Synthesis of Photoactivatable 1,2-*O*-Diacyl-*sn*-glycerol Derivatives of 1-L-Phosphatidyl-D-*myo*-inositol 4,5-Bisphosphate ($PtdInsP_2$) and 3,4,5-Trisphosphate ($PtdInsP_3$)

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Photoactivatable analogues of 1-L-phosphatidyl-D-*myo*-inositol 4,5-bisphosphate ($PtdIns(4,5)P_2$ or $PtdInsP_2$) and the corresponding 3,4,5-trisphosphate ($PtdIns(3,4,5)P_3$ or $PtdInsP_3$) were prepared from the two chiral precursors, methyl α-D-glucopyranoside and 1,2-isopropylidene-*sn*-glycerol. Two key synthetic transformations included the Ferrier rearrangement reaction to construct the optically-pure inositol skeleton and the sequential acylation of the primary and secondary hydroxyl groups on the glycerol derivatives. The *sn*-1-*O*-(6-aminohexanoyl) $PtdInsP_2$ and $PtdInsP_3$ derivatives were further modified to contain benzophenone photophores in unlabeled and high specific activity tritium-labeled forms.

Introduction

Hydrolysis of $PtdInsP_2$ by phospholipase C (PLC) generates the now ubiquitous second messenger $Ins(1,4,5)P_3$[1−4] (Scheme 1). $PtdInsP_2$ itself functions in the recruitment of proteins to membranes[5] via pleckstrin homology (PH) domains.[6] Indeed, the PH domain of PLC allows membrane localization via the 4,5-bisphosphate of $PtdInsP_2$,[7] while the catalytic domain cleaves the P−O bond of an adjacent $PtdInsP_2$ molecule. Three-dimensional structures of the β-spectrin−$Ins(1,4,5)P_3$ complex and the PLC PH domain−complex have verified the importance of the 4,5-bisphosphate interaction with hydrogen bonding and protonated basic residues.[8] In addition, $PtdInsP_2$ sequesters profilin to the inner surface of the phospholipid bilayer, facilitating the polymerization of actin.[5,9] Release of profilin following $PtdInsP_2$ hydrolysis suppresses further cytoskeletal reorganization.

In addition to its role as $Ins(1,4,5)P_3$ precursor and membrane protein anchor, $PtdIns(4,5)P_2$ 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.[11,12] PI 3-K catalyzes the transfer of the γ phosphate of ATP to the D-3 hydroxyl of PtdIns, PtdIns(4)P, and $PtdIns(4,5)P_2$ to form PtdIns(3)P, $PtdIns(3,4)P_2$, and $PtdIns(3,4,5)P_3$, respectively. PI 3-K is a heterodimer with a native molecular weight of 190 kDa and consists of an 85 kDa regulatory subunit (p85) and a 110 kDa catalytic subunit (p110).[13] The p85 subunit has been found to contain two SH2 domains and one SH3 domain, while kinase activity has been demonstrated to reside exclusively in the p110 subunit.[14−16] PI 3-K is translocated from the cytosol to the plasma membrane where it is recruited to activated protein tyrosine kinase receptors (PTKs) upon stimulation by various growth factors.[12,17,18] This association is believed to occur through phosphotyrosine-SH2 domain interactions and serves to bring the enzyme in proximity to its substrate(s), facilitating the production of second messenger molecules.

Recent studies have identified $PtdIns(3,4,5)P_3$ as a putative second messenger molecule acting independently of the classical inositol polyphosphate pathway.[19−21] Although the molecular action of $PtdIns(3,4,5)P_3$ is unclear, several putative targets for this phosphoinositide have been reported: the serine/threonine kinase Akt/PKB,[22,23] protein kinase C,[24] and a mediator of the

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(1) Berridge, M. J.; Irvine, R. F. *Nature* **1984**, *312*, 315−321.
(2) Berridge, M. J. *Ann. Rev. Biochem.* **1987**, *56*, 159−193.
(3) Berridge, M. J. *Nature* **1993**, *361*, 315−325.
(4) Nishizuka, Y. *Science* **1992**, *258*, 607−614.
(5) (a) Janmey, P. A. *Chem. Biol.* **1995**, *2*, 61−65. (b) Janmey, P. A. *Annu. Rev. Physiol.* **1994**, *56*, 169−191.
(6) Saraste, M.; Hyvönen, M. *Curr. Opin. Struct. Biol.* **1995**, *5*, 403−408.
(7) Rebecchi, M.; Peterson, A.; McLaughlin, S. *Biochemistry* **1992**, *31*, 12742−12747.
(8) (a) Hyvönen, M.; Macias, M. J.; Nilges, M.; Oschkinat, H.; Saraste, M.; Wilmanns, M. *EMBO J.* **1995**, *14*, 4676−4685. (b) Ferguson, K. M.; Lemmon, M. A.; Schlessinger, J.; Sigler, P. B. *Cell* **1995**, *83*, 1037−1046.
(9) Lambrechts, A.; Vandamme, J.; Goethals, M.; Vandekerckhove, J.; Ampe, C. *Eur. J. Biochem.* **1995**, *230*, 281−286.
(10) Stephens, L.; Cooke, F. T.; Walters, R.; Jackson, T.; Volinia, S.; Gout, I.; Waterfield, M. D.; Hawkins, P. T. *Curr. Biol.* **1994**, *4*, 203−214.
(11) Stephens, L.; Jackson, T.; Hawkins, P. T. *J. Biol. Chem.* **1993**, *268*, 17162−17172.
(12) Stephens, L. R.; Jackson, T. R.; Hawkins, P. T. *Biochim. Biophys. Acta* **1993**, *1179*, 27−75.
(13) Carpenter, C. L.; Duckworth, B. C.; Auger, K. R.; Cohen, B.; Schaffhausen, B. S.; Cantley, L. C. *J. Biol. Chem.* **1990**, *265*, 19704−19711.
(14) Escobedo, J. A.; Navankasattusas, S.; Kavanaugh, W. M.; Milfay, D.; Fried, V. A.; Williams, L. T. *Cell* **1991**, *65*, 75−82.
(15) Hiles, I. D.; Otsu, M.; Volinia, S.; Fry, M. J.; Gout, I.; Dhand, R.; Panayotou, G.; Ruiz-Larrea, F.; Thompson, A.; Totty, N., F.; Hsuan, J. J.; Courtneidge, S. A.; Parker, P. J.; Waterfield, M. D. *Cell* **1992**, *70*, 419−429.
(16) Otsu, M.; Hiles, I.; Gout, I.; Fry, M. J.; Ruiz-Larrea, F.; Panayotou, G.; Thompson, A.; Dhand, R.; Hsuan, J.; Totty, N.; Smith, A. D.; Morgan, S. J.; Courtneidge, S. A.; Parker, P. J.; Waterfield, M. D. *Cell* **1991**, *65*, 91−104.
(17) Susa, M.; Keeler, M.; Varticovski, L. *J. Biol. Chem.* **1992**, *267*, 22951−22956.
(18) Kapeller, R.; Cantley, L. C. *Bioessays* **1994**, *16*, 565−576.
(19) Eberle, M.; Traynor-Kaplan, A. E.; Sklar, L. A.; Norgauer, J. *J. Biol. Chem.* **1990**, *265*, 16725−16728.
(20) Traynor-Kaplan, A. E.; Harris, A. L.; Thompson, B. L.; Taylor, P.; Sklar, L. A. *Nature* **1988**, *334*, 353−356.
(21) Traynor-Kaplan, A. E.; Thompson, B. L.; Harris, A. L.; Taylor, P.; Omann, G. M.; Sklar, L. A. *J. Biol. Chem.* **1989**, *264*, 15668−15673.
(22) Burgering, B. M. T.; Coffer, P. J. *Nature* **1995**, *376*, 599−602.
(23) Franke, T. F.; Yang, S.; Chan, T. O.; Datta, K.; Kazlauskas, A.; Morrison, D. K.; Kaplan, D. R.; Tsichlis, P. N. *Cell* **1995**, *81*, 727−736.
(24) Toker, A.; Meyer, M.; Reddy, K. K.; Falck, J. R.; Aneja, R.; Aneja, S.; Parra, A.; Burns, D. J.; Ballas, L. M.; Cantley, L. C. *J. Biol. Chem.* **1994**, *269*, 32358−32367.

