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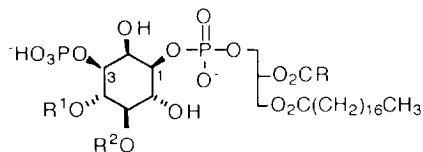
SYNTHESIS OF 1*D*-DISTEAROYLPHOSPHATIDYL-*MYO*-INOSITOL  
3,4,5-TRIS(DIHYDROGEN PHOSPHATE)

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**Abstract:** Distearoylphosphatidyl-*myo*-inositol 3,4,5-tris(dihydrogen phosphate) (PIP<sub>3</sub>) with the natural configuration was synthesized concisely by regioselective protection and phosphorylation starting from enzymatically resolved 1*D*-1,2-cyclohexylidene-*myo*-inositol.

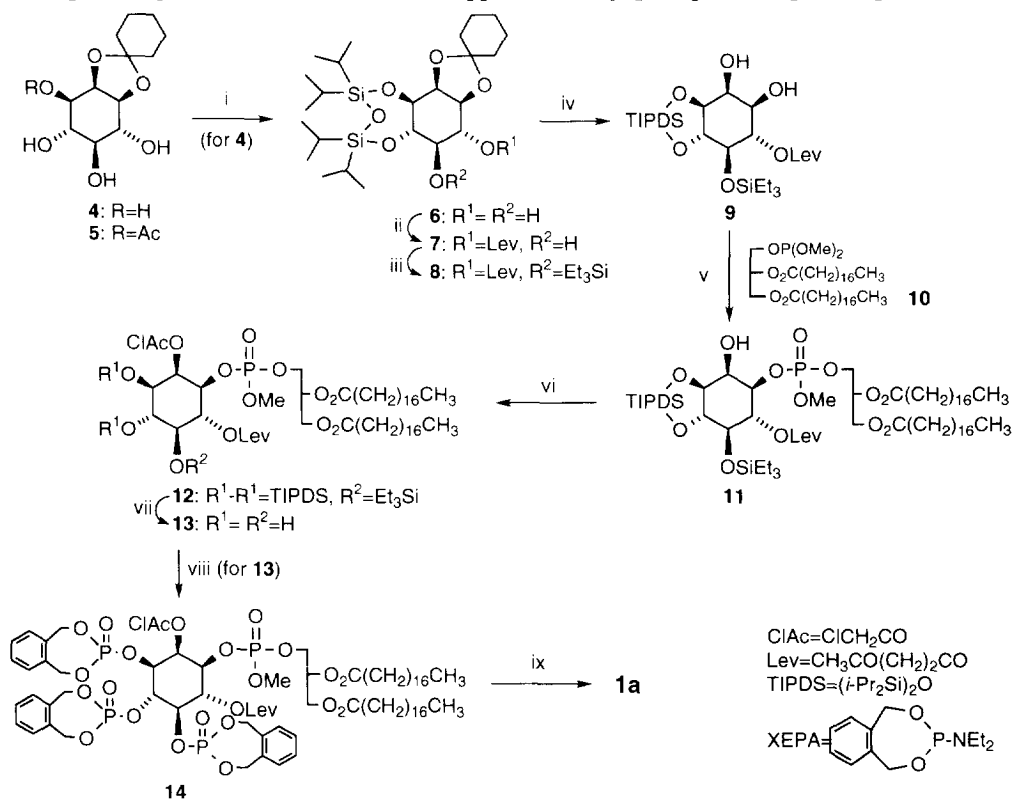
Phosphatidylinositols **1**, **2**, and **3** bearing the phosphate function at the 3 position are formed in a plasma membrane from the corresponding 3-*O*-unsubstituted derivatives by phosphatidylinositol 3-kinase,<sup>1</sup> the activation of which is closely associated with receptors of growth factors linked to the activation of tyrosine kinase.<sup>2</sup> Thus, these lipids are supposed to be connected with cell proliferation and oncogenesis.<sup>3</sup> Their chemical synthesis and supply have been required for biological investigation of their role because of their difficult acquisition from nature. Falck presented the synthesis of distearoyl analog **1a** of natural **1** using (-)-quinic acid as the chiral starting material for the construction of the *myo*-inositol skeleton at the National Meeting of the American Chemical Society in 1990.<sup>4</sup> We also recently reported the synthesis of **1a** where the inositol moiety was racemic.<sup>5</sup> More recently, Gou and Chen have completed the synthesis of **1b** using enzymatically resolved 1*D*-1,2:5,6-dicyclohexylidene-*myo*-inositol.<sup>6</sup> Furthermore, Bruzik and Kubiak synthesized the dipalmitoyl analogs of **1**, **2**, and **3** starting from the camphor ketal of *myo*-inositol.<sup>7</sup> As part of a program directed towards the preparation of 3-*O*-phosphorylated phosphatidylinositols, we describe here the synthesis of **1a** in an optically active form starting from a pivotal synthetic intermediate **6** which was also employed in the previous synthesis of **1a**.<sup>5</sup> In the present strategy, the phosphatidyl group at C-1 was introduced earlier than the three phosphoric monoester functions at C-3, -4, and -5 and differing from our old methodology<sup>5</sup> where the protected monoesters were first constructed.



