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Synthesis of phospholipids on a glyceric acid scaffold: design and preparation of phospholipase A_2 specific substrates

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ABSTRACT

Synthesis of a new series of phospholipid analogues to serve as activity-based probes of secretory phospholipase A_2 enzymes is reported. The synthesis is based upon (1) preparation of long-chain esters and amides of glyceric acid, followed by (2) regioselective derivatization of the diol function of the molecule to achieve phosphorylation at the primary hydroxyl group, and to introduce the incipient *sn*-2-ester group of the target compounds. The sequence has been shown to allow incorporation of fluorescent, paramagnetic, and redox-active reporter groups, leading to phospholipid analogues applicable to detect and measure enzyme activity, to develop highly specific, real-time spectroscopic assay of phospholipase A_2 enzymes, as well as to track the metabolic fate of the hydrolysis products. The synthetic method has a great deal of flexibility to open the way to the design and synthesis of activity-probes for other phospholipid metabolizing enzymes as well.

1. Introduction

Development of new synthetic methods for the preparation of phospholipids is one of the key steps in advancing membrane chemistry and biochemistry today.[1] The compounds are needed for structural and dynamic studies of biomembranes and membrane-bound enzymes with particular emphasis on establishing structure–activity relationships with respect to phospholipid–phospholipid and phospholipid–protein interactions, as well as for mechanistic elucidation of phospholipid metabolizing enzymes.[1–3] Specifically, phospholipases A_2 (PLA_2s) comprise a large group of intracellular and secreted enzymes that catalyze the hydrolysis of the *sn*-2-ester bond of glycerophospholipids (Fig. 1), yielding fatty acids such as arachidonic acid, and lysophospholipids.[4] The products are precursors of signaling molecules with a broad range of biological functions.[5] For example, arachidonic acid is converted to eicosanoids that have been shown to be involved in immune response, inflammation, pain perception and sleep regulation,[6] while lysophospholipids are precursors of lipid mediators such as lysophosphatidic acid (LPA)[7] and platelet activating factor (PAF).[8] Lysophosphatidic acid has been shown to be involved in cell proliferation, survival and migration, while PAF is particularly involved in inflammatory processes.[8]

Secreted phospholipases A_2 ($sPLA_2$s) are widespread in nature.[9] To date more than 30 isozymes have been identified in mammals,

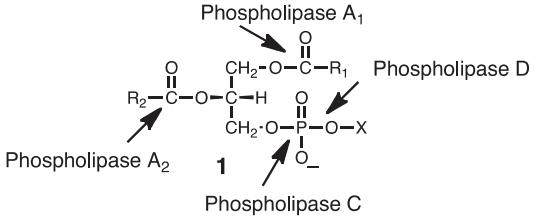

Fig. 1. Hydrolytic cleavage of naturally occurring phospholipids by the four phospholipases.

and they have been classified based on their structures, catalytic mechanisms, localizations, and evolutionary relationships.[10] Specifically, the mammalian $sPLA_2$ family includes 10 catalytically active isoforms.[9] They are low-molecular weight (14–18 kDa) secreted proteins, with a compact structure stabilized by six conserved disulfide bonds and two additional disulfides that are unique to each member.[3] Studies focusing on their mechanism of action have shown that an active site histidine and a highly conserved neighboring aspartate form the catalytic dyad involved in the reaction, with absolute dependence on Ca^{2+} for activation.[4]

The synthesis here reported focuses on preparation of phospholipase A_2 substrates including phospholipid compounds with chain-terminal reporter groups, *specifically directed at $sPLA_2$ enzymes*. The synthetic method was designed to provide access to

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compounds that will be able (1) to detect and measure phospholipase A_2 activity, (2) to develop real-time spectrophotometric assay of the enzyme, and (3) to serve as markers tracking the metabolic fate of the fatty acids and lysophospholipids released on catalytic hydrolysis by the enzyme. The underlying working hypothesis in the design of the target compounds (Fig. 2) was inspired by our earlier discovery that structural modification of the *sn*-1-position of the natural phospholipid, introducing an inverse ester function to prevent PLA_1 activity, yielded analogues that could readily be hydrolyzed at the *sn*-2-ester group by $sPLA_2$ enzymes.[11] Indeed, studies in other laboratories have also shown, that secretory PLA_2s well tolerate a range of structural modifications at the neighboring *sn*-1-substitution[12] using analogues that readily undergo catalytic hydrolysis at the *sn*-2-position by the enzyme. Thus, we have designed two principal target structures **3** and **4** as platforms for preparation of the PLA_2-directed substrates: incorporating at the *sn*-1-position glyceric acid derivatives of an ester group in **3**, and an amide group in **4**, to prevent cleavage by PLA_1, as well as by other, non-specific esterase enzymes.[13]

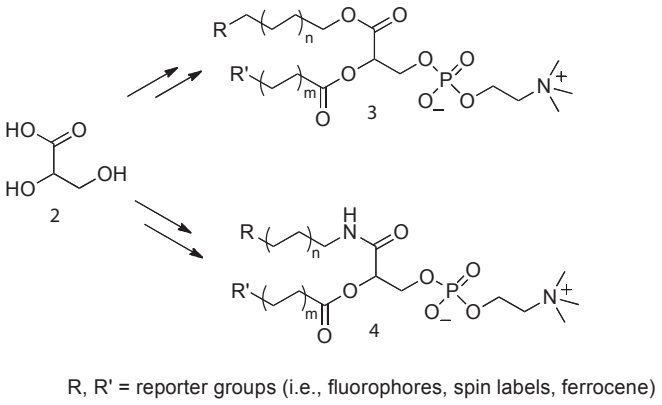


Fig. 2. Structures of the glyceric acid precursor and the phospholipid targets.

Moreover, in designing the PLA_2 targeted phospholipid probes we relied on using chain-terminal reporter groups of small size to minimize the impact on the physicochemical properties of the fatty acyl side-chains, including their effect on the packing in phospholipid bilayers and micelles. Specifically, we have shown recently, that phospholipid probes with coumarin-labeled hydrocarbon side-chains could readily be incorporated into micellar interfaces of natural phospholipids, showing near ideal mixing behavior with the unlabeled phospholipid components in micellar aggregates.[14]

2. Results and discussion

2.1. Syntheses

Our synthetic approach to the preparation of phospholipid compounds derived from glyceric acid involved three strategic components. The first phase of the synthesis, shown in Scheme 1, started with introduction of the long-chain ester or amide functions at the incipient *sn*-1-position of the target phospholipids. Since glyceric acid itself is quite insoluble in organic solvents we relied on its isopropylidene protected methyl ester **5** as the source of the required three-carbon scaffold to construct the phospholipid molecules. Thus, base-catalyzed hydrolysis of the commercially available methyl ester of 2,3-*O*-isopropylidene-L-glyceric acid **5**, followed by treatment with Dowex-H^+ ion exchange resin in aq dioxane yielded the acetonide protected glyceric acid **6** as a key intermediate for subsequent structural derivatization. Condensation of compound **6** with the respective long-chain alcohol or amine, using DCC/DMAP afforded the desired *sn*-1-substituted products **7** and **8** in good overall yields (70–80%). Specifically, we have found that the carboxylic group of glyceric acid is quite reactive, in that the corresponding amide **8** could be prepared from the acid directly, without having to synthesize an active ester, most likely due to the rapid formation of the corresponding anhydride intermediate.

