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L-α-Phosphatidyl-D-*myo*-inositol 3,5-bisphosphate: total synthesis of a new inositol phospholipid *via myo*-inositol orthoacetate

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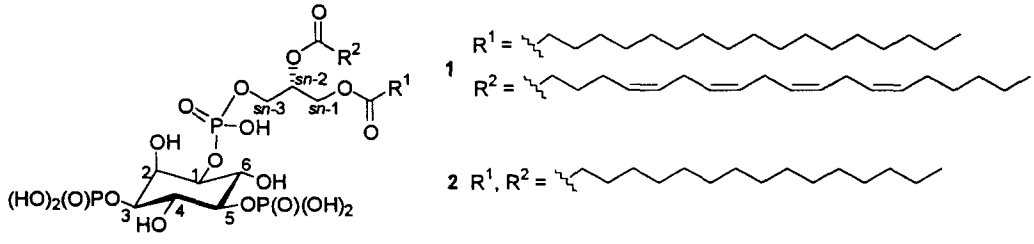
Abstract: The synthesis from *myo*-inositol of a newly-discovered inositol phospholipid, phosphatidylinositol 3,5-bisphosphate [PtdIns(3,5)P_2], is described. The synthetic strategy, employing *inter alia*, a trimethylaluminium-mediated regioselective cleavage of a protected *myo*-inositol orthoacetate followed by an optical resolution using (*R*)-(−)-5-oxo-2-tetrahydrofurancarboxylate esters, allows rapid access to dipalmitoyl PtdIns(3,5)P_2.

Phosphorylation of the hydroxyl groups in phosphatidylinositol [PtdIns] at one or a combination of positions D-3, 4 and 5 of the inositol head group generates a family of phosphatidylinositol phosphates [PtdIns(P)$_n$s], which have been identified as components of the lipid bilayer of cell membranes. The biological functions of PtdIns(P)$_n$s in signal transduction, exocytosis and the regulation of membrane trafficking are currently the subject of intense interest in cell biology.[1] Recently, the previously unknown phosphatidylinositol 3,5-bisphosphate [PtdIns(3,5)P_2, **1**] was reported to occur in mammalian cell lines[2] and a second study has shown that PtdIns(3,5)P_2 is widespread among eukaryotes.[3] There now exists compelling evidence that PtdIns(3,5)P_2 may be at the centre of a previously uncharacterised regulatory pathway,[3,4] but attempts to identify the cellular function of PtdIns(3,5)P_2 will require much larger quantities of phospholipid than can be obtained from natural sources, as well as routes adaptable to the preparation of other pharmacological probes. We therefore report here a concise and versatile synthetic route to dipalmitoyl PtdIns(3,5)P_2 (**2**).



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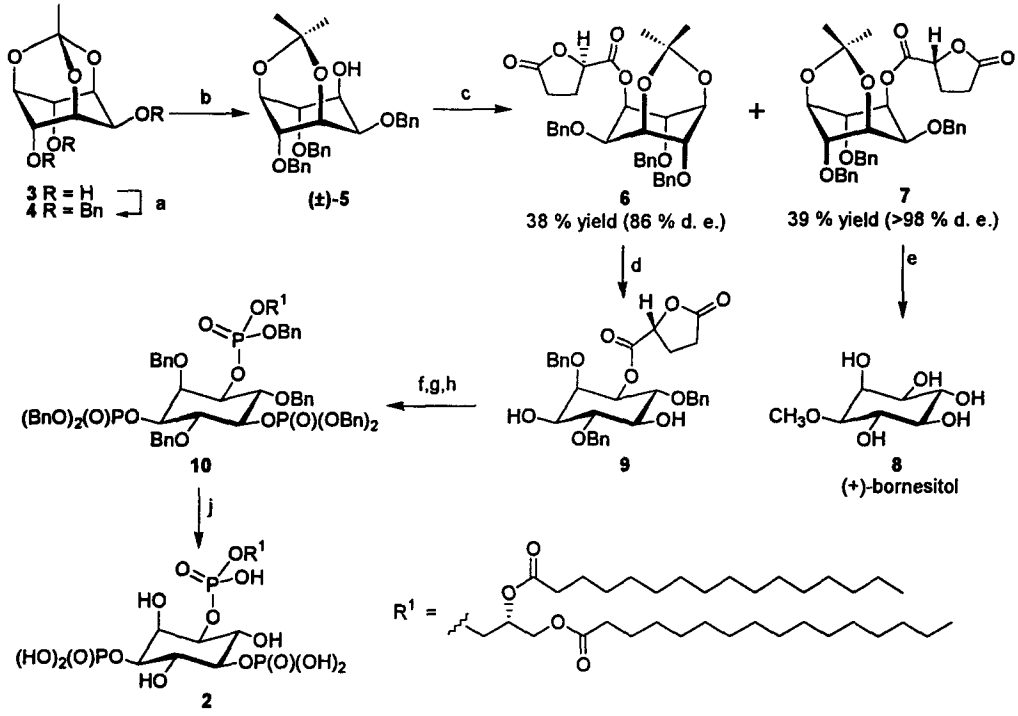
The route begins with *myo*-inositol orthoacetate (**3**).[5] Conventional benzylation of **3** gave the highly crystalline tri-*O*-benzyl derivative **4**[6] in 87 % yield without recourse to chromatography. It had previously been reported that treatment of 2,4,6-tri-*O*-benzyl-*myo*-inositol 1,3,5-orthoformate with trimethylaluminium gave (±)-2,4,6-tri-*O*-benzyl-1,5-*O*-ethylidene *myo*-inositol[7], and we reasoned that application of a similar procedure to the orthoacetate ester **4** should give the more useful isopropylidene acetal (±)-**5**. The advantages of employing the orthoacetate ester are two-fold: first, the resulting isopropylidene acetal does not contain a new stereogenic centre at the bridging carbon, and second, this acetal should be more acid-labile than the corresponding ethylidene, enabling its removal under mild conditions.



