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A Unified Approach to Unambiguous Synthesis of the Phosphatidylinositol-3-phosphates Involved in Intracellular Signal Transduction

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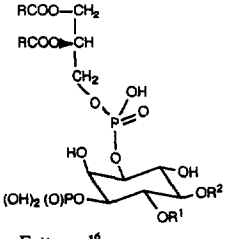
Abstract: A unified approach to unambiguous synthesis of the phosphatidylinositol-3-phosphates involved in intracellular signalling is illustrated by the synthesis of 1D-1-(1′,2′-dihexadecanoyl-*sn*-glycero-3′-phospho)-*myo*-inositol-3,4,5-trisphosphate.

The 3-phosphate derivatives of 1D-1-(1′,2′-di-*O*-fattyacyl-*sn*-glycero-3′-phospho)-*myo*-inositols (PtdIns) including phosphatidylinositol-3-phosphate, PtdIns(3)P, and the bis- and tris-phosphate derivatives PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3, have been found in eukaryotic cells, and the occurrence of PtdIns(3,5)P_2 has been suggested.[1,2] These compounds have been demonstrated as activators of protein kinase C isoforms δ, ϵ, and ζ,[3a] and are putative messengers in cellular signal cascades pertinent to inflammation, cell proliferation, transformation, protein kinesis, and cytoskeletal assembly.[4] Minute quantities are found in cells and therefore synthetic methods are needed to obtain samples for establishing the putative roles.[5]

Continuing our studies on the synthesis and functions of the cellular phosphoinositides,[3] we report on a unified approach which is suitable for facile synthesis of all cellular PtdIns-3-phosphates, provides unambiguous structural and stereochemical control in the *myo*-inositol as well as the *sn*-glycerol moieties, and is applicable for both short and long chain fattyacyl types required for cytophysiological studies. We illustrate by the synthesis of 1D-(1′,2′-dihexadecanoyl-*sn*-glycero-3′-phospho)-*myo*-inositol-3,4,5-trisphosphate (+)-**12**.

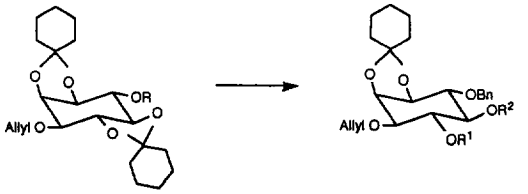


RCO: Fattyacyl[6]
PtdIns(3)P: $R^1 = R^2 = H$
PtdIns(3,4)P_2: $R^1 = P(O)(OH)_2$, $R^2 = H$
PtdIns(3,5)P_2: $R^1 = H$, $R^2 = P(O)(OH)_2$
PtdIns(3,4,5)P_3: $R^1 = R^2 = P(O)(OH)_2$

The approach has several novel features. One, it uses 1D-1,2:4,5-di-*O*-cyclohexylidene-3-*O*-allyl-*myo*-inositol (−)-**1**[7] as purposely designed starting material[7] and 1D-1,2-*O*-cyclohexylidene-3-*O*-allyl-6-*O*-benzyl-*myo*-inositol (+)-**3** as the key *myo*-inositol synthon. Two, it incorporates strategic *O*-protection by and sequentially invariant removal of allyl, 4-methoxybenzyl, and benzyl protecting groups from the inositol hydroxyls destined to appear in the target structures as phosphate, phosphatidyl, and free hydroxyl respectively. Three, it employs preformed 1,2-di-*O*-fattyacyl-*sn*-glycero-3-phosphoric acid (*sn*-3-phosphatidic acid) as the lipid synthon for coupling to appropriately *O*-protected *myo*-inositol by a phosphodiester condensation.

