A New Approach to the Stereospecific Synthesis of Phospholipids. The Use of L-Glyceric Acid for the Preparation of Diacylglycerols, Phosphatidylcholines, and Related Derivatives

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Received March 8, 1999

A new stereospecific synthesis of phospholipid derivatives of 1,2-diacyl-*sn*-glycerols is reported. The synthesis is based on (1) the use of L-glyceric acid as the stereocenter for construction of the optically active phospholipid molecule, (2) preparation of 3-triphenylmethyl-*sn*-glycerol as the key intermediate for sequential introduction of the primary and secondary acyl functions leading to the chiral diglycerides, and (3) elaboration of the *sn*-3-phosphodiester headgroup via phosphorylation using 2-chloro-2-oxo-1,3,2-dioxaphospholane, followed by ring opening of the five-membered phosphorus heterocycle with trimethylamine, ammonia, as well as oxygen and sulfur nucleophiles. The sequence has been shown to be suitable for the preparation of both symmetric and mixed-chain diacylglycerols with saturated and unsaturated acyl substituents. Phospholipid headgroups including phosphocholine, phosphoethanolamine, phosphoethanol, and phosphoethylthioacetate functions have been prepared. Application of the method to the synthesis of functionalized phosphatidylcholines has also been demonstrated by incorporating spectroscopically active spin-labeled and fluorescent reporter groups via postsynthetic derivatization of chain terminal ω-aminoalkyl functions of the acyl substituents of the compounds. The synthetic methods developed have a great deal of flexibility, providing convenient routes to a wide range of structurally variable phospholipids for physicochemical, enzymological, and cell-biological studies.

The development of new synthetic methods for the preparation of structurally well-defined phospholipid compounds has become a timely and important goal since it was discovered that in addition to their role as a major component in all biological membranes[1,2] phospholipids are also involved in a wide range of physiological and regulatory processes.[3−12] Phospholipids are essential for the functioning of a number of membrane-bound enzymes such as protein kinase C[5,10,11] and play an important role in signal transduction[4−6] as substrates for the production of second messengers[5,12] (inositol-1,4,5-trisphophate, diacylglycerol) and in the release of arachidonic acid. Significantly, some of the most potent phospholipid compounds, which are active below the micromolar[8] and nanomolar[3] levels, occur in low concentrations in the cell, such that preparation of synthetic derivatives represents a clear prerequisite to elucidation of their biochemical mechanism of action. The compounds are required for both structural and dynamic studies of biomembranes[13−16] and membrane-bound enzymes,[5,10,11,17] with particular emphasis on establishing structure−activity relationships with respect to phospholipid−phospholipid[13−16] and phospholipid−protein[17] interactions. Furthermore, delineation of the structural requirements for the biological activities of phospholipids will not only advance the current level of understanding of the chemistry and biology of these compounds but also provide important insight into the design of new target molecules with the desired activity and potency.

Despite growing recognition of the importance and timeliness of the problem, relatively few synthetic methods of general significance[18−23] have been developed for

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10.1021/jo990414e CCC: $18.00
Published on Web 09/23/1999

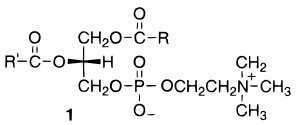
the preparation of structurally variable phospholipid derivatives with particular attention to (1) incorporation of different acyl chains at the primary vs secondary glycerol positions, including both saturated and unsaturated substituents and (2) variation of the phosphodiester function at the polar headgroup of the molecule.[24−26]

Most chemical approaches developed to date seem to rely on either using the traditional chiral pool of 1,2-isopropylidene-*sn*-glycerol/D-mannitol synthons[19−21] as phospholipid precursors or employing derivatives of enantiomerically pure (*S*)-glycidol[22,23] that more recently became available from stereospecific epoxidation of allylic alcohols.[27] The key issue in all phospholipid syntheses has been the generation of a well-defined stereocenter at the incipient glycerol 2-position followed by regiospecific introduction of the target substituents in such a manner that preserves the stereochemical integrity of the central carbon.[22] Consequently, most stereospecific schemes that have been developed, particularly those which aimed at preparation of phospholipids with two different acyl groups, require multiple protection−deprotection steps.[18,22]

As part of our research in this area we recently focused our efforts on the development of a new stereospecific synthesis of symmetric and mixed-chain diglycerides and phospholipids, with special attention to compounds incorporating unsaturated and functionalized acyl substituents and variable headgroup functions. In an attempt to devise a new strategy to minimize the number of protecting groups required for the synthesis we turned to L-glyceric acid to develop the sequence here presented. Because we have shown this readily available three-carbon synthon to be highly suitable for the preparation of alkyl ether and thioether phospholipids,[28] it appeared a promising strategy to explore its use for the synthesis of diacyl glycerols and their respective phospholipid derivatives.

Results and Discussion

Our synthetic approach outlined in Scheme 1 is based on the recognition that chirality of the stereocenter in the target compound **1** is similar to that of the optically active α-carbon in L-glyceric acid acid **2**. Thus, our



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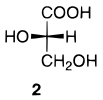

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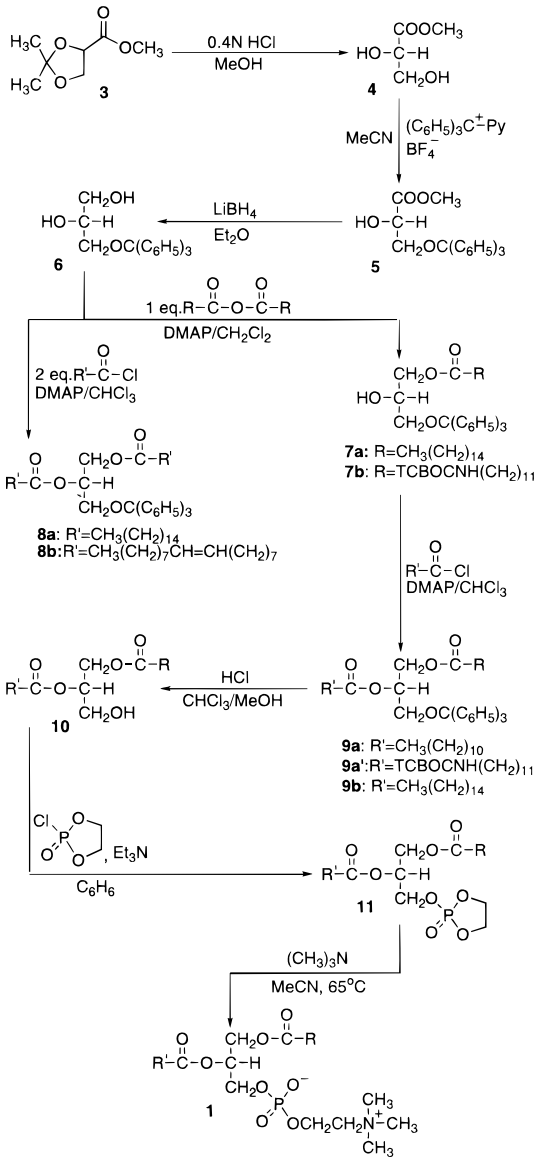
strategy is based on constructing the phospholipid skeleton around the glyceric acid nucleus. As the structural relationship between compounds **1** and **2** indicates, the synthetic procedure must involve (1) reduction of the carboxylic group without destroying the chirality of the *sn*-2-carbon and (2) sequential acylation of the incipient primary and existing secondary alcohol functions, followed by (3) introduction of the desired phosphodiester function to obtain the *sn*-3-phospholipid headgroup.

Scheme 1



To implement this strategy we first prepared the 3-triphenylmethyl derivative **5** of L-methyl glycerate **4** whose carbomethoxy group could subsequently be reduced without loss of the optical activity at the *sn*-2-carbon. Thus, compound **5** was obtained from commercially available 2,3-isopropylidene methyl-L-glycerate **3** in a two-step sequence in 79% overall yield, following a procedure previously reported.[28] It was then reduced with excess lithium borohydride to give 3-trityl-*sn*-glycerol **6** isolated and purified by silica gel chromatography as a white crystalline solid in good yield (85%).[29] Compound **6** turned out to be the key intermediate in the sequence, showing significantly higher reactivity at the primary vs secondary alcohol function: it could be selectively acylated at the *sn*-1-position using 1 equiv of the anhydride

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(29) The use of $LiBH_4$ rather than lithium aluminum hydride as reducing agent greatly improved the recovery of the product **6**. Although reduction of **5** with $LiAlH_4$ could also be accomplished in high yield, substantial losses occurred on isolation of the product, most likely as a result of chelation of aluminum by the bis-diol function of compound **6**.

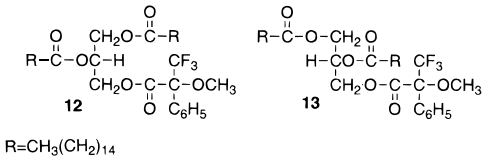
of the corresponding fatty acid in 67−71% yield. Along these lines reaction of 3-trityl-*sn*-glycerol **6** with 1 equiv of palmitic anhydride in the presence of 4-(dimethylamino)pyridine (DMAP) in anhydrous chloroform at 0 °C for 24 h afforded the *sn*-1-palmitoyl compound **7a** as a low-melting crystalline solid (mp 45−47°).[30]

Characterization of the *sn*-1-monoacyl compounds **7** and determination of their regioisomeric purity could readily be established by high-field ^{1}H NMR spectroscopy.[31] Specifically, the chemical shift of the C−H proton at the glycerol 2-position is shifted from δ 4.10 to 5.09 ppm on acylation, giving rise to a nearly symmetrical multiplet (being split by two sets of nonequivalent neighboring CH_2 protons) such that in the presence of even traces of the *sn*-2-regioisomer a new peak at 5.09 ppm becomes apparent.

Introduction of the second acyl substituent was carried out in a rather straightforward manner, using acid chloride, anhydride, or the *p*-nitrophenyl ester of the desired fatty acid with DMAP catalysis, in nearly quantitative yield. Subsequent deprotection of the *sn*-3-alcohol function was accomplished by acid-catalyzed methanolysis using 1 equiv of HCl for 24 h. Significantly, the diacyl glycerol products **10** obtained under these experimental conditions could readily be isolated and purified by silica gel chromatography; the analytically pure diglycerides were obtained as crystalline solids in 70−73% yield.[32] The corresponding symmetric diacyl glycerols were prepared via intermediates **8a** and **8b** in a similar series of reactions. Thus, single-step diacylation of compound **6** using 2 equiv of acid halide gave the symmetric 1,2-diacyl-3-triphenylmethyl-*sn*-glycerol **8** in 86−90% yield. Acid-catalyzed deprotection as outlined above gave saturated and unsaturated 1,2-diglycerides that were isolated and purified by silica gel chromatography, as before.

