Synthesis of Phosphotriester Analogues of the Phosphoinositides PtdIns(4,5)P_2 and PtdIns(3,4,5)P_3

Qu-Ming Gu and Glenn D. Prestwich*,†

Department of Chemistry and Department of Biochemistry and Cell Biology, State University of New York at Stony Brook, Stony Brook, New York 11794

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A synthetic route was developed for the preparation of novel *O*-(3-aminopropyl) tethered phosphotriester analogs (**5**) of phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5,)P_2, or PIP_2) and phosphatidylinositol 3,4,5-trisphosphate (PtdIns(3,4,5)P_3, or PIP_3) using the coupling reagent 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite. The phosphotriester ligand design introduced a reactive aminopropyl group at the polar lipid head of the ring-phosphorylated phosphoinositides, allowing a reporter moiety to be positioned at the surface of the bilayer and in the vicinity of the phosphorylated inositol. Such reporter groups may interact with membrane-proximal regions of PIP_2- and PIP_3-binding proteins recruited to membrane sites by electrostatic interactions between the phosphates of the phospholipid and basic regions of the proteins. Following a convergent strategy, phosphitylation of an optically-pure 1,2-*O*-diacyl-*sn*-glycerol with 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite was followed by coupling with protected inositol precursors to give adducts **8** in 80% to 95% yield. The 2-cyanoethyl phosphotriester was stable during the subsequent reaction steps and could be conveniently converted to the 3-aminopropyl group during the final hydrogenolysis of the benzyl protecting groups. Benzophenone-containing photoaffinity probes of the phosphotriester **11a** and **11b** were also synthesized. Alternatively, the versatile cyanoethyl group could be removed using diisopropylethylamine prior to hydrogenolysis, thereby furnishing the corresponding phosphodiesters, PIP_3 and PIP_2 (**13a** and **13b**).

Introduction

L-α-Phosphatidyl-D-*myo*-inositol 3,4,5-trisphosphate (PtdIns(3,4,5)P_3, or PIP_3) (**2**) and L-α-phosphatidyl-D-*myo*-inositol 4,5-bisphosphate (PtdIns(4,5,)P_2, or PIP_2) (**1**) play a variety of essential roles in biological processes. PIP_2 was first observed to be a substrate for phospholipase C (Scheme 1), and the receptor-activated action of PLC on PIP_2 generated the second messengers Ins(1,4,5)P_3 (**3**) and 1,2-diacylglycerol (**4**).[1−4] More recently, PIP_2 has been recognized to be a crucial element in the recruitment of signaling proteins to membranes,[5] as mediated by the pleckstrin homology domains.[6] Indeed, the recruitment of the PLC δ_1 isozyme is facilitated by the interaction of its PH domain with 4,5-bisphosphate of PIP_2.[7] A three-dimensional structure of the β-spectrin-Ins(1,4,5)P_3 complex has verified the importance of the 4,5-bisphosphate interaction with hydrogen bonding and protonated basic residues.[8] In addition, PIP_2 affects the organization of the cytoskeleton by sequestering profilin, thereby preventing the association of profilin with monomeric F-actin and thus permitting the polymerization of actin.[5,9]

PtdIns(4,5,)P_2 can be converted by agonist-stimulated, receptor-mediated activation of phosphoinositide 3-kinase (PI 3-K)[10] to PtdIns(3,4,5,)P_3, the key element in a new intracellular signaling system (Scheme 1).[11] Recent efforts to understand the role of PI 3-K in cell signaling have focused on possible targets for PIP_3, in particular, activation of Akt serine/threonine kinase,[12,13] activation of protein kinase C,[14] and phosphorylation of pleckstrin in platelets.[15] For both PIP_2 and PIP_3, the question remains as to what role the diacylglycerol moiety might have in the interaction of these phosphoinositides with hydrophobic regions of PIP_n binding proteins. We report herein the synthesis of novel triester analogues of PIP_n with pendant functionality for attaching reporter groups that would enable investigation of protein−PIP_n interactions either in solution or at bilayer interfaces.

A number of published reports have described strategies for the syntheses of diester and diether analogues of PIP_2 and PIP_3.[16−19] A typical synthetic strategy

† Current address: Department of Medicinal Chemistry, 308 Skaggs Hall, The University of Utah, Salt Lake City, Utah 84112. Phone: 801-585-9051. Fax: 801-585-9053. E-Mail: gprestwich@deans.pharm.utah.edu.

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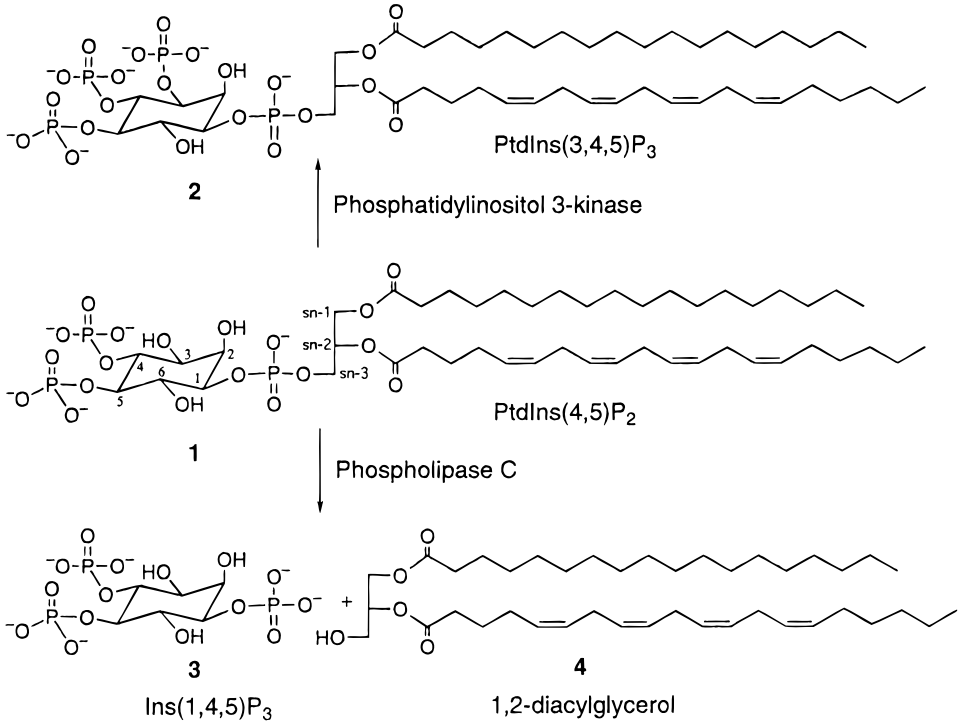
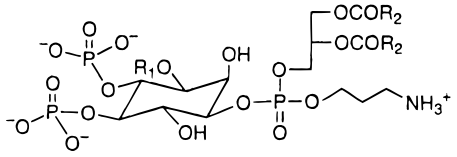
Scheme 1. Metabolic Pathways for PtdIns(4,5)P_2 in Eukaryotic Cells



