

## Synthesis of phospholipids with fatty acid chains containing aromatic units at various depths

M. Subramanian, V. Praveen Kumar, and Santanu Bhattacharya\*

*Department of Organic Chemistry, Indian Institute of Science, Bangalore, India 560 012.*

*E-mail: [sb@orgchem.iisc.ernet.in](mailto:sb@orgchem.iisc.ernet.in)*

**Dedicated to Professor S. V. Kessar on the occasion of his 70<sup>th</sup> birthday**

**(received 03 Apr 02; accepted 06 Aug 02; published on the web 14 Aug 02)**

---

### Abstract

A series of diacyl phosphatidylcholine lipid derivatives, which contain aromatic units at various depths of their fatty acid chains, have been synthesized. These lipids produced stable aqueous suspensions. Electron microscopy revealed the presence of vesicular aggregates in the suspensions of these newly synthesized lipids. These membranes were oxidatively stable and maintained fluid character at ambient temperature making them ideal candidates for membrane protein reconstitution studies.

**Keywords:** Phosphatidylcholine lipids, aromatic unsaturations, membranes, vesicles and fluidity

---

### Introduction

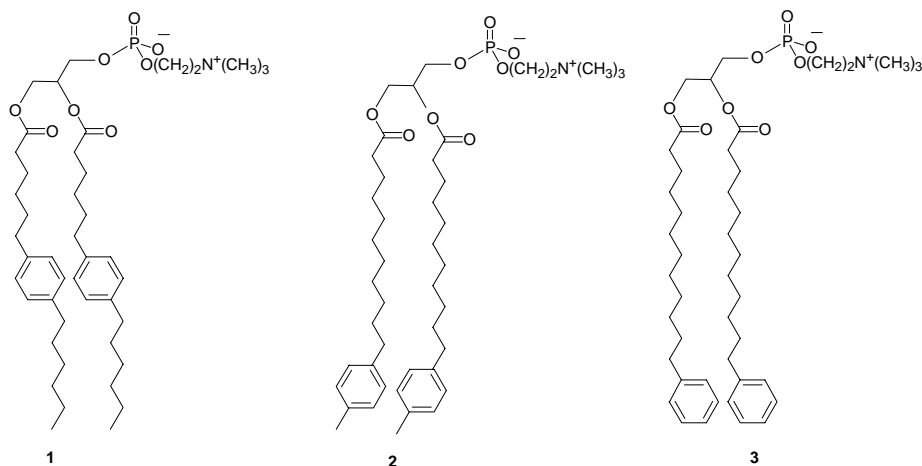
Phospholipids are important building blocks of the biological membranes that constitute the cellular bodies.<sup>1</sup> The lipophilic parts of such molecules are generally composed of fatty acid chains, which are often connected to the glycerol based backbones via ester or ether linkages.<sup>2</sup> A number of phospholipid analogues have been synthesized incorporating modified lipophilic chains, due to their importance in the investigations of the structure and function of biological membranes. Examples include ones containing sulfur-substituted fatty acids,<sup>3a</sup> vicinal-chain tethered phospholipids with large macrocyclic rings,<sup>3b-d</sup> chains incorporating aromatic fluorenyl residues,<sup>3e</sup> or chains with acetylenic residues,<sup>3f</sup> or terminal acryloyl groups,<sup>3g</sup> and phospholipids with methyl-substituted chains.<sup>3h-i</sup> Phospholipid analogues also exhibit useful pharmaceutical and medicinal activities. Towards this end, several phospholipids have been synthesized that exhibit significant antifungal,<sup>4a</sup> antitumor,<sup>4b</sup> antihypertensive,<sup>4c</sup> and anti-inflammatory<sup>4d</sup> properties. As a result there have been numerous attempts to find novel routes to achieve the synthesis of various phospholipid derivatives.<sup>5</sup>

## Results and Discussion

In the reconstitution of membrane bound proteins or enzymes *in vitro*, phospholipids bearing unsaturated hydrocarbon chains are particularly useful.<sup>6</sup> However, in order to keep the protein in their functionally active, correctly folded state, it is essential to maintain the membrane in its *disordered* state with significant dynamic fluidity. Hydrocarbon chains with *cis*-olefin containing unsaturations allow preservation of the membrane in a fluidized state, which is necessary for optimal signal transduction activity by the membrane bound protein.<sup>6</sup> Phospholipids containing naturally occurring unsaturated fatty acids bearing *cis*-double bonds such as oleic acid (*cis*-9-octadecenoic acid), linoleic acid (*cis,cis*-9,12-octadecadienoic acid), or arachidonic acid (*cis,cis,cis,cis*-5,8,11,14-eicosatetraenoic acid) are generally therefore used for the studies of membrane protein reconstitution. However, the membranes prepared from such phospholipids, invariably suffer from facile lipid peroxidation<sup>7</sup> limiting their usefulness for such biochemical studies. In addition, these lipids are also photochemically sensitive, restricting the shelf life and utility of the resulting formulations even further.

