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

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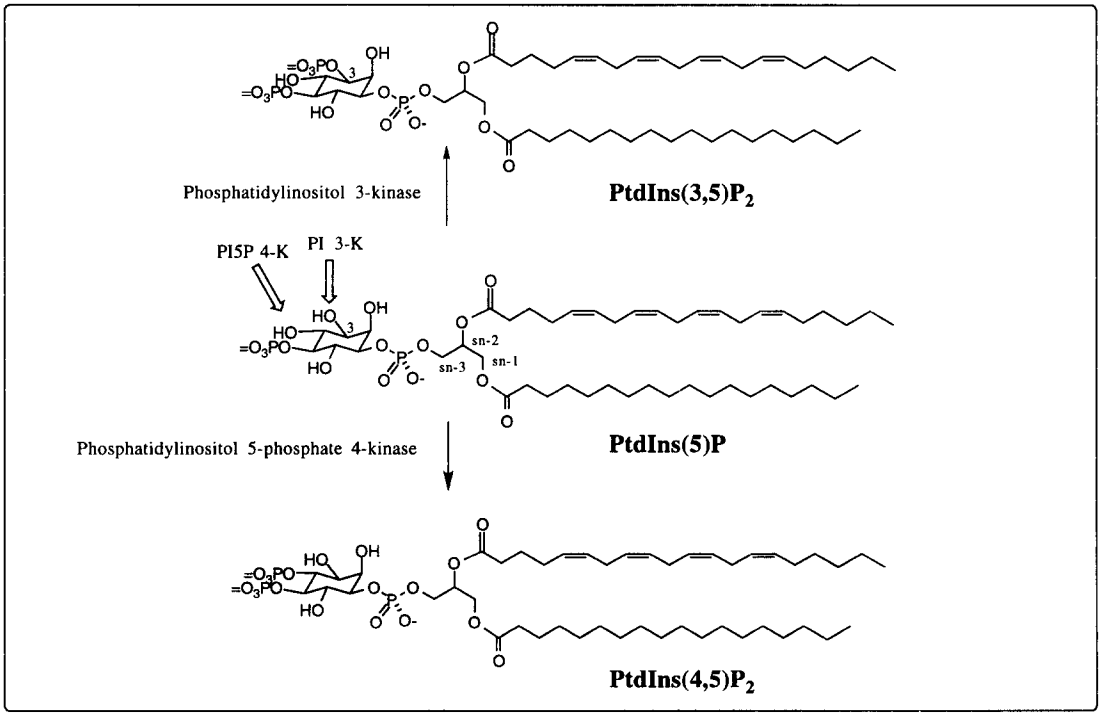
Summary: Two new 5-phosphorylated phosphatidylinositols have been synthesized from methyl α-D-glucopyranoside as the chiral precursor. These new PtdInsP$_n$s have been recently detected in mammalian cells.

To meet the rapidly increasing demands of various phosphatidylinositol polyphosphates (PtdInsP$_n$s) 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$_n$s 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)P$_2$ (**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)P$_2$ from PtdIns(5)P via a substrate-specific PtdIns(5)P 4-kinase and (**ii**) the putative formation of PtdIns(3,5)P$_2$ 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]



Scheme 1. Two products from PtdIns(5)P.

