Enantiomerically Convergent Synthesis of Phosphatidyl-D-*myo*-inositol 3,5-Bisphosphate from Both L- and D-1,2-*O*-Cyclohexylidene-*myo*-inositol

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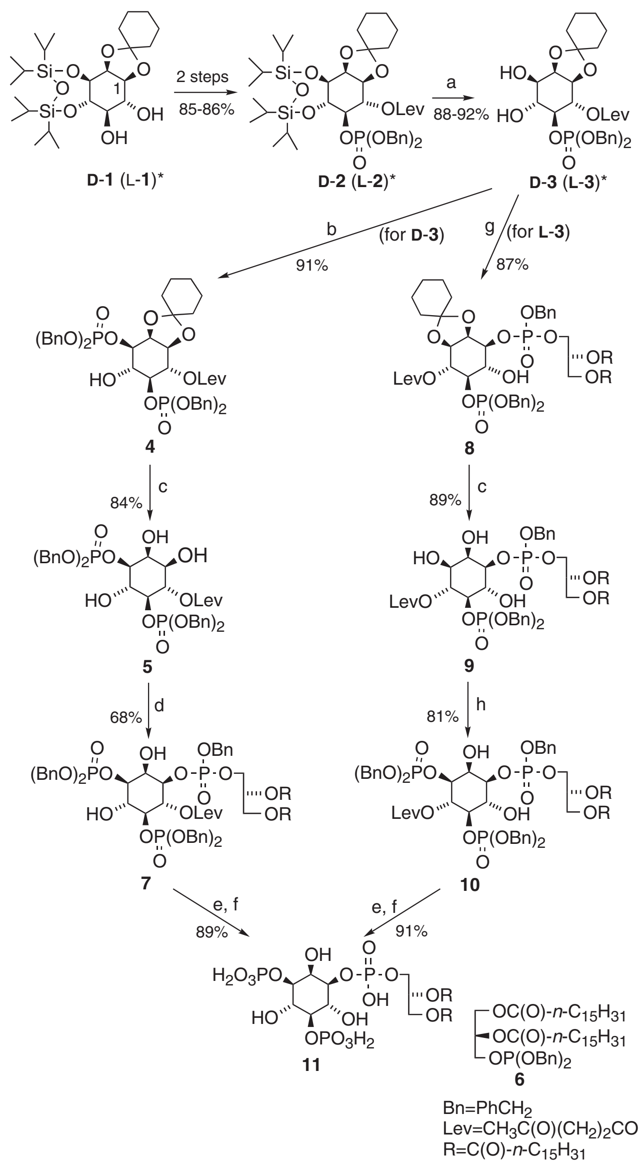
The synthesis of phosphatidyl-D-*myo*-inositol 3,5-bisphosphate [PtdIns(3,5)P2] has been conveniently accomplished via convergent routes starting from both enantiomers, 1,2-*O*-cyclohexylidene-*myo*-inositol. The synthetic strategy involves completely regioselective phosphorylation of 3,4-diol and 2,3,6-triol of the suitably protected inositols with the corresponding phosphite in the presence of pyridinium tribromide and 2,6-lutidine, resulting in the formation of 3-*O*-phosphorylated products, respectively.

A general problem in synthetic phosphatidylinositol phosphates (PtdInsPns) and inositol phosphates (InsPns) from *myo*-inositol is only half of the starting material can be converted to the target molecules. To overcome this problem, we are exploring new methodologies aimed at synthesizing the same chiral PtdInsPns and/or InsPns molecules from both enantiomers of *myo*-inositol derivative.