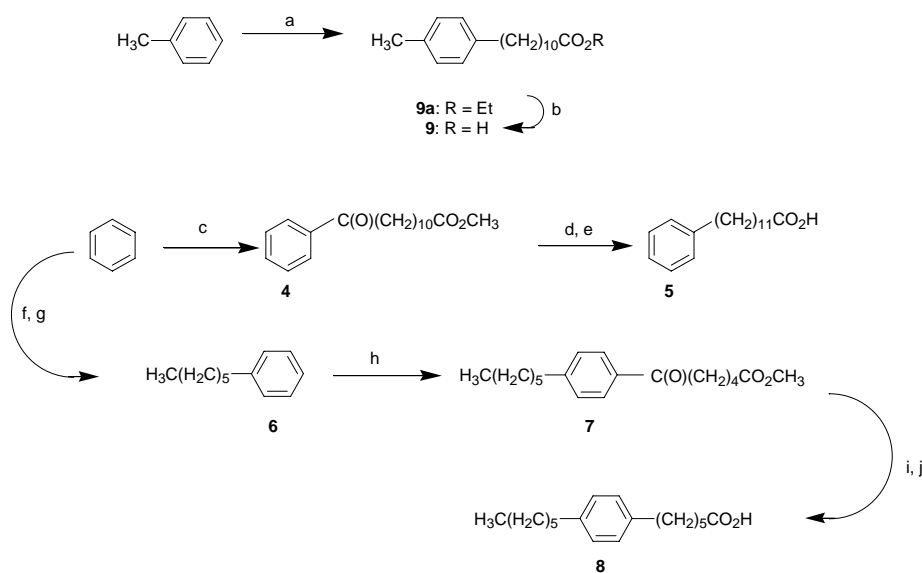
It occurred to us that by designing phospholipids with chains bearing oxidatively stable unsaturation units one might provide a way to circumvent this problem. Unlike their olefinic counterparts, aromatic systems are known for their chemical stability. As a part of our ongoing chemical biology program<sup>8</sup> focused on optimizing lipid lead structures which offer suitable membranes for reconstitution of membrane proteins at ambient conditions, we embarked upon the syntheses of a series of phospholipids incorporating aromatic units in their hydrophobic fatty acid segments.<sup>9</sup> In this paper we present in detail the synthesis of three such phospholipid analogues **1** - **3**, in which unsaturation in the form of aromatic units forms part of the acyl chains at specific locations along the chains. We also demonstrate that incorporation of such aromatic units into hydrocarbon chains leads to remarkable fluidization of the resulting membranes.

## Synthesis



The synthesis of the individual phospholipids **13** started with the preparation of fatty acids containing aromatic units at specified locations of the hydrocarbon chain (Scheme 1). Friedel-

Crafts acylation of benzene with caproyl chloride in the presence of anhydrous  $\text{AlCl}_3$ , under reflux for ~10 h furnished *n*-pentyl phenyl ketone in ~58% yield. This molecule upon reduction under Huang-Minlon conditions<sup>10</sup> gave *n*-hexyl benzene, **6**, which was isolated in ~60% yield as a colorless liquid, upon distillation under reduced pressure. The *n*-hexyl benzene was then acylated using methyl adipoyl chloride in the presence of anhydrous  $\text{AlCl}_3$  in dry carbon disulfide under refluxing conditions to give **7** in ~58% isolated yield. The ketoester, **7** was finally converted to 6-(4-*n*-hexylphenyl)hexanoic acid, **8** in ~60% yield first upon reduction under refluxing conditions with  $\text{NH}_2\text{NH}_2/\text{KOH}$  in ethylene glycol followed by work up and acidification.



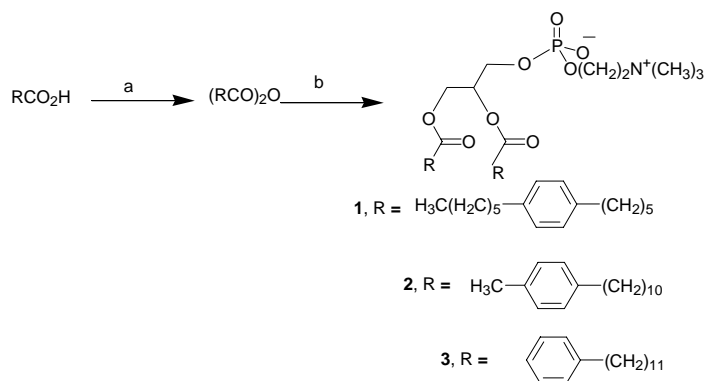
<sup>a</sup> *Reagents, conditions and yields:* a) ethyl 11-bromoundecanoate, anhyd.  $\text{AlCl}_3$ , RT, 4 h; HCl-ice water, 60%; b) 10% KOH-MeOH, reflux, 6 h;  $\text{H}_3\text{O}^+$ -ice water, 95%; c)  $\text{MeO}_2\text{C}(\text{CH}_2)_{10}\text{COCl}$ , anhyd.  $\text{AlCl}_3$ , in benzene, 4 °C (1 h) → RT (12 h) work-up in dil. ice-cold HCl, 58%; d)  $\text{NH}_2\text{NH}_2$ -KOH, ethylene glycol, reflux, 4 h; e)  $\text{H}_3\text{O}^+$ , ice-water, 90%; f) caproyl chloride, anhyd.  $\text{AlCl}_3$ , reflux, 10 h, 58%; g)  $\text{NH}_2\text{NH}_2$ -KOH, ethylene glycol, reflux, 4 h, 60%; h)  $\text{MeO}_2\text{C}(\text{CH}_2)_4\text{COCl}$ , anhyd.  $\text{AlCl}_3$ ,  $\text{CS}_2$ , reflux, 12 h, 58%; i)  $\text{NH}_2\text{NH}_2$ -KOH, ethylene glycol, reflux, 4h, 66%; j)  $\text{H}_3\text{O}^+$ , ice-water, 90%.

### Scheme 1

The synthesis of 12-phenyldodecanoic acid, **5** also started with the acylation of benzene using methyl 12-chloro-12-oxo-dodecanoate in the presence of anhydrous  $\text{AlCl}_3$ . Work up afforded the keto ester, **4** in 58% isolated yield. Then the keto group in **4** also reduced again under Huang-Minlon conditions to furnish **5** in ~70% yield. The synthesis of 11-(4-methylphenyl)undecanoic acid, **9**, employed toluene, which was alkylated using ethyl-11-bromoundecanoate under Friedel-Crafts conditions to furnish ethyl 11-(4-methylphenyl)

undecanoate, **9a**, in ~57% yield. Work up followed by saponification and acidification afforded **9** in ~95% isolated yield.

