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A New Synthesis of Lysophosphatidylcholines and Related Derivatives. Use of *p*-Toluenesulfonate for Hydroxyl Group Protection

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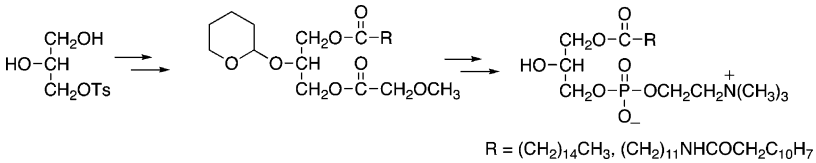

A new stereoselective synthesis of lysophosphatidylcholines is reported. The synthesis is based upon (1) the use of 3-*p*-toluenesulfonyl-*sn*-glycerol to provide the stereocenter for construction of the optically active lysophospholipid molecule, (2) tetrahydropyranylation of the secondary alcohol function to achieve orthogonal protection of the *sn*-2- and *sn*-3-glycerol positions, and (3) elaboration of the phosphodiester headgroup using a 2-chloro-1,3,2-dioxaphospholane/trimethylamine sequence. In the course of developing the synthesis it has been discovered that methoxyacetate displacement of the *sn*-3-*p*-toluenesulfonate yields a reactive methoxyacetyl ester, which in turn can be selectively cleaved with methanol/*tert*-butylamine, while the ester group at the *sn*-1-position remains unaffected. The sequence has been shown to be suitable for preparation of spectroscopically labeled lysophosphatidylcholines. One of these compounds was readily converted to a double-labeled mixed-chain phosphatidylcholine applicable for real-time fluorescence resonance energy transfer (FRET) assay of lipolytic enzymes. In addition, the work led to new synthetic strategies based on chemoselective manipulation of the tosyl group in the presence of other base-labile groups such as FMOC derivatives that are often used for the protection of amino and hydroxyl groups in syntheses.

Introduction

Lysophospholipids are membrane-derived signaling molecules produced by phospholipases that exhibit a wide range of diverse biological activities.[1−3] They have recently been recognized as highly potent extracellular regulators of cell growth, differentiation, migration, adhesion, invasion, and morphogenesis through G protein-coupled receptors.[2,3] These functions influence a broad spectrum of physiological and pathophysiological processes including neurogenesis, angiogenesis, wound healing, immunity, and carcinogenesis.[2] Specifically, lysophosphatidylcholine **1** has been shown to be involved in regulation of gene transcription, mitogenesis, monocyte chemotaxis, smooth muscle relaxation, and platelet activation.[4] Among its neurotropic effects, lysophosphatidylcholine facilitates synaptic vesicle fusion and is involved in dopamine turnover.[5] In endothelial cells lysophosphatidylcholine modulates calcium signals[6] and inhibits the phosphoprylation of nitric oxide synthase and cytosolic phospholipase A_2.[7] It activates protein kinase C,[6] protein kinase A,[5] and c-Jun N-terminal kinase[8] and stimulates phospholipase D,[9] adenylate cyclase, [4] and cyclooxygenase.[5,10] In addition, a key

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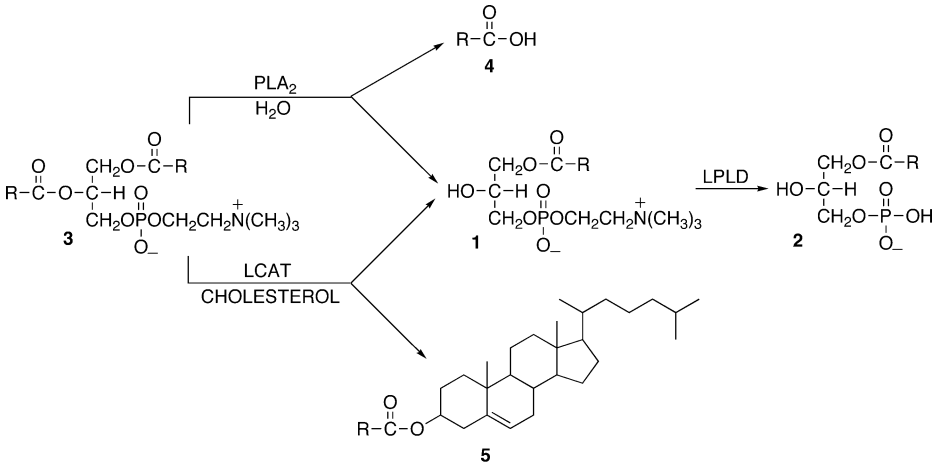
SCHEME 1



role has been attributed to lysophosphatidylcholine as a major antigenic component of oxidized low-density lipoprotein (ox-LDL) implicated in atherosclerosis, the primary cause of heart disease and stroke.[11] When released from the liver as a product of phospholipase A_2 (PLA_2) or produced by the enzyme lecithin/cholesterol acyl transferase (LCAT) in the plasma, lysophosphatidylcholine is converted by lysophospholipase D (LPLD) to lysophosphatidic acid **2**,[12] a highly potent inducer of cell proliferation, migration, and survival (Scheme 1).[13]

Despite ongoing vigorous investigation of the biochemistry and cell biology of lysophospholipids, details of their mechanism of action remain to be elucidated. Development of new synthetic methods for preparation of structurally variable lysophospholipid compounds is an important prerequisite to establishment of structure−activity relationships toward better understanding the enzymological, cell-biological, and membrane-biophysical properties of the compounds.[14] Along these lines, spectroscopically labeled lysophosphatidylcholines should become useful mechanistic probes for (1) studying the enzymes involved in lysophospholipid biosynthesis and degradation, (2) understanding lysophospholipid self-assembly, including their interaction with membrane bilayers, and (3) monitoring the fate of the compounds in cell cultures and tissues. Specifically, availability of lysophospholipids with fluorescent reporter groups could offer an attractive alternative to the use of radioactively labeled analogues.

To date relatively few synthetic methods have been developed for the preparation of lysophospholipids, mainly due to difficulties associated with acyl- and phosphoryl-group migration in the course of the syntheses, leading to regioisomeric mixtures in the products.[15,16] One recently developed synthesis, relying on nucleophilic ring opening of phosphorylated glycidol derivatives, yielded only minor amounts of the migrated *sn*-2-acyl byproduct and provided a highly efficient sequence to lysophospholipids.[17] However, preparation of functionalized analogues, for example, phospholipid compounds incorporating photoactivable functional groups, has been shown to require extensive use of protection/deprotection strategies.[18]

Results and Discussion

As part of our research in this area we turned our attention to development of a new synthesis of spectroscopically labeled lysophosphatidylcholines with fluorescent reporter groups at the *sn*-1-chain terminals. In an attempt to devise a new strategy we employed 3-(*p*-toluenesulfonyl)-*sn*-glycerol as chiral precursor for preparation of the target lysophospholipids, as it is readily available from commercial starting materials. Specifically, we used the *p*-toluenesulfonyl ester function as a base-labile hydroxyl protecting group as one of the key features of the synthesis. In the past, toluenesulfonylation strategies were mainly employed for activation of the hydroxyl group toward nucleophilic displacement and rarely for protection[19] because of lack of efficient displacement reactions of the tosylate by suitable oxygen nucleophiles. We have now discovered that tetraethylammonium methoxyacetate reacts readily with the *p*-toluenesulfonate ester of glycerol in anhydrous acetonitrile to afford the corresponding methoxyacetyl ester, which on subsequent treatment with *tert*-butylamine in methanol yields the deprotected alcohol. The two-step sequence proceeds with high yield and selectivity in that other carboxylic ester groups in the molecule remain unaffected. Thus, while methoxyacetate turns out to be sufficiently nucleophilic in dipolar aprotic media