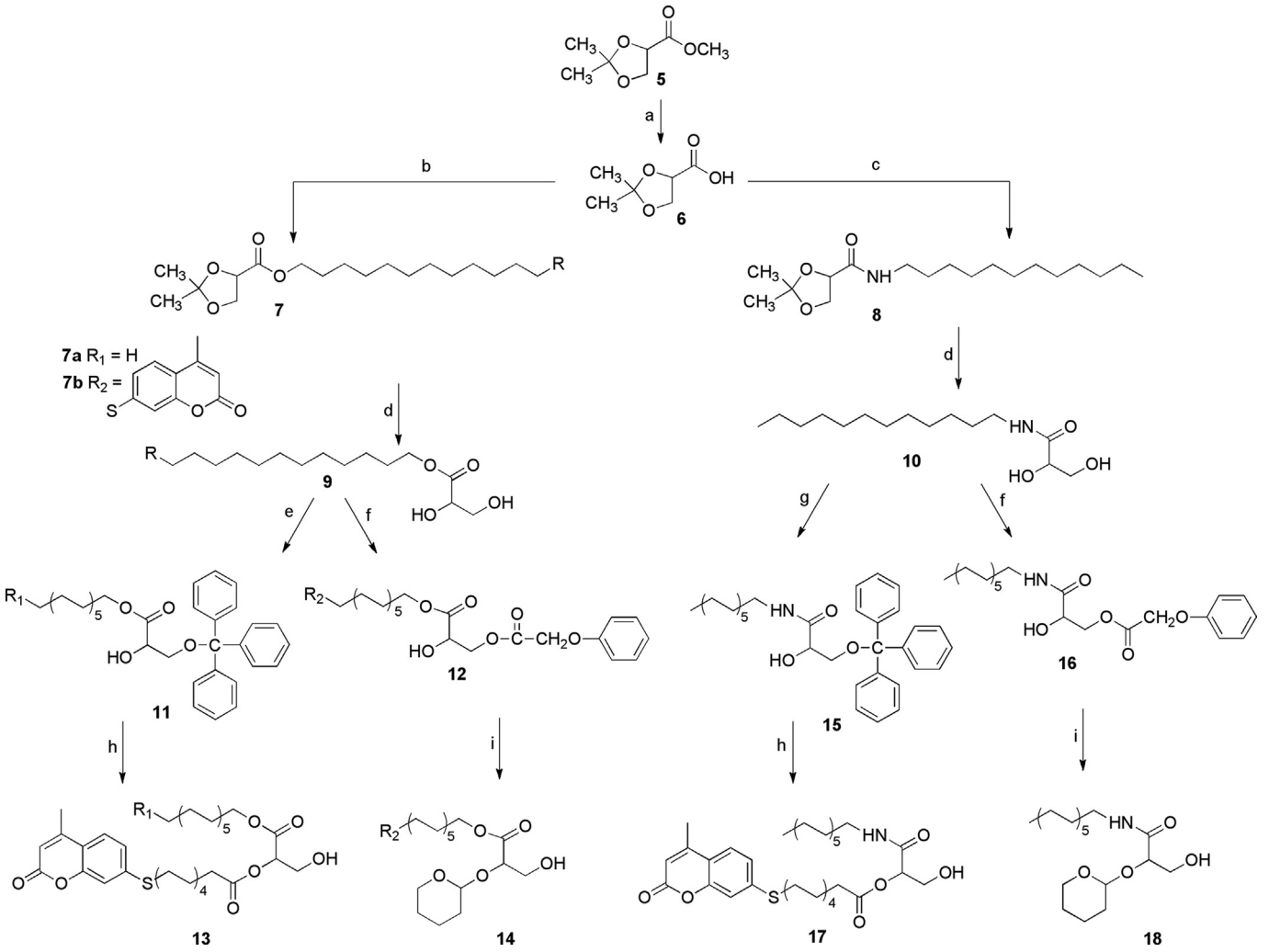
Acid-catalyzed hydrolytic cleavage of the isopropylidene protecting group in the series **7** and **8** was next accomplished using 0.4 M hydrochloric acid solution in aq dioxane. In order to achieve efficient isopropylidene cleavage, it turned out to be quite important to control the acidity of the solution as higher acidity led to partial decomposition, while at lower acid concentrations incomplete hydrolysis occurred. Consequently, freeze-drying the solution, rather than evaporating the solvent was used to isolate the product. Subsequent silica gel chromatography afforded the deprotected diols **9** and **10** in high purity and in good yields (78–91%).

We used a two-prong approach to the regioselective manipulation of the *sn*-2 and *sn*-3 hydroxyl groups of compounds **9** and **10** for preparation of the functionalized/substituted *sn*-3-carbinols (Scheme 1). One method relied on a sequence of reactions that included (1) tritylation of the primary hydroxyl group, (2) acylation of the secondary alcohol function to introduce the *sn*-2-ester group, and (3) acid-catalyzed cleavage of the trityl group to obtain the desired *sn*-3-alcohol for the subsequent phosphorylation step. Along these lines, reaction of the dodecyl ester of glyceric acid **9** with triphenylmethyl chloride, in the presence of collidine, in dichloromethane yielded the corresponding trityl ether **11** (80%), which was subsequently acylated with coumarin-labeled decanoic acid using DCC/DMAP (72%), and finally detritylated to produce the *sn*-3-carbinol **13** (61%). In a similar series of reactions dodecylamide of glyceric acid **10** was first converted to **15** by tritylation of the primary hydroxyl group (72%), followed by acylation at the *sn*-2-position (95%) and detritylation to obtain the substituted glycerol **17** in 79% yield.

This three-step sequence readily produced the desired glyceric acid derivatives as the precursors needed for phosphorylation, however, application of the method required development of rigorous experimental conditions, specific for each substrate, in the last step of the sequence, in the course of the detritylation reaction. Specifically, as it has recently been well demonstrated, the experimental conditions needed to prevent acid-catalyzed acyl migration to a neighboring hydroxyl group is very much dependent on the structure of the acyl group involved.[15] Along these lines it has been shown that even minor structural variations (such as the chain-length of the acyl group) make a significant difference in terms of the specific experimental conditions that need to be employed to prevent acyl migration.[15b]

Consequently, in order to develop a more robust method for regioselective manipulation of the diol portion of the molecule, we turned to an alternative strategy relying on orthogonal protection at the *sn*-2- and *sn*-3-positions. To that effect, we used the base-labile phenoxyacetyl group for protection of the primary alcohol function,[16] and the acid-labile tetrahydropyranyl function to protect the secondary hydroxyl group. First the phenoxyacetyl group was introduced in reaction of compounds **9** and **10** with phenoxyacetyl chloride in chloroform at 0°C, using 2,4,6-collidine as catalyst.[16] The *sn*-3-phenoxyacetyl compounds **12** and **16** were obtained in 68% yield.[17] The regioselectivity of the reaction could readily be confirmed by the observation that both *sn*-3-phenoxyacetyl esters showed base-line ^1H NMR absorption in the δ 5.00–5.10 range, which is consistent with the presence of the free *sn*-2-hydroxyl group in the products.[15]

In the next step, tetrahydropyranylation of the hydroxyl group at the *sn*-2-position was carried out using excess of 3,4-dihydro-2*H*-



Scheme 1. Reagents and conditions: (a) 1 M KOH/MeOH; (b) ROH, DCC/DMAP, CH_2Cl_2; (c) $CH_3(CH_2)_{11}NH_2$, DCC/DMAP, CH_2Cl_2; (d) HCl, aq dioxane; (e) $(C_6H_5)_3CCl$, 2,4,6-trimethylpyridine, CH_2Cl_2; (f) $C_6H_5OCH_2COCl$, 2,4,6-trimethylpyridine, $CHCl_3$; (g) $(C_6H_5)_3CCl$, pyridine, $CHCl_3$; (h) (i) 10-(7′-mercapto-4′-methylcoumarin)decanol, DCC/DMAP, $CHCl_3$, (ii) HCl, aq dioxane); (i) (I) DHP-PPTS, CH_2Cl_2, (II) *tert*.-butylamine, ($CHCl_3$/MeOH 1:1).