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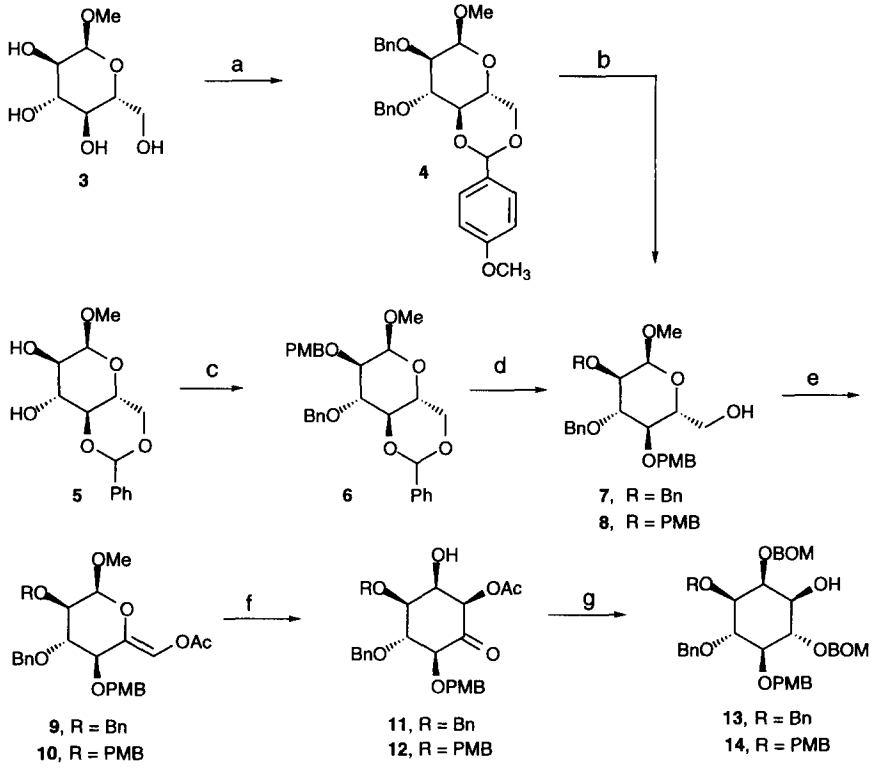
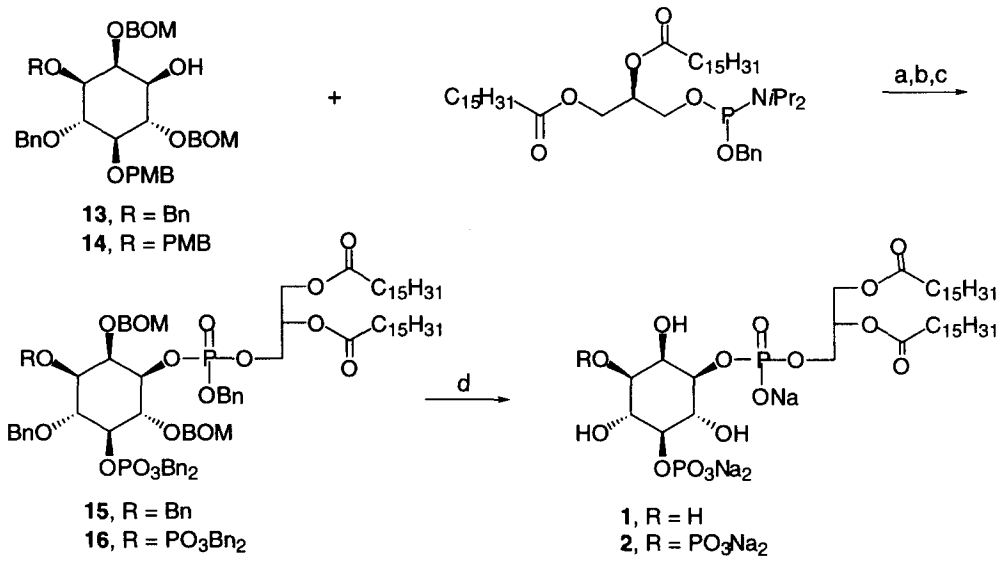
The selectively-protected precursor **7** for PtdIns(5)P was obtained from α-D-methyl glucopyranoside (Scheme 2). Treatment of pyranoside **3** with *p*-anisaldehyde dimethyl acetal in DMF in the presence of catalytic amount of *p*-toluenesulfonic acid followed by benzylation provided the fully-protected 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 Ac_2O and K_2CO_3 gave the enol acetate **9** in 80% yield. Ferrier rearrangement[4] 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 triacetoxyborohydride[5] 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**) $LiAlH_4$, $AlCl_3$, Et_2O, CH_2Cl_2, 0 °C, 3 h; (**c**) (i) Bu_2SnO, PMBCl, Bu_4NBr, toluene, reflux, 4 h; (ii) NaH, BnBr, DMF, rt, 10 h; (**d**) (i) *p*-TsOH, CH_3OH, rt, 4 h; (ii) TrCl, DMF, Et_3N, DMAP, rt, 4 h; (iii) NaH, PMBCl, DMF, rt, 16 h; (iv) *p*-TsOH, CH_3OH, rt, 14 h; (**e**) (i) $(COCl)_2$, DMSO, CH_2Cl_2, -78 °C, 0.5 h; (ii) Et_3N; (iii) Ac_2O, K_2CO_3, CH_3CN, rt, 20 h; (**f**) $Hg(OAc)_2$, NaCl, acetone, H_2O, rt, 20 h; (**g**) (i) $NaB(OAc)_3H$, HOAc, CH_3CN, rt, 1 h, (ii) BOMCl, H^+Sponge, Bu_4NI, CH_3CN, 40 °C, 48 h; (iii) NaOH, THF, H_2O, rt, 16 h.

The synthesis of PtdIns(3,5)P_2 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 Bu_4NBr, 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)P_2 inositol skeleton moiety **14**[6] was obtained in six steps in 21% overall yield.



Scheme 3. (**a**) (i) tetrazole, CH_2Cl_2, rt, 5 h; (ii) *m*-CPBA, -40 °C, 0.5 h; (**b**) DDQ, CH_2Cl_2, H_2O, rt, 3 h; (**c**) (i) $(BnO)_2PNiPr_2$, tetrazole CH_2Cl_2, rt, 5 h; (ii)*m*-CPBA, -40 °C, 0.5 h; (**d**) (i) 50 psi H_2, 10% Pd/C, CH_3OH, H_2O, 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 CH_2Cl_2, 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)P_2 precursor **16**.[6] Hydrogenation of **15** or **16** over 10% Pd/C in 80% MeOH[1] afforded PtdIns(5)P (**1**) and PtdIns(3,5)P_2 (**2**), respectively. Synthetic PtdIns(5)P (**1**) was accepted as a substrate for PtdIns(5)P 4-kinase, yielding the dihexadecanoyl PtdIns(4,5)P_2.[2a] Dihexadecanoyl PtdIns(3,5)P_2 (**2**) was obtained as the product of the reaction of dihexadecanoyl PtdIns(3)P[7] with PtdIns(3)P 5-kinase.[2b]

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6. (**a**) Spectral data for **13**: ^{1}H 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 $C_{44}H_{47}O_9$ $(M-H)^+$, 719.3220; found, 719.3243.

(**b**) Spectral data for **14**: ^{1}H 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 $C_{45}H_{50}O_{10}Na$ $(M + Na)^+$, 773.3302; found, 773.3314.

(**c**) Spectral data for **15**: ^{1}H 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 $C_{92}H_{126}O_{18}P_2Na$ $(M + Na)^+$, 1603.8317; found, 1603.8354.

(**d**) Spectral data for **16**: ^{1}H 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 $C_{99}H_{133}O_{21}P_3Na$ $(M + Na)^+$, 1773.8450; found, 1773.8511.

(**e**) Spectral data for **1**: ^{31}P NMR and ^{1}H 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 $C_{41}H_{80}O_{16}P_2$, 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: ^{1}H 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**: ^{1}H 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 $C_{41}H_{81}O_{19}P_3$, 971.01; Found for $(M-H)^-$, 970.34.

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