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Practical unequivocal synthesis of phosphatidyl-*myo*-inositols

Rajindra Aneja * and Sarla G. Aneja

Functional Lipids Division, Nutrimed Biotech, Langmuir Laboratory, Cornell University Research Park, Ithaca, NY 14850-1257, USA

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Abstract

The direct phosphatidylation of 1D-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol with *sn*-3-phosphatidic acid and subsequent hydrogenolytic debenzylation produces 1D-1-(*sn*-3-phosphatidyl)-*myo*-inositol in excellent yield (>90%) and unequivocal structural and stereochemical purity, and, is readily adaptable for large scale production.

1D-1-(1-Fattyacyl′-2-fattyacyl′′-*sn*-glycero-3-phospho)-*myo*-inositols **1a** based on diverse long chain fattyacyls, commonly referred to as phosphatidylinositols (PtdIns), constitute an important group of biological phospholipids.[1] As the parent participant in the phosphatidylinositol metabolic and signaling cycle, PtdIns regulate vital normal cellular functions including growth, multiplication, survival, and apoptosis, as well as abnormal states exemplified by cancers and diabetes.[2] PtdIns are important also for the development of diagnostics and therapeutics based on signaling modalities,[3] and bio-compatible amphiphiles for skincare and intravenous drug delivery formulations.[4] Large quantities of individual molecular species of PtdIns with saturated fattyacyls are required for the latter applications. Hence there is a considerable interest and all recent syntheses have targeted PtdIns with saturated fattyacyls.[5] However, no practical approach adaptable for economical large scale synthesis is available. Moreover, the molar rotation values of some synthetic PtdIns differ significantly from the bench mark value provided by natural PtdIns.

* Corresponding author.

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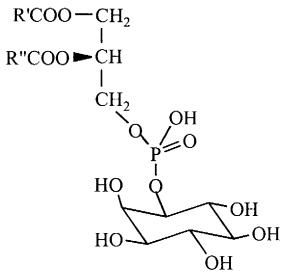
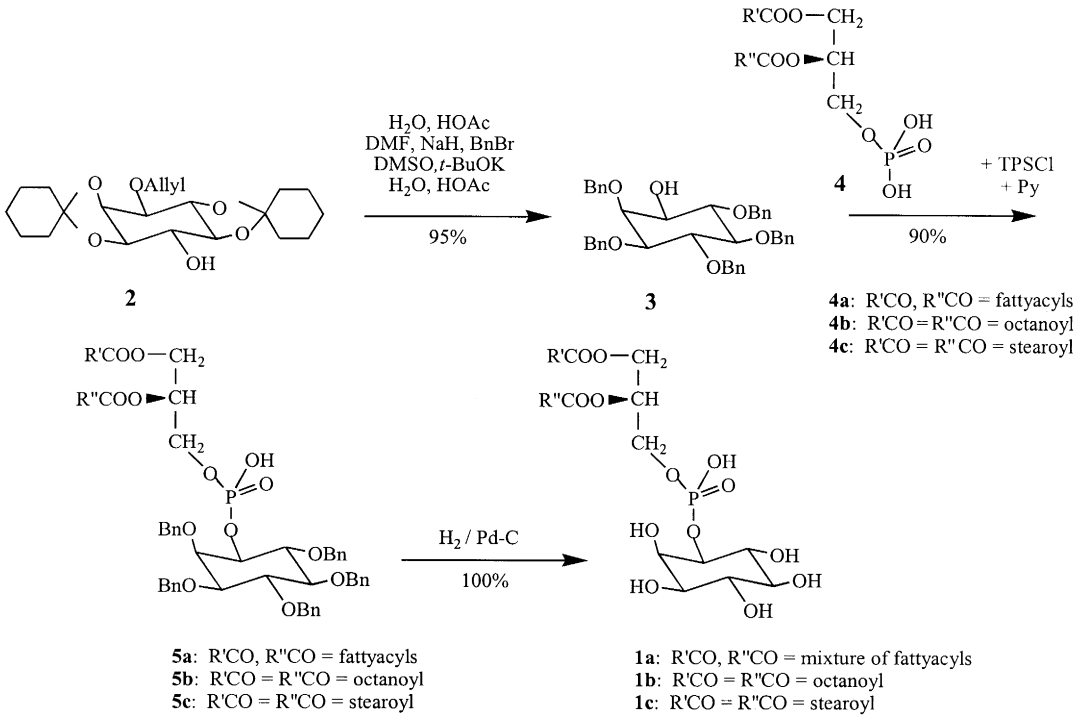