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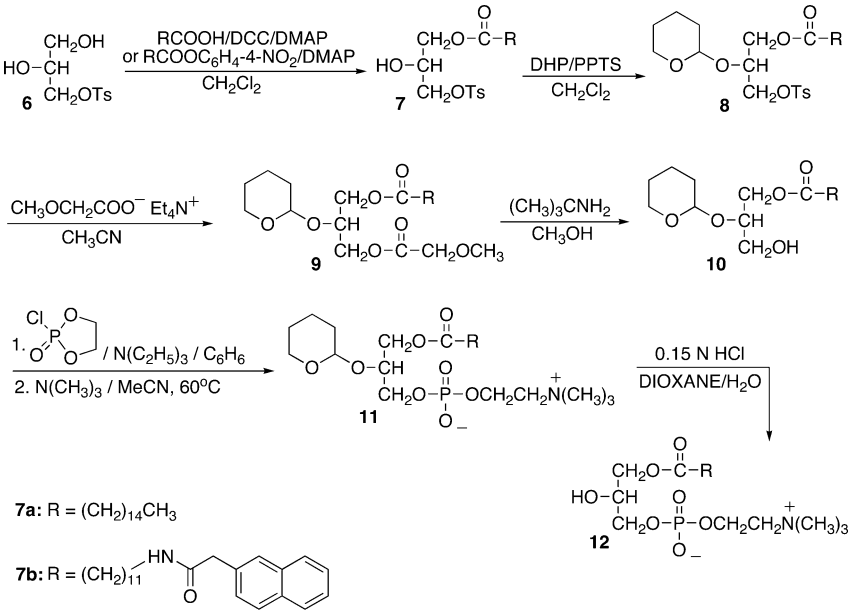
SCHEME 2



to displace *p*-toluenesulfonate, the resulting methoxyacetyl ester is found to be much more reactive toward base-catalyzed cleavage than the corresponding acetyl or other related carboxylic ester functions. These findings make the synthetic strategy feasible. Our synthetic approach to the preparation of functionalized lysophosphatidylcholines outlined in Scheme 2 is based on the following elements: (1) 3-(*p*-toluenesulfonyl)-*sn*-glycerol is used to provide the chiral center for construction of the optically active target phospholipid molecule, (2) the order in which the functional groups are introduced requires minimal use of protecting groups, and (3) phosphorylation is carried out using a cyclic phosphochloridate (2-chloro-2-oxo-1,3,2-dioxaphospholane) whose five-membered heterocyclic ring can be readily cleaved to afford the polar portion of the phosphodiester function directly.

To implement this strategy, compound **6**[20] was prepared from acid-catalyzed deprotection of commercially available 1,2-*O*-isopropylidene-*sn*-glyceryl-3-tosylate using 0.4 N HCl in methanol in essentially quantitative yield. Monoacylation of the primary hydroxyl group of 3-(*p*-toluenesulfonyl)-*sn*-glycerol **6** was achieved in reaction with the palmitic acid using dicyclohexyl carbodiimide as condensing agent, with a catalytic amount of 4-(dimethylamino)pyridine in dichloromethane, at room temperature for 4 h. The resulting *sn*-1-ester **7a** was isolated and purified by silica gel chromatography as a white solid in 72% yield. Specifically, we found that using an approximately 2-fold excess of the diol **6** completely avoided formation of the 1,2-diacyl compound. The regioselectivity of the reaction could readily be ascertained by high-field ^{1}H NMR spectroscopy: absence of a ^{1}H NMR signal in the spectrum of compound **7a** in the δ 5.00−5.09 range indicates that there is no *sn*-2-ester group in the molecule. Alternatively, acylation of compound **6** with a stoichiometric amount of the *p*-nitrophenyl ester of 12-(2′-naphthylacetyl)aminododecanoic acid in the presence of DMAP as catalyst yielded the *sn*-1-ester **7b** (58%) with complete regioselectivity as well, as shown by ^{1}H NMR. Next, the tetrahydropyranyl function was introduced at the *sn*-2-position in reaction of **7** with excess 3,4-dihydropyran in dichloromethane, in the presence of pyridinium *p*-toluenesulfonate as catalyst, in 89−95% yield.

Compound **8** turned out to be a useful intermediate for preparation of the target lysophospholipids in the next phase of the synthesis, in that (1) the tetrahydropyranyl and *p*-toluenesulfonyl groups provide orthogonal protection for independent manipulation of the *sn*-2- and *sn*-3-glycerol positions, and (2) the reaction conditions required for removal of these protecting groups do not interfere with the integrity of the chain-terminal spectroscopic labels. Although installation of the tetrahydropyranyl function introduces a new asymmetric center, because this protecting group is removed later in the synthesis, it creates no problem in carrying compound **8** further along the sequence, without having to separate the stereoisomeric components. Furthermore, an important advantage of using the tetrahydropyranyl protecting group in phospholipid synthesis is that it can be cleaved under mild acidic conditions, preventing acyl and phosphoryl group migration to the deprotected alcohol function.[21] On the other hand, the reason for selecting the *p*-toluenesulfonyl group to protect the primary hydroxyl group becomes apparent from the reactions outlined in Scheme 3. Specifically, we have found that the tosylate function can be selectively manipulated in the presence of other base-labile protecting groups. For example, it is stable in the course of DBU-catalyzed cleavage of the FMOC group (**14** → **15**), whereas it can be easily displaced with nucleophiles as shown in Scheme 2 (**8** → **9**) that are unreactive toward FMOC or other conventional base-labile functional groups that are normally employed to protect amino groups and/or alcohols.

For displacement of the *sn*-3-tosyl group of compounds **8a** and **8b** we first tried using anhydrous tetraethylammonium acetate in dry acetonitrile at room temperature.[22] Indeed, the acetoxy compounds were obtained in high yield (>90%); however, attempts to achieve subsequent chemoselective cleavage of the acetyl group in the presence of the *sn*-1-carboxylic

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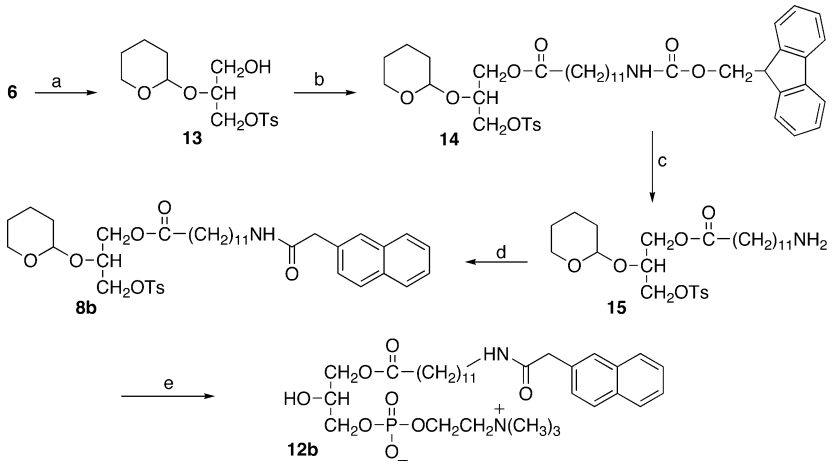
SCHEME 3[a]