The key starting material for the synthesis of phospholipid, free *sn*-glycerophosphocholine was obtained<sup>11</sup> from natural lecithin. This was first extracted from egg yolk and then subjected to hydrolysis using 0.1M Bu<sub>4</sub>N<sup>+</sup>OH<sup>-</sup> in MeOH to afford free *sn*-glycerophosphocholine (GPC) after chromatographic purification. The resulting material was then converted to a complex with CdCl<sub>2</sub> (GPC.CdCl<sub>2</sub>) to make it soluble in CHCl<sub>3</sub>.<sup>9</sup> Finally the syntheses of the desired phospholipids from the newly synthesized fatty acids containing aromatic units were accomplished by full acylation of GPC.CdCl<sub>2</sub> with respective anhydride of the appropriate fatty acid in ethanol-free, freshly dry CHCl<sub>3</sub> in the presence of dicyclohexylcarbodiimide (DCC) and *N,N*-4-dimethylaminopyridine (DMAP). This is schematically presented in Scheme 2. All the new intermediates and final compounds were characterized by IR, NMR spectroscopy and elemental analysis. Spectral data for these compounds are presented in the Experimental Section.



<sup>a</sup> *Reagents, conditions and yields*: (a) i) RCO<sub>2</sub>H (1 equiv.) Et<sub>3</sub>N, -20 °C, 1 h; add ClCO<sub>2</sub>Et-THF, stir at -20 °C, 2h, then at RT, 1 h. ii) Cool to -20 °C, add a solution of RCO<sub>2</sub>H (1 equiv.)/Et<sub>3</sub>N in THF at -20 °C, 1 h; stir overnight at RT, (isolated yields: 90-95% ); (b) DMAP, GPC.CdCl<sub>2</sub>, CHCl<sub>3</sub>, stir at RT, 48 h (isolated yields: 72, 55, 63% for **1-3** respectively).

## Scheme 2

Brief bath sonication (10 min) above 50 °C, of the thin film prepared from each of the above phospholipid in aqueous HEPES (5 mM) buffer containing 1 mM EDTA at pH 7.4 afforded stable, translucent lipid suspensions. The existence of multi-walled vesicular organizations (MLVs) in such aggregates was evident from negative stain transmission electron microscopy studies (not shown) with each of these lipid samples.<sup>8</sup> Differential scanning calorimetric studies of these aggregates from 5 to 90 °C did not show the presence of any peak due to main-chain melting transition suggesting that the membranes remained in their fluid state already at 5 °C. Inclusion of any of these lipids **13** into a sample containing dipalmitoyl phosphatidylcholine (DPPC) however, reduced the main chain melting transition of the DPPC by several degrees

depending on the mol% of the synthetic phospholipids incorporated. Taken together it is evident that these newly synthesized phospholipids retain a fluid like melted state (liquid-crystalline like phase) at ambient temperatures in their membranes. These lipid suspensions remain unscathed in ambient conditions for several months demonstrating high chemical stability associated with such lipid aggregates.

In summary, a general and convenient method has been developed for the synthesis of phospholipid analogues bearing acyl chains with aromatic type unsaturations, which form the first examples of this class of compounds that are also oxidatively and photochemically stable. Such synthetic phospholipids are now being exploited for membrane protein reconstitution studies in our laboratory.

## Experimental Section

**General Procedures.** Unless stated otherwise, all general chemicals were obtained from commercial grades of the highest purity available and used without further purification. Solvents were dried and distilled prior to use. Dicyclohexylcarbodiimide (DCC), *N,N*-4-dimethylaminopyridine (DMAP), adipic acid, dodecanoic acid, hexanoic acid, were purchased from Aldrich. The *sn*-Glycero-3-phosphocholine- $\text{CdCl}_2$  complex was prepared from egg yolk by procedure as described.<sup>11</sup> IR spectra were recorded using a Perkin-Elmer 781 IR spectrophotometer. NMR spectra were obtained on either a Bruker WH-400 (400 MHz) or Jeol-90Q (90 MHz) or Varian-T60 (60 MHz) spectrometers. The chemical shifts for NMR spectra are reported in parts per million (ppm) relative to  $\text{SiMe}_4$  as internal standard and J values are reported in Hz. Column chromatography was performed using silica gel of 60-120 mesh procured from Merck. Elemental analyses were performed by using a Carlo Erba Elemental Analyzer Model 1106.

### Synthesis of fatty acids

**1-Phenylhexane (6).** Friedel-Crafts acylation of benzene with caproyl chloride (0.266 g, 2 mmol) in excess dry benzene (2.5 ml) afforded hexanophenone after acidic work-up and purification. This material was finally isolated upon distilled at  $\sim 265^\circ\text{C}$  in 58% yield (0.2 g). This hexanophenone on treatment with hydrazine hydrate and KOH by refluxing at  $180^\circ\text{C}$  for 4h in ethylene glycol yielded 1-phenylhexane (0.11 g, 60%) as a colorless liquid upon purification followed by distillation at  $226^\circ\text{C}$ .  $^1\text{H-NMR}$  ( $\text{CCl}_4$ , 60 MHz)  $\delta$  (ppm) 0.90 (*t*, 3H,  $-\text{CH}_3$ ), 1.26 (br *m*, 8H,  $-(\text{CH}_2)_4-$ ), 2.52 (*t*, 2H,  $-\text{CH}_2-\text{C}_6\text{H}_5$ ), 7.1 (*s*, 5H,  $-\text{C}_6\text{H}_5$ ). The properties of this compound are in agreement with that reported in literature.<sup>12</sup>

