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The total synthesis of 2-*O*-arachidonoyl-1-*O*-stearoyl-*sn*-glycero-3-phosphocholine-1,3,1'-¹³C₃ and -2,1'-¹³C₂ by a novel chemoenzymatic method

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Abstract

2-*O*-Arachidonoyl-1-*O*-stearoyl-*sn*-glycero-3-phosphocholine was synthesized with carbon-13 enrichment of the three glycerol carbons and the carbonyl of the stearoyl group. Phospholipase A₂ was utilized to give optically pure *lys*-PC, and only 3% acyl migration occurred during reacylation with arachidonic acid anhydride. This phospholipid is an important biosynthetic precursor of arachidonic acid metabolites as well as the endocannabinoid 2-arachidonoylglycerol (2-AG), and is now available for NMR studies.

Keywords

2-*O*-arachidonoyl-1-*O*-stearoyl-*sn*-glycero-3-phosphocholine; 2-*O*-arachidonoyl-1-*O*-stearoyl-*sn*-glycerol; ¹³C-labeled; PLA₂; PLC

1. Introduction

Probing model lipid membranes at the molecular level with NMR has utilized 1,2-diacyl-*sn*-glycero-3-phosphocholines (PCs) isotopically labeled in the acyl groups (Stockton, et al., 1974; Schmidt, et al., 1977; Seelig, 1977; Davis, 1979; Wittebort, et al., 1981; Blume, et al., 1982; Wittebort, et al., 1982; Perly, et al., 1984; Ruocco, et al., 1985; Makriyannis, et al., 1986; Makriyannis and Mavromoustakos, 1989; Makriyannis, et al., 1990; Yang, et al., 1992; Huang, et al., 1993; Rajamoorthi, et al., 2005) and the phosphocholine head group (Stockton, et al., 1974; Seelig, 1977; London, et al., 1979; Roux, et al., 1983; Ruocco, et al., 1985; Yang, et al., 1992; Lin, et al., 1997). In addition to the phosphorus nuclei (Yeagle, 1978; Gorenstein, 1984; Burt, 1987; Deihl, 2002; Seydel and Wiese, 2002), the stable isotopically labeled nuclei of these lipids have been utilized to provide the molecular details of structural and dynamic properties, such as lipid phase transitions of model membranes and the perturbation effects of drug molecules. Labeled membrane components are extremely useful in the determination of the depth of insertion, preferred orientation, and conformation of drug molecules and integral membrane proteins present within the lipid matrix of such model membranes using solid state NMR (Makriyannis, et al., 1986; Makriyannis, et al., 1989;

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Makriyannis, et al., 1990; Yang, et al., 1992; Holzgrabe, et al., 1999; Toke, et al., 2004; Tian, et al., 2005; Tang, et al., 2007; Su, et al., 2009).

Stable isotopic labeling of the glycerol backbone of PCs would be particularly useful for studying the critical interfacial region of membranes. Such novel glycerol-labeled PCs have the potential to reveal information by probing the region between the polar membrane surfaces and the central hydrophobic environment of model membrane bilayers. Introducing stable isotopic labels in the glycerol backbone represents the next challenge for lipid synthetic chemistry. The only previous synthetic approach to glycerol-labeled lipids utilized glycerol kinase in a chemoenzymatic synthesis of a perdeuterated 1,2-diacyl-*sn*-glycero-3-phosphocholine (Kingsley and Feigenson, 1979). Also, Pasquaré utilized tissue incubations in the related preparations of [*sn*-2-³H]glycerophospholipids (Pasquaré de Garcia and Giusto, 1986; Pasquaré and Giusto, 1993; Pasquaré, et al., 2006).

This report details an alternative approach developed to produce optically pure diacyl-*sn*-glycero-3-phosphocholines on a 300 mg scale that are labeled in the glycerol portion of the molecule. Of particular interest are 2-*O*-arachidonoyl-1-*O*-stearoyl-*sn*-glycero-3-phosphocholine (Hindeney, et al., 2000) and the corresponding diglyceride 2-*O*-arachidonoyl-1-*O*-stearoyl-*sn*-glycerol that is the predominant diacylglycerol (DG) component in brain (Jung, et al., 2007) and neural tissue (Eichberg and Zhu, 1992). Thus, the phosphocholine group is also utilized as a protecting group (Duclos, et al., 2009) for synthetic 1,2-diacyl-*sn*-glycerols that are isotopically labeled in the glycerol backbone. As a protecting group, the phosphocholine group is easily removed enzymatically (Zwaal, et al., 1971; Mavis, et al., 1972; Majerus and Prescott, 1982; Dennis, 1983; Duclos, et al., 2009), and it is particularly suited to arachidonoyl glycerides where reductive or acidic deprotection conditions and extensive chromatographic purifications cannot be used.

2. Results and Discussion

Consideration of the optimal signal-to-noise intensities of the carbon-13 NMR signals from the three glycerol resonances required a strategy such that there were no adjacent labeled carbons where ¹³C-¹³C couplings (¹*J*_{CC}) would occur. This coupling would be particularly problematic for the *sn*-2 carbon resonance if it were to have two adjacent carbon-13 labels in addition to the strong ³*J*_{CP} coupling of glycerol-3-phospholipids. The intensities of the glycerol carbon resonances were optimized by starting with a 50:50 mixture of glycerol-1,3-¹³C₂ (**1**) and glycerol-2-¹³C (**2**) (see Scheme 1) which was first converted to the corresponding trityl derivative **3**. This mono-protected glycerol **3** was prepared from the relatively insoluble labeled glycerols **1** and **2** with one recycle of ditrityl byproduct to improve the conversion yield to *rac*-3-tritylglycerol **3**. The racemic trityl-protected 1,2-diacylglycerol **4** was prepared by diesterification of diol **3** with stearic-1-¹³C acid. Deprotection by the method of Kodali and Duclos (Kodali and Duclos, 1992) gave the racemic 1,2-diacylglycerol **5** in 90% yield. The *rac*-1,2-diacylglycerol **5** had 100% ¹³C enrichment in the two carbonyls, and as for all subsequent compounds, labeling enrichment of 50% in each of the three glycerol backbone positions. The 1,2-diacylglycerol **5** was used promptly due to the possibility of rearrangement (Mattson and Volpenhein, 1962; Kodali, et al., 1990; Stella, et al., 1997). Any 1,3-diacylglycerol byproduct can be seen by 1D and 2D TLC (Thomas III, et al., 1965; Pollack, et al., 1971; Kodali, et al., 1990) and by the attenuation of the δ 5.08 and 3.72 resonances in the ¹H NMR with the appearance of the rearrangement product resonances between δ 4.05 and 4.21 PPM. Conversion to the corresponding racemic phosphatidylcholine **7** (*rac*-DSPC-¹³C₅) was performed in 63% by the mild method that we have previously used for phosphocholines (Duclos and Makriyannis, 1992; Duclos, 1993; Duclos, et al., 1994; Lin, et al., 2001). The absolute stereospecificity of the PLA₂ enzyme (Gupta, et al., 1977; Dennis, 1994) was utilized to perform the hydrolysis of the *sn*-2 acyl chain to give only the desired enantiomer of the corresponding

