

Synthesis of L- α -Phosphatidyl-D-*myo*-inositol 5-Phosphate and L- α -Phosphatidyl-D-*myo*-inositol 3,5-Bisphosphate

Jirong Peng and Glenn D. Prestwich*

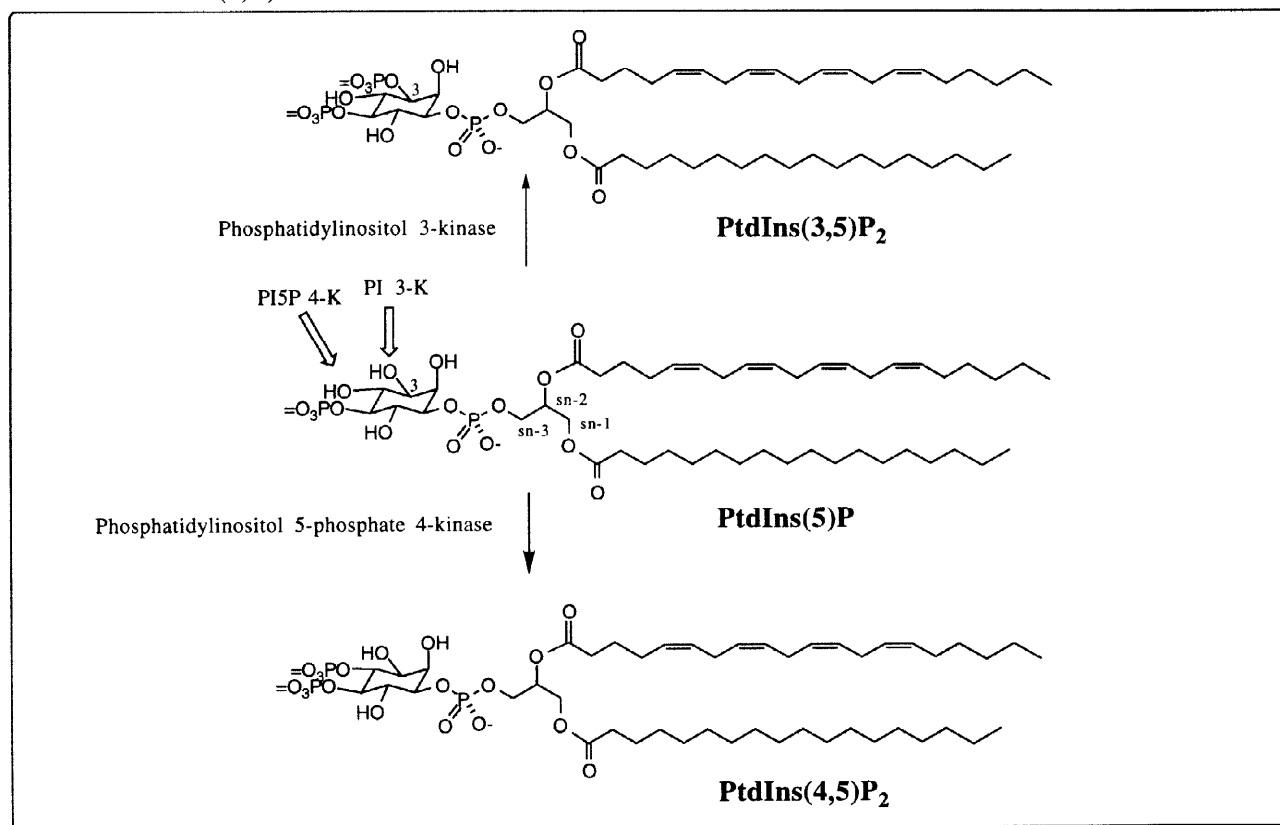
Department of Medicinal Chemistry
The University of Utah, 30 South, 2000 East, Room 201
Salt Lake City, Utah 84112-5820