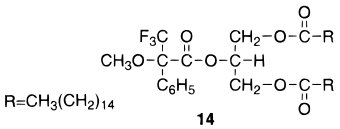
The stereochemistry of the synthetic diacyl glycerols was established by two different methods. First, the Mosher esters[33] were prepared from both D- and L-enantiomers, and the resulting triesters were characterized by high-field ^{1}H NMR spectroscopy. The second method involved preparation of the corresponding phosphatidylcholine **1** and its characterization by enzymatic hydrolysis using bee venom phospholipase A_2.[34] The optical rotation of the product **1c** was also determined and compared to that of a phosphatidylcholine reference standard.

Along these lines 1,2-dipalmitoyl-*sn*-glycerol and separately prepared 2,3-dipalmitoyl-*sn*-glycerol were allowed to react with (*S*)-(+)-MPTA chloride using DMAP as catalyst in chloroform to obtain the corresponding Mosher esters **12** and **13**. The 500 MHz ^{1}H NMR spectra of the



diastereoisomeric MTPA esters **12** and **13** turned out to be sufficiently different from each other in the 4.00−4.60 ppm region, allowing us to readily distinguish between the compounds. Most notably, the high-field proton of the *sn*-1-methylene group of compound **12** prepared from the L-enantiomer showed a four-line multiplet (doublet of doublets) in the 4.06−4.09 ppm range, whereas the chemical shifts of both *sn*-1-glycerol CH_2 protons of compound **13** derived from the D-enantiomer occurred between δ 4.11−4.14. Absence of the latter (at baseline level separation) from the ^{1}H NMR spectrum of compound **12** confirmed the enantiomeric purity of the product (and consequently that of the diglyceride) at the NMR detection level.

To rule out the possibility of acyl migration in the course of manipulation of the diacylglycerol compounds we have also prepared the MTPA ester from 1,3-dipalmitoyl glycerol **14** for comparison. The 500 MHz ^{1}H NMR



spectrum of this compound **14** showed a set of chemical shifts in the 4.15−4.55 ppm range that appeared distinctly different from and nonoverlapping with the spectrum of either compound **12** or **13**.

Independent evidence for stereospecifity of the synthesis was obtained from complete enzymatic hydrolysis of phosphatidylcholine **1a** by bee venom phospholipase A_2.[34] The lysophosphatidylcholine and fatty acid degradation products were identified in comparison with the corresponding reference standards. As expected, the enantiomeric *sn*-1-phosphatidylcholine prepared from 2,3-dipalmitoyl-*sn*-glycerol turned out to be resistant to enzymatic hydrolysis since phospholipase A_2 is known to be specific for *sn*-3-glycerophospholipids.[34] Finally, we have also compared the optical rotation of our synthetic dipalmitoyl phosphatidylcholine **1c** to that of standard dipalmitoyl phosphatidylcholine (DPPC) and found those to be identical within experimental error (see the Experimental Section). Therefore, by analogy, the series of other phospholipid compounds that were prepared in a similar sequence of reactions shown in Scheme 1 are also expected to be optically pure.

In addition to providing a facile and efficient scheme for the preparation of phosphatidylcholine **1**, a number of useful synthetic strategies have emerged from the sequence. The first one concerns the use of 3-triphenylmethyl-*sn*-glycerol **6** as a chiral precursor for the synthesis of 1,2-diacyl-*sn*-glycerols **10** and the corresponding phosphatidylcholines **1**. Specifically, compound **6** can be as readily and selectively either monoacylated at the *sn*-1-position or diacylated as the more conventional 3-ben-

(30) We have examined a series of alternative reaction conditions in an attempt to determine the best method to achieve regiospecific monoacylation of 3-trityl-*sn*-glycerol. Specifically, using equimolar palmitoyl chloride in place of the anhydride lowered the yield of **7a** from 67% to 36%, while variation of the solvent from chloroform to CH_2Cl_2 and CCl_4 resulted in decrease from 67% to 50% and 21%, respectively. Furthermore, acylation of **6** with free fatty acid via dicyclohexylcarbodiimide (DCC) coupling in the presence of DMAP[19−21] resulted in lower yields as well: compound **7b** was obtained in 43% along with the *sn*-2-regioisomer (10%) and the 1,2-diacyl product (8.1%), compared to the 71% yield obtained under the reaction conditions shown in Scheme 1.

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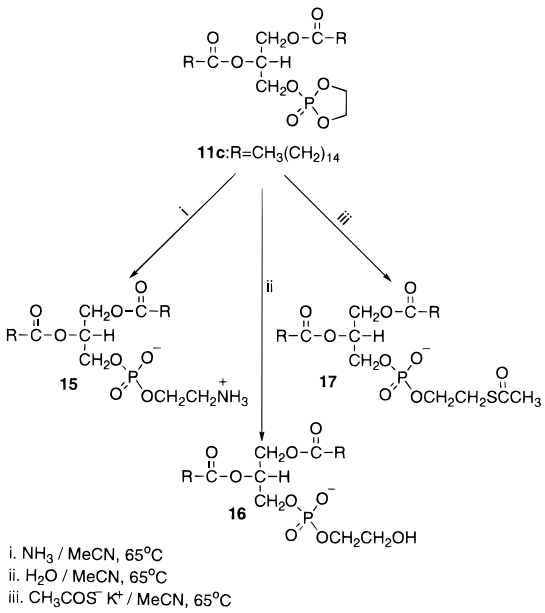
zyl/(4′-methoxy)benzyl-*sn*-glycerol analogues,[19,20] and deprotection of the *sn*-3-alcohol function of intermediate **9** becomes feasible under very mild experimental conditions such that the product **10** can be flash chromatographed without any appreciable acyl migration. This represents a substantial improvement in protection−deprotection techniques and addresses one of the most common problems encountered in phospholipid/diglyceride synthesis.[18−21] Because acyl migration is subject to both acid- and base-catalysis, alternative deprotection methods under neutral conditions, relying on catalytic hydrogenation[20] or oxidation,[35,36] have largely been limited in scope of the acyl substituents to saturated derivatives. Even recently developed low-temperature Lewis acid hydrolysis[19,21] appears to have significant limitations, as it has been reported that the *sn*-2-acyl substituted deprotected glycerol products could not be chromatographed on either silica gel or alumina without extensive isomerization.[19] In contrast, diglycerides available from Scheme 1 can be readily purified, and the sequence is applicable to the synthesis of saturated, unsaturated, symmetric, and mixed-chain diglycerides. Because 1,2-diacyl-*sn*-glycerols are important biologically active lipids in their own right,[37,38] availability of the synthetic analogues should contribute to elucidation of their biochemical/regulatory mechanism of action.

Our approach to elaboration of the phospholipid headgroup focused on the use of 2-chloro-2-oxo-1,3,2,-dioxaphospholane as phosphorylating agent to obtain the substituted glycerophosphoryl intermediate **11**, whose reactive five-membered heterocyclic ring was then subjected to nucleophilic cleavage by anhydrous trimethylamine. Both reactions occurred readily because the substituents on the glycerol skeleton carried no nucleophilic group that could interfere with either the phosphorylation step or the ring opening involved in elaboration of the phosphodiester function.[24] Successful implementation of this procedure opened the way to preparation of a new series of phospholipids incorporating different polar headgroups.

Synthesis of Related Analogues. Because of the well-known importance of the hydrophilic component of the phosphodiester function in determining the physicochemical and biological properties of phospholipids,[39] we have explored the feasibility of extending the scope of our synthesis to the preparation of related derivatives incorporating other nitrogen, oxygen, and sulfur atoms at the polar phosphoester portion of the molecule. Our target

Scheme 2



compounds included phosphatidylethanolamine, as this ubiquitous phospholipid is known to contribute to biological membrane structure in providing specific microenvironments to modulate the level of activity of membrane-bound enzymes.[10,40] The compound has also been shown recently to be required for conformational maturation of the polytopic membrane protein lactose permease both in vivo and in vitro, acting as a molecular chaperone.[41] Other polar headgroup substituents aimed at preparation of hydroxyethyl and thioethyl phosphoester derivatives because synthetic phospholipid analogues incorporating such headgroups have been shown to act as potent inhibitors of lipolytic enzymes.[42,43]

To realize these objectives we have carried out a series of ring-opening reactions of 2-(1′,2′-dipalmitoyl-*sn*-glycero)-2-oxo-1,3,2-dioxaphospholane intermediate **11c** available from Scheme 1 using N-, O-, and S-nucleophiles. As shown in Scheme 2, in the first reaction compound **11c** was allowed to react with anhydrous ammonia in acetonitrile solution to obtain the corresponding phosphatidylethanolamine **15**. The reaction was conducted in a pressure bottle at 65 °C for 24 h. The product **15** that crystallized on cooling was obtained in high yield; however, as silica gel chromatography of phospholipids frequently results in losses on recovery, the final product after purification was isolated in 56% yield. Next, compound **11c** was allowed to react with water in acetonitrile solution to give 1,2-dipalmitoyl-*sn*-glycerophosphoethanol **16** in 62% yield, and finally, cleavage of the five-membered dioxaphospholane ring of **11c** using potassium thioacetate under similar conditions afforded the sulfur-substituted phospholipid **17** in 45% yield following silica gel column chromatography. The synthesis of compounds **15**−**17** indicates that analogous ring-opening reactions using other nucleophiles are likely to yield a series of related phospholipid derivatives.