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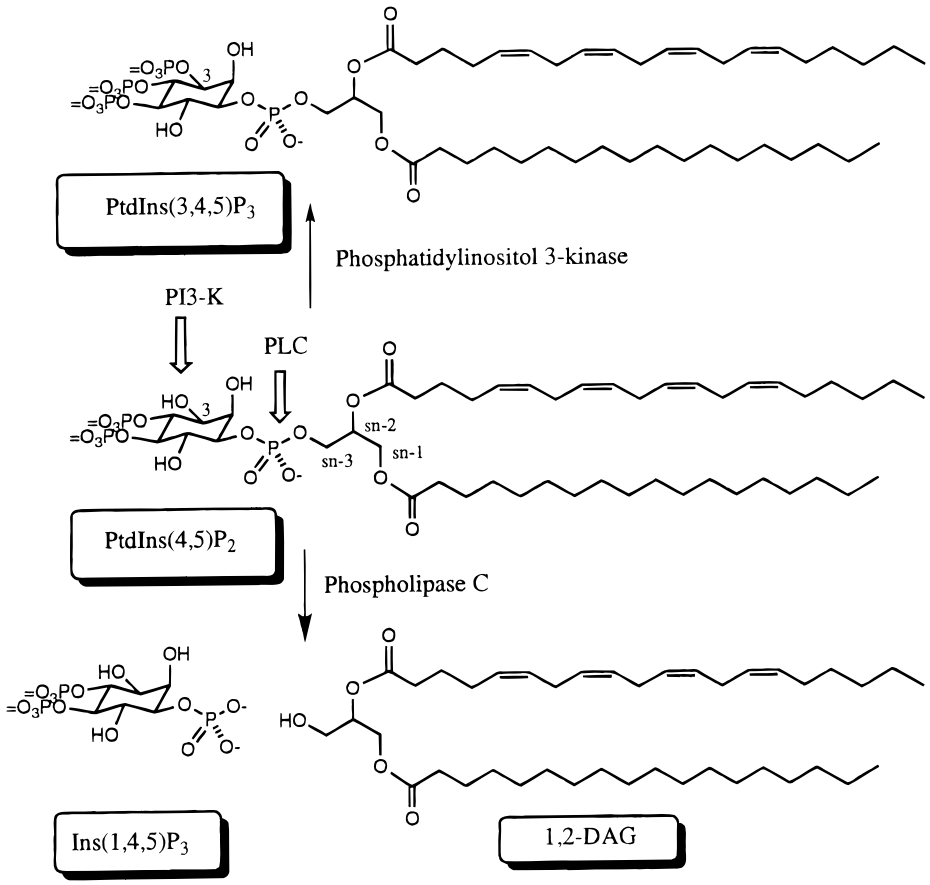
Scheme 1. Phosphatidylinositol 4,5-Bisphosphate ($PtdInsP_2$) as a Substrate for Phospholipase C and for Phosphoinositide 3-Kinase



phosphorylation of platelet p47 (pleckstrin).[25] In addition, a new 46-kDa protein, centaurin,[26] has been purified using a P-1-tethered $Ins(1,3,4,5)P_4$ affinity column[27] and labeled by a photolabile $Ins(1,3,4,5)P_4$ analogue[28,29] and appears to be a $PtdInsP_3$ target.

In order to clarify the physiological role of the inositol polyphosphate and phosphoinositide polyphosphates, we have pursued a program of preparing P-1-tethered inositol polyphosphate derivatives.[30] Scheme 2 illustrates the parent ligands $Ins(1,4,5)P_3$ and $Ins(1,3,4,5)P_4$ and their respective P-1-tethered photoaffinity reagents.[28,31,32] The selection of the benzophenone moiety optimized ease of biochemical use, chemical stability, high specific activity compatible with protein sequencing, and most importantly, nearly quantitative photocovalent modification of the target proteins.[33,34] For example, benzoyldihydrocinammoyl-IP_3 (BZDC-IP_3) has been employed to identify the active site of the rat brain type I $InsP_3$ receptor[31] and the PH domain of PLC δ_1.[35] We recognized that the lipid group appended to the aminopropyl tether on the P-1 phosphate resembled the lipidic moiety provided by a diacylglycerol. In several cases, proteins labeled by [^{3}H]BZDC-IP_3 and [^{3}H]BZDC-IP_4 probes are in fact high affinity targets for the $PtdInsP_2$ and $PtdInsP_3$ ligands, respectively. Thus, we designed analogues that more accurately mimic the $PtdInsP_2$ and $PtdInsP_3$ structures by incorporating the photoactivatable BZDC amide moiety in the backbone of the 1-*O*-acyl chain in unlabeled or radiolabeled form.[36] In this paper, we describe the synthesis of $PtdIns(4,5)P_2$ analogues **13a** and **13b** and $PtdIns(3,4,5)P_3$ analogues **13c** and **13d** pursuant to this strategy.

Results and Discussion

The convergent synthetic strategy (Schemes 3 and 4) combined a simple but concise route to the mixed 1,2-*O*-diacyl-*sn*-glycerols[37] with differentially-protected intermediates obtained via Ferrier rearrangements of glucose derivatives.[30] Thus, protection of (+)-1,2-*O*-isopropylidene-*sn*-glycerol (**1**) was performed with *p*-methoxyl-

(25) Toker, A.; Bachelot, C.; Chen, C. S.; Falck, J. R.; Hartwig, J. H.; Cantley, L. C.; Kovacsovics, T. J. *J. Biol. Chem.* **1995**, *270*, 29525−29531.

(26) Hammonds-Odie, L. P.; Jackson, T. R.; Profit, A. A.; Blader, I. J.; Turck, C.; Prestwich, G. D.; Theibert, A. B. *J. Biol. Chem.* **1996**, in press.

(27) Theibert, A. B.; Estevez, V. A.; Ferris, C. D.; Danoff, S. K.; Barrow, R. K.; Prestwich, G. D.; Snyder, S. H. *Proc. Natl. Acad. Sci., U.S.A.* **1991**, *88*, 3165−3169.

(28) Estevez, V. A.; Prestwich, G. D. *J. Am. Chem. Soc.* **1991**, *113*, 9885−9887.

(29) Theibert, A. B.; Estevez, V. A.; Mourey, R. J.; Marecek, J. F.; Barrow, R. K.; G. D. Prestwich; Snyder, S. H. *J. Biol. Chem.* **1992**, *267*, 9071−9079.

(30) Prestwich, G. D. *Acc. Chem. Res.* **1996**, in press.

(31) Mourey, R. J.; Estevez, V. A.; Marecek, J. F.; Barrow, R. K.; Prestwich, G. D.; Snyder, S. H. *Biochemistry* **1993**, *32*, 1719−1726.

(32) Prestwich, G. D.; Marecek, J. F.; Mourey, R. J.; Theibert, A. B.; Ferris, C. D.; Danoff, S. K.; Snyder, S. H. *J. Am. Chem. Soc.* **1991**, *113*, 1822−1825.

(33) Dormán, G.; Prestwich, G. D. *Biochemistry* **1994**, *33*, 5661−5673.

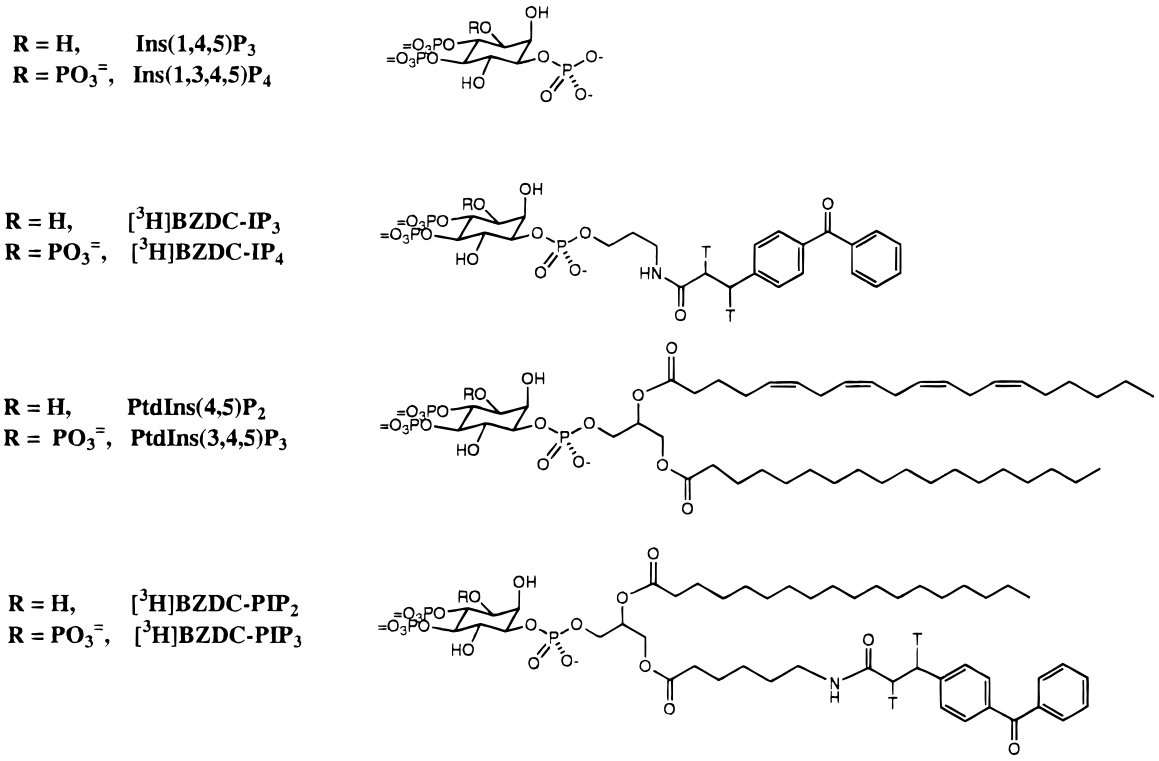
(34) Prestwich, G. D.; Dormán, G.; Elliott, J. T.; Marecak, D. M.; Chaudhary, A. *Photochem. Photobiol.* **1996**, in press.