involves incorporation of benzyl 1,2-diacylglyceryl (*N*,*N*-diisopropylamino)phosphoramidite to a variety of protected inositols derived from different chiral synthons. The bifunctional phosphitylating reagent benzyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite has been used most frequently for tetrazole-mediated phosphitylation of 1,2-diacylglycerol, an approach that was first effectively employed[20] in the synthesis of PIP_2 analogues. The required 1,2-diacylglyceryl (*N*,*N*-diisopropylamino)phosphoramidite could also be prepared from benzyl chloro (*N*,*N*-diisopropylamino)phosphoramidite.[21]

In order to gain further insight into the electrostatic and hydrophobic interactions important in phosphoinositide recognition and signal transduction, a strategy has been developed that allows synthetic access to PIP_3 and PIP_2 analogues with an *O*-(3-aminopropyl) phosphate linker group at polar lipid head. This "triester strategy" provides a different spatial orientation for affinity probes and reporter groups as compared to the modification of one of the acyl chains of the 1,2-diacylglycerol moiety.[21] Thus, the aminopropyl tethered triester PIP_2 and PIP_3 analogues should permit normal incorporation of the diacylglycerol moiety in the phospholipid bilayer as well as the presence of the unmodified 4,5-bisphosphate or 3,4,5-trisphosphate to recruit target proteins. The reporter group would reside at the interface of the lipid bilayer and the aqueous environment, in an orientation to potentially provide important information on PIP_2 and PIP_3 binding proteins. In this article, we present a facile synthesis of these novel phosphotriesters (**5**) of PIP_3 and PIP_2. The synthetic strategy using 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite as a bifunctional phosphitylating reagent also permits the preparation of several other phosphatidylinositol analogues.



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a, $R_1 = PO_2^{-2}$, $R_2 = (CH_2)_{14}CH_3$
b, $R_1 = H$, $R_2 = (CH_2)_{14}CH_3$

Results and Discussion

The synthesis of the 1,2-*O*-diacyl 3-*O*-PMB-*sn*-glycerols (PMB = *p*-methoxybenzyl) was achieved from (+)-1,2-*O*-isopropylidene-*sn*-glycerol as previously described.[21,22] The selection of the PMB protecting group minimized 2-3 acyl migration. Thus, removal of the PMB group[21] from the *sn*-3-position of 1,2-*O*-diacyl 3-*O*-PMB-glycerols could be achieved by either catalytic hydrogenolysis at 1 atm or by oxidative cleavage using DDQ at 24 °C. Hydrogenolysis of the PMB group using Pd/C (10%) was achieved without detectable 2-3 acyl migration (98% yield, recrystallized from EtOH). Pure 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite[23] was condensed with 1,2-*O*-dipalmitoyl-*sn*-glycerol in the presence of 1*H*-tetrazole to give 1,2-dipalmitoyl-*sn*-glyceryl 2-cyanoethyl (*N*,*N*-diisopropylamino)phosphoramidite (**6**) in 90−95% yield after SiO_2 chromatography.

For the PIP_3 analogues, differentially protected inositol **7a**,[24] an intermediate used in the synthesis of P-1-modified D-*myo*-Ins(1,3,4,5)P_4 *via* a Ferrier rearrangement,[25] was employed. For the PIP_2 analogues, intermediate **7b**, a precursor for D-*myo*-Ins(1,4,5)P_3 affinity