1a: R'CO, R"CO = mixture of fattyacyl

We now report a new convergent synthesis based on the direct phosphatidylation of a penta-*O*-protected *myo*-inositol with *sn*-phosphatidic acid as the lipid synthon. This contrasts with the previous syntheses which all use 1,2-diacyl-*sn*-glycerol, a synthon prone to racemization via neighboring acyl migrations, and, introduce the phosphate group in multiple steps using trivalent and pentavalent phosphorus reagents.[6] The three key steps in the new synthesis are outlined in Scheme 1. The starting material 1D-1-*O*-allyl-2,3:5,6-di-*O*-cyclohexylidene-*myo*-inositol **2** was obtained from *myo*-inositol in four steps as described.[7] The reaction of **2** successively with (i) acetic acid–water (80:20) at 100°C, evaporation to dryness, (ii) complete benzylation in DMF using NaH/BnBr and evaporation under 2 millitorr to remove all volatiles, and (iii) deallylation by successive heating with *t*-BuOK in DMSO followed by acetic acid–water (80:20), in a one pot procedure, gave (−)-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol **3** in 95% yield. The absolute configuration of **3** has been established unequivocally as 1D-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol.[8] The chiral lipid synthons 1,2-difattyacyl-*sn*-glycero-3-phosphoric acids **4a**–**c** (*sn*-3-PA) were prepared from the corresponding *sn*-phosphatidylcholines by lipolysis with phospholipase D.[9] The crucial esterification reaction of the secondary 1-hydroxyl group in **3** with the phosphoric acid in **4** gave less than 50% yield when phosphatidylation was conducted under conditions recommended for primary alcohols[10] but a dramatic, highly reproducible increase in yield to 90% was achieved by the following protocol. Typically, 0.15 mmole of *sn*-3-PA **4** was added gradually over 1 h at 35–37°C to a stirred solution of the alcohol **3** (0.1 mmole) and triisopropylbenzene sulfonyl chloride (TPSCl, 0.4 mmole) in anhydrous pyridine. Thus, reaction with dioctanoyl *sn*-3-PA **4b**, work-up by addition of water and purification by flash chromatography on silica, gave 1D-1-(1,2-dioctanoyl-*sn*-glycero-3-phospho)-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol **5b**,[11] $[\alpha]_D$ +12.79 (c 1.04, $CHCl_3$), in 90% yield. Reaction of **5b** in *tert*-butanol with H_2 at 45 psi and Pd–C catalyst caused complete debenzylation and produced a quantitative yield of the short acyl chain 1D-1-(1,2-dioctanoyl-*sn*-glycero-3-phospho)-*myo*-inositol (dioctanoyl PtdIns) **1b**,[12] $[\alpha]_D$ +8.80 (c 0.45, $CHCl_3$:CH_3OH 4:1), molar rotation [Φ] +51.57. Similarly, use of distearoyl *sn*-3-PA **4c** in Scheme 1 yielded the long acyl chain distearoyl PtdIns **1c**, $[\alpha]_D$ +5.97 (c 0.55, $CHCl_3$:CH_3OH 4:1), [Φ] +51.70, in yield comparable with **1b**.

The absolute configurations of **3** and **4** are well established and are expected to be delivered unambiguously to the target *sn*-3-phosphatidyl-D-*myo*-inositols during coupling by esterification, and retained during hydrogenolysis, leading unequivocally to 1D-1-(*sn*-3-phosphatidyl)-*myo*-inositol absolute stereochemistry for **1b** and **1c**. The ^1H, ^{13}C and ^{31}P NMR spectra are consistent with this. The newly prepared compounds **1b** and **1c** and other PtdIns differ in fattyacyl type and hence molecular weight. To normalize these differences in the homologous series, we compared the molar rotations of our synthetics with the values for natural PtdIns as the bench mark for 1D-1-(*sn*-3-phosphatidyl)-*myo*-inositol absolute configuration. The molar rotation values of **1b** [Φ] +51.57 and **1c** [Φ] +51.70 are virtually identical with the values for PtdIns from soybean [Φ] +51.77, $[\alpha]_D$ +6.20 (c 0.51, $CHCl_3$:CH_3OH 4:1), and bovine brain [Φ] +51.01, $[\alpha]_D$ +5.75 (c 0.40, $CHCl_3$:CH_3OH 4:1).