lyso-PC- $^{13}\text{C}_4$ **8**. The *lyso*-PC- $^{13}\text{C}_4$ **8** was separated from the unhydrolyzed enantiomer *ent*-DSPC- $^{13}\text{C}_5$ **9** and from free stearic- I - ^{13}C acid by a variation of a reported method using DEAE-cellulose column chromatography (Barlow, et al., 1988). The ^{13}C NMR spectrum demonstrated the presence of 10% of the isomeric *lyso*-PC, labeled 2-*O*-stearoyl-*sn*-glycero-3-phosphocholine, however. A small amount of acyl rearrangement (Kodali, et al., 1990), along with the possibility of some phosphoester rearrangement (Lammers, et al., 1978; Plückthun and Dennis, 1982), occurred during the lengthy purification of *lyso*-PC- $^{13}\text{C}_4$ **8**. This necessitated the facile re-esterification to give pure 1,2-di-*O*-stearoyl-*sn*-glycero-3-phosphocholine (**10**, DSPC- $^{13}\text{C}_5$) in 98% yield. The PLA₂ enzymatic hydrolysis (100% yield) followed immediately by reacylation (Mason, et al., 1981) with arachidonic anhydride (84% yield) gave the optically pure mixed-chain phosphatidylcholine product, labeled 2-*O*-arachidonoyl-1-*O*-stearoyl-*sn*-glycero-3-phosphocholine (**11**, SAPC- $^{13}\text{C}_4$). The C4 and C16 protons of the arachidonoyl chain (see Fig. 1) were easily assigned based on the reported TOCSY of arachidonic acid (Singer, et al., 1996). The carbon resonances (see Fig. 2) were assigned based on the work of Gundstone (Gunstone). The three glycerol carbon resonances were readily distinguished by their chemical shifts and couplings. The carbonyl region (see Fig. 3) shows the enriched carbonyl of the *sn*-1 stearoyl group at δ 174.27 with its satellite peaks at δ 174.51 and 174.06 (each approximately 0.55%) resulting from coupling ($^1J_{\text{CC}} = 55$ Hz) to the 1.1% natural abundance of ^{13}C in the C2'-methylene carbon alpha to the carbonyl. The 1''-carbonyl of the *sn*-2 arachidonoyl group appeared at δ 173.63, identical to unlabeled SAPC (Avanti Polar Lipids, Inc.). The carbonyl resonance at δ 173.91 indicated the presence of approximately 3% of the isomeric 1-*O*-arachidonoyl-2-*O*-stearoyl-*sn*-glycero-3-phosphocholine-1,3,1''- $^{13}\text{C}_3$ and -2,1''- $^{13}\text{C}_2$, an isomeric PC that typically results during the acylation of *lyso*-PCs.

The ^{13}C enrichment of four carbons was accomplished in this synthesis of a 1:1 mixture of the title compounds 2-*O*-arachidonoyl-1-*O*-stearoyl-*sn*-glycerol-1,3,1'- $^{13}\text{C}_3$ and -2,1'- $^{13}\text{C}_2$. This labeled SAPC- $^{13}\text{C}_4$ **11** will be useful for NMR and mass spectral studies of hydrolysis by phospholipases A₂, C, D, and arachidonic acid utilization (Nicolaou and Kokotos, 2004). There is considerable recent interest in the phospholipase C hydrolysis to give the corresponding 1,2-diglyceride that is the biosynthetic precursor of the endocannabinoid 2-AG (Sugiura, et al., 2006; Ahn, et al., 2008; Duclos, et al., 2009). Preliminary experiments with unlabeled 2-*O*-arachidonoyl-1-*O*-stearoyl-*sn*-glycero-3-phosphocholine run on a 10 mg scale gave a quantitative conversion to unlabeled 2-*O*-arachidonoyl-1-*O*-stearoyl-*sn*-glycerol.

3. Experimental

3.1. General

The CH_2Cl_2 , CHCl_3 (amylene stabilized), and CCl_4 used for reaction solvents were distilled twice from P_2O_5 immediately prior to use. Diisopropylethylamine and pyridine were distilled from CaH_2 . Et_2O was freshly distilled from benzophenone ketyl. Glycerol-1,3- $^{13}\text{C}_2$ (**1**), glycerol-2- ^{13}C (**2**), and stearic- I - ^{13}C acid were obtained from Cambridge Isotope Laboratories (Andover, MA). The stearic- I - ^{13}C acid contained less than 1% of palmitic acid as the only impurity by GC (5% DEGS-PS on 100/120 supelcoport, 160°C, FID) following methylation. All other reagents and solvents were purchased from Sigma-Aldrich (Milwaukee, WI) and were used without further purification. All reactions were performed with magnetic stirring. Organic phases were dried over Na_2SO_4 , solvents removed by rotary evaporation under reduced pressure, and flash column chromatography employed silica gel 60 (230-400 mesh, E. Merck). All compounds were demonstrated to be homogeneous by analytical thin-layer chromatography on aluminum pre-coated silica gel TLC plates (UV₂₅₄, layer thickness 250 μm , E. Merck), and chromatograms were visualized under ultraviolet light or by charring (Bitman and Wood, 1982). Phosphocholines were readily identified on duplicate plates with molybdc acid reagent (Dittmer and Lester, 1964; Ryu and MacCoss, 1979). All solvent ratios

are by volume. Melting points were determined on a micro apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on Bruker AMX-500 spectrometer. Unless otherwise stated, all NMR spectra were recorded using CDCl_3 as solvent which shows a residual proton resonance at δ 7.26 and a carbon triplet centered at δ 77.02. However, in 2:1 $\text{CDCl}_3/\text{CD}_3\text{OD}$ the chloroform residual proton resonance is observed at δ 7.49 and the carbon triplet is centered at δ 77.71. Chemical shifts are reported in ppm relative to tetramethylsilane as an internal standard with multiplicities indicated as br (broadened), s (singlet), d (doublet), t (triplet), q (quartet), quintet (quintet), and m (multiplet).

