

Synthesis of dipalmitoyl phosphatidylinositol 3,4-bis(phosphate) and 3,4,5-tris(phosphate) and their enantiomers

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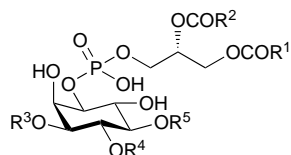
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The dipalmitoyl derivatives **4** and **5** of 3-phosphorylated *myo*-inositol phospholipids **2** and **3** and their enantiomers are synthesised from homochiral *myo*-inositol precursors **6** and **11**; they serve as biological probes for cell signal transduction.

The group of *myo*-inositol phospholipids PtdIns(3)P **1**, PtdIns(3,4)P₂ **2** and PtdIns(3,4,5)P₃ **3** are believed to have a role in the mechanisms by which cell surface receptors for antigens,



1 R¹ = C₁₉H₃₁, R² = C₁₇H₃₅, R³ = P(O)(OH)₂, R⁴ = R⁵ = H

2 R¹ = C₁₉H₃₁, R² = C₁₇H₃₅, R³ = R⁴ = P(O)(OH)₂, R⁵ = H

3 R¹ = C₁₉H₃₁, R² = C₁₇H₃₅, R³ = R⁴ = R⁵ = P(O)(OH)₂

4 R¹ = R² = C₁₅H₃₁, R³ = R⁴ = P(O)(OH)₂, R⁵ = H

5 R¹ = R² = C₁₅H₃₁, R³ = R⁴ = R⁵ = P(O)(OH)₂

inflammatory stimuli and growth factors control a variety of cellular functions.^{1–3} These lipids appear to be resistant to hydrolysis by phosphatidyl inositol-specific phospholipase C (PI-PLC),⁴ implying an independent cell signalling pathway from that already established for PtdIns(4,5)P₂.⁵ In order to probe the function of the phospholipids **2** and **3** in signal transduction we proposed to synthesise the functionally similar dipalmitoyl derivatives **4** and **5** and their enantiomers for use in biological assays. The synthetic work presented here utilises differentially protected homochiral *myo*-inositol derivatives **6** and **11** which were obtained efficiently from *myo*-inositol orthoformate.⁶

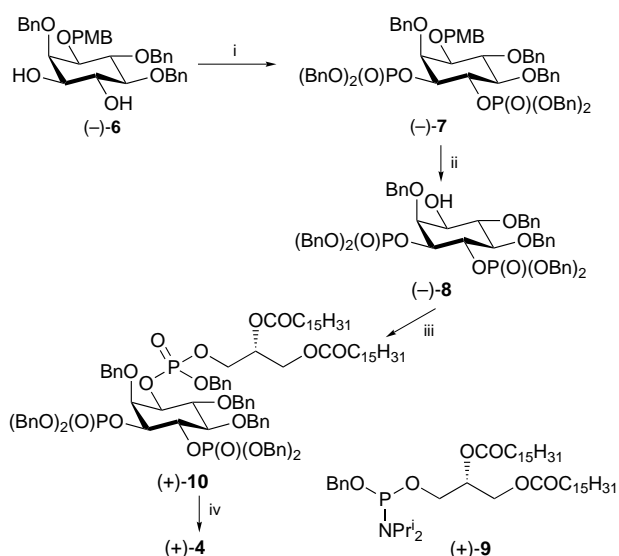
Dipalmitoyl PtdIns(3,4)P₂ **4** was prepared from (–)-**6** in four synthetic steps with an overall yield of 72% (Scheme 1). The 3,4-bisphosphorylated derivative (–)-**7** was prepared *via* phosphorylation of (–)-**6** with bis(benzyloxy)(*N,N*-diisopropylamino)phosphine⁷ and 1*H*-tetrazole followed by *in situ* oxidation with MCPBA. After removal of the *p*-methoxybenzyl ether with ceric ammonium nitrate the 1-hydroxy group was coupled with the phosphoramidite (+)-**9**.^{8,9} *In situ* oxidation of the phosphorus(III) species afforded the fully protected phospholipid (+)-**10**. Reductive debenzoylation was readily effected using the conditions reported¹⁰ by Kozikowski *et al.*, furnishing dipalmitoyl PtdIns(3,4)P₂ (+)-**4**.[†]

Elaboration of the antipodal diol (+)-**6** *via* the same reaction sequence as shown in Scheme 1 and coupling with (–)-**9**,[‡] afforded (–)-**4**,[†] the enantiomer of dipalmitoyl PtdIns(3,4)P₂.

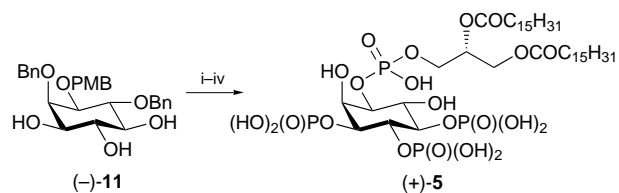
The use of similar reagents and reaction conditions as outlined for the synthesis of (+)-**4** afforded dipalmitoyl PtdIns(3,4,5)P₃ (+)-**5**[†] in 70% yield from the 3,4,5-triol (–)-**11**

(Scheme 2). Similarly (–)-**5**[†] was synthesised from (+)-**11** and (–)-**9** by the route used in Scheme 2.

