A Short Synthesis of Dipalmitoylphosphatidylinositol 4,5-Bisphosphate via 3-*O*-Selective Phosphorylation of a 3,4-Free Inositol Derivative

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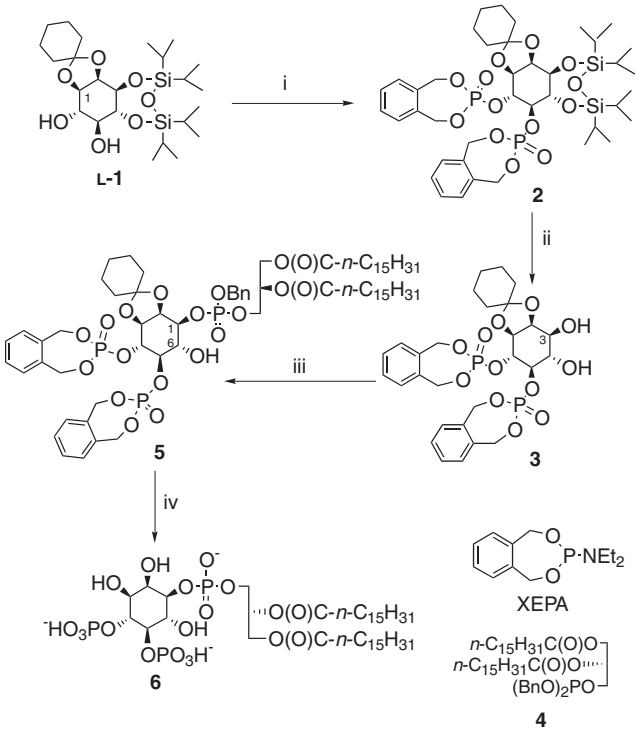
Dipalmitoylphosphatidylinositol 4,5-bisphosphate was conveniently synthesized via the regioselective phosphorylation of L-1,2-*O*-cyclohexylidene-5,6-di-*O*-(*o*-xylylene phosphoryl)-*myo*-inositol derived from 1,2-*O*-cyclohexylidene-3,4-*O*-(tetraisopropyldisiloxane-1,3-diyl)-*myo*-inositol.

Phosphatidylinositol 4,5-bisphosphate [PI(4,5)P2, **6**] is a key phosphoinositide due to its role as the precursor of at least three second-messenger molecules,[1] such as *myo*-inositol 1,4,5-trisphosphate, PI(3,4,5)P3, and diacylglycerol. More recent studies have proved PI(4,5)P2 itself is also a second messenger.[2] In addition, PI(4,5)P2 may also be involved in several other cellular processes, including exocytosis, cytoskeletal regulation and intracellular trafficking of vesicles.[3] Natural PI(4,5)P2 from bovine brain is commercially available. To modify PI(4,5)P2 as a biological tool, aiming at disclosing its physiological functions, chemical synthesis is indispensable. Many PI(4,5)P2 analogs have been synthesized so far from *myo*-inositol,[4] L-(−)-quebrachitol,[5] and D-glucose.[6] In these cases, however, long routes were required and/or the overall yield was low because many protection-deprotection sequences by using monofunctional protecting groups to differentiate the hydroxyls have been adopted. Therefore, developing a more practical and efficient strategy for the synthesis of PI(4,5)P2 is still significant.

In this communication, we report a concise synthesis of a D-PI(4,5)P2 dipalmitoyl analog, that, as a key reaction, involves the regioselective phosphorylation of the vicinal 3,4-diol in L-1,2-*O*-cyclohexylidene-5,6-di-*O*-(*o*-xylylene phosphoryl)-*myo*-inositol (**3**). The simultaneous deprotection of phosphates and diol moieties at the final stage is also noteworthy to accomplish the convenient synthesis.

