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SYNTHESIS OF PI(3,4,5)P3 WITH UNSATURATED AND SATURATED FATTY ACID CHAINS

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Abstract: Synthesis of three PI(3,4,5)P3s, *sn*-1-*O*-stearoyl-*sn*-2-*O*-arachidonoyl, stearoyl-linolenoyl, and distearoyl phosphatidyl-*myo*-inositol 3,4,5-trisphosphate with the natural configuration for the arachidonoyl version was established by employing 9-fluorenylmethyl-protected phosphate derivatives.

Keywords: *phosphatidyl inositol, phospholipid, natural product, protecting group*

In various intracellular signals relating to cell proliferation, oncogenesis, insulin action, and so on,[1] phosphatidylinositol-specific 3-kinases (PI 3-kinases),[2] the activation of which is closely associated with receptors of growth factors linked to the activation of tyrosine kinase,[3] have been recognized to play a key role. They produce 3-phosphorylated phosphoinositides, phosphatidylinositol 3,4,5-trisphosphate, phosphatidylinositol 3,4-bisphosphate, and phosphatidylinositol 3-phosphate, respectively, from the corresponding D-3-free phosphatidylinositols.

There have been reported several papers concerning synthesis of PI(3,4,5)P3. In most of the papers, were prepared the analogs bearing saturated fatty acid chains such as stearic,[4-6] palmitic,[7] and octanoic acid.[8] Saturated analogs can be prepared much more easily than the unsaturated version, since the former synthetic procedures allow the use of common benzylic protecting groups which are removed by hydrogenolysis. Very recently, natural stearoyl-arachidonoyl-PI(3,4,5)P3 have been synthesized by Gaffney and Reese,[9] and us.[10] Our synthetic study on unsaturated PI(3,4,5)P3 is reported here in detail.

Results and Discussion

The synthetic process to unsaturated- and saturated-PI(3,4,5)P3 is summarized in Scheme 1. The present synthetic route is essentially the same as that for the racemic PI(3,4,5)P3 reported in 1994.[4] As described below, choice of the protecting group for phosphoric esters in the unsaturated-lipid synthesis was most crucial, and a novel one, 9-fluorenylmethyl (Fm), was found to be promising for our purpose.[11]

When *myo*-inositol is used as the starting material, optical resolution at an adequate stage is essential since it is a *meso*-compound. We employed so far two resolution procedures to obtain an optically active 3,4-*O*-disiloxanyl derivative. Enantio- and regioselective acetylation of DL-1,2-*O*-cyclohexylidene-*myo*-inositol **17** at the 3-position using Lipase CES from *Pseudomonas sp.* (Amano Pharmaceutical Co. Ltd.) as a biocatalyst,[12] deacylation, and disiloxanylation gave **D-1** with 98% ee. A chemical method derivatizing to diastereomers with an appropriate chiral auxiliary is frequently more reliable because 1) optically pure substance can be obtained; 2) a chiral chemical is available constantly whereas an uncommon enzyme is difficult to obtain. From these

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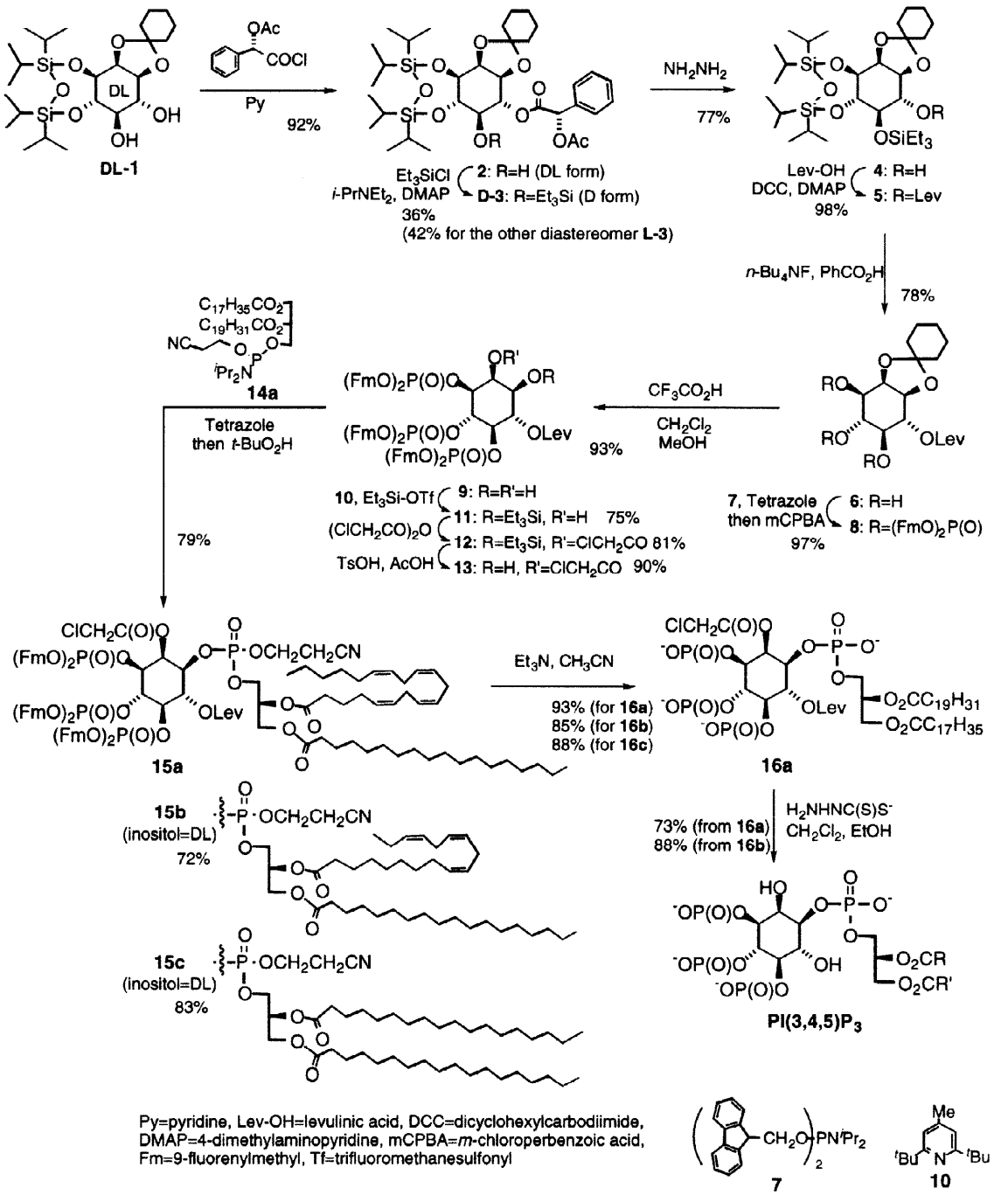
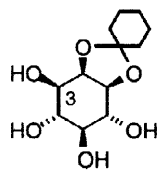
standpoints, we had previously derived **DL-1** to *l*-menthyloxyacetic ester[13] **18a** and its silyl derivative **18b** was separated to two diastereomers. However, careful treatment of **18a** was required to prevent the acyl migration to the 5-position. To avoid such a problem, a more bulky *O*-acetylmandelic ester (**2**)[14,15] was employed this time and its diastereomeric silyl ethers **3** were readily separated by flash column chromatography to afford **D-3** (36%) and **L-3** (42%), although their R_f values [0.25 and 0.30 (AcOEt/*n*-C_6H_{14}, 1:12)] were close. Optical purity of each diastereomer was confirmed by NMR, TLC, and HPLC (SiO_2) analysis. The silylation was accomplished by the reaction with triethylsilyl chloride in the presence of DMAP and ethyl(diisopropyl)amine as a bulky tertiary

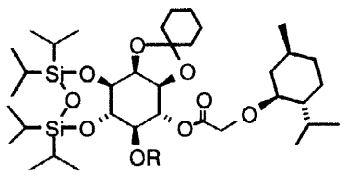


Scheme 1

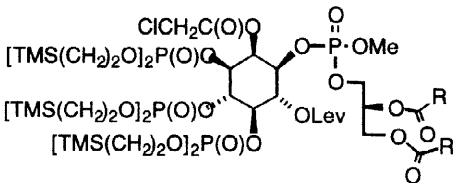
amine, while a less bulky amine, triethylamine, accelerated the migration of the acyl group in **2**.