- 1:** R<sup>1</sup>=R<sup>2</sup>=PO<sub>3</sub>H<sup>-</sup>, R=(CH<sub>2</sub>)<sub>2</sub>(CH<sub>2</sub>CH=CH)<sub>4</sub>(CH<sub>2</sub>)<sub>4</sub>CH<sub>3</sub>  
**1a:** R<sup>1</sup>=R<sup>2</sup>=PO<sub>3</sub>H<sup>-</sup>, R=(CH<sub>2</sub>)<sub>16</sub>CH<sub>3</sub>  
**1b:** R<sup>1</sup>=R<sup>2</sup>=PO<sub>3</sub>H<sup>-</sup>, R=(CH<sub>2</sub>)<sub>14</sub>CH<sub>3</sub>  
**2:** R<sup>1</sup>=PO<sub>3</sub>H<sup>-</sup>, R<sup>2</sup>=H, R=(CH<sub>2</sub>)<sub>2</sub>(CH<sub>2</sub>CH=CH)<sub>4</sub>(CH<sub>2</sub>)<sub>4</sub>CH<sub>3</sub>  
**3:** R<sup>1</sup>=R<sup>2</sup>=H, R=(CH<sub>2</sub>)<sub>2</sub>(CH<sub>2</sub>CH=CH)<sub>4</sub>(CH<sub>2</sub>)<sub>4</sub>CH<sub>3</sub>

## Results and Discussion

Optically active 1,2-*O*-cyclohexylidene-D-*myo*-inositol (**4**, 96% ee), which is now readily accessible by the enzymatic enantio- and regioselective acetylation of racemic **4** at the 3 position leading to **5** and subsequent deacetylation,<sup>8</sup> was converted into **7** in two steps in a completely regioselective manner as reported in the case of the racemic derivative.<sup>5</sup> After triethylsilylation of **7** (quant.), the cyclohexylidene group was removed carefully by treatment with limited amounts of distilled ethylene glycol (1.2 equiv) and anhydrous *p*-toluenesulfonic acid (0.05 equiv) in purified chloroform. The resulting diol **9** was successfully converted to the 1-phosphate **11** in a quantitative yield by the regioselective phosphorylation via the phosphite-phosphonium salt approach<sup>9</sup> employing glyceryl dimethyl phosphite **10**. The phosphorylation site could not be determined at this stage because signals corresponding to H-1 and -2 overlapped four glyceryl methylene protons at 4.1-



- i) TIPDS-Cl<sub>2</sub>, Py (94%); ii) Lev-OH, DCC, DMAP (84%); iii) Et<sub>3</sub>SiCl, Et<sub>3</sub>N (100%); iv) (CH<sub>2</sub>OH)<sub>2</sub>, TsOH (73%),  
 v) PyHBr<sub>3</sub>, Et<sub>3</sub>N (96%); vi) (ClCH<sub>2</sub>CO)<sub>2</sub>O, 1,8-bis-(dimethylamino)naphthalene (100%); vii) 47% aq. HF (69%); viii)  
 XEPA, tetrazole then mCPBA (89%); ix) 1. H<sub>2</sub>, 5% Pd-C, 2. H<sub>2</sub>NNHC(S)S<sup>-</sup>HEt<sup>i</sup>-Pr<sub>2</sub>N<sup>+</sup>, 3. PhSH, Et<sub>3</sub>N,  
 4. NH<sub>2</sub>NH<sub>2</sub> (21%)