Reagents and conditions: a) NaH, BnBr, DMF, 87% b) Me_3Al (2.5 - 3.0 equiv.), CH_2Cl_2, hexane, –78°C, 91%; c) (*R*)-(–)-5-oxo-2-tetrahydrofurancarboxylic acid, DCC, DMAP, CH_2Cl_2, –78°C to rt; d) 80% acetic acid, rt, then recrystallise from propan-2-ol, 78%; e) i) NaOH, MeOH, reflux; ii) NaH, MeI, DMF; iii) CH_2Cl_2 / CF_3COOH / H_2O 80:19:1; iv) H_2, 50 p.s.i., Pd-C, EtOH; 71% yield for 4 steps; f) $(BnO)_2PNPr^i_2$, 1*H*-tetrazole, CH_2Cl_2; ii) *m*-CPBA, –40°C to rt, 96%; g) NH_3 / MeOH, rt, 91%; h) $R^1OP(OBn)NPr^i_2$, 1*H*-tetrazole, CH_2Cl_2; ii) *m*-CPBA, – 40°C to rt, 83%; j) H_2, 50 p.s.i., $Pd(OH)_2$-C, Bu^{t}OH, 85%. Bn = benzyl.

Optimised conditions involved the use of 2.5 to 3.0 equivalents of trimethylaluminium at –78°C, followed by an alkaline work-up. Under these conditions the alcohol (±)-**5** was obtained in high yields on a multigram scale. Higher temperatures gave substantial amounts of the unwanted 2,4,6-tri-*O*-benzyl-*myo*-inositol. The isopropylidene acetal of (±)-**5** proved to be highly labile, and this property was exploited later in the synthesis.

Attempts to resolve (±)-**5** employing the widely used (*S*)-(–)-camphanate esters were unsuccessful, as was the use of acetylmandelate esters,[8] but DCC-promoted esterification with (*R*)-(–)-5-oxo-2-tetrahydrofurancarboxylic [(*R*)-(–)-TOF] acid[9] gave the diastereoisomeric esters **6** and **7**[10] which were separable by flash chromatography. The less polar ester was obtained pure (as judged by ^{1}H NMR) in this way, and was converted in four steps to (+)-bornesitol[11] (**8**), identifying the ester as **7**. The more polar diastereoisomer **6** was obtained contaminated with some **7**. For analytical purposes, pure **6** could be isolated by further chromatography, but for the present route, it was convenient to proceed directly to the next step using partially purified **6**. Removal of the isopropylidene acetal from **6** by mild acid treatment (acetic acid at room temperature) followed by a single crystallisation from propan-2-ol gave the single diastereoisomer **9**[12] in 78% yield. Highly crystalline **9** could routinely be obtained on a gram scale in this way. Benzylphosphate groups were then introduced at positions 3 and 5 by phosphitylation using bis(benzyloxy)(*N,N*-diisopropylamino)phosphine[13] and 1*H*-tetrazole followed by *in situ* oxidation with *m*-CPBA, and the rather labile (*R*)-(–)-TOF ester was cleaved using ammonia-saturated dry methanol. Reaction at the exposed 1-OH group with benzyl 1,2-*O*-dipalmitoyl-*sn*-glyceryl *N,N*-diisopropylphosphoramidite[14] in the presence of 1*H*-tetrazole, gave **10** as a mixture of diastereoisomers after *m*-CPBA oxidation. Finally, deprotection by hydrogenation over palladium hydroxide on carbon in *tert*-butyl alcohol[15] gave dipalmitoyl PtdIns(3,5)P_2 (**2**).[16]