[a] Reagents and conditions: (a) (i) $(CH_3)_3CCOOH$/DMAP, DCM, (ii) DHP/PPTS, DCM, (iii) $LiBH_4$, THF/Et_2O; (b) FMOC-NH$(CH_2)_{11}$COOH/DCC/DMAP, DCM; (c) DBU, DCM; (d) *p*-nitrophenyl 2-naphthylacetate/DMAP, DCM; (e) (i) $CH_3OCH_2COO^-Et_4H^+$, MeCN, (ii) Bu^tNH_2, MeOH/$CHCl_3$, (iii) ethylene chlorophosphate/Et_3N, C_6H_6, (iv) $(CH_3)_3N$, MeCN, 60 °C, (v) 0.15 M HCl, dioxane/water.

ester group turned out to be difficult, resulting in low yields of the desired products (30−35%).[23] Thus, in order to achieve better selectivity, we sought to increase the hydrolytic reactivity of the intermediate that forms on tosylate displacement. Specifically, we prepared anhydrous tetraethylammonium methoxyacetate to replace the corresponding acetate nucleophile. Although a weaker nucleophile, methoxyacetate was found capable of displacing tosylate under similar experimental conditions, except that the reaction times were somewhat longer, taking 48−72 h to achieve complete conversion. The resulting methoxyacetyl esters **9a** and **9b** were readily purified by silica gel chromatography and obtained as oily products (in 95 and 84% yields, respectively).

Selective cleavage of the *sn*-3-methoxyacetyl group was achieved with *tert*-butylamine in chloroform/methanol (4:1) solution at 0−10 °C for 1 h in 92−98% yield.[24] The reaction seems to involve base-catalyzed methanolysis rather than direct acyl transfer to the amine, because in the absence of methanol no reaction takes place under similar experimental conditions.

Phosphorylation of the alcohol **10** was carried out in sodium-dried benzene, using 1 equiv of 2-chloro-2-oxo-1,3,2-dioxaphospholane in the presence of a stoichiometric amount of triethylamine.[25] The cyclic phosphotriester intermediate (obtained in essentially quantitative yield as a single phosphate-positive product) was readily isolated from the reaction mixture and treated directly with anhydrous trimethylamine in dry acetonitrile, at 65 °C (in a pressure bottle) for 24 h. The phospholipid **11** that separated from the reaction mixture on cooling was purified by silica gel chromatography using chloroform/methanol/water (65:25:4) in 61−62% yield. Finally, acid-catalyzed hydrolysis of the tetrahydropyranyl group was accomplished in 0.15 N HCl solution in dioxane/water (1:1).[26] The lysophospholipid products were purified by chromatography, using either a Sephadex LH-20 column, (**12a**, 80% yield) or silica gel (**12b**, 69% yield).

Since the synthesis of the functionalized fatty acid derivatives often involves extensive use of protection/deprotection strategies requiring rather elaborate/circuitous sequences,[18] we developed an alternative synthetic strategy in which the chain-terminal fluorophore is installed *after* the *sn*-1-ester linkage to the long-chain carboxylic acid substituent has already been elaborated. Using the chemistry developed for the transformations outlined in the sequence shown in Scheme 2, our strategy called for preparation of *N*-protected aminododecanoic acid to be linked at the *sn*-1-position of the incipient phospholipid molecule, followed by replacing the amino protecting group with the desired fluorescent label.

Thus, reaction of 12-aminododecanoic acid with FMOC-chloroformate in aqueous dioxane in the presence of 1 equiv of sodium hydroxide gave the corresponding fluorenyl-9-methoxycarbonylaminododecanoic acid in high yield (90%). On the other hand, we have found that acylation of 3-(*p*-toluenesulfonyl)-*sn*-glycerol **6** with FMOC-protected aminododecanoic acid yielded a mixture of the *sn*-1- and the *sn*-2-substituted monoesters under a wide variety of different experimental conditions. In addition, the two regioisomers exhibited very similar chromatographic behavior on silica gel, such that isolation of the desired *sn*-1-ester from the mixture turned out to be quite difficult. Therefore, it seemed that changing the synthetic sequence by introducing the tetrahydropyranyl group at the *sn*-2-position *before* the acylation step offered a more promising approach to solve the problem. Along these lines, we prepared compound **13** in a rapid three-step sequence: (1) tosyl glycerol **6** was first converted to the *sn*-1-pivaloyl ester with complete regioselectivity (due to the presence of the bulky *tert*-butyl group in the molecule[27]), followed by (2) tetrahydropyranylation of the *sn*-2-hydroxyl group, and at last (3) the ester function at the *sn*-1-position was reduced with lithium borohydride to give the alcohol **13** in an overall yield of 74%. Since

(23) The low yield clearly results from hydrolysis of both ester groups. These groups have shown quite similar reactivities in reaction with a series of base catalysts that were used in an attempt to achieve chemoselective cleavage (via base-catalyzed elimination) of the acetyl group. Along these lines both DBU and Verkade's superbase (Ilancumaran, P.; Verkade, J. G. *J. Org. Chem*. **1999**, *64*, 3086−3089) failed to improve the yield of compound **10**.

(24) We have found that the combination of *tert*-butylamine/methanol gives better results than the methods previously used for hydrolysis of methoxyacetyl esters, including catalysis with ytterbium(III) triflate: Hanamoto, T.; Sugimoto, Y.; Yokoyama, Y.; Inanaga, J. *J. Org. Chem*. **1996**, *61*, 4491−4492.

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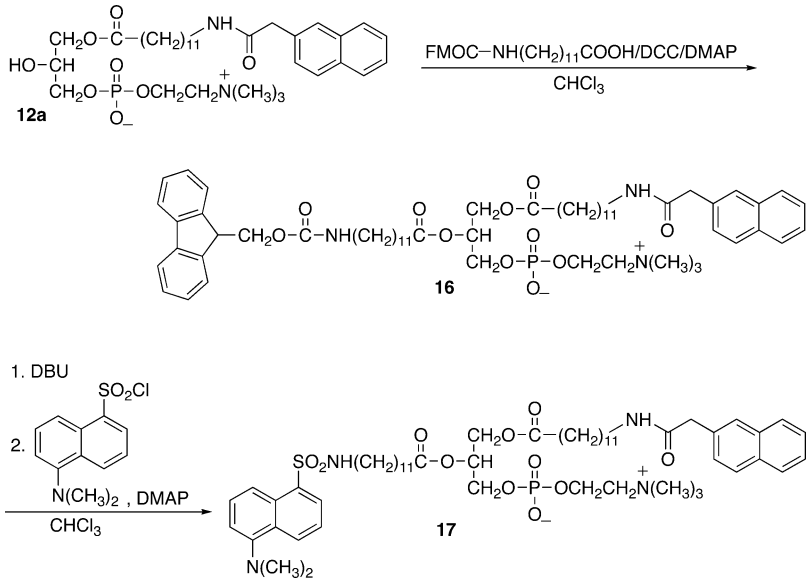
SCHEME 4



acylation of compound **13** can only occur at the free hydroxyl group at the *sn*-1-position, the regiochemistry of the product **14** in this case is unambiguously established.

Replacement of the FMOC protecting group with the target fluorophore at the *sn*-1-chain terminal was carried out in a two-step/one-pot sequence. Specifically, DBU-catalyzed elimination of the fluorenylmethyl group, followed by acylation of the deprotected amino terminal upon addition of *p*-nitrophenyl 2′-naphthylacetate/DMAP to the reaction mixture led to the 2′-naphthylacetyl derivative **8b** in an overall 80% yield. Conversion of **8b** to the target lysophosphatidylcholine **12b** was carried out as before (shown in Scheme 3).