**Methyl 6-(4-*n*-hexylphenyl)-6-oxo-hexanoate (7).** Methyl hydrogen adipate was prepared by refluxing adipic acid (0.57 g, 2.5 mmol) with absolute methanol (10 mL) in the presence of conc. HCl for 8h on a waterbath. This was isolated as a viscous liquid upon distillation at reduced

pressure (~159 °C at 10 mm) in (0.27 g) 45% yield. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 90 MHz) δ (ppm) 1.7 (br *m*, 4H, -(CH<sub>2</sub>)<sub>2</sub>-), 2.35 (*t*, 4H, -(CH<sub>2</sub>)<sub>2</sub>-), 3.6 (*s*, 3H, -OCH<sub>3</sub>).<sup>13</sup>

This half-acid ester (0.4 g, 2.5 mmol) was dissolved in freshly distilled thionyl chloride and refluxed on waterbath for 2h. Removal of the excess thionyl chloride gave methyl adipoyl chloride, which was used for the next step. To the stirred suspension of anhydrous AlCl<sub>3</sub> (0.32 g, 2.4 mmol) in dry CS<sub>2</sub> (10 mL), was added 1-phenylhexane (0.324 g, 2 mmol) dissolved in CS<sub>2</sub> at +4 °C. To this ice cooled reaction mixture, methyl adipoyl chloride (0.4 g, 2.5 mmol) dissolved in dry CS<sub>2</sub> (10 mL), was added dropwise over a period of 1h. During the addition the temperature was kept at 5 °C and then the reaction was continued with stirring for 6h at room temperature. Then the reaction mixture was poured into ice-cold dilute HCl to decompose the AlCl<sub>3</sub>-complex. The organic layer was separated, the solvent was evaporated to leave a crude residue, which was extracted several times with ether (3 × 25 mL), and washed with successively dilute sodium bicarbonate (10 mL) and water (25 mL). The ether layer was then dried over anhydrous sodium sulphate and filtered. The crude solid obtained on removal of the solvent was subjected to column chromatography over silica gel using petroleum ether-ethyl acetate (80:20) to give methyl 6-(4-*n*-hexylphenyl)-6-oxo-hexanoate (0.35 g), yield: 58%. IR 1740 (ester carbonyl) and 1630 (keto carbonyl) cm<sup>-1</sup>. <sup>1</sup>H-NMR (CCl<sub>4</sub>, 60 MHz) δ (ppm) 0.91 (*t*, 3H, -CH<sub>3</sub>), 1.36 (br *m*, 8H, -(CH<sub>2</sub>)<sub>4</sub>-), 1.69 (br *m*, 8H, -(CH<sub>2</sub>)<sub>4</sub>-), 2.3 – 2.8 (mixture of *t*, -CH<sub>2</sub>-C<sub>6</sub>H<sub>4</sub>-, C(O)-CH<sub>2</sub>-, -CH<sub>2</sub>-COOCH<sub>3</sub>), 3.56 (*s*, 3H, -COOCH<sub>3</sub>), 7.25 (*d*, 2H, aromatic-H), 7.75 (*d*, 2H, aromatic-H). Anal. Calcd. for C<sub>19</sub>H<sub>28</sub>O<sub>3</sub>: C, 74.96, H, 9.27. Found: C, 75.23, H, 9.5.

**6-(4-*n*-Hexylphenyl)hexanoic acid (8).** The methyl ester, **7**, (0.3 g, 1 mmol) was then subjected to reduction using hydrazine hydrate (0.1 g, 2 mmol), KOH (0.11 g, 2 mmol) in ethylene glycol (5 mL) under reflux for 4h. Ethylene glycol was then removed by distillation and the reaction mixture was poured to crushed ice and neutralized with HCl (1 M, 2 mL). The crude solid that separated out was extracted ethyl acetate (4 × 25 mL), and washed with dilute aq. NaHCO<sub>3</sub> (25 mL) and water (2 × 25 mL). Ethyl acetate extract was dried over anhydrous sodium sulphate and the solvent was removed. Solid material obtained was subjected to column chromatography over silica gel and eluted with petroleum ether-ethyl acetate (90:10) gave 6-(4-*n*-hexylphenyl) hexanoic acid (0.18 g) as a colorless low melting solid in 60% yield. IR 1700 cm<sup>-1</sup> (-COOH), <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 90 MHz) δ (ppm) 0.92 (*t*, 3H, -CH<sub>3</sub>), 1.3 (br *m*, 8H, -(CH<sub>2</sub>)<sub>4</sub>-CH<sub>2</sub>-C<sub>6</sub>H<sub>4</sub>-), 1.61 (*m*, 6H, -(CH<sub>2</sub>)<sub>3</sub>-CH<sub>2</sub>-COOH), 2.36 (*t*, 2H, -CH<sub>2</sub>-COOH), 2.58 (*t*, 4H, -CH<sub>2</sub>-C<sub>6</sub>H<sub>4</sub>-CH<sub>2</sub>), 7.08 (*s*, 4H, aromatic-H), 9.1 (br peak, 1H, -COOH); LR-MS: *m/z* 276 (M<sup>+</sup>). Anal. Calcd. for C<sub>18</sub>H<sub>28</sub>O<sub>2</sub>: C, 78.21, H, 10.21. Found: C, 78.1, H, 10.43.