4.4 ppm. The problem was solved by an NMR analysis of the 2-*O*-chloroacetyl derivative **12** obtained during the next stage. Thus, in the  $^1\text{H}$  NMR spectra, two diastereomeric triplets for H-2 (5.62 and 5.63 ppm) clearly appeared at about 1 ppm lower field than those of **10**, suggesting that the phosphorylation occurred at C-1. Chloroacetylation of **10** was performed by the reaction with chloroacetic anhydride in the presence of 1,8-bis(dimethylamino)naphthalene to give 2-*O*-chloroacetate **12** quantitatively. This strong base was essential to prevent decomposition of the triethylsilyl ether and migration of the phosphatidyl moiety in **11** which were conducted presumably by the chloroacetic acid generated during the reaction. If the hydroxyl group at C-2 could be protected with the same 4-oxopentanoyl (levulinoyl) group as that at C-6 in place of the chloroacetyl, both hydroxyls would be deprotected simultaneously. However, levulinoylation was unsuccessful. The silyl protecting groups in **12** were removed with aqueous HF solution in acetonitrile to afford triol **13** which was then phosphorylated by using a cyclic amidite, XEPA (*o*-xylylene *N,N*-diethylphosphoramidite)<sup>10</sup> to afford the fully protected derivative **14**. During the final stage, **14** was deprotected by the following four-step procedure; the catalytic hydrogenation on Pd-C, removal of the chloroacetyl with the ethyldiisopropylammonium salt of hydrazinodithio-carbonate,<sup>11</sup> demethylation of the methyl phosphate with phenylthiolate,<sup>12</sup> and then removal of the levulinoyl with hydrazine in acetic acid and pyridine,<sup>13</sup> giving the desired **1a**. The removal of the chloroacetyl was performed under anhydrous conditions to avoid the undesired reactions such as liberation of distearoyl glycerol which occurred in a reported aqueous medium ( $\text{H}_2\text{O}$ -EtOH-dioxane).<sup>11</sup> The dithiocarbonate reagent was also effective for the removal of the levulinoyl function in a simple derivative **7**, but in the real case, the reaction was not complete. The final product was isolated as the triethylammonium salt by precipitation from chloroform and methanol. Its structure was characterized by  $^{31}\text{P}$ - and  $^{13}\text{C}$ -NMR spectroscopic data and FAB-MS as well as a comparison on TLC with the diastereomeric specimen previously prepared<sup>5</sup> while  $^1\text{H}$ -NMR data did not provide a structurally useful information. In summary, an efficient access to the optically active synthetic intermediate **4**, regioselective introduction of protecting groups and the phosphoryl function at C-1 have produced a practical procedure for obtaining a phosphatidylinositol with saturated fatty acid moieties.

### Experimental

NMR spectra (JEOL JNM GSX270) were recorded in  $\text{CDCl}_3$  unless otherwise noted. As references for the  $^1\text{H}$ -,  $^{13}\text{C}$ -, and  $^{31}\text{P}$  NMR measurements, TMS ( $\delta=0.0$ ),

$\text{CDCl}_3$  ( $\delta=77.0$ ), and 85%  $\text{H}_3\text{PO}_4$  ( $\delta=0.0$ , external) were used, respectively.  $^{13}\text{C}$ - and  $^{31}\text{P}$  NMRs were all taken under  $^1\text{H}$ -decoupled conditions. IR spectra were recorded using a Horiba FT-IR FT-210. 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 Wako Pure Chemical Industries silica gel, Wakogel C-300. Thin layer chromatographic analyses were 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. Anhydrous solvents used were prepared in the usual manner. Extracts obtained after work-up were dried over  $\text{MgSO}_4$  or  $\text{Na}_2\text{SO}_4$ . 1D-1,2-Cyclohexylidene-*myo*-inositol **4** with 96% ee<sup>7</sup> was used.