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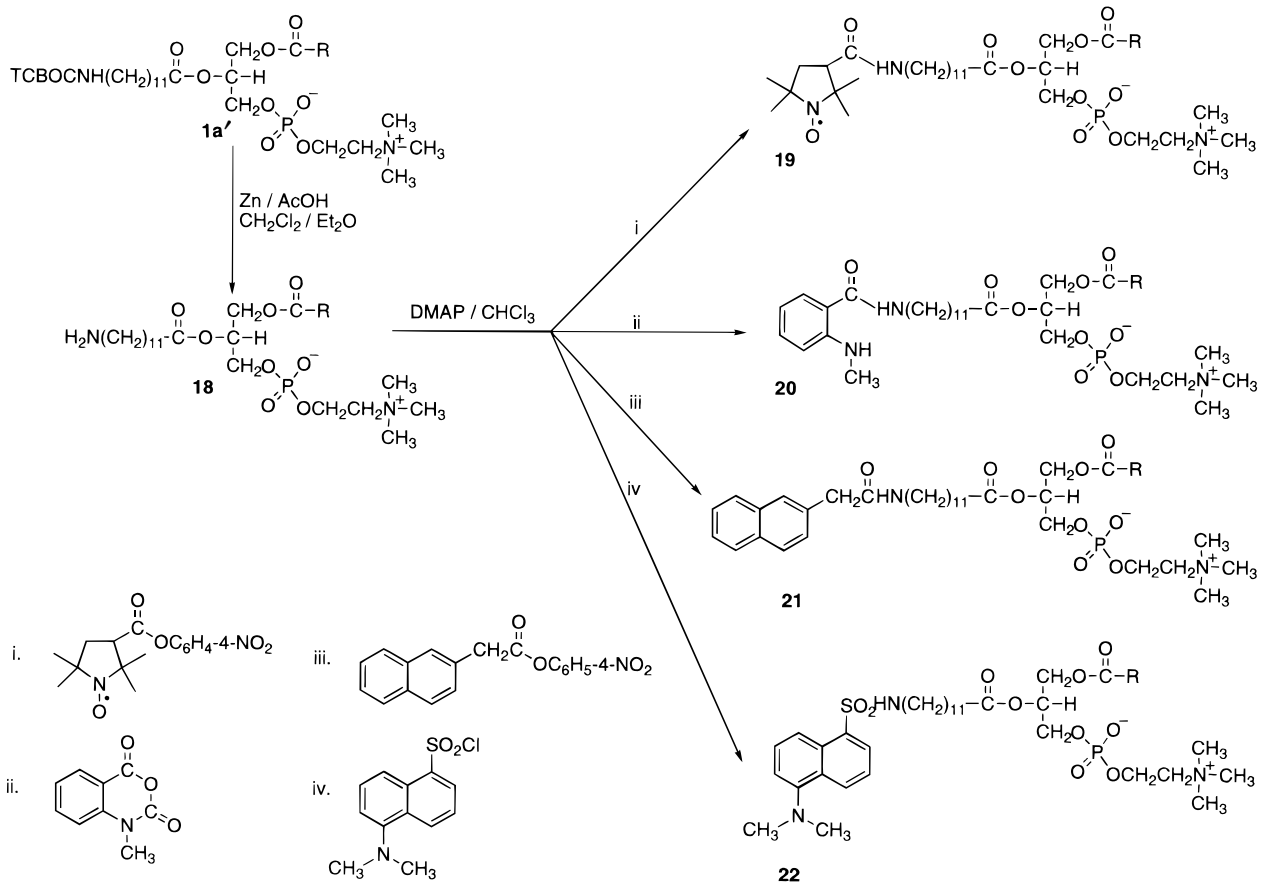
Scheme 3



Synthesis of Functionalized Phosphatidylcholines. Structurally modified phospholipid analogues incorporating spectroscopically active reporter groups have been shown to be valuable structural probes to study the organization (location and dynamics) of phospholipids in aggregates such as micelles, bilayers, and vesicles.[15,44,45] In extending our synthetic method to the preparation of such probes we focused on the synthesis of mixed-chain phosphatidylcholines with reporter-group-carrying acyl substituents. The specific target functions introduced include the solvent-sensitive fluorophore 5-(dimethylamino)-1-naphthalenesulfonyl (dansyl) group, which should be suitable for studying the local polarity at the sulfonamide-linked chain-terminal.[46] Furthermore, the same functional group (dansyl) and the *N*-methylanthraniloyl group can also function as acceptors in fluorescence energy transfer experiments[44] in conjunction with β-naphthylacetyl[47] as donor to measure proximity and orientation between the respective phospholipid side chains.[45] Synthesis of the *N*-terminal spin label derivative **19** was inspired by recent advances in electron spin resonance spectroscopy[48] indicating that measurement of the collision rates between spin-labeled phospholipids and hydrophilic vs hydrophobic paramagnetic "indicator broadeners" could be used to determine if the spin label function is to be found at the surface or alternatively oriented toward the core of the aggregate.[48] In addition, because nitroxides are also known to act as fluorescence quenchers,[45] the same spin label such as in **19** could be used in independent time-resolved fluorescence-quenching (TRFQ) experiments to study the location and dynamics of the fluorophores complementing the ESR measurements.

Our synthetic strategy is outlined in the sequence presented in Scheme 3. Specifically, to provide a suitable function for introduction of the reporter groups we have used ω-NH_2-substituted fatty acid derivatives. The chain-terminal amino group had been protected during phospholipid synthesis (cf. Scheme 1) by the 2,2,2-trichloro-*tert*-butoxycarbonyl (TCBOC) function,[25,26] which as a result of its electron-withdrawing chlorine atoms interfered less with the phosphorylation/ring-opening steps than the corresponding *t*-BOC group possessing a more nucleophilic carbonyl oxygen. Reductive deprotection of the NH_2 group following assembly of the phospholipid molecule could readily be accomplished using zinc/acetic acid/ether/methylene chloride, as shown in Scheme 3, in good yield (78%), and the product **18** was purified by silica gel and Sephadex LH-20 chromatography. Introduction of the desired chromophore/fluorophore/spin label was carried out by DMAP-catalyzed acylation/sulfonyla-

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(47) (a) Lee, Y. C.; Lee, R. T. *Acc. Chem. Res.* **1995**, *28*, 321. (b) The absorption spectrum of the dansyl chromophore overlaps with the emission spectra of β-naphthylacetyl group. The *N*-methylanthranoyl fluorophore also absorbs at the emission maximum of the β-naphthylacetyl sprctrum (see the Experimental Section).

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tion of intermediate **18**. This approach allowed incorporation of functional groups *after* the phospholipid skeleton had been assembled, with particular attention to enabling inclusion of those derivatives (e.g., spin label functions[49]) that would not survive the reaction conditions involved in the total synthesis of phospholipids shown in Scheme 1.

In conclusion, the main significance of the synthesis here presented is in providing a facile and efficient method for the preparation of a wide spectrum of diacyglycerols and phospholipids, including symmetric, mixed-chain, saturated, unsaturated, and functionalized phosphatidylcholines, phosphatidylethanolamines, and related derivatives. The strengths of the method are in its (1) simplicity and efficiency, (2) flexibility with respect to the substituent groups that can be introduced, and (3) applicability to the development of new phospholipid analogues with desired target structures for biological and physicochemical studies. Synthetic work along these lines is currently under way in our laboratory.

Experimental Section

General Methods. ^{1}H NMR spectra were recorded at 200 or 500 MHz. Methyl-α,β-isopropylidene-L-glycerate, pyridinum triphenylmethyl tetrafluoroborate, palmitic acid, 4-(dimethylamino)pyridine, 12-aminolauric acid, *N*,*N*′-dicyclohexlcarbodiimide, 2-chloro-2-oxo-1,3,2-dioxaphospholane, *p*-nitrophenol, potassium thioacetate, and *S*(+)-α-methoxy-α-trifluoromethylphenylacetyl chloride were purchased from Fluka and were used as received. Phosphorus pentoxide, calcium hydride, 2 M lithium borohydride in tetrahydrofuran, 4 N HCl in dioxane, di-*tert*-butyl dicarbonate, 2,2,2-trichloro-1,1-dimethylethyl chloroformate, 1,3-dipalmitin, 3-carboxyl-PROXYL, 2-naphthylacetic acid, and *N*-methyl isatoic anhydride were purchased from Aldrich and used as received. Oleoyl chloride was obtained from Nu-Chek Prep, Elysian, MN. Bee venom phospholipase A_2 was obtained from Boehringer Mannheim Biochemicals and dialyzed as described elsewhere,[34b] and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine was purchased from Avanti Polar-Lipids, Inc. Anhydrous trimethylamine (Kodak) was used directly as received. Triton X-100 and 1-dimethylaminonaphthalene-5-sulfonyl chloride were obtained from Sigma. Reagent grade chloroform and dichloromethane (Fisher) were freshly distilled from P_2O_5. Benzene (Sigma-Aldrich, HPLC) was dried over sodium wire and distilled from calcium hydride just prior to use. Acetonitrile (Burdick & Jackson) and triethylamine (Fluka) were dried over freshly activated Linde 3 Å molecular sieves (Aldrich). Methanol, diethyl ether, ethyl acetate, and hexane were obtained from Fisher Scientific, and diethyl ether was kept over sodium wire. Dioxane was obtained from J. T. Baker. Methanol-d_4 was purchased from Aldrich. Deuterated chloroform was obtained from Cambridge Isotope Laboratories and was dried over freshly activated Linde 3 Å molecular sieves. Regular column chromatography was carried out with silica gel 60 (70−230 mesh ASTM, E. M. Science), and flash column chromatography was carried out with silica gel 60 (230−400 mesh ASTM, E. M. Science). The silica gel was stored at 120 °C and cooled to room temperature prior to use in a desiccator over P_2O_5. Thin-layer chromatography was carried out on Whatman diamond MK6F silica gel 60 Å plates (layer thickness, 250 μm). AG 50W−X8 resin (100−200 mesh) was obtained from Bio-Rad Laboratories, and Sephadex LH-20 (25−100 μm beads) was obtained from Pharmacia. The TLC plates were visualized by iodine vapor and UV light where appropriate. The phospholipids were visualized by molybdenum spray,[50] and the primary amines were sprayed by 0.25% ninhydrin in acetone solution. Trityl compounds were visualized by concentrated hydrochloric acid solution vapor. Elemental analyses were performed by Desert Analytics, Tucson, AZ and by Galbraith Laboratories, Inc. Fast atom bombardment (FAB) mass spectra were obtained at the University of California Riverside Mass Spectrometry Facility.

L-Glyceric Acid Methyl Ester (4). To mixture of 90 mL of absolute methanol and 10 mL of 4 N HCl in dioxane was added **3** (6.00 g, 37.5) mmol), and the resulting solution was stirred for 2 h at room temperature. The solvent was removed in a vacuum, and the yellow oily product was dried over KOH in a vacuum desiccator for 3 h. The compound was then dissolved in dry acetonitrile (100 mL) and kept over activated molecular sieves overnight. The acidity of the resulting solution was checked with pH paper, which showed that it was neutral. Evaporation of the solvent gave 4.4 g (36.67 mmol, 97.8%) of **4** as a yellow liquid. It was used as soon as possible for the next reaction: ^{1}H NMR ($CDCl_3$) δ 3.80 (s, 3H), 3.90−4.40 (m, 3H), 4.60 (d, 2H).

3-Triphenylmethyl-L-glyceric Acid Methyl Ester (5). To a stirred solution of **4** (4.0 g, 35.0 mmol) in 200 mL of dry acetonitrile was added pyridinium triphenylmethyl tetrafluoroborate, and the resulting mixture was stirred at room temperature for 24 h. The solvent was then evaporated and replaced by chloroform (100 mL). The pyridinium tetrafluoroborate that precipitated was filtered and washed with chloroform, and the combined filtrate was evaporated to dryness. The residue was chromatographed in four portions on freshly activated silica gel (40 g silica gel each time) using hexane/ethyl acetate (3:1) as eluent, and the product was freeze-dried from benzene to give a combined 9.95 g (27.5 mmol, 78.6%) of **5** as a colorless product that solidified on storage at 0−4 °C: mp 73−75 °C; IR (CH_2Cl_2) 3546, 1742 cm^{-1}; ^{1}H NMR ($CDCl_3$) 3.13−3.17 (d, 1H), 3.33−3.52 (m, 2H), 3.78 (s, 3H), 4.26−4.30 (m, 1H), 7.24−7.45 (m, 15H); R_f (hexane/EtOAc 3:1) 0.27; [α_D^{25} +6.8 (*c* 1.66, $CH_3OH/CHCl_3$ 1:4).