Synthesis of Related Derivatives: Preparation of Double-Labeled Mixed-Chain Phosphatidylcholines. Phospholipid analogues incorporating spectroscopically active reporter groups are useful structural probes to study the location and dynamics of phospholipids in self-assembled systems such as bilayers, micelles, and vesicles.[28] Furthermore, experiments involving techniques of fluorescence resonance energy transfer (FRET) between two fluorophores or between a fluorophore and a fluorescence quencher have been shown to be applicable to develop real-time assay systems for the study of lipolytic enzymes.[29] With the fluorescently labeled lysophosphatidylcholines in hand, both types of double-labeled phospholipids should be accessible. In extending our synthetic method to the preparation of such probes we focused on the synthesis of compound **17** carrying a donor−acceptor pair. Specifically, the fluorescence emission of the 2-naphthylacetyl group as donor overlaps with the absorption of the 5-(dimethylamino)-1-naphthalenesulfonyl (dansyl) group acting as the acceptor[30] to conduct fluorescence resonance energy transfer experiments to study the proximity and orientation between the respective phospholipid chain terminals both in solution and in self-assembly. On the other hand, monitoring the changes in energy transfer (decrease in fluorescence emission of the acceptor and increase in fluorescence of the donor) in the course of hydrolysis of one of the carboxylic ester functions (for example, by phospholipase A_2)[29] should provide a useful method to measure in real-time the rate of enzyme catalysis.

Our synthetic strategy is outlined in the sequence presented in Scheme 4. Specifically, lysophosphatidylcholine **12b**, prepared as described in Scheme 2, was acylated at the *sn*-2-position using FMOC-protected aminododecanoic acid/DCC with DMAP catalysis. The reaction was carried out at 25 °C to prevent intramolecular acyl migration.[31] In additon, we used sonication rather than stirring of the reaction mixture and added glass beads to increase the glass surface in the reaction vessel.

Under these conditions the reaction reached completion in 6 h, requiring only 2.3 equiv of acylating agent.[32] The product **16** was isolated by silica gel chromatography in good yield (77%) and in high regioisomeric purity.[33] DBU-catalyzed deprotection of the amino group in chloroform, followed by addition of 1.2 equiv of dimethylaminonaphthalenesulfonyl chloride/DMAP gave the desired double-labeled phosphatidylcholine **17**, which was purified by silica gel chromatography, in 52% yield. Significantly, since introduction of the *sn*-2-chain terminal fluorescent label takes place after the phospholipid skeleton has been assembled, the method should allow incorporation of spectroscopic labels, including functional groups that would otherwise not survive the conditions of phospholipid total synthesis.

In conclusion, the significance of the synthesis here presented is in providing a facile and efficient method for the preparation of a wide range of functionalized phospholipids, including fluorescently labeled lysophosphatidylcholines with chain-terminal reporter groups, and double-labeled mixed-chain phos-

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(32) The reaction conditions that have been reported for acylation of lysophosphatidylcholines generally required a large (5- to 10-fold) excess of acylating agent; see ref 18a,b for detailed discussion.

(33) Compound **17** was completely hydrolyzed by phospholipase A_2 from bee venom to give lysophosphatidylcholine **12b** and 12-*N*-(5′-dimethylaminonaphthalene-1′-sulfonyl)aminododecanoic acid under the assay conditions previously reported: Roodsari, F. S.; Wu, D.; Pum, G. S.; Hajdu, J. *J. Org. Chem.* **1999**, *64*, 7727−7737.

phatidylcholines. The strength of the methods introduced lie in their simplicity and flexibility. They are likely to become applicable to the preparation of additional types of phospholipid compounds as well, including paramagnetic and photoactivable derivatives for biological and physicochemical studies. Work toward this goal is underway in our laboratory.

Experimental Section

1-Palmitoyl-3-(*p*-toluenesulfonyl)-*sn*-glycerol (7a). To a stirred solution of 3-(*p*-toluenesulfonyl)-*sn*-glycerol **6**[20] (1.67 g, 5.83 mmol) in 25 mL of freshly distilled dichloromethane were added palmitic acid (0.58 g, 2.26 mmol), DCC (0.47 g, 2.30 mmol), and DMAP (5 mg, 0.041 mmol) at 0 °C. The reaction mixture was then stirred at room temperature for 4 h. The DCC-urea precipitate was filtered with suction, and the solvent was removed by rotatory evaporation. The residue was chromatographed on activated silica gel using cyclohexane/ethyl acetate 3:2 as eluant and dried overnight in a desiccator over P_2O_5 to give 0.83 g (72%) of **7a** as a white solid. IR ($CHCl_3$) 1732 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 0.85 (t, J = 6.8 Hz, 3H), 1.22 (br s, 24H) 1.42−1.62 (m, 2H), 2.23 (t, J = 6.7 Hz, 2H), 2.41 (s, 3H), 4.07−4.12 (m, 5H), 7.32 (d, J = 8.2 Hz, 2H), 7.8 (d, J = 8.2 Hz, 2H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.2, 19.2, 22.0, 25.4, 29.6, 30.6, 32.0, 34.3, 63.1, 64.3, 71.9, 127.9, 130.0, 133.0, 145.1, 173.5; R_f (cyclohexane/EtOAc 3:2) = 0.71; $[\alpha]^{23}_D$ = −3.5 (*c* 1.07, $CHCl_3/CH_3OH$ 4:1). Anal. Calcd for $C_{26}H_{44}O_6S$: C, 64.38; H, 9.15. Found: C, 64.23; H, 9.27.