**1D-1,2-O-Cyclohexylidene-3,4-O-(tetraisopropylidisiloxane-1,3-diyl)-*myo*-inositol (6).** Distillation of a pyridine solution of cyclohexylidene inositol **4** (7.09 g, 27 mmol) under reduced pressure was repeated three times to remove a trace of  $\text{H}_2\text{O}$ . Its pyridine (82 ml) solution was cooled at 0 °C and 1,3-dichloro-1,1,3,3-tetraisopropylidisiloxane (10.01 ml, 31.3 mmol) was added dropwise. The mixture was stirred for 13 h at room temperature and after addition of  $\text{H}_2\text{O}$ , poured into an ice-water solution. The mixture was extracted with ether and the extract was washed with a saturated aq.  $\text{KHSO}_4$  and aq.  $\text{NaHCO}_3$  solution, and then brine, dried, and evaporated. The residue was chromatographed on silica gel (EA-Hex 1:3) to afford **6** (12.89 g, 94%):  $R_f=0.3$  (EA-Hex 1:2);  $[\alpha]_D^{23} -14.3^\circ$  ( $c$  0.98,  $\text{CH}_2\text{Cl}_2$ );  $^1\text{H}$  NMR( $\text{CDCl}_3$ - $\text{D}_2\text{O}$ , 270 MHz)  $\delta$  0.94-1.15 (complex, 28 H), 1.25-1.80 (br, 10 H), 3.23 (dd, 1 H,  $J=10.23$  and 9.15 Hz), 3.67 (dd, 1 H,  $J=10.23$  and 7.48 Hz), 3.88 (complex, 2 H), 3.95 (dd, 1 H,  $J=7.48$  and 4.57 Hz), 4.21 (dd, 1 H,  $J=4.57$  and 3.67 Hz); IR(neat,  $\text{cm}^{-1}$ ) 3430; Anal. Calc. for  $\text{C}_{24}\text{H}_{46}\text{O}_7\text{Si}_2 \cdot 1/4\text{H}_2\text{O}$ : C, 56.82; H, 9.24%. Found: C, 56.83; H, 9.47%.

**1D-1,2-O-Cyclohexylidene-6-O-(4-oxopentanoyl)-3,4-O-(tetraisopropylidisiloxane-1,3-diyl)-*myo*-inositol (7).** A  $\text{CH}_2\text{Cl}_2$  (40 ml) solution of **6** (2.0 g, 3.98 mmol), *N,N'*-dicyclohexylcarbodiimide (1.07 g, 5.17 mmol), and 4-(dimethylamino)pyridine (DMAP, catalytic) was cooled at 0 °C and 4-oxopentanoic acid (0.51 ml, 4.97 mmol) was added. After being stirred overnight, the mixture was treated with  $\text{H}_2\text{O}$  for 20 min, filtered, concentrated, and dissolved in AcOEt. The solution was washed with a saturated  $\text{KHSO}_4$  solution,  $\text{NaHCO}_3$  solution, and then brine, dried, and evaporated. A column chromatography on silica gel ( $\text{CH}_2\text{Cl}_2$ -EA 25:1) gave ester **7** (2.01 g, 84%):  $R_f=0.5$  ( $\text{CH}_2\text{Cl}_2$ -EA 7:1);  $[\alpha]_D^{23} -24.0^\circ$  ( $c$  1.0,  $\text{CH}_2\text{Cl}_2$ );  $^1\text{H}$  NMR (270 MHz)  $\delta$  0.89-1.10 (complex, 28 H), 1.19-1.84 (br, 10 H), 2.12 (s, 3 H), 2.50-2.85 (complex, 4 H), 2.55 (d, 1 H,  $J=1.82$  Hz) 3.33 (ddd, 1 H,  $J=10.67$ , 8.85, and 1.82 Hz), 3.85 (dd, 1 H,  $J=8.85$  and 4.27 Hz), 3.94 (t, 1 H,  $J=8.85$  Hz), 3.99

(dd, 1 H,  $J=7.63$  and  $4.27$  Hz),  $4.24$  (t, 1 H,  $J=4.27$  Hz),  $5.09$  (dd, 1 H,  $J=10.67$  and  $7.63$ ); IR(neat,  $\text{cm}^{-1}$ )  $3500$ ,  $1710$ ; Anal. Calc. for  $\text{C}_{29}\text{H}_{52}\text{O}_9\text{Si}_2$ : C,  $57.97$ ; H,  $8.72\%$ . Found: C,  $57.60$ ; H,  $8.90\%$ .

