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Synthesis of 1-lyso-2-palmitoyl-*rac*-glycero-3-phosphocholine and its regioisomers and structural elucidation by NMR spectroscopy and FAB tandem mass spectrometry

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Abstract—Three regioisomers of a naturally occurring lysophosphatidylcholine were chemically synthesized from glycerol. The phosphocholine moiety of the molecule was introduced by sequentially reacting with ethylene chlorophosphite, bromine, water, and trimethyl amine. Removal of a silyl protecting group of the hydroxyl group in the glycerol backbone was achieved without any accompanying acyl migration in the final stage of the synthesis by using NBS in a dimethyl sulfoxide–water cosolvent system. Structures of all regioisomers were compared by NMR spectroscopy and FAB tandem mass spectrometry.

Lysophospholipids can be produced through the action of phospholipase on cell membranes and they function as key intermediates in diverse biological processes. Recently, lysophospholipids were reported to exist in the marine sponge *Spirastrella abata*[1] and deer antler.[2] These compounds display inhibition of cholesterol biosynthesis in Chang liver cells and also exhibit antifungal activity. In addition, lysophosphatidylcholine plays an important role in signal transduction pathway by mediating protein kinase C[3,4] and inhibition of platelet aggregation.[5] Lysophosphatidylcholine also stimulates the release of arachidonic acid in endothelial cells[6] and polyamine synthesis in vascular smooth muscle cells.[7] As diverse properties of phospholipase A_1 were investigated in bovine and rat brains[8,9] as well as fresh bonito white muscle,[10] the role of 1-lysophosphatidylcholine has drawn considerable attention. The 1-lysophosphatidylcholine bound to albumin could be an efficient delivery form of fatty acid to the brain, compared with unesterified fatty acid.[11] In particular, 1-lysophosphatidylcholine is preferentially transferred over 2-lysophosphatidylcholine in vivo.[9] In addition, 1-lysophosphatidylcholine is more prone to reacylation[12] and transacylation[10] than 2-lysophosphatidylcholine, and this result is in good agreement with the predominance of 2-lysophosphatidylcholine in cells. Due to the interesting biological activity of lysophospholipids, extensive studies have resulted in semi-synthetic and chemical procedures of the 2-lysophospholipids[13] as well as their derivatives.[14] However, no such studies have been reported for 1-lysophospholipids. In this study, we synthesized 1-lyso-2-palmitoyl-*rac*-glycero-3-phosphocholine **1** (lysoPC **1**) as well as two regioisomers, 1-palmitoyl-2-lyso-*rac*-glycero-3-phosphocholine **2** (lysoPC **2**), and 1-lyso-3-palmitoyl-*rac*-glycero-2-phosphocholine **3** (lysoPC **3**), as shown in Figure 1. After synthesis, the structures of **1**–**3** were confirmed by NMR spectroscopy and FAB tandem mass spectrometry. This study has demonstrated the development of general synthetic protocols and structural elucidation of

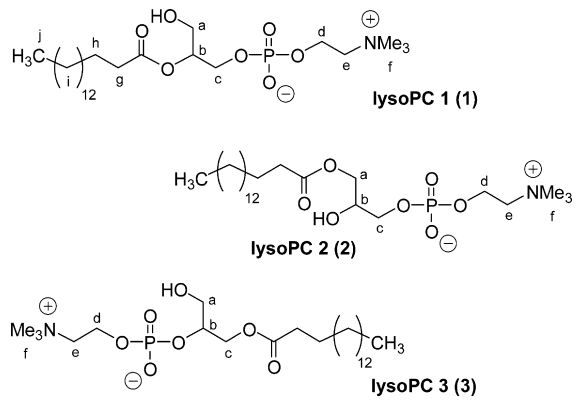

Figure 1. Structures of 1-lyso-2-palmitoyl-*rac*-glycero-3-phosphocholine **1** (lysoPC **1**), 1-palmitoyl-2-lyso-*rac*-glycero-3-phosphocholine **2** (lysoPC **2**), and 1-lyso-3-palmitoyl-*rac*-glycero-2-phosphocholine **3** (lysoPC **3**) with atom labeling used in NMR spectral data.

Keywords: 1-lysophosphatidylcholine; acyl migration; regioisomer; NMR (nuclear magnetic resonance); FAB-MS (fast atom bombardment mass spectrometry); MS/MS (tandem mass spectrometry); CID (collision-induced dissociation).

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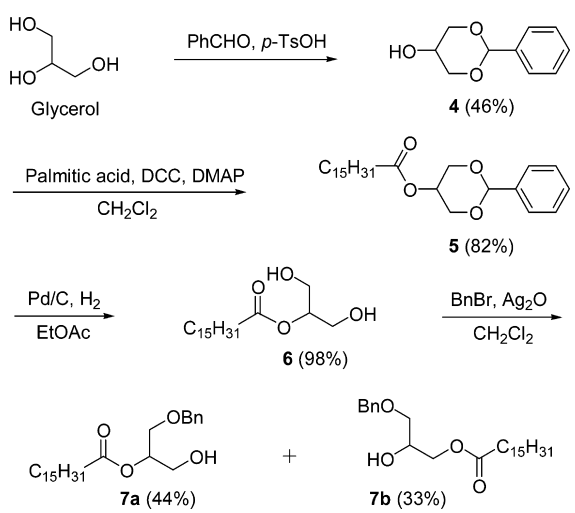
all three possible regioisomers of lysophosphatidylcholine and thus provides the tools for clear assignment of these biologically important organic lysophosphatidylcholines.