17

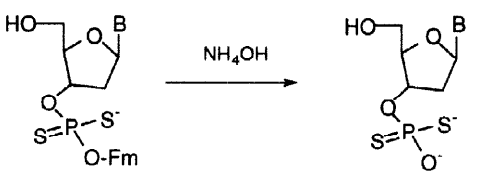


18a: R=H
18b: R=Et_3Si



19 (R=$C_{17}H_{35}$)

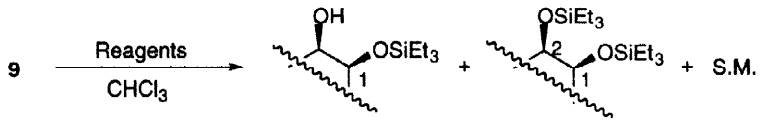
The chiral auxiliary from **D-3** was removed by the reaction with hydrazine at low temperature (0 °C or lower) to afford the 6-*O*-free derivative **4**. Levulinoylation of **4** followed by removal of the silyl functions gave triol **6**. In the next phosphorylation, various phosphate protecting groups were examined. When benzyl phosphate, which has been almost invariably employed in saturated PIPs syntheses, was used, various deprotection trials based on trimethylsilyl bromide and Na in liquid NH_3 to remove the benzyl groups from the unsaturated acyl phosphatidylinositol phosphates at the final stage were all unsuccessful. We then searched for protecting groups which may be removed by a β-elimination mode. Deprotection of the 2-(trimethylsilyl)ethyl-protected PIP_3 derivative **19** failed. The 2-cyanoethyl[9] and 2-(*p*-nitrophenyl)ethyl[16] esters corresponding to **8** were also not suitable for our purposes. In the former case, phosphate migration occurred during removal of the cyclohexylidene group. In the latter, the same process was resistant to various acidic reaction conditions. We turned our attention to finding a new phosphate protecting group, and Fm was found to be promising.[11] Caruthers and co-workers used this group to obtain phosphorodithioates **21** via deprotection of the transient monofluorenylmethyl esters **20** with concentrated ammonia .[17] Since their report, Fm has not been utilized for phosphate protection. Thus, triol **6** was transformed quantitatively via the phosphoramidite procedure using di-(9-fluorenylmethyl) *N,N*-diisopropylphosphoramidite **7** to 3,4,5-trisphosphate **8**, which was then converted to 1,2-diol **9**.



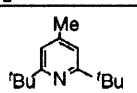
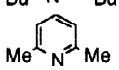
20 21 (B=nucleoside bases T and A)

Since 1,2-diol derivatives are readily available from the parent *myo*-inositol, selective phosphorylation of them at the 1-position is a convenient procedure for the synthesis of phosphatidylinositol derivatives.[18] Such a useful strategy was realized by the reaction of a 1,2-diol with a phosphite in the presence of pyridinium tribromide, and the methodology has been demonstrated in the synthesis of phosphatidylinositol phosphates. However, its application to **9** failed, while the benzyl-protected triphosphate derivative was phosphorylated albeit in low yield.[4] Diol **9** was consequently transformed into the 2-*O*-protected derivative **13** via 1-*O*-silyl ether **11**. Thus, temporary protection of the equatorial hydroxyl group in **9** with the triethylsilyl group was achieved in 75% (87% yield based on the recovered starting material) by using a highly reactive silylating reagent, triethylsilyl triflate, and a bulky base, 2,6-di-*t*-butyl-4-methylpyridine **10**. Usual procedures using triethylsilyl chloride yielded a small amount of 1,2-disilyl ether **22** together with a large amount of the starting material (run 3 in Scheme 2), and none of the desired 1-monosilyl ether **11** was observed. A combination of TESOTf and lutidine also induced silyl migration to finally furnish a serious amount of **22** (run 2). These observations

indicate that the silyl group in **11** first formed was extraordinarily prone to migrate to 2-*O*-silyl ether, which was then silylated resulting in the formation of **22**.



11 **22** **9**

Run	Reagents	Yield, % 11	22	9
1	Et_3Si-OTf, 	75	-	14
2	Et_3Si-OTf, 	38	19	32
3	Et_3Si-Cl, Pyridine	-	10	83

Scheme 2

The sequential 2-chloroacetylation and desilylation of **11** were accomplished by common procedures to give **13** in high yield. Phosphitylation of **13** with 2-*O*-arachidonoyl-1-*O*-stearoyl-*sn*-glycerol 2-cyanoethyl *N,N*-diisopropylphosphoramidite **14a**, which was derived from the corresponding 1,2-diacyl glycerol and chloro(2-cyanoethyloxy)diisopropylaminophosphine, was followed by oxidation with *t*-butyl hydroperoxide to afford smoothly 1-*O*-phosphate **15a** in good yield. In a similar manner, other 1-phosphates with the racemic inositol moiety, **15b** and **15c**, were obtained by using the corresponding (stearoyl-linolenoyl)- and distearoylglycerol phosphoramidite, **14b** and **14c**, respectively. Product analysis of the reaction for **14a** synthesis by TLC and NMR experiments suggested that acyl migration did not occurred, although 1,2-di-*O*-acylglycerols are known to readily isomerize.[7c] Careful analysis of phosphorylation products showed also that **15a**, **15b**, and **15c** were only the inositol-containing products.

Deprotection of all phosphoric esters in **15** was carried out by the reaction with a large excess of triethylamine in acetonitrile under anhydrous conditions at room temperature to give **16** (14 h, 93% for **16a**; 20 h, 85% for **16b**; 15 h, 88% for **16c**). The dechloroacetylation procedure[19] in methylenechloride and ethanol using hydrazine dithiocarbonate under anhydrous conditions[20] induced elimination of the levulinoyl group as well as the chloroacetyl in **16a** and **16b**, resulting in the formation of the final molecule, unsaturated PI(3,4,5)P3. It should be noted that, when chloroform instead of CH_2Cl_2 was used for removal of the acyl groups from **16c**, the levulinoyl remained intact (68% yield). Therefore, further treatment with hydrazine in pyridine and acetic acid[21] was necessary to provide saturated PI(3,4,5)P3 in 78% yield. The same solvent in the case of **16b** caused formation of an unidentified material insoluble in any common solvents.

In conclusion, the present procedure provides a convenient method for the synthesis of unsaturated and saturated PI(3,4,5)P3. The new phosphate protecting group, Fm was confirmed to be useful for inositol phospholipid synthesis.

Experimental

NMR spectra (JEOL JNM GSX270) were recorded in $CDCl_3$ unless otherwise noted. As references for the ^1H-, ^{13}C-, and ^{31}P NMR measurements, TMS (δ=0.0), $CDCl_3$ (δ=77.0), and 85% H_3PO_4 (δ=0.0, external) were

used, respectively. ^{13}C- and ^{31}P NMRs were all taken under ^{1}H-decoupled conditions. Optical rotations were measured using a Union PM-101. Elemental analyses were performed using a Perkin-Elmer 240C. Flash chromatography was utilized for column chromatography by using Fuji Silysia silica gel, BW-300. Thin layer chromatographic analysis was performed on Merck pre-coated plates, Silica Gel 60 F254. Solvents used here are abbreviated as follows: EA=AcOEt, Hex=hexane. An anhydrous reaction atmosphere was achieved using nitrogen gas. Extracts obtained after work-up were dried over $MgSO_4$ or Na_2SO_4.