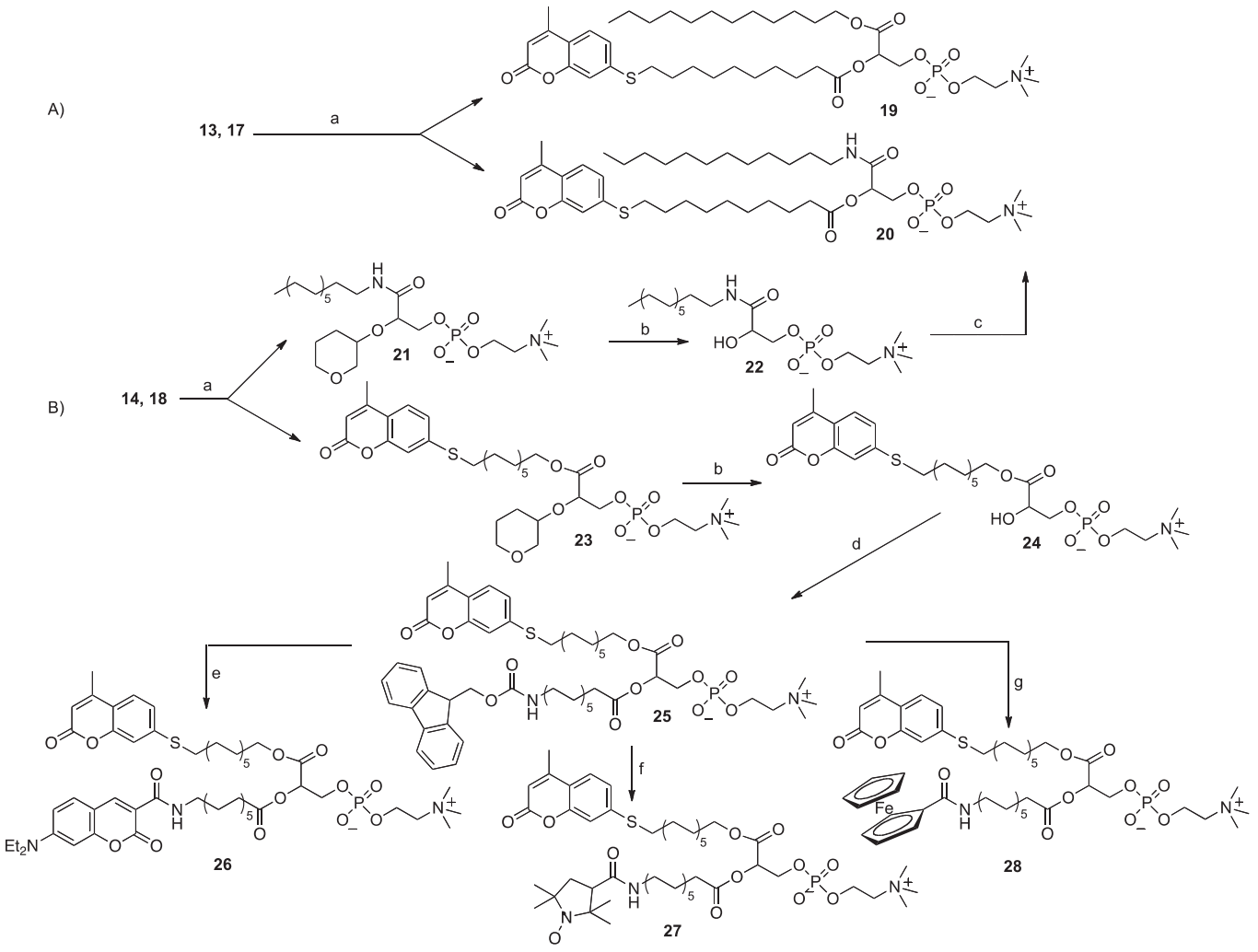
pyran with PPTS as catalyst in dichloromethane. Subsequent chemoselective base-hydrolysis of the phenoxyacetyl ester with *tert* butylamine in a mixture of methanol/chloroform afforded the *sn*-3-carbinols **14** and **18** in 91–97% overall yield. Although introduction of the tetrahydropyranyl group created a second chiral center in the molecule, it did not complicate the isolation and purification of the products.[18] It should be pointed out that one significant advantage of the phenoxyacetyl/tetrahydropyranyl strategy is that *it completely circumvents the problem of acyl migration in the sequence.*

The second phase of the synthesis, shown in Scheme 2, focused on elaboration of the *sn*-3-phosphocholine headgroup of the target compounds. To achieve efficient phosphorylation of the substituted glyceric acid derivatives we used 2-chloro-2-oxo-1,3,2-dioxaphospholane, as this reagent has been shown to produce phosphocholine derivatives in good yields and with few byproducts, even in reactions with substrates that carry nucleophilic amide–carbonyl groups that are widely regarded as rather difficult substrates to phosphorylate.[19] Thus, reaction between ethylene chlorophosphate and the substituted glyceric acid series of **13** and/or **17** in benzene, in the presence of triethylamine as catalyst, followed by nucleophilic ring-opening of the cyclic phosphodiester intermediates with trimethylamine in anhydrous acetonitrile produced the corresponding phosphocholine compounds (Scheme 2).

The phospholipid products **19** and **20** were purified by silica gel chromatography, and isolated in 48–54% overall yield.[20] Similarly, phosphorylation of the *sn*-2-tetrahydropyranyl protected glyceric acid derivatives **14** and **18** afforded the corresponding phosphorylcholines **21** and **23** in somewhat higher overall yields (67–74%).

The third and final phase of the synthesis focused on developing a method to replace the *sn*-2-tetrahydropyranyl protecting group in compounds **21** and **23** with the series of *sn*-2-ester groups of the target phospholipids. The sequence shown in Scheme 2 proceeded via formation of lysophospholipid intermediates **22** and **24** that were prepared by acid-catalyzed cleavage of the *sn*-2-tetrahydropyranyl group of the phosphorylated compounds. Thus, phospholipid **21** treated with dilute hydrochloric acid in aq dioxane yielded lysophospholipid **22** (94%), and deprotection of **23** was accomplished using a biphasic system comprised of aq HCl and chloroform that produced the corresponding lysophospholipid **24** isolated by silica gel chromatography in 57% yield.

With compounds **22** and **24** in hand, we proceeded to introduce a series of *sn*-2-fatty acyl groups for preparation of mixed-chain double labeled phospholipid compounds, including those that would carry chain-terminal reporter groups. We have used two methods for introduction of the *sn*-2-substituents: (1) acylation of the lysophospholipid analogues with fatty acyl groups already



Scheme 2. Reagents and conditions: (a) (i) ethylene chlorophosphate, Et_3N, benzene, (ii) $(CH_3)_3N$, MeCN, 65 °C; (b) HCl, aq dioxane; (c) 10-(7′-mercapto-4′-methylcoumarin) decanoic acid/DCC/DMAP, $CHCl_3$; (d) 12-(FMOC)-aminododecanoic acid/DCC/DMAP, $CHCl_3$; (e) (i) DBU, $CHCl_3$, (ii) *p*-nitrophenyl-7-mercapto-4-methylcoumarin-3-carboxylate, DMAP, $CHCl_3$; (f) (i) as in (e), then (ii) *p*-nitrophenyl-PROXYL-3-carboxylate, DMAP, $CHCl_3$; (g) (i) as in (e), then ferrocenecarbonyl fluoride, DMAP, $CHCl_3$.

labeled at their chain-end, and (2) incorporating fatty acyl groups with a chain-terminal protecting group, to elaborate the reporter group after the acylation step. Thus, reaction between lysophospholipid **22** and 7-mercapto-4-methylcoumarin-labeled decanoic acid, using DCC/DMAP in chloroform, with added glass beads and sonication to increase the glass surface in the reaction vessel,[21] yielded the phospholipid product **20** (62%), with an *sn*-2-acyl group carrying the chain-terminal fluorophore.

In an alternative sequence, lysophospholipid **24** was first acylated in reaction with FMOC-protected 12-aminododecanoic acid using DCC/DMAP in chloroform, producing phospholipid **25** that was isolated by silica gel chromatography in 60% yield. Replacement of the FMOC protecting group with the desired chain-terminal reporter group was carried out in a two-step/one-pot sequence. Along these lines, base-catalyzed elimination of the fluorenylmethoxycarbonyl protecting group with excess of DBU in chloroform, followed by addition of the *p*-nitrophenyl active ester of 7-diethylaminocoumarin-3-carboxylate and DMAP yielded the desired *sn*-2-fatty acyl ester with the fluorescent chain-terminal **26**. The product was purified by silica gel chromatography and isolated in 66% overall yield. The phospholipid compounds carrying the *sn*-2-chain-terminal paramagnetic spin label **27** (52%), and the redox-active ferrocene group **28** (58%), were obtained under similar experimental conditions.