In conclusion, we have described an expedient route to a recently-discovered inositol phospholipid, employing a novel regioselective protecting group strategy and a chiral auxiliary new to the field of inositol chemistry. The enantiomer of **2**, potentially of use in investigating the specificity of putative target proteins for PtdIns(3,5)P_2, can be synthesised either from **7** or by the use of (*S*)-(+)-5-oxo-2-tetrahydrofurancarboxylic acid. Finally, intermediate **9**, rapidly accessible in high purity from *myo*-inositol orthoacetate, provides a suitable starting point for the synthesis of other analogues of PtdIns(3,5)P_2.

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References and Notes

1. For reviews see: (a) Divecha, N. and Irvine, R. F. *Cell* **1995**, *80*, 269-278; (b) De Camilli, P.; Emr, S. D.; McPherson, P. S., Novick, P. *Science* **1996**, *271*, 1533-1539.
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6. Compound **4** had R_f 0.36 (EtOAc / hexane 1:3), mp 77.5 - 78.5°C (from hexane). All new compounds exhibited satisfactory spectroscopic and analytical data.

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9. Purchased from Aldrich and recrystallised once from ether / hexane before use. The enantiomer, (*S*)-(+)-5-oxo-2-tetrahydrofurancarboxylic [(*S*)-(+)-TOF] acid, also available from Aldrich, has been used as a chiral derivatising reagent for alcohols (Doolittle, R. E. and Heath, R. R. *J. Org. Chem.* **1984**, *49*, 5041-5050). (*S*)-(+)-TOF esters have also been employed for the optical resolution of (±)-4-benzyloxy-tetrahydrofuran-3-ol (Altenbach, H.-J. and Wolf, E. *Tetrahedron: Asymmetry* **1993**, *4*, 2155-2158).

10. Data for **6**: $[\alpha]_D^{20}$ –21 (*c* 1.5, $CHCl_3$); R_f 0.40 ($CHCl_3$/EtOAc 10:1); δ_H (270 MHz, $CDCl_3$, TMS) 1.38 (3 H, s, CH_3), 1.43 (3 H, s, CH_3), 2.07 - 2.16 (1 H, m), 2.24 - 2.40 (2 H, m), 2.42 - 2.56 (1 H, m), 4.05 (1 H, br d, *J* ~ 5.5 Hz), 4.27-4.30 (2 H, m), 4.38 - 4.54 (5 H, m), 4.62 - 4.72 (3 H, m), 4.92 (1 H, dd, *J* 8.4, 4.4 Hz, C-2′-H), 5.50 (1 H, dd, *J* 7.1 5.5 Hz, C-1-H), 7.20 - 7.32 (15 H, 3 × C_6H_5).

    Data for **7**: $[\alpha]_D^{16}$ –4 (*c* 1, $CHCl_3$); R_f 0.48 ($CHCl_3$/EtOAc 10:1); δ_H (400 MHz, $CDCl_3$, TMS) 1.39 (3 H, s, CH_3), 1.49 (3 H, s, CH_3), 1.95 - 2.04 (1 H, m), 2.08 - 2.18 (1 H, m), 2.23 - 2.35 (2 H, m), 3.94 (1 H, br s), 4.24 (1 H, dd, *J* 6.1 2.1 Hz), 4.38 (1 H, br s), 4.41 - 4.54 (4 H, m), 4.62 - 4.76 (4 H, m), 4.87 (1 H, dd, *J* 8.2, 4.3 Hz, C-2′-H), 5.65 (1 H, dd, *J* 6.1 3.1 Hz, C-1-H), 7.20 - 7.33 (15 H, m, 3 × C_6H_5).