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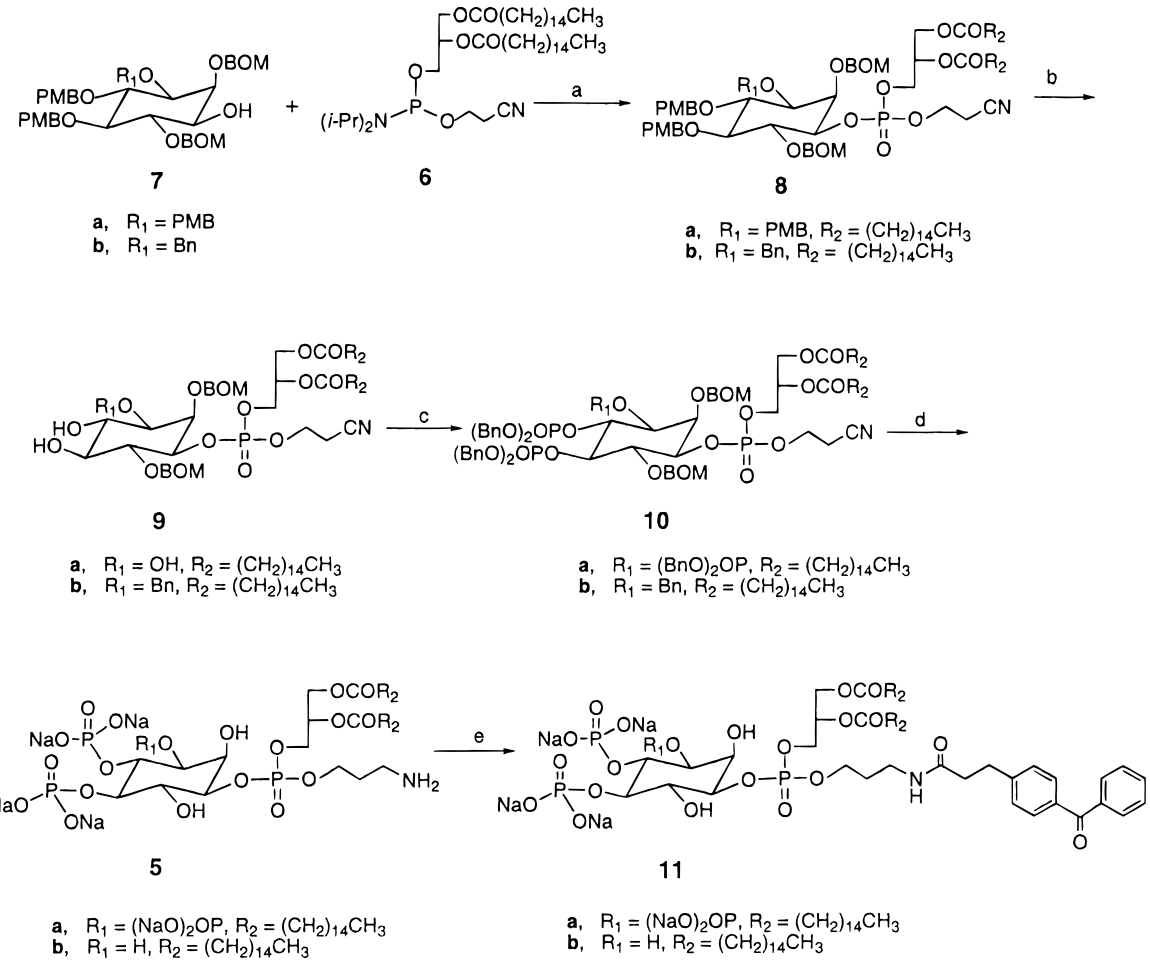
Scheme 2[a]



[a] (a) 1*H*-tetrazole, CH_2Cl_2, 24 °C, *m*-CPBA, −20 °C; (b) DDQ, $CH_2Cl_2-H_2O$, 24 °C; (c) $(i\text{-Pr})_2NP(OBn)_2$, 1*H*-tetrazole, 24 °C; *m*-CPBA; (d) H_2, Pd/C (10%), *t*-BuOH−H_2O (6:1), $NaHCO_3$, 50 psi, 24 °C; (e) BZDC−NHS, DMF, 0.2 M Et_3NHCO_3, pH 8.5, 24 °C.

probes[26] and PIP_2 analogues,[21] was prepared. In each of these precursors, the PMB ethers mask the inositol hydroxyls destined for phosphorylation, while the benzyl (Bn) and benzyloxymethyl (BOM) ethers mask hydroxyls that will remain as hydroxyl groups in the final phosphoinositides. As shown in Scheme 2, coupling of protected inositol **7a** with phosphoramidite **6** followed by the low-temperature oxidation of the resulting phosphite provided the fully-protected inositol phosphotriester **8a** in 82% yield. Only a single isomer was detected based on TLC and ^{31}P NMR analysis. Note that the inositol moiety is optically-pure, arising from α-D-glucose, and the 1,2-diacylglycerol portion is also optically-pure. The remaining stereogenic site is the phosphorus of the triester, and in the absence of any asymmetric induction, the product would consist of a diastereomeric mixture differing only in the absolute stereochemistry at phosphorus. It is likely that neither technique could separate these diastereomers, as has been observed for other diastereomeric phosphoinositide intermediates.[21]

Similarly, reaction of protected inositol **7b**[26] with **6** at rt gave the protected inositol **8b** in 93% yield. Removal of the PMB protecting groups from the protected inositols **8** by the oxidation with DDQ in wet CH_2Cl_2 provided triol **9a** and diol **9b**, in 85% and 95% yields, respectively. Subsequent phosphitylation using dibenzyl *N*,*N*-diisopropylphosphoramidite followed by the *m*-CPBA oxidation furnished 2,6-*O*-bis(benzyloxymethyl)-3,4,5-*O*-tris(dibenzylphosphoryl)-D-*myo*-inositol 1,2-*O*-diacyl-*sn*-glyceryl 2-cyanoethyl phosphate **10a** in 73% yield. Under the same reaction conditions, **10b** was obtained in 98% yield. Removal of Bn and BOM protecting groups as well as conversion of the 2-cyanoethyl to 3-aminopropyl group were accomplished simultaneously by catalytic hydrogenation (Pd/C, 50 psi) to give the 3-aminopropyl tethered phosphotriesters of PIP_3 (**5a**) and PIP_2 (**5b**) in 60% and 75% yields, respectively. Hydrogenation of the nitrile group under neutral or acidic conditions was sufficiently rapid that no remaining 2-cyanoethyl group was observed based on the ^1H NMR analysis. Reaction of the 3-(*p*-benzoyldihydrocinnamoyl)−NHS reagent (BZDC−NHS)[27] with **5a** and **5b** provided BZDC-derivatized photoaffinity probes **11a** and **11b** in 91% and 85% yields, respectively.

Alternatively, the removal of 2-cyanoethyl groups from the protected PIP_3 and PIP_2 derivatives **10a** and **10b** could be accomplished by heating the compounds in a 1:40 mixture of diisopropylethylamine and acetonitrile at 60 °C for 12 h, producing phosphodiesters **12a** and **12b** in 85% and 90% yields, respectively (Scheme 3). Cleavage of 2-cyanoethyl group from the phosphate moiety via a β-elimination[28] could also be achieved by treatment with other tertiary amines, e.g., triethylamine or tri-

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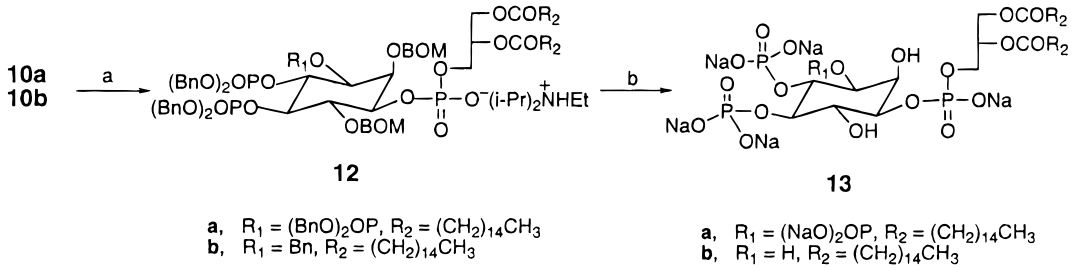
Scheme 3[a]