3-Triphenylmethyl-L-glycerol (6). To a stirred solution of **5** (4.95 g, 13.7 mmol) in 100 mL of anhydrous ether at 0 °C was added 8.20 mL of 2 M lithium borohydride (16.4 mmol) in THF in one portion. The reaction mixture was stirred at 0 °C for 10 min followed by stirring at room temperature for 1 h. Excess hydride was then decomposed by cautious addition of a solution of $NaHCO_3$ (2.0 g in 50 mL of H_2O) followed by stirring for 15 min. The product was then extracted with ether (2 × 50 mL). The combined etheral solution was washed with saline (2 × 10 mL) and dried over anhydrous Na_2SO_4. The crude material obtained after evaporation of the solvent was chromatographed over freshly activated silica gel (40 g) using hexane/EtOAc (1:1) to give **6** (3.87 g, 11.6 mmol, 84.7%) as white crystals. The product was freeze-dried from benzene to give a white solid: mp 92−94 °C; IR ($CHCl_3$) 3395 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.20−2.80 (br m, 2H), 3.23−3.27 (m, 2H), 3.61−3.68 (m, 2H), 3.88 (m, 1H), 7.23−7.49 (m, 15H); R_f (1:1 hexane/EtOAc) 0.42; [α_D^{25} +5.86 (*c* 1.45, $CH_3OH/CHCl_3$ 1:4). Anal. Calcd for $C_{22}H_{22}O_3$: C, 79.02; H, 6.63. Found: C, 78.98; H, 6.67.

1-Palmitoyl-3-triphenylmethyl-*sn*-glycerol (7a). To a stirred solution of **6** (1.96 g, 5.85 mmol) in 50 mL of chloroform at 0 °C were added palmitic anhydride[51] (3.18 g, 6.44 mmol) and DMAP (0.80 g, 6.44 mmol). The reaction mixture was stirred at 0 °C for 48 h. The excess anhydride was then decomposed by addition of $NaHCO_3$ (1.5 g in 50 mL of H_2O), followed by stirring for 15 min. The product was then extracted with chloroform (2 × 50 mL), and the combined chloroform solution was washed with saline (2 × 10 mL) and dried over Na_2SO_4. The crude material obtained after evaporation of the solvent was chromotographed over freshly activated silica gel (40 g) with hexane/EtOAc (3:1) to give **7a** (2.24 g, 3.91 mmol, 66.8%) as a white solid. The product was freeze-dried from benzene as a white powder: mp 45−47 °C; IR (Nujol) 3482, 1731 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.89 (br t, 3H), 1.26 (br s, 24H), 1.50−1.60 (br m, 2H), 2.29 (t, 2H), 2.38 (br m, 1H), 3.21−3.23

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(d, 2H), 4.02 (m, 1H), 4.18−4.19 (m, 2H), 7.25−7.46 (m, 15H); FAB-MS MNa^+ calcd 595.3763, found 595.3743; R_f (hexane/EtOAc 3:1) 0.49; [α_D^{25} +4.84 (*c* 1.24, $CH_3OH/CHCl_3$ 1:4). Anal. Calcd for $C_{38}H_{52}O_4$: C, 79.68; H, 9.15. Found: C, 79.79; H, 9.18.

1-(12′-TCBOC-aminolauroyl)-3-triphenylmethyl-*sn*-glycerol (7b) was prepared in a three step sequence: *(i) 12-TCBOC-aminolauric acid*. To a white cloudy suspension 12-aminolauric acid (4.32 g, 20.0 mmol) in 40 mL of 1 N NaOH and 40 mL of dioxane was added dropwise TCBOC-chloroformate (4.80 g, 20.0 mmol) in 40 mL of dioxane, and the resulting solution was stirred at room temperature for 10 min. As TLC showed that the reaction was completed, a 1 N HCl solution was added dropwise until the resulting solution became acidic (pH ≈ 2). The resulting white suspension was stirred for 10 min, it was then vacuum filtered, and the precipitate was washed with water until the filtrate became neutral. The solid was air-dried to give 7.83 g pure product (88.7%): mp 90−91 °C; IR (Nujol) 3276, 3120, 1713, 1661 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.28 (br s, 14H), 1.40−1.65 (m, 4H), 1.95 (s, 6H), 2.31−2.40 (t, 2H), 3.05−3.10 (m, 2H), 4.80 (b m, 1H); R_f ($CHCl_3$/MeOH 5:1) 0.54. *(ii) 12-TCBOC-aminolauric anhydride*. To a solution of 12-TCBOC-aminolauric acid (8.14 g, 18.4 mmol) in 80 mL freshly distilled $CHCl_3$ was added DCC (1.90 g, 9.2 mmol), and the mixture was stirred at room temperature overnight. The precipitate was filtered, the solvent was evaporated, and the product was chromatographed over 40 g of freshly baked silica gel with EtOAc/$CHCl_3$ 1:10. Subsequent freeze-drying from benzene gave 5.82 g anhydride as a white solid (73.1%): mp 64−65 °C; IR (Nujol) 3315, 1818, 1717, 1036 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.28 (br s), 1.40−1.80 (m, 8H), 1.92 (s, 12H), 2.48−2.52 (t, 4H), 3.10−3.21 (q, 4H), 4.80 (br m, 2H); R_f (EtOAc/$CHCl_3$ 1:10) 0.82. *(iii)* To a stirred solution of **6** (1.18 g, 3.53 mmol) in 50 mL of freshly distilled chloroform were added 12-TCBOC-aminolauric anhydride prepared above (2.59 g, 3.18 mmol) and DMAP (0.52 g, 4.23 mmol) at 0 °C. The reaction mixture was stirred at 4 °C for 24 h. The excess anhydride was then decomposed by addition of $NaHCO_3$ (1.5 g in 50 mL of H_2O), followed by stirring for 15 min. The product was extracted with chloroform (2 × 50 mL), and the combined chloroform solution was washed with saline (2 × 10 mL) and dried over anhydrous Na_2SO_4. The solvent was then evaporated in vacuo, and the crude material was chromatographed on 40 g of freshly baked silica gel with hexane/EtOAc 2:1 to yield the pure product **7b** (1.67 g, 2.27 mmol, 71.4%). Freeze-drying from benzene gave a white oil: IR 1716, 1598 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.27 (br s, 14H), 1.40−1.70 (m, 4H), 1.92 (s, 6H), 2.30 (t, 2H), 2.40 (d, 1H), 3.10−3.20 (m, 2H), 3.25 (d, 2H), 4.00 (m, 1H), 4.20 (m, 2H), 4.80 (br m, 1H), 7.17−7.32 (m, 15H); [α_D^{25} +2.6 (*c* 0.990, $CHCl_3$/MeOH 4:1); R_f (hexane/EtOAc 2:1) 0.35; FAB-MS MNa^+ calcd 756.2601, found 756.2604. Anal. Calcd for $C_{39}H_{50}NO_6Cl_3$: C 63.72; H 6.85; N 1.91. Found: C 63.85; H 6.96; N 2.03.

1,2-Dipalmitoyl-3-triphenylmethyl-*sn*-glycerol (8a). To a stirred solution of **6** (2.23 g, 6.68 mmol) in 50 mL of $CHCl_3$ were added palmitoyl chloride (4.58 g, 16.7 mmol) and DMAP (2.06 g, 16.7 mmol). The resulting solution was stirred overnight at room temperature. The excess palmitoyl chloride was decomposed by addition of 1.5 g of $NaHCO_3$ in 50 mL of H_2O to the solution, and it was stirred for 15 min. Then it was extracted with 2 × 50 mL of $CHCl_3$, and the combined chloroform solution was washed with 2 × 10 mL of saline and dried over Na_2SO_4. Evaporation of the solvent followed by chromatography over freshly activated SiO_2 with $CHCl_3$ gave 4.85 g (5.99 mmol, 89.6%) of **8a** as a white solid: mp 40−41 °C; IR (Nujol) 2911, 1733 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (br t, 6H), 1.25 (br s, 48H), 1.56−1.60 (m, 4H), 2.20−2.34 (m, 4H), 3.24 (m, 2H), 4.18 (dd, 1H), 4.35 (dd, 1H), 5.25 (m, 1H), 7.24−7.35 (m, 15H); [α_D^{25} +11.58 (*c* 1.01, $CHCl_3/CH_3OH$ 4:1); R_f ($CHCl_3$) 0.88. Anal. Calcd for $C_{54}H_{82}O_5$: C, 79.95; H, 10.19. Found: C, 79.74; H, 10.33.

1,2-Dioleoyl-3-triphenylmethyl-*sn*-glycerol (8b) was prepared under the same experimental conditions as **8a** and was obtained as a colorless oil (86%): IR ($CDCl_3$) 2925, 2851, 1738 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (br t, 6H), 1.29 (br s, 40H) 1.58−1.62 (m, 4H), 2.02 (m, 8H), 3.24 (m, 2H), 4.22 (dd, 1H) 4.32 (dd, 1H), 5.25 (m, 1H), 5.31−5.34 (m, 4H,), 7.25−7.37 (m, 15H); [α_D^{25} +12.46 (*c* 1.035, $CHCl_3/CH_3OH$ 4:1); R_f ($CHCl_3/C_6H_{14}$ 1:1) 0.89. Anal. Calcd for $C_{58}H_{86}O_5$: C, 80.69; H, 10.04. Found: C, 80.60; H, 9.97.

1-Palmitoyl-2-lauroyl-triphenylmethyl-*sn*-glycerol (9a). To a stirred solution of compound **7a** (0.870 g, 1.52 mmol) in 60 mL of chloroform were added lauroyl chloride (0.99 g, 4.56 mmol) and 4-(dimethylamino)pyridine (0.63 g, 5.17 mmol). The reaction mixture was stirred at room temperature for 48 h. Excess lauroyl chloride was then decomposed by addition of $NaHCO_3$ (1.5 g in 50 mL of H_2O), followed by stirring for 15 min. The product was then extracted with $CHCl_3$ (3 × 50 mL), and the combined chloroform solution was washed with saline (3 × 10 mL) and dried over Na_2SO_4. The solvent was then evaporated, and the residue was chromatographed on freshly activated silica gel with chloroform to give 1.10 g (96%) of **9a** as a viscous oil: IR ($CHCl_3$) 2925, 2851, 1738 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (br t, 6H), 1.26 (s, 40H), 1.60 (m, 4H), 2.33 (m, 4H) 3.25 (m, 2H), 4.22 (dd 1H CH_2O), 4.33 (dd, 1H), 5.23 (m, 1H), 7.24−7.35 (m, 15H); [α_D^{25} +12.74 (*c* 1.24, $CHCl_3/CH_3OH$ 4:1); R_f ($CHCl_3$) 0.76; Anal. Calcd for $C_{50}H_{74}O_5$: C, 79.53; H, 9.88. Found: C, 79.83; H, 9.93.

