Synthesis of L-α-Phosphatidyl-D-*myo*-Inositol 3,4,5-Trisphosphate, an Important Intracellular Signalling Molecule

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A facile synthesis of L-α-phosphatidyl-D-*myo*-inositol 3,4,5-trisphosphate is described.

L-α-Phosphatidyl-D-*myo*-inositol 3,4,5-trisphosphate [PtdIns-(3,4,5)P_3], an *in vivo* product of phosphoinositide 3OH-kinase (PI3K), was first isolated by Traynor-Kaplan *et al.* from agonist stimulated human neutrophils in 1988.[1] Studies have suggested that PtdIns(3,4,5)P_3 and PI3K are both involved in a new intracellular signalling system independent of the PtdIns(4,5)P_2-based pathway.[2] This premise is corroborated by the correlation between the stimulated accumulation of PtdIns(3,4,5)P_3 and the agonist-activated translocation of PI3K activity to protein tyrosine kinases (PTKs). While the role of PtdIns(3,4,5)P_3 in signal transduction remains to be assessed, this D-3-phosphoinositide has been implicated in an array of biological events including cytoskeletal rearrangement, actin assembly, mitogenesis, *etc.*

One of the major obstacles to unveiling the biological function of PtdIns(3,4,5)P_3 is the lack of ready access to this molecule. Previously, Falck and Abdali reported an elegant synthetic scheme for this molecule from (−)-quinic acid.[3] More recently, Ozaki and coworkers described a concise synthesis of the compound, however, in a racemic form.[4] As part of our effort to explore the biological relevance of PI3K catalysis, we report herein a facile synthesis of PtdIns(3,4,5)P_3 from (−)-3,4,5-tri-*O*-allyl-6-*O*-benzyl-*myo*-inositol (−)-**2** (Scheme 1)† that was also an intermediate in our previous synthesis of D-*myo*-inositol 1,3,4,5-tetrakisphosphate.[5,6]

The optically active diol **2** was prepared from (−)-1,2:5,6-di-*O*-cyclohexylidene *myo*-inositol **1** (≥0.98% *ee*) in four steps with 55% yield.[5,6] Regioselective introduction of a methoxyethoxymethyl (MEM) group to the C-1 hydroxy function of **2** (235 mg, 0.6 mmol) *via* the corresponding stannylene acetal, followed by *O*-benzylation at the C-2, gave the fully protected derivative (+)-**4** in 88% yield. Displacement of the MEM function of **4** (300 mg, 0.53 mmol) with an acetate to afford (−)-**6** was accomplished by treating **4** with $ZnBr_2$ at room temperature to cleave the MEM ether and then, without purification, with acetic anhydride in 87% combined yield. Removal of the allyl groups of (−)-**6** (240 mg, 0.45 mmol) with Pd/C and *p*-toluenesulfonic acid (PTSA) under reflux yielded the triol (−)-**7** in 88% yield. Phosphorylation of **7** (160 mg, 0.4 mmol) to furnish the corresponding 3,4,5-tris(dibenzyl phosphate) (−)-**8** was achieved by exposure to dibenyl *N,N*-di-isopropylphosphoramidite and 1*H*-tetrazole, followed by MCPBA, in 90% yield. The 1-*O*-acetyl function of **8** (242 mg, 0.2 mmol) was then hydrolysed by NaOH to furnish (−)-**9** in 84% yield in order for the subsequent formation of the phosphodiester linkage at the C-1 position. Reaction of **9** (100 mg, 0.1 mmol) with 1,2-di-*O*-palmitoyl-*sn*-glycerol 3-(benzyl *N,N*-diisopropylphosphoramidite)[7] **10** in the presence of 1*H*-tetrazole, followed by MCPBA, gave the perbenzylated derivative (−)-**11**. Subsequent debenzylation of **11** (138 mg, 0.07 mmol) was effected by hydrogenolysis to afford PtdIns(3,4,5)P_3 in a nearly quantitative yield. The validity of this synthetic approach was proven by ^1H and ^{31}P NMR and FAB mass spectral analysis of the final product. The ^1H and ^{31}P NMR spectra conformed to those reported by Ozaki *et al.*[4] for the racemic compound. This synthetic PtdIns(3,4,5)P_3 displayed interesting properties of modulating the activities of enzymes that play key roles in signal transduction (unpublished data). Use of this methodology to prepare other 3-phosphorylated phosphatidylinositides is currently underway in this laboratory.