DL-6-*O*-[(*S*)-(+)-*O*-Acetylmandeloyl]-1,2-*O*-cyclohexylidene-3,4-*O*-(tetraisopropyldisiloxane-1,3-diyl)-*myo*-inositol (2). To a solution of 1,2-*O*-cyclohexylidene-3,4-*O*-(tetraisopropyldisiloxane-1,3-diyl)-*myo*-inositol (11.4 g. 22.7 mmol) in $CHCl_3$ (100 mL) was added dropwise at 0 °C pyridine (26.9 g, 34 mmol) and then (*S*)-(+)-*O*-acetylmandeloyl chloride[22] (6.3 g, 29.4 mmol). After removal of the cooling bath, the mixture was stirred for 2 h at room temperature and AcOEt was added. The resulting organic solution was washed successively with H_2O, saturated aqueous $KHSO_4$ solution, H_2O, saturated aqueous $NaHCO_3$ solution, and saturated aqueous NaCl solution, dried, filtered, and evaporated. The residue was chromatographed on silica gel eluting with AcOEt and Hexane (1:5) to give **2** (14.3 g, 92% yield): R_f=0.6 (EA/Hex,1:3); ^{1}H NMR (270 MHz) δ 0.90-1.10 (28H, complex, isopropyl H), 1.46-1.72 (10H, complex, cyclohexylidene H), 2.15 (3H, s, Ac), 3.25 & 3.44 (1H, ddx2, *J*=9.2 and 8.9 Hz, Ins H_5), 3.82-3.87 (1.5H, complex, Ins $H_{1,3}$), 3.96 & 4.00 (1H, t x2, *J*=9.2 Hz, Ins H_4), 4.06 (0.5H, dd, *J*=8.2 and 4.3 Hz, Ins H_1), 4.21 & 4.28 (1H, tx2, *J*=4.3 Hz, Ins H_2), 5.18 & 5.21 (1H, ddx2, *J*=8.9 and 8.2 Hz, Ins H_6), 6.05 & 6.09 (1H, sx2, benzylic H), 7.38-7.48 (5H, complex, aromatic H); ^{13}C NMR (68 MHz) δ 12.09-12.85, 17.09-17.51 (12C, TIPDS C), 20.67 & 20.73 (Ac), 23.58, 23.63, 23.74, 23.78, & 24.96 (3C, cyclohexylidene C), 35.00, 35.11, 37.49, &3 7.58 (2C, cyclohexylidene C), 71.87 & 72.07 (Ins C_2), 73.12 & 73.14 (Ins C_4), 74.35 & 74.43 (benzylic C), 75.50 & 75.58 (Ins C_3), 75.72 & 75.75 (Ins C_6), 76.31 (Ins C_5), 76.64 & 76.70 (Ins C_1), 110.57 & 110.69 (cyclohexylidene quaternary C), 128.01 & 128.10 (2C, aromatic $C_{3,5}$), 128.58 & 128.61 (2C, aromatic $C_{2,6}$), 129.04 & 129.08 (2C, aromatic C_4), 133.76 & 134.13 (2C, aromatic C_1), 167.94, 168.34, 170.02, & 170.20 (2C, CO).

1D- and 1L-6-*O*-[(*S*)-(+)-*O*-Acetylmandeloyl]-1,2-*O*-cyclohexylidene-3,4-*O*-(tetraisopropyldisiloxane-1,3-diyl)-5-*O*-triethylsilyl-*myo*-inositol (D-3 and L-3). To a solution of diastereomeric **2** (14.0 g, 20.6 mmol) in $CHCl_3$ (100 mL) were added successively triethylsilyl chloride (12.4 g, 82.2 mmol), ethyldiisopropylamine (16.0 g, 123.4 mmol), and DMAP (1.3 g, 10.3 mmol) at 0 °C and the mixture was stirred for 2 h at room temperature. After addition of AcOEt, the organic solution was washed sequentially with H_2O, saturated $KHSO_4$ solution, H_2O, saturated $NaHCO_3$ solution, and saturated NaCl solution, dried, filtered, and evaporated. Chromatography of the residue (Et_2O-hexane, 1:2) gave diastereomers **D-3** (5.9 g, 36%) and **L-3** (6.8 g, 42%) accompanied with fractions including both isomers (0.8 g, 5%): **D-3:** R_f=0.25 (EA/Hex, 1:12); ^{1}H NMR (270 MHz) δ 0.45 (6H, q, *J*=7.9 Hz, CH_2), 0.84 (9H, t, *J*=7.9 Hz, CH_3), 0.96-1.11 (28H, complex, isopropyl H), 1.24-1.73 (10H, complex, cyclohexylidene H), 2.17 (3H, s, Ac), 3.43 (1H, t, *J*=5.0 Hz, Ins H_5), 3.79 (1H, dd, *J*=9.7 and 3.5 Hz, Ins H_3), 4.04 (1H, dd, *J*=9.7 and 5.0 Hz, Ins H_4), 4.17 (1H, t, *J*=5.0 Hz, Ins H_1), 4.37 (1H, dd, *J*=5.0 and 3.5 Hz, Ins H_2), 5.05 (1H, t, *J*=5.0 Hz, Ins H_6), 6.00 (1H, s, benzylic H), 7.34-7.45 (5H, complex, aromatic H); ^{13}C NMR (68 MHz) δ 4.73 (3C, CH_2), 6.76 (3C, CH_3), 12.08-12.81 & 17.16-17.56 (12C, TIPDS C), 20.67 (Ac), 23.64, 23.71, & 25.14 (3C, cyclohexylidene C), 34.17 & 36.09 (2C, cyclohexylidene C), 72.56 (Ins C_2), 74.39 (benzylic C), 75.08 (Ins C_4), 75.92 (Ins C_3), 76.23 (Ins C_6), 76.61 (Ins C_5), 76.69 (Ins C_1), 110.38 (cyclohexylidene C), 127.80 (2C, aromatic $C_{3,5}$),

128.65 (2C, aromatic $C_{2,6}$), 129.30 (aromatic C_4), 133.75 (aromatic C_1), 167.50 & 169.89 (2C, CO); $[\alpha]_D^{23}$ +1.3° (*c* 1.75, $CHCl_3$). **L-3:** R_f=0.30 (EA/Hex, 1:12); ^1H NMR (270 MHz) δ 0.63 (6H, q, *J*=7.6 Hz, CH_2), 0.92 (9H, t, *J*=7.6 Hz, CH_3), 0.99-1.12 (28H, complex, isopropyl H), 1.22-1.80 (10H, complex, cyclohexylidene H), 2.17 (3H, s, Ac), 3.46 (1H, t, *J*=8.4 Hz, Ins H_5), 3.59 (1H, dd, *J*=7.0 and 4.0 Hz, Ins H_1), 3.76 (1H, dd, *J*=9.3 and 4.0 Hz, Ins H_3), 3.95 (1H, dd, *J*=9.3 and 8.4 Hz, Ins H_4), 4.12 (1H, t, *J*=4.0 Hz, Ins H_2), 5.07 (1H, dd, *J*=7.0 and 8.4 Hz, Ins H_6), 6.15 (1H, s, benzylic H), 7.35-7.47 (5H, complex, aromatic H); ^{13}C NMR (68 MHz) δ 5.03 (3C, CH_2), 6.90 (3C, CH_3), 11.82-12.83 & 17.06-17.55 (12C, TIPDS C), 20.71 (Ac), 23.59, 23.86, & 25.09 (3C, cyclohexylidene C), 34.92 & 36.91 (2C, cyclohexylidene C), 73.32 (Ins C_2), 73.60 (benzylic C), 74.02 (Ins C_4), 75.50 (Ins C_3), 75.85 (Ins C_6), 76.32 (Ins C_5), 77.41 (Ins C_1), 110.31 (cyclohexylidene C), 128.16 (2C, aromatic $C_{3,5}$), 128.57 (2C, aromatic $C_{2,6}$), 129.05 (aromatic C_4), 134.31 (aromatic C_1), 167.59 & 169.64 (2C, CO); $[\alpha]_D^{23}$ +4.5° (*c* 2.07, $CHCl_3$).