Specifically, compound **26** with a fluorescent donor–acceptor pair, and compound **27** with fluorophore-quencher reporter groups were prepared to detect and measure phospholipase A_2 activity following the changes in fluorescence resonance energy transfer (FRET), and fluorescence de-quenching on hydrolysis by the enzyme. In case of compound **26** the fluorescence emission peak of the donor, (7-mercapto-4-methylcoumarin), at 390 nm shows substantial overlap with the excitation spectrum of the acceptor (7-diethylaminocoumarin) at 405 nm, whose emission is observed at 462 nm. Thus, PLA_2 catalyzed cleavage results in increase of the fluorescence by the donor, and in loss of FRET (i.e., emission of the acceptor) that can be used to follow the hydrolysis by the enzyme. Along the same line, hydrolysis of the *sn*-2-ester bond in **27** causes an increase in fluorescence at 390 nm via de-quenching due to the departure of the paramagnetically labeled fatty acid quencher[22] from the substrate. Finally, we have applied the synthetic method to prepare compound **28** incorporating a chain-terminal ferrocene reporter group that was recently introduced as a redox-active label of phospholipids.[23] Thus, the sequence here described provides a method that should be widely applicable to the synthesis of phospholipid compounds including acyl groups with chain-terminal spectroscopic labels that could be introduced later in the synthesis such as after the phosphorylation step, either because they would not survive the phosphorylation conditions or are not

readily available in the amounts required to carry out multi-step syntheses.

2.2. Enzymatic hydrolysis

Catalytic hydrolysis of the synthetic phospholipid substrates **19**, **20**, **26**–**28** was carried with bee-venom phospholipase A_2, a widely used, readily available representative of the low-molecular weight secretory PLA_2 enzymes.[24] In an assay mixture containing Triton X-100/phospholipid mixed micelles,[25] in the presence of catalytically essential Ca^{2+}, each one of the phospholipid compounds was completely hydrolyzed by the enzyme, producing the lysophospholipid analogues, and the corresponding fatty acids **29**–**32** labeled with chain-terminal reporter groups, shown in Fig. 3

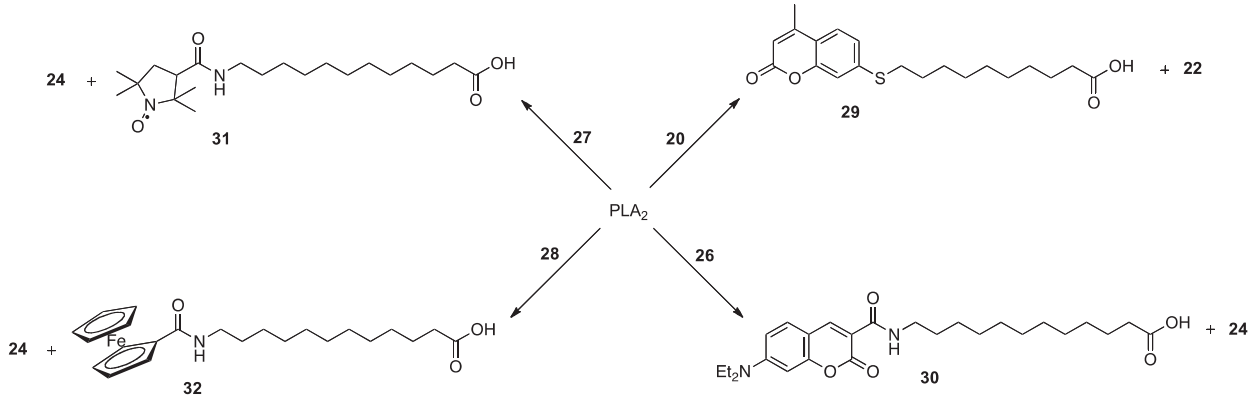


Fig. 3. Hydrolysis of the synthetic probes by PLA_2.

The rates of enzymatic hydrolysis of the coumarin-labeled compounds were well within the same order of magnitude as the naturally occurring phospholipids. Specifically, preliminary results showed that compound **26** was cleaved by bee-venom PLA_2 only 2.3 times slower compared to the hydrolysis of dipalmitoyl phosphatidylcholine by the same enzyme under similar conditions. These results indicate that introduction of chain-terminal coumarin labels maintain good substrate quality, in good agreement with what we observed with coumarin-labeled fluorescent glycerophospholipids.[14]

3. Conclusions

The main significance of the synthesis here reported is in providing a facile and efficient method for preparation of a new class of phospholipid compounds, including functionalized phospholipid analogues with fluorescent, paramagnetic, and redox-active reporter groups. 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 the desired target structures for biological and physicochemical studies. Availability of the new probes opens the way for future in vivo experiments for detection and measurement of phospholipase A_2 activity, and for the design and development of highly specific activity-based probes for related studies of phospholipid hydrolyzing enzymes. Work toward these goals is under way in our laboratory.

4. Experimental section

4.1. General methods

Proton NMR spectra were recorded at 200 MHz, carbon NMR spectra were recorded at 50 MHz; both are reported in δ units relative to TMS. IR spectra were measured on a Fourier Transform infrared spectrometer in chloroform solution. Optical rotations were measured using sodium light (D line at 589 nm) on a Perkin Elmer 341 polarimeter. High resolution mass spectra for determination of exact mass of the new compounds were determined at the Mass Spectrometry Facility at University of California, Riverside. Elemental analyses were determined by Desert Analytics, Tucson AZ, and Galbraith Laboratories Knoxville, TN. Column chromatography was carried out using silica gel 60 (230–400 mesh, ASTM, E.M. Science). Reactions were monitored by thin layer chromatography using MK6F silica gel 60 plates. The compounds were visualized on the TLC plates by iodine vapor and UV light, where appropriate. Phospholipids were visualized by molybdenum spray[26] and the primary amines were sprayed with 0.25% ninhydrin in acetone solution, followed by heating the plates at 120 °C. Ion exchange resin AG 50W-X8 (100–200 mesh) was obtained from Bio-Rad Laboratories. Commercially available reagents used were reagent grade or better and used as obtained. Dichloromethane and chloroform were freshly distilled from P_2O_5; benzene was kept over sodium wire and distilled from CaH_2 before use. Acetonitrile (spectrograde, Burdick & Jackson) was dried over activated molecular sieves (3 Å). Bee-venom phospholipase A_2 was obtained from Sigma, it was dialyzed against 0.05 M phosphate buffer, pH 8.00, and stored at 4 °C.

4.2. General procedures for preparation of 1,2-disubstituted glyceric acid derivatives

*4.2.1. Method A: (1) preparation of glyceric esters **9** and amides **10***

*4.2.1.1. Dodecyl-2,3-dihydroxypropanoate (**9a**). 4.2.1.1.1. 2,2-Dimethyl-1,3-dioxolane carboxylic acid (**6**).* To a solution of methyl-2,2-dimethyl-1,3-dioxalane-4-carboxylate **5** (2.057 g, 12.8 mmol) in 5 mL MeOH, kept in an ice-water bath was added drop-wise 15 mL 1 M potassium hydroxide in methanol. After addition of KOH, the ice-bath was removed and the mixture was stirred at room temperature for 45 min. To this solution were added 15 mL of MeOH and 30 mL of Dowex-H^+ ion exchange resin, and then the mixture was stirred for 3 min. The mixture was then filtered and the resin was washed with MeOH (40 mL). The solvent was evaporated, the residue was dispersed in benzene and freeze-dried to give compound **6** (1.695 g, 90.5%) as colorless oil. IR (neat): 3180br m, 1736vs, 1220s, 1104s, 1069s, 837m cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.32 (s, 3H), 1.42 (s, 3H), 4.04–4.12 (dd, 1H, J=8.7, 5.1 Hz), 4.16–4.24 (dd, 1H, J=7.3, 8.7 Hz), 4.51–4.57 (dd, 1H, J=5.1, 7.3 Hz), 9.45 (s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 25.2, 25.7, 67.1, 73.5, 111.7, 175.8. $[\alpha]_D^{20}$ −25.2 (*c* 1.1, $CHCl_3$), known compound.

