Synthesis of Lysophosphatidylcholine Analogues Using D-Mannitol as a Chiral Template and Their Biological Activity for Sepsis

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Received March 13, 2006

LPC analogues including natural and unnatural LPC, 3-L-2-PC, acetylated LPC and ethylene glycol derivative are prepared from D-mannitol using in convenient procedures by only changing the synthetic sequences, and their protective activities against cecal ligation and puncture (CLP)-induced severe sepsis are compared. The chirality at C2 position in LPC is found to be required as (S)-configuration for sepsis inhibition, comparing from the protection activity between LPC **6** and unnatural LPC **8**. The hydroxyl functionality is also very important and required at C2 or C3 position as shown in the protection activities of ethylene glycol analogue **11** and 3-L-2-PC **9**.

Key Words : Lysophosphatidylcholine (LPC), Sepsis, D-Mannitol, Phosphocholine

Introduction

Sepsis shows an increasing cause of death in intensive care units resulting from immunosuppression.[1] Severe sepsis is defined by signs of systemic inflammation and organ dysfunction, including abnormalities in body temperature, heart rate, respiratory rate and leukocyte count, elevated liver enzymes and altered cerebral function.[2] Recently, it was reported that administration of lysophosphatidylcholine (LPC) protects mice against lethal sepsis, even when the first dose was given as late as ten hours after the onset of disease.[3] It was found that LPC protects against lethal sepsis by enhancing the bactericidal function of neutrophils, and by effectively pre-empting the production of causative inflammatory mediators such as TNF.[3] LPC is normally found at high levels in the circulation, and decreases significantly in patients with sepsis.[4] In this paper, a convenient synthesis of LPC analogues including natural and unnatural LPC, 3-L-2-PC, acetylated LPC and ethylene glycol derivative is reported, and their protective activities against cecal ligation and puncture (CLP)-induced severe sepsis are compared.