1-Palmitoyl-2-(12′-TCBOC-aminolauroyl)-3-triphenylmethyl-*sn*-glycerol (9a′). To a stirred solution of **7a** (2.02 g, 3.53 mmol) in 50 mL of freshly distilled chloroform were added DMAP (0.52 g, 4.23 mmol) and *p*-nitrophenyl-12-TCBOC-aminolaurate[52] (2.28 g, 4.23 mmol). The reaction mixture was stirred for 48 h at room temperature. The excess active ester was then decomposed by addition of $NaHCO_3$ (1.5 g in 50 mL of H_2O), followed by stirring for 15 min. The product was extracted with chloroform (2 × 50 mL), and the combined chloroform solution was washed with saline (2 × 10 mL) and dried over anhydrous Na_2SO_4. The solvent was then evaporated in vacuo, and the crude material was chromatographed on 40 g of activated silica gel with hexane/EtOAc 3:1 to give the pure product **9a′** (3.08 g, 3.16 mmol, 89.5%). It was freeze-dried from benzene to give a white oil: IR 3379, 1740, 1598 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (br t, 3H), 1.20 (br s, 38H), 1.57−1.64 (m, 6H), 1.83 (s, 6H), 2.20−2.35 (m, 4H), 3.00−3.05 (m, 2H), 3.05−3.20 (m, 2H), 4.18−4.33 (m, 2H), 4.65 (br m, 1H), 5.20 (m, 1H), 7.17−7.32 (m, 15H); R_f (hexane/EtOAc 3:1) 0.55; [α_D^{25} +9.6 (*c* 0.995, $CHCl_3$/MeOH 4:1). Anal. Calcd for $C_{55}H_{80}NO_7Cl_3 \cdot 0.25\ C_6H_{14}$: C, 68.23; H, 8.41; N, 1.44. Found: C, 68.24; H, 8.72; N, 1.47. FAB-MS $C_{55}H_{80}NO_7Cl_3\ MNa^+$ calcd 994.4898, found 994.4912.

1-(12′-TCBOC-aminolauroyl-2-palmitoyl-3-triphenylmethyl-*sn*-glycerol (9b) was prepared under the same experimental conditions as **9a** and was obtained as a white oil (89.5%): IR (Nujol) 3379, 1740, 1598 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (br t, 3H), 1.20 (br s, 38H), 1.57−1.64 (m, 6H), 1.83 (s, 6H), 2.20−2.35 (m, 4H), 3.00−3.05 (m, 2H), 3.05−3.20 (m, 2H), 4.18−4.33 (m, 2H), 4.65 (br m, 1H), 5.20 (m, 1H), 7.17−7.32 (m, 15H). R_f (hexane/EtOAc 2:1) 0.68; [α_D^{25} +9.6 (*c* 1.01, $CHCl_3$/MeOH 4:1); FAB−MS MNa^+ calcd 994.4898, found 994.4875. Anal. Calcd for $C_{55}H_{80}NO_7Cl_3$: C, 67.85; H, 8.28; N, 1.44. Found: C, 67.64; H, 8.18, N, 1.48.

1-Palmitoyl-2-lauroyl-*sn*-glycerol (10a). Compound **9a** (0.40 g, 0.53 mmol) in 20 mL of chloroform−methanol (1:1) was added dropwise to a mixture of hydrochloric acid (12 N, 44 μL) in chloroform−methanol (20 mL, 1:1) at 0 °C. The resulting solution was stirred at 0 °C for 18 h. The excess acid was then neutralized by addition of $NaHCO_3$ (1.0 g in 50 mL of H_2O), followed by stirring for 15 min. The product was extracted with chloroform (3 × 50 mL), and the combined chloroform solution was washed with saline (3 × 10 mL) and dried over Na_2SO_4. The solvent was then evaporated in vacuo, and the crude material was flash chromatographed on silica gel to give 0.200 g (73%) of **10a**. The product was freeze-dried

(52) The *p*-nitrophenyl ester of 12-TCBOC-aminolauric acid was prepared in reaction between 12-TCBOC-aminolauric acid and *p*-nitrophenol using DCC as coupling agent.

from benzene to give white crystals: mp 53−55 °C. IR ($CHCl_3$) 3499, 2911, 2845, 1725, 1174, cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.89 (br t, 6H), 1.26 (s, 40H), 1.58−1.64 (m, 4H), 3.74 (m, 2H), 4.24 (dd, 1H,), 4.33 (dd, 1H), 5.10 (m, 1H); [α_D^{25} −0.30 (*c* 1.0, $CHCl_3$/CH_3OH 4:1) R_f ($CHCl_3$) 0.32. Anal. Calcd for $C_{31}H_{60}O_5 \cdot H_2O$: C, 70.14; H, 11.39. Found: C, 69.92; H, 11.44.

1-Palmitoyl-2-(12′-TCBOC-aminolauroyl-*sn*-glycerol (10a′) was prepared under the same experimental conditions as **10a** and was obtained as a white powder (69.5%): mp 49−51 °C; IR (Nujol) 3334, 1738, 1694 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.89 (br t, 6H), 1.26 (br s, 38H), 1.42−1.70 (br m, 6H), 1.93 (s, 6H), 2.33−2.35 (m, 4H), 3.10−3.20 (m, 2H), 3.74 (br d, 2H), 4.20−4.30 (m 2H), 4.80 (br m, 1H), 5.10 (m, 1H); R_f (EtOAc/$CHCl_3$ 5:100) 0.15; [α_D^{25} +0.67 (*c* 1.020, $CHCl_3$/MeOH 4:1); FAB−MS MH^+ calcd 730.3983, found 730.3947. Anal. Calcd for $C_{36}H_{66}O_7NCl_3$: C, 59.13; H, 9.10; N, 1.92. Found: C, 59.08; H, 9.19, N, 1.81.

1-(12′-TCBOC-aminolauroyl)-2-palmitoyl-*sn*-glycerol (10b) was prepared under the same experimental conditions as **10a** and was obtained as a white powder (56.3%): mp 35−36 °C; IR (Nujol) 3334, 1738, 1694 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.89 (br t, 3H), 1.26 (br s, 38H,), 1.42−1.70 (br m, 6H), 1.93 (s, 6H), 2.33−2.35 (m, 4H), 3.10−3.20 (m, 2H), 3.74 (br d, 2H), 4.20−4.30 (m, 2H), 4.80 (br m, 1H), 5.10 (m, 1H); R_f (EtOAc/$CHCl_3$ 5:100) 0.16; [α_D^{25} +0.63 (*c* 1.020, $CHCl_3$/MeOH 4:1). Anal. Calcd for $C_{36}H_{66}NO_7Cl_3$: C, 59.13; H, 9.10; N, 1.92. Found: C, 59.33; H, 9.11, N, 2.09.

1,2-Dipalmitoyl-*sn*-glycerol (10c) was prepared under the same experimental conditions as **10a** and was obtained as a white powder (74%): mp 65−67 °C, lit.[20] mp 64.5−66.5; IR (Nujol) 3501, 2912, 1733 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (t, 6H), 1.25 (br s, 48H), 1.58−1.64 (m, 4H), 2.04 (br t, 1H), 2.33−2.36 (m, 4H), 3.74 (br t, 2H), 4.23 (dd, 1H), 4.33 (dd, 1H), 5.08 (m 1H); [α_D^{25} +1.17 (*c* 0.94, $CHCl_3$/CH_2OH 4:1); R_f ($CHCl_3$) 0.28. Anal. Calcd for $C_{35}H_{68}O_5$: C, 73.89; H, 12.05. Found: C, 73.90; H, 12.33.

1,2-Dioleoyl-*sn*-glycerol (10d) was prepared under the same experimental conditions as **10a** in 73% yield: IR ($CHCl_3$) 3472, 2925, 2852, 1734 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (t, 6H), 1.26−1.30 (br s, 16H), 1.60−1.64 (br s, 4H), 2.00−2.03 (m, 8H), 2.32−2.36 (m, 4H), 3.73 (br m, 2H), 4.25 (dd, 1H), 4.32 (dd, 1H), 5.08 (m, 1H), 5.34−5.39 (m, 4H); [α_D^{25} +1.7 (*c* 1.00, $CHCl_3$/CH_3OH 4:1); R_f ($CHCl_3$) 0.33. Anal. Calcd for $C_{39}H_{72}O_5$: C, 75.43; H, 11.68. Found: C, 75.59; H, 11.60.

2-(1-Palmitoyl-2-lauroyl-*sn*-glycerol)-2-oxo-1,3,2-dioxaphospholane (11a). To a solution of **10a** (0.900 g, 1.76 mmol) in 60 mL of dry benzene was added triethylamine (0.197 g, 1.96 mmol), and the mixture was cooled to 0 °C. To this was added 2-chloro-2-oxo-1,3,2-dioxaphospholane (0.28 g, 1.96 mmol) in 40 mL of benzene in one portion. The mixture was stirred at room temperature for 16 h. The crystalline $(C_2H_5)_3N \cdot HCl$ that precipitated was filtered off, and the solvent was removed in vacuo to give **11a** as a single phosphate-positive product in the form of a colorless semisolid (1.0 g, 92%): ^1H NMR ($CDCl_3$) δ 0.86 (br t, 6H), 1.24 (s, 40H) 1.50−1.60 (m, 4H,), 2.28−2.40 (m, 4H), 4.00−4.15 (m, 2H), 4.20−4.30 (m, 2H), 4.30−4.40 (m, 4H), 5.15 (m, 1H); R_f ($CHCl_3$/CH_3OH 95:5) 0.57. This compound was used as soon as possible for the next reaction without further treatment.

1-Palmitoyl-2-lauroyl-*sn*-glycerol-3-phosphocholine (1a). Compound **11a** was transferred into a pressure bottle (1.0 g, 1.62 mmol) with dry acetonitrile (30 mL). It was cooled in a dry ice bath, and to this was added 1.5 mL of anhydrous trimethylamine. The bottle was sealed and then heated at 65 °C for 24 h. The pressure bottle was cooled in a dry ice bath, and the product that precipitated was filtered, yielding 1.0 g of phosphatidylcholine (84% from **10a**) as a white hygroscopic solid. The product was chromatographed on activated silica gel with chloroform/methanol/water (65:25:4 v/v) to give 0.60 g of pure phospholipid **1a** (55%): IR (KBr) 3411, 2912, 2848, 1725, 1247, 1055) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (br t, 6H), 1.27 (s, 40H), 1.40−1.60 (m, 4H), 2.18−2.29 (m, 4H), 3.37 (s, 9H), 3.60 (m, 2H), 3.95 (m, 2H), 4.10 (dd, 1H), 4.20−4.30 (m, 2H), 4.30−4.40 (dd, 1H), 5.26 (m, 1H); [α_D^{25} +5.10 (*c* 1.021, 1:4 CH_3OH_3/$CHCl_3$); R_f ($CHCl_3$/CH_3OH/H_2O 65:25:4) 0.32. Anal. Calcd for $C_{36}H_{72}O_5PN \cdot 2.5H_2O$: C, 59.81; H, 10.74; P, 4.28; N, 1.94. Found: C, 59.65; H, 10.77, P, 4.08; N, 1.82.