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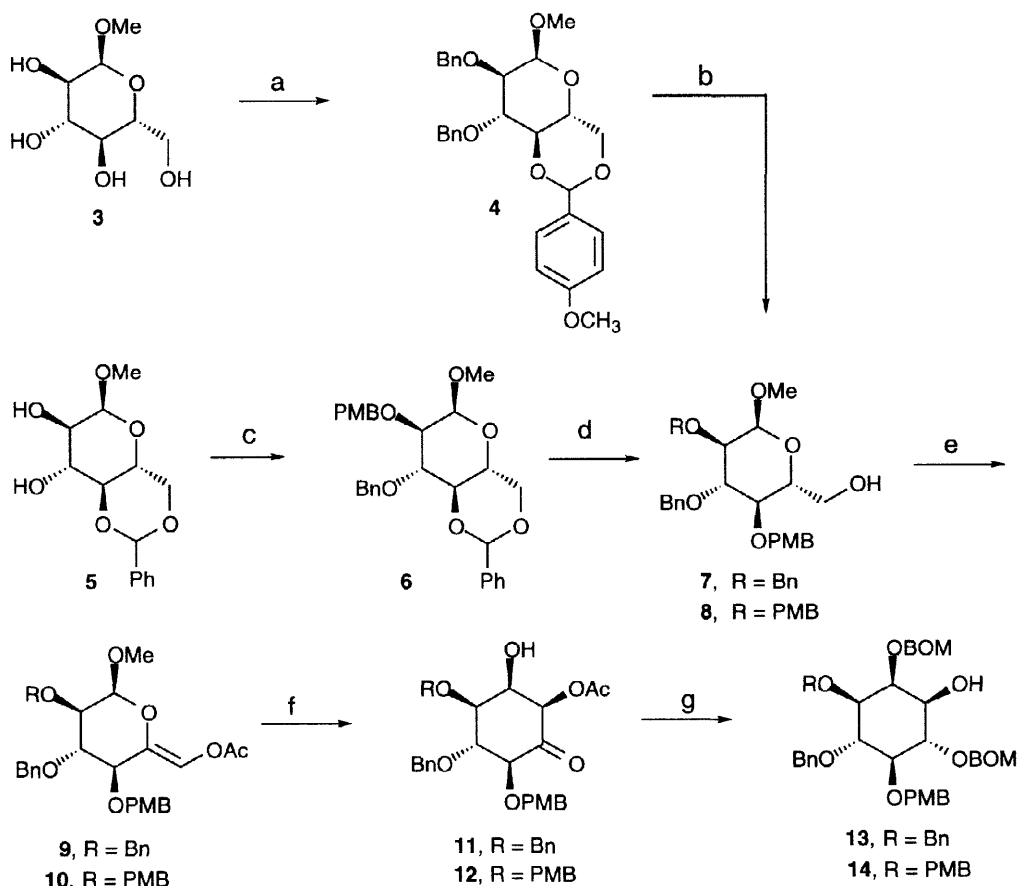
Summary: Two new 5-phosphorylated phosphatidylinositols have been synthesized from methyl α -D-glucopyranoside as the chiral precursor. These new PtdInsP_ns have been recently detected in mammalian cells.

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To meet the rapidly increasing demands of various phosphatidylinositol polyphosphates (PtdInsP_ns) and their analogs for the study of cell signaling pathways, we have recently developed general strategies for the systematic synthesis of all biologically-relevant PtdInsP_ns in enantiomerically-pure form.¹ We now extend these strategies to the synthesis of L- α -phosphatidyl-D-*myo*-inositol 5-phosphate, PtdIns(5)P (**1**) and L- α -phosphatidyl-D-*myo*-inositol 3,5-bisphosphate, PtdIns(3,5)P₂ (**2**), new naturally-occurring membrane phosphoinositides.^{2,3} Scheme 1 indicates two important new pathways involving PtdIns(5)P: (i) the generation of PtdIns(4,5)P₂ from PtdIns(5)P via a substrate-specific PtdIns(5)P 4-kinase and (ii) the putative formation of PtdIns(3,5)P₂ by the action of a PtdIns 3-kinase on PtdIns(5)P (evidence is also available for its production by the action of PtdIns 5-kinase on PtdIns(3)P).³