**1*D*-1,2-*O*-Cyclohexylidene-6-*O*-(4-oxopentanoyl)-3,4-*O*-(tetraisopropyl)disiloxane-1,3-diyl)-5-*O*-triethylsilyl-*myo*-inositol (8).** To a  $\text{CH}_2\text{Cl}_2$  (2 ml) solution of **7** (101 mg, 0.166 mmol) and triethylamine (138  $\mu\text{l}$ , 0.98 mmol) was added triethylsilylchloride (150  $\mu\text{l}$ , 1.07 mmol) and a catalytic amount of DMAP at  $0^\circ\text{C}$  and the mixture was stirred overnight at room temperature. After addition of  $\text{H}_2\text{O}$  and AcOEt, the organic layer was washed successively with aq.  $\text{KHSO}_4$ ,  $\text{NaHCO}_3$ , and  $\text{NaCl}$  solutions, dried, and evaporated. The residue was subjected to  $\text{SiO}_2$ -chromatography (EA-Hex 1:7) to give the silyl ether **8** (133 mg, quant.):  $R_f=0.5$  (EA-Hex 1:5);  $[\alpha]_D^{20} -15.3^\circ$  ( $c$  1.4,  $\text{CH}_2\text{Cl}_2$ );  $^1\text{H}$  NMR (270 MHz)  $\delta$  0.59 (q, 6 H,  $J=7.93$  Hz), 0.93 (t, 9 H,  $J=7.93$  Hz), 1.06 (complex, 27 H), 1.60 (br, 10 H), 2.20 (s, 3 H), 2.67 (m, 2 H), 2.77 (m, 2 H), 3.45 (dd, 1 H,  $J=9.46$  and  $8.70$  Hz), 3.85 (dd, 1 H,  $J=8.70$  and  $3.96$  Hz), 3.93 (dd, 1 H,  $J=7.63$  and  $3.96$  Hz), 3.96 (t, 1 H,  $J=8.70$  and  $7.63$  Hz), 4.26 (t, 1 H,  $J=3.96$  Hz), 5.06 (dd, 1 H,  $J=9.46$  and  $7.63$  Hz); IR(neat,  $\text{cm}^{-1}$ )  $1749$ ,  $1722$ ; Anal. Calc. for  $\text{C}_{35}\text{H}_{66}\text{O}_9\text{Si}_3$ : C,  $58.78$ ; H,  $9.30\%$ . Found: C,  $58.72$ ; H,  $9.43\%$ .

**1*D*-6-*O*-(4-Oxopentanoyl)-3,4-*O*-(tetraisopropyl)disiloxane-1,3-diyl)-5-*O*-triethylsilyl-*myo*-inositol (9).** A solution of **8** (105 mg, 0.148 mmol), distilled ethyleneglycol (9.9  $\mu\text{l}$ , 0.179 mmol), and anhydrous TSOH (1.4 mg, 0.008 mmol) in  $\text{CHCl}_3$  (5 ml) which was purified by passing through a column of basic  $\text{Al}_2\text{O}_3$  (Merck) was stirred at room temperature for 1.1 h and AcOEt was added. The organic layer was washed with aq.  $\text{NaHCO}_3$  and  $\text{NaCl}$  solutions, dried, and chromatographed on silica gel (EA-Hex 1:1) to give diol **9** (68.7 mg, 73%):  $R_f=0.5$  (EA-Hex 1:1);  $[\alpha]_D^{23} -15.2^\circ$  ( $c$  1.05,  $\text{CH}_2\text{Cl}_2$ );  $^1\text{H}$  NMR (270 MHz)  $\delta$  0.61 (q, 6 H,  $J=7.94$  Hz), 0.94 (t, 9 H,  $J=7.94$  Hz), 1.05 (m, 28 H), 2.20 (s, 3 H), 2.67 (m, 2 H), 2.81 (m, 2 H), 3.47 (dd, 1 H,  $J=3.05$  and  $10.07$  Hz), 3.53 (dd, 1 H,  $J=8.85$  and  $9.46$  Hz), 3.59 (dd, 1 H,  $J=3.05$  and  $8.85$  Hz), 3.93 (t, 1 H,  $J=8.85$  Hz), 4.04 (t, 1 H,  $J=3.05$  Hz), 5.21 (dd, 1 H,  $J=10.07$  and  $9.46$  Hz); IR(neat,  $\text{cm}^{-1}$ )  $3440$ ,  $1737$ .