Enzymatic Hydrolysis of 1-Palmitoyl-2-lauroyl-*sn*-glycero-3-phosphocholine. To a sample of 30.0 mg (37.5 μmol) of phosphatidylcholine **1a** was added a solution of 7.5 mL of 0.05 M Tris (pH 8.5) containing 10 mM Triton X-100 and 10 mM $CaCl_2$. The mixture was vortexed thoroughly, followed by incubation of the resulting disperson at 40 °C in a water bath for 10 min. The mixture was then once again vortexed and used for the phospholipase assay directly. The reaction between phosphatidylcholine **1a** dispersed in the mixed micellar aggregates prepared and the enzyme was initiated by addition of bee venom phospholipase A_2 (5 μg in 50 μL of buffer). The reaction was run at 40 °C for 40 min using a constant-temperature water bath. The product solution was analyzed by thin-layer chromatography. The spots were visualized by iodine absorption followed by molybdic acid spray.[50] TLC analysis (chloroform/methanol/water 65:25:4) showed complete hydrolysis of phospholipid **1a** (R_f 0.38) to 1-palmitoyl-2-lysophosphatidycholine (R_f 0.17) and lauric acid (R_f 0.85), confirming the natural *sn*-3 configuration in the synthetic phosphatidylcholine. The enantiomeric *sn*-1-phosphatidylcholine, prepared from methyl-D-glycerate in the sequence of reactions shown in Scheme 1 did not react with the enzyme; it was recovered from the corresponding assay mixture unchanged. This is in line with the well-known stereospecificity of bee venom phospholipase A_2.[34]

2-(1′-Palmitoyl-2′-(12″-TCBOC-aminolauroyl)-*sn*-glycerol)-2-oxo-1,3,2-dioxaphospholane (11a′) was prepared under the same experimental conditions as **11a** and was obtained as a single phosphate-positive product in the form of a white semisolid in 92% yield: ^1H NMR ($CDCl_3$) δ 0.81 (br t, 3H), 1.19 (br s, 38H), 1.40−1.60 (m, 6H), 2.23−2.25 (m, 4H), 3.05 (t, 2H), 4.00−4.15 (m, 2H), 4.20−4.30 (m, 2H), 4.30−4.40 (m, 4H), 5.15 (m, 1H). R_f (EtOAc/hexane 1:1) 0.50. The compound was immediately used for the synthesis of **1a′**.

1-Palmitoyl-2-(12′-TCBOC-aminolauroyl)-*sn*-glycero-3-phosphocholine (1a′). Dioxaphospholane **11a′** (1.31 g, 1.54 mmol) was transferred into a pressure bottle with dry acetonitrile (45 mL). It was cooled in a dry ice bath, and to this was added 2.0 mL of anhydrous trimethylamine. The bottle was sealed, heated at 65 °C for 48 h, and then cooled to room temperature. Evaporation of the solvent followed by chromatography over activated silica gel (20 g) with MeOH/$CHCl_3$/H_2O/HOAc 65:25:4:2 yielded 0.74 g (65.1%) of phospholipid **1a′**. It was freeze-dried from benzene to give an off-white semisolid: IR (Nujol) 3379, 1726, 1721, 1692 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.81 (br t, 3H), 1.19 (br s, 38H), 1.40−1.60 (m, 6H), 1.85−1.87 (s, 6H), 2.23−2.25 (m, 4H), 3.05 (t, 2H), 3.37 (s, 9H), 3.56 (m, 2H), 3.95 (m, 2H), 4.10 (dd, 1H), 4.15−4.30 (m, 2H), 4.30−4.40 (dd, 1H), 5.15 (m, 1H); [α_D^{25} +4.04 (*c* 0.955, $CHCl_3$/MeOH 4:1); R_f (MeOH/$CHCl_3$/H_2O/HOAc 65:25:4:2) 0.20, Anal. Calcd for $C_{54}H_{78}N_2O_{10}P \cdot 2H_2O$: C, 52.81; H, 8.86; N, 3.00. Found: C, 52.58; H, 9.02; N, 2.73. FAB−MS MH^+ calcd. 895.4538, found 895.4512.

2-(1′-(12″-TCBOC-aminolauroyl)-2′-Palmitoyl-*sn*-glycero)-2-oxo-1,3,2-dioxaphospholane (11b) was prepared under the same experimental conditions as **11a** and was obtained in 95% yield as a single phosphate-positive product in the form of a white semisolid: ^1H NMR ($CDCl_3$) δ 0.81 (br t, 3H), 1.19 (br s, 38H), 1.40−1.60 (m, 6H), 1.85−1.87 (s, 6H), 2.23−2.25 (m, 4H), 3.05 (t, 1H), 4.00−4.15 (dd, 2H), 4.20−4.30 (dd, 2H), 4.30−4.40 (m, 4H), 5.15 (m, 1H); R_f (EtOAc/hexane 1:1) 0.50. This compound was used for the next step as soon as possible without further treatment.

1-(12′-TCBOC-aminolauroyl)-2-palmitoyl-*sn*-glycero-3-phosphocholine (1b). Compound **11a** was transferred into a pressure bottle with dry acetonitrile (50 mL). It was cooled in a dry ice bath, and to this was addedd 1.3 mL of anhydrous trimethylamine. The bottle was sealed and then heated at 65 °C for 48 h. The solvent was then removed through rotary evaporation to give a crude product as a white hygroscopic oil.

It was chromatographed over activated silica gel (8 g) as soon as possible with MeOH/$CHCl_3$/H_2O/HOAc 65:25:4:2 to give 0.53 g of **1b** (0.59 mmol, 56.7%). Phospholipid **1b** was freeze-dried from benzene to give a white solid: mp 47−48 °C; IR (Nujol) 3365, 1734 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.81 (br t, 3H), 1.19 (br s, 38H), 1.40−1.60 (m, 6H), 1.85−1.87 (s, 6H), 2.23−2.25 (m, 4H), 3.05 (t, 1H), 3.37 (s, 9H), 3.56 (m, 2H), 3.95 (m, 2H), 4.10 (dd, 1H), 4.15−4.30 (m, 2H), 4.30−4.40 (dd, 1H), 5.15 (m, 1H); R_f (MeOH/$CHCl_3$/$CHCl_3$/H_2O/HOAc 65:25:4:2) 0.32; [α_D^{25} +3.92 (*c* 1.02, $CHCl_3$/MeOH 4:1). Anal. Calcd for $C_{41}H_{79}N_2O_{10}PCl\cdot 6.5HOAc\cdot H_2O$: C, 49.03; H, 8.23; N, 2.12. Found: C, 48.92; H, 7.87; N, 2.49. FAB−MS MH^+($C_{41}H_{79}N_2O_{10}PCl_3$) calcd 895.4538, found 895.4534.

2-(1′,2′-Dipalmitoyl-*sn*-glycero)-2-oxo-1,3,2-dioxaphospholane (11c) was prepared under the same experimental conditions as **11a** and was obtained as a colorless semisolid in 90% yield: ^1H NMR ($CDCl_3$) δ 0.88 (br t, 6H), 1.25 (s, 48H), 1.54−1.60 (m, 4H), 2.23−2.33 (m, 4H), 4.00−4.18 (m, 2H), 4.20−4.30 (m, 2H), 4.30−4.40 (m, 4H), 5.15 (m, 1H, CH). R_f ($CHCl_3$/CH_3OH 95:5) 0.47. This compound was used as early as possible for the next reaction without further treatment.

1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (1c) was prepared from **11c** under the same experimental conditions as **1a**. The phosphatidylcholine **1c** was obtained after silica gel chromatography in 56% yield as an analytically pure product: ^1H NMR ($CDCl_3$) δ 0.88 (t, 6H), 1.26 (s, 48H), 1.56−1.63 (m, 4H,), 2.25−2.31 (m, 4H), 3.35 (s, 9H), 3.79 (m, 2H), 3.90−3.96 (m, 2H), 4.10−4.14 (dd, 1H), 4.31 (m, 2H), 4.38−4.40 (dd, 1H), 5.21 (m, 1H); R_f ($CHCl_3$/CH_3OH/H_2O 65:25:4) 0.38; [α_D^{25} +7.15 (*c* 0.47, $CHCl_3$/CH_3OH 4:1), for an authentic reference sample from Avanti Polar Lipids [α_D^{25} +7.02 (*c* 0.47, $CHCl_3$/CH_3OH 4:1).

Mosher Ester of (L)-1,2-Dipalmitoyl-*sn*-glycerol (12). To a stirred solution of (L)-1,2-dipalmitoyl-*sn*-glycerol (0.21 g, 0.37 mmol) in 10 mL of $CHCl_3$ were added *S*(+)-α-methoxy-α-trifluoromethylphenylacetyl chloride (*S*(+)-MPTA chloride, 0.11 g, 0.44 mmol) and DMAP (0.054 g, 0.44 mmol). The mixture was stirred at room temperature overnight. To the resulting solution was added 0.5 g of $NaHCO_3$ in 20 mL of H_2O. Then it was extracted with 2 × 20 mL of $CHCl_3$, washed with 2 × 10 mL of brine, and dried over Na_2SO_4. Evaporation of the solvent gave an off-white solid, which was chromatographed on 10 g of freshly activated silica gel using chloroform as eluant, yielding 0.25 g (0.32 mmol, 87%) of product **12**: ^1H NMR (500 MHz, $CDCl_3$) δ 0.87−0.89 (br t, 6H), 1.25 (br s, 48H), 1.54−1.60 (br m, 4H), 2.23−2.31 (m, 4H), 3.53 (s, 3H), 4.06−4.09 (dd, 1H, J = 11.9, 5.5 Hz, CH_3OCOCH_2), 4.26−4.29 (dd, 1H, J = 11.9, 5.0 Hz), 4.35−4.39 (dd, 1H, J = 11.8, 5.8 Hz), 4.57−4.61 (dd, H, J = 11.8, 4.0 Hz), 5.28−5.30 (m, 1H), 7.38−7.51 (m, 5H).