Results and Discussion

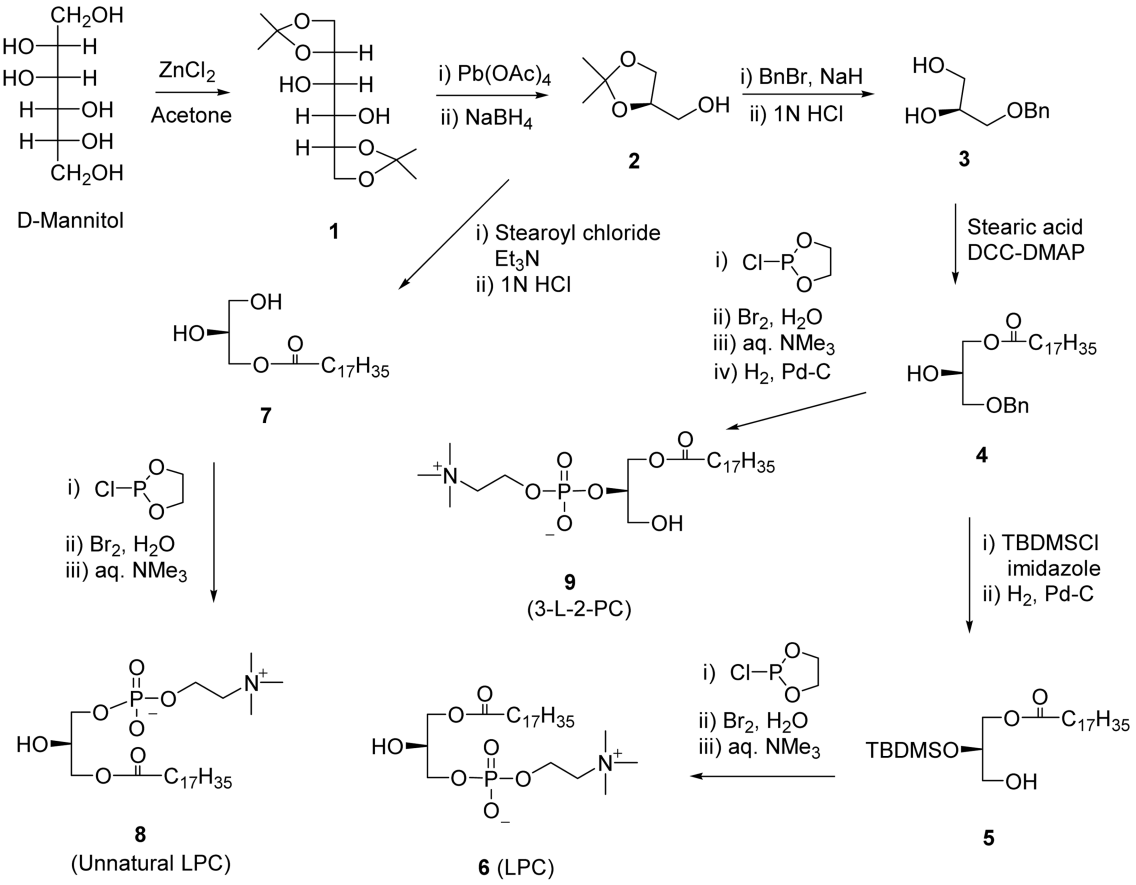
Han reported the racemic synthesis of LPC from glycerol[5] and we modified their method for the synthesis of chiral LPC and analogues. D-Mannitol has been utilized for the synthesis of key intermediate **2** *via* acetonide formation, lead tetraacetate cleavage followed by sodium borohydride reduction as shown in McClure's method (Scheme 1).[6] The isopropylidene glycerol **2** was chosen as the chiral template and protected by benzylation with BnBr/NaH and hydrolyzed with HCl to provide benzyl glycerol **3** in 86% yield. Coupling reaction between glycerol **3** and stearic acid was achieved by using DCC methodology to provide the desired ester **4** in 68% yield. Protection (88%) of secondary alcohol of benzyl ester **4** with TBDMSCl/imidazol in DMF and following debenzylation (70%) with H_2/Pd-C produced the C3-alcohol **5**. The introduction of the phosphocholine group into the C3 hydroxyl position was examined by using cholinetosylate,[7] 2-bromoethyl dichlorophosphate,[8] ethylene chlorophosphite-trimethylamine[9] protocols, but only the ethylene chlorophosphite-trimethylamine method gave the LPC **6** (same configuration with natural LPC; (S)-2-lyso-1-stearoyl-*sn*-glycero-3-phosphocholine) in 57% yield. It was noteworthy that the deprotection of the TBDMS group occurred during Br_2-H_2O reaction.

Synthesis of the unnatural LPC **8** (enantiomer of the LPC **6**, (R)-2-lyso-3-stearoyl-*sn*-glycero-1-phosphocholine) was accomplished by esterification (87%) of the isopropylidene glycerol **2** with stearoyl chloride in triethylamine followed by hydrolysis and phosphocholination using same methods but different sequences as shown in the synthesis of the LPC **6**.

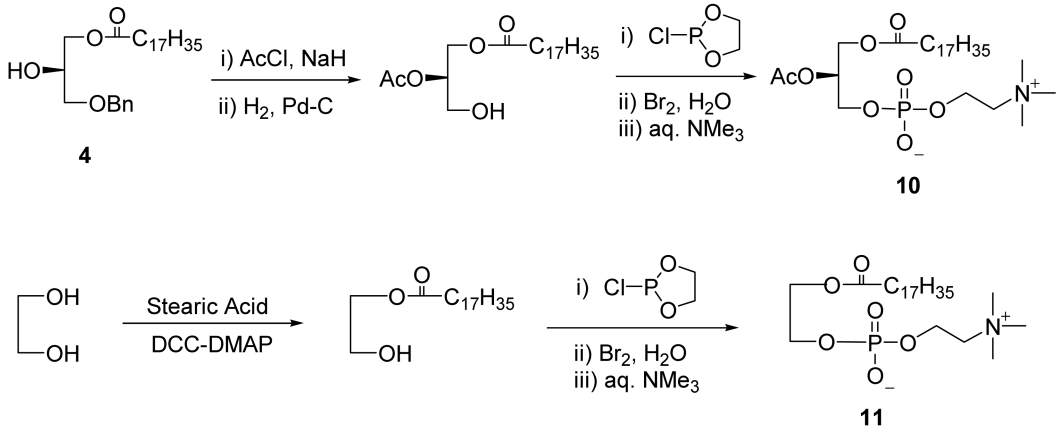
A regioisomer, (S)-3-lyso-1-stearoyl-*sn*-glycero-2-phosphocholine **9** (3-L-2-PC), was synthesized by phosphocholination (52%) from the benzyl ester **4** followed by debenzylation (70%).