1D-1,2-*O*-Cyclohexylidene-3,4-*O*-(tetraisopropyldisiloxane-1,3-diyl)-5-*O*-triethylsilyl-*myo*-inositol (4). A DMF (50 mL) solution of **D-3** (5.5 g, 6.9 mmol) was cooled to 0 °C and hydrazine hydrate (7.0 g, 140.2 mmol) in DMF (20 mL) was carefully added. The solution was stirred for an additional 2.5 h at room temperature and after addition of AcOEt, washed with H_2O (several times), saturated $KHSO_4$ solution, H_2O, saturated $NaHCO_3$ solution, and saturated NaCl solution, dried, filtered, and evaporated. The residue was chromatographed on silica gel (EA/Hex, 1:12) to give the 6-free silyl ether derivative **4** (3.8 g, 89%): R_f=0.4 (Et_2O/Hex, 1:7); ^1H NMR (270 MHz) δ 0.69 (6H, q, *J*=7.9 Hz, CH_2), 0.96 (9H, t, *J*=7.9 Hz, CH_3), 1.05-1.10 (28H, complex, isopropyl H), 1.42-1.76 (10H, complex, cyclohexylidene H), 2.23 (1H, d, *J*=3.2 Hz, OH), 3.24 (1H, ddd, *J*=10.5, 9.0, and 3.2 Hz, Ins H_5), 3.53 (1H, ddd, *J*=10.5, 7.6, and 2.1 Hz, Ins H_6), 3.82 (1H, dd, *J*=7.5 and 4.0 Hz, Ins H_3), 3.87 (1H, dd, *J*=9.0 and 7.5 Hz, Ins H_4), 3.88 (1H, dd, *J*=7.6 and 4.6 Hz, Ins H_1), 4.26 (1H, dd, *J*= 4.6 and 4.0 Hz, Ins H_2); $[\alpha]_D^{25}$ -21.6° (*c* 1.58, $CHCl_3$).

1D-1,2-*O*-Cyclohexylidene-6-*O*-levulinoyl-3,4-*O*-(tetraisopropyldisiloxane-1,3-diyl)-5-*O*-triethylsilyl-*myo*-inositol (5).[5] To a solution of **4** (1.8 g, 2.9 mmol) in $CHCl_3$ (20 mL) were added at 0 °C levulinic acid (0.7 g, 5.9 mmol), *N,N'*-dicyclohexylcarbodiimide (1.5 g, 7.3 mmol), and then 4-dimethylaminopyridine (0.07 g, 0.6 mmol). The mixture was stirred for 3 h at rt and filtered through a pad of Celite. The filtrate was filtered again after evaporation and addition of ethyl ether, and chromatographed (EA/$CHCl_3$, 1:8) to give **5** (1.9 g, 98%): $[\alpha]_D^{25}$ -16.9° (*c* 1.14, $CHCl_3$). Its spectral data were in agreement with those for PI(3,4,5)P3 reported.[5]

1D-1,2-*O*-Cyclohexylidene-6-*O*-levulinoyl-*myo*-inositol (6). To a solution of **5** (1.9 g, 2.7 mmol) in THF (20 mL) were added at 0 °C benzoic acid (1.3 g, 10.9 mmol) and *n*-$Bu_4NF{\cdot}3H_2O$ (3.3 g, 10.4 mmol), and the mixture was stirred at the same temperature for an additional 7 h. After evaporation of the solvent, the residue was chromatographed (EA/CH_3OH, 20:1) to afford 6 (758 mg, 78%): R_f=0.3 (EA/MeOH, 10:1); mp 149-151 °C (from MeOH); ^1H NMR (270 MHz) δ 1.30-1.82 (10H, complex, cyclohexylidene H), 2.19 (3H, s, CH_3), 2.50-3.00 [4H, complex, $(CH_2)_2$], 3.42 (1H, dd, *J*=9.8 and 10.1 Hz, Ins H_5), 3.78 (1H, dd, *J*=9.5 and 4.0 Hz, Ins H_3), 3.87 (1H, dd, *J*=9.8 and 9.5 Hz, Ins H_4), 4.13 (1H, dd, *J*=7.6 and 4.9 Hz, Ins H_1), 4.46 (1H, dd, *J*=4.9 and 4.0 Hz, Ins H_2), 5.10 (1H, dd, *J*=10.1 and 7.6 Hz, Ins H_6); IR (nujor, cm^{-1}) 3400, 1740, 1700; *Anal.* Calc. for $C_{17}H_{26}O_8$: C, 56.97; H, 7.31%. Found: C, 56.77; H, 7.29%.

1D-1,2-*O*-Cyclohexylidene-3,4,5-tri-*O*-[di(9-fluorenylmethyl) phosphoryl]-6-*O*-levulinoyl-*myo*-inositol (8). To a $CHCl_3$ (20 mL) solution of triol **6** (758 mg, 2.1 mmol) were added at 0 °C di(9-fluorenylmehtyl) *N,N*-diisopropylphosphoramidite **7** (5.0 g, 9.6 mmol) and 1*H*-tetrazole (703 mg, 10.0 mmol)

and the mixture was stirred for 2 h at rt. After addition of *m*-chloroperbenzoic acid (1.8 g, 10.1 mmol) at -78 °C, the resulting solution was stirred for 1.5 h at rt, and 10% Na_2SO_3 aqueous solution was added. The mixture was stirred for 30 min at rt, and AcOEt was added. The organic layer was washed sequentially with H_2O, saturated $KHSO_4$ solution, H_2O, saturated $NaHCO_3$ solution, and saturated NaCl solution, dried, filtered, and evaporated. Chromatography of the residue (EA/$CHCl_3$, 1:2) gave trisphosphate **7** (3.42 g, 97%): R_f=0.4 (EA/$CHCl_3$, 1:2); ^1H NMR (270 MHz) δ 1.20-1.73 (10H, complex, cyclohexylidene H), 2.01 (3H, s, CH_3), 2.39-2.64 [4H, complex, $(CH_2)_2$], 3.94-4.39 [22H, complex, Ins $H_{1,2,3,5}$, and (CH_2CH)x6], 4.90 (1H, q, *J*=7.6 Hz, Ins H_4), 5.26 (1H, t, *J*=6.4 Hz, Ins H_6), 7.01-7.67 (48H, complex, aromatic H); ^{13}C NMR (68 MHz) δ 23.34, 23.62, & 24.75 (3C, cyclohexylidene C), 27.79 (α-CH_2), 29.65 (CH_3), 31.54 & 33.91 (2C, cyclohexylidene C), 37.64 (β-CH_2), 47.62 & 47.71 (6C, dx2, *J*=7.0 Hz each, Fm methine), 68.92 (d, *J*=6.1 Hz, Fm methylene), 69.29-69.72 (6C, complex, Ins C_6 and Fm methylene), 72.06 (Ins C_2), 72.89 (Ins C_1), 73.54 (br, Ins C_3), 74.52 (m, Ins C_4), 75.60 (m, Ins C_5), 111.47 (cyclohexylidene C), 119.67-119.86 (12C, complex, Fm C_5), 124.76-125.35 (12C, complex, Fm C_2), 126.87-127.84 (24C, complex, Fm $C_{3,4}$), 141.14-141.26 (12C, complex, Fm C_6), 142.67-143.14 (12C, complex, Fm C_1), 171.19 (CO), 206.06 (CO); ^{31}P NMR (109 MHz) δ -0.98, -0.55, -0.10; $[\alpha]_D^{23}$ +14.3° (*c* 1.61, $CHCl_3$); *Anal.* Calc. for $C_{101}H_{89}O_{18}P_3 \cdot CHCl_3$: C, 68.55; H, 5.08%.