Scheme 1.

Unequivocal synthesis of PtdIns has been a problem. For instance, we have shown previously[8] that *myo*-inositol synthons belonging to the 1L- absolute configuration series were used in some recent syntheses[5g,5h] which claimed 1D-1 series PtdIns products. Consistent with this, the molar rotation values calculated from the published specific rotations of ditetradecanoyl PtdIns [Φ] +101.10, $[\alpha]_D$ +13.4,[5g] and diheptanoyl PtdIns [Φ] +83.21, $[\alpha]_D$ +14.2,[5h] differ significantly from the bench mark value [Φ] +51 for (*sn*-3-phosphatidyl)-*myo*-inositols. Rotation data for synthetic PtdIns were not reported in other recent literature.[5d,5e,5i]

Large quantities of the starting materials **2** and **4a–c** are readily obtained in high structural and optical purity, and the revised protocol for TPSCl mediated condensation gives very high yields. The unit operations of Scheme 1 are simple and well suited for scale up, and the TPSCl reagent is inexpensive. Thus, the synthesis described herein provides the very first practical unequivocal synthesis of saturated fattyacyl type PtdIns adaptable for large scale production. In contrast with the published syntheses of PtdIns which require oxidation of H-phosphonate or phosphite intermediates to phosphate, Scheme 1 is potentially adaptable for unsaturated fattyacyl type PtdIns using analogues of **3** with alternative protecting groups which do not require hydrogenolysis. Work on scale up and extension to unsaturated fattyacyl type PtdIns will be reported.

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11. All new compounds were characterized fully including high resolution and multidimensional ^{1}H, ^{13}C and ^{31}P NMR, ESMS, and optical rotation data. $[\alpha]_D$ is expressed without units, and the actual units, deg mL/(g dm), are understood; all measurements are at rt (20–22°C). Molar rotations of natural PtdIns are based on molecular weights calculated from their fattyacyl compositions.
12. $DiC_{8:0}$PtdIns (+)-**1b**: ES(−) MS *m/z* 585.0 $(M-H)^-$; ^{1}H NMR (400 MHz, $CDCl_3$:CD_3OD, 2:1) δ ppm 0.90 (t, 6H, CH_3), 1.29 (br s, 16H, $(CH_2)_8$), 1.61 (*m*, 4H, C*H*$_2$CH$_2$C=O), 2.29–2.36 (m, 4H,C*H*$_2$C=O), 3.30 (*t*, 1H, inositol 5-*H*), 3.46–3.48 (dd, 1H, inositol 3-*H*), 3.67(m, 1H, inositol 4-*H*), 3.80 (m, 1H, inositol 6-*H*), 3.92 (t, 1H, inositol 2-*H*), 4.05–4.17 (m, 2H, *sn*-3 C*H*$_2$), 4.28, 4.45 (m, 2H, *sn*-1 C*H*$_2$), 4.28 (t, 1H, inositol 1-*H*), 5.26 (m, 1H, *sn-2* C*H*); ^{13}C NMR (100 MHz, $CDCl_3$:CD_3OD, 2:1) 173.69 and 173.35 (2 C=O), 76.24 (inositol C-2), 74.12 (inositol C-5), 72.10 (inositol C-4), 71.35 (inositol C-6), 71.19 and 70.98 (inositol C-1), 70.14 and 70.06 (*sn*-2 glycerol C), 63.46 (*sn*-3 glycerol C), 62.39 (*sn*-1 glycerol C), 33.82 (acyl chain *sn*-2 α-CH_2), 33.68 (acyl chain *sn*-1 α-CH_2), 31.29, 31.28, 24.51 and 24.45 ($(CH_2)_n$), 22.19 (acyl chain ω-CH_2), 13.46 (acyl chain CH_3); ^{31}P NMR (162 MHz, $CDCl_3$:CD_3OD, 2:1) δ ppm (external H_3PO_4 ref.) −0.258 (s).