In order to study the structure-activity relationship for the compounds, we made acetylated derivative of the LPC **6**. Ester **4** was acetylated with acetyl chloride/NaH (65%) followed by debenzylation (84%) and phosphocholination (60%) to give the acetyl product **10** (Scheme 2). Ethylene glycol derivative **11** was also prepared by esterification (71%) and phosphocholination (35%) sequentially from the ethylene glycol.

All the LPC analogues, **6**, **8**, **9**, **10** and **11**, were testified for the protective activity against sepsis (Figure 1). CLP was performed on albino ICR (Institute of Cancer Research) mice, as described in experimental section. Animals were subcutaneously injected with various doses of LPC analogues or with vehicle (PBS containing 2% BSA). Injections



Scheme 1



Scheme 2

were given four times at different adjacent sites, at 12 h intervals beginning 2 h after CLP. Mice were then observed for up to 10 days after CLP. A natural 18 : 0 (one-position fatty acid chain length/degree of saturation) LPC **6** was known to give the significant protection against CLP-induced lethality.[3] 3-L-2-PC **9** showed a comparable activity with the LPC **6** on CLP mice survival at a dose of 10 mg/kg as shown in Figure 1a. But, there was no additive effect between LPC and 3-L-2-PC **9** (Figure 1a). 3-L-2-PC provided significant protection against CLP-induced lethality even at a dose of 3 mg/kg (Figure 1b). Unnatural LPC **8**, acetylated derivative **10** and ethylene glycol analogue **11** provided no significant protection against CLP-induced lethality as shown in Figure 1c, 1d and 1e respectively.

In conclusion, LPC analogues are prepared from D-mannitol using in convenient procedures by only changing the synthetic sequences. The chirality at C2 position in LPC is found to be required as (S)-configuration for sepsis inhibition, comparing from the protection activity between LPC **6** and unnatural LPC **8**. The hydroxyl functionality is also very