1D-3,4,5-Tri-*O*-[di(9-fluorenylmethyl) phosphoryl]-6-*O*-levulinoyl-*myo*-inositol (9). A solution of **7** (3.35 g, 2.0 mmol), methanol (0.34 g, 10.0 mmol), and trifluoroacetic acid (5.70 g, 25.0 mmol) in $CHCl_3$ (35 ml) was stirred around -5 to 0 °C for 8 h, and AcOEt was added. The organic layer was washed sequentially with H_2O, saturated $NaHCO_3$ solution, and saturated NaCl solution, dried, filtered, and evaporated. Chromatography of the residue (EA/$CHCl_3$, 5:2) gave 1,2-diol **9** (2.97 g, 93%): R_f=0.2 (EA/$CHCl_3$, 1:1); ^1H NMR (270 MHz) δ 2.07 (3H, s, CH_3), 2.13-2.73 [4H, complex, $(CH_2)_2$], 2.83 & 3.03 (2H, br, OH), 3.22 (1H, br, Ins H_1), 3.75 (1H, ddd, *J*=7.0, 6.4, and 2.7 Hz, Ins H_3), 3.82 (1H, br, Ins H_2), 3.83-4.36 [19H, complex, Ins H_5, and (CH_2CH)x6], 4.75 (1H, q, *J*=7.0 Hz, Ins H_4), 5.24 (1H, t, *J*=9.8 Hz, Ins H_6), 6.91-7.72 (48H, complex, aromatic H); ^{13}C NMR (100 MHz) δ 28.15 (lev C_2), 29.96 (CH_3), 38.10 (lev C_3), 47.62, 47.72, & 47.80 (6C, dx3, *J*=8.3 Hz each, Fm methine), 68.15 (Ins C_6), 68.71 (d, *J*=6.4 Hz, Fm methylene), 69.19-69.57 (7C, complex, Ins $C_{1,2}$ and Fm methylene), 72.55 (Ins C_3), 75.70 (m, Ins C_4), 76.23 (m, Ins C_5), 119.68-120.05 (12C, complex, Fm C_5), 124.61-125.37 (12C, complex, Fm C_2), 126.90-128.79 (24C, complex, Fm $C_{3,4}$), 141.12-141.36 (12C, complex, Fm C_6), 142.64-143.19 (12C, complex, Fm C_1), 172.61 (CO), 207.63 (CO); ^{31}P NMR (109 MHz) δ -1.75, -0.66, -0.49; $[\alpha]_D^{23}$ +1.3° (*c* 23.1, $CHCl_3$); *Anal.* Calc. for $C_{95}H_{82}O_{17}P_3 \cdot 3/2CHCl_3$: C, 65.61; H, 4.71%. Found: C, 65.95; H, 4.77%.

1D-3,4,5-Tri-*O*-[di(9-fluorenylmethyl) phosphoryl]-6-*O*-levulinoyl-1-*O*-triethylsilyl-*myo*-inositol (11). To a solution of **9** (2.75 g, 1.7 mmol) in $CHCl_3$ (30 mL) were added 2,6-di-*t*-butyl-4-methylpyridine (0.90 g, 4.4 mmol) and triethylsilyl trifluoromethanesulfonate (2.45 g, 9.3 mmol), and the mixture was stirred for 2 h between -42 and -30 °C. The solution was treated with methanol for 30 min to destroy an excess of the triflate and AcOEt was added. The organic solution was washed sequentially with H_2O, saturated $KHSO_4$ solution, H_2O, saturated $NaHCO_3$ solution, and saturated NaCl solution, dried, filtered, and evaporated. Chromatography of the residue (EA/$CHCl_3$, 1:10 then acetone/$CHCl_3$, 1:2) gave silyl ether **11** (2.21 g, 75%) and the starting material **9** (0.42 g, 14%): R_f=0.65 (acetone/$CHCl_3$, 1:5); ^1H NMR (270 MHz) δ 0.58 (6H, q, *J*=7.9 Hz, Si CH_2), 0.91 (9H, t, *J*=7.9 Hz, Si CH_3), 1.98 (3H, s, lev CH_3), 2.25-2.62 [4H, complex, lev $(CH_2)_2$], 3.60 (1H, dd, *J*=9.8 and 2.7 Hz, Ins H_1), 3.87-4.38 [21H, complex, Ins $H_{2,3,5}$, and

Fm (CH_2CH)x6], 4.97 (1H, q, *J*=9.8 Hz, Ins H_4), 5.38 (1H, t, *J*=9.8 Hz, Ins H_6), 6.91-7.72 (48H, complex, aromatic H); ^{13}C NMR (100 MHz) δ 4.70 (3C, Si CH_3), 6.56 & 6.62 (3C, Si CH_2), 27.85 (lev C_2), 29.78 (lev CH_3), 37.39 (lev C_3), 47.52, 47.65, & 47.77 (6C, Fm methine), 69.33-69.79 (7C, complex, Ins C_6 and Fm methylene), 70.29 (Ins C_2), 70.85 (Ins C_1), 72.09 (Ins C_3), 75.15 (m, Ins C_4), 75.72 (br, Ins C_5), 119.66-119.82 (12C, Fm C_5), 124.96-125.16 (12C, Fm C_2), 126.88-127.75 (24C, Fm $C_{3,4}$), 141.10-141.28 (12C, Fm C_6), 142.72-143.10 (12C, Fm C_1), 171.98 (CO), 206.09 (CO); ^{31}P NMR (109 MHz) δ -1.71, -1.29, -1.15; $[\alpha]_D^{23}$ +2.3° (*c* 2.45, $CHCl_3$).

1D-2-*O*-Chloroacetyl-3,4,5-tri-*O*-[di(9-fluorenylmethyl) phosphoryl]-6-*O*-levulinoyl-1-*O*-triethylsilyl-*myo*-inositol (12). After addition of pyridine (650 μL, 8.0 mmol), chloroacetic anhydride (0.99 g, 5.8 mmol), and then 4-dimethylaminopyridine (30.5 mg, 0.3 mmol) at 0 °C to a solution of **11** (1.95 g, 1.2 mmol) in $CHCl_3$ (20 mL), the mixture was stirred for 30 min at rt, and AcOEt was added. The organic layer was treated by a usual procedure and **12** (1.65 g, 81%) was isolated by SiO_2-chromatography (EA/$CHCl_3$, 1:8): R_f=0.7 (AcOEt/$CHCl_3$,1:3); ^1H NMR (270 MHz) δ 0.62 (6H, q, *J*=8.2 Hz, Si CH_2), 0.92 (9H, t, *J*=8.2 Hz, Si CH_3), 2.20 (3H, s, lev CH_3), 2.60 (2H, t, *J*=6.1 Hz, lev C_2), 2.87 (2H, t, *J*=6.1 Hz, lev C_3), 3.69 (1H, dd, *J*=9.8 and 2.3 Hz, Ins H_1), 3.77-4.41 [22H, complex, Ins $H_{3,5}$, Fm (CH_2CH)x6, and CH_2Cl], 4.76 (1H, q, *J*=9.8 Hz, Ins H_4), 5.29 (1H, t, *J*=9.8 Hz, Ins H_6), 5.74 (1H, t, *J*=2.3 Hz, Ins H_2), 7.00-7.65 (48H, complex, aromatic H); ^{31}P NMR (109 MHz) δ -0.10, -0.08, -0.03; $[\alpha]_D^{23}$ +7.6° (*c* 1.91, $CHCl_3$); *Anal.* Calc. for $C_{103}H_{96}ClO_{18}P_3Si{\cdot}CHCl_3$: C, 65.82; H, 5.15%. Found: C, 65.81; H, 5.31% .