1. Results and discussion

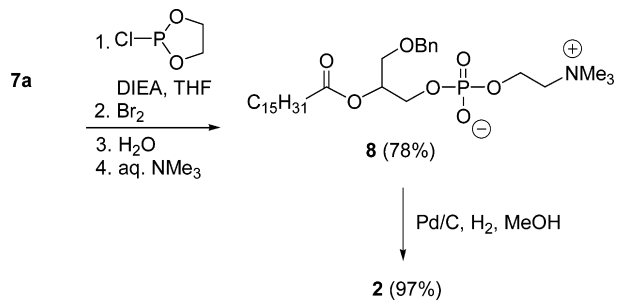
1.1. Synthesis

At first we envisioned synthesis of lysoPC **1** as one of three target molecules. The key intermediates, **7a** and **7b**, were prepared as outlined in Scheme 1. Synthesis began with commercially available glycerol. The 1,3-diol of glycerol was protected as 1,3-benzylideneacetal **4** by treatment with *p*-TsOH and benzaldehyde. The remaining hydroxyl group of **4** was treated with palmitic acid in the presence of DCC and DMAP to afford ester **5**, which was then deprotected by using H_2/Pd/C in EtOAc over the course of 3 days. The resulting diol **6** was selectively monobenzylated with BnBr and Ag_2O[15] to give two benzylated products, C2-hexadecanoate **7a** and C3-hexadecanoate **7b**. These regioisomers were chromatographically separated and the starting material **6** was recovered. Structure of the desired C2-hexadecanoate **7a** was clearly distinguished from its C2,C3-acyl group migrated byproduct **7b** by comparison of the chemical shifts at C1 and C3 of the glycerol backbone in ^{1}H NMR spectra, as we previously reported for a monoacetyldiglyceride system.[16]

Insertion of the phosphocholine moiety was achieved using a four step sequence as shown in Scheme 2.[17] Compound **7a** was treated with ethylene chlorophosphite in the presence of DIEA in THF at −20°C to produce cyclic phosphite intermediate. After ring opening with bromine and subsequent hydrolysis, the resulting phosphoethanolamine was quaternized with 40% aqueous trimethylamine in $CHCl_3$/^{i}PrOH/CH_3CN to afford compound **8** in good yield (78% from **7a**). Interestingly, we found that catalytic hydrogenation of compound **8** proceeded exclusively with unexpected acyl migration. Thus, our second target, lysoPC **2**, was obtained instead of lysoPC **1** in 97% yield from **8**. Both the proton and carbon NMR spectra of lysoPC **2** were identical with those of 2-lysophosphatidylcholine purchased from Sigma.

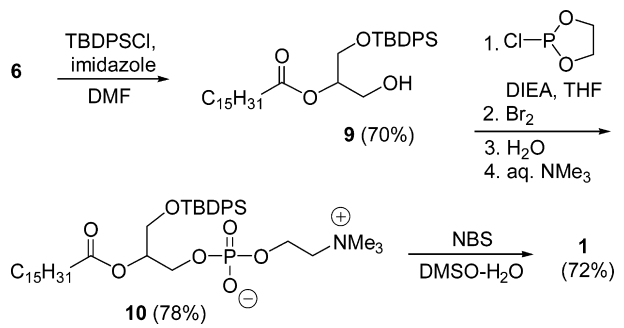


Scheme 1.

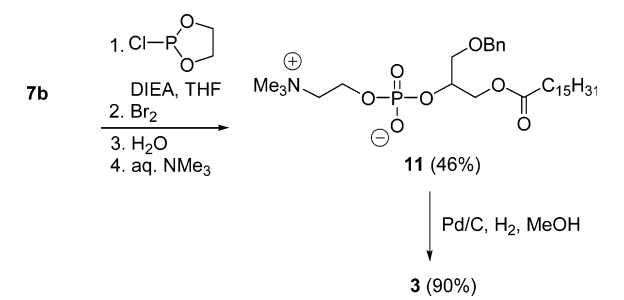


Scheme 2.

To prevent acyl migration, we next chose a silyl protecting group in place of a benzyl group at the C1 position (Scheme 3). Diol **6** was selectively monosilylated by treatment with TBDPSCl in the presence of imidazole to afford compound **9** in 70% yield. The reaction was conducted carefully and monitored closely by TLC in order to obtain C1-monosilylated derivative as the major product. Introduction of the phosphocholine moiety successfully furnished **10** in good yield. We found that TBDMS protecting group was not suitable for our synthesis, because it was removed in the presence of aqueous trimethylamine during phosphocholine formation. Desilylation method of **10** was investigated in depth to avoid acyl migration. It is noteworthy to mention that desilylation was only successful with NBS in DMSO/H_2O to provide the desired compound, lysoPC **1**, according to the procedure reported by Johnson.[18] The trace amount of byproduct could be removed successfully by silica-gel flash column chromatography. In comparison, we found that the use of TBAF or HF–pyridine to deprotect the TBDPS group gave exclusively lysoPC **2**.



Scheme 3.



Scheme 4.

The third target, lysoPC **3**, was prepared from **7b** via the same synthetic procedure as for lysoPC **2** (Scheme 4). Sequential reaction of **7b** with ethylene chlorophosphite, bromine, and water, followed by aqueous trimethylamine gave **11** without accompanying phosphoryl migration.[19] Subsequently, palladium-catalyzed hydrogenolysis of **11** afforded lysoPC **3**.

1.2. Structural elucidation

We have previously determined the structure of the 2-lysophosphatidylcholine isolated from deer antler using a combination of NMR spectroscopic and FAB tandem mass spectrometric analysis.[2] However, several spectral analyses were not sufficient to provide a complete assignment of carbons on the glycerol backbone. Thus, all three regioisomers of lysoPCs **1**–**3** have been synthesized and characterized by ^{1}H, ^{13}C, DEPT 135, $^{1}H-^{1}H$ COSY, and $^{1}H-^{13}C$ COSY NMR as well as FAB tandem mass spectrometric methods. These data were used to establish a relationship to the regiospecificity of the fatty acyl group and the polar head group on the glycerol backbone of lysophosphatidylcholine.