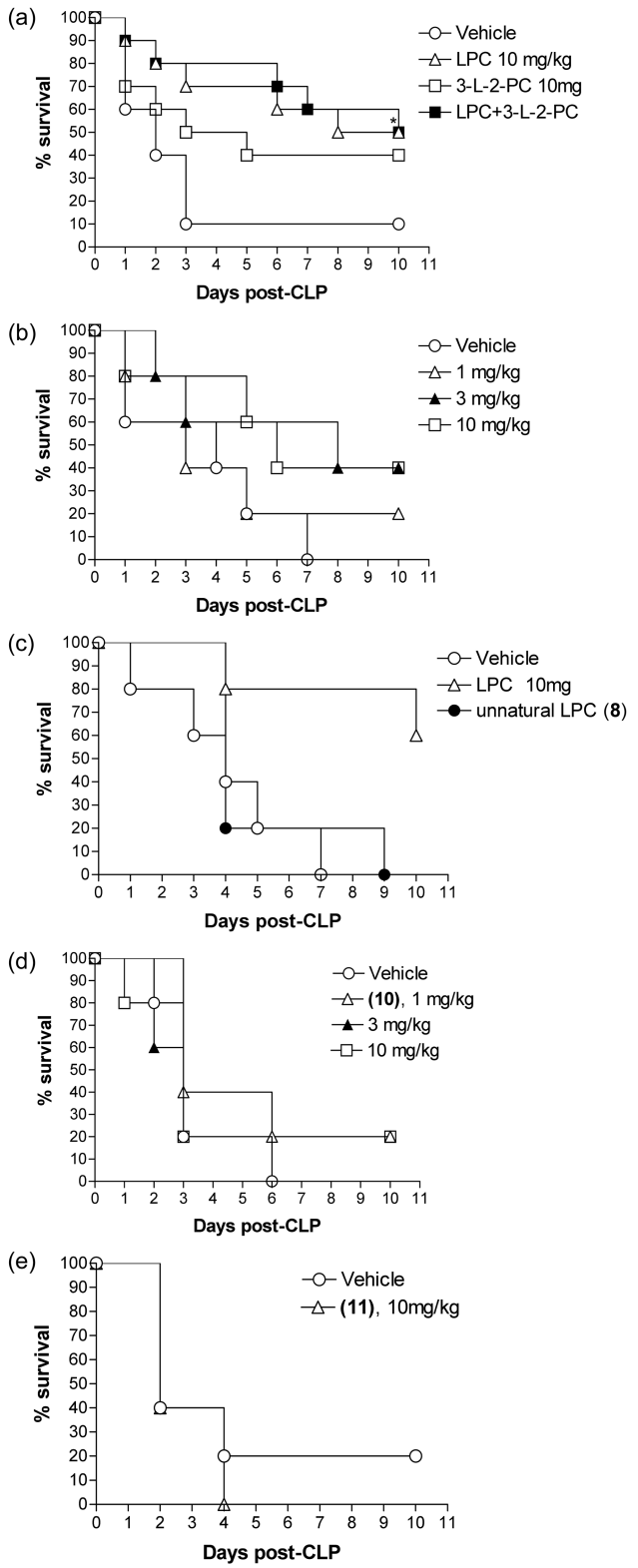


Figure 1. LPC protects against sepsis-induced lethality. (a) Effects of LPC **6**, 3-L-2-PC **9** and their mixture on CLP mice survival. (b) CLP mice were administered with various doses of 3-L-2-PC. (c) Effects of LPC **6** and unnatural LPC **8** on CLP mice survival (10 mg/kg). (d) CLP mice were administered with various doses of acetylated LPC **10**. (e) Effect of ethylene glycol derivative **11** on CLP mice survival. *, $p < 0.01$ compared with vehicle. n = 5-10 mice per group.

important and required at C2 or C3 position as shown in the protection activities of ethylene glycol analogue **11** and 3-L-2-PC **9**.

Experimental Section

Animals and the Sepsis Model. We used male ICR mice. Procedures for animal experiments were approved by the Animal Experimentation Committee at Hallym University. For CLP, mice were anesthetized with pentobarbital (50 mg/kg, i.p.), a small abdominal midline incision was made, and the cecum was exposed. The cecum was mobilized and ligated below the ileocecal valve, punctured through both surfaces twice with a 22-gauge needle, and the abdomen was closed. Survival was monitored once daily for 10 days.

(S)-3-Benzyl-*sn*-glycerol (3). To a solution of (S)-1,2-*O*-isopropylidene-*sn*-glycerol **2** (1.00 g, 8.00 mmol) in dry THF (10 mL) under nitrogen atmosphere was added sodium hydride (*ca*. 60% in oil, 0.26 g, 17.5 mmol) with benzyl bromide (1.40 g, 8.80 mmol) and heated for 3 h at 70-80 °C. The reaction was quenched by the addition of aqueous sodium bicarbonate at rt. The organic product was extracted with ethyl acetate, washed with brine, dried and concentrated to give the liquid. Acetone with 1 N HCl was added to this liquid and stirred for 30 min at rt. Evaporation and column chromatography (EtOAc : hexane = 1 : 15) gave the product **3** (1.20 g, 86%). R_f 0.26 (EtOAc : hexane = 2 : 1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.49 (2H, br s), 3.52-3.74 (4H, m), 3.91 (1H, m), 4.56 (2H, s), 7.33 (5H, m).