3.2. rac-3-O-Tritylglycerol-1,3- $^{13}\text{C}_2$ and -2- ^{13}C (3)

A 1:1 mixture of labeled glycerols (0.954 g, 10.2 mmol) from 0.5 g of glycerol-1,3- $^{13}\text{C}_2$ (**1**) and 0.5 g of glycerol-2- ^{13}C (**2**) with 2.66 mL (1.97 g, 15.2 mmol, 150 mol%) of Hunig's base in 20 mL of CH_2Cl_2 was stirred vigorously during the addition of 2.84 g (10.2 mmol, 100 mol %) of trityl chloride in 10 mL of CH_2Cl_2 over 30 min. Some fuming was observed, and the reaction became homogeneous, clear, and yellow during an additional 2 hr of stirring. Chromatography (95:4.5:0.5 $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{Et}_3\text{N}$) separated ditrityl byproduct (R_f 0.80) from the product **3** (R_f 0.27) by TLC (95:4.5:0.5 $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{Et}_3\text{N}$). The byproduct was de-tritylated with 0.86 mL (0.99 g) of BF_3 -etherate in 15 mL of 2:1 $\text{CH}_2\text{Cl}_2/\text{MeOH}$, the recovered labeled glycerol was triturated with benzene, and recycled one additional time to improve the monotritylation yield. The product **3** (1.40 g, 4.17 mmol, 41%) was a white crystalline solid: mp 97-98°C; ^1H NMR δ 6.90-7.60 (m, 15 H, aromatics), 3.60-4.30 (m, 1 H, 2), 3.30-3.70 (m, 2 H, 1), 3.10-3.40 (m, 2 H, 3); ^{13}C NMR δ 143.72, 128.67, 127.96, and 127.22 (aromatics), 87.02 (C- Ph_3), 71.18, 65.07, and 64.32 (glycerol).

3.3. rac-1,2-Di-O-stearoyl-3-O-tritylglycerol-1,3,1',1''- $^{13}\text{C}_4$ and -2,1',1''- $^{13}\text{C}_3$ (4)

To a stirred solution of 1.40 g (4.17 mmol, 100 mol%) of diol **3**, 2.50 g (8.76 mmol, 210 mol %) of stearic-1- ^{13}C acid, and 1.15 g (9.41 mmol, 225 mol%) of 4-dimethylaminopyridine (DMAP) in 50 mL of CCl_4 was added a solution of 2.15 g (10.4 mmol, 250 mol%) of dicyclohexylcarbodiimide (DCC) in 10 mL of CCl_4 dropwise over 10 min. The reaction became cloudy and was allowed to stir overnight, during which time a precipitate of dicyclohexylurea had formed. The reaction was warmed to 30-35°C, the byproduct was removed by filtration through a 10-20 μm fritted glass funnel, and the CCl_4 filtrate and washings were combined and concentrated. Chromatography on a warmed column (wrapped with heating tape from Ace Glass, Cat. No. 12063) eluting with 30-35°C 49.5:50:0.5 $\text{CH}_2\text{Cl}_2/\text{hexanes}/\text{Et}_3\text{N}$ gave 3.04 g (3.49 mmol, 84%) of diester **4** as a white solid which was homogeneous by TLC (49.5:50:0.5 $\text{CH}_2\text{Cl}_2/\text{hexanes}/\text{Et}_3\text{N}$, R_f 0.19): mp 33.5°C (soften), 48.0-48.5°C (melt); ^1H NMR δ 7.10-7.70 (m, 15 H, aromatic), 5.25 (m; and, d, $^1J_{\text{CH}} = 150$ Hz, 1 H, 2), 4.31 (m; and, d, $^1J_{\text{CH}} = 150$ Hz, 2 H, 1), 3.59 (br s; and, br d, $^1J_{\text{CH}} = 144$ Hz, 2 H, 3), 2.33 and 2.22 (two dt, 4 H, $J_{2,3} = 7.3$, $^2J_{\text{CH}} = 7.3$ Hz, 2' and 2''), 1.40-1.70 (m, 4 H, 3' and 3''), 0.90-1.40 (m, 56 H, 4'-17' and 4''-17''), 0.88 (t, 6 H, $J = 6.3$ Hz, 18' and 18''); ^{13}C NMR δ 173.35 and 172.99 (1' and 1'' carbonyls), 143.66, 128.67, 127.87, and 127.14 (aromatics), 86.73 (C- Ph_3), 70.47, 62.85, and 62.29 (glycerol), 34.42 and 34.14 (two d, $^1J_{\text{CC}} = 57.5$ Hz, 2' and 2''), 31.96 (16' and 16''), 29.11-29.73 (4'-15' and 4''-15''), 24.99 and 24.89 (3' and 3''), 22.71 (17' and 17''), 14.12 (18' and 18'').

3.4. rac-1,2-Di-O-stearoylglycerol-1,3,1',1''- $^{13}\text{C}_4$ and -2,1',1''- $^{13}\text{C}_3$ (5, rac-DG- $^{13}\text{C}_5$)

A stirred solution of 2.92 g (3.35 mmol, 100 mol%) of trityl compound **4** in 40 mL of dry CH_2Cl_2 under argon was cooled to -20°C (N_2/CCl_4). A solution of 1.21 g (10.0 mmol, 300 mol%) of bromodimethylborane (*caution: Me_2BBr is spontaneously flammable in air*) in 4.3 mL (2.5 M) CH_2Cl_2 was added dropwise over 15 min. After 3 hr, the reaction was quenched by the dropwise addition of 27 mL of cold saturated aqueous NaHCO_3 . Then 200 mL of Et_2O

was added followed by shaking, and two washes with 75 mL H₂O. The Et₂O phase was dried and evaporated. Column chromatography on 250 g of boric acid treated silica gel Boric acid-silica gel (Kodali and Duclos, 1992; Paltauf and Hermetter, 1994) prepared according to Buchnea (Buchnea, 1974) and Bergelson (Bergelson, 1980) gave 1.90 g (3.02 mmol, 90%) of 1,2-diglyceride **5** as a white solid that was nearly homogeneous by TLC (5:95 acetone CH₂Cl₂, *R_f* 0.40 with E. Merck silica gel, *R_f* 0.37 with Analtech silica gel G treated with boric acid). Two dimensional TLC demonstrated that the 1,3-diglyceride rearrangement byproduct (5:95 acetone CH₂Cl₂, *R_f* 0.52 with E. Merck silica gel, *R_f* 0.52 with Analtech silica gel G treated with boric acid) forms to a very small extent under the analytical TLC conditions: mp 70-71°C; ¹H NMR δ 5.08 (m; and, br d, ¹*J*_{CH} = 144 Hz, 1 H, 2), 4.27 (m; and, br d, ¹*J*_{CH} = 150 Hz, 2 H, 1), 3.72 (br s; and, br d, ¹*J*_{CH} = 144 Hz, 2 H, 3), 2.34 (apparent quintet, 4 H, 2' and 2''), 1.50-1.70 (m, 4 H, 3' and 3''), 1.15-1.40 (m, 56 H, 4'-17' and 4''-17''), 0.88 (t, 6 H, *J* = 6.6 Hz, 18' and 18'').