As a key intermediate protected by two bifunctional protecting groups, L-1,2-*O*-cyclohexylidene-3,4-*O*-(tetraisopropyldisiloxane-1,3-diyl)-*myo*-inositol (**L-1**) was chosen, this time. The enantiomer, **L-1** has been the ''waste'' one until now, whereas both enantiomers of **1** can be readily derived from the corresponding D- and L-1,2-cyclohexylidene-*myo*-inositol obtained by the enzyme-aided optical resolution with excellent yields.[7] The opposite D-isomer has been demonstrated to be useful for the synthesis of natural D-series of various inositol phosphates such as PI(3,4,5)P3[8] and phosphatidylinositol dimannopyranoside (PIM2).[9] Thus, phosphorylation of diol **L-1** {$[\alpha]_D^{26}$ +13.2 (*c* 1.0, CH_2Cl_2)},[10] using *o*-xylylene *N*,*N*-diethylphosphoramidite (XEPA)[11] afforded bisphosphate **2** {$[\alpha]_D^{26}$ −3.9, (*c* 3.0, $CHCl_3$)}, which was subsequently converted to diol **3** {$[\alpha]_D^{26}$ = +4.23, (*c* 1.56, $CHCl_3$)} by employing TBAF and acetic acid at −20 °C. Such reagents as well as low temperature were necessary to prevent the migration of phosphate groups.



Scheme 1. Reagents and conditions: (i) *o*-xylylene *N*,*N*-diethylphosphoramidite (XEPA) (3.2 eq.), tetrazole (5 eq.), CH_2Cl_2, r.t., 1 h, then *m*CPBA (4 eq.), −78–0 °C, 45 min, 95%; (ii) TBAF·$3H_2O$ (3 eq.), AcOH (4 eq.), THF, −20 °C, 12 h, 81%; (iii) **4** (3 eq.), 2,6-lutidine (5 eq.), pyridinium tribromide (4 eq.), −42–0 °C, 1.5 h, 88%; (iv) H_2, 10%-Pd/C (25 wt%), AcOEt, r.t., 2.5 d, quant.

Our attention was then turned to the regioselective phosphorylation of **3**. Thus, phosphorylation of diol **3** with 1,2-di-*O*-palmitoyl-*sn*-glycerol phosphite **4** in the presence of pyridinium tribromide and 2,6-lutidine[12] was found to exclusively occur at the OH-3 position to afford **5** in high yield.[13] The phosphorylation position was confirmed by transforming **5** into its 6-*O*-chloroacetyl derivative and analyzing its ^{1}H NMR spectrum, combined with H–H COSY analysis.

The unprecedented finding of the selective phosphorylation of the 3,4-diol opens a concise way to prepare not only PI(4,5)P2 but also other PIPn and IPn derivatives using suitably protected 3,4-free inositol derivatives. There have rarely been reports on

their selective reaction and such derivatives were restricted to 1, 2 : 5, 6-diketals such as dicyclohexylidene and diisopropylidene. Their acylation[14] and carbonylation,[15] mainly via the stannylene derivatives were shown to proceed regioselectively at the 3-position, although silylation and benzylation were unsuccessful.[16] The diketals were distorted by the *trans*-ketal function, therefore, the reactivity of **3** seems to be different from these diketals. Indeed, 1,2-*mono*ketals bearing the 3,4-dihydroxyl moiety similar to **3** were subjected to regioselective silylation as well as acylation.[17] According to coupling constants of inositol methine protons in the NMR[18] for **3**, the conformation of **3** was suggested to deviate from a normal chair form to some extent, making 3-OH less crowded and 4-OH sterically hindered, while the conformation of 1,2-*O*-isopropylidene-*myo*-inositol and its cyclohexylidene analog[18] takes the chair form.

The final deprotection of **5** was eventually performed at a single procedure, when hydrogenolysis was carried out in a commercial grade of AcOEt as a solvent, giving rise to the target PI(4,5)P2 {$[\alpha]_D^{26}$ +5.41, (triethylammonium salt, *c*0.61, $CHCl_3$)} in quantitative yield. In place of AcOEt, MeOH as a protic medium, that is commonly employed for the final deprotection of PIPns,[6b,c] was insufficient to remove the cyclohexylidene group. The details of such different results will be discussed elsewhere.