[a] (a) (*i*-Pr)$_2$NEt, CH_3CN, 60 °C; (b) H_2, Pd/C (10%), *t*-BuOH-H_2,O (6:1), $NaHCO_3$, 50 psi, 24 °C.

methylamine. The cleavage rate was dramatically enhanced when a more polar organic solvent such as methanol or ethanol was used instead of acetonitrile. Compounds **12a** and **12b** could be either purified on silica gel or directly subjected to catalytic hydrogenolysis after removal of the solvents in vacuo. Thus, Pd/C-catalyzed hydrogenolysis of **12a** and **12b** provided corresponding phosphodiesters, dipalmitoyl PIP_3 (**13a**), and dipalmitoyl PIP_2 **(13b)** in overall yields of 60% and 70% from **10a** and **10b**, respectively.

Conclusion

Novel affinity probes based on phosphotriester analogues of PIP_3 and PIP_2 offer the opportunity to examine PIP_n−protein interactions at membrane surfaces. In preparing these affinity probes, we found that the use of 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite provided a general approach to the synthesis of novel 3-aminopropyl phosphotriesters of phosphatidylinositides in excellent overall yield. In particular, we have demonstrated that this methodology can be applied to the synthesis of two aminopropyl tethered phosphatidylinositides as well as to other analogues of PIP_2 and PIP_3. Preparation and biological function of specific affinity probes derived from these aminopropyl and aminoacyl derivatives will be reported elsewhere.[29] In addition, the synthetic approach described herein is extremely versatile, allowing access to the synthesis of additional PIP_n and phosphatidic acid derivatives that will be reported in due course.[29a]

Experimental Section

General procedures were described previously.[21]

1,2-*O*-Dipalmitoyl-*sn*-glycerol. To a solution of 3-*O*-(*p*-methoxybenzyl)-*sn*-glycerol[21] (0.89 g, 4.2 mmol), DMAP (0.24 g, 2 mmol), and palmitic acid (2.18 g, 8.5 mmol) in dry CH_2Cl_2 (50 mL) was added a solution of DCC (1.85 g, 9 mmol) in 40 mL of dry CH_2Cl_2 (20 mL). The resulting mixture was stirred at 24 °C for 12 h. The mixture was filtered through Celite, concentrated in vacuo, and purified on SiO_2 (EtOAc/hexane, 1:3) to give 2.54 g of product (88% yield). TLC (SiO_2) EtOAc/hexane (1:2), $R_f \sim 0.8$.

A mixture of the PMB ether (2.0 g, 2.9 mmol) and 5% Pd/C (250 mg) in 50 mL of EtOH/AcOH (19:1) was shaken under H_2 at atmosphere for 3 h. The catalyst was removed by filtration on Celite (1 g), concentrated in vacuo, and purified on SiO_2 (EtOAc/hexane, 1:1) to give 1.48 g of product (98% yield) as a white solid. TLC (SiO_2) EtOAc/hexane (1:2), $R_f \sim 0.20$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.12−5.08 (m, 1H), 4.30−4.22 (m, 2H), 3.72 (t, 2H, J = 6.0 Hz), 2.30 (q, 4H), 1.60−1.50 (m, 4H), 1.38−1.20 (m, 48H), 0.80 (t, 3H, J = 7.2 Hz). FAB MS: 551 (M^+ − OH, 24.3); 569 (MH^+, 2.1). Anal. Calcd for $C_{35}H_{68}O_5$: C, 73.88; H, 12.05. Found: C, 74.02; H, 11.81.

2-Cyanoethyl *N*,*N*,*N*′,*N*′-Tetraisopropylphosphorodiamidite. To a solution of PCl_3 (13.7 g, 8.55 mL, 0.1 mol) and dry pyridine (8.1 mL, 0.1 mol) in 20 mL of dry Et_2O under nitrogen at −78 °C was added dropwise over 1 h 3-hydroxypropanenitrile (7.1 g, 6.8 mL, 0.1 mol) in 40 mL of dry Et_2O. The mixture was warmed to rt and stirred for an additional 1 h at 24 °C. Precipitates were removed by filtration under nitrogen and washed twice with dry Et_2O (2 × 40 mL). The solvent was evaporated in vacuo, and the oily residue was dried for 2 h at 20 Torr. Next, a solution of this oily residue (7.4 g, 43 mmol) in 30 mL of dry Et_2O (20 mL) under nitrogen was treated with (*i*-Pr)$_2$NH (53.6 mL, 0.38 mol) over 10 min at −20 °C. The mixture was stirred at 24 °C for 1.5 h, and precipitated solids were removed by filtration under nitrogen and washed twice with Et_2O (2 × 20 mL). After evaporation of solvent, the oily residue was distilled over a 10-cm Vigreux column to give 8.6 g of the phosphoramidite reagent (70% yield), bp 115 °C/0.6 Torr.

1,2-*O*-Dipalmitoyl-*sn*-glyceryl 2-Cyanoethyl (*N*,*N*-Diisopropylamino)phosphoramidite (6). To a mixture of the 1,2-*O*-dipalmitoyl-*sn*-glycerol (150 mg, 0.26 mmol) and 1*H*-tetrazole (36.4 mg, 0.52 mmol) in dry CH_2Cl_2 (1 mL) under nitrogen was added a solution of the phosphoamidite (157 mg, 0.52 mmol) in CH_2Cl_2 (1 mL). The mixture was stirred at 24 °C for 40 min, concentrated in vacuo, and purified on SiO_2 (EtOAc/hexane/Et_3N, 1:5:0.1) to give 190 mg of product (94% yield) as a colorless oil. TLC (SiO_2) EtOAc/hexane (1:2), $R_f \sim 0.9$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.20 (br, s, 1H), 4.85−4.20 (m, 4H), 3.80−3.70 (t, 2H, J = 1.5 Hz), 3.65−3.60 (m, 2H), 3.14 (m, 2H), 2.65 (t, 2H, J = 1.5 Hz), 2.35−2.20 (m, 4H), 1.60−1.50 (m, 4H), 1.30−1.20 (m, 60H), 0.88−0.82 (t, 6H).