3.5. *rac*-1,2-Di-O-stearoylglycero-3-phospho-β-bromoethanol-1,3,1',1''-¹³C₄ and -2,1',1''-¹³C₃(**6**)

Pyridine (3.64 mL, 3.56 g, 45 mmol) in 5 mL of Et₂O was added dropwise to a stirred solution of 2.54 g (10.5 mmol, 350 mol%) of β-bromoethyldichlorophosphate (Duclos and Makriyannis, 1992; Duclos, 1993; Duclos, et al., 1994; Lin, et al., 2001) in 10 mL of Et₂O under argon over 5 min. After stirring for 15 min, a solution of 1.89 g (3.01 mmol, 100 mol%) of *rac*-DG-¹³C₅ **5** in 40 mL of Et₂O was added over 15 min. The reaction mixture was then refluxed gently for 4 hr using an oil bath at 55°C. The reaction mixture was cooled to 0°C, and 12 mL of H₂O was added dropwise over 2 min. After 5 min, 20 mL of Et₂O was added and the reaction was allowed to stir overnight. The reaction mixture was partitioned between 60 mL of 2 N HCl and 90 mL of 10:90 MeOH/CH₂Cl₂. The organic phase was washed with 100 mL of water, dried, and the solvent removed to give a white solid foam. Chromatography on 225 g of silica gel eluting with a gradient of 8% to 49% methanol in CH₂Cl₂ gave 1.69 g (2.08 mmol, 69%) of β-bromoethylphosphodiester **6** as a white solid (20:80 MeOH/CH₂Cl₂, *R_f* 0.23; 60:30:4 CHCl₃/MeOH/H₂O, *R_f* 0.57): mp 72-4°C; ¹H NMR (2:1, CDCl₃/CD₃OD) δ 5.24 (m; and, br d, ¹*J*_{CH} = 144 Hz, 1 H, 2), 4.40 (m; and, br d, ¹*J*_{CH} = 150 Hz, 2 H, 1), 4.15 (m, 2 H, P-O-CH₂), 4.02 (br s; and, br d, ¹*J*_{CH} = 144 Hz, 2 H, 3), 3.54 (t, 2 H, *J* = 6.2 Hz, CH₂-Br), 2.33 (apparent quintet, 4 H, 2' and 2''), 1.50-1.70 (m, 4 H, 3' and 3''), 1.15-1.50 (m, 56 H, 4'-17' and 4''-17''), 0.89 (t, 6 H, *J* = 6.9 Hz, 18' and 18''); ¹³C NMR (2:1, CDCl₃/CD₃OD) δ 174.25 and 173.91 (1' and 1'' carbonyls), 70.85 (d, ³*J*_{CP} = 6.9 Hz, 2), 65.85 (d, ²*J*_{CP} = 4.9 Hz, P-O-CH₂), 64.11 (d, ²*J*_{CP} = 5.5 Hz, 3), 62.98 (1), 34.59 and 34.42 (two d, ¹*J*_{CC} = 57.3 Hz, 2' and 2''), 32.24 (16' and 16''), 31.19 (m, CH₂-Br), 29.48-30.02 (4'-15' and 4''-15''), 25.21 (3' and 3''), 22.97 (17' and 17''), 14.21 (18' and 18'').

3.6. *rac*-1,2-Di-O-stearoylglycero-3-phosphocholine-1,3,1',1''-¹³C₄ and -2,1',1''-¹³C₃ (**7**, *rac*-DSPC-¹³C₅)

Excess trimethylamine was added to a solution of bromide **6** (1.68 g, 2.06 mmol) in 25 mL of 2:2:1 CHCl₃/*i*-propanol/*N,N*-dimethylformamide in a thick walled pressure tube with a PTFE-lined screw top. The reaction mixture was stirred magnetically and heated with a 50°C oil bath for one day. The solvents were removed under reduced pressure to give a white solid. Chromatography (warmed column) eluting with a 30-35°C gradient of 60:30:0 to 60:30:4 CHCl₃/MeOH/H₂O gave a white solid which was re-dissolved in CHCl₃ and filtered through a 0.5 μm pore PTFE membrane. Solvent removal and lyophilization from *t*-butanol gave 1.53 g (1.88 mmol, 91%) of racemic phosphocholine **7** (*rac*-DSPC-¹³C₅) as a fluffy white solid that was homogeneous by TLC (60:30:5 CHCl₃/MeOH/H₂O, *R_f* 0.24; 25:15:4:2 CHCl₃/MeOH/HOAc/H₂O, *R_f* 0.28; 60:30:5 CHCl₃/MeOH/NH₄OH_{aq}, *R_f* 0.20): mp 80-82°C; ¹H NMR (2:1, CDCl₃/CD₃OD) δ 5.24 (m; and, br d, ¹*J*_{CH} = 144 Hz, 1 H, 2), 4.40 (m; and, br d, ¹*J*_{CH} = 150 Hz, 1 H, 1a), 4.26 (m, 2 H, P-O-CH₂), 4.16 (m; and, br d, ¹*J*_{CH} = 150 Hz, 1 H, 1b), 4.00 (m;

and, br d, $^1J_{\text{CH}} = 144$ Hz, 2 H, 3), 3.62 (m, 2 H, $\text{CH}_2\text{-N}$), 3.23 (m, 9 H, $\text{N-(CH}_3)_3$), 2.32 (apparent quintet, 4 H, 2' and 2''), 1.55-1.65 (m, 4 H, 3' and 3''), 1.20-1.45 (m, 56 H, 4'-17' and 4''-17''), 0.89 (t, 6 H, $J = 6.9$ Hz, 18' and 18''); ^{13}C NMR (2:1, $\text{CDCl}_3/\text{CD}_3\text{OD}$) δ 174.36 and 174.01 (1' and 1'' carbonyls), 70.92 (br s, 2), 66.93 (br s, $\text{CH}_2\text{-N}$), 64.08 (br s, 3), 63.13 (1), 59.57 (d, $^2J_{\text{CP}} = 4.2$ Hz, P-O-CH_2), 54.49 ($\text{N-(CH}_3)_3$), 34.62 and 34.47 (two d, $^1J_{\text{CC}} = 57.4$ Hz, 2' and 2''), 32.31 (16' and 16''), 29.54-30.07 (4'-15' and 4''-15''), 25.33 and 25.28 (3' and 3''), 23.02 (17' and 17''), 14.20 (18' and 18''); and was a broad doublet resonance with unreferenceed ^{31}P NMR in 2:1 $\text{CDCl}_3/\text{CD}_3\text{OD}$.