(S)-3-Benzyl-1-stearoyl-*sn*-glycerol (4). The mixture of stearic acid (1.11 g, 3.92 mmol), DCC (1.47 g, 1.91 mmol) and DMAP (0.02 g, 0.05 mmol) in dry methylene chloride (30 mL) under nitrogen atmosphere was stirred for 30 min at rt. (S)-3-benzyl-*sn*-glycerol **3** (0.68 g, 3.73 mmol) was added and stirred for 12 h at 45 °C. Filtration, evaporation and column chromatography (EtOAc : hexane = 1 : 15) gave the product **3** (1.10 g, 68%). R_f 0.27 (EtOAc : hexane = 1 : 4); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (3H, t, J = 6.5 Hz), 1.25 (28H, s), 1.61 (2H, m), 2.33 (2H, t, J = 7.4 Hz), 2.52 (1H, d, J = 4.7 Hz), 3.53 (2H, m), 4.04 (1H, m), 4.16 (2H, m), 4.56 (2H, s), 7.33 (5H, m).

(S)-3-Benzyl-2-*tert*-butyldimethylsilyl-1-stearoyl-*sn*-glycerol. To a solution of (S)-3-benzyl-1-stearoyl-*sn*-glycerol **4** (0.45 g, 1.04 mmol) in DMF was added TBDMSCl (0.17 g, 1.14 mmol) with imidazole (0.14 g, 2.08 mmol) and stirred for 2 h at rt. The reaction mixture was extracted with methylene chloride, washed with brine and 1 N HCl, dried, concentrated and column chromatography (EtOAc : hexane = 1 : 20) gave the product (0.50 g, 88%). R_f 0.78 (EtOAc : hexane = 1 : 4); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.10 (6H, s), 0.87 (12H, br s), 1.24 (28H, br s), 1.60 (2H, br s), 2.32 (2H, t, J = 7.4 Hz), 3.44 (1H, br d, J = 5.2 Hz), 3.62 (1H, dd, J = 4.8, 1.9 Hz), 3.74 (1H, dd, J = 5.2, 1.0 Hz), 4.02 (1H, m), 4.52 (2H, br s), 5.05 (1H, quintet, J = 5.2 Hz), 7.31 (5H, br s).

(S)-2-*tert*-Butyldimethylsilyl-1-stearoyl-*sn*-glycerol (5). To a solution of (S)-3-benzyl-2-*tert*-butyldimethylsilyl-1-

stearoyl-*sn*-glycerol (0.59 g, 1.05 mmol) in THF-EtOH (15 mL, 4 : 1) under nitrogen was added 10% Pd-C (0.33 g) with hydrogen gas bubbling and stirred for 12 h at rt. Filtration, concentration and column chromatography (EtOAc : hexane = 1 : 20) gave the product **5** (0.34 g, 70%). R_f 0.55 (EtOAc : hexane = 1 : 4); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.10 (6H, br s), 0.87 (12H, m), 1.25 (28H, br s), 1.60 (2H, m), 2.32 (2H, t, J = 7.4 Hz), 3.56 (1H, dd, J = 7.7, 4.2 Hz), 3.71 (2H, dd, J = 14.1, 6.9 Hz), 3.90 (1H, m), 4.06 (1H, dd, J = 5.8, 2.8 Hz).

(S)-2-Lyso-1-stearoyl-*sn*-glycero-3-phosphocholine (6). To a solution of (S)-2-*tert*-butyldimethylsilyl-1-stearoyl-*sn*-glycerol **5** (0.30 g, 0.63 mmol) in dry THF (13 mL) under nitrogen at −20 °C was added DIPEA (0.44 mL, 2.54 mmol) with ethylene chlorophosphite (0.17 mL, 1.90 mmol) and stirred for 30 min at same temperature. Br_2 (0.10 mL, 1.90 mmol) was added to the reaction mixture at −50 °C and stirred for 20 min. Water (2.2 mL) was added to the reaction bottle and stirred for 1 h at rt. 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, 13 mL) at 0 °C was added 30% aqueous trimethylamine (13 mL). The reaction mixture was stirred at 0 °C for 1 h followed by stirring at rt for 12 h. Concentration and column chromatography (CH_2Cl_2 : MeOH = 5 : 1 → 1 : 3 → 0 : 1) gave the white solid **6** (0.18 g, 57%). R_f 0.26 (CH_2Cl_2 : MeOH : H_2O = 2 : 1 : 0.2); mp 100-200 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89 (3H, t, J = 6.5 Hz), 1.28 (28H, br s), 1.62 (2H, br s), 2.35 (2H, t, J = 7.4 Hz), 3.22 (9H, s), 3.32 (1H, m), 3.62 (2H, m), 3.90 (2H, m), 3.97 (1H, m), 4.14 (2H, m), 4.28 (2H, m).