1-(12′-*N*-[2″-Naphthylacetyl]aminolauroyl)-3-(*p*-toluenesulfonyl)-*sn*-glycerol (7b) was prepared in a three-step sequence: (i) 12-(*N*-naphthyl-2′-acetyl)aminolauric acid. To a solution of 12-aminolauric acid (1.00 g, 4.64 mmol) in 40 mL of 0.5 M KOH in methanol/water (3:1) was added 2-naphthylacetyl chloride (1.42 g, 6.96 mmol), followed by stirring at room temperature for 1 h. To this reaction mixture were added 100 mL of chloroform, followed by dropwise addition of a 1.0 M aqueous HCl until the solution became acidic (pH ∼4). After phase separation the organic layer was extracted, and the solvent was evaporated. The residue was washed with methanol (5 × 20 mL), the solvent was removed by rotatory evaporation, and the light yellow residue was chromatographed on silica gel using $CHCl_3/CH_3OH$ (9:1) as eluant. The fractions of the product were collected, the solvent was evaporated, and the residue was freeze-dried from benzene to give a light yellow powder (1.432 g, 81%). IR ($CHCl_3$) 1660, 1743 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.15−1.50 (br m, 18H), 1.64 (m, 2H), 2.39 (t, J = 7.47 Hz, 2H), 3.20 (m, 2H), 3.76 (s, 2H), 5.41 (br s, 1H), 7.37 (m, 1H), 7.51 (m, 2H), 7.72 (br s, 1H), 7.84 (m, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 24.6, 26.5, 28.3, 29.0, 29.2, 29.3, 29.4, 29.5, 29.8, 33.9, 39.6, 43.1, 125.0, 125.7, 127.0, 127.6, 131.6, 133.5, 135.2, 169.5, 177.2; R_f ($CHCl_3/CH_3OH$ 9:1) = 0.73. FAB-MS [M + Na^+] calcd for $C_{24}H_{33}NO_3Na^+$ 406.2358, found 406.2345. (ii) *p*-Nitrophenyl-12-*N*-(2′-naphthylacetyl)aminolaurate. To a stirred solution of 12-*N*-(2′-naphthylacetyl)aminolauric acid (1.43 g, 3.75 mmol) in 25 mL of chloroform were added *p*-nitrophenol (0.63 g, 4.53mmol) and DCC (0.93 g, 4.51 mmol). The resulting solution was stirred overnight at room temperature. The dicyclohexylurea was filtered, and the solvent was evaporated. The residue was passed through a silica gel column using chloroform/ethyl acetate (9:1) as the eluant to give the *p*-nitrophenyl ester as a white solid (1.48 g, 78%). IR ($CHCl_3$) 1732, 1738 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.20−1.35 (m, 18H), 2.07 (t, J = 7.7 Hz, 2H), 3.09 (m, 2H), 3.45 (s, 2H), 5.45 (br s, 1H), 7.37 (m, 3H), 7.49 (m, 2H), 7.80 (m, 6H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 24.5, 26.7, 29.3, 33.7, 40.1, 44.0, 125.8, 126.0, 126.1, 126.4, 127.3, 127.6, 128.1, 128.2, 129.8, 132.6, 133.5, 135.6, 144.9, 147.7, 170.8, 172.7; R_f ($CHCl_3$/EtOAc 9:1) = 0.88. FAB-MS [M + H^+] calcd 505.2702, found 505.2705. Anal. Calcd for $C_{30}H_{36}N_2O_5$: C, 71.40; H, 7.19; N, 5.55. Found: C, 71.11; H, 7.41; N, 5.61. (iii) **7b**. To a stirred solution of **6** (1.00 g, 4.06 mmol) and DMAP (0.5 g, 4.06 mmol) was added *p*-nitrophenyl 12-*N*-(2′-naphthylacetyl)aminolaurate (2.29 g, 4.06 mmol) in dichloromethane, and the reaction mixture was stirred for 4 days at room temperature. The solvent was evaporated, and the product was isolated by silica gel chromatography using cyclohexane/ethyl acetate (2:3) as the eluant to give analytically pure **7b** (1.43 g, 58%). IR ($CHCl_3$) 1660, 1732 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.19−1.45 (m, 18H), 2.29 (t, J = 7.7 Hz, 2H), 2.45 (s, 3H), 3.17−3.20 (m, 2H), 3.40−3.69 (m, 2H), 3.74 (s, 2H), 4.06−4.32 (m, 5H), 5.40−5.45 (br s, 1H), 7.26−7.37 (m, 3H), 7.47−7.52 (m, 3H), 7.71−7.86 (m, 6H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 20.5, 24.5, 26.6, 29.0, 33.9, 39.7, 44.1, 64.3, 66.4, 126.0, 126.4, 127.3, 127.6, 128.1, 128.8, 129.9, 132.4, 133.2, 136.6, 145.1, 170.7, 173.7; R_f (cyclohexane/ethyl acetate 2:3) = 0.35. FAB-MS [M + Na^+] calcd for $C_{34}H_{45}NNaO_7S$ 634.2809, found 634.2822. Anal. Calcd for $C_{34}H_{45}NO_7S$: C, 66.75; H, 7.41, N, 2.29. Found: C, 67.00; H, 7.70; N, 2.16.

1-Palmitoyl-2-tetrahydropyranyl-3-(*p*-toluenesulfonyl)-*sn*-glycerol (8a). To a stirred solution of **7a** (1.53 g, 3.16 mmol) in 30 mL of freshly distilled dichloromethane was added dihydro-2*H*-pyran (0.80 g, 9.48 mmol), followed by PPTS (0.240 g, 0.95 mmol). The reaction mixture was stirred for 3.5 h at room temperature. The solvent was evaporated, and the oily residue was dried in a desiccator and then passed through an activated silica gel column using cyclohexane/ethyl acetate (3:2) to give the tetrahydropyranyl product **8a** (1.65 g, 95%) as a low melting solid. IR ($CHCl_3$) 1732 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 0.85 (t, J = 6.8, 3H), 1.22 (br s, 24H), 1.53−1.67 (m, 6H), 1.69−1.76 (m, 2H), 2.27−2.35 (dt, J = 7.7, 2.1 Hz, 2H), 2.45 (s, 3H), 3.40-.3.48 (m, 1H) 3.65−3.78 (m, 1H), 4.07−4.18 (m, 5H), 4.66 (m, 0.5H), 4.72 (m, 0.5H), 7.32 (d, J = 8.2 Hz, 2H), 7.74 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.1, 19.1, 22.7, 24.9, 25.3, 29.1, 29.2, 29.3, 29.4, 29.6, 30.5, 31.9, 34.1, 61.7, 62.3, 63.1, 69.6, 71.8, 98.2, 127.7, 127.8, 129.6. 129.7, 132.5, 144.8, 173.4; R_f (cyclohexane/ethyl acetate 3:2) = 0.77. Anal. calcd for $C_{31}H_{52}O_7S$: C, 65.46; H, 9.21. Found: C, 65.60; H, 8.96; FAB-MS [M + Na^+] calcd 591.3332, found 591.3354.

1-(12′-*N*-[2″-Naphthylacetyl]aminolauroyl)-2-tetrahydropyranyl-3-(*p*-toluenesulfonyl)-*sn*-glycerol (8b). To a solution of **7b** (0.84 g, 1.38 mmol) in 30 mL of freshly distilled dichloromethane were added 3,4-dihydro-2*H*-pyran (0.34 g, 4.14 mmol) and PPTS (0.10 g, 0.414 mmol), and the reaction mixture was stirred at room temperature for 24 h. The solvent was evaporated, and the residue was chromatographed on a silica gel column using cyclohexane/ethyl acetate (2:3) as the eluant. The fractions of the product were combined, and the solvent was evaporated to give **8b** (0.85 g, 89%) as a white solid. IR ($CHCl_3$) 1660, 1732 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.19−1.58 (m, 24H), 2.26−2.33 (t, J = 7.7 Hz, 2H), 2.45 (s, 3H), 3.17−3.20 (m, 2H), 3.40−3.54 (m, 1H), 3.70−3.89 (m, 1H), 3.74 (s, 2H), 4.06−4.32 (m, 5H), 4.66−4.69 (m, 1H) 5.40−5.45 (br s, 1H), 7.26−7.37 (m, 3H), 7.47−7.52 (m, 2H), 7.71−7.86 (m, 6H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 19.0, 20.5, 24.5, 24.8, 26.6, 29.0, 33.9, 39.7, 44.1, 62.1, 64.3, 66.4, 67.6, 94.6, 126.0, 126.4, 127.3, 127.6, 128.1, 128.8, 129.9, 132.4, 133.2, 136.6, 145.1, 170.7, 173.7; R_f (cyclohexane/ethyl acetate 2:3) = 0.51. Anal. Calcd for $C_{39}H_{53}NO_8S$: C, 67.31; H, 7.68. Found: C, 67.33; H, 7.36. FAB-MS [M + Na^+] calcd 718.3377, found 718.3384.