3.7. 1-O-Stearoyl-*sn*-glycero-3-phosphocholine-1,3,1'- $^{13}\text{C}_3$ and -2,1'- $^{13}\text{C}_2$ (8, lyso-PC- $^{13}\text{C}_4$); and, 2,3-Di-O-stearoyl-*sn*-glycero-1-phosphocholine-1,3,1'',1'''- $^{13}\text{C}_4$ and -2,1'',1'''- $^{13}\text{C}_3$ (9, ent-DSPC- $^{13}\text{C}_5$) via PLA₂ hydrolysis

According to the reported method (Mason, et al., 1981), 1.49 g (1.84 mmol) *rac*-DSPC- $^{13}\text{C}_5$ **7** was dispersed in 56 mL of buffer (100 mM NaEDTA, 100 mM Tris, pH 8.1) and mixed by rotation on a rotary evaporator for 1 hr. The mixture was extracted with 150 mL of 30-35°C 2:1 $\text{CHCl}_3/\text{MeOH}$, followed by two additional extractions of 50 mL each. The combined warm extracts were dried and rotary evaporated. The white residue was given one chase with CHCl_3 , and was lyophilized from *t*-butanol to give 1.44 g of a fluffy white solid suitable for dispersion in solution and enzymatic hydrolysis. The phosphocholine **7** (1.43 g, 1.76 mol) was dissolved in 120 mL of 1:5 $\text{MeOH}/\text{Et}_2\text{O}$ and stirred vigorously magnetically with heating from a 29°C bath. A solution of 185 mg of *Crotalus adamanteus* snake venom in 15 mL of buffer (220 mM NaCl, 20 mM CaCl_2 , 1 mM Na_2EDTA , 50 mM Tris, pH 7.4) was added in five 3 mL portions at 20 min intervals, then the mixture was stirred for an additional 1 hr 40 min (total of 3 hr). The organic phase was separated and saved. The (15 mL) aqueous phase was extracted with four 15 mL portions of 2:1 $\text{CHCl}_3/\text{MeOH}$, and the combined organic phases were rotary evaporated without heating above 35°C to give a white foam. The foam was dissolved in CHCl_3 , some water was separated, then the CHCl_3 solution was dried, and rotary evaporated to give 1.42 g of a mixture of lysophosphocholine **8**, phosphocholine **9**, and stearic- I - ^{13}C acid. The chromatographic separation was performed using the method of Sigler (Barlow, et al., 1988). A 1500 cc column of microgranular preswollen DEAE-cellulose (DE-52, Whatman) in H_2O was poured and washed with 4200 mL of HOAc , 8400 mL of MeOH , 4200 mL of 1:1 $\text{CHCl}_3/\text{MeOH}$, followed by 5500 mL of CHCl_3 . A solution of the crude product mixture in 5 mL of CHCl_3 was loaded and eluted with a stepwise gradient of methanol in CHCl_3 . The *ent*-DSPC- $^{13}\text{C}_5$ **9** byproduct (1.05 g) eluted between 4-7% methanol and was identical by NMR to *rac*-DSPC- $^{13}\text{C}_5$ **7**. The gradient was then steeply increased to 33% MeOH and then eluted isocratically to give 0.33 g (0.61 mmol, 69% theoretical yield) of lysophosphocholine **8** as a white solid following lyophilization from cyclohexane. The product lyso-PC- $^{13}\text{C}_4$ **8** (R_f 0.21) was free of phosphatidylcholine (R_f 0.36) by TLC (60:35:8 $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$) and was used promptly: ^1H NMR (2:1, $\text{CDCl}_3/\text{CD}_3\text{OD}$) δ 4.34 (m, P-O-CH_2), 4.14 (m; and, br d, $^1J_{\text{CH}} = 150$ Hz, 2 H, 1), 3.98-4.04 (m; and, br d, $^1J_{\text{CH}} = 150$ Hz, 2 H, 2 and 3a), 3.95 (m; and, br d, $^1J_{\text{CH}} = 144$ Hz, 1 H, 3b), 3.65 (m, 2 H, $\text{CH}_2\text{-N}$), 3.23 (m, 9 H, $\text{N-(CH}_3)_3$), 2.35 (dt, 4 H, $J_{2,3} = 7.4$, $^2J_{\text{CH}} = 7.4$ Hz, 2'), 1.55-1.64 (m, 2 H, 3'), 1.20-1.40 (m, 28 H, 4'-17'), 0.88 (t, 3 H, $J = 7.1$ Hz, 18'); ^{13}C NMR (2:1, $\text{CDCl}_3/\text{CD}_3\text{OD}$) δ 174.83 (1' carbonyl), 68.88 (d, $^3J_{\text{CP}} = 6.9$ Hz, 2), 67.03 (br s, 3 and $\text{CH}_2\text{-N}$), 65.17 (1), 59.33 (d, $^2J_{\text{CP}} = 4.5$ Hz, P-O-CH_2), 54.23 (t, $^1J_{\text{CN}} = 4.0$ Hz, $\text{N-(CH}_3)_3$), 34.18 (d, $^1J_{\text{CC}} = 57.0$ Hz, 2'), 32.04 (16'), 29.29-29.81 (4'-15'), 24.98 (3'), 22.79 (17'), 14.10 (18'). The presence of 10% isomeric 2-*O*-stearoyl-*sn*-glycero-3-phosphocholine-1,3,1''- $^{13}\text{C}_3$ and -2,1''- $^{13}\text{C}_2$ was indicated by enriched nuclei resonance signals at δ 174.36 (1'' carbonyl), 73.26 (d, $^3J_{\text{CP}} = 7.3$ Hz, 2), 63.35 (d, $^2J_{\text{CP}} = 5.3$ Hz, 3), 60.01 (1); and, a second small broad doublet resonance that was observed upfield of the broad doublet resonance with unreferenceed ^{31}P NMR in 2:1 $\text{CDCl}_3/\text{CD}_3\text{OD}$.

