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Synthesis of Natural PI(3,4,5)P3

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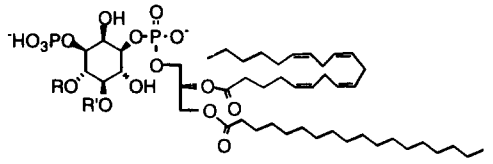
Abstract: Natural phosphatidylinositol 3,4,5-trisphosphate which has been believed to have stearoyl and arachidonoyl groups at the *sn*-1 and -2 positions, respectively, has been synthesized using 1,2-*O*-cyclohexylidene-3,4-*O*-disiloxanyl-*myo*-inositol as the pivotal intermediate.

Since the discovery of phosphatidylinositol specific 3-kinase which is activated by tyrosine kinase, the biological importance of its 3-phosphorylation products, PI(3,4,5)P_3, PI(3,4)P_2, and PI(3)P has been emphasized.[1] However, the scarcity of the natural products has delayed investigation of their physiological roles. While saturated acyl chain analogs of PI(3,4,5)P_3 and PI(3,4)P_2 prepared chemically[2] have contributed to disclosure of the roles, supply of natural and closely related unsaturated chain substances would be much more useful for the study, considering the facts that: 1) saturated long chain analogs are sparingly soluble in water, 2) the role of fatty acid moieties in physiological action of the phosphoinositides is unclear. From these standpoints, we have tried to prepare unsaturated phosphatidylinositol phosphates. During this project, the 9-fluorenylmethyl group (Fm) was recently found to be quite promising as a phosphate protecting group.[3] Using this strategy, synthesis of natural PI(3,4,5)P_3 which has been believed to have the stearoyl and arachidonoyl groups at the *sn*-1 and-2 positions in the glycerol moiety, respectively, will be reported here. Very recently, Reese's group has completed the synthesis of the same molecule.[4]



PI(3,4,5)P_3: R=R'=PO_3H^-
PI(3,4)P_2: R=PO_3H^-, R'= H
PI(3)P: R=R'= H

The useful synthetic intermediate **1**, which can be derived readily in two steps from *myo*-inositol,[5] was transformed to diastereomeric 6-mandelates by the regioselective reaction with (S)-(+)-*O*-acetylmandelyl chloride.[6] The two diastereomers **2** were isolated after 5-*O*-triethylsilylation by flash column chromatography [R_f values (AcOEt/*n*-C_6H_{14}, 1:12) and yield for **3** and the other diastereomer: 0.25, 36% and 0.30, 42%] , while **2** could not be separated. The silyl mandelate **3** with a lower R_f value had the desired absolute configuration.[7] Hydrazinolysis of **3** followed by levulinoylation yielded **5** which was then desilylated to give a triol. Phosphorylation of the triol was performed via phosphitylation with difluorenylmethyl phosphoramidite[3] **6** which was developed recently to prepare unsaturated-type phosphoinositides, giving trisphosphate **7**. The cyclohexylidene group in **7** was removed to phosphorylate the resultant 1,2-diol **8** regioselectively using the