2,6-*O*-Bis(benzyloxymethyl)-3,4,5-*O*-tris(*p*-methoxybenzyl)-D-*myo*-inositol 1-*O*-(1,2-*O*-Dipalmitoyl)-*sn*-glyceryl 2-Cyanoethyl Phosphate (8a). To a mixture of **6** (171 mg, 0.22 mmol) and 1*H*-tetrazole (15.4 mg, 0.22 mmol) in dry CH_2Cl_2 (1 mL) was added a solution of 2,6-*O*-bis(benzyloxymethyl)-3,4,5-*O*-tris(*p*-methoxybenzyl)-D-*myo*-inositol **7a** (79 mg, 0.1 mmol) in CH_2Cl_2 (1 mL). After the mixture was stirred at 24 °C for 1 h under nitrogen, TLC analysis showed that the reaction was complete (R_f of the coupled product: 0.80; SiO_2; EtOAc/hexane 1:1). The reaction mixture was then cooled to −40 °C, and a solution of *m*-CPBA (43 mg, 0.25 mmol) in CH_2Cl_2 was added. After stirring at 24 °C for an additional 1 h, the mixture was diluted with CH_2Cl_2 (40 mL), washed once with 10% aqueous $NaHCO_3$ solution (20 mL) and brine (20 mL), dried over $MgSO_4$, concentrated in vacuo, and purified on SiO_2 (EtOAc/hexane, 1:1) to give 121 mg of product **8a** (82% yield) as a colorless oil. TLC (SiO_2) EtOAc/hexane (1:1), $R_f \sim 0.55$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.29−7.10 (m, 22H), 6.80−6.75 (m, 6H), 5.20 (br, s, 1H), 4.95−4.50 (m, 10H), 4.40−4.05 (m, 4H), 3.80 (s, 9H), 3.75 (t, 2H), 2.70 (t, 2H), 2.30−2.20 (m, 4H), 1.60−1.50 (m, 4H), 1.30−1.20 (m, 48H), 0.88−0.82 (t, 6H). ^{31}P NMR ($CDCl_3$, 101 MHz) δ 0.03. FAB MS: 1487 (MNa^+, 0.8). Anal. Calcd for $C_{84}H_{122}NO_{18}P$: C, 68.86; H, 8.40; N, 0.96. Found: C, 68.82; H, 8.21; N, 1.04.

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2,6-*O*-Bis(benzyloxymethyl)-3-*O*-(benzyloxy)-4,5-*O*-bis-(*p*-methoxybenzyl)-D-*myo*-inositol 1-*O*-(1,2-*O*-Dipalmitoyl)-*sn*-glyceryl 2-Cyanoethyl Phosphate (8b). To a mixture of **6** (64 mg, 0.083 mmol) and 1*H*-tetrazole (7 mg, 0.10 mmol) in dry CH_2Cl_2 (1 mL) was added a solution of 2,6-*O*-bis-(benzyloxymethyl)-3-*O*-(benzyloxy)-4,5-*O*-bis(*p*-methoxybenzyl)-D-*myo*-inositol **7b** (60 mg, 0.075 mmol) in CH_2Cl_2 (1 mL). After stirring at 24 °C for 30 min under nitrogen, the reaction mixture was cooled to −40 °C and a solution of *m*-CPBA (21 mg, 0.12 mmol) in 2 mL of CH_2Cl_2 was added. After 20 min, the mixture was diluted with CH_2Cl_2 (20 mL), washed with 10% aqueous $NaHCO_3$ solution (20 mL) and brine (20 mL), dried over $MgSO_4$, concentrated in vacuo, and purified on SiO_2 (EtOAc/hexane, 1:2) to give 101 mg of product **8b** (93% yield) as a colorless syrup. TLC (SiO_2) EtOAc/hexane (1:2), $R_f \sim 0.25$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.45−7.20 (m, 23H), 6.85−6.75 (m, 4H), 5.15−5.12 (m, 1H), 4.85−4.50 (m, 11H), 4.42−4.20 (m, 6H), 3.79 (s, 6H), 3.75−3.60 (t, 2H), 2.45 (t, 2H), 2.35−2.25 (m, 4H), 1.55−1.50 (m, 4H), 1.30−1.10 (m, 48H), 0.88−0.75 (t, 6H, J = 7.5 Hz). ^{31}P NMR ($CDCl_3$, 101 MHz) δ 0.03. FAB MS: 1457 (MNa^+, 2.4). Anal. Calcd for $C_{83}H_{120}NO_{17}P$: C, 69.46; H, 8.43; N, 0.98. Found: C, 69.10; H, 8.80; N, 1.22.

2,6-*O*-Bis(benzyloxymethyl)-D-*myo*-inositol 1-*O*-(1,2-*O*-Dipalmitoyl)-*sn*-glyceryl 2-Cyanoethyl Phosphate (9a). A mixture of **8a** (100 mg, 0.068 mmol) and DDQ (45.5 mg, 0.21 mmol) in 50 mL of $CH_2Cl_2-H_2O$ (100:0.5) was stirred at 24 °C for 3 h. The resulting solution was diluted with 50 mL of CH_2Cl_2 and washed with $NaHCO_3$ (10%) (2 × 30 mL) and brine (40 mL), dried over $MgSO_4$, and concentrated in vacuo. The residue was purified on SiO_2 (EtOAc/hexane, 5:1) to give 64 mg of product **9a** (85% yield) as a white solid:. TLC (SiO_2) EtOAc/hexane (5:1), $R_f \sim 0.20$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.30−7.21 (m, 10H), 5.20 (br s, 1H), 4.90−4.50 (m, 8H), 4.45−4.05 (m, 4H), 3.75 (t, 2H), 3.55 (m, 2H), 2.65 (t, 2H), 2.30−2.25 (m, 4H), 1.66−1.45 (m, 4H), 1.35−1.10 (m, 48H), 0.80 (t, 6H). ^{31}P NMR ($CDCl_3$, 101 MHz) δ 0.03. FAB MS: 1127 (MNa^+, 7.7). Anal. Calcd for $C_{60}H_{98}NO_{15}P$: C, 65.24; H, 8.95; N, 1.27. Found: C, 65.12; H, 8.98; N, 1.26.