3.8. Stearic-1,1'-¹³C₂anhydride

The labeled anhydride was prepared by the method used for the unlabeled compound (Selinger and Lapidot, 1966). A slightly warm (30-35°C) solution of 2.02 g (7.08 mmol, 200 mol%) of stearic-1-¹³C acid in 30 mL of CCl₄ and 0.723 g (3.50 mmol, 100 mol%) of dicyclohexylcarbodiimide in 10 mL of CCl₄ were shaken in a 125 mL separatory funnel for 5 min, then the reaction was allowed to proceed for 18 h. The mixture was filtered through Whatman #1 filter paper, and the white dicyclohexylurea precipitate was washed with three 5 mL portions of CCl₄ that were also filtered and combined. The solvent was removed to give 1.99 g (3.60 mmol, quantitative) of anhydride that was used without further purification: ¹H NMR δ 2.44 (dt, 4 H, *J*_{2,3} = 7.4, ²*J*_{CH} = 7.4 Hz, 2), 1.60-1.70 (m, 4 H, 3), 1.20-1.40 (m, 56 H, 4-17), 0.88 (t, 6 H, *J* = 6.9 Hz, 18); ¹³C NMR δ 169.64 (1), 35.31 (d, ¹*J*_{CC} = 58 Hz, 2), 31.96 (16), 28.90-29.75 (4-15), 24.26 (3), 22.72 (17), 14.13 (18).

3.9. 1,2-Di-O-stearoyl-sn-glycero-3-phosphocholine-1,3,1',1''-¹³C₄ and -2,1',1''-¹³C₃ (10, DSPC-¹³C₅)

To a cloudy solution of *lyso*-PC-¹³C₄ **8** (0.321 g, 0.590 mmol) and 0.979 g (1.77 mmol, 300 mol%) of stearic-1,1'-¹³C₂ anhydride in 5 mL of CHCl₃ was added a solution of 0.175 g (1.18 mmol, 200 mol%) of 4-pyrrolidinopyridine (4-PPY) in 2 mL of CHCl₃. The reaction was initially warmed for 10 min to 30-35°C with a water bath and became homogeneous after three hours. TLC of samples of the homogeneous reaction mixture showed that the reaction was essentially complete after stirring overnight. The volume was adjusted to 20 mL with CHCl₃ and 10 mL of MeOH was then added. The organic solution was washed with 15 mL of 0.25 N HCl. The aqueous extract was backwashed twice with CHCl₃ and the backwashes were combined with the organic phase, dried, and the solvent removed. Column chromatography was performed with a wrapped warmed column and elution with 30-35°C solvents. Byproducts were first eluted with a gradient of 60:30:0 to 60:30:5 CHCl₃/MeOH/H₂O. The pure product DSPC-¹³C₅ **10** subsequently eluted and the solvent was removed. The DSPC-¹³C₅ **10** was dissolved in 90:10 CHCl₃/MeOH and filtered through a 0.5 μm pore PTFE filter, the solvent was removed, and lyophilization from *t*-butanol gave 0.407 g (0.501 mmol, 98%) of a fluffy white solid that was homogeneous by TLC (*R*_f 0.28, 25:15:4:2 CHCl₃/MeOH/HOAc/H₂O): mp 83-85°C; ¹H, ¹³C, and ³¹P NMR were identical to the racemic material *rac*-DSPC-¹³C₅ **7** detailed above and to *ent*-DSPC-¹³C₅ **9**; [*α*]_D²⁴ +6.8° (c 4.59, 1:1 CHCl₃/MeOH) [rotations for unlabeled DSPC: Lit. (Patel, et al., 1979) [*α*]_D +6.4° (c 4.59, 1:1 CHCl₃/MeOH); Lit. (Baer and Buchnea, 1959) [*α*]_D²⁹ +6.2° (c 10, 1:1 CHCl₃/MeOH)].

3.10. Arachidonic Anhydride

Anhydrous CCl₄ was vacuum degassed, then further degassed by argon bubbling and 20 mL was used to dissolve 1.00 g (3.28 mmol, 200 mol%) of arachidonic acid and 0.376 g (1.82 mmol, 100 mol%) of dicyclohexylcarbodiimide. The reaction mixture was allowed to stand overnight under an argon atmosphere. The white precipitate that formed was removed by paper filtration and the filtrate washed with additional CCl₄. The solvent was removed to give a quantitative yield of anhydride as a clear very faint yellow liquid that was used without further purification: ¹H NMR δ 5.30-5.45 (m, 16 H, 5,6,8,9,11,12,14,15), 2.80-2.85 (m, 12 H, 7,10,13), 2.46 (t, 4 H, *J* = 7.4 Hz, 2), 2.15 (dt, 4 H, *J* = 7.2, 7.2 Hz, 4), 2.05 (dt, 4 H, *J* = 7.1, 7.1 Hz, 16), 1.71-1.77 (m, 4 H, 3), 1.20-1.60 (m, 12 H, 17,18,19), 0.89 (t, 6 H, *J* = 6.8 Hz, 20); ¹³C NMR δ 169.31 (1), 130.52, 129.36, 128.64, 128.46, 128.37, 128.02, 127.83, 127.55 (olefinics), 34.59 (2), 31.55 (18), 29.35 (17), 27.25 (16), 26.25 (4), 25.66 (br s, 7,10,13), 24.05 (3), 22.60 (19), 14.08 (20).