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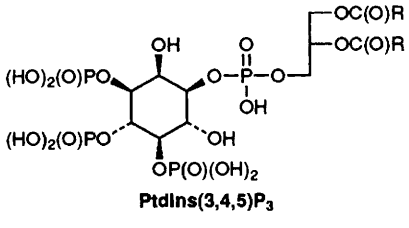
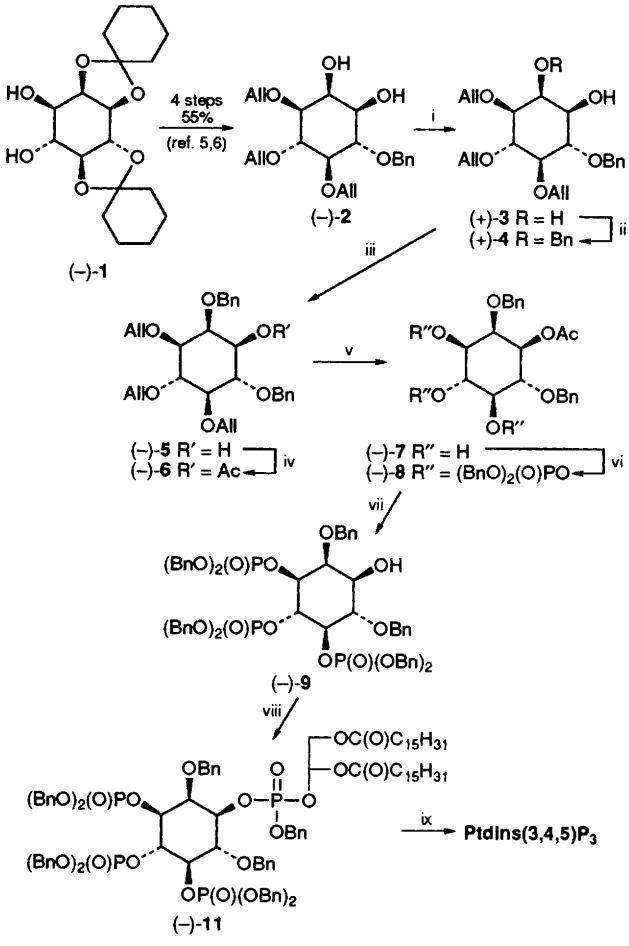


PtdIns(3,4,5)P_3



Scheme 1 *Reagents and conditions*: i, Bu_2SnO (1 equiv.)–toluene, 1 h, heat, then MEMCl (1.1 equiv.), CsF (4.5 equiv.)–DMF, 23 °C, 16 h; ii, BnBr (1.5 equiv.) NaH (3 equiv.)–DMF, 23 °C, 12 h; iii, $ZnBr_2$, CH_2Cl_2, 23 °C, 20 h; iv, Ac_2O (1.5 equiv.), DMAP (1.5 equiv.), CH_2Cl_2, 23 °C, 1 h; v, Pd/C, PTSA, MeOH–H_2O (4:1), heat, 2 h; vi, $(BnO)_2PN(Pr^i)_2$ (3.3 equiv.), 1*H*-tetrazole (12 equiv.), CH_2Cl_2, 23 °C, 12 h, then MCPBA (10 equiv.), 23 °C, 10 min; vii, NaOH (1 equiv.), MeOH–H_2O (10:1), 23 °C, 30 min; viii, **10** (2.5 equiv.), 1*H*-tetrazole (12 equiv.), CH_2Cl_2, 23 °C, 12 h, then MCPBA (3 equiv.), 23 °C, 10 min; ix, Pd blank, H_2 (50 psi), EtOH–H_2O (4:1), 6 h. All = $-CH_2-CH=CH_2$.