*4.2.1.1.2. Dodecyl-2,2-dimethyl-1,3-dioxolane-4-carboxylate (**7a**).* To a solution of compound **6** (1.6213 g, 11.1 mmol) in 35 mL

of $CHCl_3$ were added 12-dodecanol (2.2920 g, 12.3 mmol), DMAP (1.5027 g, 12.3 mmol), and DCC (2.5379 g, 12.3 mmol) and the mixture was stirred for 7 h. The DCC–urea that formed was filtered off, and the solvent was evaporated. The residue was dissolved in CH_2Cl_2 and purified on a silica gel column packed and eluted with CH_2Cl_2. The fractions containing the product were combined, evaporated, dissolved in benzene, and freeze-dried to give **7a** (2.8202 g, 76.4%) as colorless wax. IR ($CHCl_3$): 1740br cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 0.86 (br t, 3H), 1.24 (br s, 18H), 1.39 (s, 3H), 1.48 (s, 3H), 1.64 (m, 2H), 4.18 (m, 4H), 4.56 (t, 1H, J=6.2 Hz). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 14.2, 22.8, 25.7, 25.9, 26.0, 28.7, 29.3, 29.5, 29.6, 29.7, 29.7, 32.0, 65.6, 67.5, 74.3, 111.4, 171.4. R_f (CH_2Cl_2) 0.69. $[\alpha]_D^{20}$ −10.4 (*c* 1.02, $CHCl_3$/MeOH 4:1), known compound.

*4.2.1.1.3. 12-[(4′-Methyl-2′-oxo-2H′-chromen-7′-yl)thio]dodecyl-2,2-dimethyl-1,3-dioxolane-4-carboxylate (**7b**).* To a solution of **6** (0.400 g, 2.7 mmol) in 25 mL $CHCl_3$ were added 7-[(12-hydroxydodecyl)thio]-4-methyl-2*H*-chromen-2-one (0.8010 g, 2.1 mmol), DMAP (0.2598 g, 2.1 mmol), and DCC (0.5614 g, 2.1 mmol). The mixture was stirred for 6 h, then the DCC–urea that formed was filtered and the solvent was evaporated. The residue was re-dissolved in $CHCl_3$ and purified on a silica gel column packed with $CHCl_3$ and eluted with $CHCl_3$/EtOAc (20:1). The fractions containing the product were combined, evaporated, dissolved in benzene, and freeze-dried to give **7b** (0.9342 g, 88.2%) as a white solid. IR ($CHCl_3$): 3257, 1732br, 1621, 1246 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.17 (br s, 16H), 1.30 (s, 3H), 1.39 (s, 3H), 1.56 (m, 4H), 2.28 (s, 3H), 2.87 (t, 2H, J=7.2 Hz), 4.05 (m, 4H), 4.48 (t, 1H, J=5.4 Hz), 6.06 (s, 1H), 7.03 (m, 2H), 7.33 (d, 1H, J=8.1 Hz). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 18.2, 25.2, 25.4, 25.5, 28.2, 28.3, 28.6, 28.8, 29.1, 31.7, 65.1, 67.0, 73.8, 110.9, 113.2, 116.5, 122.4, 124.2, 143.5, 151.9, 153.5, 160.1, 171.0. R_f ($CHCl_3$/EtOAc 20:1) 0.67. $[\alpha]_D^{20}$ −9.7 (*c* 0.99, $CHCl_3$), known compound.

*4.2.1.1.4. Compound (**9a**).* To a solution of **7a** (1.7502 g, 5.26 mmol) in 25 mL of 1,4-dioxane was added a solution of HCl (1.6 mL of 12 M aq HCl diluted with 23.5 mL 1,4-dioxane) at room temperature. The reaction was stopped after 2 h. To this mixture was added 30 mL benzene and then freeze-dried to give a white residue. The solid was re-dissolved in $CHCl_3$ and purified on a silica gel column loaded with $CHCl_3$ and eluted with $CHCl_3$/EtOAc (2:1). The fractions corresponding to the product were combined and evaporated, dissolved in benzene, and freeze-dried to give **9a** (1.3134 g, 91%) as white solid. IR ($CHCl_3$): 3340, 1742br cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) 0.86 (br t, 3H), 1.24 (br s, 18H), 1.57 (m, 2H), 3.84 (m, 2H), 4.16 (t, 2H, J=6.8 Hz), 4.25 (m, 1H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 14.2, 22.8, 25.8, 29.1, 29.3, 29.5, 29.7, 29.7, 29.7, 32.1, 65.8, 67.6, 74.5, 173.0. R_f ($CHCl_3$/EtOAc 1:1) 0.36. $[\alpha]_D^{20}$ −8.4 (*c* 1.02, $CHCl_3$/MeOH 4:1), known compound.

*4.2.1.2. 12-[(4-Methyl-2-oxo-2H-chromen-7-yl)thio]dodecyl-2,3-dihydroxypropanoate (**9b**).* To a solution of **7b** (0.9304g, 1.84 mmol) in 25 mL 1,4-dioxane was added a solution of HCl (1.6 mL of 12 M aq HCl diluted with 23.5 mL 1,4-dioxane) at room temperature. The reaction was stopped after 2 h. To this mixture 30 mL of benzene were added and then freeze-dried to give a white residue. The solid was dissolved in $CHCl_3$ and purified on a silica gel column loaded with $CHCl_3$ and eluted with $CHCl_3$/EtOAc (5:1). The fractions corresponding to the product were combined and evaporated, dissolved in benzene and freeze-dried to give **9b** (0.6612 g, 78%) as white solid. IR ($CHCl_3$): 3330 br, 1740 br, 1614, 1215 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.24 (br s, 16H), 1.62 (m, 4H), 2.36 (s, 3H), 2.94 (t, 2H, J=7.2 Hz), 3.20 (br s, 2H), 3.84 (m, 2H), 4.16 (t, 2H, J=6.8 Hz), 4.24 (m, 1H), 6.16 (s, 1H), 7.08–7.12 (m, 2H), 7.41 (d, 1H, J=8.8 Hz). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 18.4, 25.6, 28.3, 28.5, 28.7, 29.0, 30.0, 29.2, 29.3, 32.0, 64.0, 65.9, 71.6, 113.4, 113.6, 116.7, 122.7, 124.4, 143.7, 152.3, 153.7, 160.7, 172.9. R_f ($CHCl_3$/EtOAc 1:1) 0.23. Anal. Calcd for $C_{25}H_{36}O_6S$: C, 64.63; H, 7.81. Found: C, 64.84; H, 7.50. MS MH^+ $C_{25}H_{36}O_6SH$ calcd: 465.2310, found: 465.2307. $[\alpha]_D^{20}$ −14.2 (*c* 0.96, $CHCl_3$).