In conclusion, the successful regioselective phosphorylation of **3** at the OH-3 position and the spontaneous deprotection at the final stage made the present methodology advantageous. The method provides the shortest route with good yield (68% based on the **L-1**) for the synthesis of PI(4,5)P2 as compared with the reported methods. It could be scaled up to gram scale. In addition, the distinct reactivity of 3- and 4-hydroxyls makes 1,2-*O*-cyclohexylidene-3,4-*O*-(tetraisopropyl disiloxane-1,3-diyl)-*myo*-inositol more versatile as a synthetic intermediate for the synthesis of inositol phosphates and phosphatidyl inositols.

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13 Physical and spectral data of **5**, that consists of two diastereomers based on the phosphorus at the D-1 position: *Rf* = 0.65 (Hex : AcOEt = 1 : 3); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.30–7.39 (m, 26H), 5.58 (complex, 4H, Ar$\underline{CH_2}$O), 5.30 (t, 2H, J = 13.6 Hz, Ar$\underline{CH_2}$O), 5.04–5.23 (complex, 22H in Ar$\underline{CH_2}$O, glyceryl *sn*-2-H), 4.85 (br q, 2H, InsH-4), 4.60–4.71 (complex, 3H, InsH-1 & H-2), 4.55 (br t, 1H, J = 4.0 Hz, InsH-2), 4.40 (br t, 2H, InsH-5), 4.22–4.32 (complex, 8H, InsH-6, H-3, glyceryl *sn*-3-H or *sn*-1-H), 4.12 (dd, 4H, J = 14.0, 7.2 Hz, glyceryl *sn*-3-H or *sn*-1-H), 2.29 (m, 8H, Pal H-2), 1.82 (m, 4H, cyclohexylidene H), 1.57–1.65 (br, 24H, Pal H-3 and cyclohexylidene H), 1.25 (br, 96H, Pal H4–15), 0.88 (t, 12H, J = 6.2 Hz, CH_3); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ: 173.36, 173.22, 173.10, 172.84 (C=O), 128.30–135.89 (complex, aromatic C), 112.10 (spiral C), 79.57–80.13 (complex, 4C, InsC-5, C-4), 75.90–76.26 (m, 2C, InsC-1), 74.57 (m, 2C, InsC-2), 68.65–71.70 (complex, 14C, InsC-6, glyceryl *sn*-2-C and Ar$\underline{CH_2}$O), 65.90 (m, 2C, glyceryl *sn*-3-C), 61.80, 61.67 (s each, 2C, glyceryl *sn*-1-C), 37.45, 37.41 (s each, 2C, cyclohexylidene C), 34.98 (2C, cyclohexylidene C), 34.15, 34.11, 34.40 (s each, 4C, C-2 in Pal), 31.91, 31.57 (s each, 4C, C-14 in Pal), 29.08–29.69 (complex, C4–13 in Pal), 24.82 (C-3 in Pal), 23.79, 23.65, 22.67, 22.64 (s each, cyclohexylidene C), 21.04 (C-15 in Pal), 14.11 (C-16 in Pal), signals for InsC-3 was overlapped with those of $CDCl_3$; ^{31}P NMR ($CDCl_3$, 162 MHz) δ: −0.28 (1P), −0.32 (1P), −0.46 (1P), −0.60 (2P), −0.68 (1P); Anal. Calc. for $C_{70}H_{107}O_{19}P_3$: C, 62.48; H, 8.02%; Found: C, 62.10; H, 8.02%.
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18 Coupling constants (J, Hz) of inositol methine protons H1–H2, H2–H3, H3–H4, H4–H5, H5–H6, H6–H1: compound **3** (in $CDCl_3$): 5.6, 3.7, 7.8, 7.4, 8.8, 6.4; 1, 2 : 4, 5-di-*O*-cyclohexylidene-*myo*-inositol (in $CDCl_3$): 4.6, 4.8, 9.3, 9.3, 10.8, 6.3 (see also reference 16 for 1, 2 : 4, 5- and 1, 2 : 5, 6-di-*O*-isopropylidene-*myo*-inositol); 1,2-*O*-cyclohexylidene-*myo*-inositol (in CD_3OD): 4.9, 4.2, 9.5, 9.3, 10.1, 7.3 (see also reference 7b).