1D-2-*O*-Chloroacetyl-3,4,5-tri-*O*-[di(9-fluorenylmethyl) phosphoryl]-6-*O*-levulinoyl-*myo*-inositol (13). After addition of 80% aqueous acetic acid (9.0 mL) and *p*-toluenesulfonic acid hydrate (0.76 g, 4.0 mmol) to a solution of **12** (5.3 g, 3.0 mmol) in $CHCl_3$ (4.5 mL) at 0 °C, the mixture was stirred for 5 h at rt and AcOEt was added. The organic solution was washed continuously with H_2O and then with saturated $NaHCO_3$ solution and saturated NaCl solution, dried, filtered, and evaporated. The residue was chromatographed (SiO_2, acetone/$CHCl_3$, 1:5) to give **13** (4.49 g, 90%): R_f=0.4 (acetone/$CHCl_3$, 1:3); ^1H NMR (270 MHz) δ 2.09 (3H, s, CH_3), 2.18-2.78 [4H, complex, lev $(CH_2)_2$], 3.56 (1H, *J*=9.8 and 2.4 Hz, Ins H_1), 3.64 (1H, br, OH), 3.79-4.41 [22H, complex, Ins $H_{3,5}$, Fm (CH_2CH)x6, and CH_2Cl], 4.75 (1H, q, *J*=9.8 Hz, Ins H_4), 5.28 (1H, t, *J*=9.8 Hz, Ins H_6), 5.61 (1H, t, *J*=2.4 Hz, Ins H_2), 6.99-7.72 (48H, complex, aromatic H); ^{13}C NMR (68 MHz) δ 28.07 (α-CH_2), 29.62 (CH3), 38.25 (β-CH_2), 40.68 (CH2Cl), 47.42-47.75 (6C, complex, Fm methine), 67.94 (InsC6), 68.87 (d, *J*=5.5 Hz, Fm methylene), 69.26-69.63 (5C, complex, Fm methylene), 72.39 (InsC2), 72.55 (InsC1), 73.28 (InsC3), 75.17 (m, InsC4), 76.08 (InsC5), 119.70-119.90 (12C, Fm C5), 124.67-125.34 (12C, Fm C2), 126.75-127.95 (24C, Fm C3,4), 141.03-141.35 (12C, Fm C6), 142.60-143.22 (12C, Fm C1), 166.34 (CO), 172.35 (CO), 208.18 (CO); ^{31}P NMR (109 MHz) δ -0.90, -0.51, -0.21; $[\alpha]_D^{23}$ +5.5° (*c* 2.13, $CHCl_3$); *Anal.* Calc. for $C_{97}H_{82}ClO_{18}P_3$: C, 70.01; H, 4.97%. Found: C, 69.93; H, 5.14%.

1D-1-*O*-[(2-*O*-Arachidonoyl-1-*O*-stearoyl-*sn*-glycero)-2-cyanoethyloxyphosphoryl]-2-*O*-chloroacetyl-3,4,5-tri-*O*-[di-(9-fluorenylmethyloxy)phosphoryl]-6-*O*-levulinoyl-*myo*-inositol (15a). 2-*O*-Arachidonoyl-1-*O*-stearoyl-*sn*-glycerol 2-cyanoethyl *N,N*-diisopropylphosphoramidite (**14a**) was first prepared as follows: A solution of 2-*O*-arachidonoyl-1-*O*-stearoyl-*sn*-glycerol[23] (451 mg, 0.73 mmol) in ethyl ether (7 mL) was cooled to -78 °C, and then triethylamine (210 mg, 2.08 mmol) and chloro(2-cyanoethyloxy)diisopropylaminophosphine (236 mg, 1.0 mmol) were added. The solution was stirred at the same temperature for an additional 5 min and then at rt for 1 h. After addition of ethyl ether, the organic solution

was washed with saturated $NaHCO_3$ solution and saturated NaCl solution, dried, filtered, and evaporated. The residue was chromatographed eluting with Et_2O and hexane (1:3) including 5% of triethylamine to give amidite **14a** (469 mg, 79%): ^1H NMR (270 MHz) δ 0.88 & 0.89 (6H, tx2, *J*=6.8 Hz, ste and ara CH_3), 1.17 & 1.83 (6H, dx2, *J*=6.8 Hz, *i*Pr CH_3), 1.25 (34H, br, ste and ara CH_2), 1.60 (2H, br, ste H_3), 1.70 (2H, q, *J*=7.3 Hz, ara H_3), 2.0-2.16 (4H, complex, terminal allylic CH_2), 2.30, 2.33 & 2.34 (2H, tx3, *J*=7.3 Hz, ste and ara H_2), 2.63 (2H, t, *J*=6.3 Hz, CH_2CN), 2.73-2.90 (6H, complex, inner allylic CH_2), 3.50-3.90 (6H, complex, *i*Pr CH, gly *sn*-H_3, OCH_2), 4.15 & 4.18 (1H, ddx2, *J*=11.7 and 7.3 Hz, gly *sn*-H_1), 4.32 & 4.37 (1H, ddx2, *J*=11.7 and 3.7 Hz, gly *sn*-H_1), 5.19 (1H, br, gly *sn*-H_2), 5.28-5.45 (8H, complex, vinyl H); ^{31}P NMR (109 MHz) δ 150.72 (int, 100%), 150.57 (80%).

The amidite **14a** (469 mg, 0.57 mmol) and 1*H*-tetrazole (67 mg, 0.96 mmol) were added to a $CHCl_3$ (7 mL) solution of **13** (627 mg, 0.38 mmol), and the resulting clear solution was stirred for 2.5 h at rt, cooled to -78 °C and then *t*-butyl hydrogen peroxide (63 mg, 0.7 mmol) was added. After being stirred for 2 h at rt, the solution was treated with 10% aqueous Na_2SO_3 for 30 min and AcOEt was added. The organic layer was washed with H_2O, saturated $NaHCO_3$ solution, and saturated NaCl solution, dried, filtered, and evaporated. Chromatography of the residue (EA/$CHCl_3$, 1:4 then acetone/$CHCl_3$, 1:4) afforded **15a** (713 mg, 79%): R_f=0.4 (Acetone/$CHCl_3$, 1:4); ^1H NMR (270 MHz) 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 H_3), 1.67 (2H, m, ara H_3), 1.99 & 2.00 (3H, sx2, lev CH_3), 2.00-2.08 (4H, complex, terminal allylic CH_2), 2.09-2.59 [8H, complex, ste and ara H_2 and lev $(CH_2)_2$], 2.69 (1H, t, *J*=7.8 Hz, CHCN), 2.80 (7H, complex, CHCN, inner allylic CH_2), 3.72-4.38 [28H, complex, Ins $H_{3,5}$, gly *sn*-$H_{1,3}$, Fm (CH_2CH)x6, OCH_2, -CH_2Cl], 4.47 (1H, m, Ins H_1), 4.71 (1H, m, Ins H_4), 5.20-5.44 (10H, complex, InsH_6, gly *sn*-H_2, vinyl H), 5.96 & 5.98 (1H, tx2, *J*=3.9 Hz, Ins H_2), 7.01-7.74 (48H, complex, aromatic H); ^{13}C NMR (68 MHz) δ 14.00 & 14.05 (2C, ste and ara CH_3), 19.27 & 19.38 (CCN), 20.48 & 20.61 (2C, ste and ara CCH_3), 24.59, 24.64 & 24.72 (2C, ste and ara C_3), 25.53, 26.39, & 27.13 (5C, allylic C), 27.14 & 27.32 (lev C_2), 29.04-29.75 (13C, complex, ste and ara CH_2 and lev CH_3), 31.41 (ara C_{18}), 31.83 (ste C_{16}), 33.43, 33.82, & 33.87 (2C, ste and ara C_2), 36.87 & 37.02 (lev C_3), 40.38 (CCl), 47.55 & 47.67 (6C, dx2, *J*=7.3 Hz, Fm methine), 61.50 (m, OCH_2), 62.52 & 62.88 (m, gly *sn*-C_1), 66.27 & 66.63 (m, gly *sn*-C_3), 69.07-69.84 (8C, complex, InsC_6, gly *sn*-C_2, Fm methylene), 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 & 206.31 (CO); ^{31}P NMR (109 MHz) δ -1.69 (1/2P), -1.59 (1/2P), -1.32 (1P), -0.86 (2P); *Anal.* Calc. for $C_{141}H_{156}ClNO_{25}P_4 \cdot 2H_2O$: C, 68.84; H, 6.56; N, 0.57%. Found: C, 68.64; H, 6.52; N, 0.72%.