Reaction of (-)-1[7] with excess BnBr/NaH in DMF at R.T. overnight gave in quantitative yield its 6-*O*-benzyl derivative (-)-**2** $[\alpha]_D$ -51.6° (c 1.1, $CHCl_3$). Transketalization under kinetic control by reaction of (-)-**2** with ethylene glycol (1.2 mole)/catalytic *p*-TSA in CH_2Cl_2 at R.T. for 3 hr. gave the key synthon (+)-**3**, yield 81%, $[\alpha]_D$ +26.2° (c 1.0, $CHCl_3$).[8] Reaction of (+)-**3** in DMF at R.T. for 8 hr. with 1.2 moles of allyl bromide and NaH yielded the complete set of intermediates required for all four known PtdIns-3-phosphates. By chromatography on silica, the following pure compounds were obtained (Scheme 1): in 28% yield, 1D-1,2-*O*-cyclohexylidene-3,4,5-tri-*O*-allyl-6-*O*-benzyl-*myo*-inositol (-)-(**4**) $[\alpha]_D$ -11.3° (c 1.0, $CHCl_3$), Lit. $[\alpha]_D$ -9.2°, (c 1.5, $CHCl_3$)[9]; in 26% yield, 1D-1,2-*O*-cyclohexylidene-3,4-di-*O*-allyl-6-*O*-benzyl-*myo*-inositol (+)-(**4a**)[10] $[\alpha]_D$ +11.6° (c 0.82, $CHCl_3$); in 24% yield, 1D-1,2-*O*-cyclohexylidene-3,5-di-*O*-allyl-6-*O*-benzyl-*myo*-inositol (-)-(**4b**)[10] $[\alpha]_D$ -13.5° (c 0.96, $CHCl_3$); and, in 22% yield, unchanged starting material (+)-**3**. The overall utilization of (+)-**3** is 90% considering that the recovered compound is converted into (-)-**4e** in the next step (complete benzylation). Alternatively, reaction of (+)-**3** as above but using an excess of allyl bromide/NaH yielded (-)-(**4**) in quantitative yield. Compounds (+)-**4a**, (-)-**4b**, and (+)-**3** each were treated with an excess of BnBr and NaH in DMF at R.T. for 16 hr. and gave quantitative yields of the fully *O*-protected *myo*-inositols (-)-**4c** $[\alpha]_D$ -5.6° (c 1.43, $CHCl_3$), (-)-**4d** $[\alpha]_D$ -21.3° (c 1.23, $CHCl_3$), and (-)-**4e** $[\alpha]_D$ -25.3° (c 2.0, $CHCl_3$).