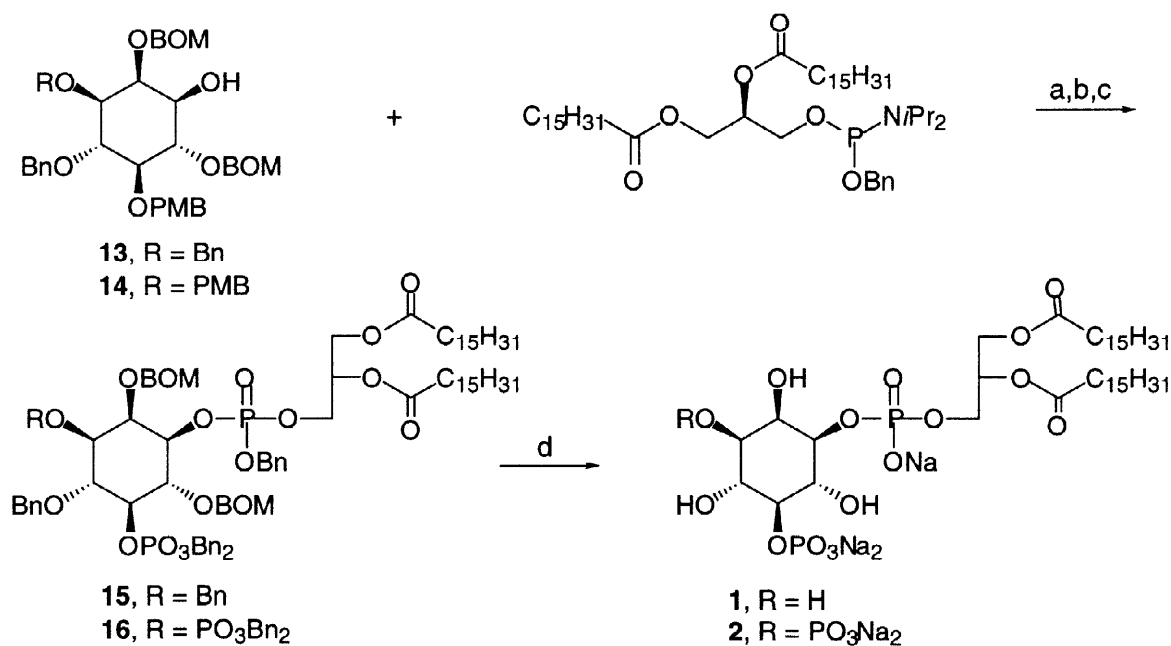


The selectively-protected precursor **7** for PtdIns(5)P was obtained from α -D-methyl glucopyranoside (Scheme 2). Treatment of pyranoside **3** with *p*-anisaldehyde dimethyl acetal in DMF in the presence of catalytic amount of *p*-toluenesulfonic acid followed by benzylation provided the fully-protected acetal **4** in 75% overall yield. Cleavage of the *p*-methoxybenzylidene acetal with lithium aluminum hydride-aluminum chloride at 0 °C generated the desired regioisomer **7** in 95% yield. Swern oxidation followed by treatment with Ac₂O and K₂CO₃ gave the enol acetate **9** in 80% yield. Ferrier rearrangement⁴ of the enol acetate **9** using mercuric acetate and aqueous NaCl afforded the inosose **11** with the desired configuration at C-2 as the major product in 75% isolated yield. Stereoselective reduction of the carbonyl group with sodium triacetoxyborohydride⁵ gave the *D*-myo-inositol backbone in 63% yield. Sequential protection of C-2, C-6 hydroxyl groups with benzyloxymethyl ether (BOM) and deprotection of C-1 acetate provided precursor **13** with desired protecting groups.



Scheme 2. (a) (i) *p*-anisaldehyde dimethyl acetal, DMF, *p*-TsOH, 65 °C, 6 h; (ii) BnBr, NaH, DMF, rt, 5 h; (b) LiAlH₄, AlCl₃, Et₂O, CH₂Cl₂, 0 °C, 3 h; (c) (i) Bu₂SnO, PMBCl, Bu₄NBr, toluene, reflux, 4 h; (ii) NaH, BnBr, DMF, rt, 10 h; (d) (i) *p*-TsOH, CH₃OH, rt, 4 h; (ii) TrCl, DMF, Et₃N, DMAP, rt, 4 h; (iii) NaH, PMBCl, DMF, rt, 16 h; (iv) *p*-TsOH, CH₃OH, rt, 14 h; (e) (i) (COCl)₂, DMSO, CH₂Cl₂, -78 °C, 0.5 h; (ii) Et₃N; (iii) Ac₂O, K₂CO₃, CH₃CN, rt, 20 h; (f) Hg(OAc)₂, NaCl, acetone, H₂O, rt, 20 h; (g) (i) NaB(OAc)₃H, HOAc, CH₃CN, rt, 1 h, (ii) BOMCl, H+Sponge, Bu₄NI, CH₃CN, 40 °C, 48 h; (iii) NaOH, THF, H₂O, rt, 16 h.