3.11. 2-O-Arachidonoyl-1-O-stearoyl-sn-glycero-3-phosphocholine-1,3,1'-¹³C₃ and -2,1'-¹³C₂ (**11**, SAPC-¹³C₄)

According to the reported method (Mason, et al., 1981), 0.393 g (0.484 mmol) of optically pure labeled DSPC-¹³C₅ **10** was dispersed in 15 mL of buffer (100 mM NaEDTA, 100 mM Tris, pH 8.1) and mixed by rotation on a rotary evaporator for 1 hr. The mixture was extracted with 30 mL of 30°C 2:1 CHCl₃/MeOH, followed by two additional extractions of 15 mL each. The combined extracts were dried and rotary evaporated. The white residue was given one chase with CHCl₃, and was lyophilized from *t*-butanol to give 0.388 g of a fluffy white solid suitable for dispersion in solution and enzymatic hydrolysis. The phosphatidylcholine DSPC-¹³C₅ **10** (0.388 g, 0.478 mol) was dissolved in 36 mL of 1:5 MeOH/Et₂O and stirred vigorously magnetically with heating from a 29°C bath. A solution of 49.4 mg of *Crotalus adamanteus* snake venom in 4 mL of buffer (220 mM NaCl, 20 mM CaCl₂, 1 mM Na₂EDTA, 50 mM Tris, pH 7.4) was added in five 0.8 mL portions at 20 min intervals, then the mixture was stirred for an additional 1 hr 40 min (total of 3 hr). The organic phase was separated and the solvent removed. The (4 mL) aqueous phase was extracted with six 5 mL portions of Et₂O. The Et₂O extracts were combined with the residue from the first organic phase, concentrated to 5 mL, and backwashed twice with 2 mL portions of water. The three aqueous phases were combined (8 mL). (The combined Et₂O extracts contained 0.126 g of fatty acid and very little *lyso*-PC.) The aqueous phase was extracted with three 10 mL portions of 4:1 CHCl₃/MeOH with phase separation performed rapidly using a tabletop centrifuge. The combined organic phases were dried, rotary evaporated without heating above 35°C, given one chase with CHCl₃, lyophilized from *t*-butanol, re-lyophilized from cyclohexane, and then dried at high vacuum overnight in the presence of P₂O₅ to give 0.262 g (quantitative yield) of a fluffy white powder that was nearly homogeneous by TLC (*R*_f 0.07, 60:30:5 CHCl₃/MeOH/H₂O) containing only trace amounts of PC (*R*_f 0.27) and fatty acid (*R*_f 0.86): mp 116-120°C soften, 240-245°C melt.

To a cloudy solution of the lysophosphocholine intermediate (0.260 g, 0.478 mmol, 100 mol %) and 0.970 g (1.64 mmol, 343 mol %) of arachidonic anhydride in 5 mL of CHCl₃ was added a solution of 0.142 g (0.958 mmol, 200 mol %) of 4-pyrrolidinopyridine (4-PPY) in 2 mL of CHCl₃. The reaction mixture became homogeneous within a half hour. TLCs (60:30:5 CHCl₃/MeOH/H₂O and 25:15:4:2 CHCl₃/MeOH/HOAc/H₂O) of samples of the homogeneous reaction mixture showed that the starting material was completely consumed after stirring overnight. The reaction mixture was washed with 5.6 mL of 0.25 N HCl. The aqueous extract was backwashed twice with CHCl₃ and the backwashes were combined with the organic phase, dried, and the solvent removed. Column chromatography with argon-degassed solvent mixtures first eluted byproducts with a gradient of 60:30:0 to 60:30:3 CHCl₃/MeOH/H₂O. The product SAPC-¹³C₄ **11** subsequently eluted and the solvent was removed. TLC (25:15:4:2 CHCl₃/MeOH/HOAc/H₂O) showed 4-pyrrolidinopyridine (*R*_f 0.56) to be the only impurity present with the product (*R*_f 0.31). The SAPC-¹³C₄ **11** was dissolved in 30 mL of 90:10 CHCl₃/MeOH, washed with 5 mL of 0.25 M HCl, the phases separated using centrifugation, the organic phase dried, and the solvent removed. The product was re-chromatographed isocratically with argon-degassed 60:30:3 CHCl₃/MeOH/H₂O, then dissolved in 90:10 CHCl₃/MeOH, filtered through a 0.5 μm pore PTFE membrane, the solvent was removed, and then lyophilization from *t*-butanol gave 0.334 g (0.402 mmol, 84%) of an off-white solid that was homogeneous by TLC (*R*_f 0.34, 25:15:4:2 CHCl₃/MeOH/HOAc/H₂O): mp 62-64°C; ¹H NMR (2:1, CDCl₃/CD₃OD) δ 5.30-5.45 (m, 8 H, 5'',6'',8'',9'',11'',12'',14'',15''), 5.24 (m; and, br d, ¹*J*_{CH} = 144 Hz, 1 H, 2), 4.42 (br d, ²*J*_{1a,1b} = 15 Hz; and, br d, ¹*J*_{CH} = 145 Hz, 1 H, 1a), 4.25 (m, 2 H, P-O-CH₂), 4.16 (m; and, br d, ¹*J*_{CH} = 150 Hz, 1 H, 1b), 4.00 (m; and, br d, ¹*J*_{CH} = 149 Hz, 2 H, 3), 3.61 (m, 2 H, CH₂-N), 3.23 (m, 9 H, N-(CH₃)₃), 2.75-2.90 (m, 6 H, 7'',10'',13''), 2.36 (t, 2 H, *J*_{2,3} = 7.4, 2''), 2.31 (dt, 2 H, *J*_{2,3} = 7.4, ²*J*_{CH} = 7.4 Hz, 2'), 2.13 (dt, 4 H, *J* = 7.0, 7.0 Hz, 4''), 2.06 (dt, 4 H, *J* = 7.1, 7.0 Hz, 16''), 1.65-1.75 (m, 2 H, 3''),

1.55-1.65 (m, 2 H, 3'), 1.20-1.45 (m, 34 H, 4'-17' and 17''-19''), 0.89 (apparent q, 6 H, 18' and 20''); ^{13}C NMR (2:1, $\text{CDCl}_3/\text{CD}_3\text{OD}$) δ 174.27 (1'), 173.91 (1'' of the rearrangement byproduct), 173.63 (1''), 130.74, 129.31, 129.04, 128.90, 128.61, 128.36, 128.11, 127.85 (olefinics), 70.87 (d, $^3J_{\text{CP}} = 7.6$ Hz, 2), 66.86 (br s, $\text{CH}_2\text{-N}$), 63.92 (d, $^2J_{\text{CP}} = 4.2$ Hz, 3), 63.01 (1), 59.34 (d, $^2J_{\text{CP}} = 5.1$ Hz, P-O- CH_2), 54.43 (N-(CH_3) $_3$), 34.36 (d, $^1J_{\text{CC}} = 57.4$ Hz, 2'), 33.96 (2''), 32.22 (16'), 31.82 (18''), 29.43-29.98 (4'-15' and 17''), 27.50 (16''), 26.80 (4''), 25.91 (s, 7'', 10'', 13''), 25.17 and 25.11 (3' and 3''), 22.95 and 22.84 (17' and 19''), 14.19 and 14.16 (18' and 20''); and was a broad doublet resonance with unreference ^{31}P NMR in 2:1 $\text{CDCl}_3/\text{CD}_3\text{OD}$.