1.2.1. NMR spectroscopic analysis. The ^{1}H and ^{13}C NMR spectra of lysoPCs **1**–**3** are shown in Figure 2. LysoPC **1** contains palmitic acid at the C2 position and phosphocholine at the C3 position of the glycerol backbone, whereas lysoPC **2** and lysoPC **3** contain palmitic acid at C1 in addition to phosphocholine at C3 and C2, respectively. The fatty acyl group could be readily distinguished as being in the upfield region in the ^{1}H (Fig. 2(a)–(c)) and ^{13}C NMR (Fig. 2(d)–(f)) spectra on the basis of previous studies.[2,20] Additionally, lysoPCs **1**–**3** displayed several characteristic signals, belonging to the glycerol backbone and polar head group. As a representative example, the methyl protons H_f on the polar head group of lysoPC **1** displayed a signal at 3.23 ppm, whereas the methylene protons H_d and H_e were observed at 4.28 and 3.67 ppm. For the remaining peaks, we assumed that the peaks at 3.67 and 3.96 ppm correspond to the methylene protons, H_a and H_c, and the peak at 5.00 ppm to the methine proton H_b on the glycerol backbone (Fig. 2(a)). Three carbons C_d, C_e, and C_f attached directly to the polar head group protons displayed carbon signals at 60.7, 67.7, and 55.0 ppm based on the $^{1}H-^{13}C$ COSY experiment. The methine carbon C_b at 74.9 ppm was correlated to the proton H_b at 5.00 ppm. The two remaining peaks at 61.5 and 65.0 ppm were assigned as the backbone carbons, C_a and C_c, which were associated with the protons, H_a and H_c, at 3.67 and 3.96 ppm, respectively (Fig. 2(d)). Further analysis of the ^{13}C NMR spectrum of lysoPC **1** in comparison with those of its regioisomers, lysoPCs **2** and **3**, in detail enabled us to fully assign all carbon signals corresponding to protons. The ^{1}H and ^{13}C NMR data of their representative regions of lysoPCs **1**–**3** are listed in Tables 1 and 2, respectively. An important issue in the NMR analyses was the effect of the specific position of the fatty acid on the glycerol backbone on chemical shift in order to confirm the structures of synthetic lysoPCs. In the ^{1}H NMR of lysoPC **1**, the fatty acyl chain at the C2 position caused the downfield shift of H_b from 3.97 to 5.00 ppm compared with that of lysoPC **2**, as well as the upfield shift of H_a from 4.14 to 3.69 ppm (Table 1). In addition, we found that structural

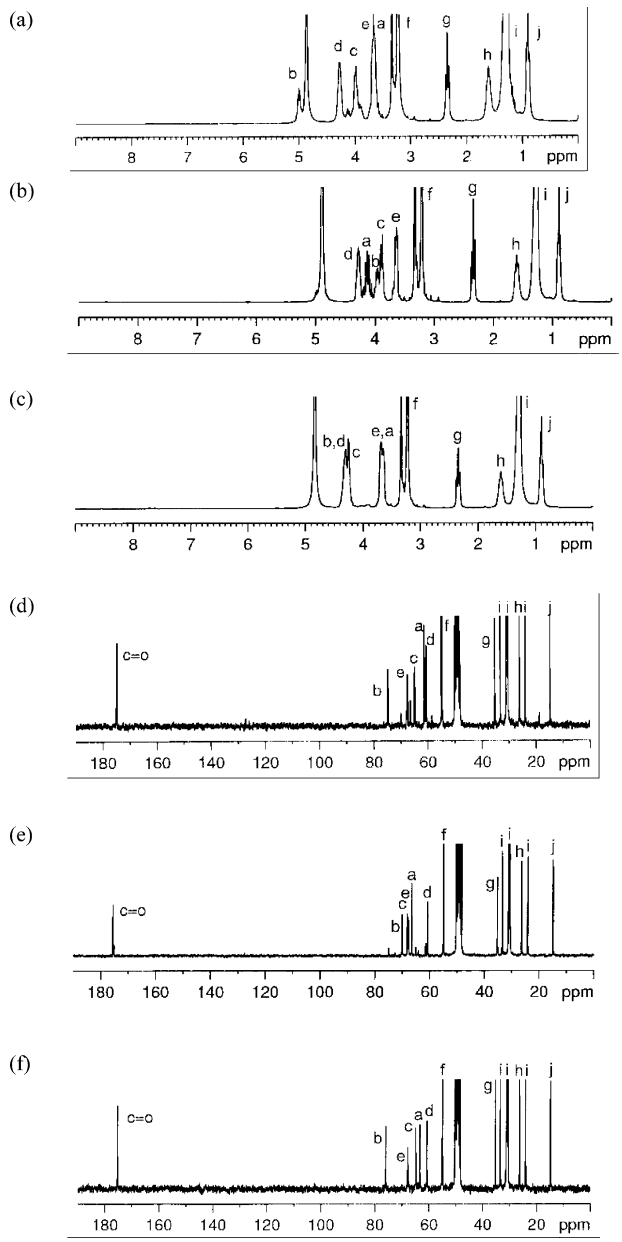


Figure 2. ^{1}H NMR spectra (250 MHz, CD_3OD) of (a) lysoPC **1** (b) lysoPC **2** (c) lysoPC **3**, and ^{13}C NMR spectra (63 MHz, CD_3OD) of (d) lysoPC **1** (e) lysoPC **2** (f) lysoPC **3**.

Table 1. ^{1}H NMR spectral data for the representative regions on lysoPCs **1**–**3** (250 MHz, CD_3OD)

Assignment	Chemical shifts (δ)		
	1	**2**	**3**
a	3.67	4.14	3.70
b	5.00	3.97	4.31
c	3.96	3.89	4.26
d	4.28	4.30	4.31
e	3.67	3.65	3.70
f	3.23	3.23	3.23

Structures are drawn in Figure 1.

Table 2. ^{13}C NMR spectral data for the representative regions on lysoPCs **1**–**3** (63 MHz, CD_3OD)

Assignment	Chemical shifts (δ, mult.[a], J[b])		
	1	**2**	**3**
a	61.5 (s)	66.5 (s)	63.3 (d, 4.4)
b	74.9 (d, 8.2)	70.1 (d, 7.6)	76.1 (d, 6.3)
c	65.0 (d, 5.0)	68.1 (d, 5.0)	64.9 (d, 5.0)
d	60.7 (d, 4.4)	60.7 (d, 4.4)	60.8 (d, 5.0)
e	67.7 (m)	67.7 (m)	67.8 (m)
f	55.0 (m)	55.0 (m)	55.0 (m)

Structures are drawn in Figure 1 and spectra in Figure 3.
[a] Singlet, s; doublet, d; multiplet, m.
[b] The C–P coupling constants.

changes in carbon skeleton of the glycerol backbone did not affect the chemical shifts of the polar head group protons (H_d, H_e and H_f) as they consistently appeared in the spectra of compounds **1**–**3** (Table 1).