*4.2.1.3. N-Dodecyl-2,3-dihydroxypropanamide (**10**). 4.2.1.3.1. N-Dodecyl-2,2-dimethyl-1,3-dioxolane-4-carboxamide (**8**).* To a solution of **6** (1.5009 g, 0.0103 mmol) in 40 mL of $CHCl_3$ were added 12-dodecylamine (2.2909 g, 12.4 mmol), DMAP (1.3812 g, 11.3 mmol), and DCC (2.3315 g, 11.3 mmol) and the mixture was stirred at room temperature for 16 h. The DCC–urea that formed was filtered and the solvent was evaporated. The residue was re-dissolved in $CHCl_3$ and purified on a silica gel column packed with $CHCl_3$ and eluted with $CHCl_3$/EtOAc (10:1). The fractions containing the product were combined, evaporated, re-dissolved in benzene, and freeze-dried to give **8** (2.9162 g, 90.3%) as colorless oil that solidified in the freezer. IR ($CHCl_3$): 3345, 1680br cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 0.83 (br t, 3H), 1.21 (br s, 18H), 1.35 (s, 3H), 1.42 (s, 3H), 1.69 (m, 2H), 3.24 (m, 2H), 4.04 (m, 1H), 4.23 (br t, 1H), 4.43 (br t, 1H), 6.58 (m, 1H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 14.0, 22.5, 24.5, 24.9, 26.0, 26.7, 29.1, 29.2, 29.4, 29.5, 30.6, 31.8, 38.8, 67.6, 74.9, 110.9, 170.9. R_f ($CHCl_3$/EtOAc 5:1) 0.80. $[\alpha]_D^{20}$ −12.4 (*c* 1.03, $CHCl_3$), known compound.[11]

*4.2.2. Compound (**10**).* To a solution of **8** (2.8241 g, 9 mmol) in 25 mL of 1,4-dioxane was added a solution of HCl (1.6 mL of 12 M aq HCl diluted in 23.5 mL of 1,4-dioxane) at room temperature. After 2.5 h, the mixture became cloudy and formed a white precipitate. To this mixture was added 40 mL benzene, the precipitate was filtered, and washed with benzene. The solid was then dispersed in benzene and freeze-dried to give **8** (1.5584 g, 5.7 mmol, 63%) as white powder. The filtrate was collected, freeze-dried, dissolved in $CHCl_3$/EtOAc (1:1), and purified on a silica gel column loaded and eluted with $CHCl_3$/EtOAc (1:1). The fractions corresponding to the product were combined, evaporated, and dispersed in benzene, and freeze-dried to give an additional crop of **10** (0.6398 g, 26%) to a combined yield of 89%. IR ($CHCl_3$): 3380br, 1683br cm^{-1}; ^1H NMR ($CDCl_3$+CD_3OD, 200 MHz) δ 0.82 (br t, 3H), 1.20 (br s, 18H), 1.45 (m, 2H), 3.19 (t, 2H, J=7.32 Hz), 3.37 (br s, 2H) 3.70 (m, 2H), 4.01 (br t, 1H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 14.0, 22.5, 26.2, 27.0, 29.1, 29.2, 29.4, 29.5, 30.6, 31.8, 38.8, 67.9, 75.0, 170.7. R_f ($CHCl_3$/EtOAc 1:1) 0.21. Anal. Calcd for $C_{15}H_{31}NO_3 \cdot 1/3CHCl_3$: C, 58.80; H, 10.08; N, 0.47, Found: C, 58.83; H, 9.77; N, 4.96. MS MH^+ $C_{15}H_{31}NO_3H$ calcd: 274.2382, found: 274.2378. $[\alpha]_D^{20}$ −9.4 (*c* 1.01, $CHCl_3$/MeOH 4:1).

4.2.3. Method A: (2) protection of the primary hydroxyl group of glyceric acid derivatives via tritylation

*4.2.3.1. Dodecyl-2-hydroxy-3-(trityloxy)propanoate (**11**).* To a solution of **9a** (1.3502 g, 4.6 mmol) in 30 mL of CH_2Cl_2 were added collidine (0.64 mL, 4.8 mmol) and trityl chloride (1.4003 g, 4.8 mmol). The reaction mixture was kept at room temperature for 26 h. The volume of the solution was reduced to one-third by evaporation and it was then loaded onto a silica gel column and eluted with $CHCl_3$. The fractions corresponding to the product were combined and evaporated, dissolved in benzene, and freeze-dried to give **11** (1.9008g, 80.4%) as a white waxy solid. IR ($CHCl_3$): 3370, 1736 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 0.88 (br t, 3H), 1.25 (br s, 18H), 1.58 (m, 2H), 3.25 (d, 1H, J=9.4 Hz), 3.33 (dd, 1H, J=3, 9.4 Hz), 3.49 (dd, 1H, J=3, 9.4 Hz), 4.17 (t, 2H, 6.7 Hz), 4.26 (br m, 1H), 7.18–7.44 (m, 15H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 14.1, 22.6, 25.7, 28.5, 29.2, 29.3, 29.4, 29.6, 31.9, 65.3, 65.9, 70.7, 86.3, 127.0, 127.8, 128.3, 143.6, 173.2. R_f ($CHCl_3$) 0.55. Anal. Calcd for $C_{34}H_{44}O_4 \cdot 1/3CHCl_3$: C, 77.83; H, 8.45. Found: C, 77.85; H, 8.40. MS MNa^+ $C_{34}H_{44}O_4Na$ calcd: 539.3137, found: 539.3125. $[\alpha]_D^{20}$ −5.7 (*c* 1.13, $CHCl_3$/MeOH 4:1).

*4.2.3.2. N-Dodecyl-2-hydroxy-3-(trityloxy)propanamide (**15**).* To a solution of **10** (0.5547 g, 2 mmol) in 35 mL of $CHCl_3$ were added trityl chloride (1.0201 g, 3.66 mmol) and pyridine (0.5 mL), and the reaction mixture was kept under reflux for 24 h. The mixture was then directly loaded on a silica gel column packed with $CHCl_3$ and eluted with $CHCl_3$/EtOAc (9:1). The fractions containing the product were combined, evaporated, and the residue re-dissolved in benzene and freeze-dried to give the product **15** (0.7427 g, 72%) as a white waxy solid. IR ($CHCl_3$): 3340br m, 1657, 1236 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 0.90 (br t, 3H), 1.27 (br s, 18H), 1.48 (m, 2H), 3.30 (m, 4H), 4.14 (dd, 1H, J=5.4, 9.4 Hz), 4.16 (m, 1H), 6.81 (m, 1H), 7.25–7.45 (m, 15H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 14.1, 22.7, 26.9, 29.3, 29.2, 29.4, 29.5, 29.6, 29.7, 29.6, 31.9, 39.3, 65.0, 70.4, 87.3, 127.3, 127.9, 128.9, 129.0, 128.5, 143.3, 171.5. R_f ($CHCl_3$/EtOAc 4:1) 0.78. Anal. Calcd for $C_{34}H_{45}NO_3 \cdot 1/3H_2O$: C, 77.38; H, 8.85; N, 2.65. Found: C, 77.06; H, 8.82; N, 2.78. MS MNa^+ $C_{34}H_{45}NO_3Na$ calcd: 538.3297, found: 538.3317. $[\alpha]_D^{20}$ −2.4 (*c* 0.91, $CHCl_3$/MeOH 4:1).

*4.2.4. Method A: (3) acylation at the secondary hydroxyl group and detritylation to produce compounds **13** and **17***

*4.2.4.1. 1-(Dodecyloxy)-3-hydroxy-1-oxopropan-2-yl(10-(4-methyl-2-oxo-2H-chromen-7-yl)thio)decanoate (**13**). 4.2.4.1.1. 1-(Dodecyloxy)-1-oxo-3-(trityloxy)propan-2-yl-10-[(4-methyl-2-oxo-2H-chromen-7-yl)thio]decanoate.* To a solution of **11** (1.0012 g, 1.93 mmol) in 30 mL of $CHCl_3$ were added (4′-methyl-7′-mercaptocoumarin-7′-yl)-10-decanoic acid (0.7002 g, 1.93 mmol), DCC (0.4008 g, 1.93 mmol), and DMAP (50 mg, 0.41 mmol). The reaction was stopped after 4 h stirring at room temperature. The mixture was filtered and the solvent was evaporated. The residue was re-dissolved in $CHCl_3$ and purified on silica gel column packed with $CHCl_3$/hexane (1:1) and eluted with a stepwise gradient of $CHCl_3$/hexane (1:1, 7:5, 2:1 and 5:1). The fractions corresponding to the product were isolated, evaporated, dissolved in benzene, and freeze-dried to give the analytically pure product (1.1903 g, 72%) as a colorless wax. IR ($CHCl_3$): 3340, 1735br, 1625, 1210 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 0.91 (br t, 3H), 1.28 (br s, 28H), 1.65 (m, 6H), 2.32 (s, 3H), 2.50 (t, 2H, J=7.4 Hz), 2.94 (t, 2H, J=6.8 Hz), 3.52 (br s, 2H), 4.16 (m, 2H), 5.25 (m, 1H), 6.16 (s, 1H), 7.10–7.49 (m, 18H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 13.9, 18.2, 22.5, 24.6, 25.5, 28.3, 28.4, 28.6, 28.9, 29.0, 29.1, 29.2, 29.4, 31.7, 31.8, 33.8, 62.9, 65.3, 71.63, 86.4, 113.4, 116.6, 122.5, 124.3, 126.9, 127.6, 128.0, 128.4, 143.3, 143.6, 151.9, 153.6, 160.2, 167.93, 172.70. R_f ($CHCl_3$) 0.27. Anal. Calcd for $C_{54}H_{68}O_7S$: C, 75.31; H, 7.96. Found: C, 75.71; H, 7.66. MS MNa^+ $C_{54}H_{68}O_7SNa$ calcd: 883.4583, found: 883.4575. $[\alpha]_D^{20}$ −7.2 (*c* 1.21, $CHCl_3$/MeOH 4:1).