**Ethyl 11-(4-methylphenyl)undecanoate (9a).** Anhydrous AlCl<sub>3</sub> (0.16 g, 1.2 mmol) was suspended in excess dry toluene (6 mL) and kept at 4 °C with stirring. To this stirred suspension, ethyl 11-bromoundecanoate (0.29 g, 1 mmol) was added slowly for a period of 45 min keeping the temperature at 4 °C during the addition. The reaction mixture was thawed to room temperature and stirring was continued at room temperature for an additional 4h. The AlCl<sub>3</sub>-complex was then decomposed by pouring the reaction mixture into crushed ice and cold dilute HCl. This was followed up by the work-up as described above. The solid material thus obtained

on removal of the solvent purified through column chromatography over silica gel via elution with petroleum ether-ethyl acetate solvent mixture (8:2) to give ethyl 11-(4-methylphenyl) undecanoate (0.17 g) in ~57% yield. IR 1740  $\text{cm}^{-1}$  (ester carbonyl).  $^1\text{H-NMR}$  ( $\text{CCl}_4$ , 60 MHz)  $\delta$  (ppm) 1.3 (*t* + *br m*, 19H,  $-\text{CH}_2-\text{CH}_3$ ,  $-(\text{CH}_2)_8-$ ), 2.3 - 2.4 (*s* + *t*, 5H,  $\text{CH}_3-\text{C}_6\text{H}_4-\text{CH}_2-$ ), 4.0 (*q*, 2H,  $\text{OCH}_2\text{CH}_3$ ), 6.95 (*m*, 4H, aromatic-H).

**11-(4-Methylphenyl) undecanoic acid (9).** Ethyl ester, **9a** (0.152 g, 0.5 mmol) was dissolved in 10% methanolic NaOH solution and refluxed on a water bath for 6h. Methanol was evaporated and the reaction mixture was poured into crushed ice and neutralized with dil. HCl (1 M, 1 mL). This furnished a gummy solid material which was separated from the reaction mixture via extraction with ether ( $4 \times 25$  mL). The organic layer was washed with 5% aq.  $\text{NaHCO}_3$  (25 mL) and water ( $4 \times 25$  mL), and dried over anhydrous sodium sulphate. Removal of the solvent yielded 0.13 g of 11-(4-methylphenyl) undecanoic acid (95%). IR 1700  $\text{cm}^{-1}$  (acid carbonyl).  $^1\text{H-NMR}$  (90 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm) 1.32 (*br m*, 8H,  $-(\text{CH}_2)_4-$ ), 1.63 (*m*, 8H,  $-(\text{CH}_2)_4-$ ), 2.3 (*br m*, 7H,  $-\text{C}_6\text{H}_5-\text{CH}_3$ ,  $-\text{CH}_2-\text{C}_6\text{H}_4-$ , and  $-\text{CH}_2-\text{COOH}$ ), 7.0 (*m*, 4H, aromatic-H). Anal. Calcd. for  $\text{C}_{18}\text{H}_{28}\text{O}_2$ : C, 78.21, H, 10.21. Found: C, 77.93, H, 10.41.

**Methyl 11-oxo-phenyldodecanoate (4).** To 1,10-decanedicarboxylic acid (0.46 g, 2 mmol) was added dry methanol (9 mL) and the solution was heated on a water bath for 5h. Excess methanol was removed under reduced pressure. The reaction mixture was passed through column chromatography over silica gel and eluted with petroleum ether-ethyl acetate (7:3) to give the desired methyl hydrogen dodecanoate (0.32 g) as a colorless solid in 65% yield.<sup>13</sup> This acid ester (0.24 g, 1 mmol) was dissolved in freshly distilled thionyl chloride (3 mL) and refluxed for 4h on a water bath. Excess thionyl chloride was removed under vacuum to produce crude methyl hydrogen decanoyl chloride as residue. This was then directly used for Friedel-Crafts acylation with dry benzene by a procedure similar to one described above to give methyl 11-oxo-phenyldodecanoate, **4**, (0.17 g) in 58% yield.  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 90 MHz),  $\delta$  (ppm) 1.35 - 1.6 (*br m*, 16H,  $-(\text{CH}_2)_8-$ ), 2.3 (*t*, 2H,  $-\text{CH}_2-\text{CO}$ ), 2.97, (*t*, 2H,  $-\text{CH}_2-\text{COOCH}_3$ ), 3.62 (*s*, 3H,  $\text{COOCH}_3$ ), 7.4 - 8.1 (*m*, 5H, aromatic-H).

**12-Phenyldodecanoic acid (5).** This keto ester was refluxed with hydrazine hydrate (0.06 g, 1.2 mmol) and KOH (0.067 g, 1.2 mmol) in ethylene glycol (5 mL) for 6h. The reaction mixture was then subjected to work-up as described above to afford 12-phenyldodecanoic acid, **5**, (0.11 g) in 70% yield. IR 1700  $\text{cm}^{-1}$ .  $^1\text{H-NMR}$  (90 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm) 1.28 (*s*, 14H), 1.59 (*m*, 4H), 2.32 (*t*, 2H,  $-\text{CH}_2-\text{COOH}$ ), 2.58 (*t*, 2H,  $-\text{CH}_2-\text{C}_6\text{H}_5$ ), 7.18 (*s*, 5H, aromatic-H). Anal. Calcd. for  $\text{C}_{18}\text{H}_{28}\text{O}_2$ : C, 78.21, H, 10.21. Found: C, 78.61, H, 10.05.

### Preparation of the fatty acid anhydrides

**6-(4-*n*-Hexylphenyl)hexanoic anhydride.** To a solution of 6-(4-*n*-hexylphenyl)hexanoic (0.27 g, 1 mmol) in dry THF (5 mL) was added 1 mL of triethylamine and cooled to  $-20$   $^\circ\text{C}$ . To this, a solution of ethyl chloroformate (0.11 mL, 1.2 mmol) in dry THF (5 mL) was added by dropwise with stirring. Stirring was continued at  $-20$   $^\circ\text{C}$  for 2h. Then the reaction mixture was warmed to room temperature and stirred for half an hour. Again the reaction mixture was cooled to  $-20$   $^\circ\text{C}$