The synthesis of PtdIns(3,5)P₂ inositol skeleton **14** followed a similar route (Scheme 2). Refluxing 4,6-*O*-benzylidene methyl glucopyranoside **5** with dibutyltin oxide in toluene in the presence of Bu₄NBr, followed by adding *p*-methoxybenzyl chloride generated a mixture of two regioisomers. The desired C-2 *p*-methoxybenzyl (PMB) ether isomer **6** could be separated from the C-3 PMB ether isomer by flash chromatography in 36% yield. The C-3 hydroxyl group was then protected as a benzyl ether. Sequential hydrolysis of benzylidene acetal, selective tritylation of C-6 primary alcohol, protection of C-4 with PMB ether, and detritylation afforded intermediate **8** in 36% overall yield. Then, under the same conditions as in the synthesis of precursor **13**⁶, PtdIns(3,5)P₂ inositol skeleton moiety **14**⁶ was obtained in six steps in 21% overall yield.



Scheme 3. (a) (i) tetrazole, CH₂Cl₂, rt, 5 h; (ii) *m*-CPBA, -40 °C, 0.5 h; (b) DDQ, CH₂Cl₂, H₂O, rt, 3 h; (c) (i) (BnO)₂PNiPr₂, tetrazole CH₂Cl₂, rt, 5 h; (ii) *m*-CPBA, -40 °C, 0.5 h; (d) (i) 50 psi H₂, 10% Pd/C, CH₃OH, H₂O, rt, 24 h; (ii) Chelex resin, Na⁺ form.

The coupling reactions (Scheme 3) of the protected inositol precursors **13** and **14** with 1,2-di-*O*-palmitoyl-*sn*-glycerol-3-(benzyl *N,N*-diisopropylphosphoramidite)^{1b} were performed in the presence of tetrazole, followed by oxidation with *m*-CPBA at low temperature. After removal of PMB protecting groups with DDQ in wet CH₂Cl₂, the free hydroxyl groups were phosphorylated with dibenzyl *N,N*-diisopropylphosphoramidite-tetrazole, and the phosphite intermediates were oxidized with *m*-CPBA to provide fully-protected PtdIns(5)P precursor **15** and the PtdIns(3,5)P₂ precursor **16**.⁶ Hydrogenation of **15** or **16** over 10% Pd/C in 80% MeOH¹ afforded PtdIns(5)P (**1**) and PtdIns(3,5)P₂ (**2**), respectively. Synthetic PtdIns(5)P (**1**) was accepted as a substrate for PtdIns(5)P 4-kinase, yielding the dihexadecanoyl PtdIns(4,5)P₂.^{2a} Dihexadecanoyl PtdIns(3,5)P₂ (**2**) was obtained as the product of the reaction of dihexadecanoyl PtdIns(3)P⁷ with PtdIns(3)P 5-kinase.^{2b}