Mosher Ester of (D)-1,2-Dipalmitoyl-glycerol (13). To a solution of 2,3-dipalmitoyl-*sn*-glycerol, prepared in a similar fashion as the (L)-enantiomer **10c** from 2,3-isopropylidene-D-methylglycerate, (0.10 g, 0.18 mmol) in 10 mL of $CHCl_3$ were added *S*(+)-MTPA chloride (0.053 g, 0.21 mmol) and DMAP (0.026 g, 0.21 mmol). The mixture was stirred at room temperature overnight. To the resulting solution was added 0.5 g of $NaHCO_3$ in 10 mL of H_2O. Then it was extracted with 2 × 20 mL of $CHCl_3$, washed with 2 × 10 brine, and dried over Na_2SO_4. Evaporation of the solvent followed by chromatography on 10 g of freshly activated silica gel using $CHCl_3$ afforded 0.12 g (0.15 mmol, 87%) of **13** as an off-white solid: ^1H NMR (500 MHz, $CDCl_3$) δ 0.87−0.90 (br t, 6H), 1.26 (br s, 48H), 1.54−1.61 (br m, 4H), 2.24−2.32 (m, 4H), 3.53 (s, 3H), 4.11−4.14 (dd, 1H, J = 11.9, 5.5 Hz), 4.26−4.29 (dd, 1H, J = 11.9, 4.7 Hz), 4.35−4.39 (dd, 1H, J = 11.9, 5.5 Hz), 4.58−4.61 (dd, 1H, J = 11.9, 3.9 Hz), 5.30−5.32 (m, 1H), 7.39−7.52 (m, 5H).

Mosher Ester of 1,3-Dipalmitoyl-*sn*-glycerol (14). *S*(+)-MTPA chloride (0.040 g, 0.14 mmol) was added to a solution of 1,3-dipalmitoyl-*sn*-glycerol (Aldrich) (0.041 g, 0.07 mmol) in 5.0 mL of $CHCl_3$ followed by addition of DMAP (0.018 g, 0.14 mmol and stirring for 24 h. To the resulting solution was added 0.25 g of $NaHCO_3$ in 5 mL of H_2O. Then it was extracted with 2 × 10 mL of $CHCl_3$, washed with 2 × 5 mL of brine, and dried over Na_2SO_4. Evaporation of the solvent gave 0.25 g of crude product. This yellowish solid was chromatographed over 10 g of freshly baked SiO_2 with $CHCl_3$. Evaporation of the solvent gave 0.045 g of **14** (0.057 mmol, 81%) as an off-white solid: ^1H NMR (500 MHz, $CDCl_3$) δ 0.87−0.90 (br t, 6H), 1.26 (br s, 48H), 1.55−1.60 (br m, 4H), 2.21−2.31 (m, $4H_2$), 3.57 (s, 3H), 4.11−4.14 (dd, 1H, J = 12.2, 6.5 Hz), 4.17−4.21 (dd, 1H, J = 12.2, 7.0 Hz), 4.33−4.36 (dd, 1H, J = 12.2, 3.8 Hz), 4.42−4.45 (dd, 1H, J = 12.2, 3.6 Hz), 5.53−5.54 (m, 1H), 7.38−7.55 (m, 5H).

2-(1′,2′-Dioleoyl-*sn*-glycero)-2-oxo-1,3,2-dioxaphospholane (11d) was prepared under the same experimental conditions as **11a** and was obtained as a single phosphate-positive product in the form of a colorless semisolid in 93% yield: ^1H NMR ($CDCl_3$) δ 0.88 (br t, 6H), 1.27 (s, 16H), 1.58−1.62 (m, 4H), 1.98−2.02 (m, 8H), 2.28−2.32 (m, 4H), 4.12−4.21 (m, 2H), 4.26−4.32 (m, 2H), 4.37−4.47 (m, 4H), 5.15 (m, 1H); R_f ($CHCl_3$/CH_2OH 95:5) 0.58. This compound was used as soon as possible for the next reaction without further treatment.

1,2-Dioleoyl-*sn*-glycerol-3-phosphocholine (1d) was prepared under the same experimental conditions as **1a** and was obtained from **10d** as a hygroscopic white solid. Chromatography on activated silica gel with chloroform/methanol/water (65:25:4) afforded analytically pure **1d** in 64% isolated yield: IR ($CHCl_3$) 2918, 2851, 1732, 1234, 1087 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (br t, 6H), 1.28 (br s, 16H), 1.59 (m, 4H), 2.01 (m, 8H), 2.20−2.30 (m, 4H), 3.35 (s, 9H), 3.78 (m, 2H), 3.95 (m, 2H), 4.13 (dd, 1H), 4.30 (m, 2H), 4.40 (dd, 1H), 5.20 (m, 1H), 5.30−5.40 (m, 4H); R_f ($CHCl_3$/CH_3OH/H_2O 65:25:4) 0.36; [α_D^{25} +6.07 (*c* 0.56, $CHCl_3$−CH_3OH 4:1). Anal. Calcd for $C_{44}H_{84}O_8PN\cdot 2H_2O$: C, 62.39; H, 10.47; N, 1.82; P, 4.02. Found C, 62.44; H, 10.69; N, 1.71; P, 3.98.

1,2-Dipalmitoyl-*sn*-glycerol-3-phosphoethanolamine (15). A solution of phosphotriester **11c** (0.960 g, 1.42 mmol) in 25 mL of anhydrous acetonitrile was placed in a pressure bottle cooled in a dry ice bath, and to this was added 25 mL of a saturated solution of ammonia in acetonitrile. The pressure bottle was sealed and then heated in an oil bath at 65 °C for 24 h. The reaction mixture was then cooled, and the product **15** crystallized, yielding 0.96 g (87% overall from alcohol **10c**) as a white hygroscopic solid. Chromatography of the product on activated silica gel eluting with chloroform/methanol/water (65:25:4) gave analytically pure phosphatidylethanolamine 0.55 g (56%), which was freeze-dried from a suspension of 30 mL of benzene as a white solid: IR ($CHCl_3$) 3367, 2915, 2848, 1732, 1221, 1066 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (br t, 6H), 1.26 (s, 48H), 1.58−1.60 (m, 4H), 2.28−2.33 (m, 4H), 3.20 (m, 2H), 4.03 (m, 4H), 4.17 (dd, 1H), 4.38 (dd, 1H), 5.25 (m, 1H); [α_D^{25} +6.74 (*c* 0.45, 1:9 $CH_3OH/CHCl_3$); R_f ($CHCl_3$/CH_3OH/H_2O 65:25:4) 0.59. Anal. Calcd for $C_{37}H_{74}O_8PN\cdot H_2O$: C, 62.59; H, 10.50; P, 4.30; N, 1.97. Found C, 62.49; H, 10.20; P, 4.00; N, 1.85.

1,2-Dipalmitoyl-*sn*-glycero-3-phosphoethanol (16). Dioxaphospholane **11c** (0.700 g, 1.04 mmol) was transferred to a pressure bottle with dry acetonitrile (40 mL), and to this was added 1.5 mL of double-distilled water while cooling in a dry ice bath. The pressure bottle was sealed and then heated at 65 °C for 24 h. The reaction mixture was cooled, and the product **16** precipitated as a white hygroscopic solid. It was then chromatographed on activated silica gel eluting with chloroform/methanol/water (65:25:4) and freeze-dried from benzene to give analytically pure phospholipid **16** (0.45 g, 62%): IR ($CHCl_3$) 3345, 2913, 2849, 1732, 1224, 1069 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (br t, 6H), 1.25 (s, 48H), 1.58−1.60 (m, 4H), 2.26−2.32 (m, 4H), 3.70−3.74 (m, 2H), 3.93−3.98 (m, 4H), 4.18 (dd, 1H), 4.40 (dd, 1H), 5.25 (m, 1H); [α_D^{25} +6.54 (*c* 0.52, 1:4 $CH_3OH/CHCl_3$); R_f ($CHCl_3$/CH_3OH/H_2O 65:25:4) 0.30. Anal. Calcd for $C_{37}H_{73}O_9P\cdot 1.25\ H_2O$: C, 62.11; H, 10.62; P, 4.47; Found: C, 62.08; H, 10.69, P, 4.44.

1,2-Dipalmitoyl-*sn*-glycerol-3-phosphoethylthioacetate (17). To a mixture of compound **11c** (0.800 g, 1.41 mmol) and 40 mL of anhydrous acetonitrile in a pressure bottle was

added potassium thioacetate (0.200 g, 1.72 mmol). The pressure bottle was sealed and kept at 65 °C for 24 h. The product that precipitated on cooling was filtered, yielding 0.80 g (72% from alcohol **10c**) of a light yellow hygroscopic solid. This product was chromatographed on activated silica gel with chloroform/methanol/water (65:25:4) and finally freeze-dried from benzene to afford 0.50 g (45%) pure phospholipid: IR ($CHCl_3$) 2912, 2845, 1735, 1688, 1070 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (br t, 6H), 1.25 (s, 48H), 1.56−1.58 (m, 4H), 2.28−2.30 (m, 4H), 2.32 (s, 3H), 3.12 (t, 2H), 3.86−3.94 (m, 4H), 4.17 (dd, 1H), 4.40 (dd, 1H), 5.24 (m, 1H); [α_D^{25} +8.75 (*c* 0.48, 1:4 $CH_3OH/CHCl_3$); R_f ($CHCl_3/CH_3OH/H_2O$ 65:25:4) 0.58. Anal. Calcd for $C_{39}H_{74}O_9PSK\cdot H_2O$: C, 58.03; H, 9.24; P, 3.84; S 3.94. Found: C, 58.18; H, 9.55; P, 3.64; 3.99.

1-Palmitoyl-2-(12′-aminolauroyl)-*sn*-glycero-3-phosphocholine (18). To a stirred solution of **1a′** (0.800 g, 0.89 mmol) in 20 mL of freshly distilled dichloromethane, 10 mL of diethyl ether, and 1.5 mL of acetic acid in an atmosphere of nitrogen cooled to below 10 °C (ice−water bath) was added zinc (flakes, −325 mesh, 1.1 μm thick, Alfa-Aesar) in three 0.5 g portions in 20 min intervals. The mixture was stirred for 2.5 h. When TLC ($CHCl_3/CH_3OH/H_2O$ 65:25:4) showed no more changes in the progress of the reaction, the zinc was removed by centrifugation and washed first with 20 mL of methanol and then 20 mL of chlorofom. The combined solvent was evaporated, and the oily residue was freeze-dried from benzene for chromatography. The product was purified first on Sephadex LH-20 ($CHCl_3/CH_3OH$ 1:1) followed by silica gel (5 g, $CHCl_3/CH_3OH/H_2O$/AcOH 65:25:4:2) and subsequent freeze-drying from benzene gave **18** (540 mg, 78%) as a white solid. For deprotonation of the amino group the product **18** was dissolved in 100 mL of chloroform and stirred with a solution of 1.5 g of $NaHCO_3$ in 50 mL of water for 15 min. Evaporation of the solvent, followed by one more chromatography on Sephadex LH-20 and one more freeze-drying from benzene, yielded the free amine **18** (0.51 g, 73.6% yield from **1a′**) as an analytically pure phospholipid: ^1H NMR ($CDCl_3$) δ 0.88 (br t, 3H), 1.26 (br s, 38H), 1.50−1.70 (m, 6H), 2.28−2.40 (m, 4H), 2.65−2.70 (m, 2H), 3.35 (br s, 9H), 3.85 (m, 2H), 4.00 (m, 2H), 4.10−4.20 (dd, 1H), 4.30−4.40 (m, 2H), 4.41−4.50 (dd, 1H), 5.20 (m, 1H); FAB−MS MH^+ ($C_{36}H_{74}N_2O_8P$) calcd 693.5183, found 693.5156; R_f ($CHCl_3CH_3OH/H_2O$) 0.05; [α_D^{25} +5.8 (*c* 1.00, $CHCl_3$/MeOH 4:1).