and to this added a solution of 6-(4-*n*-hexylphenyl) hexanoic (0.27 g, 1 mmol) and triethylamine (1 mL) dissolved in dry THF (12 mL) in a dropwise fashion using a pressure-equalizing dropping funnel and stirred overnight at room temperature. The solvent was removed under reduced pressure and the crude solid material obtained was extracted with solvent ether. Ether layer was washed with water and dried over anhydrous sodium sulphate. Removal of the solvent furnished 6-(4-*n*-hexylphenyl) hexanoic anhydride (0.47 g) in 90% yield. IR (neat) 1735, 1815  $\text{cm}^{-1}$  (anhydride).  $^1\text{H-NMR}$  (200 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm) 0.87 (*t*, 6H,  $-\text{CH}_3$ ), 1.29 (*m*, 12H), 1.62 (*m*, 12H), 2.34 (*t*, 4H,  $-\text{CH}_2\text{C}(\text{O})-\text{O}-\text{C}(\text{O})-\text{CH}_2-$ ), 2.39 (*t*, 4H), 2.56 (*m*, 8H,  $-\text{CH}_2-\text{C}_6\text{H}_4-\text{CH}_2-$ ), 7.07 (*s*, 8H, aromatic-H). Anal. Calcd. for  $\text{C}_{36}\text{H}_{54}\text{O}_3$ : C, 80.85, H, 10.18. Found: C, 80.5, H, 10.41.

**11-(4-Methylphenyl)undecanoic anhydride.** Using the procedure described as above the 11-(4-methylphenyl) undecanoic (0.27 g, 1 mmol) was converted into the corresponding anhydride (0.47 g) in a 90% yield. IR (neat) 1735 and 1810  $\text{cm}^{-1}$ .  $^1\text{H-NMR}$  (90 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm) 1.29 (*br m*, 16H), 1.58 (*br s*, 8H), 2.35 (*s*, 6H,  $-\text{C}_6\text{H}_5-\text{CH}_3$ ), 2.55 (*m*, 4H), 7.12 (*m*, 8H). Anal. Calcd. for  $\text{C}_{36}\text{H}_{54}\text{O}_3$ : C, 80.85, H, 10.18. Found: C 81.1, H, 10.3.

**12-Phenyldodecanoic anhydride.** Using the procedure described as above the 12-phenyldodecanoic (0.27 g, 1 mmol) was converted into the corresponding anhydride (0.496 g) in a 95% yield. IR (neat) 1735 and 1810  $\text{cm}^{-1}$ .  $^1\text{H-NMR}$  (90 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm) 1.29 (*br m*, 28H), 1.5 (*m*, 8H), 2.35 (*t*, 4H,  $\text{CH}_2-\text{C}(\text{O})-\text{O}-\text{C}(\text{O})-\text{CH}_2-$ ), 2.56 (*t*, 4H,  $-\text{CH}_2-\text{C}_6\text{H}_5$ ), 7.19 (*m*, 10H, aromatic-H). Anal. Calcd. for  $\text{C}_{36}\text{H}_{54}\text{O}_3$ : C, 80.85, H, 10.18. Found: C, 81.2, H, 10.12.

### Preparation of sn-glycero-3-phosphocholine

**1,2-Bis[6-(4-*n*-hexylphenyl)hexanoyl]-sn-glycero-3-phosphocholine (1).** To a dry 50 mL single-neck round bottom flask were added sn-glycero-3-phosphocholine- $\text{CdCl}_2$  and (0.32 g, 0.6 mmol) of 6-(4-*n*-hexylphenyl)hexanoic anhydride. The solid mixture was degassed with nitrogen and added freshly distilled  $\text{CHCl}_3$  (25 mL) and stirred. To this stirred solution was added DMAP (0.07 g, 0.6 mmol), in the form of a solution in dry  $\text{CHCl}_3$  (2 mL). The reaction mixture was stirred at room temperature for 48h. Precoated TLC plates with silica gel were used to perform TLC using  $\text{CHCl}_3/\text{CH}_3\text{OH}/\text{H}_2\text{O}$  (65:25:4) as a developing solvent to follow the course of reaction. The solvent was removed under reduced pressure and the residue obtained was dissolved in of 4:5:1  $\text{CHCl}_3/\text{CH}_3\text{OH}/\text{H}_2\text{O}$  (2-5 mL), and passed through an AG 501-X8 resin column (1 $\times$ 20 cm). The column was again eluted the same solvent mixture (10 mL) to remove the remaining adsorbed lipid. The eluted reaction product was combined and the solvent was removed under reduced pressure. The solid obtained was finally purified by chromatography over silica gel column (1 $\times$ 20 cm) using  $\text{CHCl}_3$ ,  $\text{CHCl}_3/\text{CH}_3\text{OH}$  (1:1),  $\text{CHCl}_3/\text{CH}_3\text{OH}$  (1:9) and  $\text{CH}_3\text{OH}$  as eluent. Solvent containing  $\text{CHCl}_3/\text{CH}_3\text{OH}$  (1:9) showed a TLC spot that was similar in nature that of dipalmitoyl phosphatidylcholine solvent from solution was removed under reduced pressure and dried under high vacuum (24h, 0.5 mm) to afford (0.33 g) (72%) of 1,2-Bis[6-(4-*n*-hexylphenyl)hexanoyl]-sn-glycero-3-phosphocholine, **1**.  $^1\text{H-NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm) 0.88 (*t*, 6H, 2 x  $-\text{CH}_3$ ), 1.29 (*br m*, 16H, 2 x  $(-\text{CH}_2)_4$ ), 1.58 (*br m*, 12H, 2 x  $(-\text{CH}_2)_3$ ), 2.29