In the ^{13}C NMR spectra, characteristic couplings between carbon and phosphorus as well as carbon and nitrogen were observed. The corresponding segments of spectra are shown in Figure 3. Analysis of these coupling patterns played a crucial role in the structural elucidation. For lysoPCs **1** and **2** (Fig. 3(a) and (b)), the two-bond carbon–phosphorus couplings involved the methylene carbon C_c of the glycerol backbone and the methylene carbon C_d of the polar head group. On the other hand, the backbone carbon C_b and the polar head group carbon C_e exhibited the three-bond carbon–phosphorus couplings. For lysoPC **3** (Fig. 3(c)), the methine carbon C_b of the glycerol backbone and the methylene carbon C_d of the polar head group exhibited the two-bond carbon–phosphorus couplings, whereas the backbone carbons, C_a and C_c, and the polar head group carbon C_e displayed the three-bond carbon–phosphorus couplings. Therefore, lysoPCs **1** and **2** exhibited a sharp singlet for carbon C_a in ^{13}C NMR spectra. In comparison, lysoPC **3** showed a doublet for carbon C_a with a $^3J_{cp}$ coupling value of 4.4 Hz. The J_{cp} coupling constants are given in Table 2. In case of lysoPCs **1** and **2**, the $^2J_{cp}$ coupling values were smaller than the $^3J_{cp}$, in agreement with the literature values.[21] An extraordinary coupling pattern of the carbon C_e in the polar head group of lysoPCs **1**–**3** demonstrated the existence of carbon–nitrogen coupling, thus distinguishing the carbon C_e from the carbon C_d. The ^{13}C NMR spectra of lysoPCs **1** and **2** showed identical coupling patterns. However, careful investigation of the spectra suggested that the relative changes of chemical shifts in lysoPC **1** and lysoPC **2** demonstrated the deshielding contribution[22] from the fatty acyl groups at C1 and C2 positions. The ^{13}C NMR spectrum of lysoPC **1** showed the signal of the backbone carbon C_b at 74.9 ppm shifted downfield in comparison with that of lysoPC **2** at 70.1 ppm. Therefore, by combining this information we could confirm the structures of lysoPCs **1**–**3**.

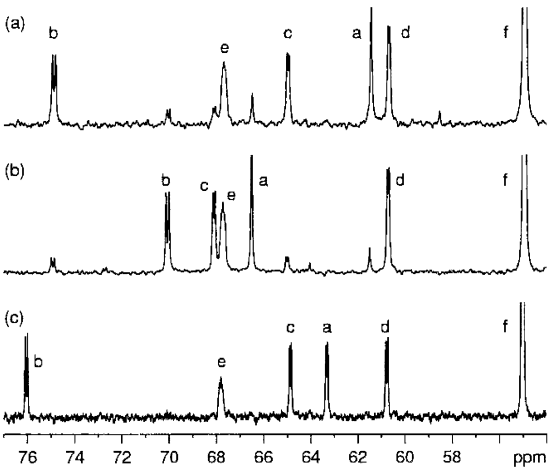


Figure 3. ^{13}C NMR spectra (63 MHz, CD_3OD) of the glycerol backbone and phosphocholine region of (a) lysoPC **1** (b) lysoPC **2** (c) lysoPC **3**.

Based on these observations, we suggest that the substituent pattern affects the ^1H and ^{13}C chemical shifts of the glycerol backbone, but not the ^{13}C chemical shifts of the polar head group.

1.2.2. FAB tandem mass spectrometric analysis. The FAB tandem mass spectra provide useful structural information based on understandable fragmentation patterns. LysoPC **1** was subjected to FAB-MS in combination with tandem mass spectrometry (MS/MS) and compared to that of regioisomers, lysoPCs **2** and **3**. FAB mass spectrum showed the prominent peak at *m/z* 518 corresponding to the sodium-adducted molecule $[M+Na]^+$

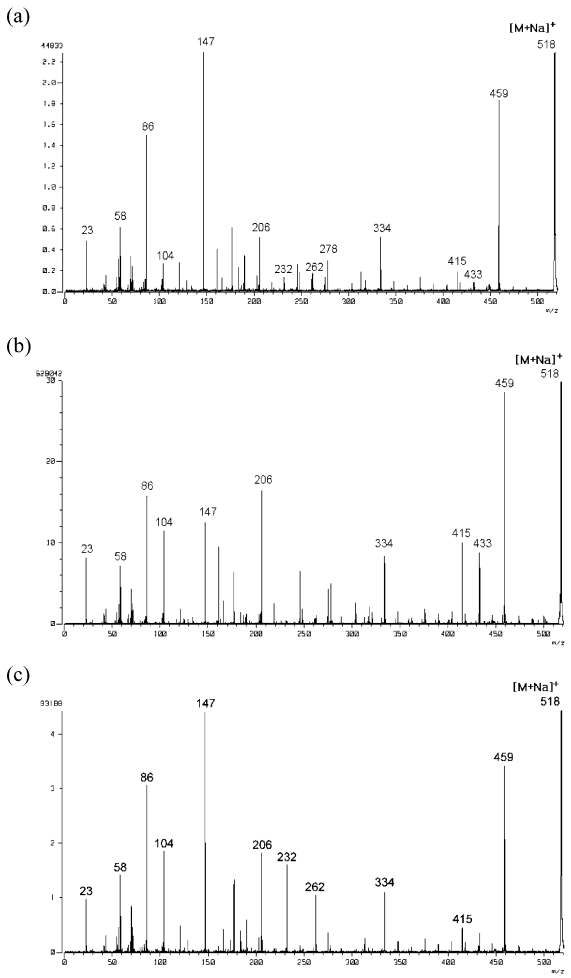


Figure 4. Positive-ion FAB tandem mass spectra of $[M+Na]^+$ ion of (a) lysoPC **1** (b) lysoPC **2** (c) lysoPC **3**, observed at *m/z* 518.

