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Synthesis of dipalmitoyl–phosphatidylinositol 5-phosphate and its modified biological tools

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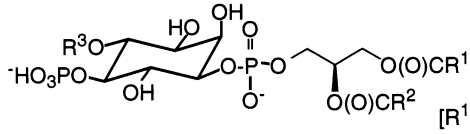
Abstract

Synthesis of a dipalmitoyl analog of phosphatidylinositol 5-phosphate with the racemic inositol skeleton was achieved via a key intermediate, 1,2-cyclohexylidene-3,4-tetraisopropyldisiloxanyl-*myo*-inositol. Probes bearing a fluorophore, NBD on a fatty acid chain and a resin for affinity chromatography were also prepared due to biological interest in cell division.

Keywords: phosphatidylinositol 5-phosphate; fluorescent analog; affinity chromatography.

Phosphatidylinositol 4,5-bisphosphate (PI4,5–P2) is a key substance in the phosphatidylinositol cell signaling pathway. It is converted to two second messengers, *myo*-inositol 1,4,5-trisphosphate and diacylglycerol, and is also transformed to 3-*O*-phosphorylated PI3,4,5–P3 that engages in a variety of signal cascades.[1] In addition, PI4,5–P2 itself acts as a modulator for regulatory proteins. PI4,5–P2 is known to be formed generally from PI4–P by phosphatidylinositol 4-phosphate specific 5-kinase (PI4P–5K) catalyzed phosphorylation. Recently, PI5–P, the content of which was 1/100 of that of PI4–P, was discovered in mammalian fibroblasts and shown to be transformed into PI4,5–P2 by phosphorylation with PI5P–4K.[2] Although the physiological role of the new pathway is still unclear, its significance is anticipated due to the low concentration of PI4,5–P2. Chemical synthesis of PI5–P with dipalmitoyl groups was reported by the Prestwich[3a] and Falck[3b] groups. In this communication, we describe a convenient alternative synthesis of a dipalmitoyl analog[4] of natural PI5–P and an analog bearing a fluorescent group, NBD. Preparation of a PI5–P attached resin for affinity chromatography is also reported.



PI4,5-P2: R^3=PO_3H^-
PI5-P: R^3=H
dipalmitoyl-PI5-P: R^1=R^2=$C_{15}H_{31}$, R^3=H
[R^1CO=stearoyl, R^2CO=arachidonoyl (for natural product)]

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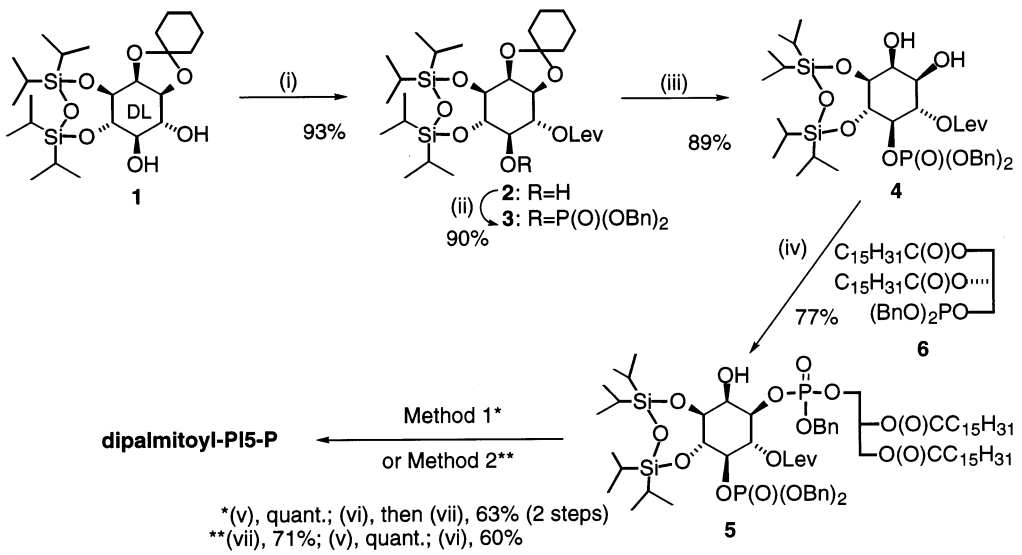
A pivotal intermediate, diol **1** with DL-form, derived regioselectively from *myo*-inositol in two steps,[5] was acylated exclusively at the 6 position with levulinic acid in the presence of DCC and DMAP, followed by phosphorylation via a phosphoramidite method to give **3** in good yield. Removal of the cyclohexylidene group in **3** was achieved by the action of pyridinium poly(hydrogen fluoride)[6] (PPF) in an anhydrous dichloromethane solution, affording 1,2-diol **4** in 89% yield. The TIPDS group was intact in this reaction whereas PPF was reported to decompose TIPDS ethers in sugar derivatives.[7] The scope and limitation of the PPF-promoted deacetalization will be described elsewhere. A conventional decyclohexylidenation using ethylene glycol and *p*-toluenesulfonic acid resulted in the formation of ethylene ketals on the levulinoyl groups of the starting material and the desired product (14% each) along with the diol **4** (48%).