Scheme 1

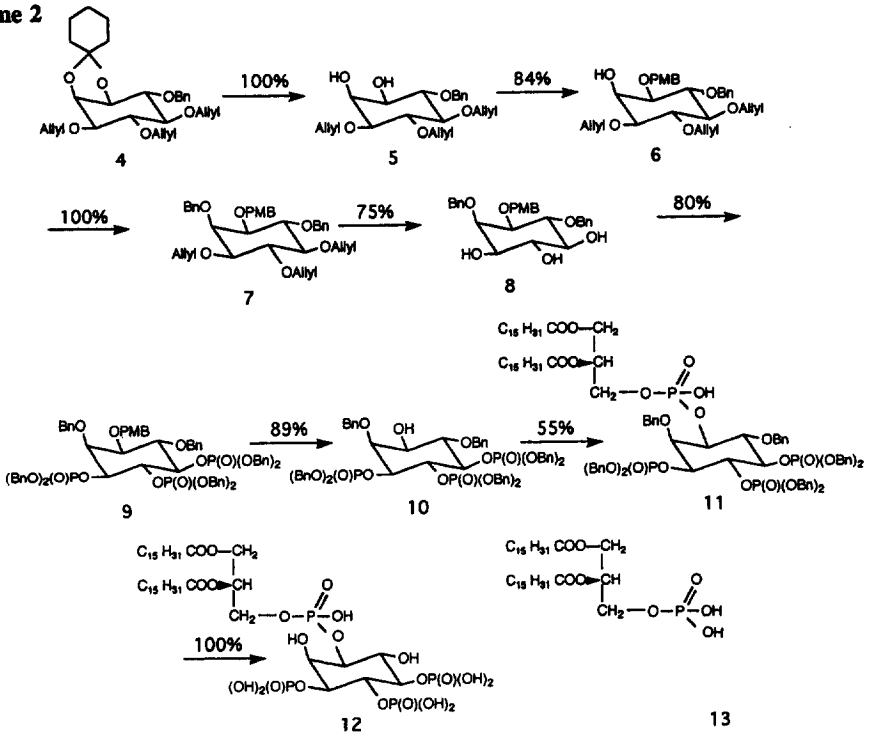


1: R = H **2**: R = Bn

3: $R^1 = R^2 = H$
4: $R^1 = R^2$ = Allyl
4a: R^1 = Allyl, R^2 = H
4b: R^1 = H, R^2 = Allyl
4c: R^1 = Allyl, R^2 = Bn
4d: R^1 = Bn, R^2 = Allyl
4e: $R^1 = R^2$ = Bn

Compounds (-)-**4**, (-)-**4c**, (-)-**4d**, and (-)-**4e** are intermediates respectively for the synthesis of PtdIns(3,4,5)P_3, PtdIns(3,4)P_2, PtdIns(3,5)P_2, and PtdIns(3)P, by the sequence of reactions illustrated for PtdIns(3,4,5)P_3 (Scheme 2). On heating at 95 °C for 3 hr. with acetic acid-water (80:20), (-)-**4** lost the *O*-cyclohexylidene protection and gave the 1,2-diol (-)-**5** $[\alpha]_D$ -16.2° (c 1.0, $CHCl_3$), Lit. $[\alpha]_D$ -10° (c 2, $CHCl_3$).[9] Reaction of (-)-**5** with Bu_2SnO in toluene with azeotropic removal of H_2O, rotary evaporation, solvent change to DMF and treatment with 4-methoxybenzyl chloride at 50 °C for 8 hr. provided high selectivity for reaction at the equatorial 1-OH over axial 2-OH (91:9) and gave after chromatography on silica (+)-**6** $[\alpha]_D$ +6.8° (c 1.0, $CHCl_3$).[11] On treatment with excess BnBr/NaH in DMF at R.T. for 16 hr., (+)-**6** produced 1D-1-*O*-(4′-methoxybenzyl)-3,4,5-*O*-tri-*O*-allyl-2,6-di-*O*-benzyl-*myo*-inositol (-)-**7** $[\alpha]_D$ -8.0° (c 1.0, $CHCl_3$). Compound (-)-**7** incorporates 3 types of blocking groups arranged for selective and successive deblocking and liberation of hydroxyls, from *O*-allyls for dibenzylphos-phorylation, from the 1-*O*-(4′-methoxybenzyl) for phosphatidylation, and the *O*-benzyls to regenerate the free hydroxyls in the target structure. Reaction of (-)-**7** with 10% Pd-C in methanol-acetic acid-water (98:2:0.1) under reflux caused complete *O*-deallylation to yield (-)-**8** $[\alpha]_D$ -7.5° (c 1.0, $CHCl_3$). Reaction of (-)-**8** in DMF with NaH and tetrabenzyl pyrophosphate[12] produced the 3,4,5-tris-*O*-(dibenzyl phosphate) derivative (-)-**9** $[\alpha]_D$ -9.5° (c 2.9, $CHCl_3$). The treatment of (-)-**9** with DDQ in CH_2Cl_2 yielded the 1D-2,6-*O*-dibenzyl-*myo*-inositol 3,4,5-tris-(dibenzylphosphate) (-)-**10** $[\alpha]_D$ -6.5° (c 0.2, $CHCl_3$).[3a] Reaction of (-)-**10** with 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoric acid[13] (**13**) in anhydrous pyridine and triisopropyl-benzenesulfonyl chloride as condensing agent[14] at R.T. for 18 hr. gave the phosphodiester product 1D-(1′,2′-dihexadecanoyl-*sn*-glycero-3′-phospho)-*myo*-inositol-3,4,5-tris-(dibenzylphosphate) (+)-**11** $[\alpha]_D$ +4.0° (c 0.3, $CHCl_3$). Hydrogenolysis of (+)-**11** in ethanol using Pd-black and H_2 gas at 45 psi yielded 1D-(1′,2′-dihexadecanoyl-*sn*-glycero-3′-phospho)-*myo*-inositol-3,4,5-trisphosphate, PtdIns(3,4,5)P_3, (+)-**12** $[\alpha]_D$ +5.8° (c 0.2, $CHCl_3$-MeOH-H_2O, 2:1:0.1), Lit. $[\alpha]_D$ +3.7 (c 0.5, $CHCl_3$).[5b]