(35) Tall, E.; Dormán, G.; Garcia, P.; Shah, S.; Prestwich, G. D.; Rebecchi, M. J. *J. Biol. Chem.* **1996**, submitted.

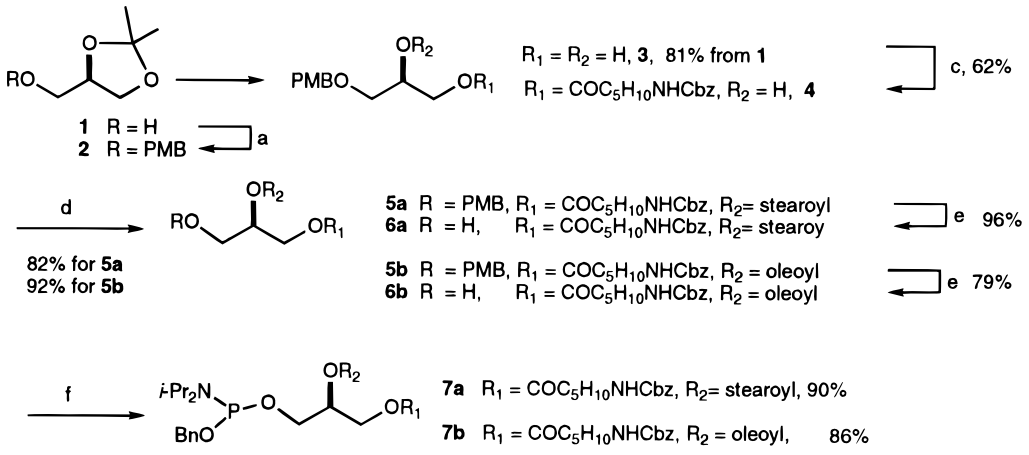
(36) Olszewski, J. D.; Dormán G.; Elliott, J. T.; Hong, Y.; Ahern, D. G.; Prestwich, G. D. *Bioconjugate Chem.* **1995**, *6*, 395−400.

(37) Martin, S. F.; Josey, J. A.; Wong, Y.-L.; Dean, D. W. *J. Org. Chem.* **1994**, *59*, 4805−4820.

Scheme 2. Structures of Inositol Polyphosphates (InsP$_n$s), InsP$_n$ Photoprobes, Phosphoinositide Polyphosphates PtdIns(4,5)P$_2$ and PtdIns(3,4,5)P$_3$, and PtdInsP$_n$ Photoprobes



Scheme 3[a]

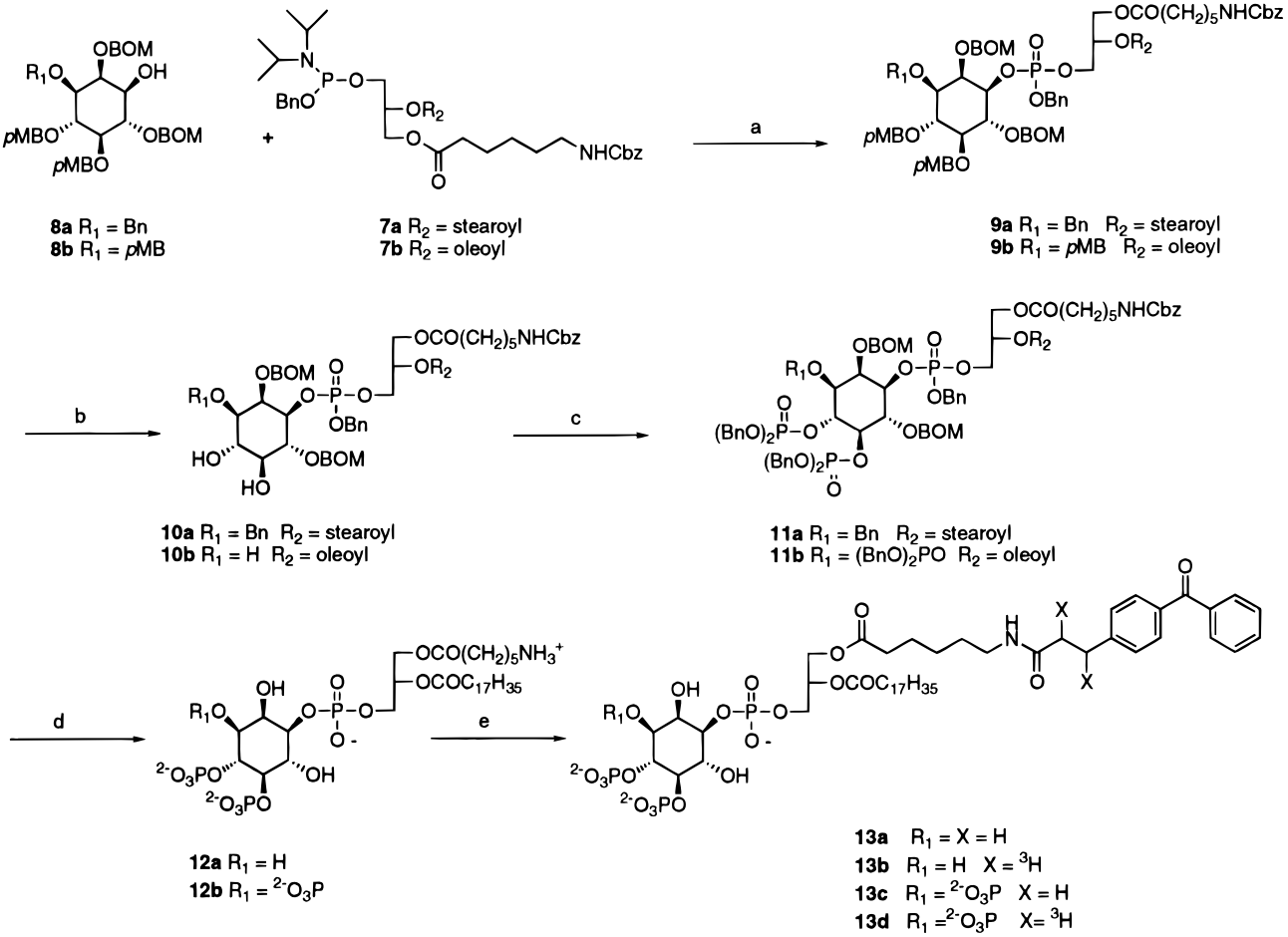


[a] Reagents: (a) PMBCl/NaH, DMF, rt, 1 h; (b) 5% (wt) *p*-TsOH, MeOH, rt, 1 h; (c) CbzNHC$_5$H$_{10}$COOH, DMAP, DCC, CH_2Cl_2, 0 °C, overnight; (d) stearic acid or oleic acid, DMAP, DCC, CH_2Cl_2, rt, overnight; (e) DDQ, wet CH_2Cl_2, rt, overnight; (f) (BnO)P(Cl)(NPr$_2$-*i*), *i*-Pr$_2$NEt, CH_2Cl_2, 0 °C to rt, 2 h.

benzyl chloride (PMB-Cl)[38] to give PMB ether **2**, which was transketalized (10 mol % of *p*-TsOH in MeOH) to the 1,2-diol **3** in 83% isolated yield.[39] The mixed 1,2-*O*-diacylated glycerol was prepared by DCC/DMAP-mediated sequential acylation.[38] Thus, diol **3** was condensed with 6-(*N*-Cbz-amino)hexanoic acid in the presence of DCC and DMAP at 0 °C to give three products. The desired 1-acylated compound **4** was the main product obtained in 62% isolated yield; this regioisomer was readily distinguished from the 2-acylated isomer by the ^{1}H resonance of the methine proton (δ = 5.2 ppm (m) for the undesired 2-acyl material). Esterification of the remaining secondary hydroxyl with either stearic acid or oleic acid provided diacylglycerols **5a** or **5b** in 82% and 92% yield, respectively. The use of oleic acid at this step set the stage for introduction of tritium into the fatty acyl group during hydrogenolytic deprotection in the final step. Removal of PMB with DDQ in wet CH_2Cl_2 (0.5% water in volume) to give 1-[6-(*N*-Cbz-amino)hexanoyl]-2-stearoylglycerol (**6a** and **6b**) (in 96% and 79% yield) occurred without any detectable migration of the stearoyl group from the 2-position to the 3-position. Reaction of diacylglycerol **6a** with (benzyloxy)(*N*,*N*-diisopropylamino)chlorophosphine[28] gave phosphatidylating reagent **7a** in good isolated yield. This reagent was found to be unstable even when stored at −20 °C because of facile air-oxidation detected by ^{31}P NMR. Thus, the glyceryl

(38) Hebert, N.; Beck, A.; Lennox, R. B.; Just, G. *J. Org. Chem.* **1992**, *57*, 1777−1783.
(39) Manley, P. W.; Tuffin, D. T.; Allanson, N. M.; Buckle, P. E.; Lad, N.; Lai, S. M. F.; Lunt, D. O.; Porter, R. A.; Wade, P. J. *J. Med. Chem.* **1987**, *30*, 1812−1818.