On the other hand, phosphatidylinositol 3,5-bisphosphate [PtdIns(3,5)P2] was found to occur in mammalian cell lines[1] and is widespread among eukaryotes.[2] Its biological function identification is presently of keen interest to biochemists. Although its biological role has not yet been well recognized, several investigations have shown that PtdIns(3,5)P2 is biologically important, for instance, in sorting membrane proteins into the lumen of the yeast vacuole and maintaining the vacuolar size.[3] To date, several reports on the chemical syntheses of PtdIns(3,5)P2 analogs have appeared employing glucose,[4] and *myo*-inositol derivatives such as orthoacetate,[5] orthoformate[6] and camphor ketal.[7] The most rapid route described hitherto is that of Falck,[6a] involving 8 steps. This route, however, is not entirely satisfactory where the yield for the preparation of the ultimate starting material is less than 50% based on the approach they used,[8] in addition to the general problem we mentioned above.

We communicate here a convenient and convergent method to synthesize dipalmitoyl D-PtdIns(3,5)P2 (**11**) from both D- and L-1,2-*O*-cyclohexylidene-3,4-*O*-(tetraisopropyl disiloxane-1,3-diyl)-*myo*-inositol (**1**), which can be very readily available in three steps including the enzyme-aided resolution from *myo*-inositol[9] and, may serve as a versatile intermediate to access various PtdInsPns and InsPns.[10] The striking features of the method described here involved: (1) both D- and L-**1** were used to arrive at the same goal; (2) regioselective phosphorylation of triols **5** and **9** is unprecedented, and remarkably limits the step numbers; (3) the convergent synthetic method is amenable to other PtdInsPns and/or InsPns molecules.

As outlined in Scheme 1, both enantiomers of diol **1** were smoothly converted to the fully protected **2** according to the re-



Scheme 1. Conditions and reagents: (a) TBAF·3H_2O, AcOH, THF, −15 to −10 °C; (b) $(BnO)_3P$, pyridinium tribromide, 2,6-lutidine, CH_2Cl_2, −42 to 0 °C; (c) Py(HF)*n*, ethylene glycol, CH_2Cl_2, 0 °C to r.t.; (d) **6**, pyridinium tribromide, 2,6-lutidine, pyridine/CH_2Cl_2 (v/v=1.1/1), −22 °C to r.t.; (e) hydrazine monohydrate, pyridine/AcOH (v/v=4/1), 0 °C to r.t.; (f) 5% Pd/C, H_2, AcOEt/MeOH (v/v=1/1), r.t.; (g) **6**, pyridinium tribromide, 2,6-lutidine, CH_2Cl_2, −42 to 0 °C; (h) $(BnO)_3P$, pyridinium tribromide, 2,6-lutidine, pyridine/CH_2Cl_2 (v/v 1:1), −22 to 0 °C. *L-Isomers of **1**, **2**, and **3** are not illustrated.

ported approach.[10h] **2** was transformed into diol **3** by desilylation. To regioselectively introduce the phosphate group at the 3-position in 3,4-diol D-**3**, the phosphite–pyridinium tribromide approach[11] was employed as reported in the synthesis of PtdIns(4,5)P2.[12] Thus, D-**3** was subjected to phosphorylation with tribenzyl phosphite, resulting in the formation of the desired **4** in a complete selective manner. Neither its regioisomer nor diphosphorylated product was isolated. The 3,5-diphosphate derivative **4** was then transformed smoothly to triol **5** by the cleavage of the cyclohexylidene ketal with pyridinium poly(hydrogen fluoride) [Py(HF)$_n$], which was used to decompose the isopropylidene group without the migration of the adjacent phosphate function.[13] Triol **5** was converted to its trichloroacetate in order to confirm no migration of the three substituents during the reaction through the ^{1}H NMR and ^{1}H-^{1}H COSY analyses.