Scheme 2



Our choice of preformed *sn*-3-phosphatidic acid as the lipid synthon merits special comment. It contrasts with the related syntheses which all utilize *sn*-1,2-diacylglycerol in tetrazole-catalyzed reaction with (benzyloxy)bis(*N,N*-diisopropylamino)-phosphine, $BnOP(NCH(CH_3)_2)_2$, or related phosphoramidite.[5] The use of *sn*-3-phosphatidic acid prepared from natural *sn*-glycero-3-phosphocholine[13] avoids problems endemic to the chemistry of 1,2-diacylglycerol. The latter isomerize readily via neighboring *O*-acyl migration to equilibrium mixtures comprising the 1,2-, 1,3- and 2,3-diacylglycerols,[15] and indeed 1,3-dihexadecanoylglycerol is detected by TLC in the tetrazole-catalyzed reaction of *sn*-1,2-dihexadecanoylglycerol with $BnOP(NCH(CH_3)_2)_2$.[16] This equilibration is tantamount to racemization which is virtually complete for the reaction of *sn*-1,2-dihexanoylglycerol.[16] Such propensity for racemization is absent from *sn*-3-phosphatidic acids. This is critically important for synthesis of PtdIns-3-phosphates with hexanoyl or shorter chain acyls. In contrast with the long chain acyl derivatives which are self-aggregating in water, the short chain analogues are expected to form monomeric solutions and are considered advantageous as biochemical probes.[3a,4] The absolute configuration of *sn*-3-phosphatidic acids is well established,[13] and that of our key *myo*-inositol synthons is derived unequivocally based on their preparation from (-)-**1**.[7] The one-step esterification of the *sn*-3-phosphatidic acid and the *myo*-inositol synthon is stereochemically innocuous. Thus, our approach ensures that the structural and stereochemical integrity of the lipid and the *myo*-inositol synthons is conveyed faithfully and unambiguously to the target phosphatidylinositol-3-phosphates.[17]