(S)-1,2-*O*-Isopropylidene-3-stearoyl-*sn*-glycerol. To a solution of (S)-1,2-*O*-isopropylidene-*sn*-glycerol **2** (0.30 g, 2.27 mmol) in dry methylene chloride (10 mL) under nitrogen was added stearoyl chloride (0.75 mL, 2.25 mmol) with triethylamine (0.47 mL, 3.40 mmol) and stirred for 30 min at rt. Extraction with methylene chloride, evaporation and concentration gave the product (0.79 g, 87%). R_f 0.70 (EtOAc : hexane = 1 : 3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87 (3H, br t), 1.24 (28H, s), 1.36 (3H, s), 1.42 (3H, s), 1.61 (2H, br s), 2.33 (2H, t, J = 7.4 Hz), 3.72 (1H, dd, J = 7.9, 6.6 Hz), 4.04-4.20 (3H, m), 4.30 (1H, quintet, J = 5.5 Hz).

(S)-3-Stearoyl-*sn*-glycerol (7). To a solution of (S)-1,2-*O*-isopropylidene-3-stearoyl-*sn*-glycerol (0.20 g, 0.50 mmol) in acetone (50 mL) was added aqueous 3 N HCl (10 mL) and stirred for 1 h at rt. Evaporation and column chromatography (EtOAc : hexane = 1 : 4) gave the product **7** (0.15 g, 85%). R_f 0.32 (EtOAc : hexane = 1 : 1); mp 72-73 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.81 (3H, t, J = 6.5 Hz), 1.18 (28H, s), 1.55 (2H, br s), 2.27 (2H, t, J = 7.4 Hz), 3.37 (2H, br s), 3.51 (1H, dd, J = 11.5, 5.8 Hz), 3.62 (1H, dd, J = 11.6, 3.9 Hz), 3.74 (1H, d, J = 4.9 Hz), 3.86 (1H, br quintet, J = 4.2 Hz), 4.09 (1H, m).

(R)-2-Lyso-3-stearoyl-*sn*-glycero-1-phosphocholine (8). To a solution of (S)-3-stearoyl-*sn*-glycerol **7** (0.20 g, 0.56 mmol) in dry THF (12 mL) under nitrogen at −20 °C was added DIPEA (0.39 mL, 2.23 mmol) with ethylene chlorophosphite (0.15 mL, 1.67 mmol) and stirred for 30 min at same temperature. Br_2 (0.10 mL, 1.90 mmol) was added to the reaction mixture at −50 °C and stirred for 20 min. Water (2.0 mL) was added to the reaction bottle and stirred for 1 h at rt. 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, 12 mL) at 0 °C was added 30% aqueous trimethylamine (12 mL). The reaction mixture was stirred at 0 °C for 1 h followed by stirring at rt for 12 h. Concentration and column chromatography (CH_2Cl_2 : MeOH = 5 : 1 → 1 : 3 → 0 : 1) gave the white solid **8** (0.03 g, 11%). R_f 0.26 (CH_2Cl_2 : MeOH : H_2O = 2 : 1 : 0.2); mp 100-200 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89 (3H, t, J = 6.5 Hz), 1.28 (28H, br s), 1.62 (2H, br s), 2.35 (2H, t, J = 7.4 Hz), 3.22 (9H, s), 3.32 (1H, m), 3.62 (2H, m), 3.90 (2H, m), 3.97 (1H, m), 4.14 (2H, m), 4.28 (2H, m).