2,6-*O*-Bis(benzyloxymethyl)-3-*O*-(benzyloxy)-D-*myo*-inositol 1-*O*-(1,2-*O*-Dipalmitoyl)-*sn*-glyceryl 2-Cyanoethyl Phosphate (9b). A mixture of **8b** (90 mg, 0.062 mmol) and DDQ (32.5 mg, 0.15 mmol) in 6 mL of $CH_2Cl_2-H_2O$ (100:0.5) was stirred at 24 °C for 3 h. The resulting solution was diluted with 50 mL of CH_2Cl_2 and washed with $NaHCO_3$ (10%) (2 × 30 mL) and brine (40 mL), dried over $MgSO_4$, and concentrated in vacuo. The residue was purified on SiO_2 (EtOAc/hexane, 5:1) to give 71 mg of product **9b** (95% yield) as a white solid. TLC (SiO_2) EtOAc/hexane (5:1), $R_f \sim 0.40$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.35−7.20 (m, 15H), 5.15 (br, s, 1H), 5.12−4.55 (m, 8H), 4.44−3.85 (m, 8H), 2.66 (t, 2H), 2.25 (m, 4H), 1.55−1.45 (m, 4H), 1.30−1.10 (m, 48H), 0.82 (t, 6H, J = 8.0 Hz). ^{31}P NMR ($CDCl_3$, 101 MHz) δ −0.58. FAB MS: 1217 (MNa^+, 4.4).

2,6-*O*-Bis(benzyloxymethyl)-3,4,5-*O*-tris(dibenzylphosphoryl)-D-*myo*-inositol 1-*O*-(1,2-*O*-dipalmitoyl)-*sn*-glyceryl 2-Cyanoethyl Phosphate (10a). To a mixture of **9a** (64 mg, 0.057 mmol) and 1*H*-tetrazole (40 mg, 0.57 mmol) in dry CH_2Cl_2 (1 mL) was added a solution of dibenzyl *N*,*N*-diisopropylphosphoramidite (117 mg, 0.34 mmol) in CH_2Cl_2 (2 mL). After stirring at 24 °C for 1 h, the reaction mixture was cooled to −40 °C and a solution of *m*-CPBA (60 mg, 0.35 mmol) in CH_2Cl_2 (2 mL) was added. After an additional 1 h, the mixture was diluted with CH_2Cl_2 (20 mL), washed once with 10% aqueous $NaHCO_3$ solution (20 mL) and brine (20 mL), dried over Na_2SO_4, concentrated in vacuo, and purified on SiO_2 (EtOAc/hexane, 1:3) to give 79 mg of product **10a** (73% yield) as a colorless syrup. TLC (SiO_2) EtOAc/hexane (2:1), $R_f \sim 0.32$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.45−7.05 (m, 40H), 5.15−4.60 (m, 22H), 4.55−4.05 (m, 6H), 2.42 (t, 2H), 2.25−2.10 (m, 4H), 1.60−1.50 (m, 4H), 1.40−1.20 (48H), 0.88−0.85 (t, 6H). ^{31}P NMR ($CDCl_3$, 101 MHz) δ 0.15, −0.03, −0.47, −1.06. FAB MS: 1907 (MNa^+, 4.7). Anal. Calcd for $C_{102}H_{137}NO_{24}P_4$: C, 64.97; H, 7.33; N, 0.74. Found: C, 64.82; H, 7.36; N, 0.92.

2,6-*O*-Bis(benzyloxymethyl)-3-*O*-(benzyloxy)-4,5-*O*-bis-(dibenzylphosphoryl)-D-*myo*-inositol 1-*O*-(1,2-*O*-Dipalmitoyl)-*sn*-glyceryl 2-Cyanoethyl Phosphate (10b). To a mixture of **9b** (70 mg, 0.058 mmol) and 1*H*-tetrazole (80 mg, 1.14 mmol) in dry CH_2Cl_2 (2 mL) was added a solution of dibenzyl *N*,*N*-diisopropylphosphoramidite (79 mg, 0.23 mmol) in CH_2Cl_2 (2 mL). After stirring at 24 °C for 1 h, the reaction mixture was cooled to −40 °C, and a solution of *m*-CPBA (60 mg, 0.35 mmol) in CH_2Cl_2 (2 mL) was added. After an additional 1 h, the mixture was diluted with CH_2Cl_2 (20 mL), washed once with 10% aqueous $NaHCO_3$ solution (20 mL) and brine (20 mL), dried over Na_2SO_4, concentrated in vacuo, and purified on SiO_2 (EtOAc/hexane, 1:2) to give 92 mg of product **10b** (98% yield) as a colorless syrup. TLC (SiO_2) EtOAc/hexane (1:2), $R_f \sim 0.35$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.45−7.05 (m, 35H), 5.20−4.75 (m, 16H), 4.65−3.75 (m, 8H), 3.50−3.45 (t, 2H), 2.42 (t, 2H), 2.25−2.10 (m, 4H), 1.60−1.50 (m, 4H), 1.35−1.10 (m, 48H), 0.88−0.85 (t, 6H, J = 7.0 Hz). ^{31}P NMR ($CDCl_3$, 101 MHz) δ 0.045, −0.20, −0.65. FAB MS: 1737 (MNa^+, 6.6). Anal. Calcd for $C_{95}H_{130}NO_{21}P_3$: C, 66.52; H, 7.64; N, 0.82. Found: C, 66.82; H, 7.66; N, 1.04.