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- (a) Spectral data for **13**: ^1H NMR (200 MHz, CDCl_3) δ 7.10-7.45 (m, 22H), 6.70-6.85 (m, 2H), 4.50-5.00 (m, 14H), 4.10-4.20 (m, 1H), 3.96 (dd, $J = 9.4$, 9.4 Hz, 1H), 3.83 (dd, $J = 9.5$, 9.5 Hz, 1H), 3.78 (s, 1H), 3.60-3.80 (m, 1H), 3.35-3.50 (m, 2H), 1.60 (b, 2H) ppm; ^{13}C NMR (50 MHz, CDCl_3) δ 138.9, 137.9, 137.5, 137.2, 130.7, 129.3, 128.3, 128.2, 128.1, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 96.4, 95.7, 82.7, 82.5, 81.6, 79.8, 76.2, 75.6, 75.4, 72.3, 71.0, 69.9, 69.5 ppm. HRMS (FAB) Calcd. for $\text{C}_{44}\text{H}_{47}\text{O}_9$ (M-H) $^+$, 719.3220; found, 719.3243.
 (b) Spectral data for **14**: ^1H NMR (200 MHz, CDCl_3) δ 7.15-7.40 (m, 19H), 6.70-6.85 (m, 4H), 4.50-5.00 (m, 12H), 4.15-4.20 (m, 1H), 3.96 (dd, $J = 9.5$, 9.5 Hz, 1H), 3.84 (dd, $J = 9.5$, 9.5 Hz, 1H), 3.70-3.80 (m, 1H), 3.78 (s, 6H), 3.44 (dd, $J = 9.6$, 2.3 Hz, 1H), 3.43 (dd, $J = 9.1$, 9.1 Hz, 1H), 1.63 (b, 1H) ppm; ^{13}C NMR (50 MHz, CDCl_3) δ 139.7, 137.5, 137.2, 130.7, 130.0, 129.3, 128.3, 128.2, 128.1, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3, 126.8, 96.4, 95.9, 82.6, 81.6, 79.5, 76.3, 75.5, 75.4, 72.1, 71.0, 69.9, 69.6, 64.9 ppm. HRMS (FAB) Calcd. for $\text{C}_{45}\text{H}_{50}\text{O}_{10}\text{Na}$ (M + Na) $^+$, 773.3302; found, 773.3314.
 (c) Spectral data for **15**: ^1H NMR (200 MHz, CDCl_3) δ 7.00-7.40 (m, 35H), 3.90-5.00 (m, 27H), 3.40-3.50 (m, 2H), 2.15-2.30 (m, 4H), 1.10-1.65 (m, 52H), 0.88 (dd, $J = 6.1$, 6.1 Hz, 6H) ppm; ^{31}P NMR (81 MHz, CDCl_3) δ -0.39, -0.47 ppm. HRMS (FAB) Calcd. for $\text{C}_{92}\text{H}_{126}\text{O}_{18}\text{P}_2\text{Na}$ (M + Na) $^+$, 1603.8317; found, 1603.8354.
 (d) Spectral data for **16**: ^1H NMR (200 MHz, CDCl_3) δ 7.00-7.40 (m, 40H), 3.50-5.10 (m, 31H), 2.10-2.30 (m, 4H), 1.16-1.65 (m, 52h), 0.85-1.00 (m, 6H) ppm; ^{13}C NMR (50 MHz, CDCl_3) δ 173.0, 137.9, 137.6, 135.8, 135.7, 135.6, 135.5, 135.4, 128.6, 128.4, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.2, 96.0, 79.7 (m), 77.9 (m), 76.5 (m), 75.7 (m), 74.5 (m), 70.3, 70.2, 70.0, 69.8 (m), 69.5, 69.4, 69.3, 69.2, 65.6 (m), 61.5, 33.9, 33.8, 31.6, 29.6, 29.4, 29.3, 29.2, 29.0, 28.9, 24.7, 22.6, 14.0 ppm; ^{31}P NMR (81 MHz, CDCl_3) δ -0.42, -0.59, -0.67 ppm. HRMS (FAB) Calcd. for $\text{C}_{99}\text{H}_{133}\text{O}_{21}\text{P}_3\text{Na}$ (M + Na) $^+$, 1773.8450; found, 1773.8511.
 (e) Spectral data for **1**: ^{31}P NMR and ^1H NMR showed only extremely broad, unresolved peaks in CDCl_3 or D_2O ; mixed solvents were unsuccessful, and material was insoluble in DMSO. MALDI-MS Calcd. for $\text{C}_{41}\text{H}_{80}\text{O}_{16}\text{P}_2$, 891.02; found for (M-H) $^-$, 890.24. As anticipated, the dibutyryl ester (synthesis not shown; J. Peng, unpublished results) was water-soluble and gave high resolution NMR spectra: ^1H NMR (200 MHz, D_2O) δ 5.10-5.25 (m, 1H), 4.31 (dd, $J = 9$, 3 Hz), 4.05-4.25 (m, 2H), 3.55-4.00 (m, 6H), 3.48 (dd, 9, 3 Hz), 2.15-2.35 (m, 4H), 1.35-1.55 (m, 4H), 0.79 (dd, $J = 7$, 7 Hz), 0.77 (dd, $J = 7$, 7 Hz). ^{31}P NMR (81 MHz, D_2O) δ 6.22, 2.65.
 (f) Spectral data for **2**: ^1H NMR (200 MHz, D_2O) δ 5.10-5.30 (br.), 3.60-4.50 (br.), 2.10-2.40 (br.), 0.90-1.60 (br.), 0.6-0.9 (br.). MALDI-MS Calcd for $\text{C}_{41}\text{H}_{81}\text{O}_{19}\text{P}_3$, 971.01; Found for (M-H) $^-$, 970.34.
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