Scheme 4[a]



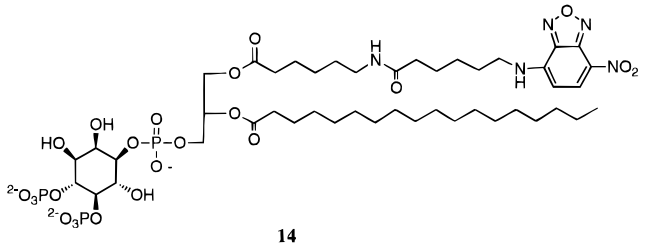
[a] Reagents: (a) 1*H*-tetrazole, *m*-CPBA, −40 °C to rt; (b) DDQ, wet CH_2Cl_2; (c) $(BnO)_2PNPr_2$-*i*, 1*H*-tetrazole then *m*-CPBA; (d) 50 psi H_2, Pd/C, *t*-BuOH−H_2O, $NaHCO_3$; (e) BZDC-NHS or [^{3}H]BZDC-NHS, DMF 0.25 M TEAB, rt, overnight.

phosphoramidite reagent was used immediately after preparation for coupling to the protected inositol phosphate precursor.

Construction of 2,6-bis-*O*-(benzyloxymethyl)-3-*O*-benzyl-4,5-bis-*O*-(4-methoxybenzyl)-*myo*-inositol (**8a**) from methyl α-D-glucose was accomplished as described for the synthesis of a P-1-tethered Ins(1,4,5)P_3 photoaffinity label.[40] Coupling of intermediate **8a** with phosphatidylating reagent **7a** in the presence of 1*H*-tetrazole followed by *m*-CPBA oxidation gave the protected phosphoinositide precursor **9a** in 87% yield. Removal of two PMB groups from compound **9a** with DDQ in wet CH_2Cl_2 gave diol **10a** that was further phosphorylated to give the PtdInsP_2 analogue precursor **11a**. Deprotection of the benzyl and BOM groups was carried out at an initial pressure of 50 psi of H_2 in 2-methyl-2-propanol−H_2O containing $NaHCO_3$ using 5% Pd/C as catalyst at rt for 4 h. After removal of the catalyst and concentration in vacuo, an amorphous solid was obtained. Purification by Chelex chromatography afforded sodium salt **12a** in 78% yield. This solid was partially dissolved in water to yield a cloudy solution.

Reaction of the aminohexanoyl PtdInsP_2 analogue **12** with *N*-hydroxysuccinimidyl benzoyldihydrocinnamic acid ester (BZDC-NHS ester)[36] in 1:1 (v/v) DMF−0.25 M triethylammonium bicarbonate (TEAB) buffer at rt for overnight, followed by purification on DEAE-cellulose, gave the cold BZDC derivative **13a** in 70% yield. After the reaction was scaled down to the microgram scale, the [^{3}H]BZDC derivative **13b** was prepared similarly.

In the same way, when reacted with *N*-hydroxysuccinimidoyl 6-[(7-nitro-2-oxa-1,3-diazobenz-4-yl)amino]-hexanoate (NBD aminohexanoic NHS ester), a fluorescent PtdInsP_2-NBD (**14**) was obtained. This fluorescent analogue has been used to demonstrate the effect of a highly basic peptide in sequestering PtdInsP_2 into two-dimensional lateral domains in phospholipid vesicles.[41]



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The synthetic strategy for the 1-L-phosphatidyl-D-*myo*-inositol 3,4,5-trisphosphate analogues parallels that of our PtdInsP_2 compounds, except that the PtdInsP_3 prod-

(40) Dormán, G.; Chen, J.; Prestwich, G. D. *Tetrahedron Lett.* **1995**, *36*, 8719−8722.

(41) Glaser, M.; Wanaski, S.; Buser, C. A.; Boguslavsky, V.; Rashidzada, W.; Morris, A.; Rebecchi, M.; Scarlata, S.; Runnels, L. W.; Prestwich, G. D.; Chen, J.; Aderem, A.; Ahn, J.; McLaughlin, S. *J. Biol. Chem.* **1996**, in press.

uct requires an inositol intermediate (obtained via a Ferrier rearrangement) equipped with PMB ethers protecting the C3, -4, and -5 hydroxyl groups (**8b**).[28] Phosphitylation of **8b** with glycerol phosphoramidite **7b** in the presence of 1*H*-tetrazole followed by oxidation with *m*-CPBA furnished the protected phosphatidylinositol **9b** in 88% yield. Cleavage of the PMB groups with DDQ afforded triol **10b** in 61% yield, and phosphorylation was achieved using dibenzyl *N*,*N*-diisopropylphosphoramidite followed by oxidation to give compound **11b** (83% yield). Removal of all benzyl-derived protecting groups was accomplished by catalytic hydrogenation on 10% Pd/C in 2-methyl-2-propanol/H_2O with seven equivalents of $NaHCO_3$, giving the PtdInsP$_3$ analogue **12b** as a hexasodium salt. As with the PtdInsP$_2$ analogue, condensation of the primary amine with BZDC-NHS ester afforded the benzophenone-based photoaffinity probe in both radiolabeled and nonlabeled forms (**13c** and **13d**).

Earlier syntheses of a number of phosphatidylinositol polyphosphates have been reported, and these synthetic compounds have been employed to verify specific biological roles for these ligands. Falck and co-workers described the synthesis of the *sn*-1,2-distearoyl analogue of PtdInsP$_3$ using (−)-quinic acid as the chiral starting material for the construction of D-*myo*-inositol skeleton.[42−44] Gou and Chen have completed an analogous synthesis from enzymatically-resolved 1-D-dicyclohexylidene-*myo*-inositol,[45] while Bruzik and Kubiak started from the camphor ketal of *myo*-inositol.[46] Watanabe et al. reported concise routes to the racemic form of PtdIns(3,4,5)P$_3$ and recently the optically pure form from the enzymatically resolved 1-D-1,2-cyclohexylidene-*myo*-inositol.[47,48]

The syntheses of modified PtdInsP$_2$ and PtdInsP$_3$ analogues described herein offer three unique features. First, the Ferrier rearrangement[49] of an inexpensive α-D-glucose derivative provides the enantiomerically-pure inositol framework. Second, the selective esterification of the *sn*-1 position with an amino-functionalized ester allows introduction of a variety of reporter groups. Third, the syntheses described herein represent the first high specific activity, tritium-labeled photoactivatable analogues of the phosphoinositide polyphosphates available for exploration of lipid binding regions of PtdInsP$_2$ and PtdInsP$_3$ biochemical targets. These photoactivatable acyl-modified analogues, as well as a novel series of phosphotriester head group-modified analogues[50] of PtdInsP$_2$ and PtdInsP$_3$, have been evaluated in biochemical experiments and appear to be readily recognized as PtdInsP$_2$ or PtdInsP$_3$ mimics. These results will be reported elsewhere in due course.

Experimental Section

^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded in $CDCl_3$ or D_2O on a QE-300 or AC-250 NMR spectrometer and reported relative to δ(TMS) = 0 ppm. When necessary, solvents and reagents were dried using standard procedures. FAB HRMS were performed at the University of California at Riverside, using NBA matrix to obtain MNa^+ as formula peak.

3-(4-Methoxybenzyl)-*sn*-glycerol (3). To a solution of 1,2-*O*-isopropylidene-*sn*-glycerol (**1**, 1000 mg, 7.6 mmol) in 100 mL of dry DMF was added NaH (455 mg, 60%, 11.4 mmol), followed by addition of *p*-methoxybenzyl chloride (1,188 mg, 9.1 mmol). The mixture was stirred at rt for 1 h (TLC, **2** R_f = 0.82, 33% ethyl acetate/hexane). Excess NaH was destroyed by dropwise addition of MeOH, and CH_2Cl_2 was added to extract. The CH_2Cl_2 layer was washed with brine and dried over Na_2SO_4. After concentration, an oil was obtained and redissolved in 20 mL of MeOH containing *p*-toluenesulfonic acid monohydrate (70 mg, 0.05 equiv, 0.37 mmol). The mixture was stirred at rt for 1 h (TLC, **3** R_f = 0.24, 75% ethyl acetate/hexane). Next, $NaHCO_3$ (100 mg) was added, and the solvent was evaporated. The residue was purified by flash chromatography on silica gel ("purified on SiO_2") with 75% ethyl acetate/hexane to give 1.322 g of 3-(4-methoxybenzyl)-*sn*-glycerol (**3**) (83% yield from **1**): mp 40−42 °C (lit.[6] mp 40−41 °C). ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 7.25, 7.22, 6.89, 6.85 (4s, 4H, phenyl), 4.46 (s, 2H, PMBCH_2O), 3.90−3.75 (s, 1H, H-2), 3.80 (s, 3H, MeO), 3.64−3.45 (m, 4H, H-1 and H-3), 2.86 (s and br, 1H, OH), 2.42 (s and br, 1H, OH) ppm. ^{13}C NMR ($CDCl_3$, 63 MHz) δ: 129.46, 113.91, 73.24, 71.48, 70.64, 64.01, 55.29 ppm.