(not shown). It was proven that the collision-induced dissociation (CID) of this sodium-adducted molecule generated a wide variety of product ions. These ions provide information about the regiospecificity of fatty acyl linkages on the glycerol backbone, as well as on fatty acid composition and polar head group.[23] The tandem mass spectra (Fig. 4) of fragment ions produced by CID of $[M+Na]^+$ ions provided significant information about the structures of lysoPCs **1**–**3**. The product ions appearing below *m/z* 250 are formed by the fragmentation of the phosphocholine head group, supporting the presence of phosphocholine moiety. There are $[CH_2{=}NMe_2]^+$ (*m/z* 58), $[CH_2{=}CHNMe_3]^+$ (*m/z* 86), $[HOCH_2CH_2NMe_3]^+$ (*m/z* 104), and $[HOP(O)(ONa)OCH_2CH_2NMe_3]^+$ (*m/z* 206). An intense peak was also observed at *m/z* 147, which corresponds to the sodiated five-membered cyclophosphane. In addition, the neutral losses of trimethylamine, dehydrocholine and choline resulted in the generations of sodiated 1-acyl- or 2-acyl-*sn*-glycero-3-cyclo(1′,2′-ethylene glycol)phosphodiester (i.e. $[M+Na-59]^+$ at *m/z* 459), sodiated 1-acyl- or 2-acyl-*sn*-glycerol-3-phosphate (i.e. $[M+Na-85]^+$ at *m/z* 433) and sodiated monoacyl glycerophosphodiester (i.e. $[M+Na-103]^+$ at *m/z* 415), respectively. These product ions were also observed in their electrospray ionization (ESI) tandem mass spectra reported previously.[24] The structure of the fatty acyl group can be determined from analysis of the spectral pattern of the homologous ions formed by charge-remote fragmentation occurring along the long chain of fatty acyl group.[23] The palmitoyl group was confirmed by separation of 14 Da between the neighboring peaks due to the neutral loss of C_nH_{2n+2} along the fatty acyl chain from the alkyl-terminus.

On the other hand, several product ions informative on the regiospecificity of the fatty acyl group on the glycerol backbone were observed in their tandem mass spectra. Especially, the ratios of the peak intensities of specific product–ion pairs from lysoPC **1** and lysoPC **2** were significantly different (Fig. 4(a) and (b)). For example, the peak intensity ratio of product ions at *m/z* 104 and 147 differed by about tenfold in individual regioisomers. Furthermore, the product ions observed at *m/z* 415 and 433 in the MS/MS spectra of sodiated lysoPC **1** were less abundant than that from sodiated lysoPC **2**. These results are consistent with previous data obtained from low-energy CID of sodiated lysophosphocholine regioisomers produced by electrospray ionization.[24] In addition, in the MS/MS spectrum of lysoPC **3** (Fig. 4(c)), two characteristic peaks corresponding to $[CH_2{=}CHP(O)(ONa)OCH_2CH_2NMe_3]^+$ (*m/z* 232) and the fragment ion after the neutral loss of palmitic acid (*m/z* 262) have greater abundance than those from other regioisomers, lysoPCs **1** and **2**.

Thus, the regiospecificity of the fatty acyl linkage in the lysoPCs **1**–**3** can be easily determined from the ratios of the peak intensities of these product ions observed in CID spectra of sodium-adducted molecules of individual lysophosphocholine regioisomers.

2. Conclusions

We efficiently synthesized all three regioisomers of racemic lysoPC from glycerol. During the synthesis of the first target molecule, lysoPC **1**, we were unexpectedly confronted with acyl migration within the molecule. It was found that the benzyl protecting group was not suitable for the C1 hydroxyl group of the glycerol backbone because catalytic hydrogenolysis of the benzyl moiety resulted in only unexpected acyl-migrated product **2**. Thus, the second target molecule, lysoPC **2**, was easily obtained from glycerol in nine steps and 12% overall yield. However, this acyl migration was eliminated by use of TBDPS protecting group as well as careful deprotection of the silyl group with NBS in $DMSO/H_2O$ in the last step. This afforded lysoPC **1** in nine steps and overall 15% yield. Therefore, the choice of protecting group played a major role in the synthetic strategy. The third target molecule, lysoPC **3**, was also synthesized in a similar manner in overall 9% yield. Structures of all three isomers were confirmed by spectroscopic analysis. We determined the carbon–phosphorus and carbon–nitrogen coupling patterns were important criteria for complete assignment of the ^{13}C NMR spectra. The two and three bond C–P coupling and the shield effect both contributed to the coupling pattern and chemical shift differences between lysoPC **1** and lysoPC **2**. Additionally, the FAB tandem mass spectra were highly informative for structural elucidation. In particular, the CID of sodium-adducted molecules generated product ions, which were informative about the regiospecificity of the fatty acyl group on the glycerol backbone.

These results provide valuable information for the preparation and structural elucidation of the lysophosphatidylcholine as well as other lysophospholipids in order to study their biochemical mechanism of action.

3. Experimental

3.1. General

Melting points were determined on Fisher–Johns melting point apparatus and are uncorrected. Optical rotation was determined on a Jasco DIP-140 Digital Polarimeter. All anhydrous reaction performed in the oven-dried glassware under Ar condition. THF was distilled from sodium benzophenone ketyl, while MeOH, CH_2Cl_2 and EtOAc were distilled from calcium hydride. Benzaldehyde and DMF were vacuum distilled. Ethylene chlorophosphite and 40% aqueous trimethylamine were obtained from Aldrich Chemical Co. Br_2 was purchased from Junsei Chemical Co. Merck silica gel 60 (230–400 mesh) was used for flash column chromatography. TLC was performed on Merck silica gel 60 F-254 plates (0.25 mm). NMR spectra were recorded on a Bruker DPX 250 FT NMR and chemical shifts are expressed in parts per million (δ) relative to trimethylsilane (TMS, 0 ppm), chloroform ($CHCl_3$, 7.24 ppm for ^1H and 77.0 ppm for ^{13}C) or methanol (CH_3OH, 4.8 ppm for ^1H and 49.0 ppm for ^{13}C) as internal reference. Peak assignments based on DEPT 135, $^1H-^1H$ COSY and $^1H-^{13}C$ COSY experiments. IR spectra were recorded on a Bio-Rad FTS 165 spectrometer. Fast atom bombardment mass spectra and tandem mass spectra were obtained on JMS-HX110/110A tandem mass spectrometer (JEOL, Tokyo, Japan), a four-sector instrument of $E_1B_1E_2B_2$ configuration

described previously,[25] using the positive-ion mode with a 3-nitrobenzyl alcohol (NBA) as the matrix. CID of the sodium-adducted molecules selected with the first mass spectrometer occurred in the collision cell located between B_1 and E_2 and floated at 3.0 kV. The resultant product ions were analyzed by the B/E linked scan method in the second mass spectrometer. The collision gas, N_2, was introduced into the collision chamber at a pressure sufficient to reduce the precursor ion signal by 70%.