3.12. Unlabeled 2-O-Arachidonoyl-1-O-stearoyl-sn-glycerol (DG) via phospholipase C

To a 1.5 mL screw-top Eppendorf tube containing 10.1 mg (1.22×10^{-5} mol) of 2-O-arachidonoyl-1-O-stearoyl-sn-glycero-3-phosphocholine (SAPC, Avanti 850469C) in 150 μL of buffer (100 mM NaCl, 50 mM Tris, 10 mM CaCl_2 , 0.1 mM ZnCl_2 , pH 7.0) was added 1.2 mL of hexane. A solution of phospholipase C (Sigma P 9439 from *Bacillus cereus*, 50 Units) in 250 μL of buffer was delivered by pipette to the bottom (aqueous) phase in 50 μL portions at 20 min intervals. With the screw cap in place and under an argon atmosphere, the reaction was gently stirred magnetically. A sample of the hexane phase was removed after 2 hr and found to contain >95% product 1,2-diacyl-sn-glycerol (DG) by TLC (R_f 0.50, 30:70 acetone/hexane) with very little unlabeled DSPC starting material (R_f 0.00, 30:70 acetone/hexane). The bottom (aqueous) phase was removed by pipette and was discarded. The top (hexane) phase was centrifuged at $10K \times g$ for 2 min. The top (hexane) phase was transferred to a clean PTFE-lined screw-top vial and was dried briefly over Na_2SO_4 . The dry hexane solution was transferred to a new vial, and to this solution (250 μL) of crude product was added 107 μL of acetone. A plug of silica gel (150 mg, 0.3 cc) over a bed of glass wool and sand in a disposable 1 mL syringe body fitted with a 0.2 μm PTFE filtration membrane was thoroughly washed with 30:70 acetone/hexane and used to remove origin material. The solution of crude product in 30:70 acetone/hexane was rapidly forced through the column and immediately washed with an additional 400 μL portion of 30:70 acetone/hexane. The column elution and wash fractions were combined to give a quantitative yield of 2-O-arachidonoyl-1-O-stearoyl-sn-glycerol (DG) that was free of starting phosphatidylcholine (SAPC). There was no evidence of the rearrangement 1,3-diacyl-sn-glycerol byproduct that would have had a slightly higher R_f than that for the 1,2-diacyl-sn-glycerol (R_f 0.50, 30:70 acetone/hexane) (Gaffney and Reese, 1997; Hindenes, et al., 2000), and the stability of the 2-O-arachidonoyl-1-O-stearoyl-sn-glycerol was demonstrated by 2D TLC. The product was stored in the acetone/hexane solution under an argon atmosphere at -20°C when not in use: ^1H NMR (CD_3OD) δ 5.25-5.40 (m, 8 H, 5'', 6'', 8'', 9'', 11'', 12'', 14'', 15''), 5.00-5.10 (m, 1 H, 2), 4.34 (dd, 1 H, $J = 12.0, 3.4$ Hz, 1a), 4.10 (dd, 1 H, $J = 12.0, 6.7$ Hz, 1b), 3.59-3.65 (m, 2 H, 3), 2.78-2.85 (m, 6 H, 7'', 10'', 13''), 2.33 and 2.27 (two t, 4 H, $J = 7.4$ Hz, 2' and 2''), 2.11 (dt, 2 H, $J = 6.9, 6.9$ Hz, 4''), 2.04 (dt, 2 H, $J = 7.0, 7.0$ Hz, 16''), 1.55-1.86 (m, 4 H, 3' and 3''), 1.25-1.37 (m, 34 H, 4'-17' and 17''-19''), 0.87 and 0.86 (two t, 6 H, $J = 6.8$ Hz, 18' and 20''); ^{13}C NMR δ 173.79 and 173.16 (1' and 1''), 130.54, 129.06, 128.79, 128.65, 128.34, 128.12, 127.86, 127.56 (olefinics), 72.25, 61.98, 61.57 (glycerol), 34.12 and 33.66 (2' and 2''), 31.95 (16'), 31.54 (18''), 29.15-29.72 (4'-15' and 17''), 27.25 (16''), 26.51 (4''), 25.67, 25.65, and 25.64 (7'', 10'', 13''), 24.91 and 24.78 (3' and 3''), 22.71 and 22.59 (17' and 19''), 14.12 and 14.08 (18' and 20'').

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Abbreviations

DG	diacylglycerol
DSPC	1,2-di- <i>O</i> -stearoyl- <i>sn</i> -glycero-3-phosphocholine
PC	1,2-diacyl- <i>sn</i> -glycero-3-phosphocholine
PLA ₂	phospholipase A ₂
PLC	phospholipase C
SAPC	2- <i>O</i> -arachidonoyl-1- <i>O</i> -stearoyl- <i>sn</i> -glycero-3-phosphocholine

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Fig. 1.
 ^1H NMR spectrum of 2-*O*-arachidonoyl-1-*O*-stearoyl-*sn*-glycero-3-phosphocholine-1,3,1'- $^{13}\text{C}_3$ and -2,1'- $^{13}\text{C}_2$ (**11**, SAPC- $^{13}\text{C}_4$).

Fig. 2.
 ^{13}C NMR spectrum of 2-*O*-arachidonoyl-1-*O*-stearoyl-*sn*-glycero-3-phosphocholine-1,3,
1'- $^{13}\text{C}_3$ and -2,1'- $^{13}\text{C}_2$ (**11**, SAPC- $^{13}\text{C}_4$).

Fig. 3.

The carbonyl region ^{13}C NMR of 2-*O*-arachidonoyl-1-*O*-stearoyl-*sn*-glycero-3-phosphocholine-1,3,1'- $^{13}\text{C}_3$ and -2,1'- $^{13}\text{C}_2$ (**11**, SAPC- $^{13}\text{C}_4$) demonstrates the presence of 3% of the 1-*O*-arachidonoyl-2-*O*-stearoyl-PC- $^{13}\text{C}_4$ isomer formed during reacylation of *lyso*-PC- $^{13}\text{C}_4$.

Scheme 1.

Synthesis of 2-*O*-arachidonoyl-1-*O*-stearoyl-*sn*-glycero-3-phosphocholine-1,3,1'- $^{13}\text{C}_3$ and -2,1'- $^{13}\text{C}_2$ (**11**, SAPC- $^{13}\text{C}_4$).