1-[6-(*N*-Cbz-amino)hexanoyl]-3-(4-methoxybenzyl)-*sn*-glycerol (4). To a solution of the 3-(4-methoxybenzyl)-*sn*-glycerol (**3**, 772 mg, 3.64 mmol) and 6-[(carbobenzyloxy)amino]-hexanoic acid (927 mg, 3.5 mmol) in 40 mL of dry CH_2Cl_2 at 0 °C was added dropwise a solution of DCC (865 mg, 4.2 mmol) and DMAP (512 mg, 4.2 mmol) in 10 mL of dry CH_2Cl_2. The solution was stirred at 0 °C for 16 h and concentrated. The residue was redissolved in 20 mL of dry ethyl acetate, filtered, concentrated in vacuo, and purified on SiO_2 (25% ethyl acetate in CH_2Cl_2, R_f = 0.7; the R_f of the other isomer is 0.65, and the R_f of the diester is 0.95) giving 1.041 g (62% yield) of glycerol derivative **4**. ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 7.40−7.30 (m, 5H, Ph), 7.25, 7.21, 6.89, 6.85 (4s, 4H, PMB), 5.08 (s, 2H, Bn), 4.84 (s and br, 1H, NH), 4.47 (s, 2H, PMBCH_2), 4.20−4.09 (m, 2H, H-1 and H-2), 4.08−3.97 (m, 1H, H-1), 3.79 (s, 3H, MeO), 3.53−3.40 (m, 2H, H-3), 3.27 (q, J = 6.5 Hz, CH_2N), 2.61 (d, J = 4.5 Hz, 1H, OH), 2.31 (t, J = 7.2 Hz, 2H, CH_2CO), 1.70−1.30 (m, 6H) ppm. ^{13}C NMR ($CDCl_3$, 63 MHz) δ: 173.64, 159.36, 129.74, 129.46, 128.52, 128.12, 113.87, 73.15, 70.51, 68.85, 66.62, 65.48, 55.29, 40.77, 33.94, 29.57, 26.08, 24.44 ppm. HRMS: calcd for $C_{25}H_{34}NO_7(MH^+)$ 460.2335, found 460.2344.

1-*O*-[6-(*N*-Cbz-amino)hexanoyl]-2-*O*-stearoyl-3-*O*-(4-methoxybenzyl)-*sn*-glycerol (5a). A solution of DCC (453 mg, 2.2 mmol) and DMAP (112 mg, 1 mmol) in 5 mL of CH_2Cl_2 was added to a solution of glycerol **4** (841 mg, 1.83 mmol) and stearic acid (624 mg, 2.2 mmol) in 10 mL of dry CH_2Cl_2. Stirring was continued overnight at rt. Similar workup as above and purification on SiO_2 (20% ethyl acetate/hexane, R_f = 0.55) gave 1.225 g (92% yield) of product **5a**. ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 7.40−7.30 (m, 5H, Ph), 7.25, 7.21, 6.89, 6.85 (4s, 4H, PMB), 5.25−5.17 (m, 1H, H-2), 5.08 (s, 2H, Bn), 4.84 (s and br, 1H, NH), 4.45 (AB, J_{AB} = 11.8 Hz, 2H, PMBCH_2), 4.32 (dd, J = 3.7 Hz, 11.8 Hz, 1H, H-1), 4.12 (dd, J = 11.8 Hz, 3.7 Hz, 1H, H-1), 3.78 (s, 3H, MeO), 3.53 (d, J = 5.1 Hz, 2H, H-3), 3.17 (q, J = 6.5 Hz, CH_2N), 2.28 (2t, J = 7.2, 8.9 Hz, 4H, CH_2CO), 1.70−1.10 (m, 36H), 0.88 (t, J = 6.2 Hz, 3H CH_3) ppm. ^{13}C NMR ($CDCl_3$, 63 MHz) δ: 173.16, 173.10, 159.31, 156.39, 136.65, 129.74, 129.32, 128.51, 128.08, 113.82, 72.94, 70.00, 67.87, 66.56, 62.84, 55.26, 40.82, 33.94, 29.51, 29.30, 29.09, 26.15, 24.96, 24.41, 22.72, 14.14 ppm. HRMS: calcd for $C_{43}H_{67}NNaO_8(MNa^+)$ 748.4764, found 748.4746.

1-[6-(*N*-Cbz-Amino)hexanoyl]-2-stearoyl-*sn*-glycerol (6a). To a solution of glyceryl ether **5** (1.152 mg, 1.59 mmol) in 50 mL of wet CH_2Cl_2 was added DDQ (750 mg, 3.30 mmol). The mixture was stirred at rt overnight, diluted with CH_2Cl_2, washed with 10% $NaHCO_3$, dried over Na_2SO_4, concentrated, and purified on SiO_2 (30% ethyl acetate/hexane, R_f = 0.55) to give compound **6a** (930 mg, 96% yield). ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 7.35−7.25 (m, 5H, Ph), 5.15−5.00 (m, 3H, H-2 and

(42) Falck, J. R.; Abdali, A. *BioMed. Chem. Lett.* **1993**, *3*, 717−720.
(43) Reddy, K. K.; Falck, J. R.; Capdevila, J. *Tetrahedron Lett.* **1993**, *34*, 7869−7872.
(44) Reddy, K. K.; Saady, M.; Falck, J. R.; Whited, G. *J. Org. Chem.* **1995**, *60*, 3385−3390.
(45) Gou, D.-M.; Chen, C.-S. *J. Chem. Soc., Chem. Commun.* **1994**, 2125−2126.
(46) Bruzik, K. S.; Kubiak, R. J. *Tetrahedron Lett.* **1995**, *36*, 2415−2418.
(47) Watanabe, Y.; Hirofuji, H.; Ozaki, S. *Tetrahedron Lett.* **1994**, *35*, 123−124.
(48) Watanabe, Y.; Tomioka, M.; Ozaki, S. *Tetrahedron* **1995**, *51*, 8969−8976.
(49) Ferrier, R. J.; Middleton, S. *Chem. Rev.* **1993**, *93*, 2779−2831.
(50) Gu, Q.-M.; Prestwich, G. D. *J. Org. Chem.* **1996**, submitted.

BnCH_2), 4.86 (s and br, 1H, NH), 4.32 (dd, J = 3.7 Hz, 11.8 Hz, 1H, H-1), 4.12 (dd, J = 11.8, 3.7, 1H, H-1), 3.69 (dd, J = 5.6, J = 5.7 Hz, 2H, H-3), 3.17 (q, J = 6.5 Hz, CH_2N), 2.41−2.30 (m, 4H, CH_2CO), 1.70−1.10 (m, 36H), 0.88 (t, J = 6.2 Hz, 3H CH_3) ppm. ^{13}C NMR ($CDCl_3$, 63 MHz) δ: 173.43, 156.45, 136.56, 128.52, 128.10, 72.02, 66.63, 62.17, 61.40, 40.82, 33.94, 29.51, 29.30, 29.09, 26.15, 24.96, 24.41, 22.72, 14.14 ppm. HRMS: calcd for $C_{35}H_{60}NO_7(MH^+)$ 606.4370, found 606.4384.

(Benzyloxy)3-[1-[6-(*N*-Cbz-amino)hexanoyl]-2-stearoyl-*sn*-glyceryl](*N*,*N*-diisopropylamino)phosphine (7a). Glycerol derivative **6a** (240 mg, 0.04 mmol) was dissolved in 10 mL of CH_2Cl_2, and diisopropylethylamine (103 mg, 0.04 mmol) was added. The solution was cooled to 0 °C under N_2, and a solution of (benzyloxy)(*N*,*N*-diisopropylamino)chlorophosphine (110 mg, 0.04 mmol) in 2 mL of CH_2Cl_2 was added. The mixture was stirred at 0 °C for 10 min and at rt for 75 min, diluted with CH_2Cl_2, washed with 10% aqueous $NaHCO_3$, dried (Na_2SO_4), concentrated, and purified on SiO_2 (ethyl acetate/hexane/triethylamine (20:10:1) R_f = 0.96) to give 300 mg (90% yield) of product **7a** as a colorless oil. ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 7.34−7.24 (m, 10H, phenyl), 5.15−5.00 (m, 5H, H-2 and BnCH_2), 4.77−3.34 (m, 15H), 3.17 (q, J = 6.5 Hz, CH_2N), 2.41−2.30 (m, 4H, CH_2CO), 1.70−1.10 (m, 48H), 0.88 (t, J = 6.2 Hz, 3H, CH_3) ppm. ^{13}C NMR ($CDCl_3$, 63 MHz) δ: 173.07, 156.45, 141.96, 128.52, 128.28, 128.11, 127.31, 126.95, 70.79, 66.60, 62.17, 61.87, 43.15, 42.95, 40.82, 33.94, 29.51, 29.30, 29.09, 26.15, 24.96, 24.41, 22.72, 14.17 ppm. ^{31}P NMR (101 MHz, $CDCl_3$) δ: 150.07, 149.94 (1:1) ppm.