1-Palmitoyl-2-[12′-(PROXYL-3″-carbamoyl)aminolauroyl]-*sn*-3-glycero-phosphocholine (19) was prepared in two steps: *(i) 3-Carboxyl-PROXYL p-nitrophenyl ester.* To a solution of 3-carboxyl-PROXYL (1.0 g, 5.36 mmol) in 40 mL of freshly distilled $CHCl_3$ was added *p*-nitrophenol (0.82 g, 5.89 mmol) followed by addition of DCC (1.22 g, 5.89 mmol). The mixture was stirred at room temperature overnight. The precipitate was then filtered, and the solvent was evaporated. Chromatography of the crude product on 40 g of freshly baked silica gel with $CHCl_3$/EtOAc 10:1 followed by freeze-drying from benzene gave 1.38 g (83.8%) of a light yellow solid: mp 68−70 °C; IR (Nujol) 3389, 1765 cm^{-1}; R_f ($CHCl_3$/EtOAc 3:100) 0.72. *(ii) To a stirred solution of* **18** (0.46 g, 0.66 mmol) in 45 mL of freshly distilled $CHCl_3$ was added DMAP (0.18 g, 1.45 mmol) followed by addition of 3-carboxyl-PROXYL *p*-nitrophenyl ester prepared above (0.45 g, 1.45 mmol). This solution was stirred overnight. The solvent was evaporated, and the residue was passed through a cation-exchange resin (Bio Rad AG 50-X8, 30 mL bed volume) eluting it with methanol/chloroform 1:1. Next the crude product was chromatographed over 8 g of freshly activated silica gel with $CHCl_3$/MeOH/H_2O 65:25:4. Evaporation of the solvent and freeze-drying from benzene gave 0.39 g (0.48 mmol, 72.7%) of a yellow solid: IR (Nujol) 3389, 1756 cm^{-1}; [α_D^{25} +5.5 (*c* 1.100, $CHCl_3$/MeOH 4:1). Anal. Calcd for $C_{48}H_{81}N_2O_9P\cdot 2.5H_2O$: C, 63.62; H, 9.57; N, 3.09. Found: C, 63.46; H, 9.61; N, 3.11. FAB−MS MH^+ calcd 861.5758, found: 861.5739; R_f ($CHCl_3$/MeOH/H_2O 65:25:4) 0.50.

1-Palmitoyl-2-(12′-*N*-methylanthraniloylaminolauroyl)-*sn*-glycero-3-phosphocholine (20). To a stirred suspension of **18** (0.510 g, 0.74 mmol) in 30 mL of freshly distilled chloroform were added *N*-methyl isatoic anhydride (0.26 g, 1.48 mmol and DMAP (0.18 g, 1.48 mmol). The progress of the reaction was monitored by TLC ($CHCl_3/CH_3OH/H_2O$/AcOH 65:25:4:2), and after 2 h an additional 0.5 equiv of anhydride and 0.5 equiv of DMAP were added. The reaction mixture was stirred for 12 h, the solvent was then evaporated, and the residue was freeze-dried from benzene. Chromatography on Sephadex LH-20 with $CHCl_3/CH_3OH$ 1:1 achieved good separation, and after one more freeze-drying from benzene analytically pure **20** (0.39 g, 63.7%) was obtained: ^1H NMR ($CDCl_3$) δ 0.84 (br t, 3H), 1.21−1.30 (br s, 38H), 1.51−1.55 (m, 6H), 2.23−2.30 (m, 4H), 2.81 (s, 3H), 3.27 (s, 9H), 3.33 (br t, 2H), 3.95−3.99 (m, 4H), 4.10−4.15 (dd, 1H), 4.20−4.25 (m, 2H), 4.35−4.40 (dd, 1H), 5.20 (m, 1H), 6.54−7.33 (m, 4H); R_f ($CHCl_3/CH_3OH/H_2O$ 65:25:4) 0.36; [α_D^{25} +6.25 (*c* 1.00, $CHCl_3/CH_3OH$ 4:1); FAB−MS MH^+ calcd 826.5710, found 826.5732. Anal. Calcd for $C_{44}H_{80}H_3O_9P\cdot H_2O$: C, 62.53; H, 9.78; N, 4.97. Found: C, 62.76; H, 9.89; N, 4.77; UV spectrum λ_1 = 258 nm, λ_2 = 340 nm; fluorescence emission was at 424 nm with excitation at 340 nm.

1-Palmitoyl-2-[12′-(2″-naphthylacetyl)aminolauroyl]-*sn*-glycero-3-phosphocholine (21) was prepared in two steps: *(i) 2-Naphthylacetic acid p-nitrophenyl ester.* To a suspension of 2-naphthylacetic acid (1.86 g, 10.0 mmol) in 50 mL of chloroform was added *p*-nitrophenol (1.53 g, 11.0 mmol) followed by addition of DCC (2.27 g, 11.0 mmol). The reaction mixture was warmed to 35−40 °C to obtain a clear solution, and then it was stirred overnight. The precipitate was then filtered, and evaporation of the solvent gave the crude product, which was chromatographed over 30 g of freshly activated silica gel with EtOAc/$CHCl_3$ 3:100. Evaporation of the solvent and freeze-drying from benzene gave 2.49 g (8.10 mmol, 81.0%) of pure product: mp 95−97 °C; IR (Nujol) 3536, 1760 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.06 (s, 2H), 7.25 (m, 2H), 7.40−7.90 (m, 6H), 8.25−8.30 (m, 2H); R_f (EtOAc/$CHCl_3$ 3:100) 0.72. *(ii)* To a stirred solution of **18** (0.40 g, 0.58 mmol) in 50 mL of freshly distilled $CHCl_3$ was added DMAP (0.086 g, 0.69 mmol) followed by addition of 2-naphthylacetic acid *p*-nitrophenyl ester (0.21 g, 0.69 mmol) to form a clear yellow solution. After overnight stirring, the solvent was removed in vacuo to give a yellow solid residue. It was first passed through a 25 mL Bio-Rad AG 50-X8 cation-exchange resin, eluting with MeOH/$CHCl_3$ 1:1. Evaporation of the solvent gave the crude product, which was then chromatographed over 10 g of freshly activated silica gel with $CHCl_3/CH_3OH/H_2O$ (65:25:4). The solvent was removed in vacuo, and the residue was passed through a Sephadex LH-20 gel filtration column (70 g), eluting with $CHCl_3/CH_3OH$ (1:1), followed by freeze-drying from benzene to obtain pure **21** (0.15 g, 30%) as a white solid: IR (Nujol) 3355, 1755 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.80 (br t, 3H), 1.10−1.25 (m, 38H), 1.40−1.50 (m, 6H), 2.28−2.30 (m, 4H), 3.10 (m, 2H), 3.30−3.50 (br s, 9H), 3.60 (m, 2H), 3.65 (s, 2H), 3.90 (m, 2H), 4.00−4.10 (dd, 1H), 4.20−4.30 (m, 2H), 4.30−4.40 (dd, 1H), 5.15 (m, 1H), 7.20−7.80 (m, 6H). Anal. Calcd for $C_{45}H_{87}N_3O_{10}\cdot 4H_2O\cdot 0.5C_6H_6$: C, 59.30; H, 10.16; N, 4.32. Found: C, 59.05; H, 9.87; N, 4.55. FAB−MS MH^+ calcd 861.6207, found 861.6184; R_f ($CHCl_3$/MeOH/H_2O 65:25:4) 0.49; [α_D^{25} +4.5 (*c* 1.020, $CHCl_3$/MeOH 4:1); UV spectrum λ_1 = 237 nm, λ_2 = 277 nm, ($CHCl_3$/MeOH 4:1); fluorescence emission maximum was at 340 nm with excitation at 280 nm ($CHCl_3$/MeOH 4:1).

1-Palmitoyl-2-[12′-(5″-dimethylaminonaphthalene-1″-sulfonyl)aminolauroyl]-*sn*-glycero-3-phosphocholine (22). To the stirred solution of **18** (0.50 g, 072 mmol) in 40 mL of freshly distilled $CHCl_3$ was added DMAP (0.11 g, 0.86 mmol) followed by addition of dansyl chloride (0.23 g, 0.86 mmol) to form a clear yellow solution. After 1 h of stirring, the solvent was removed in vacuo, and the solid residue was passed through a 40 mL Bio-Rad AG 50-X8 exchange resin with MeOH/$CHCl_3$ (1:1) to remove the catalyst. The solid obtained after evaporation of the solvent was passed through a Sephadex LH-20 gel filtration column (70 g) eluting with MeOH/$CHCl_3$ (1:1) to give the crude product, which was finally chromatographed over 7 g of freshly activated silica gel with

$CHCl_3$/MeOH/H_2O (65:25:4). The solvent was removed in vacuo, and the product was freeze-dried from benzene to give 0.27 g (0.29 mmol, 40.3%)of light green solid **22**: IR (Nujol) 3365, 1745 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.86 (br t, 3H), 1.25 (br s, 38H), 1.52−1.64 (m, 6H), 2.24−2.34 (m, 4H), 2.85−3.00 (m, 8H), 3.34 (br s, 9H), 3.78−3.82 (m, 2H), 3.95−4.05 (m, 2H), 4.15−4.20 (dd, 1H), 4.30 (m, 2H), 4.40−4.45 (dd, 1H), 5.24 (m, 1H), 7.14−8.56 (m, 6H). Anal. Calcd for $C_{48}H_{84}O_{10}N_3PS \cdot H_2O \cdot 1.5HOAc$: C, 59.22; H, 8.97; N, 4.06. Found: C, 59.07; H, 8.98; N, 4.08. FAB−MS MH^+ calcd 926.5693, found, 926.5723; R_f ($CHCl_3$/MeOH/H_2O 65:25:4) 0.47; $[\alpha]_D^{25}$ +4.5 (*c* 1.005, $CHCl_3$/MeOH 4:1); UV spectrum $\lambda_1 = 254$ nm, $\lambda_2 = 340$ nm, ($CHCl_3$/MeOH 4:1); fluorescence emission was at 498 nm with excitation maxima at 340 nm ($CHCl_3$/MeOH 4:1).

Acknowledgment. This work was supported by the National Institutes of Health, including grants GM41452, S06 GM/HD48680, and T34 GM08395. We also thank the Research and Grants Committee of California State University, Northridge for support.

JO990414E