**1*D*-6-*O*-(4-Oxopentanoyl)-3,4-*O*-(tetraisopropyl)disiloxane-1,3-diyl)-5-*O*-triethylsilyl-*myo*-inositol 1-[(1,2-di-*O*-octadecanoyl-*sn*-glyceryl) methyl phosphate] (11).** A mixture of 1,2-di-*O*-octadecanoyl-*sn*-glycerol (583 mg, 0.93 mmol), dimethyl *N,N*-diethylphosphoramidite (277  $\mu\text{l}$ , 1.67 mmol), and 1*H*-tetrazole (131 mg, 1.87 mmol) in  $\text{CH}_2\text{Cl}_2$  (15 ml) was stirred for 30 min at room temperature. After addition of anhydrous ethyl ether, the organic solution was washed with a saturated aq.  $\text{NaCl}$  solution, dried, and evaporated. A benzene solution of **10** thus obtained was evaporated under reduced pressure to remove a trace of  $\text{H}_2\text{O}$  and after addition of diol **9** (198

mg, 0.311 mmol) the same azeotropic distillation procedure was again carried out. To the mixture was added  $\text{CH}_2\text{Cl}_2$  (5 ml), pyridine (0.5 ml), and triethylamine (216  $\mu\text{l}$ ) and the resulting solution was cooled to  $-20^\circ\text{C}$  by a dry ice- $\text{CCl}_4$  bath. After addition of pyridinium bromide perbromide (398 mg, 1.24 mmol) the mixture was stirred vigorously at the same temperature for 1 min and at  $0^\circ\text{C}$  for an additional 30 min. Ethyl acetate was added and the organic layer was washed with  $\text{H}_2\text{O}$ , aq.  $\text{KHSO}_4$ , aq.  $\text{NaHCO}_3$ , and brine, dried, and evaporated. The residue was chromatographed on silica gel (EA-Hex 2:3) to give the phosphate (412 mg, 96% yield):  $R_f=0.5$  (EA-Hex 2:3);  $^1\text{H NMR}$ (270 MHz)  $\delta$  0.59(q, 6 H,  $J=7.93$  Hz), 0.88 (t, 9 H,  $J=7.93$  Hz), 1.06 (m, 34 H), 1.25 (br, 56 H), 1.60 (br, 4 H), 1.75 (br, 1 H), 2.20 (s, 3 H), 2.30 (br, 4 H), 2.70 (complex, 4 H), 3.55 (t, 1 H,  $J=8.85$  Hz), 3.63 (dd, 1 H,  $J=2.75$  and 8.85 Hz), 3.77 & 3.79 (d, 3 H,  $J=11.29$  Hz), 3.97 (t, 1 H,  $J=8.85$  Hz), 4.1-4.4 (complex, 6 H), 5.38 (m, 1 H), 5.42 (t, 1 H,  $J=8.85$  Hz);  $^{31}\text{P NMR}$ (109 MHz)  $\delta$  -0.50 & -0.29 (diastereomeric); IR(neat,  $\text{cm}^{-1}$ ) 3400, 1740, 1720; Anal. Calc. for  $\text{C}_{69}\text{H}_{135}\text{O}_{16}\text{PSi}_3$ : C, 62.03; H, 10.24%. Found: C, 61.94; H, 10.32%.

**1D-2-Chloroacetyl-6-O-(4-oxopentanoyl)-3,4-O-(tetraisopropyl-disiloxane-1,3-diyl)-5-O-triethylsilyl-myo-inositol 1-[(1,2-di-octadecanoyl-sn-glyceryl) methyl phosphate] (12).** A mixture of phosphate 11 (314.7 mg, 0.23 mmol), 1,8-bis(dimethylamino)naphthalene (734 mg, 3.43 mmol), chloroacetic anhydride (1.17 g, 6.84 mmol), and DMAP (catalytic) in pyridine was stirred for 2.5 h at room temperature. After addition of AcOEt, the organic solution was washed with aq.  $\text{KHSO}_4$ ,  $\text{NaHCO}_3$  solutions, and brine, dried, and evaporated. The residue was chromatographed on silica gel (EA-Hex 1:3) to give chloroacetate (347 mg, 100%):  $R_f=0.5$  (EA-Hex 1:3);  $^1\text{H NMR}$  (270 MHz)  $\delta$  0.61 (q, 6 H,  $J=7.94$  Hz), 0.85-0.98 (complex, 15 H), 1.04 (m, 28 H), 1.25 (br, 56 H), 1.56 (br, 4 H), 2.20 (s, 3 H), 2.31 (m, 4 H), 2.73 (m, 4 H), 3.58 and 3.59 (t x 2, 1 H,  $J=9.07$  Hz, diastereomeric), 3.73 (d, 3 H,  $J=11.29$  Hz), 3.73 (m, 1 H), 3.91 (t, 1 H,  $J=8.85$  Hz), 4.14 (complex, 5 H), 4.31 (m, 1 H), 4.45 (m, 1 H), 5.21 (br, 1 H), 5.30 (t, 1 H,  $J=9.76$  Hz), 5.62 and 5.63 (t x 2, 1 H,  $J=2.75$  Hz, diastereomeric);  $^{31}\text{P NMR}$ (109 MHz)  $\delta$  0.095, 0.33 (diastereomeric); IR(neat,  $\text{cm}^{-1}$ ) 1745; Anal. Calc. for  $\text{C}_{71}\text{H}_{136}\text{O}_{17}\text{ClPSi}_3\cdot\text{H}_2\text{O}$ : C, 59.61; H, 9.72%. Found: C, 59.91; H, 9.63%.