Benzyl 3-[1-[6-(*N*-Cbz-amino)hexanoyl]-2-stearoyl-*sn*-glyceryl] 1-[2,6-Bis-*O*-[(benzyloxy)methyl]-3-*O*-benzyl-*myo*-inosityl] Phosphate (9a). A mixture of protected **8a** (150 mg, 0.20 mmol) in 8 mL of CH_2Cl_2 and 1*H*-tetrazole (56 mg, 0.8 mmol) was stirred at rt, while a solution of glyceryl phosphoramidite **7a** (202 mg, 0.24 mmol) in 2 mL of CH_2Cl_2 was added in one portion. The mixture was stirred at rt for 1 h and then cooled to −40 °C, and *m*-CPBA (70 mg, 0.4 mmol) was added while stirring continued at this temperature for 5 min. Next, the mixture was stirred at 0 °C for 30 min and rt for 30 min. The mixture was diluted to 50 mL with CH_2Cl_2, washed with 10% aqueous Na_2SO_3, 10% $NaHCO_3$, and water, dried ($MgSO_4$), and concentrated. The residue was purified by chromatography on silica gel using 30% ethyl acetate/hexane (R_f = 0.50) to give 160 mg (87% yield based on consumed starting material) of compound **9a** as a colorless syrup. Unreacted inositol **8a** (58 mg, 39% yield) was recovered. ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 7.40−7.15 (m, 29H, phenyl and PMB), 6.83, 6.81, 6.79, 6.77 (4s, 4H, PMB), 5.20−4.45 (m, 20H), 4.30−3.90 (m, 6H), 3.77 (s, 6H, MeO), 3.55−3.30 (m, 2H), 3.16 (q, J = 6.5 Hz, 2H, CH_2N), 2.36−2.16 (m, 4H, CH_2CO), 1.70−1.20 (m, 36H), 0.87 (t, J = 6.2 Hz, 3H, CH_3) ppm. ^{13}C NMR ($CDCl_3$, 63 MHz) δ: 173.04, 159.67, 137.83, 129.67, 129.13, 128.58, 1128.37, 128.23, 127.97, 127.86, 127.71, 127.59, 127.45, 127.37, 113.73, 96.04, 70.79, 66.60, 62.17, 61.87, 43.15, 42.95, 40.82, 33.94, 29.51, 29.30, 29.09, 26.15, 24.96, 24.41, 22.72, 14.17 ppm. ^{31}P NMR (250 MHz, $CDCl_3$) δ: 9.74, 0.27 (1:1) ppm. (The minor impurity ^{31}P NMR was readily removed after the next step.) HRMS: calcd for $C_{87}H_{114}NNaO_{19}P(MNa^+)$ 1530.7620, found 1530.7703.

Benzyl 1-[6′-(*N*-Cbz-amino)hexanoyl]-2-stearoyl-*sn*-glyceryl 1-*O*-[2,6-Bis-*O*-[(benzyloxy)methyl]-3-*O*-benzyl-D-*myo*-inositol] Phosphate (10a). A mixture of the protected coupled phosphoinositide **9a** (160 mg, 0.113 mmol) and DDQ (100 mg, 0.44 mmol) in 10 mL of wet CH_2Cl_2 was stirred at rt for 4 h. TLC showed the reaction was complete. (R_f (**9a**) = 0.50; R_f (**10a**) = 0.22, 50% ethyl acetate/hexane). Usual workup followed by purification of SiO_2 (50% ethyl acetate/hexane) gave 100 mg of pure compound **10a** (75% yield). ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 7.35−7.15 (m, 25H, phenyl), 5.20−4.45 (m, 18H), 4.30−3.90 (m, 8H), 3.55−3.30 (m, 2H), 3.16 (q, J = 6.5 Hz, 2H, CH_2N), 2.36−2.16 (m, 4H, CH_2CO), 1.70−1.20 (m, 36H), 0.87 (t, J = 6.2 Hz, 3H, CH_3) ppm. ^{13}C NMR ($CDCl_3$, 63 MHz) δ: 172.83, 170.46, 144.50, 137.76, 128.71−127.57 (m), 121.27, 96.55, 95.31, 76.54, 73.65, 73.13, 72.14, 70.49, 69.55, 69.30, 69.17, 66.60, 65.32, 61.61, 58.11, 40.82, 34.08, 33.70, 31.94, 29.72, 29.52, 29.39, 29.33, 29.10, 26.15, 24.96, 24.41, 22.72, 14.17 ppm. ^{31}P NMR (101 MHz, $CDCl_3$) δ: 0.15, 0.04 (near 1:1) ppm. HRMS: calcd for $C_{71}H_{98}NNaO_{17}P(MNa^+)$ 1290.6470, found 1290.6399.

2,6-Bis-*O*-[(benzyloxy)methyl]-3-*O*-benzyl-4,5-bis-*O*-(dibenzylphosphonyl)-D-*myo*-inosityl 1-*O*-[1-[6′-(*N*-Cbz-amino)hexanoyl]-2-stearoyl-*sn*-glyceryl Benzyl Phosphate (11a). A mixture of diol **10a** (90 mg, 0.076 mmol) in 8 mL of CH_2Cl_2 and 1*H*-tetrazole (43 mg, 0.61 mmol) was stirred at rt, while a solution of dibenzyl *N*,*N*-diisopropylphosphoramidite (105 mg, 0.305 mmol) in 2 mL of CH_2Cl_2 was added in one portion. The mixture was stirred at rt for 30 min, and then *m*-CPBA (65 mg, 0.38 mmol) was added to effect oxidation as described for compound **9a**. Similar workup gave 109 mg of fully-protected PtdInsP_2 **11a** by chromatography on silica gel using 30% ethyl acetate/hexane (R_f = 0.21). ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 7.40−7.15 (m, 45H, phenyl), 5.20−4.45 (m, 27H), 4.30−3.90 (m, 6H), 3.50−3.44 (m, 1H), 3.16 (q, J = 6.5 Hz, 2H, CH_2N), 2.36−2.16 (m, 4H, CH_2CO), 1.70−1.20 (m, 36H), 0.87 (t, J = 6.2 Hz, 3H, CH_3) ppm. ^{13}C NMR ($CDCl_3$, 63 MHz) δ: 172.86, 171.77, 144.50, 133.20, 130.10−127.50 (m), 96.53, 95.46, 76.54, 73.65, 73.13, 72.14, 70.49, 69.55, 69.30, 69.17, 66.60, 65.32, 61.61, 58.11, 40.82, 34.08, 33.70, 31.94, 29.72, 29.52, 29.39, 29.33, 29.10, 26.15, 24.96, 24.41, 22.72, 14.14 ppm. ^{31}P NMR (101 MHz, $CDCl_3$) δ: 0.09, −0.03, -0.39 ppm (near 1:3:2, corresponding to two diastereoisomers). HRMS: calcd for $C_{99}H_{124}NNaO_{23}P_3$ 1810.7675, found 1810.7672.

4,5-Di-*O*-phosphoryl-D-*myo*-inosityl 1-*O*-[1-(6′-Aminohexanoyl)-2-stearoyl-*sn*-glyceryl] Phosphate Tetrasodium Salt (12a). A mixture of protected PtdInsP_2 **11a** (130 mg, 0.074 mmol), 5% Pd/C (190 mg), and $NaHCO_3$ (43 mg, 0.51 mmol) in 55 mL of *t*-BuOH-H_2O (6:1) was shaken under H_2 (50 psi) at rt for 4 h. The catalyst was filtered through Celite, and the Celite pad was washed with 10 mL of ethyl acetate, 10 mL of EtOH, 10 mL of EtOH−H_2O, and 10 mL of H_2O. The combined filtrate was concentrated in vacuo giving a solid, which made a cloudy solution when dissolved in water. This solution was adsorbed to a Chelex column (Na^+ form), and the phosphoinositide product was eluted with water. Concentration in vacuo gave an amorphous solid of compound **12a** (56 mg, 78% yield). ^{1}H NMR (D_2O, 250 MHz) δ: 5.20−5.00 (m, 1H), 4.30−3.50 (m, 10H), 2.90 (m, 2H, CH_2N), 2.36−2.16 (m, 4H, CH_2CO), 1.70−1.20 (m, 36H), 0.87 (m, 3H, CH_3) ppm. ^{31}P NMR (D_2O, 101 MHz) δ: 8.70, 8.60, 3.29 (near 1:1:1) ppm.