(*m*, 4H, 2 x -OC(O)CH<sub>2</sub>) 2.54 (*m*, 8H, 2 x (-CH<sub>2</sub>-C<sub>6</sub>H<sub>4</sub>CH<sub>2</sub>-) 3.32 (*s*, 9H, 3 x N<sup>+</sup>(CH<sub>3</sub>)<sub>3</sub>), 3.74 (*t*, 2H, -CH<sub>2</sub>N<sup>+</sup>(CH<sub>3</sub>)<sub>3</sub>), 3.92 (*m*, 2H, -CH-CH<sub>2</sub>OC(O)), 4.11 (*m*, 1H, -CH-CH<sub>a</sub>H<sub>b</sub>OP(O)), 4.28 (*m*, 3H, OCH<sub>2</sub>CH<sub>2</sub>N<sup>+</sup>Me<sub>3</sub> + -CH-CH<sub>a</sub>H<sub>b</sub>OP(O)), 5.19 (*m*, 1H, -CH-CH<sub>2</sub>O), 7.05 (*m*, 8H, 2 x -C<sub>6</sub>H<sub>4</sub>-). <sup>13</sup>C-NMR (67.5 MHz, CDCl<sub>3</sub>) δ (ppm) 13.85, 22.38, 24.55, 26.67, 30.96, 31.31, 31.53, 33.86, 34.03, 35.13, 35.37, 54.19, 59.22, 62.83, 63.39, 66.23, 70.29, 70.43, 127.98, 128.05, 139.34, 139.99, 172.97, 173.26. IR (cm<sup>-1</sup>): 1735 (ester C=O), 1460, 1230 (P=O), 820 (P-O). Anal. calcd for C<sub>44</sub>H<sub>72</sub>NO<sub>8</sub>P.H<sub>2</sub>O: C, 66.72, H, 9.42, N, 1.77. Found: C, 66.4, H, 9.72, N, 1.54.

**1,2-Bis[11-(4-methylphenyl)undecanoyl]-sn-glycero-3-phosphocholine (2).** Reaction of 1,2-Bis[11-(4-methylphenyl)undecanoic anhydride (0.32g, 0.6 mmol) employing the same procedure as above 1,2-bis[11-(4-methylphenyl)undecanoyl]-sn-glycero-3-phosphocholine, **2**, was obtained (0.25 g) in 55% yield. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>) δ (ppm) 1.18 (*br m*, 24H, 2 x (-CH<sub>2</sub>)<sub>6</sub>), 1.5 (*br m*, 8H, 2 x (-CH<sub>2</sub>)<sub>2</sub>), 2.4 (mixture of *br m*, 14H, 2 x -OC(O)CH<sub>2</sub>) and 2 x (-CH<sub>2</sub>-C<sub>6</sub>H<sub>4</sub>-CH<sub>3</sub>), 3.22 (*s*, 9H, 3 x N<sup>+</sup>(CH<sub>3</sub>)<sub>3</sub>), 3.65 (*t*, 2H, -CH<sub>2</sub>N<sup>+</sup>(CH<sub>3</sub>)<sub>3</sub>), 3.83 (*m*, 2H, -CH-CH<sub>2</sub>OC(O)), 4.05 (*m*, 1H, -CH-CH<sub>a</sub>H<sub>b</sub>OP(O)), 4.29 (*m*, 1H, -CH-CH<sub>a</sub>H<sub>b</sub>OP(O)), 4.31 (*m*, 2H, OCH<sub>2</sub>CH<sub>2</sub>N<sup>+</sup>Me<sub>3</sub>), 5.1 (*m*, 1H, -CH-CH<sub>2</sub>O), 6.98 (*m*, 8H, 2 x -C<sub>6</sub>H<sub>4</sub>-). <sup>13</sup>C-NMR (67.5 MHz, CDCl<sub>3</sub>) δ (ppm) 20.96, 21.47, 22.28, 24.88, 26.19, 27.63, 29.31, 29.49, 31.53, 34.11, 34.29, 35.5, 35.91, 36.89, 38.41, 50.5, 53.86, 54.46, 59.25, 62.96, 63.44, 66.48, 70.57, 115.23, 124.67, 125.4, 126.32, 126.51, 128.12, 128.92, 129.22, 173.21, 173.57. IR (cm<sup>-1</sup>): 1730 (ester C=O), 1230 (P=O), 810 (P-O). Anal. calcd for C<sub>44</sub>H<sub>72</sub>NO<sub>8</sub>P: C, 68.27, H, 9.38, N, 1.81. Found: C, 67.9, H, 9.71, N, 1.6.

**1,2-Bis[12-(4-phenyl)dodecanoyl]-sn-glycero-3-phosphocholine (3).** Using the procedure described above the 1,2-Bis[12-(4-phenyl)dodecanoic anhydride (0.32 g, 0.6 mmol) afforded 1,2-bis[12-(4-phenyl)dodecanoyl]-sn-glycero-3-phosphocholine, **3**, (0.29 g) in 63% yield. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>) δ (ppm) 1.25 (*br m*, 28H, 2 x (-CH<sub>2</sub>)<sub>7</sub>), 1.59 (*m*, 8H, 2 x (-CH<sub>2</sub>)<sub>2</sub>), 2.29 (*m*, 4H, 2 x -OC(O)CH<sub>2</sub>), 2.58 (*m*, 4H, 2 x -CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>), 3.33 (*s*, 9H, 3 x N<sup>+</sup>(CH<sub>3</sub>)<sub>3</sub>), 3.76 (*br t*, 2H, -CH<sub>2</sub>N<sup>+</sup>(CH<sub>3</sub>)<sub>3</sub>), 3.93 (*m*, 2H, -CH-CH<sub>2</sub>OC(O)), 4.12 (*m*, 1H, -CH-CH<sub>a</sub>H<sub>b</sub>OP(O)), 4.30 (*m*, 1H, -CH-CH<sub>a</sub>H<sub>b</sub>OP(O)), 4.39 (*m*, 2H, OCH<sub>2</sub>CH<sub>2</sub>N<sup>+</sup>Me<sub>3</sub>), 5.19 (*m*, 1H, -CH-CH<sub>2</sub>O) 7.17 – 7.25 (mixture of *m*, 10H, aromatic protons). <sup>13</sup>C-NMR (50 MHz, CDCl<sub>3</sub>) δ (ppm) 24.9, 24.99, 29.24, 29.37, 29.64, 31.47, 34.16, 34.31, 35.97, 50.29, 54.31, 59.43, 63.02, 63.56, 63.62, 65.32, 66.37, 70.44, 70.56, 125.56, 128.21, 128.35, 142.86, 173.46, 173.76. IR (cm<sup>-1</sup>): 1740 (ester C=O), 1400, 1220 (P=O), 820 (P-O). Anal. calcd for C<sub>44</sub>H<sub>72</sub>NO<sub>8</sub>P.0.5H<sub>2</sub>O: C, 67.49, H, 9.4, N, 1.79. Found: C, 67.23, H, 9.76, N, 1.49.

