98%): $[\alpha]^{23}_D = -4°$ (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 3.14 (dd, J = 2.3, 9.4 Hz, 1 H), 3.21−3.30 (m, 2 H), 3.78−3.84 (m, 4 H), 3.91−3.97 (m, 2 H), 4.04−4.08 (m, 2 H), 4.25−4.35 (m, 4 H), 4.50−4.59 (m, 2 H), 4.77−4.87 (m, 4 H), 5.12−5.17 (m, 2 H), 5.23−5.31 (m, 2 H), 5.83−6.02 (m, 2 H), 6.81−6.86 (m, 2 H), 7.21−7.43 (m, 12 H); MS (EI) *m*/*z* (rel inten) 600 (M$^+$, 18), 560 (29), 510 (28), 480 (17), 420 (9), 121 (100).

(−)-2,6-Di-*O*-benzyl-1-*O*-(*p*-methoxybenzyl)-*myo*-inositol (25). Deallylation of (−)-**24** (120 mg, 0.20 mmol) with Pd/C and PTSA, as described for (+)-**10**, gave (−)-**25** (amorphous, 79 mg, 82%): $[\alpha]^{23}_D = -15°$ (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.6 (br s, 1 H), 2.4 (br s, 1 H), 2.7 (br s, 1 H), 3.45 (t, J = 9.2 Hz, 1 H), 3.54 (m, 1 H), 3.74−3.81 (m, 4 H), 3.89 (t, J = 9.2 Hz, 1 H), 4.06 (t, J = 2.7 Hz, 1 H), 4.65−4.87 (m, 5 H), 6.81−6.86 (m, 2 H), 7.25−7.37 (m, 12 H); MS (EI) *m*/*z* (rel inten) 479.3 [M$^+$ − H, 4], 389.3 (6), 359.1 (28), 299.6 (4), 121 (100).

Anal. Calcd for $C_{28}H_{32}O_7$: C, 69.98; H, 6.71. Found: C, 69.90, H 6.46.

(−)-2,6-Di-*O*-benzyl-1-*O*-(*p*-methoxybenzyl)-*myo*-inositol 3,4,5-Tris(dibenzyl phosphate) (26). Phosphorylation of (−)-**25** (60 mg, 0.125 mmol) via the phosphoramidite method, as described for (−)-**11**, provided (−)-**26** (syrup, 150 mg, 95%): $[\alpha]^{23}_D = -12.3°$ (*c* 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 3.38−3.49 (m, 3 H), 3.78−3.84 (m, 4 H), 3.96−4.04 (m, 1 H), 4.19−4.28 (m, 1 H), 4.42−4.48 (m, 2 H), 4.65−5.10 (m, 16 H), 6.81−6.86 (m, 2 H), 7.24−7.37 (m, 42 H); ^{31}P NMR ($CDCl_3$, H_3PO_4 as external reference) δ −2.23, −1.76, −1.56; MS (FAB) *m*/*z* (rel inten) 1260 (M, 100), 1170 (40), 1140 (33), 1080 (19), 1050(8).

(−)-2,6-Tri-*O*-benzyl-*myo*-inositol 3,4,5-Tris(dibenzyl phosphate) (27). Removal of the *p*-methoxybenzyl group of (−)-**26** (140 mg, 0.11 mmol) with TFA, as described for (−)-**12**, gave (−)-**27** (syrup, 120 mg, 95%): $[\alpha]^{23}_D = -8.2°$ (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.95 (d, J = 9.6 Hz, 1 H), 3.45−3.55 (m, 1 H), 3.81 (t, J = 9.6 Hz, 1 H), 4.76−4.86 (m, 2 H), 4.87−5.12 (m, 13 H), 7.09−7.43 (m, 40 H); ^{31}P NMR ($CDCl_3$, H_3PO_4 as external reference) δ −2.12, −1.55, −1.43; MS (FAB) *m*/*z* (rel inten) 1141.3 (M + H, 100), 1051.4 (50), 961.4 (16), 871 (2).

(−)-1-*O*-(1,2-Di-*O*-palmitoyl-*sn*-glycerol-3-benzyloxyphosphoryl)-2,6-di-*O*-benzyl-*myo*-inositol 3,4,5-Tris(dibenzyl phosphate) (28). Coupling of (−)-**27** (102 mg, 0.09 mmol) with the 1,2-di-*O*-palmitoyl-*sn*-glycerophosphoryl moiety, as described for (−)-**14**, yielded (−)-**28** (syrup, 143 mg, 86%): $[\alpha]^{23}_D = -2°$ (*c* 0.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.87 (t, J = 6.2 Hz, 6 H), 1.21−1.29 (m, 48 H), 1.42−1.51 (m, 4 H), 2.11−2.22 (m, 4 H), 3.72−4.08 (m, 5 H), 4.24−4.69 (m, 3 H), 4.71−5.07 (m, 21 H), 6.85−7.42 (m, 45 H); ^{31}P NMR ($CDCl_3$, H_3PO_4 as external reference) δ −2.28 (1P), −2.05 (0,25 P), −1.93 (0.5 P), −1.84 (0.25 P), −1.75 (1P), −1.52 (1 P); MS (FAB) *m*/*z* (rel inten) 1862 (M + 2 H, 9), 1772 (5), 1681.6 (1), 1311 (8), 551 (100).

L-α-Phosphatidyl-D-*myo*-inositol 3,4,5-trisphosphate, Dipalmitoyl [PtdIns(3,4,5)P_3]. The perbenzylated deriva-

tive (−)-**28** (130 mg, 0.07 mmol) was subjected to hydrogenolysis, as described for PtdIns(3)P, to afford PtdIns(3,4,5)P_3 (lyophilized powder, 72 mg, 98%): $[\alpha]^{23}_D = +3.7°$ (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, $J = 7.2$ Hz, 6 H), 1.15−1.41 (m, 48 H), 1.53−1.68 (m, 4 H), 2.37−2.66 (m, 4 H), 3.07−3.20 (m, 1 H), 3.57−3.74 (m, 2 H), 3.86−4.21 (m, 6 H), 4.37−4.46 (m, 1 H), 5.05 (br s, 1 H); ^{31}P NMR ($CDCl_3$, H_3PO_4 as external reference) δ −1.66 (br, 1 P), −1.17 (1 P), −0.50 (1 P), −0.31 (br, 1 P); MS (negative ion FAB) *m/z* (rel inten) 1049.4 (M − H, 5), 811.1 (100), 793.1 (8), 498.9 (20).

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Supporting Information Available: Copies of the ^{1}H NMR spectra for compounds **3**−**12**, **14**−**28**, **PtdIns(3)P**, **PtdIns(3,4)P_2**, and **PtdIns(3,4,5)P_3** (28 pages). This material is contained in 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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