4,5-Di-*O*-phosphoryl-D-*myo*-inosityl 1-*O*-[1-[6′-[(*p*-Benzoyldihydrocinnamoyl)amino]hexanoyl]-2-stearoyl-*sn*-glyceryl] Phosphate Tetrakis(triethylammonium) Salt (PtdInsP_2-BZDC, 13a). PtdInsP_2 analogue **12a** (8 mg, 8.3 μmol) was dissolved in 1.2 mL of 0.25 M TEAB buffer, and a solution of *N*-hydroxysuccinimidyl *p*-benzoyldihydrocinnamic acid ester (BZDC-NHS ester) (6 mg, 17.1 μmol) in 400 μL of DMF was added. The mixture was stirred at rt overnight and concentrated in vacuo, and the residue was redissolved in 1 mL of water and reconcentrated to remove the remaining triethylammonium salt. Acetone was added, the precipitate formed was centrifuged, and the acetone was decanted. The solid was washed with acetone and centrifuged five times, until the acetone solution showed no UV-active materials. The solid, which was found to be free of *N*-hydroxysuccinimide and *p*-benzoylhydrocinnamic acid, was then dissolved in 1 mL of water and applied to a DEAE cellulose column (55 × 10 mm, HCO_3^- form). The column was eluted with two 3-mL portions of water, and then with 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, and 1.0 M TEAB (3 mL for each concentration), and finally with three 3-mL portions of 1.28 M TEAB. The product eluted in 1.0 and 1.28 M buffer; fractions were concentrated and analyzed by TLC developed in $CHCl_3$:MeOH:10 N NH_4OH = 6:4:1. The fractions were lyophilized to give 8 mg (80% yield) of PtdInsP_2-BZDC (**13a**). ^{1}H NMR (250 MHz, D_2O) δ: 7.70−7.55 (m, 5H), 7.40 (t, 2H), 7.26 (t, 2H), 5.2 (m, 1H), 4.8−3.4 (m, 10H), 3.06 (t, J = 6.5 Hz, 2H), 2.90 (t, J = 7.5 Hz, 2H), 2.45 (t, J = 7.5 Hz, 2H), 2.1 (m, 4H), 1.4−1.0 (m, 36H), 0.8(br s, 3H) ppm. ^{31}P NMR δ: 4.97 (s), 4.37 (s), 3.71 (s) (1:1:1) ppm. MALDI-TOF MS: 1131 (M^- + Na − 1).

4,5-Di-*O*-phosphoryl-D-*myo*-inosityl 1-*O*-[1-[6′-[(*p*-Benzoylditritiocinnamoyl)-amino]hexanoyl]-2-stearoyl-*sn*-glyceryl] Phosphate Tetrakis(triethylammonium) salt ([^{3}H]PtdInsP_2-BZDC, 13b). Aminohexanoyl derivative **12a**

(0.25 mg/1 mL of 0.25 M TEAB buffer stock solution, 100 μL) was added to 30 μL of DMF containing [^{3}H]BZDC-NHS ester (2 mCi) and stirred overnight at rt. The solvents were evaporated in vacuo, and the residue was evaporated with 100 μL of water to remove the remaining triethylammonium salt. The residue was dissolved in 1 mL of water and applied to DEAE cellulose column (15 × 4 mm, HCO_3^- form). The column was eluted with two 1-mL portions of water, and then with 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, and 1.0 M TEAB (1 mL for each concentration), and finally with three 1-mL portions of 1.28 M TEAB. A 1-μL aliquot of each fraction was analyzed by liquid scintillation counting, the 1.0 M and 1.28 M fractions contained 120 μCi of **13b** (6% radiochemical yield).[51]

4,5-Di-*O*-phosphoryl-D-*myo*-inosityl 1-*O*-[1-[6′-[[6-[(7-nitro-2-oxa-1,3-diazolobenz-4-yl)amino]hexanoyl]amino]-hexanoyl]-2-stearoyl-*sn*-glyceryl] Phosphate Tetrakis-(triethylammonium) Salt (PtdInsP$_2$-NBD, 14). Aminohexanoyl derivative **12a** (7 mg, 7.3 μmol) was dissolved in 1200 μL of 0.25 M TEAB buffer, and a solution of *N*-hydroxysuccinimidyl 6-[7-nitro-2-oxa-1,3-diazolobenz-4-yl)amino]-hexanoate (NBD-aminocaproic NHS ester, 5 mg, 12.8 μmol) in 400 μL of DMF was added. The mixture was stirred at rt overnight and concentrated in vacuo, and the residue was redissolved in 1 mL of water and reconcentrated to remove the remaining triethylammonium salt. Acetone was added, the precipitate formed was centrifuged, and the acetone was decanted. The solid was washed with acetone and centrifuged five times, until the acetone solution showed no fluorescent-active (NBD-aminocaproic acid) or UV-active (*N*-hydrosuccinimide) materials. The solid, now free of *N*-hydroxysuccinimide and NBD-aminocaproic acid by TLC was dissolved in 1 mL of water and applied to a 55 mm × 10 mm column of DEAE cellulose (HCO_3^- form). The column was eluted with two 3-mL portions of water, and then 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, and 1.0 M TEAB (3 mL for each concentration), and three 3-mL portions of 1.28 M TEAB. The product was in the 1.0 and 1.28 M TEAB fractions that were yellow and showed fluorescence. The fractions were lyophilized to give 7 mg (78% yield) of yellow PtdInsP$_2$-NBD **14**. ^{1}H NMR (250 MHz, D_2O) δ: 8.43 (m, 1H, very weak), 61.5 (m, 1H, very weak), 5.20 (m, 1H), 4.4−3.8 (m, 10H), 3.1−3.0 (m, 4H), 2.1 (m, 6H), 1.4−1.0 (m, 42H), 0.8 (br s, 3H) ppm. ^{31}P NMR δ: 4.80 (s), 4.17 (s), 3.34 (s) (1:1:1) ppm. MALDI-TOF MS: found 1151, required for free acid 1148 ($M^- - 1$).

(Benzyloxy) 3-[1-[6-(*N*-Cbz-amino)hexanoyl]-2-oleoyl-*sn*-glyceroxy](*N*,*N*-diisopropylamino)phosphine (7b). 1-[6-[(Carbobenzyloxy)amino]hexanoyl]-2-oleoyl-*sn*-glycerol (600 mg, 0.993 mmol) was dissolved in 10 mL of dry CH_2Cl_2, and diisopropylethylamine (346 μL, 1.99 mmol) was added. The mixture was cooled to 0 °C under an atmosphere of N_2 and (benzyloxy)(*N*,*N*-diisopropylamino)chlorophosphine (326 mg, 1.19 mmol) dissolved in 15 mL CH_2Cl_2 added dropwise over 30 min. The mixture was kept at 0 °C for an additional 15 min. Next, the cooling bath was removed and the reaction allowed to proceed at rt for 2 h. The mixture was evaporated to dryness, and the crude product was purified on SiO_2 with 33% ethyl acetate/hexane containing 5% triethylamine (R_f = 0.84). The purified product **7b** (721 mg, 86% yield) was obtained as a colorless oil. ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 7.45−7.25 (m, 10H), 5.40−5.28 (m, 2H), 5.20−5.14 (m, 1H), 5.08 (s, 2H), 4.77−4.58 (m, 3H), 4.39−4.30 (m, 1H), 4.19−4.11 (m, 1H), 3.83−3.55 (m, 4H), 3.18−3.15 (q, J = 6.1 Hz, 2H), 2.32−2.26 (t, J = 7.4 Hz, 4H), 2.01−1.98 (q, J = 5.5 Hz, 4H), 1.67−1.14 (m, 6H), 1.28−1.26 (m, 22H), 1.19 −1.16 (2d, 12H), 0.89−0.84 (t, J = 6.3 Hz, 3H) ppm. ^{31}P NMR (101 MHz, $CDCl_3$) δ: 150.18, 150.07 (near 1:1) ppm.

Benzyl 3-[1-[6-(*N*-Cbz-amino)hexanoyl]-2-oleoyl-*sn*-glyceroxy] 1-[2,6-Bis-*O*-(benzyloxymethyl)-3,4,5-tris-*O*-(*p*-methoxybenzyl)-*myo*-inosityl) Phosphate (9b). A mixture of protected inositol **8b** (171 mg, 0.219 mmol) and 1*H*-tetrazole (107.3 mg, 1.53 mmol) was coevaporated three times with 1 mL of dry benzene (azeotropic removal of water) and dried under vacuum for approximately 3 h. The dry mixture was placed under an atmosphere of N_2, and (benzyloxy)3-[1-[6-[(carbobenzyloxy)amino]hexanoyl]-2-oleoyl-*sn*-glyceroxy](*N*,*N*-diisopropylamino)phosphine (556 mg, 0.661 mmol) dissolved in 4 mL of dry CH_2Cl_2 was added. The reaction was stirred for 1 h at rt, the mixture was cooled to −40 °C, and *m*-CPBA (203 mg of 57−85% pure material, a minimum of 0.809 mmol) was added. Oxidation was allowed to proceed at −40 °C for 5 min and at 0 °C for 30 min. The mixture was diluted with 80 mL of CH_2Cl_2 and extracted with 2 × 30 mL 10% Na_2SO_3 followed by 2 × 30 mL 10% $NaHCO_3$. The organic phase was dried over $MgSO_4$, filtered, concentrated to a yellowish oil, and purified on SiO_2 with 33% ethyl acetate/hexane (R_f = 0.25) to yield the desired product **9b** (297 mg, 88%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 7.33−7.17 (m, 26H), 6.82−6.77 (m, 6H), 5.33−5.31 (m, 1H), 5.29 (s, 2H), 5.14−4.44 (m, 18H), 4.26−3.91 (m, 8H), 3.77 (s, 9H), 3.48−3.35 (m, 2H), 3.20−3.15 (q, 2H), 2.8 (br s, 1H), 2.26−2.17 (m, 4H), 2.04−1.10 (m, 4H), 1.60−1.27 (m, 28H), 0.90−0.85 (t, J = 6.1 Hz, 3H) ppm. ^{31}P NMR (101 MHz, $CDCl_3$) δ: 0.23 ppm. HRMS: calcd for $C_{88}H_{114}NO_{20}NaP$ 1558.7570, found 1558.7660.