## Acknowledgments

This work was supported by the Swarnjayanti Fellowship grant of the Department of Science and Technology, Government of India. VPK is thankful to C.S.I.R. for the award of a Senior Research Fellowship.

## References and Notes

1. See: Biochemistry Voet, D.; Voet, J.G. *cf.* 2<sup>nd</sup> Edn; Wiley: New York, 1995; p 280.
2. For reviews, see: Cevc, G. Ed. *Phospholipid Handbook*; Marcel Dekker: New York, 1993.
3. (a) Li, R.; Pascal, R. A. *J. Org. Chem.* **1993**, *58*, 1952. (b) Ladika, M.; Fisk, T. E.; Wu, W. W.; Jons, S. D. *J. Am. Chem. Soc.* **1994**, *116*, 12093. (c) Menger, F. M.; Chen, X. Y. *Tetrahedron Lett.* **1996**, *37*, 323. (d) Patwardhan, A. P.; Thompson, D.H. *Organic Lett.* **1999**, *1*, 241. (e) Starck, J.-P.; Nakatani, Y.; Ourisson, G. *Tetrahedron* **1995**, *51*, 2629. (f) Rürup, J.; Mannova, M.; Brezesinski, G.; Schmid, R. D. *Chem. Phys. Lipids* **1994**, *70*, 187. (g) Sisiri, W.; Lee, Y.-S.; O'Brien, D. F. *Tetrahedron Lett.* **1995**, *36*, 8945. (h) Menger, F. M.; Wood, M. G.; Zhou, Q. Z.; Hopkins, H. P.; Fumero, J. *J. Am. Chem. Soc.* **1988**, *110*, 6804. (i) Morr, M.; Fortkamp, J.; Rühle, S. *Angew Chem., Int. Ed.* **1997**, *36*, 2460.
4. (a) Lu, Q.; Ubillas, R. P.; Zhou, Y.; Dubenko, L. G.; Dener, J. M.; Litvak, J.; Phuan, P.-W.; Flores, M.; Ye, Z.; Gerber, E. Y.; Truong, T.; Bierer, D. E. *J. Nat. Prod.* **1999**, *62*, 824. (b) Hilgard, P.; Klenner, T.; Stekar, J.; Unger, C. *Cancer Chemother. Pharmacol.* **1993**, *32*, 90. (c) Wissner, A. Schaub, R. E.; Sum, P. E.; Kohler, C. A.; Goldstein, B. M. *J. Med. Chem.* **1986**, *29*, 328. (d) Shen, T. Y. *J. Med. Chem.* **1981**, *24*, 1.
5. (a) Martin, S. F. Josey, J. A.; Wong, Y.-L.; Dean, D. W. *J. Org. Chem.* **1994**, *59*, 4805. (b) Erukulla, R. K.; Byun, H. S.; Bittman, R. *Tetrahedron Lett.* **1994**, *35*, 5783. (c) Garigapati, V. R.; Roberts, M. F. *Tetrahedron Lett.* **1993**, *34*, 769. (d) Kazi, A. B.; Hajdu, J. *Tetrahedron Lett.* **1992**, *33*, 2291. (e) Herbert, N.; Beck, A.; Lennox, R. B.; Just, G. *J. Org. Chem.* **1992**, *57*, 1777.
6. Bhattacharya, S.; Marti, T.; Otto, H.; Heyn, M. P.; Khorana, H. G. *J. Biol. Chem.* **1992**, *267*, 6757.
7. Gilbertson, S. M.; Porter, N. A. *J. Am. Chem. Soc.* **2000**, *122*, 4032.
8. For representative examples of other lipid design, see: (a) Bhattacharya, S.; De, S. *Chem. Eur. J.* **1999**, *5*, 2335. (b) Bhattacharya, S.; Dileep, P. V. *Tetrahedron Lett.* **1999**, *40*, 8167. (c) Bhattacharya, S.; De, S.; Subramanian, M. *J. Org. Chem.* **1998**, *63*, 7640. (d) Bhattacharya, S.; Ghosh, S.; Easwaran, K. R. K. *J. Org. Chem.* **1998**, *63*, 9232. (e) Bhattacharya, S.; Acharya, S. N. G. *Langmuir* **2000**, *16*, 87. (f) Bhattacharya S.; De, S. *Chem. Commun.* **1995**, 651.
9. Bhattacharya, S.; Subramanian, M. *Tetrahedron Lett.* **2002**, *43*, 4203.
10. Huang-Minlon, *J. Am. Chem. Soc.* **1946**, *68*, 2487.
11. For details, see: Bergelson, L. D. Ed. *Lipid Biochem. Prep.*; Elsevier: Amsterdam, 1980; Ch. II.3, p 125.
12. (a) Hauser, C. R.; Humphlett, J.; Weiss, M. J. *J. Am. Chem. Soc.* **1948**, *70*, 426. (b) Williams, J. W.; Dickert, Y. J.; Krynitsky, J. A. *J. Am. Chem. Soc.* **1941**, *63*, 2510.
13. Ogawa, H.; Chihara, T.; Taya, K. *J. Am. Chem. Soc.* **1985**, *107*, 1365 references cited therein.