11. ^{1}H NMR data for **8** agreed with those published for 1D-(–)-1-*O*-methyl *myo*-inositol, (Jaramillo, C.; Chiara, J.-L. and Martín-Lomas, M. *J. Org Chem.* **1994**, *59*, 3135-3141) but **8** had $[\alpha]_D^{23}$ +32 (*c* 2, H_2O), identifying it as 1L-1-*O*-methyl *myo*-inositol [(+)-bornesitol]; mp 203-205°C (from MeOH / EtOH); Lit. values: + 31.9 (*c* 1, H_2O), mp 205-207°C (Gigg, J.; Gigg, R.; Payne, S. and Conant, R. *J. Chem. Soc. Perkin Trans. 1* **1987**, 1757-1762. See also references therein).

12. Data for **9**: mp 121.5 - 123.5°C from propan-2-ol; $[\alpha]_D^{24}$ –28° (*c* 1, $CHCl_3$); δ_H (^{1}H - ^{1}H COSY, 400MHz, $CDCl_3$, TMS) 1.93 - 2.05 (1 H, m, C-3′-H_{2A}), 2.26 - 2.37 (3 H, m, C-4′-H_2 and C-3′-H_{2B}), 2.39 (1 H, d, *J* 5.2 Hz, D_2O ex., C-3-OH), 2.59 (1 H, d, *J* 2.4 Hz, D_2O ex., C-5-OH), 3.58 - 3.64 (2 H, m, C-3-H and C-5-H), 3.73 (1 H, dd, *J* 9.5, 9.3 Hz, C-4-H), 3.95 (1 H, dd, *J* 10.1, 9.2 Hz, C-6-H), 4.06 (1 H, dd, *J* 2.7, 2.5 Hz, C-2-H), 4.65 - 4.71 (3 H, m, OCH_2Ar and C-2′-H), 4.75 - 4.90 (4 H, m, OCH_2Ar), 4.87 (1 H, dd, partly buried, *J* 10.3, 2.7 Hz C-1-H), 7.24 - 7.40 (15 H, m, 3 × C_6H_5).

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15 Kozikowski, A. P.; Tückmantel, W. and Powis, G. *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 1379 - 1381. Deprotection using hydrogenation over palladium on carbon in *tert*-butyl alcohol / water / $NaHCO_3$ was unsatisfactory because the product could not be adequately characterised: the sodium salt of **2** gave only broad, unresolved peaks in the ^{1}H NMR spectrum, and a ^{31}P NMR spectrum could not be obtained.

16. Although the free acid **2** was poorly soluble in water, and NMR spectra taken in chloroform / methanol mixtures were broad, well-resolved NMR spectra could be obtained in [$^2H_7$]DMF.
    Data for **2**: δ_H (^{1}H - ^{1}H COSY, 400 MHz, [$^2H_7$]DMF, TMS) 0.88 (6 H, t, *J* 7 Hz, palmitoyl CH_3), 1.22 - 1.38 (48 H, m, palmitoyl CH_2), 1.54 - 1.64 (4 H, m, palmitoyl β-CH_2), 2.32 (2 H, t, *J* 7.6 Hz, palmitoyl α-CH_2), 2.34 (2 H, t, *J* 7.6 Hz, palmitoyl α-CH_2), 3.98 (2 H, br t, *J* 9 Hz, inositol C-4-H, C-6-H), 4.10 (1 H, br q, *J* 8 Hz, inositol C-5-H), 4.18 - 4.27 (5 H, m, inositol C-1-H and C-3-H, glyceryl *sn*-1-CH_{2A}, *sn*-3-CH_2), 4.42 (1 H, dd, *J* 11.6 Hz, 2.4 Hz, glyceryl *sn*-1-CH_{2B}), 4.52 (1 H, br s, inositol C-2-H), 5.25 (1 H, m, glyceryl *sn*-2-H); δ_P (162 MHz, [$^2H_7$]DMF, external H_3PO_4) –1.70 (1P), –0.02 (1P), 1.73 (1P). Negative FABMS (cyclohexylammonium salt, *m*-NBA): ***m/z*** 969.5 [$(M–H)^-$, 100%], 647.5 [$C_{15}H_{31}COOCH_2CH(OCOC_{15}H_{31})CH_2OPO_3H^-$, 80%], 255.2 [$C_{15}H_{31}COO^-$, 60%]. 97.0 [$H_2PO_4^-$, 40%].