*4.2.4.1.2. Compound (**13**).* To a solution of the product 1-(dodecyloxy)-1-oxo-3-(trityloxy)propan-2-yl-10-((4-methyl-2-oxo-2*H*-chromen-7-yl)thio)decanoate (0.8012 g, 0.93 mmol) in 25 mL of 1,4-dioxane was added a solution of HCl (0.25 mL 1 M aq HCl diluted with 5 mL of 1,4-dioxane). The reaction mixture was kept at room temperature overnight. To this mixture was added 30 mL benzene and then freeze-dried. The white residue obtained was dissolved in $CHCl_3$ and purified on silica gel column packed with $CHCl_3$ and eluted with $CHCl_3$/EtOAc (9:1). The fractions containing the product were isolated, evaporated, re-dissolved in benzene, and freeze-dried to give **13** (0.3402 g, 61%) as white solid. IR (Nujol): 3350, 1738br, 1630 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 0.91 (br t, 3H), 1.26 (br s, 30H), 1.65 (m, 4H), 2.35 (m, 5H), 2.93 (t, 2H, J=6.8 Hz), 3.94 (br m, 2H), 4.11 (t, 3H, J=6.8 Hz), 5.12 (t, 1H, J=4 Hz), 6.15 (s, 1H), 7.07–7.43 (m, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 14.0, 18.4, 22.5, 24.6, 25.6, 28.3, 28.5, 28.7, 28.9, 29.0, 29.1, 29.2, 29.3, 29.4, 29.5, 31.8, 32.0, 33.7, 62.0, 65,6, 73.1, 113.5, 113.6, 116.7, 122.7, 124.4, 143.7, 152.2, 153.7, 160.6, 168.2, 172.8. R_f ($CHCl_3$/EtOAc 9:1) 0.49. Anal. Calcd for $C_{35}H_{54}O_7S \cdot 1/4C_6H_6$: C, 68.67; H, 8.76. Found: C, 68.57; H, 8.50. MS MNa^+ $C_{35}H_{54}O_7S$ calcd: 618.3590, found: 618.3583. $[\alpha]_D^{20}$ −8.9 (*c* 1.23, $CHCl_3$/MeOH 4:1).

*4.2.4.2. 1-(Dodecylamino)-3-hydroxy-1-oxopropan-2-yl-10-((4-methyl-2-oxo-2H-chromen-7-yl)thio)decanoate (**17**). 4.2.4.2.1. 1-(Dodecylamino)-1-oxo-3-(trityloxy)propan-2-yl-10-[(4-methyl-2-oxo-2H-chromen-7-yl)thio]decanoate.* To a solution of **15** (0.5402 g, 1.05 mmol) in 30 mL of $CHCl_3$ were added (4′-methyl-7′-mercaptocoumarin)-10-decanoic acid (0.4567 g, 1.26 mmol), DCC (0.3362 g, 1.26 mmol), and DMAP (32 mg, 0.26 mmol, 20 mol %). The reaction was over after 1 h stirring at room temperature. The DCC–urea that formed was filtered and the solvent was evaporated. The residue was re-dissolved in $CHCl_3$ and purified on a silica gel column packed with $CHCl_3$ and eluted with $CHCl_3$/EtOAc (9:1). The fractions containing the product were combined, evaporated, re-dissolved in benzene, and freeze-dried to give the analytically pure product (0.8602 g, 95%) as pale yellow wax. IR ($CHCl_3$): 3330, 1731br, 1672 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 0.88 (br t, 3H), 1.25 (br s, 28H), 1.66 (m, 6H), 2.39 (m, 5H), 2.97 (t, 2H, J=7.2 Hz), 3.30 (m, 2H), 3.42 (dd, 1H, J_1=3.3 Hz, J_2=9.9 Hz), 3.54 (dd, 1H, J_1=4.8 Hz, J_2=9.9 Hz), 5.34 (br t, 1H, J_1=3.3 Hz), 6.20 (s, 1H), 6.29 (m, 1H), 7.14–7.47 (m, 18H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 14.1, 18.5, 22.6, 24.8, 26.8, 26.9, 28.6, 28.8, 28.9, 29.0, 29.1, 29.2, 29.2, 29.3, 29.5, 29.6, 31.8, 32.1, 34.2, 39.4, 63.3, 73.0, 86.6, 113.6, 116.9, 122.9, 124.5, 127.1, 127.8, 128.6, 143.4, 152.1, 152.2, 153.9, 160.6, 167.7, 172.0. Anal. Calcd for $C_{54}H_{69}NO_6S \cdot 1/2H_2O$: C, 74.62; H, 8.12; N, 1.61. Found: C, 74.22; H, 8.23; N, 1.74. MS MNa^+ $C_{54}H_{69}NO_6SNa$ calcd: 882.4738, found: 882.4774. R_f ($CHCl_3$/EtOAc 9:1) 0.78. $[\alpha]_D^{20}$ −7.4 (*c* 1.19, $CHCl_3$/MeOH 4:1).

*4.2.4.2.2. Compound (**17**).* To a solution of 1-(dodecylamino)-1-oxo-3-(trityloxy)propan-2-yl-10-[(4-methyl-2-oxo-2*H*-chromen-7-yl)thio]decanoate (0.6502 g, 0.76 mmol) in 30 mL 1,4-dioxane was added 0.2 mL of 12 M HCl. The reaction was stopped after 5 h stirring at room temperature. To this mixture was added 30 mL benzene and then freeze-dried. The white residue obtained was dissolved in $CHCl_3$ and purified on a silica gel column packed with $CHCl_3$ and eluted with $CHCl_3$/EtOAc (5:2). The fractions containing the product were combined, evaporated, re-dissolved in benzene, and freeze-dried to give **17** (0.3662 g, 79%) as white solid. IR ($CHCl_3$): 3335 m, 1729, 1666, 1209 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 0.87 (br t, 3H), 1.26 (br s, 30H), 1.66 (m, 4H), 2.35 (m, 5H), 2.98 (t, 2H, J=6.8 Hz), 3.22 (m, 2H), 3.93 (m, 2H), 5.26 (t, 1H, J=4 Hz), 6.28 (s, 1H), 6.33 (m, 1H), 7.12–7.20 (m, 2H), 7.43 (d, 1H, J=8 Hz). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 14.1, 18.5, 22.6, 24.8, 26.8, 28.6, 28.7, 29.0, 29.1, 29.1, 29.2, 29.3, 29.37, 29.58, 31.9, 32.1, 34.1, 39.3, 62.5, 67.1, 71.5, 73.6, 113.6, 117.2, 123.0, 124.5, 143.1, 152.3, 153.5, 160.2, 168.4, 172.4. R_f ($CHCl_3$/EtOAc 5:2) 0.38. Anal. Calcd for $C_{35}H_{55}NO_6S \cdot 1/2H_2O$: C, 67.06; H, 9.00; N, 2.23. Found: C, 67.04; H, 8.47; N, 2.22. MS MH^+ $C_{35}H_{55}NO_6SNa$ calcd: 618.3828, found: 618.3795. $[\alpha]_D^{20}$ −6.1 (*c* 1.21, $CHCl_3$/MeOH 4:1).