Benzyl 3-[1-[6′-(*N*-Cbz-amino)hexanoyl]-2-oleoyl-*sn*-glyceroxy] 1-[2,6-Bis-*O*-(benzyloxymethyl)-*myo*-inosityl] Phosphate (10b). A mixture of coupled derivatives **9b** 148 mg, 0.0964 mmol) and DDQ (140 mg, 0.617 mmol) in 15 mL of wet CH_2Cl_2 was stirred for 5 h at rt. Next, the mixture was diluted with 85 mL CH_2Cl_2 and extracted 2 × 35 mL of 10% $NaHCO_3$, followed by 35 mL of saturated brine. The organic phase was dried over $MgSO_4$, filtered, concentrated to an oily residue, and purified on SiO_2 with ethyl acetate (R_f = 0.42) to give triol **10b** (68.9 mg, 61%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 7.45−7.30 (s, 20H), 5.41−5.30 (m, 2H), 5.15−4.51 (m, 13H), 4.28−4.04 (m, 8H), 3.82−3.69 (br s, 1H), 3.46−3.31 (m, 3H), 3.22−3.11 (q, J = 6.1 Hz, 2H), 2.89 (br s, 1H), 2.72 (br s, 1H), 2.29−2.27 (t, J = 6.8 Hz, 4H), 2.04−1.94 (m, 4H), 1.61−1.22 (m, 28), 0.89−0.84 (t, J = 6.7 Hz, 3H) ppm. ^{31}P NMR (101 MHz, $CDCl_3$) δ: −0.09, −0.3 (near 1:1) ppm. HRMS: calcd for $C_{64}H_{90}NO_{17}NaP$ 1198.5844, found 1198.5774.

2,6-Bis-*O*-[(benzyloxy)methyl]-3,4,5-tris-*O*-(dibenzylphosphonyl)-D-*myo*-inosityl 1-*O*-[1-[6′-(*N*-Cbz-amino)-hexanoyl]-2-oleoyl-*sn*-glyceryl Benzyl Phosphate (11b). Triol **10b** (63.9 mg, 0.054 mmol) along with 1*H*-tetrazole (52.3 mg, 0.747 mmol) was coevaporated twice with 1 mL of dry benzene and dried under vacuum for approximately 2 h. Bis-(benzyloxy) *N*,*N*-diisopropylphosphoramidite (122 mg, 0.354 mmol) dissolved in 2 mL of CH_2Cl_2 was then added, and the reaction was allowed to proceed for 2.5 h. The mixture was cooled to −40 °C, and *m*-CPBA (142 mg of 57−85% pure material, a minimum of 0.469 mmol) was added. Oxidation proceeded at −40 °C for 5 min, at 0 °C for 30 min, and at rt for an additional 30 min. The mixture was diluted with 120 mL of CH_2Cl_2 and extracted twice with 40 mL of 10% Na_2SO_3, followed by two extractions with 50 mL of 10% $NaHCO_3$. The organic phase was dried over $MgSO_4$, filtered, concentrated to a cloudy oil, and purified on SiO_2 with 50% ethyl acetate/hexane (R_f = 0.14) to give 88 mg (83% yield) of the compound **11b** as a colorless oil. ^{1}H NMR (250 MHz, $CDCl_3$) δ: 7.31−7.18 (m, 50H), 5.06−3.99 (m, 35H), 3.12 (m, 2H), 2.91 (m, 2H), 2.19 (m, 4H), 1.85−1.25 (m, 32H), 0.87−0.79 (t, 3H) ppm. ^{31}P NMR (101 MHz, $CDCl_3$) δ: 0.334, 0.142, −0.390 (1:2:1) ppm.

3,4,5-Tris-*O*-phosphoryl-D-*myo*-inosityl 1-*O*-[1-(6′-Aminohexanoyl)-2-steroyl-*sn*-glyceryl] Phosphate Hexasodium Salt (12b). A mixture of fully-protected PtdInsP$_3$ derivative **11b** (71.3 mg, 0.037 mmol), $NaHCO_3$ (28.2 mg, 0.336 mmol), and 233 mg of 10% Pd/C catalyst in 30 mL of *t*-BuOH/H_2O 6:1 was shaken under an atmosphere of H_2 (50 psi) in a Parr apparatus for 24 h. The catalyst was removed by filtration through a bed of Celite and washed with 10 mL of *t*-BuOH, 2 × 10 mL of *t*-BuOH/H_2O 1:1, and 4 × 10 mL of H_2O. The filtrate was concentrated in vacuo and lyophilized, leaving the PtdInsP$_3$ derivative **12b** as a white solid. ^{1}H NMR (250 MHz, D_2O) δ: 5.35 (m, 1H), 4.51−3.97 (m, 10H), 3.02 (m, 2H), 2.72 (m, 4H), 1.70−1.30 (m, 36H), 0.89 (m, 3H) ppm. ^{31}P

(51) Radiochemical yields for carrier-free materials, particularly the detergent-like phosphoinositides, are invariably lower than chemical yields. Since reactions are performed at higher dilution and with 100- to 1,000-fold less mass, adsorptive losses and incomplete conversion are common occurrences.

NMR (101 MHz, D_2O) δ: 8.25, 7.02, 5.21, 3.65 ppm. MALDI-TOF MS: 952.4 (M − 1), calcd 952.8.

3,4,5-Tris-*O*-phosphoryl-D-*myo*-inosityl 1-O-[1-[6′-[(*p*-benzoyldihydrocinnamoyl)amino]hexanoyl]-2-stearoyl-*sn*-glyceryl] Phosphate Hexakis(triethylammonium) Salt (13c). A suspension of aminohexanoyl $PtdInsP_3$ analogue **12b** (4.88 mg, 4.51 μmol) in 200 μL of 0.25 M TEAB buffer was stirred at rt for 3 h until a clear solution was obtained. BZDC-NHS ester (5.05 mg, 14.4 μmol) dissolved in 200 μL of DMF was added and the resulting cloudy solution stirred at rt overnight. The mixture was evaporated to dryness, and residual buffer salts were removed by the addition and evaporation twice with 0.5 mL of MeOH. The residue was washed by centrifugation with four portions of acetone and dried, leaving 5.85 mg of photolabel **13c** as a white solid. ^1H NMR (D_2O, 250 MHz) δ: 7.81−7.50 (m, 9H), 4.45−3.86 (m, 10H), 3.06−3.00 (t, J = 7.5 Hz, 2H), 2.61−2.55 (t, J = 7.5 Hz, 2H), 2.47−2.14 (m, 6H), 1.87−1.23 (m, 36H), 0.89 (m, 3H) ppm. MALDI-TOF MS: 1189.4 (M − 1), calcd 1188.1 (free acid).

3,4,5-Tris-*O*-phosphoryl-D-*myo*-inosityl 1-*O*-[1-[6′-[(*p*-benzoylditritiumcinnamoyl)amino]caproyl]-2-stearoyl-*sn*-glyceryl] Phosphate Hexakis(triethylammonium) Salt (13d). Aminohexanoyl $PtdInsP_3$ analogue **12b** (17 μL of a 3.3 mg/1 mL of stock solution in H_2O, 0.056 mg, 52 nmol) was added to 33 μL of 0.38 M TEAB buffer (final TEAB concentration, 0.25 M). The resulting phospholipid solution was added to 70 μL of DMF containing [^{3}H]BZDC-NHS ester (2 mCi, 58 nmol) and the mixture stirred overnight at rt. The solvent was evaporated in vacuo, and residual DMF and buffer salts were removed by the addition and evaporation of 100 μL of H_2O. The residue was dissolved in 1 mL of H_2O and applied to a Pasteur pipet column of DEAE cellulose. The column was washed twice with 1-mL portions of H_2O and eluted with a linear gradient of TEAB buffer (0.1−1.3 M). One microliter aliquots from each fraction were subjected to liquid scintillation counting, and the radioligand **13d** was determined to be present in the 1.0−1.3 M TEAB fractions (2.6% radiochemical yield).

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