Footnote

† All compounds exhibited satisfactory spectral and analytical data. (+)-**3**: $[\alpha]_D$ + 30.6 (*c* = 1.2, CH_3Cl); ^1H NMR ($CDCl_3$) δ 2.75 (br s, 1 H), 3.20–3.65 (m, 2 H), 3.65 (s, 3 H), 3.46–3.52 (m, 3 H), 3.67–3.74 (m, 2 H), 3.81–3.87 (m, 2 H), 4.15–4.18 (m, 2 H), 4.26–4.32 (m, 5 H), 4.78 (q, 2 H, *J* = 5.31, 11.2 Hz), 4.87 (q, 2 H, *J* = 10.73, 23.02 Hz), 5.12–5.20 (m, 3 H), 5.23–5.33 (m, 3 H), 5.88–6.00 (m, 3H), 7.26–7.34 (m, 5 H). (+)-**4**: $[\alpha]_D$ + 10.5 (*c* = 0.95, CH_3Cl); ^1H NMR ($CDCl_3$) δ 3.20–3.26 (m, 2 H), 3.41 (s, 3 H), 3.36–3.50 (m, 3 H), 3.60–3.65 (m, 2 H), 3.78–3.92 (m, 2 H), 4.02 (t, 1 H, *J* = 2.3 Hz), 4.10–4.13 (m, 2 H), 4.25–4.33 (m, 4 H), 4.71–4.29 (m, 6 H), 5.11–5.16 (m, 3 H), 5.22–5.34 (m, 3 H), 5.86–6.02 (m, 3 H), 7.24–7.43 (m, 10 H). (−)-**6**: $[\alpha]_D$ −34 (*c* = 0.9, CH_3Cl); ^1H NMR ($CDCl_3$) δ 1.89 (s, 3 H), 3.25–3.32 (m, 2 H), 3.77–3.84 (m, 1 H), 3.92–3.99 (m, 2 H), 4.10–4.13 (m, 2 H), 4.24–4.38 (m, 4 H), 4.06–4.69 (m, 3 H), 4.80–4.86 (q, 2 H, *J* = 6.85, 10.1 Hz), 5.12–5.19 (m, 3 H), 5.23–5.34 (m, 3 H), 5.84–6.04 (m, 3 H), 7.24–7.35 (m, 10 H). (−)-**7**: m.p. 108–109 °C; $[\alpha]_D$ −49, (*c* = 1, CH_3Cl); ^1H NMR ($CDCl_3$) δ 1.98 (s, 3 H), 2.60 (br s, 1 H), 2.7 (br s, 2 H), 3.45 (t, 1 H, *J* = 9.3 Hz), 3.52 (dd, 1 H, *J* = 3, 4.5 Hz), 3.78 (t, 1 H, *J* = 9.4 Hz), 3.89 (t, 1 H, *J* = 9.4 Hz), 4.06 (t, 1 H, *J* = 2.7 Hz), 4.72 (q, 2 H, *J* = 11.6, 36 Hz), 4.76 (t, 1 H, *J* = 10 Hz), 4.83–4.87 (dd, 2 H, *J* = 2.7, 9.0 Hz), 7.29–7.38 (m, 10 H); Calcd for $C_{22}H_{26}O_7$: C, 65.65; H, 6.51. Found: C, 65.48; H, 6.60%. (−)-**8**: $[\alpha]_D$ −15.7 (*c* = 1, CH_3Cl); ^1H NMR ($CDCl_3$) δ 2.05 (s, 3 H), 4.05 (t, 1 H, *J* = 12 Hz), 4.24–4.36 (m, 1 H), 4.41–4.60 (m, 1 H), 4.70–4.83 (m, 4 H), 4.88–5.11 (m, 16 H), 7.05–7.35 (m, 40 H). ^{31}P NMR ($CDCl_3$, external H_3PO_4) −2.40, −1.85 and −1.53. (−)-**9**: $[\alpha]_D$ −8.2 (*c* = 1, CH_3Cl); ^1H NMR ($CDCl_3$) δ 1.95 (d, 1 H, *J* 6.6 Hz), 3.45–3.55 (m, 1 H), 3.81 (t, 1 H, *J* = 9.6 Hz), 4.23–4.28 (m, 2 H), 4.42 (q, 1 H, *J* = 9.2, 13.7 Hz), 4.61–4.71 (m, 2 H), 4.76–4.86 (m, 2 H), 4.87–5.12 (m, 13 H), 7.09—7.43 (m, 40 H); ^{31}P NMR ($CDCl_3$, external H_3PO_4) −2.12, −1.55 and −1.43. (−)-**11**: $[\alpha]_D$ −2 (*c* = 0.8, CH_3Cl); ^1H NMR ($CDCl_3$) δ 0.87 (t, 6 H, *J* = 6.2 Hz), 1.21–1.29 (m, 48 H), 1.42–1.51 (m, 4 H), 2.11–2.22 (m, 4 H), 3.72–4.08 (m, 5 H), 4.24–4.69 (m, 3 H), 4.71–5.07 (m, 21 H), 6.85–7.42 (m, 45 H); ^{31}P NMR ($CDCl_3$, external H_3PO_4) −2.28 (1 P), −2.06 (0.25 P), −1.93 (0.5 P), −1.84 (0.25 P), −1.75 (1 P), −1.52 (1 P). **PtdIns(3,4,5)P_3**: $[\alpha]_D$ +3.7 (*c* = 0.5, $CHCl_3$); ^1H NMR (D_2O–$CDCl_3$–CD_3OD, 1 : 2 : 4) δ 0.88 (t, 6 H, *J* = 7.2 Hz), 1.26 (br s, 48 H), 1.60 (br s, 4 H), 2.66–2.37 (m, 4 H), 3.07–3.20 (m, 1 H), 3.57–3.74 (m, 2 H), 3.86–4.21 (m, 6 H), 4.37–4.46 (m, 1 H), 5.05 (br s, 1 H); ^{31}P NMR $[^2H]_6Me_2SO$, external H_3PO_4, 346 K) −1.66 (br, 1 P), −1.17 (1 P), −0.50 (1 P), −0.31 (br, 1 P); MS (negative ion FAB) *m/z* 1049.4 (M − 1), 811.1 (M − $C_{15}H_{31}CO$), 793.1, 498.9 [M − $C_{15}H_{31}COOCH_2CH(OCOC_{15}H_{31})CH_2$].

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