3,4,5-Tri-*O*-phosphoryl-D-*myo*-inositol 1-*O*-(1,2-*O*-Dipalmitoyl)-*sn*-glyceryl 3-Aminopropyl Phosphate Sodium Salt (5a). A mixture of **10a** (60 mg, 0.032 mmol), $NaHCO_3$ (16 mg, 0.19 mmol), and Pd/C (10%) (120 mg) in 20 mL of *t*-BuOH/H_2O (6:1) was shaken under H_2 (51 psi) for 21 h. The catalyst was removed by filtration over 1 g of Celite, and the filter was washed thoroughly with water (20 mL) and EtOH (20 mL). The combined filtrates were lyophilized to give 30 mg of product **5a** (80% yield) as a solid, which was positive to ninhydrin. TLC (SiO_2) CH_2Cl_2/MeOH/concd NH_4OH (5:4:1), $R_f \sim 0.70$. ^{1}H NMR (D_2O, 300 MHz) δ 5.20−5.00 (m, 1H), 4.40−4.10 (m, 4H), 4.06−3.60 (m, 2H), 3.15 (m, 2H), 2.20−2.10 (m, 4H), 1.78 (m, 2H), 1.50 (m, 4H), 1.30−1.10 (m, 48H), 0.88−0.75 (t, 6H). ^{31}P NMR (D_2O, 101 MHz) δ 8.1, 7.8, 5.1, 1.1. Mass (MALDI-TOF) 1106 ($M^+ - 1$); 1128 ($MNa^+ - 1$); calcd: 1106.

4,5-Di-*O*-phosphoryl-D-*myo*-inositol 1-*O*-(1,2-*O*-Dipalmitoyl)-*sn*-glyceryl 3-Aminopropyl Phosphate Sodium Salt (5b). A mixture of **10b** (72 mg, 0.045 mmol), $NaHCO_3$ (15 mg, 0.18 mmol), and Pd/C (10%) (100 mg) in 20 mL of *t*-BuOH/H_2O (6:1) was shaken under H_2 (51 psi) for 22 h. The catalyst was removed by filtration over 1 g of Celite, and the filter was washed thoroughly with water (20 mL) and EtOH (20 mL). The combined filtrates were lyophilized to give 35 mg of crude product **5b** (72% yield) as a solid, which was positive to ninhydrin. TLC (SiO_2) CH_2Cl_2/MeOH/concd NH_4OH (5:4:1), $R_f \sim 0.80$. ^{1}H NMR (D_2O, 300 MHz) δ 5.15−5.00 (s, 1H), 4.40−4.15 (m, 4H), 4.00−3.90 (m, 2H), 3.20 (m, 2H), 2.15−2.05 (m, 4H), 1.78 (m, 2H), 1.60−1.45 (m, 4H), 1.40−1.05 (m, 48H), 0.85−0.70 (t, 6H). ^{31}P NMR (D_2O, 101 MHz) δ 7.01, 6.60, 0.8. Mass (MALDI-TOF), 1027 ($M^+ - 1$); 1049 ($MNa^+ - 1$), calcd: 1027.

3,4,5-Tri-*O*-phosphoryl-D-*myo*-inositol 1-*O*-(1,2-*O*-Dipalmitoyl)-*sn*-glyceryl (3-BZDC-amido)propyl Phosphate Sodium Salt (11a). PIP_3 derivative **5a** (10 mg, 9 μmol) was suspended in a solution of 0.2 M Et_3NHCO_3 buffer, pH 8.8 (0.8 mL), and DMF (0.8 mL) and stirred at 24 °C for 3 h until a clear solution was obtained. To the resulting solution was added BZDC−NHS[27] (5 mg, 0.014 mmol) in DMF (0.2 mL), and the mixture was stirred for 16 h at 24 °C. Solvents were evaporated in vacuo, and the residue was washed with acetone (4 × 1 mL) to remove most of the unreacted or hydrolyzed BZDC derivatives. The residue was then dissolved in 1 mL of 0.1 M Et_3NHCO_3 buffer, pH 8.8, and loaded onto a DEAE-cellulose column (3 cm × 0.5 cm i.d.) preequilibrated with same buffer. The column was eluted using a linear gradient of Et_3NHCO_3 buffer (pH 8.8) from 0.1 M to 1.2 M, and the product was eluted out at a concentration between 1.0 to 1.2 M. The fractions were collected and concentrated to give 11 mg of product **11a** (91% yield) as a solid. ^{1}H NMR (D_2O, 300 MHz) δ 7.72−7.35 (m, 9H), 4.40−3.50 (m, 10H), 3.15−3.00 (m, 4H), 2.50−2.40 (m, 4H), 2.20−2.10 (m, 4H), 1.80−1.70 (m, 2H), 1.50−1.45 (m, 4H), 1.30−1.10 (m, 48H), 0.88−0.82 (t, 6H). ^{31}P NMR (D_2O, 101 MHz) δ 7.2, 5.6, 5.1, 1.9. Mass (MALDI-TOF), 1342 ($M^+ - 1$); 1364 ($M^+ + Na - 1$); 1386 ($M^+ + 2Na - 1$); 1408 ($M^+ + 3Na - 1$); calcd: 1342.

4,5-Di-*O*-phosphoryl-D-*myo*-inositol 1-*O*-(1,2-*O*-Dipalmitoyl)-*sn*-glyceryl (3-BZDC-amido)propyl Phosphate Sodium Salt (11b). PIP_2 analogue **5b** (5 mg, 4.9 μmol) was