1-Palmitoyl-2-tetrahydropyranyl-3-methoxyacetyl-*sn*-glycerol (9a). To a solution of **8a** (0.912 g, 1.60 mmol) in 5 mL of dry acetonitrile was added 35 mL of 0.3 M tetraethylammonium methoxyacetate in acetonitrile, and the mixture was stirred at room temperature for 48 h. The reaction mixture was kept over 3 Å molecular sieves to ensure completely anhydrous conditions. When the reaction reached completion, the molecular sieves were filtered, the solvent was evaporated, and the yellow oil was purified by silica gel chromatography with hexane/ethyl acetate 3:1 to give the methoxyacetate **9a** as a colorless oil (0.739 g, 95% yield). IR ($CHCl_3$) 1731, 1738, 3456 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 0.85 (t, 6.8 Hz, 3H), 1.22 (s, 24H), 1.53−1.67 (m, 6H), 1.74−1.77 (m, 2H), 2.27−2.35 (dt, J = 7.7, 2.1 Hz, 2H), 3.44 (s, 3H), 3.48−

3.56 (m, 1H), 3.81−3.89 (m, 1H), 4.03−4.10(m, 2H), 4.12−4.18 (m, 2H), 4.22−4.26 (m, 2H), 4.28−4.36 (m, 1H), 4.78 (m, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.0, 19.0, 22.6, 24.9, 25.3, 29.1, 29.3, 29.4, 29.6, 30.4, 31.9, 34.1, 59.3, 62.1, 63.1, 64.2, 69.6, 71.7, 97.9, 170.0, 173.4; R_f (hexane/EtOAc 3:1) = 0.49. Anal. Calcd for $C_{27}H_{50}O_7$: C, 66.63: H, 10.36. Found: C, 66.68; H, 10.33. FAB-MS [M + Na^+] calcd 509.3454, found 509.3471.

1-(12′-*N*-[2″-Naphthylacetyl]aminolauroyl-2-tetrahydropyranyl-3-methoxyacetyl-*sn*-glycerol (9b). To a solution of **8b** (2.20 g, 3.16 mmol) in 10 mL of anhydrous acetonitrile was added 50 mL of 0.6 M tetraethyammonium methoxyacetate solution in acetonitrile. The acetonitrile solutions were dried over 3 Å molecular sieves. The reaction mixture was stirred at room temperature for 72 h. The solvent was evaporated to dryness, and the residue was chromatographed on a silica gel column with cyclohexane/ethyl acetate (2:3) to give the product **9b**, as a clear oily liquid (1.63 g, 84% yield). IR ($CHCl_3$) 1660, 1732 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.19−1.58 (m, 24H), 2.26−2.33 (t, J = 7.7 Hz, 2H), 3.17−3.20 (m, 2H), 3.41 (s, 3H), 3.45−3.56 (m, 1H), 3.74 (s, 2H), 3.75−3.89 (m, 1H) 4.06−4.32 (m, 7H), 4.75−476 (m, 1H), 5.60−5.75 (m, 1H), 7.32−7.47 (m, 3H), 7.68−7.82 (m, 4H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 19.0, 24.5, 24.8, 25.5, 26.6, 29.0, 33.9, 39.7, 44.1, 48.8, 62.1, 64.3, 66.4, 67.6, 97.8, 126.0, 126.4, 127.1, 127.4, 128.1, 128.5, 132.4, 133.2, 169.9,170.7, 173.7; R_f (cyclohexane/ethyl acetate 3:2) = 0.24. Anal. Calcd for $C_{35}H_{51}NO_8$: C, 68.49; H, 8.38; N, 2.28. Found: C, 68.33; H, 8.29; N, 2.51; FAB-MS [M + Na^+] calcd for $C_{35}H_{51}NNaO_8$ 636.3507, found 636.3524.

1-Palmitoyl-2-tetrahydropyranyl-*sn*-glycerol (10a).[26] To a solution of **9a** (0.680 g, 1.4 mmol) in 10 mL of chloroform/methanol (1:4) was added *tert*-butylamine (1.73 mL, 16.4 mmol) at 0 °C, and the reaction mixture was stirred at 0−10 °C for 1 h. The solvent was reduced to one-half of its original volume and passed through a silica gel column (EtOAc/hexane 1:3) to give a fluffy white product **10a** (0.569 g, 98%). IR ($CHCl_3$) 1732 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.86 (t, J = 6.8 Hz, 3H), 1.24 (s, 24H), 1.50−1.58 (m, 6H), 1.59−1.61 (m, 2H), 2.26−2.31 (dt, J = 7.7, 2.7 Hz, 2H), 3.58−3.68 (m, 3H), 3.88−3.98 (m, 2H) , 4.13−4.35 (m, 2H), 4.59 (m, 0.5H), 4.78 (m, 0.5H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.0, 19.0, 22.6, 24.9, 25.3, 29.1, 29.2, 29.3, 29.4, 29.6, 30.4, 31.7, 34.2, 58.6, 62.1, 64.2, 70.0, 97.9, 173.4; R_f (hexane/EtOAc 3:1) = 0.37. Anal. Calcd for $C_{24}H_{46}O_5$: C, 69.52; H, 11.38. Found: C, 69.66; H, 11.58. FAB-MS [M + NH_4^+] calcd 432.3689, found 432.3698.

1-(12′-*N*-[2″-Naphthylacetyl]aminolauroyl)-2-tetrahydropyranyl-*sn*-glycerol (10b). To a stirred solution of **9b** (1.63 g, 2.66 mmol) in 20 mL of chloroform/methanol (1:4) was added *tert*-butylamine (3.29 mL, 31.2 mmol) at 0 °C, and the reaction mixture was stirred at 0 °C for 1 h. The solvent was then evaporated to one-third volume, and the solution was passed through a silica gel column using cyclohexane/ethyl acetate (3:2) to remove the impurities, followed by ethyl acetate to elute the product. The solvent was evaporated, and the product was freeze-dried from benzene to give **10b** (1.32 g, 92%) as a fluffy white solid. IR ($CHCl_3$) 1660, 1732 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.19−1.64 (m, 24H), 2.26−2.36 (t, J = 7.7 Hz, 2H), 3.17−3.20 (m, 2H), 3.45−3.59 (m, 1H), 3.74 (s, 2H), 3.78−3.84 (m, 1H), 4.06−4.32 (m, 5H), 4.52 (m, 0.5H), 4.81 (m, 0.5H), 5.4−5.6 (m, 1H), 7.32−7.47 (m, 3H), 7.68−7.82 (m, 4H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 19.0, 24.5, 24.8, 26.6, 29.0, 33.9, 39.7, 44.1, 62.1, 64.3, 66.4, 67.6, 97.8, 126.0, 126.4, 127.1, 127.4, 128.1, 128.5, 132.4, 133.2, 170.7, 173.7; R_f (cyclohexane/ethyl acetate 2:3) = 0.42. Anal. Calcd for $C_{32}H_{47}NO_6 \cdot {}^1/_2H_2O$: C, 69.79; H, 8.78; N, 2.54. Found: C, 69.86; H, 8.52; N, 2.55. FAB-MS [M + Na^+] calcd 564.3296, found 564.3275.

1-Palmitoyl-2-tetrahydropyranyl-*sn*-glycero-3-phosphocholine (11a).[26] To a solution of **10a** (0.610 g, 1.47 mmol) in 25 mL of freshly distilled benzene was added triethylamine (0.31 mL, 2.21 mmol), followed by 2-chloro-2-oxo-1,3,2-dioxaphospholane (0.2 mL, 2.21 mmol) at 0 °C. The reaction mixture was stirred overnight at room temperature. The white solid (Et_3N·HCl) was filtered, and the solvent was evaporated. The oily residue was dissolved in 30 mL of anhydrous acetonitrile and was transferred to a pressure bottle. The solution was cooled in a dry ice−acetone bath below −10 °C, and 1 mL of precooled trimethylamine was added. The pressure bottle was sealed and kept at 65 °C with stirring for 24 h. The solution was then cooled to 0 °C, and the resulting white precipitate was filtered and purified by silica gel column chromatography using chloroform/methanol/water (65:25:4) to yield the phospholipids **11a** isolated as a white powder after freeze-drying from benzene (0.51 g, 61%). IR ($CHCl_3$) 1732 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.78 (t, J = 6.8 Hz, 3H), 1.15 (br s, 24H), 1.27−1.30 (m, 4H), 1.41−1.44 (m, 4H), 2.22 (dt, J = 7.5, 2.6 Hz, 2H), 3.14 (s, 9H), 3.55 (m, 2H), 3.80−3.95 (m, 4H), 4.12−4.38 (m, 4H), 4.62 (m, 1H, 4.74 (m, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 13.9, 20.0, 22.6, 24.8, 25.1, 29.0, 29.2, 29.3, 29.4, 29.5, 29.6, 30.7, 31.8, 34.0, 48.3, 62.12, 63.62, 64.8, 67.4, 68.2, 69.4, 98.1, 174.2; R_f ($CHCl_3/CH_3OH/H_2O$ 65:25:4) = 0.29. Anal. Calcd for $C_{29}H_{58}NO_8P$: C, 60.08; H, 10.08; N, 2.42; P, 5.34. Found: C, 59.87; H, 10.40; N, 2.41; P, 5.32. FAB-MS [M + H^+] calcd 580.3978, found 580.3986.