DL-2-*O*-Chloroacetyl-3,4,5-tri-*O*-[di-(9-fluorenylmethyloxy)phosphoryl]-6-*O*-levulinoyl-1-*O*-[(2-*O*-linolenoyl-1-*O*-stearoyl-*sn*-glycero)-2-cyanoethyloxyphosphoryl]-*myo*-inositol (15b). First, the corresponding phosphoramidite **14b** was prepared as described above for **14a** in 84% and the same phosphitylation and subsequent oxidation afforded 1-*O*-phosphorylation product **15b** in 72% yield: ^1H NMR (270 MHz) δ 0.88 (3H, t, *J*=6.7 Hz, ste CH_3), 0.97 (3H, t, *J*=7.6 Hz, lin CH_3), 1.25 (36H, br, ste and lin CH_2), 1.57 (4H, br, ste and lin H_3), 1.99 & 2.00 (3H, sx2, lev CH_3), 2.00-2.09 (4H, complex, terminal allylic H), 2.20-2.72 [10H, complex, ste and lin H_2, lev $(CH_2)_2$, CH_2CN], 2.79 (4H, br, inner allylic H), 3.68-4.38 (26H, complex, Ins $H_{3,5}$, gly *sn*-$H_{1,3}$, Fm (CH_2CH)x6, and OCH_2), 4.43 (1H, m, Ins H_1), 4.69 (1H, m,

Ins H_4), 5.20-5.41 (8H, complex, Ins H_6, gly *sn*-H_2, vinyl H), 5.97 (1H, m, Ins H_2), 7.03-7.70 (48H, complex, aromatic H); ^{13}C NMR (68 MHz) δ 14.05 & 14.12 (2C, ste and lin C_{18}), 19.25 & 19.36 (CCN), 20.45 (lin C_{17}), 22.60 (ste C_{17}), 24.69 (2C, ste and lin C_3), 25.45 (2C, inner allylic C), 27.12 (2C, terminal allylic C), 28.96-29.74 (18C, complex, ste and lin CH_2 and lev $C_{2,5}$), 31.83 (ste C_{16}), 33.84 & 33.98 (2C, ste and lin C_2), 36.85 &3 7.00 (lev C_3), 40.38 (CCl), 47.58 & 47.65 (6C, dx2, *J*=7.3 Hz, Fm methine), 61.54 (m, OCH_2), 62.52 & 62.89 (mx2, gly *sn*-C_1), 66.31 & 66.62 (mx2, gly *sn*-C_3), 69.02-69.67 (8C, complex, Ins C_6, gly *sn*-C_2, Fm methylene), 71.02 (Ins C_2), 72.3 (m, Ins C_3), 72.65 (m, Ins C_1), 74.40 (m, Ins C_4), 75.32 (m, Ins C_5), 116.39 & 116.46 (CN), 119.72-120.13 (12C, complex, Fm C_5), 124.46-125.30 (13C, complex, Fm C_2 and vinyl C), 126.80-127.71 (25C, complex, Fm $C_{3,4}$, vinyl C), 128.18, 128.41, 130.14, & 131.83 (4C, vinyl C), 141.09-141.30 (12C, complex, Fm C_6), 142.55-143.25 (12C, complex, Fm C_1), 166.14, 166.15, 166.26, & 166.58 (chloroac CO), 171.57 & 171.90 (lev CO), 172.75 & 173.15 (2C, ste and lin CO), 205.86 & 206.31 (CO); ^{31}P NMR (109 MHz) δ -1.84 (1/2P), -1.70 (1/2P), -1.47 (1P) -1.09 (1P), -1.01 (1P).

DL-2-*O*-Chloroacetyl-3,4,5-tri-*O*-[di-(9-fluorenylmethyloxy)phosphoryl]-6-*O*-levulinoyl-1-*O*-[(1,2-di-*O*-stearoyl-*sn*-glycero)-2-cyanoethyloxyphosphoryl]-*myo*-inositol (15c). The same procedure as above except for the use of mCPBA instead of *t*-BuO_2H gave **15c** in 88% yield (96% yield for amidite **14c**): R_f=0.5 (Acetone/$CHCl_3$, 1:5); ^1H NMR (270 MHz) δ 0.88 (3H, t, *J*=6.6 Hz, ste H_{18}), 1.25 (56H, br, ste CH_2), 1.57 (4H, br, H_3), 1.99 & 2.00 (3H, sx2, lev CH_3), 2.16-2.58 [8H, complex, ste H_2 and lev $(CH_2)_2$], 2.69 & 2.81 (2H, tx2, *J*=6.1 Hz, CH_2CN), 3.74-4.38 [26H, complex, Ins $H_{3,5}$, gly *sn*-$H_{1,2}$, Fm (CH_2CH)x6, and OCH_2], 4.46 (1H, m, Ins H_1), 4.74 (1H, m, Ins H4), 5.30 (2H, complex, Ins H_6, and gly *sn*-H_2), 5.98 (1H, m, Ins H_2), 6.97-7.76 (48H, complex, aromatic H); ^{31}P NMR (109 MHz) δ -0.78 (1/2P), -0.67 (1/2P), -0.45 (1P), -0.06 (2P)

Deprotection of 15a (two steps). To an CH_3CN (4.5 mL) solution of **15a** (438 mg, 0.18 mmol) was added triethylamine (741 mg, 7.32 mmol) at 0 °C and the mixture was stirred for 14 h at rt. To remove benzoflubene formed by β-elimination of Fm, the residue obtained by evaporating the reaction mixture at rt was washed with hexane (3 mL each, ten times) and then AcOEt (3 mL each, ten times) to give chromatographically (SiO_2) and spectroscopically (^1H-, ^{31}P-, and ^{13}C-NMR) pure **16a** (286 mg, 93%): R_f=0.4 ($CHCl_3$/acetone/CH_3OH/AcOH/H_2O, 25:12:13:7:10); ^1H NMR (270 MHz, partial) δ 0.77 (6H, br, ara and ste CH_3), 2.97 (24H, br, NCH_2), 5.10 (1H, br, gly *sn*-H_2), 5.24(9H, br, Ins H_6, vinyl H), 5.80 (1H, br, Ins H_2); ^{13}C NMR (68 MHz) δ 7.77 (12C, CH_3 in Et_3N), 13.43 (2C, ara and ste CH_3), 22.03 (ara C_{19}), 22.15 (ste C_{19}), 24.35 (2C, ara and ste C_3), 25.10, 25.99, & 26.69 (5C, allylic C), 27.70 (lev C_2), 28.64, 28.82, 29.00, & 29.16 (14C, ara and ste CH_2 and lev CH_3), 31.00 (ara C_{18}), 31.40 (ste C_{16}), 33.13 (ara C_2), 33.52 (ste C_2), 37.43 (lev C_3), 40.31 (CCl), 45.14 (12C, CH_2 in Et_3N), 62.36 (br, gly *sn*-C_1), 63.36 (br, gly *sn*-C_3), 70.16 (br, gly *sn*-C_2), 70.83 (br, Ins C_6), 71.11 (br, Ins C_2), 72.09 (Ins C_3), 73.60 (Ins C_1), 75.46 (Ins C_4), 76.19 (Ins C_5), 127.04, 127.30, 127.57, 127.75, 128.06, 128.36, 128.68, & 129.91 (8C, vinyl C), 165.92 (chloroac CO), 172.01 (lev CO), 172.65 (ara CO), 173.31 (ste CO), 208.17 (br, CO); ^{31}P NMR [109 MHz, 65.8 mg, $CDCl_3$ (2.4 mL) + CD_3OD (0.4 mL) + NEt_3 (0.4 mL)] δ -0.62, 0.06, 0.30, 2.30.