suspended in a solution of 0.2 M Et_3NHCO_3 buffer, pH 8.8 (0.6 mL), and DMF (0.6 mL), and stirred at 24 °C for 1 h. To the resulting solution was added BZDC−NHS[27] (2.6 mg, 7.4 μmol) in DMF (0.2 mL), and the mixture was stirred for 16 h at 24 °C. Solvents were evaporated in vacuo, and the residue was washed with acetone (4 × 1 mL) to remove most of soluble BZDC derivatives. The residue was then dissolved in 1 mL of 0.1 M Et_3NHCO_3 buffer, pH 8.8, and loaded onto a DEAE-cellulose column (3 cm × 0.5 cm i.d.) preequilibrated with the same buffer. The column was eluted with a gradient of Et_3NHCO_3 buffer, pH 8.8, from 0.1 to 1.2 M concentration. The product was eluted at a concentration between 0.9 to 1.0 M. The fractions were collected and dried to give 5.3 mg of product **11b** (85% yield) as a solid. TLC (SiO_2) CH_2Cl_2/MeOH/concd NH_4OH (5:4:1), $R_f \sim 0.65$. ^{1}H NMR (D_2O, 300 MHz) δ 7.82−7.30 (m, 9H), 5.20 (m, 1H), 4.40−4.10 (m, 4H), 4.06−3.60 (m, 7H), 3.12 (m, 2H), 2.20−2.05 (m, 4H), 1.75 (m, 2H), 1.50−1.35 (m, 4H), 1.30−1.10 (m, 48H), 0.88−0.80 (t, 6H, J = 7.0 Hz). ^{31}P NMR (D_2O, 101 MHz) δ 8.1, 7.2, 3.1. Mass (MALDI-TOF), 1263 ($M^+ - 1$); calcd: 1263.

2,6-*O*-Bis(benzyloxymethyl)-3,4,5-*O*-tris(dibenzylphosphoryl)-D-*myo*-inositol 1-*O*-(1,2-*O*-Dipalmitoyl)-*sn*-glyceryl Diisopropylethylammonium Phosphate (12a). A mixture of **10a** (150 mg, 0.080 mmol) and diisopropylethylamine (100 μL) in dry CH_3CN (4 mL) was heated to 60 °C with stirring for 12 h. Solvents were evaporated under reduced pressure, and the residue was then purified on SiO_2 (MeOH/CH_2Cl_2, 1:1) to give 148 mg of product **12a** (97% yield) as a colorless syrup. TLC (SiO_2) MeOH/CH_2Cl_2 (1:1), $R_f \sim 0.25$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.30−7.15 (m, 40H), 5.21 (m, 1H), 5.04−4.72 (m, 21H), 4.70−3.70 (m, 8H), 3.50−3.47 (m, 3H), 2.92−2.90 (m, 2H), 1.60−1.50 (m, 4H), 1.38−1.12 (m, 60H), 0.95−0.85 (t, 6H, J = 7.0 Hz). ^{31}P NMR ($CDCl_3$, 101 MHz) δ 0.33, 0.21, −0.45, −1.59. FAB MS: 1855 (MNa^+, 5.7).

2,6-*O*-Bis(benzyloxymethyl)-3-*O*-(benzyloxy)-4,5-*O*-bis(dibenzylphosphoryl)-D-*myo*-inositol 1-*O*-(1,2-*O*-Dipalmitoyl)-*sn*-glyceryl Diisopropylethylammonium Phosphate (12b). A mixture of **10b** (52 mg, 0.033 mmol) and diisopropylethylamine (60 μL) in CH_3OH (4 mL) was stirred at 24 °C for 12 h. Solvents were evaporated under reduced pressure, and the residue was then purified on SiO_2 (MeOH/CH_2Cl_2, 1:1) to give 48 mg of product **12b** (93% yield) as a colorless syrup. TLC (SiO_2) MeOH/CH_2Cl_2 (1:1), $R_f \sim 0.60$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.40−7.10 (m, 35H), 5.18 (m, 1H), 5.02−4.75 (m, 15H), 4.70−3.75 (m, 10H), 3.55−3.40 (m, 3H), 2.90 (m, 2H), 1.60−1.50 (m, 4H), 1.38−1.12 (m, 60H), 0.95−0.85 (t, 6H, J = 7.0 Hz). ^{31}P NMR ($CDCl_3$, 101 MHz) δ 0.26, −0.24, −0.25.

3,4,5-Tri-*O*-phosphoryl-D-*myo*-inositol 1-*O*-(1,2-*O*-dipalmitoyl)-*sn*-glyceryl Phosphate Sodium Salt (13a). A mixture of **12a** (148 mg, 0.082 mmol), $NaHCO_3$ (12.6 mg, 0.15 mmol), and Pd/C (10%) (80 mg) in 15 mL of *t*-BuOH/H_2O (6:1) was shaken under H_2 (51 psi) for 6 h. Catalyst was removed by filtration over 1 g of Celite, and the filter was washed with 0.2 M NH_4OH (2 × 20 mL). The combined filtrates were lyophilized to provide 84 mg of product **13a** (87% yield) as a white solid. ^{1}H NMR (D_2O, 300 MHz) δ 5.18−5.00 (m, 1H), 4.56−4.00 (m, 8H), 3.63−3.40 (m, 2H), 2.46−2.27 (m, 4H), 1.96−1.62 (m, 4H), 1.58−1.02 (m, 48H), 0.93 (t, 6H). ^{31}P NMR (D_2O, 101 MHz) δ 8.45, 7.52, 6.38, 3.66. Mass (MALDI-TOF), 1049 ($M^+ - 1$); calcd: 1049.

4,5-Di-*O*-phosphoryl-D-*myo*-inositol 1-*O*-(1,2-*O*-Dipalmitoyl)-*sn*-glyceryl Phosphate Sodium Salt (13b). A mixture of **12b** (47 mg, 0.030 mmol), $NaHCO_3$ (12.6 mg, 0.15 mmol), and Pd/C (10%) (80 mg) in 15 mL of *t*-BuOH/H_2O (6:1) was shaken under H_2 (51 psi) for 6 h. Catalyst was removed by filtration over 1 g of Celite, and the filter was washed with 0.2 M NH_4OH (2 × 20 mL). The combined filtrates were lyophilized to give 25 mg of product **13b** (83% yield) as a white solid. ^{1}H NMR (D_2O, 300 MHz) δ 5.20−5.00 (m, 1H), 4.40−4.10 (m, 4H), 4.06−3.60 (m, 6H), 2.30−2.10 (m, 4H), 1.85−1.75 (m, 4H), 1.55−1.05 (m, 48H), 0.85 (t, 6H). ^{31}P NMR (D_2O, 101 MHz) δ 8.9, 8.1, 4.5. Mass (MALDI-TOF), 970 ($M^+ - 1$); 992 ($MNa^+ - 1$); calcd: 970.

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