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

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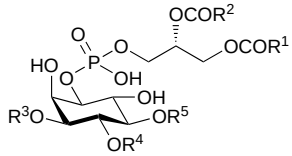
The dipalmitoyl derivatives 4 and 5 of 3-phosphorylated *myo*-inositol phospholipids 2 and 3 and their enantiomers are synthesised from homochiral *myo*-inositol precursors 6 and 11; they serve as biological probes for cell signal transduction.

The group of *myo*-inositol phospholipids PtdIns(3)P **1**, PtdIns(3,4)P_2 **2** and PtdIns(3,4,5)P_3 **3** are believed to have a role in the mechanisms by which cell surface receptors for antigens,



1 $R^1 = C_{19}H_{31}$, $R^2 = C_{17}H_{35}$, $R^3 = P(O)(OH)_2$, $R^4 = R^5 = H$
2 $R^1 = C_{19}H_{31}$, $R^2 = C_{17}H_{35}$, $R^3 = R^4 = P(O)(OH)_2$, $R^5 = H$
3 $R^1 = C_{19}H_{31}$, $R^2 = C_{17}H_{35}$, $R^3 = R^4 = R^5 = P(O)(OH)_2$
4 $R^1 = R^2 = C_{15}H_{31}$, $R^3 = R^4 = P(O)(OH)_2$, $R^5 = H$
5 $R^1 = R^2 = C_{15}H_{31}$, $R^3 = R^4 = R^5 = P(O)(OH)_2$

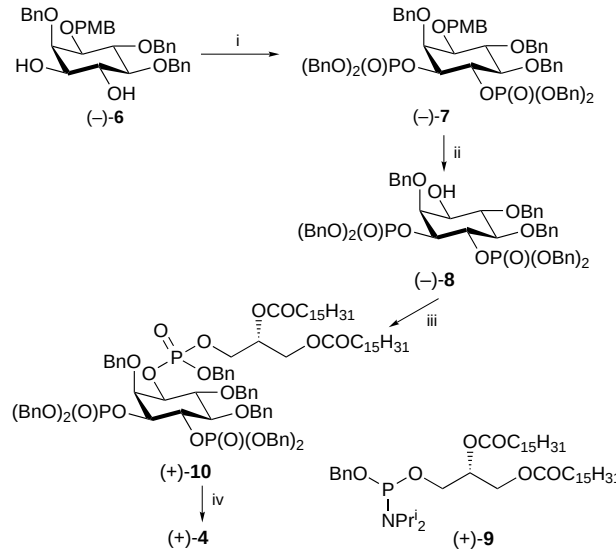
inflammatory stimuli and growth factors control a variety of cellular functions.[1–3] These lipids appear to be resistant to hydrolysis by phosphatidyl inositol-specific phospholipase C (PI-PLC),[4] implying an independent cell signalling pathway from that already established for PtdIns(4,5)P_2.[5] In 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)P_2 **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* phosphitylation of (−)-**6** with bis(benzyloxy)(*N*,*N*-diisopropylamino)phosphine[7] 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 debenzylation was readily effected using the conditions reported[10] by Kozikowski *et al.*, furnishing dipalmitoyl PtdIns(3,4)P_2 (+)-**4**.†

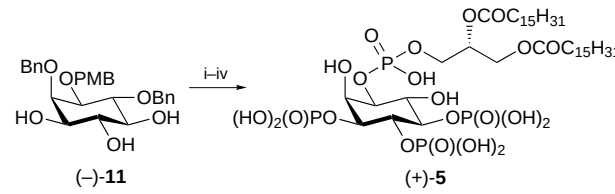
Elaboration of the antipodal diol (+)-**6** *via* the same reaction sequence as shown in Scheme 1 and coupling with (−)-**9**,‡ afforded (−)-4,† the enantiomer of dipalmitoyl PtdIns(3,4)P_2.

The use of similar reagents and reaction conditions as outlined for the synthesis of (+)-**4** afforded dipalmitoyl PtdIns(3,4,5)P_3 (+)-**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.

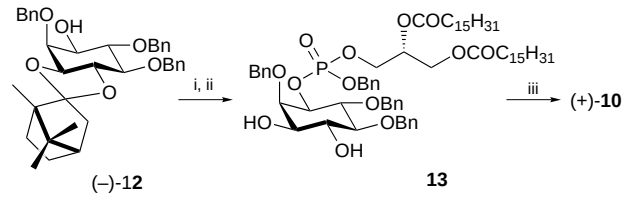
An alternative synthesis of dipalmitoyl PtdIns(3,4)P_2 (+)-**4** was also carried out from the 3,4-protected alcohol (−)-**12** (Scheme 3).[6] The alcohol was coupled directly with the



Scheme 1 *Reagents and conditions*: $(BnO)_2PNPr^i_2$, 1*H*-tetrazole, CH_2Cl_2, then MCPBA; ii, $(NH_4)_2Ce(NO_3)_6$, MeCN–H_2O (4:1); iii, (+)-**9**, 1*H*-tetrazole, CH_2Cl_2, then MCPBA; iv, $Pd(OH)_2$–C, H_2 (60 psi), Bu^tOH



Scheme 2 *Reagents and conditions*: i, $(BnO)_2PNPr^i_2$, 1*H*-tetrazole, CH_2Cl_2, then MCPBA; ii, $(NH_4)_2Ce(NO_3)_6$, MeCN–H_2O (4:1); iii, (+)-**9**, 1*H*-tetrazole, CH_2Cl_2, then MCPBA; iv, $Pd(OH)_2$–C, H_2 (60 psi), Bu^tOH



Scheme 3 *Reagents and conditions*: i, (+)-**9**, 1*H*-tetrazole, CH_2Cl_2, then MCPBA; ii, AcCl, MeOH–CH_2Cl_2 (1:1); iii, $Pd(OH)_2$–C, H_2 (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(benzyloxy)(*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)P_2: $[\alpha]_D^{22}$ +0.65 [*c* 1.1 in $CHCl_3$–MeOH (1 : 1)]; δ_H (400 MHz, [$^2H_6$]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, [$^2H_6$]DMSO) 1.64, 1.00, −0.16; Found (FAB MS): $(M + H + H)^+$, 972.4794. $C_{41}H_{83}O_{19}P_3$ requires $(M + H + H)^+$, 972.4741.

For (−)-**4**: $[\alpha]_D^{22}$ −0.75 [*c* 0.70 in $CHCl_3$–MeOH (1 : 1)].

For (+)-**5**, the dipalmitoyl analogue of PtdIns(3,4,5)P_3: $[\alpha]_D^{22}$ +1.85 [*c* 0.60 in $CHCl_3$–MeOH (1 : 1)]; δ_H (400 MHz, [$^2H_6$]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, [$^2H_6$]DMSO) 1.22, 0.98, 0.59, −0.20; Found (FAB): $(M + H)^+$, 1051.4350. $C_{41}H_{83}O_{22}P_4$ requires $(M + H)^+$, 1051.4326].

For (−)-**5**: $[\alpha]_D^{22}$ −1.75 [*c* 0.75 in $CHCl_3$–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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