1-Palmitoyl-*sn*-glycero-3-phosphocholine (12a).[17] Compound **11a** (0.300 g, 0.53 mmol) was dissolved in 7 mL of 0.15 M HCl in dioxane/water. The solution was stirred at room temperature for 2 h. Then 10 mL of dioxane was added, and the mixture was freeze-dried overnight. The crude solid was chromatographed on silica gel using chloroform/methanol/water 65:25:4 and freeze-dried from benzene to give analytically pure **12a** (0.250 g, 98%) as a white solid. IR ($CHCl_3$) 1732 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.88 (t, J = 6.8 Hz, 3H), 1.26−1.45 (br s, 26H), 2.22 (t, J = 7.7 Hz, 2H), 3.35 (s, 9H), 3.90−4.50 (m, 9H); R_f ($CHCl_3/CH_3OH/H_2O$ 65:25:4) = 0.13. The ^{1}H NMR spectrum corroborates exactly with that reported in the literature[17] and that of an authentic sample (Avanti).

1-(12′-*N*-[2″-Naphthylacetyl]aminolauroyl)-2-tetrahydropyranyl-*sn*-glycero-3-phosphocholine (11b). To a solution of **10b** (0.7503 g, 1.38 mmol) in 30 mL of benzene, cooled in an ice−water bath, was added 2-chloro-2-oxo-1,3,2-dioxaphospholane (0.25 mL, 2.76 mmol) followed by triethylamine (0.39 mL, 2.76 mmol), dropwise. The ice bath was removed, and the reaction mixture was stirred at room temperature for 9 h. The white precipitate of Et_3N·HCl was filtered and washed with benzene. The combined solvent was evaporated, and the oily residue was dissolved in 30 mL of CH_3CN. The solution was transferred to a pressure bottle and frozen at −10 °C. To this solution was added NMe_3 (3 mL), and the pressure bottle was sealed and kept at 65 °C for 36 h. The pressure bottle was then cooled in an ice bath, whereupon most of the product precipitated. This solid, combined with the oily residue obtained from evaporation of the solvent, was purified on a silica gel column with $CHCl_3$/MeOH/H_2O (65:25:4). The fractions containing the product were collected, the solvent was evaporated, and the residue was freeze-dried from benzene to give **11b** (0.576 g, 58%) as white/pale yellow solid. IR ($CHCl_3$) 1735 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.24 (br s, 16H), 1.66 (m, 10H), 2.29 (br t, 2H), 3.18 (t, 2H, J = 7.2 Hz), 3.32 (br s, 9H), 3.70 (br s, 2H), 3.90 (m, 3H), 3.99 (br m, 4H), 4.26 (m, 2H), 4.72 (m, 1H), 5.72 (m, 1H), 7.35−7.83 (m, 7H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 18.9, 19.8, 24.8, 24.9, 26.7, 29.1, 29.8, 30.8, 34.2, 39.7, 43.9, 54.2, 59.2, 61.6, 63.1, 64.3, 65.1, 66.2, 72.8, 74.22, 96.8, 99.5, 125.9, 126.3, 127.3, 127.6, 128.1, 128.6, 132.4, 133.5, 170.8, 173.7; ^{31}P NMR ($CDCl_3$, 160 MHz, pyrophosphate ext ref) δ −1.79. R_f ($CHCl_3/CH_3OH/H_2O$ 65:25:4) = 0.39. Anal. Calcd. for $C_{37}H_{59}N_2O_9P \cdot {}^5/_2H_2O$: C, 59.10; H, 8.58; N, 3.73. Found: C, 59.55; H, 8.79; N, 3.75. FAB-MS [M + H^+] calcd 707.4036, found 707.4074.

1-(12′-*N*-[2″-Naphthylacetyl]aminolauryl)-*sn*-glycero-3-phosphocholine (12b). To a cloudy solution of **11b** (0.401 g, 0.56mmol) in 45 mL of 1,4-dioxane was added 0.15 mL of 12 N aqueous HCl, and the reaction mixture was stirred for 2 h at room temperature. After addition of 30 mL more dioxane the mixture was freeze-

dried. The white residue obtained was dissolved in $CHCl_3$/MeOH/H_2O (65:25:4) and purified on a short silica gel column loaded with $CHCl_3$ and chromatographed with $CHCl_3$/MeOH/H_2O (65:25:4). The fractions corresponding to the product were collected, the solvent was evaporated, and the residue was freeze-dried from benzene to give **12b** (0.240 g, 69%) as white solid. IR ($CHCl_3$): 1755 cm^{-1}; ^1H NMR ($CDCl_3$ + CD_3OD, 200 MHz) δ 1.19 (br s, 16H), 1.55 (m, 4H), 2.29 (t, 2H, J = 7.2 Hz), 3.17 (t, 2H, J = 7.2 Hz), 3.33 (br s, 11H), 3.70 (s, 2H), 3.82−4.07 (br m, 5H), 5.99 (m, 1H), 7.27−7.82 (m, 7H); ^{13}C NMR ($CDCl_3$ + CD_3OD, 50 MHz) δ 24.7, 26.6, 28.9, 29.0, 29.2, 33.9, 39.5, 43.5, 54.0, 59.2, 64.8, 66.0, 67.0, 68.5, 125.8, 126.2, 127.1, 127.4, 127.5, 128.4, 132.3, 132.5, 133.3, 171.1, 173.8; ^{31}P NMR ($CDCl_3$, 160 MHz, pyrophosphate ext ref) δ −3.82 br. R_f ($CHCl_3$/MeOH/H_2O 65:25:4) = 0.28. Anal. Calcd for $C_{32}H_{51}N_2O_8P{\cdot}2H_2O$: C, 58.34; H, 8.42; N, 4.25. Found: C, 58.45; H, 8.09; N, 4.08. FAB-MS [M + H^+] $C_{32}H_{52}N_2O_8P$ calcd 623.3461, found 623.3439. $[\alpha]^{25}_D$ −4.7° (*c* 0.89, $CHCl_3$/MeOH 4:1).