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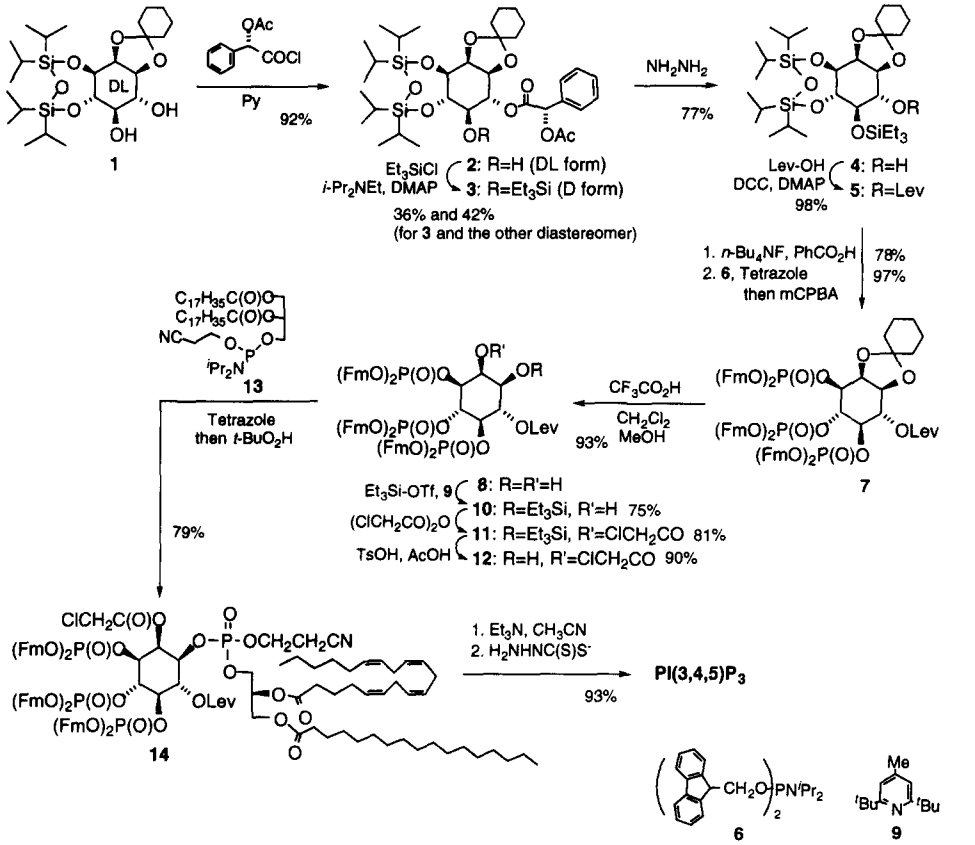
reaction with a phosphite and pyridinium tribromide,[8] which is generally applicable to the regioselective phosphorylation of 1,2-free *myo*-inositol derivatives. However, this attempt for the diol **8** failed. Therefore, **8** was transformed to 1-monool **12** via silylation, chloroacetylation, and desilylation. Selective triethylsilylation of **8** was also difficult according to common procedures,[9] by which a serious amount of the 1,2-disilyl derivative was formed. Eventually, after several experiments, we found that a highly reactive silyltriflate combined with a bulky base, 2,6-di-*t*-butyl-4-methylpyridine was effective, resulting in the formation of 1-silyl ether **10** in 75% yield. Phosphorylation of the 1-OH free derivative **12** via the amidite method using 1-stearoyl-2-arachidonoyl-*sn*-glyceryl phosphoramidite **13** gave the fully protected tetrakisphosphate derivative **14** in 79% yield.[10] Deprotection of the four phosphate functions was accomplished smoothly by treatment with triethylamine for 14 h at room temperature. Removal of the chloroacetyl group as well as the levulinoyl was achieved simultaneously by using the reported procedure for deprotection of the chloroacetate,[11] to give the final product, PI(3,4,5)P_3.[12]



Py=pyridine, Lev-OH=levulinic acid, DCC=dicyclohexylcarbodiimide, DMAP=4-dimethylaminopyridine, mCPBA=*m*-chloroperbenzoic acid, Tf=trifluoromethanesulfonyl

The product was soluble in methanol and showed relatively clear ^1H NMR signals, while the saturated distearoyl analog[2a,e] had opposite properties. Biological activities of the present synthetic natural product are now under investigation.

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References and Notes

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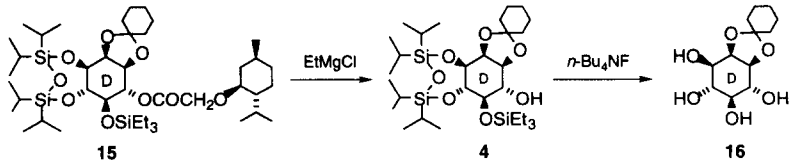
7. The hydrazinolysis product **4** with $[\alpha]^{25}_D$ -21.6° (*c* 1.58, $CHCl_3$) was in accordance with the assigned absolute configuration compared with that {$[\alpha]^{25}_D$ -19.2° (*c* 1.19, $CHCl_3$)} of the substance **4** derived by the deacylation of the 6-*l*-menthoxyacetyl derivative **15**. The specimen **4** was converted to the known 1D-1,2-*O*-cyclohexylidene-*myo*-inositol **16** [Sadovnikova, M. S.; Kuznetsova, Z. P.; Shvets, V. I.; Evstigneeva, R. P. *Zh. Org. Khim.* **1975**, *11*, 1211-1217, (1201-1206 for English version)] by treatment with tetrabutylammonium fluoride in THF and its absolute configuration was confirmed.