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8. (+)-**3**, ^{1}H-NMR (300 MHz, $CDCl_3$): δ ppm 1.54-1.71 (br m, 10 H, cyclohex-), 2.7 (br, 2H, OH), 3.38 (ψt, *J* 9.6 Hz, 1H, H-5), 3.41-3.56 (m, 2H, H-3 & H-6) , 3.89 (ψt, *J* 9.4 Hz, 1H, H-4), 4.01-4.15 (m, 1H, H-1), 4.16-4.28 (m, 2H, CH_2-C=), 4.38 (dd, *J* 4.2, 4.2 Hz, 1H, H-2), 4.81 (q, 2H, *J* 11.4 & 91.8, Phenyl-CH_2), 5.19-5.34 (m, 2H, CH_2=C), 5.89-6.03 (m, 1H, -CH=C), 7.24-7.38 (m, 5H, C_6H_5). In diacetate of (-)-**3**, 3.89 H-4, 3.38 H-5 signals shift to 5.30 and 4.99.
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10. (+)-**4a**, ^{1}H-NMR (300 MHz, $CDCl_3$): δ ppm 1.17-1.74 (br m, 10 H, cyclohex-), 2.64 (br, 1H, OH), 3.44 (ψt, *J* 9.5 Hz, 1H, H-5), 3.56-3.68 (m, 2H, H-3 and H-6) , 4.12 (ψt, *J* 5.9 Hz, 1H, H-4), 4.17-4.21 (m, 1H, H-1), 4.17-4.32 (m, 4H, 2 CH_2-C=), 4.35 (dd, *J* 4.2, 4.2 Hz, 1H, H-2), 4.80 (q, 2H, *J* 12.0 and 57.0, Phenyl-CH_2), 5.13-5.32 (m, 4H, 2 CH_2=C), 5.85-5.97 (m, 2H, -2 CH=C), 7.18-7.38 (m, 5H, C_6H_5). In the monoacetate of (+)-**4a**, the 3.44 H-5 signal shifts downfield to 4.93. The ^{1}H-NMR of (-)-**4c**, the *O*-benzyl derivative of (+)-**4a**, was identical with the spectrum of DL-**4c** prepared by complete benzylation, selective removal of 3,4-*O*-cyclohexylidene, and complete allylation from DL-1,2:3,4-di-*O*-cyclohexylidene-*myo*-inositol (Garegg, P.J; Iversen, T.; Johansson, R.; Lindberg, B. *Carbohydr. Res.* **1984**, *130*, 322-326)].

    (-)-(**4b**) ^{1}H-NMR (300 MHz, $CDCl_3$): δ ppm 1.34-1.72 (br m, 10 H, cyclohex-), 2.59 (br, 1H, OH), 3.16 (ψt, *J* 9.4 Hz, 1H, H-5), 3.48 (q, *J* 9.6 and 3.7, 1H, H-3), 3.62 (ψt, *J* 6.6 Hz, 1H, H-6), 3.93 (ψt, *J* 9.5 Hz, 1H, H-4), 4.11 (q, *J* 5.2 and 7.0 Hz, 1H, H-1), 4.17-4.38 (m, 4H, 2 CH_2-C=), 4.41 (dd, *J* 4.1, 1.1 Hz, 1H, H-2), 4.80 (q, 2H, *J* 11.4 and 35.4, Phenyl-CH_2), 5.13-5.34 (m, 4H, 2 CH_2=C), 5.87-5.98 (m, 2H, 2 -CH=C), 7.23-7.38 (m, 5H, C_6H_5). In the monoacetate of (-)-**4b**, 3.93 H-4 signal is shifted downfield to 5.33 and the latter shows spin connectivity to 3.28 H-5 and 3.58 H-3 signals observed by selective irradiation at 5.58 and ^{1}H COSY (500 MHz).
11. (+)-**6** ^{1}H-NMR (300 MHz, $CDCl_3$): δ ppm 2.54 (br, 1H, OH), 3.05 (dd, *J* 2.4 and 10.0 Hz, 1H, H-1), 3.13-3.23 (m, 2H, H-3 and H-6), 3.23-3.77 (m, 1H, H-5), 3.73 (s, 3H, OCH_3), 3.87 (ψt, *J* 10.1 Hz, 1H, H-4), 3.97-3.99 (m, 1H, H-2), 4.20-4.28 (m, 6H, 3 CH_2-C=), 4.43-4.80 (m, 4H, 2 Phenyl-CH_2), 5.05-5.25 (m, 6H, 3 CH_2=C), 5.77-5.95 (m, 3H, 3 -CH=C), 6.75-6.79 (m, 2H, aromat-), 7.13-7.35 (m, 7H, aromat-). In the monoacetate of (+)-**6**, the 3.97-3.99 H-2 signal shifted to 5.56 ppm.
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17. All new compounds were characterized fully including high resolution NMR and MS.

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