3.1.1. 1,3-Benzylideneglycerol (4). *p*-Toluenesulfonic acid (2.06 g, 10.9 mmol) was added to the solution of glycerol (10.0 g, 109 mmol) in benzaldeyde (110 mL, 1.09 mol). The solution was stirred at room temperature for 48 h and benzaldehyde was removed by distillation under reduced pressure. The residue was purified by flash column chromatography on silica gel (*n*-hexane/EtOAc, 7:1→6:1) to gave 8.50 g (46%) of product **4** as a white solid: mp 70–71°C; R_f 0.32 (*n*-hexane/EtOAc, 1:1); ^{1}H NMR (250 MHz, $CDCl_3$) δ 3.13 (d, J=8.8 Hz, 1H), 3.62 (m, 1H), 4.15 (m, 4H), 5.55 (s, 1H), 7.38 (m, 3H), 7.49 (m, 2H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 63.9, 72.2, 101.6, 125.8, 128.3, 129.1, 137.8; FTIR (KBr, cm^{-1}) 3273, 2860, 1158, 1092, 1015, 737.

3.1.2. 2-Palmitoyl-1,3-benzylideneglycerol (5). To a solution of compound **4** (8.45 g, 49.8 mmol) in CH_2Cl_2 (250 mL) was added palmitic acid (15.3 g, 59.7 mmol) and then was cooled to 0°C. To this was added 1,3-dicyclohexylcarbodiimide (12.3 g, 59.7 mmol) and 4-(*N*,*N*-dimethylamino)pyridine (1.20 g, 9.95 mmol). The reaction mixture was stirred at 0°C for 1 h followed by stirring at room temperature for 3 h. The insoluble material was filtered and the filtrate was concentrated. Chromatography on silica gel (*n*-hexane/EtOAc, 14:1→10:1) gave 16.7 g (82%) of product **5** as a white solid: mp 53–54°C; R_f 0.41 (*n*-hexane/EtOAc, 3:1); ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.88 (m, 3H), 1.25 (m, 24H), 1.67 (m, 2H), 2.44 (t, J=7.5 Hz, 2H), 4.23 (dd, J_1=12.4 Hz, J_2=30.7 Hz, 4H), 4.72 (s, 1H), 5.56 (s, 1H), 7.38 (m, 3H), 7.51 (m, 2H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 14.1, 22.7, 24.9, 29.1, 29.2, 29.3, 29.5, 29.6, 29.7, 31.9, 34.4, 65.7, 69.1, 101.2, 126.0, 128.3, 129.1, 137.8, 173.9; FTIR (KBr, cm^{-1}) 3302, 2919, 2851, 1731, 1470, 1180, 1048, 720; FABHRMS $[M+Na]^+$ m/z calcd for $C_{26}H_{42}O_4Na$ 441.3083, found 441.2982.

3.1.3. 2-Palmitoylglycerol (6). To a solution of compound **5** (3.90 g, 9.56 mmol) in EtOAc was added 10% Pd–C (0.800 g) and the mixture was stirred at a room temperature for 72 h under a hydrogen atmosphere. The reaction mixture was filtered through celite and concentrated to gave 2.74 g (98%) of product **6** as a white solid: mp 59–60°C; R_f 0.4 (*n*-hexane/EtOAc, 1:2); ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.88 (m, 3H), 1.26 (m, 24H), 1.66 (m, 2H), 2.06 (t, J=6.1 Hz, 2H), 2.38 (t, J=7.5 Hz, 2H), 3.84 (m, 4H), 4.93 (m, 1H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 14.1, 22.7, 25.0, 29.1, 29.2, 29.3, 29.4, 29.6, 29.7, 31.9, 34.3, 62.6, 75.0, 174.1; FABHRMS $[M+Na]^+$ m/z calcd for $C_{19}H_{38}O_4Na$ 353.2770, found 353.2666.

3.1.4. 1-Benzyl-2-palmitoyl-*rac*-glycerol (7a) and 1-benzyl-3-palmitoyl-*rac*-glycerol (7b). To a solution of compound **6** (1.45 g, 4.96 mmol) in CH_2Cl_2 (50 mL) was added silver(I) oxide (1.72 g, 5.46 mmol) and dropwise benzylbromide (0.7 mL, 5.46 mmol) at room temperature. After 5 h, the mixture was filtered through a pad of celite and the filtrate was concentrated. Chromatography on silica gel (*n*-hexane/EtOAc, 5:1→4:1) gave 0.480 g (44%) of product **7a** and 0.360 g (33%) of product **7b** as a colorless oil.

Compound **7a**. R_f 0.26 (*n*-hexane/EtOAc, 3:1); ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.87 (m, 3H), 1.25 (m, 24H), 1.61 (m, 2H), 2.15 (m, 1H), 2.32 (t, J=7.5 Hz, 2H), 3.69 (m, 3H), 4.23 (d, J=7.5 Hz, 2H), 4.64 (m, 2H), 7.34 (m, 5H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 14.8, 23.4, 25.6, 29.8, 30.0, 30.1, 30.2, 30.3, 30.4, 32.6, 34.9, 35.0, 62.7, 63.4, 72.8, 128.5, 128.6, 129.2, 138.5, 174.5; FTIR (KBr, cm^{-1}) 3448, 2926, 2854, 1739, 1457, 1114, 736; FABHRMS $[M+Na]^+$ m/z calcd for $C_{26}H_{44}O_4Na$ 443.3240, found 443.3132.