(S)-3-Benzyl-1-stearoyl-*sn*-glycero-2-phosphocholine. To a solution of benzyl ester **4** (0.35 g, 0.81 mmol) in dry THF (16 mL) under nitrogen at −20 °C was added DIPEA (0.56 mL, 3.24 mmol) with ethylene chlorophosphite (0.22 mL, 2.43 mmol) and stirred for 30 min at same temperature. Br_2 (0.30 mL, 2.43 mmol) was added to the reaction mixture at −50 °C and stirred for 20 min. Water (2.9 mL) was added to the reaction bottle and stirred for 1 h at rt. 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, 16 mL) at 0 °C was added 30% aqueous trimethylamine (16 mL). The reaction mixture was stirred at 0 °C for 1 h followed by stirring at rt for 12 h. Concentration and column chromatography (CH_2Cl_2 : MeOH = 5 : 1 → 1 : 3 → 0 : 1) gave the product (0.24 g, 52%). R_f 0.53 (CH_2Cl_2 : MeOH : H_2O = 2 : 1 : 0.2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89 (3H, t, J = 6.5 Hz), 1.28 (28H, br s), 1.60 (2H, m), 2.35 (2H, t, J = 7.4 Hz), 3.20 (9H, s), 3.52 (2H, m), 3.68 (2H, d, J = 5.2 Hz), 4.24 (2H, m), 4.30 (2H, m), 4.48 (1H, m), 4.54 (2H, s), 7.31 (5H, s).

(S)-3-Lyso-1-stearoyl-*sn*-glycero-2-phosphocholine (9). To a solution of (S)-3-benzyl-1-stearoyl-*sn*-glycero-2-phosphocholine (0.39 g, 0.64 mmol) in MeOH (7 mL) under nitrogen was added 10% Pd-C (0.13 g) with hydrogen gas bubbling and stirred for 12 h at rt. Filtration, concentration and column chromatography (CH_2Cl_2 : MeOH = 5 : 1 → 1 : 3 → 0 : 1) gave the product (0.23 g, 70%). R_f 0.26 (CH_2Cl_2 : MeOH : H_2O = 2 : 1 : 0.2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89 (3H, t, J = 6.5 Hz), 1.28 (28H, m), 1.60 (2H, m), 2.35 (2H, t, J = 7.4 Hz), 3.23 (9H, s), 3.67 (4H, m), 3.96 (2H, m), 4.28 (2H, m), 5.00 (1H, m).

(S)-2-Acetyl-3-benzyl-1-stearoyl-*sn*-glycerol. To a solution of (S)-3-benzyl-1-stearoyl-*sn*-glycerol **4** (1.00 g, 2.30 mmol) in THF (20 mL) was added NaH (*ca.* 60% in oil; 0.18 g, 4.60 mmol) with acetyl chloride (0.20 mL, 2.77 mmol) and stirred for 3 h at rt. The reaction mixture was extracted with chloroform, washed with saturated $NaHCO_3$ and dried, concentrated and column chromatography (EtOAc : hexane = 1 : 4) gave the product (0.71 g, 65%). R_f 0.63 (EtOAc : hexane = 1 : 4); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (3H, t, J = 6.5 Hz), 1.25 (28H, s), 1.61 (2H, m), 2.02

(3H, s), 2.32 (2H, m), 3.58 (2H, d, J = 5.2 Hz), 4.24 (2H, m), 4.52 (2H, s), 5.23 (1H, m), 7.29 (5H, m).

(S)-2-Acetyl-3-lyso-1-stearoyl-*sn*-glycerol. To a solution of (S)-2-acethyl-3-benzyl-1-stearoyl-*sn*-glycerol (0.27 g, 0.55 mmol) in MeOH (20 mL) under nitrogen was added 10% Pd-C (0.45 g) with hydrogen gas bubbling and stirred for 12 h at rt. Filtration, concentration and column chromatography (EtOAc : hexane = 1 : 4) gave the product (0.18 g, 84%). R_f 0.20 (EtOAc : hexane = 1 : 4); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (3H, t, J = 6.5 Hz), 1.26 (28H, s), 1.61 (2H, m), 2.10 (3H, s), 2.32 (2H, m), 2.69 (1H, br s), 3.70 (2H, d, J = 5.2 Hz), 4.22 (2H, m), 5.07 (1H, m).