We then turned our attention to the regioselective installation of the phosphatidyl group at the OH-1 in 1,2,4-triol **5**. The regioselective 1-*O*-phosphorylation of vicinal 1,2-diol derivatives of *myo*-inositol employing the phosphite–pyridinium tribromide method used above has been well documented by this laboratory[10c,d,h] and other group.[6a] On the other hand, our recent studies[12] showed that phosphorylation of a vicinal 1,6-diol also exclusively occurred at the 1-position. These results clearly suggest the highest reactivity of OH-1 among three hydroxyls at 1, 2, and 6 positions, therefore, the selective phosphorylation of **5** is expected to proceed at the OH-1. Indeed, phosphorylation of **5** with dipalmitoylglycerol phosphite **6** in the presence of pyridinium tribromide (PTB) proceeded in a 1.1:1 ratio of pyridine and CH_2Cl_2 to yield 1-*O*-phosphorylation product **7** in 68% yield without the formation of other possible products. It is noteworthy that the reaction was dramatically affected by the ratio of the solvents. Thus, in a 1:12 ratio of the mixed solvent,[6a,10c] the phosphorylation did not proceed at all. The reasons for such an extraordinary low-reactivity of **5** are now under investigation. To determine the exact phosphorylation site, **7** was converted into the corresponding chloroacetate, and its ^{1}H NMR and ^{1}H–^{1}H COSY analyses clearly showed that phosphorylation occurred at the OH-1 position.

With the successful technique for converting D-**1** to **7**, opposite enantiomer, L-**1** was also transformed into **10**. Thus, the phosphatidyl group at 1-position was installed through the phosphorylation of diol L-**3** with phosphite **6** prior to the installation of 3-phosphate group, as compared to the synthetic sequences from D-**3**, followed by the cleavage of the cyclohexylidene ketal to give triol **9**. Triol **9** was subjected to phosphorylation with tribenzyl phosphite to give **10** exclusively. Finally, respective removal of the Lev group from **7** and **10** by treatment with hydrazine monohydrate in the mixture of pyridine and acetic acid,[10c,14] and subsequent debenzylation by hydrogenolysis over 5% palladium on carbon afforded dipalmitoyl PtdIns(3,5)P2 (**11**)[15] as its free acid. The structure of **11** as free acid form was confirmed by its NMR and MS spectra. Further purification of the final product was not done because no other impurity was found in its NMR spectra and TLC analysis.

In conclusion, both regioselective phosphorylation reactions of diol **3**, and triol **5** and **9**, remarkably reduced laborious protection–deprotection procedures, therefore, facilitated the synthetic route to PtdIns(3,5)P2. In addition, the convergent synthetic methodology from both enantiomers can be applied to the synthesis of other PtdInsPns and/or InsPns compounds.

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15 Physical and spectra data of **11** (free acid form): $[\alpha]_D^{24}$ -1.4, [$c = 0.27$, $CHCl_3/MeOH$ 1:1 (v/v)]; δ_H (400 MHz, $CDCl_3/CD_3OD/D_2O$ 1:1:0.1) 5.27 (br, 1H, glyceryl *sn*-2-H), 4.41 (br s, 1H, InsH-2), 4.30 (br, 0.5H, glyceryl *sn*-1-H), 4.20 (m, 0.5H, glyceryl *sn*-1-H), 4.03-4.17 (m, 6H, glyceryl *sn*-1-H, *sn*-3-H, InsH-1, H-3, H-5), 3.96 (br, 2H, InsH-4, H-6), 2.34 (complex, 4H, pal α-CH_2), 1.60 (br, 4H, pal β-CH_2), 1.28 (br, 48H, pal CH_2), 0.89 (br, 6H, pal CH_3); δ_P (162 MHz, $CDCl_3/CD_3OD/D_2O$ 1:1:0.1) 5.33 (1P), 4.54 (1P), 4.12 (1P); Negative FABMS (triethylammonium salt): *m/z*: 1008 [$(M-2H+K)^-$, 25%], 992 [$(M-2H+Na)^-$, 35%], 970 [$(M-H)^-$, 100%], 648 [$C_{15}H_{31}COOCH_2CH(OCOC_{15}H_{31})\ CH_2OPO_3H^-$, 50%], 255 [$C_{15}H_{31}COO^-$, 80%]. HRMS (FAB$^-$, triethanolamine) [$M-H]^-$ Calcd. for $C_{41}H_{80}O_{19}P_3^-$, 969.4506; Found, 969.4523.

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