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9. Watanabe, Y. *Selective Reactions and Total Synthesis of Inositol Phosphates*; Rahman, A. Ed.; Elsevier: Amsterdam, 1996; Vol. 18, pp 391-456.
10. Data for **14**: R_f=0.4 ($Me_2CO/CHCl_3$, 1:4); ^1H NMR (270 MHz, $CDCl_3$) δ=0.86 (6H, complex, ste and ara CH_3), 1.17-1.40 (34H, complex, ste and ara CH_2), 1.56 (2H, br, ste β CH_2), 1.67 (2H, m, ara β CH_2),1.99 & 2.00 (3H, s x 2, lev CH_3), 2.00-2.08 (4H, complex, allylic H), 2.09-2.59 (8H, complex, ste and ara α CH_2, and lev CH_2CH_2), 2.69 (1H, t, *J*=7.8 Hz, CHCN), 2.75-2.86 (7H, complex, CHCN, CH=CHC<u>H</u>$_2$CH=CH), 3.72-4.38 (28H, complex, Ins-$H_{3,5}$, glyceryl α and γ CH_2, OCH_2CH, OCH_2CH_2CN, CH_2Cl), 4.47 (1H, m, Ins-H_1), 4.71 (1H, m, Ins-H_4), 5.20-5.44 (10H, complex, Ins-H_6, glyseryl β CH, vinyl H), 5.96 & 5.98 (1H, t x 2, *J*=3.9 Hz, Ins-H_2), 7.01-7.74 (48H, complex, aromatic H); ^{13}C NMR ($CDCl_3$, 68 MHz) δ=14.00 & 14.05 (2C, ste and ara CH_3), 19.27 & 19.38 (<u>C</u>H_2CN), 22.48 & 22.61 (2C, ste and ara <u>C</u>H_2CH_3), 24.59, 24.64, & 24.72 (2C, ste and ara β CH_2), 25.53, 26.39, & 27.13 (5C, allylic CH_2), 27.14 & 27.32 (lev α CH_2), 29.04-29.75 (13C, complex, ste and ara CH_2, and lev CH_3), 31.41 (ara <u>C</u>$H_2CH_2CH_3$), 31.83 (ste <u>C</u>$H_2CH_2CH_3$), 33.43, 33.82, & 33.87 (2C, ste and ara α CH_2), 36.87 & 37.02 (lev β CH_2), 40.38(CH_2Cl), 47.55 & 47.67 (6C, d x 2, *J*=7.3 Hz, Fm CH), 61.50 (m, -O<u>C</u>H_2CH_2CN), 62.52 & 62.88 (m x 2, glyceryl α CH_2), 66.27 & 66.63 (m x 2, glyceryl γ CH_2), 69.07-69.84 (8C, complex, Ins-C_6, glyceryl β CH, Fm CH_2), 71.02 (Ins-C_2), 72.35 (m, Ins-C_3), 72.69 (m, Ins-C_1), 74.43 (m, Ins-C_4), 75.36 (m, Ins-C_5), 116.38 & 116.47 (CN), 119.56-119.19 (12C, complex, Fm-C_5), 124.73-125.41 (12C, Fm-C_2), 126.63-130.44 (32C, Fm-$C_{3,4}$, vinyl C), 141.02-141.47 (12C, complex, Fm-C_6), 142.53-143.17 (12C, complex, Fm-C_1), 166.14 & 166.28 (chloroacetyl CO), 171.92 (lev CO), 172.55 & 173.16 (2C, ste and ara CO), 205.87, 205.94, & 206.31 (ketone CO); ^{31}P NMR($CDCl_3$, 109 MHz) δ=-1.69 (1/2P), -1.59 (1/2P), -1.32 (1P), -0.86 (2P).
11. van Boeckel C. A. A.; Beetz, T. *Tetrahedron Lett.*, **1983**, *24*, 3775-3778.
12. Data for PI(3,4,5)P3: R_f=0.3 ($CHCl_3/Me_2CO/CH_3OH/CH_3CO_2H/H_2O$, 25:12:13:7:10); ^1H NMR (270 MHz, $CDCl_3/CD_3OD$, 6:1) δ=0.95 (6H, complex, ste and ara CH_3), 1.38 (97H, br, CH_2 and $NCCH_3$), 1.58 (4H, br, ste and ara β CH_2), 2.14 (4H, m, allylic CH_2), 2.29 (4H, m, ste and ara α CH_2), 2.82 (6H, br, CH=CH-C<u>H</u>$_2$-CH=CH), 3.23 (42H, br, NCH_2), 3.96-4.65 (10H, complex, glyceryl α and γ CH_2, Ins-$H_{1,2,3,4,5,6}$), 5.21 (1H, br, glyceryl β CH), 5.38(8H, m, vinyl H); ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$, 6:1) δ=8.14 (21C, CH_3 in Et_3N), 13.60 (ara CH_3), 13.65 (ste CH_3), 22.15 (ara <u>C</u>H_2CH_3), 22.26 (ste <u>C</u>H_2CH_3), 24.42 (ara β C), 24.48 (ste β C), 25.21 (3C), 26.12 & 26.82 (5C, allylic C), 28.78, 28.94, 29.12, & 29.29 (13C, ste and ara <u>C</u>H_2), 31.12 (ara C_{18}), 31.52 (ste C_{16}), 33.29 (ara C_2), 33.68 (ste C_2), 45.93 (21C, CH_2 in Et_3N), 62.40 (br, glyceryl α C), 63.71 (br, glyceryl γ C), 69.91 (Ins-C_2), 70.14 (m, glyceryl β C), 70.58 (br, Ins-C_6), 74.95 (br d, *J*=3.2 Hz, Ins-C_3), 75.25 (br, Ins-C1), 76.49 (br, Ins-C_4), 78.86 (br, Ins-C_5), 127.15, 127.44, 127.71, 127.91, 128.23, 128.48 (2C), & 130.09 (8C, vinyl C), 172.70 (ara CO), 173.32 (ste CO); ^{31}P NMR (109 MHz, $CDCl_3/CD_3OD/Et_3N$, 6:1:0.5) δ=-0.52, 0.81, 1.88, 2.86. FAB-MS (negative, diethanolamine) *m/z* 1125 [M-1].