A solution of **16a** (85.6 mg, 0.05 mmol) in benzene was evaporated to remove azeotropically a trace of water and CH_2Cl_2 (1 mL) was added. After addition of an EtOH suspension of ethyldiisopropylammonium hydrazinedithiocarbonate (HDTC)[19] (1.02 mmol) at 0 °C, which was prepared according to a modified procedure,[5] the mixture was stirred for 2.5 h at rt, cooled to 0 °C, and then acidified with a $KHSO_4$ aqueous

solution. The mixture was extracted with chloroform twice and the combined organic extracts were dried and filtered. After addition of a small amount of triethylamine to the filtrate to form salts with phosphoric esters, the solution was evaporated and the residue was washed with AcOEt to afford *sn*-2-arachidonoyl-*sn*-1-stearoyl-PI(3,4,5)P3 (58.3 mg, 73%): R_f=0.3 ($CHCl_3$/acetone/CH_3OH/AcOH/H_2O, 25:12:13:7:10); ^1H NMR [270 MHz, 60.3 mg, $CDCl_3$ (1.2 mL) + CD_3OD (0.2 ml), partial] δ 0.95 (6H, complex, ara and ste CH_3), 1.58 (4H, br, ara and ste H_3), 2.14 (4H, m, allylic H), 2.29 (4H, m, ara and ste CH_2), 3.23 (42H, br, NCH_2), 3.96-4.65 (10H, complex, gly *sn*-$H_{1,3}$ and Ins $H_{1,2,3,4,5,6}$), 5.21 (1H, br, gly *sn*-H_2), 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 C_{19}), 22.26 (ste C_{17}), 24.42 (ara C_3), 24.48 (ste C_3), 25.21, 26.12, & 26.82 (5C, allylic C), 28.78, 28.94, 29.12, & 29.29 (13C, ara and ste CH_2), 31.12 (ara C_{18}), 31.52 (ste C_{16}), 33.29 (ara C_2), 33.68 (ste C_2), 45.93 (21C, NC), 62.40 (br, gly *sn*-C_1), 63.71 (br, gly *sn*-C_3), 69.91 (Ins C_2), 70.14 (m, gly *sn*-C_2), 70.58 (br, Ins C_6), 74.95 (brd, *J*=3.2 Hz, Ins C_3), 75.25 (br, Ins C_1), 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, 60.3 mg, CDCl3 (2.4 mL) + CD_3OD (0.4 mL) + NEt_3 (0.4 ml)] δ -0.52, 0.81, 1.88, 2.86; MASS (FAB^-, diethanolamine) *m/z* 1125 [M($C_{47}H_{86}O_{22}P_4$)-1].

Deprotection of 15b (two steps). The procedure was carried out as for **15a**: **16b**: R_f=0.5 ($CHCl_3$/acetone/CH_3OH/AcOH/H_2O, 25:12:13:7:10); ^{31}P NMR [109 MHz, 57.1 mg, $CDCl_3$ (2.4 mL) + CD_3OD (0.3 mL) + NEt_3 (0.4 mL)] δ -0.24 (1P), 1.21 (1P), 1.67 (1P), 3.64 (1P).

***sn*-2-Linolenoyl-*sn*-1-stearoyl-PI(3,4,5)P3:** R_f=0.3 ($CHCl_3$/acetone/CH_3OH/AcOH/H_2O, 25:12:13:7:10); ^1H NMR (270 MHz, $CDCl_3$/CD_3OD, 8:1) δ 0.95 (3H, , br, ste CH_3), 1.05 (3H, br, lin CH_3), 1.38 (99H, br, lin and ste CH_2 and CH_3 in Et_3N), 1.65 (4H, br, lin and ste H_3), 2.14 (4H, br, allylic H), 2.37 (4H, br, lin and ste H_2), 2.88 (4H, br, inner allylic CH_2), 3.23 (42H, br, NCH_2), 4.02-4.74 (10H, complex, gly *sn*-$H_{1,3}$ and Ins $H_{1,2,3,4,5,6}$), 5.30 (1H, br, gly *sn*-H_2), 5.42 (6H, br, vinyl H); ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD, 8:1) δ 8.14 (21C, CH_3 in Et_3N), 13.77 & 13.92 (2C, lin and ste CH_3), 20.24 (lin C_{17}), 22.38 (ste C_{17}), 24.59 & 24.61 (2C, lin and ste C_3), 25.22 & 25.32 (2C, inner allylic C), 26.93 (terminal allylic C), 28.83, 28.90, 29.00, 29.07, 29.15, 29.25, 29.41, & 29.51 (16C, lin and ste CH_2), 31.63 (ste C_{16}), 33.80 & 33.95 (2C, lin and ste C_2), 45.65 (21C, NC), 62.56 (br, gly *sn*-C_1), 63.59 (m, gly *sn*-C_3), 70.28 (2C, complex, Ins C_2 and gly *sn*-C_2), 70.75 (Ins C_6), 75.03 (Ins C_3), 75.53 (Ins C_1), 76.53 (Ins C_4), 78.86 (Ins C_5), 126.81, 127.47, 128.92, 128.01, 129.91, & 131.66 (6C, vinyl C), 173.05, 173.07, 173.45, & 173.49 (2C, lin and ste CO); ^{31}P NMR [109 MHz, 58.2 mg, $CDCl_3$ (2.4 mL) + CD_3OD (0.4 mL) + Et_3N (0.4 mL)] δ 0.56 (1P), 1.72 (1P), 2.88 (1P), 3.90 & 3.93 (1P); MASS (FAB^-, diethanolamine) *m/z* 1099 [M($C_{45}H_{84}O_{22}P_4$)-1].

Deprotection of 15c (three steps): 16c. ^1H NMR (270 MHz, $CDCl_3$/CD_3OD, 8:1, partial) δ 2.13 (3H, s, lev CH_3), 3.09 (24H, br, NCH_2), 3.93 (2H, br, gly *sn*-H_3), 4.18 (2H, br, gly *sn*-H_1), 4.73 (1H, br, Ins H_4), 5.19 (1H, br, gly *sn*-H_2), 5.38 (1H, br, Ins H_6), 6.02 (1H, br, Ins H_2); ^{31}P NMR [109 MHz, 82.6 mg, $CDCl_3$ (2.4 mL) + CD_3OD (0.3 mL)] δ -0.30 (1/2P), -0.18 (1/2P), 0.58 (1P), 1.30 (1P), 2.51 (1P).

The phosphate-deprotected product **16c** (48.7 mg, 0.03 mmol) obtained in 88% by treatment with triethylamine as above was dissolved in $CHCl_3$ (1.0 mL) and cooled to 0 °C and an EtOH suspension of HDTC (0.64 mmol) was added. The mixture was stirred for 4.5 h at rt and again cooled to 0 °C. After a work-up procedure similar to that as above, triethylammonium salt was dissolved in a small amount of $CHCl_3$ and CH_3CN was added to induce precipitation. Washing the precipitate with CH_3CN gave 2-free product (31.4 mg, 68%): ^{31}P NMR [109

MHz, 31.4 mg, $CDCl_3$ (2.4 mL) + CD_3OD (0.3 mL)] δ -0.30 (1/2P), -0.18 (1/2P), 0.58 (1P), 1.30 (1P), 2.51 (1P).

The final procedure for removing the 6-levulinoyl group was done according to the reported one,[5,21] to give distearoyl-PI(3,4,5)P_3 in 78% yield. Characterization of the compound was done by comparison with data of PI(3,4,5)P_3 already obtained by a different route:[5] R_f=0.4 ($CHCl_3$/acetone/CH_3OH/AcOH/H_2O, 25:12:13:7:10); ^{31}P NMR [109 MHz, 20.6 mg, $CDCl_3$ (2.4 mL) + CD_3OD (0.3 mL)] δ 0.98 & 1.01 (1P), 1.53 (1P), 2.36 (1P), 3.68 (1P).

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