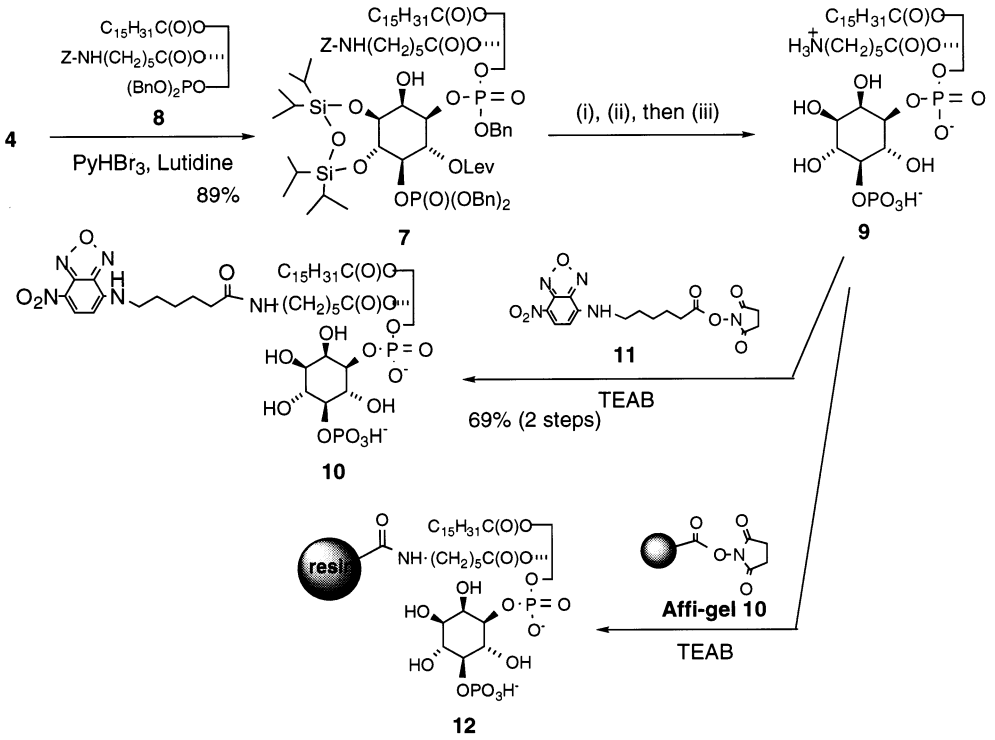
The diol **4** thus derived was regioselectively phosphorylated by reaction with *sn*-3-glyceryl phosphite **6** in the presence of pyridinium tribromide to give 1-*O*-phosphate **5** without formation of the 2-*O*-phosphoric regioisomer. The phosphorylation site was confirmed by derivatization of **5** to 2-*O*-chloracetate and its NMR analysis. The phosphate **5** was deprotected by two sequential procedures as shown in Scheme 1 to give the final product as a white solid.[8] Removal of the levulinoyl group before that of the benzyl groups in the phosphates was performed successfully (Method 2) while, in general, deprotection of the phosphate functions was carried out prior to removal of other protecting groups to avoid migration of the phosphates as done in Method 1. Since the chiral 1,2-cyclohexylidene derivative **1** is available,[9] an optically active PI5–P analog with saturated fatty acid chains has now also been formally prepared.



Scheme 1. Synthesis of dipalmitoyl–PI5–P. (i) $CH_3C(O)(CH_2)_2CO_2H$, DCC, DMAP; (ii) $(BnO)_2PNi\text{-}Pr_2$, tetrazole, then mCPBA; (iii) Py(HF)x; (iv) Lutidine; (v) H_2, Pd black, $NaHCO_3$, *t*-BuOH/H_2O (6:1 for Method 1, 4:1 for Method 2); (vi) TBAF·$3H_2O$, AcOH; (vii) $NH_2NH_2{\cdot}H_2O$, Py/AcOH (4:1). Abbreviations: DCC = dicyclohexylcarbodiimide, DMAP = 4-(dimethylamino)pyridine, Bn = benzyl, mCPBA = *m*-chloroperbenzoic acid, Py = pyridine, TBAF = tetrabutyl-ammonium fluoride

Dipalmitoyl–PI5–P thus synthesized, was assumed to be concerned with cell division due to an experiment using PI5P–4K over-expressed cells. To explore its physiological function in the system, a fluorescent probe for detection of the localized site and resin for isolation of a PI5–P

recognition protein by affinity chromatography were designed and prepared as biological tools. Thus, 1,2-diol **4** was phosphorylated with aminohexanoylglycerol phosphite **8** by a similar procedure to that used above to furnish **7** in 89% yield. A sequential procedure for deprotection of **7** involving hydrazinolysis, hydrogenolysis, and desilylation afforded **9**. On the other hand, when hydrogenolysis was first done according to Method 1 shown in Scheme 1, the reaction did not proceed smoothly under various reaction conditions because $NaHCO_3$, added to avoid reduction of the levulinoyl ketone function, retarded the reaction. Aminoacyl PI5–P **9** thus obtained was converted to fluorophore-attached PI5–P **10** by the reaction with *N*-NBD-aminohexanoic acid *N*-succinimidyl ester **11** in a 1:1 solution of DMF and aqueous triethylammonium bicarbonate (TEAB) solution (Scheme 2). The structure of **10** was principally confirmed by ^{31}P NMR and FABMS data.[10] Analogous NBD derivatives of PI4,5–P2[11] and PI[12] were reported, and similar fluorescent probes for other inositol phospholipids are now commercially available from Echeron Laboratories Inc.[13]



Scheme 2. Preparation of PI5–P based biological tools. (i) $NH_2NH_2{\cdot}H_2O$, Py/AcOH, (4:1), 86%; (ii) H_2, Pd black, *t*-BuOH/H_2O (6:1), 97%; (iii) TBAF·$3H_2O$, AcOH, 77%. Abbreviations: Z = benzyloxycarbonyl, TEAB = triethylammonium bicarbonate

On the other hand, **9** was treated with Affigel10™ involving succinimide active esters (Bio-Rad Laboratories) in a solution similar to that used above for the preparation of **10**. The reaction took place smoothly, and it was concluded the reactive sites of the resin were quantitatively converted to amides, based on the quantity of recovered **9**, giving **12**, quantitatively.

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