1-(12′-*N*-FMOC-Aminolauroyl)-2-tetrahydropyranyl-3-(*p*-toluenesulfonyl)-*sn*-glycerol (14). To a solution of **13** (2.76 g, 8.35 mmol) in 30 mL of freshly distilled dichloromethane was added FMOC-aminolauric acid (3.67 g, 8.35 mmol) followed by DCC (1.72 g, 8.35 mmol) and DMAP (0.050 g), and the reaction mixture was stirred at room temperature for 72 h.The dicyclohexyl urea was removed by vacuum filtration, and the filtrate was evaporated to dryness. The residue was chromatographed on a silica gel column using hexane/ethyl acetate (2:1) as eluant, and the product was freeze-dried from benzene to give **14** as a white powder (4.57 g, 73%). IR ($CHCl_3$) 1716, 1732 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.29−1.58 (m, 24H), 2.29 (t, J = 7.5 Hz, 2H), 2.47 (s, 3H), 3.17 (m, 2H), 3.40−3.89 (m, 2H), 4.06−4.32 (m, 6H), 4.40 (d, J = 6.8 Hz, 2H), 4.71−4.89 (m, 1H), 7.26−7.40 (m, 6H), 7.58 (d, J = 7.3), 7.74−7.83 (m, 4H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 19.0, 20.5, 24.8, 26.6, 29.0, 29.8, 33.9, 41.0, 47.2, 62.1, 64.3, 66.4, 67.6, 70.1, 94.6, 119.9, 124.9, 126.9, 127.5, 127.8, 129.9, 132.4, 141.2, 143.9, 145.1, 156.4, 173.7. R_f (hexane/EtOAc 2:1) = 0.24. Anal. Calcd for $C_{42}H_{55}NO_9S$: C, 67.31; H, 7.68; N, 2.01. Found: C, 67.19; H, 7.36; N, 1.73. FAB-MS [M + H^+] calcd 772.3490, found 772.3510.

1-(12′-*N*-[2″-Naphthylacetyl]aminolauroyl)-2-tetrahydropyranyl-3-(*p*-toluenesulfonyl)-*sn*-glycerol (8b). Method 2, from Compound 14. To a solution of **14** (4.57 g, 6.08 mmol) in 5 mL of freshly distilled dichloromethane was added 0.5 mL of DBU, and the reaction mixture was stirred at room temperature for 15 min. Next, a solution of *p*-nitrophenyl 2-naphthylacetate[33] (1.89 g, 6.08 mmol) in 25 mL of dichloromethane was added to the reaction mixture, and the stirring was continued overnight. The solvent was evaporated, and the crude product was purified by silica gel chromatography using chloroform/methanol (9:1) to obtain compound **8b** (3.38 g, 80%) as a white solid. Data for characterization of the product **8b** are given above.

1-(12′-*N*-[2″-Naphthylacetyl]aminolauroyl)-2-(12′-*N*-FMOC-aminolauroyl)-*sn*-glycero-3-phosphocholine (16). To a solution of **12b** (0.1495 g, 0.25 mmol) in 25 mL of freshly distilled chloroform were added FMOC-12-*N*-aminododecanoic acid (0.2516 g, 0.58 mmol), DCC (0.1191 g, 0.58 mmol), DMAP (71 mg, 0.58 mmol), and approximately 0.4 g of glass beads.[31] The mixture was sonicated for 6 h at 25 °C. The solvent was evaporated, and the residue was taken up in $CHCl_3$/MeOH (4:1) and loaded onto a silica gel column packed with $CHCl_3$, for chromatography. First $CHCl_3$/MeOH (4:1) was used to elute the impurities, followed by $CHCl_3$/MeOH/H_2O (65:25:4) to elute the phospholipids product. The fractions containing the product were combined, the solvent was evaporated, and the residue was freeze-dried from benzene to give **16** (0.201 g, 77%) as a white solid. IR ($CHCl_3$) 1742 br, 1690, 1246 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.25 (br s, 32H), 1.56 (m, 8H), 2.28 (br t, 4H), 3.15 (m, 4H), 3.33 (br s, 9H), 3.70 (s, 2H), 3.77 (m, 2H), 3.95 (m, 1H), 4.15−4.32 (m, 2H), 4.35 (m, 2H), 5.04 (m, 1H), 5.20 (m, 1H), 5.85 (m, 1H), 7.29−7.83 (m, 15H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 24.7, 26.7, 29.0, 29.1, 29.2, 29.4, 29.9, 34.2, 39.6, 41.0, 43.8, 47.2, 54.2, 59.2, 62.9, 63.3, 66.4, 71.0, 119.9, 125.0, 125.9, 126.3, 126.9, 127.3, 127.5, 128.0, 128.5, 132.6, 133.4, 141.2, 144.0, 156.4, 170.7, 173.1, 173.4; ^{31}P NMR ($CDCl_3$, 160 MHz, pyrophosphate ext ref) δ −0.93 br. R_f ($CHCl_3$/MeOH/H_2O 65:25:4) = 0.33. Anal. Calcd for $C_{59}H_{84}N_3O_{11}P{\cdot}H_2O{\cdot}^1/_2CHCl_3$: C, 63.81; H, 7.78; N, 3.75. Found: C, 63.60; H, 7.91; N, 3.86. FAB-MS [M + H^+] calcd 1042.5921, found 1042.5949. $[\alpha]^{20}_D$ −5.7 (*c* 1.14, $CHCl_3$/MeOH 4:1).

1-(12′-*N*-[2″-Naphthylacetyl]aminolauroyl)-2-(12′-*N*-[5‴-dimethylaminonaphthalene-1‴-sulfonyl]aminolauroyl)-*sn*-glycero-3-phosphocholine (17). To a solution of **16** (0.151 g, 0.144 mmol) in 5 mL of $CHCl_3$ was added DBU (0.110 g, 0.72 mmol) at room temperature. After the reaction mixture stirred for 1 h, dansyl chloride (47 mg, 0.17 mmol) and DMAP (21 mg, 0.17 mmol) were added. The reaction was over after 30 min, as shown by the disappearance of the ninhydrin-positive spot close to the origin on a TLC plate. The mixture was chromatographed on a silica gel column packed with $CHCl_3$ and eluted first with $CHCl_3$/MeOH (4:1) to eliminate the impurities and then with $CHCl_3$/MeOH/H_2O (65:25:4) to obtain the product. The fractions containing the product were combined, the solvent was evaporated, and the residue was freeze-dried from benzene to give **17** (0.079 g, 52%) as a yellow solid. IR ($CHCl_3$) 1742 br cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.26 (br s, 32H), 1.57 (m, 8H), 2.29 (br t, 4H), 2.88 (s, 6H), 3.17 (m, 4H), 3.23 (br s, 9H), 3.73 (s, 2H), 3.95 (m, 1H), 4.22−4.41 (m, 3H), 5.21 (m, 1H), 5.82 (m, 1H), 6.32 (m, 1H), 7.12−7.93 (m, 13H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 24.8, 26.3, 29.1, 29.3, 34.0, 39.6, 41.0, 43.1, 45.3, 51.8, 54.2, 59.5, 62.7, 63.6, 66.1, 70.2, 115.0, 119.0, 123.1, 125.8, 126.2, 127.5, 128.00, 129.0, 129.9, 132.3, 132.7, 134.3, 151.7, 170.8, 173.0, 173.4; ^{31}P NMR ($CDCl_3$, 160 MHz, pyrophosphate ext ref) δ −0.93. R_f ($CHCl_3$/MeOH/H_2O 65:25:4) = 0.37. Anal. Calcd for $C_{56}H_{85}N_4O_{11}PS$: C, 63.85; H, 8.13; N, 5.32; Found: C, 63.88; H, 8.38; N, 5.02. FAB-MS [M + H^+] calcd 1053.5751, found 1053.5733. $[\alpha]^{20}_D$ −5.2 (*c* 1.02, $CHCl_3$/MeOH 4:1).

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Supporting Information Available: Experimental procedures for preparation and characterization of compounds **6**, **13**, and additional intermediates discussed. This material is available free of charge via the Internet at http://pubs.acs.org.

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