**1D-2-Chloroacetyl-6-O-(4-oxopentanoyl)-myo-inositol 1-[(1,2-di-octadecanoyl-sn-glyceryl) methyl phosphate] (13).** To a solution of 2-chloroacetate 12 (65.5 mg, 0.045 mmol) in acetonitrile (2 ml) and THF (2 ml) was added 47% aq. hydrofluoric acid (0.2 ml) and the mixture was stirred for 16 h and evaporated. The residue was chromatographed on silica gel (EA) to give triol 13 (33.6 mg, 69%):  $R_f=0.2$  (EA);  $^1\text{H NMR}$ (270 MHz)  $\delta$  0.87 (t, 6 H,  $J=6.56$  Hz), 1.24 (br, 56 H), 1.27 (br, 4 H), 2.20 (s, 3 H), 2.30 (m, 4 H), 2.62 (m, 2 H), 2.81 (m, 2 H), 3.58 (complex, 1 H), 3.70 & 3.72 (d x 2, 3 H,

$J=11,29$  Hz, diastereomeric), 3.77 (complex, 1 H), 4.10 (complex, 1 H), 4.05-4.37 (complex, 4 H), 4.22 and 4.24 (s x 2, 2 H, diastereomeric), 4.58 (br, 1 H), 5.23 (br, 1 H), 5.25 (t, 1 H,  $J=9.86$  Hz), 5.72 (br, 1 H);  $^{31}\text{P}$  NMR(109 MHz)  $\delta$  -0.340, -0.19 (diastereomeric); IR(neat,  $\text{cm}^{-1}$ ) 1743; Anal. Calc. for  $\text{C}_{53}\text{H}_{96}\text{O}_{16}\text{ClP}\cdot 1.5\text{H}_2\text{O}$ : C, 58.90; H, 9.04%. Found: C, 58.70; H, 8.87%.

**1D-2-Chloroacetyl-6-O-(4-oxopentanoyl)-3,4,5-tri-O-(o-xylylene phosphoryl)-myo-inositol 1-[(1,2-di-O-octadecanoyl-sn-glyceryl methyl phosphate)] (14).** A  $\text{CH}_2\text{Cl}_2$  (2 ml) solution of triol **13** (88 mg, 0.08 mmol), XEPA (200 mg, 0.83 mmol), and 1H-tetrazole (117 mg, 1.67 mmol) was stirred for 25 min at room temperature and after addition of  $\text{H}_2\text{O}$  (ca. 10  $\mu\text{l}$ ) the mixture was further stirred for 15 min, cooled at  $-78^\circ\text{C}$ , and treated with mCPBA (216 mg, 1.25 mmol) for 1 min and then 30 min at ambient temperature. After addition of AcOEt, the organic layer was washed with 10% aq.  $\text{Na}_2\text{SO}_3$ , aq.  $\text{NaHCO}_3$ , and brine, dried, and evaporated. The residue was chromatographed on silica gel (EA-Hex 3:1) to give tetrakisphosphate (119 mg, 89%):  $R_f=0.3$  (EA-Hex 3:1);  $^1\text{H}$  NMR(270 MHz)  $\delta$  0.88 (t, 6 H,  $J=6.71$  Hz), 1.25 (br, 56 H), 1.59 (br, 4 H), 2.22 (s, 3 H), 2.33 (m, 4 H), 2.81 (m, 4 H), 3.78 & 3.80 (d x 2, 3 H,  $J=11.29$  Hz, diastereomeric), 4.1-4.4 (complex, 6 H), 4.63 (m, 1 H), 4.82-5.32 (complex, 12 H), 5.52 (complex, 5 H), 5.99 (br, 1 H), 7.35 (br, 12 H);  $^{31}\text{P}$  NMR (109 MHz)  $\delta$  -3.87, -3.33, -2.25 & -2.14 (diastereomeric), -0.41 & -0.06 (diastereomeric); IR( $\text{cm}^{-1}$ ) 1745; Anal. Calc. for  $\text{C}_{77}\text{H}_{117}\text{O}_{25}\text{ClP}_4\cdot 2\text{H}_2\text{O}$ : C, 56.45; H, 7.45%. Found: C, 56.23; H, 7.18%.

