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INTRACELLULAR SECOND MESSENGERS: SYNTHESIS OF L-α-PHOSPHATIDYL-D-*myo*-INOSITOL 3,4-BISPHOSPHATE AND ANALOGS

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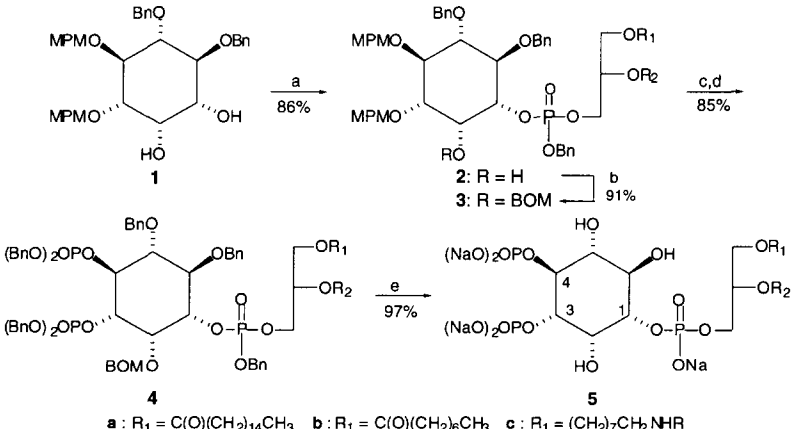
Abstract: Concise syntheses of the title phospholipid as well as a water soluble, short chain diester and a cross-linkable aminodiether analog utilized chiral inositol **1**. © 1997 Elsevier Science Ltd.

The activation of phosphatidylinositol (PtdIns) 3-kinase is now recognized as a central event in a wide spectrum of cellular processes including receptor regulation, chemotaxis, vesicle traffic, and mitogenic responses.[1] These effects are mediated via one or more of the kinase's **D**-3-phosphorylated PtdIns metabolites that, in contrast to the canonical[2] PtdIns-4-P/4,5-P_2, are not substrates for phospholipase C and are believed to interact directly with target binding sites (e.g., the pleckstrin homology domain of protein kinases).[3] Furthermore, recent studies indicate the intracellular levels of the homologous 3-phosphoinositides are independently regulated in some instances and, thus, may subserve different physiologic functions.[3] To expedite current efforts to understand the role of PtdIns 3-kinase and the actions of its lipid progeny, we report herein an asymmetric synthesis of dihexadecanoyl L-α-phosphatidyl-D-*myo*-inositol 3,4-bisphosphate[4] (**5a**) as well as water soluble, short chain[5] and cross-linkable diether analogs, **5b** and **5c** (R = H), respectively, by a concise route that complements our prior preparation[6] of PtdIns-3,4,5-P_3.

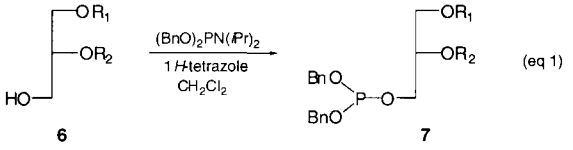
Scheme 1



Reagents and conditions: (a) **7** (3 equiv), py•HBr_3 (4 equiv), CH_2Cl_2/py/Et_3N (1:0.1:0.05), -20 °C, 2 min; 0 °C, 0.5 h. (b) $PhCH_2OCH_2Cl$/$EtN(iPr)_2$, CH_2Cl_2, 24 °C, 12 h. (c) DDQ (3 equiv), CH_2Cl_2/H_2O (20:1), 0 °C, 2 h. (d) $(BnO)_2PN(iPr)_2$ (5 equiv), 1*H*-tetrazole (10 equiv), CH_2Cl_2, 24 °C, 2 h; *m*-CPBA (7 equiv), -40 °C, 0.5 h. (e) Pd black/ H_2 (50 psi), *t*-BuOH/H_2O (7:1), 24 °C, 14 h; $NaHCO_3$.

Chiral diol **1**, readily available by modification[7] of literature procedure,[8] was smoothly transformed to phosphate triester **2** using Watanabe's pyridinium perbromide methodology[9] for the in situ activation of 1,2-di-O-hexadecanoyl-*sn*-glyceryl dibenzylphosphite (**7a**) and regioselective phosphorylation of the C(1)-alcohol (Scheme 1). The identity of **2** was confirmed by acetylation and subsequent ^{1}H NMR analysis which revealed an apparent triplet (J = 2.7 Hz) characteristic of the C(2)-methine at 6.04 ppm. The free hydroxyl in **2** was protected as a benzyloxymethyl (BOM) ether to give **3**, which was advanced to tris-phosphate **4** by sequential DDQ clevage of the 4-methoxybenzyl (MPM) ethers and bis-phosphorylation of the liberated *vic*-alcohols via phosphatidylation with *O,O*-dibenzyl-*N,N*-diisopropylphosphoramidite followed by low temperature *m*-chloroperoxybenzoic acid (*m*-CPBA) oxidation. Finally, exhaustive debenzylation by catalytic hydrogenolysis over Pd black in *t*-BuOH/H_2O afforded **5a**, isolated as its sodium salt.[10]

Phosphite **7a** (eq 1) was conveniently prepared by condensation (1*H*-tetrazole, 23°C, 0.5 h; 90%) of 1,2-dihexadecanoyl-*sn*-glycerol[6] (**6a**) with *O,O*-dibenzyl-*N,N*-diisopropylphosphoramidite (1.8 equiv); after aqueous workup, the phosphite was sufficiently pure to be used in the next step. Likewise, the known[6] glycerols **6b** and **6c** (R = Cbz) provided access to **5b** and **5c** (R = H) utilizing the above sequence.



6 7 (eq 1)

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7. Racemic **1** was esterified with (-)-camphanic chloride (Et_3N, DMAP; 81%) and the resultant diastereomeric diesters separated on SiO_2 (5% Et_2O/CH_2Cl_2). Saponification (95%) of the more polar isomer afforded (-)-**1**, $[\alpha]_D$ -20.7° (*c* 0.25, $CHCl_3$).

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10. PtdIns **5a**: ^{1}H NMR (250 MHz, D_2O) δ 0.80 (t, J = 6.9 Hz, 6 H), 1.14–1.33 (m, 48 H), 1.45–1.64 (m, 4 H), 2.34 (t, J = 7.3 Hz, 2 H), 2.38 (t, J = 7.3 Hz, 2 H), 3.49 (t, J = 9.0 Hz, 1 H), 3.75 (t, J = 9.7 Hz, 1 H), 3.81–3.99 (m, 2 H), 4.00–4.16 (m, 3 H), 4.23 (dd, J = 7.3, 12.4 Hz, 1 H), 4.42 (d, J = 14.6 Hz, 2 H), 5.20–5.34 (m, 1 H); ^{31}P NMR (202 MHz, D_2O, 85% H_3PO_4 as external reference) δ 0.20, 4.20, 5.62.

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