Compound **7b**. R_f 0.31 (*n*-hexane/EtOAc, 3:1); ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.88 (m, 3H), 1.26 (m, 24H), 1.56 (m, 2H), 2.27 (t, J=7.5 Hz, 2H), 3.28 (d, J=2.2 Hz, 1H), 3.46 (m, 2H), 3.97 (m, 1H), 4.13 (m, 2H), 4.49 (s, 2H), 7.28 (m, 5H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 14.6, 23.1, 25.3, 29.6, 29.7, 29.8, 29.9, 30.1, 30.1, 30.1, 32.4, 34.5, 65.7, 69.1, 71.4, 73.8, 127.2, 128.2, 128.8, 138.2, 174.2; FTIR (KBr, cm^{-1}) 2925, 2854, 1738, 1457, 1112, 735; FABHRMS $[M+H]^+$ m/z calcd for $C_{26}H_{45}O_4$ 421.3318, found 421.3320.

3.1.5. 1-Benzyl-2-palmitoyl-*rac*-glycero-3-phosphocholine (8). To a solution of compound **7a** (0.400 g, 0.702 mmol) in THF (14 mL) at −20°C was added *N*,*N*-diisopropylethylamine (0.5 mL, 2.81 mmol) and ethylene chlorophosphite (0.2 mL, 2.11 mmol). The mixture was stirred for 1 h and then Br_2 (0.1 mL, 2.11 mmol) was added at −20°C. After 15 min, H_2O (2.5 mL) was added at room temperature and then stirred for 1 h. To this was added NaCl and then separated. The organic layer was dried over Na_2SO_4 and concentrated. A solution of the residue in $CHCl_3$/^{i}PrOH/CH_3CN (v/v, 3/5/5, 14 mL) at 0°C was added 40% aqueous trimethylamine (8.0 mL, 135 mmol). The reaction mixture was stirred at 0°C for 1 h followed by stirring at room temperature for 12 h. The mixture was concentrated, and lyophilized. Chromatography on silica gel (CH_2Cl_2/MeOH, 3:1→0:1) gave 0.420 g (78% from **7a**) of product **8** as a white solid: R_f 0.31 (CH_2Cl_2/MeOH/H_2O, 2:1:0.2); ^{1}H NMR (250 MHz, CD_3OD) δ 0.90 (m, 3H), 1.28 (m, 24H), 1.58 (m, 2H), 2.32 (t, J=7.3 Hz, 2H), 3.14 (s, 9H), 3.52 (m, 2H), 3.87 (m, 1H), 4.00 (m, 2H), 4.21 (m, 3H), 4.34 (m, 1H), 4.67 (s, 2H), 7.32 (m, 5H); ^{13}C NMR (63 MHz, CD_3OD) δ 14.8, 24.1, 26.3, 30.5, 30.7, 30.8, 30.9, 31.1, 33.4, 35.3, 54.9, 60.7 (d, J_{cp}=5.0 Hz), 64.7, 66.0 (d, J_{cp}=5.6 Hz), 67.7, 73.4, 78.0 (d, J_{cp}=8.0 Hz), 129.1, 129.4, 129.7, 140.1, 175.5; FTIR (KBr, cm^{-1}) 3394, 2928, 2856, 1732, 1468, 1266, 1090, 970, 743; FABHRMS $[M+H]^+$ m/z calcd for $C_{31}H_{57}NO_7P$ 586.3872, found 586.3869.

3.1.6. 1-Palmitoyl-2-lyso-*rac*-glycero-3-phosphocholine (2). To a solution of compound **8** (0.160 g, 0.270 mmol) in MeOH (3.0 mL) was added 10% Pd–C (32.0 mg) and the mixture was stirred at a room temperature for 13 h under a hydrogen atmosphere. The reaction mixture was filtered through celite and concentrated. Chromatography on silica

gel (CH_2Cl_2/MeOH, 5:1→1:3→0:1) gave 0.520 g (97%) of product **2** as an white solid: R_f 0.21 (CH_2Cl_2/MeOH/H_2O, 2:1:0.2); ^{1}H NMR (250 MHz, CD_3OD) δ 0.90 (m, 3H), 1.28 (m, 24H), 1.60 (m, 2H), 2.35 (t, J=7.4 Hz, 2H), 3.23 (s, 9H), 3.65 (m, 2H), 3.89 (m, 2H), 3.97 (m, 1H), 4.14 (m, 2H), 4.30 (m, 2H); ^{13}C NMR (63 MHz, CD_3OD) δ 14.8, 24.0, 26.3, 30.5, 30.8, 30.9, 31.1, 33.4, 35.2, 35.4, 55.0, 60.7 (d, J_{cp}=4.4 Hz), 66.5, 67.7, 68.1 (d, J_{cp}=5.0 Hz), 70.1 (d, J_{cp}=7.6 Hz), 175.6; FTIR (KBr, cm^{-1}) 3242, 2918, 2851, 1736, 1468, 1241, 1092, 970; FABHRMS [M+H]$^+$ *m/z* calcd for $C_{24}H_{51}NO_7P$ 496.3403, found 496.3401.

3.1.7. 1-*tert*-Butyldiphenylsilyl-2-palmitoyl-*rac*-glycerol (9). To a solution of compound **6** (0.540 g, 1.85 mmol) in DMF (20 mL) was added imidazole (0.300 g, 4.07 mmol) and *tert*-butyldiphenylsilylchloride (0.5 mL, 2.04 mmol) at 0°C, and the mixture was stirred for 15 min. Then it was diluted with ether and washed with 5% HCl, brine and dried over Na_2SO_4. After evaporation of the solvent, the residue was purified by flash column chromatography on silica gel (*n*-hexane/EtOAc, 14:1→12:1) to give gave 0.300 g (70%) of product **9** as a colorless oil: R_f 0.39 (*n*-hexane/EtOAc, 5:1); ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.87 (m, 3H), 1.06 (m, 9H), 1.26 (m, 24H), 1.60 (m, 2H), 2.31 (m, 3H), 3.82 (m, 4H), 4.99 (m, 1H), 7.39 (m, 6H), 7.67 (m, 4H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 14.1, 19.1, 22.7, 24.9, 26.7, 29.1, 29.2, 29.3, 29.4, 29.6, 29.7, 31.9, 34.3, 62.5, 63.0, 74.5, 127.7, 129.8, 132.9, 135.5, 173.7; FTIR (KBr, cm^{-1}) 3651, 2927, 2856, 1737, 1428, 1112, 740; FABHRMS [M+H]$^+$ *m/z* calcd for $C_{35}H_{57}O_4Si$ 569.4026, found 569.4028.