**1D-1-(1,2-O-Dioctadecanoyl-3-sn-phosphatidyl)-myo-inositol 3,4,5-tris(hydrogen phosphate) (1a).** To a AcOEt (3 ml) solution of the phosphate **14** (75.6 mg) was added 5% Pd-C (80 mg) and the mixture was stirred under  $\text{H}_2$  for 5 h at room temperature, filtered, and evaporated to dryness to give product (52.7 mg, 86%). A mixture of the phosphate (27.1 mg, 0.02 mmol), an ethanol suspension of ethyldiisopropylammonium hydrazinedithiocarbonate (1.2 ml, ca. 0.4 mmol. For its preparation, see below), and chloroform (0.5 ml) was stirred for 4 h at room temperature and after further addition of 0.6 ml of the ethanol solution (0.2 mmol) the reaction was continued for an additional 2 h. After evaporation of volatile materials under reduced pressure, its chloroform and methanol (2:1) solution was washed with 1N HCl solution and evaporated to give an oily residue which was purified by precipitation from chloroform and methanol. The solid in dioxane (0.5 ml) and chloroform (0.1 ml) was treated with thiophenol (37.5  $\mu\text{l}$ , 0.36 mmol) and triethylamine (0.5 ml) for 6 h at room temperature and the mixture was evaporated to dryness under reduced pressure. To the residue were added acetic acid (0.3 ml), pyridine (1.2 ml), and hydrazine monohydrate (10  $\mu\text{l}$ ), and the mixture was stirred for 1.2 h at room temperature and evaporated. A chloroform and methanol (2:1) solution of the residue was washed with 1N HCl, concentrated after addition of

a small amount of triethylamine, and precipitated by addition of methanol to give waxy **1a** (5 mg, 21%):  $R_f=0.2$  (40:15:13:12:8 CHCl<sub>3</sub>-Me<sub>2</sub>CO-MeOH-ACOH-H<sub>2</sub>O);  $[\alpha]_D^{21} +30.43$  (triethylammonium salt,  $c$  0.7, CHCl<sub>3</sub>); FAB-MS(negative)  $m/z$  1105.6 [M-H]<sup>-</sup>; <sup>31</sup>P (109 MHz, ca. 4:1:1 CDCl<sub>3</sub>-CD<sub>3</sub>OD-Et<sub>3</sub>N)  $\delta$  -0.04, 0.80, 2.13, 3.40; <sup>13</sup>C (67.8 MHz, ca. 4:1 CDCl<sub>3</sub>-CD<sub>3</sub>OD)  $\delta$  8.41 (b, NCH<sub>2</sub>C $\overline{C}$ H<sub>3</sub>), 13.96 (s, stearoyl C-18 x 2), 22.57 (s, stearoyl C-17 x 2), 24.76 & 24.80 (s each, stearoyl C-3 x 2), 29.06-29.60 (complex, stearoyl), 31.81 (s, stearoyl C-16x2), 33.98 & 34.14 (s each, stearoyl C-2 x 2), 45.48 (b, NCH<sub>2</sub>CH<sub>3</sub>), 62.80 (s, glyceryl C-1), 63.63 (d,  $J=11.3$  Hz, glyceryl C-3), 70.30-71.07 (complex, glyceryl C-2 and C-2 and -6 in inositol), 74.90 (m, C-1 in inositol), 78.82 (m, C-5 in inositol), 173.17 & 173.57 (s each, CO). Resonance peaks corresponding to C-3 and C-4 in the inositol ring overlapped with those for CDCl<sub>3</sub>.

*Preparation of hydrazine dithiocarbonate.* To an EtOH (10 ml) solution of hydrazine hydrate (0.24 ml, 5 mmol) and ethyldiisopropylamine (0.87 ml, 5 mmol) cooled by an ice bath were added carbon disulfide (0.23 ml, 5 mmol) in EtOH (3 ml) resulting in the precipitation of the dithiocarbonate. This suspension was used for deprotection of the chloroacetic ester.

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