(S)-2-Acetyl-1-stearoyl-*sn*-glycero-3-phosphocholine (10). To a solution of (S)-2-acetyl-3-lyso-1-stearoyl-*sn*-glycerol (0.15 g, 0.37 mmol) in dry THF (7 mL) under nitrogen at −20 °C was added DIPEA (0.26 mL, 1.50 mmol) with ethylene chlorophosphite (0.10 mL, 1.12 mmol) and stirred for 30 min at same temperature. Br_2 (0.06 mL, 1.12 mmol) was added to the reaction mixture at −50 °C and stirred for 20 min. Water (1.3 mL) was added to the reaction bottle and stirred for 1 h at rt. 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, 7 mL) at 0 °C was added 30% aqueous trimethylamine (7 mL). The reaction mixture was stirred at 0 °C for 1 h followed by stirring at rt for 12 h. Concentration and column chromatography (CH_2Cl_2 : MeOH = 5 : 1 → 1 : 3 → 0 : 1) gave the product (0.12 g, 60%). R_f = 0.6 (CH_2Cl_2 : MeOH : H_2O = 2 : 1 : 0.2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (3H, t, J = 6.5 Hz), 1.25 (28H, s), 1.57 (2H, m), 2.06 (3H, s), 2.28 (2H, t, J = 7.4 Hz), 3.34 (9H, s), 3.78 (2H, m), 3.93 (2H, t, J = 6.0 Hz), 4.10-4.36 (4H, m), 5.17 (1H, m).

2-Stearoylethylene Glycol. The mixture of stearic acid (2.31 g, 7.89 mmol), DCC (1.99 g, 1.91 mmol) and DMAP (0.02 g, 1.61 mmol) in dry methylene chloride (40 mL) under nitrogen atmosphere was stirred for 30 min at rt. Ethylene glycol (0.50 g, 8.06 mmol) was added and stirred for 12 h at 45 °C. Filtration, evaporation and column chromatography (EtOAc : hexane = 1 : 15) gave the product (1.90 g, 71%). R_f 0.25 (EtOAc : hexane = 1 : 3); mp 48-51 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87 (3H, t, J = 6.3 Hz), 1.24 (28H, s), 1.62 (2H, m), 1.92 (1H, br s), 2.34 (2H, t, J = 7.4 Hz), 3.81 (2H, br s), 4.20 (2H, br s).

1-Stearoylethyleneglyco-2-phosphocholine (11). To a solution of 2-stearoylethylene glycol (0.30 g, 0.91 mmol) in dry THF (18 mL) under nitrogen at −20 °C was added DIPEA (0.64 mL, 3.65 mmol) with ethylene chlorophosphite (0.24 mL, 2.73 mmol) and stirred for 30 min at same temperature. Br_2 (0.14 mL, 2.73 mmol) was added to the reaction mixture at −50 °C and stirred for 20 min. Water (3.2 mL) was added to the reaction bottle and stirred for 1 h at rt. 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, 18 mL) at 0 °C was added 30% aqueous trimethylamine (18 mL). The reaction mixture was stirred at 0 °C for 1 h followed by stirring at rt for 12 h. Concentration and column chromatography (CH_2Cl_2 : MeOH = 5 : 1 → 1 : 3 → 0 : 1) gave the product (0.14 g, 35%). R_f = 0.41 (CH_2Cl_2 : MeOH : H_2O = 2 : 1 : 0.2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.91 (3H, t, J = 6.5 Hz), 1.29 (28H, s), 1.61 (2H, m) 2.34 (2H, t, J = 7.5 Hz), 3.23 (9H, s), 3.64 (2H, m), 4.06 (2H, m), 4.26 (4H, m).

Acknowledgments. This work was supported by the Korea Institute of Industrial Technology Evaluation & Planning (ITEP) through the Silver Biotechnology Research Center at Hallym University (RRC02725) and by the Korea Science and Engineering MRC program of MOST/KOSEF (D.-K. Song; grant: R13-2005-022-01000-0).

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