3.1.8. 1-*tert*-Butyldiphenylsilyl-2-palmitoyl-*rac*-glycero-3-phosphocholine (10). Compound **10** was prepared under the procedure outlined above for compound **8** and was obtained as a white solid (0.402 g, 78%): R_f 0.50 (CH_2Cl_2/MeOH/H_2O, 2:1:0.2); ^{1}H NMR (250 MHz, CD_3OD) δ 0.79 (m, 3H), 0.94 (s, 9H), 1.16 (m, 24H), 1.49 (m, 2H), 2.22 (m, 2H), 3.09 (s, 9H), 3.50 (m, 2H), 3.77 (d, J=4.7 Hz, 2H), 3.94 (m, 2H), 4.11 (m, 2H), 5.08 (m, 1H), 7.33 (m, 6H), 7.58 (m, 4H); ^{13}C NMR (63 MHz, CD_3OD) δ 14.8, 20.4, 24.0, 26.3, 27.6, 30.5, 30.7, 30.8, 30.9, 31.1, 31.1, 33.4, 35.5, 54.9, 60.7 (d, J_{cp}=5.0 Hz), 64.3, 65.3 (d, J_{cp}=5.3 Hz), 67.6, 75.0 (d, J_{cp}=8.3 Hz), 129.2, 131.3, 134.6, 137.0, 175.1; FTIR (KBr, cm^{-1}) 3346, 2927, 2856, 1735, 1429, 1247, 1069, 969; FABHRMS [M+H]$^+$ *m/z* calcd for $C_{40}H_{69}NO_7PSi$ 734.4581, found 734.4581.

3.1.9. 1-Lyso-2-palmitoyl-*rac*-glycero-3-phosphocholine (1). To a solution of compound **10** (0.450 g, 0.610 mmol) in DMSO/H_2O (8:1, 6.1 mL) was added *N*-bromosuccinimide (0.330 g, 1.83 mmol) under dark. The mixture was stirred for 72 h at room temperature and then lyophilized. Chromatography on silica gel (CH_2Cl_2/MeOH, 5:1→1:3→0:1) gave 0.220 g (72%) of product **1** as a white solid: R_f 0.21 (CH_2Cl_2/MeOH/$_2$O, 2:1:0.2); ^{1}H NMR (250 MHz, CD_3OD) δ 0.89 (m, 3H), 1.28 (m, 24H), 1.60 (m, 2H), 2.35 (t, J=7.4 Hz, 2H), 3.23 (s, 9H), 3.67 (m, 4H), 3.96 (m, 2H), 4.28 (m, 2H), 5.00 (m, 1H); ^{13}C NMR (63 MHz, CD_3OD) δ 14.8, 24.0, 26.3, 30.5, 30.8, 30.9, 31.1, 33.4, 35.4, 55.0, 60.7 (d, J_{cp}=4.4 Hz), 61.5, 65.0 (d, J_{cp}=5.0 Hz), 67.7, 74.9 (d, J_{cp}=8.2 Hz), 175.1; FTIR (KBr, cm^{-1}) 3377, 2919, 2851, 1729, 1468, 1233, 967; FABHRMS [M+H]$^+$ *m/z* calcd for $C_{24}H_{51}NO_7P$ 496.3403, found 496.3405.

3.1.10. 1-Benzyl-3-palmitoyl-*rac*-glycero-2-phosphocholine (11). Compound **11** was prepared under the procedure outlined above for compound **8** and was obtained as a white solid (0.189 g, 46%): R_f 0.31 (CH_2Cl_2/MeOH/H_2-O, 2:1:0.2); ^{1}H NMR (250 MHz, CD_3OD) δ 0.90 (m, 3H), 1.28 (m, 24H), 1.57 (m, 2H), 2.30 (t, J=7.4 Hz, 2H), 3.14 (s, 9H), 3.50 (m, 2H), 3.68 (d, J=5.2 Hz, 2H), 4.24 (m, 2H), 4.30 (m, 2H), 4.48 (m, 1H), 4.55 (s, 2H), 7.32 (m, 5H); ^{13}C NMR (63 MHz, CD_3OD) δ 14.7, 24.0, 26.3, 30.5, 30.7, 30.7, 30.9, 31.1, 33.4, 35.2, 55.0, 60.7 (d, J_{cp}=5.0 Hz), 65.1 (d, J_{cp}=5.0 Hz), 67.6, 71.3 (d, J_{cp}=5.3 Hz), 74.2 (d, J_{cp}=5.5 Hz), 74.7, 129.1, 129.4, 129.8, 139.9, 175.4; FTIR (KBr, cm^{-1}) 3423, 2924, 2853, 1735, 1458, 1233, 1088, 969, 749; FABHRMS [M+H]$^+$ *m/z* calcd for $C_{31}H_{57}NO_7P$ 586.3872, found 586.3873.

3.1.11. 1-Lyso-3-palmitoyl-*rac*-glycero-2-phosphocholine (3). Compound **3** was prepared under the procedure outlined above for compound **2** and was obtained as a white solid (0.120 g, 90%): R_f 0.21 (CH_2Cl_2/MeOH/H_2O, 2:1:0.2); ^{1}H NMR (250 MHz, CD_3OD) δ 0.88 (m, 3H), 1.29 (m, 24H), 1.61 (m, 2H), 2.35 (t, J=7.3 Hz, 2H), 3.23 (s, 9H), 3.66 (m, 2H), 3.70 (m, 2H), 4.26 (m, 2H), 4.31 (m, 3H); ^{13}C NMR (63 MHz, CD_3OD) δ 14.8, 24.0, 26.3, 30.5, 30.7, 30.9, 31.1, 33.4, 35.2, 55.0, 60.8 (d, J_{cp}=5.0 Hz), 63.3 (d, J_{cp}=4.4 Hz), 64.9 (d, J_{cp}=5.0 Hz), 67.8, 76.1 (d, J_{cp}=6.3 Hz), 175.4; FTIR (KBr, cm^{-1}) 3400, 2921, 2844, 1736, 1463, 1227, 1065, 949; FABHRMS [M+H]$^+$ *m/z* calcd for $C_{24}H_{51}NO_7P$ 496.3403, found 496.3414.

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