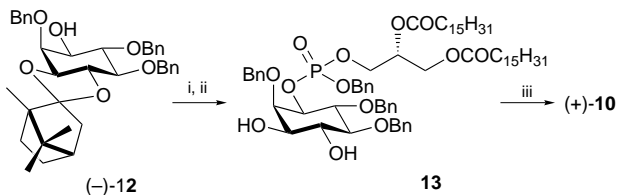
An alternative synthesis of dipalmitoyl PtdIns(3,4)P₂ (+)-**4** was also carried out from the 3,4-protected alcohol (–)-**12** (Scheme 3).⁶ The alcohol was coupled directly with the



Scheme 1 Reagents and conditions: (BnO)₂PNPr₂, 1*H*-tetrazole, CH₂Cl₂, then MCPBA; ii, (NH₄)₂Ce(NO₃)₆, MeCN–H₂O (4:1); iii, (+)-**9**, 1*H*-tetrazole, CH₂Cl₂, then MCPBA; iv, Pd(OH)₂–C, H₂ (60 psi), Bu^tOH



Scheme 2 Reagents and conditions: i, (BnO)₂PNPr₂, 1*H*-tetrazole, CH₂Cl₂, then MCPBA; ii, (NH₄)₂Ce(NO₃)₆, MeCN–H₂O (4:1); iii, (+)-**9**, 1*H*-tetrazole, CH₂Cl₂, then MCPBA; iv, Pd(OH)₂–C, H₂ (60 psi), Bu^tOH



Scheme 3 Reagents and conditions: i, (+)-**9**, 1*H*-tetrazole, CH₂Cl₂, then MCPBA; ii, AcCl, MeOH–CH₂Cl₂ (1:1); iii, Pd(OH)₂–C, H₂ (60 psi), Bu^tOH

phosphoramidite (+)-**9**, and the product was then cleaved at the 3,4-acetal with acid (AcCl–MeOH) to afford the 3,4-diol **13**. Phosphitylation of the free hydroxy groups with bis(benzyl-oxy)(*N,N*-diisopropylamino)phosphine and 1*H*-tetrazole, followed by *in situ* oxidation with MCPBA, afforded the common fully protected phospholipid (+)-**10**. This route required two fewer steps than the original procedure, as the *p*-methoxybenzyl protection and deprotection at the D-1-hydroxy are not required. The overall yield is slightly better than that of the original procedure.

In summary we have demonstrated a general synthesis of 3-phosphorylated *myo*-inositol phospholipids **4** and **5** from the homochiral precursors **6** and **11** which also allows the synthesis of enantiomeric derivatives containing the unnatural stereochemistry. These materials play an important rôle in evaluating the activation of protein kinase B.¹¹

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Footnotes and References

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† All new compounds exhibited spectroscopic and analytical data in accord with the assigned structure (*J* values in Hz). *Selected data* for (+)-**4**, the dipalmitoyl analogue of PtdIns(3,4)P₂: [α]_D²² +0.65 [*c* 1.1 in CHCl₃–MeOH (1 : 1)]; δ _H (400 MHz, [²H₆]DMSO) 5.18–5.11 (1 H, m), 4.35–4.28 (2 H, m), 4.14–4.02 (5 H, m), 3.90 (1 H, br t, *J* 9.1), 3.60 (1 H, t, *J* 9.3), 3.25 (1 H t, *J* 9.0), 2.33–2.24 (4 H, m), 1.55–1.44 (4 H, m), 1.28–1.19 (48 H, m), 0.85 (6 H, br t, *J* 6.7); δ _P (101 MHz, [²H₆]DMSO) 1.64, 1.00, –0.16; Found (FAB MS): (M + H + H)⁺, 972.4794. C₄₁H₈₃O₁₉P₃ requires (M + H + H)⁺, 972.4741.

For (–)-**4**: [α]_D²² –0.75 [*c* 0.70 in CHCl₃–MeOH (1 : 1)].

For (+)-**5**, the dipalmitoyl analogue of PtdIns(3,4,5)P₃: [α]_D²² +1.85 [*c* 0.60 in CHCl₃–MeOH (1 : 1)]; δ _H (400 MHz, [²H₆]DMSO) 5.19–5.11 (1 H, m), 4.47 (1 H, m), 4.31–3.99 (8 H, m), 3.75 (1 H, t, *J* 9.4), 2.33–2.22 (4 H, m), 1.54–1.45 (4 H, m), 1.29–1.19 (48 H, m), 0.85 (6 H, m); δ _P (101 MHz, [²H₆]DMSO) 1.22, 0.98, 0.59, –0.20; Found (FAB): (M + H)⁺, 1051.4350. C₄₁H₈₃O₂₂P₄ requires (M + H)⁺, 1051.4326.

For (–)-**5**: [α]_D²² –1.75 [*c* 0.75 in CHCl₃–MeOH (1 : 1)].

‡ Compound (–)-**9** was synthesised in a similar manner to (+)-**9** starting from (*R*)-(–)-2,2-dimethyl-1,3-dioxolane-4-methanol (Aldrich).

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