*4.2.5. Method B: preparation of 2-tetrahydropyranyl glyceric acid esters and amides (**14** and **18**)*

*4.2.5.1. 12-[(4-Methyl-2-oxo-2H-chromen-7-yl)thio]dodecyl-3-hydroxy-2-[(tetrahydro-2H-pyran-2-yl)oxy]propanoate (**14**). 4.2.5.1.1. 12-[(4-Methyl-2-oxo-2H-chromen-7-yl)thio]dodecyl-2-hydroxy-3-(2-phenoxyacetoxy)propanoate (**12**).* To a solution of **9b** (2.1021 g, 4.5 mmol) and phenoxyacetyl chloride (0.82 mL, 5.85 mmol) in 40 mL of $CHCl_3$ kept at 0 °C was slowly (0.25 mL/min) added a solution of collidine (1.1021 g, 9 mmol) in 10 mL of $CHCl_3$. After 90 min stirring, more phenoxyacetyl chloride was added (0.1 mL, 0.72 mmol) and the reaction mixture was stirred for an additional 90 min. This solution was then loaded directly on a silica gel column packed with $CHCl_3$ and eluted with $CHCl_3$/EtOAc (9:1). Two products were isolated: one corresponding to the monoacyl

and one to the diacyl compound with R_f ($CHCl_3$/EtOAc 5:1) 0.81 and 0.33, respectively, as identified by ^1H NMR. The fractions containing the monoacyl compound were combined, evaporated re-dissolved in benzene, and freeze-dried to give **12** (1.8376 g, 68.2%) as a colorless oil that turned into a white waxy solid. IR ($CHCl_3$): 3300, 1738br, 1710br, 1614 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.26 (br s, 16H), 1.66 (m, 4H), 2.39 (s, 3H), 2.97 (t, 2H, J=7.2 Hz), 4.16 (t, 2H, J=6.8 Hz), 4.48 (m, 3H), 4.65 (s, 2H), 6.19 (s, 1H), 6.87–7.47 (m, 8H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 18.5, 25.6, 28.4, 28.6, 28.8, 29.1, 29.4, 29.4, 32.1, 61.9, 65.0, 65.9, 66.5, 69.0, 113.6, 113.7, 114.6, 116.8, 121.8, 122.9, 124.4, 129.5, 143.7, 152.2, 153.8, 157.6, 160.7, 168.6, 171.7. R_f ($CHCl_3$/EtOAc 5:1) 0.33. Anal. Calcd for $C_{33}H_{42}O_8S$: C, 66.20; H, 7.07. Found: C, 66.49; H, 7.25. MS MNa^+ $C_{33}H_{42}O_8SNa$ calcd: 621.2498, found: 621.2480. $[\alpha]_D^{20}$ −7.3 (*c* 0.95, $CHCl_3$).

4.2.5.1.2. 12-[(4-methyl-2-oxo-2H-chromen-7-yl)thio]dodecyl-3-(2-phenoxyacetoxy)-2-[(tetrahydro-2H-pyran-2-yl)oxy]propanoate. To a solution of **12** (2.4 g, 4 mmol) in 20 mL of CH_2Cl_2 were added 3,4-dihydro-2*H*-pyran (1.7 g, 20 mmol) and PPTS (0.3 g, 1.2 mmol). The reaction mixture was over after 3 h. The mixture was then loaded directly on a silica gel column packed with $CHCl_3$ and eluted with $CHCl_3$/EtOAc (8:1). The fractions corresponding to the product were combined and evaporated, re-dissolved in benzene, and freeze-dried to give the tetrahydropyranyl product (2.6313 g, 96%) as white solid. IR ($CHCl_3$): 2928, 2850, 1738br, 1719, 1609, 1206 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.25 (br s, 16H), 1.68 (m, 10H), 2.38 (s, 3H), 2.96 (t, 2H, J=7.2 Hz), 3.48 (m, 1H), 3.85 (m, 1H), 4.13 (t, 2H, J=6.8 Hz), 4.41 (m, 1H), 4.56 (m, 2H), 4.62 (br s, 2H),4.70–4.81 (m, 1H), 6.18 (s, 1H), 6.86–7.46 (m, 8H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 18.5, 25.1, 25.2, 25.7, 28.4, 28.6, 28.8, 29.0, 29.1, 29.4, 29.9, 32.04, 61.85, 62.0, 64.1, 64.9, 65.0, 65.4, 65.5, 71.8, 73.5, 97.3, 98.4, 113.6, 113.7, 114.5, 114.6, 116.8, 121.7, 122.8, 124.4, 129.4, 143.7, 152.2, 153.8, 157.5, 157.6, 160.5, 168.5, 169.6. R_f ($CHCl_3$/EtOAc 5:1) 0.88. Anal. Calcd for $C_{38}H_{50}O_9S\cdot 1/12C_6H_6$: C, 67.08; H, 7.38. Found: C, 67.07; H, 7.42. MS MNa^+ $C_{38}H_{50}O_9SNa$ calcd: 705.3073, found: 705.3100.

*4.2.5.1.3. Compound (**14**).* To a solution of 12-[(4-methyl-2-oxo-2*H*-chromen-7-yl)thio]dodecyl-3-(2-phenoxyacetoxy)-2-[(tetrahydro-2*H*-pyran-2-yl)oxy]propanoate (2.5532 g, 3.73 mmol) in 8 mL mixture of $CHCl_3$/MeOH (1:1) kept at 0 °C was added *tert*-butylamine (2 mL, 19 mmol). The reaction mixture was kept at 0 °C for 14 h. The mixture was purified directly by loading it on a silica gel column packed with $CHCl_3$ and eluted with a stepwise gradient of $CHCl_3$/EtOAc (9:1 and 6:1). The fractions containing the product were combined, evaporated, re-dissolved in benzene, and freeze-dried to give **14** as a white solid (1.9821 g, 96.7%). IR ($CHCl_3$): 3300, 2855, 1735br, 1617, 1206 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.26 (br s, 16H), 1.65 (m, 10H), 2.38 (s, 3H), 2.96 (t, 2H, J=7.2 Hz), 3.51 (m, 1H), 3.85 (m, 3H), 4.16 (t, 2H, J=6.8 Hz), 4.20–4.40 (m, 1H), 4.65–4.81 (m, 1H), 6.18 (s, 1H), 7.10–7.46 (m, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 18.5, 18.7, 25.1, 25.7, 28.4, 28.6, 28.8, 29.0, 29.1, 29.4, 30.2, 30.3, 32.0, 62.6, 62.7, 62.9, 65.1, 65.2, 75.9, 77.2, 98.5, 98.8, 113.6, 116.8, 122.8, 124.4, 143.7, 152.2, 153.8, 160.6, 170.4, 170.8. R_f ($CHCl_3$/EtOAc 5:1) 0.25. Anal. Calcd for $C_{30}H_{44}O_7S$: C, 65.66; H, 8.08. Found: C, 65.89; H, 8.13. MS MNa^+ $C_{30}H_{44}O_7SNa$ calcd: 571.2705, found: 571.2687.

*4.2.5.2. N-Dodecyl-3-hydroxy-2-[(tetrahydro-2H-pyran-2-yl)oxy]propanamide (**18**). 4.2.5.2.1. 3-(Dodecylamino)-2-hydroxy-3-oxopropyl-2′-phenoxyacetate (**16**).* To a stirred suspension of **10** (1.1061 g, 4.05 mmol) in 60 mL $CHCl_3$, kept in ice-water bath was added phenoxyacetyl chloride (0.85 mL, 6.07 mmol), followed by slow drop-wise addition of a solution of 2,4,6-collidine (0.7355 g, 6.07 mmol) in 10 mL of $CHCl_3$, over 30 min. After 2 h of reaction more collidine (0.2879 g, 2.4 mmol) was added in 5 mL of $CHCl_3$. One hour later the mixture became clear. The reaction mixture was then left stirring at room temperature overnight. The solution was then loaded directly on a silica gel column packed with CH_2Cl_2 and eluted with CH_2Cl_2/EtOAc (5:1). The fractions containing the product were combined, evaporated, the residue dissolved in benzene and freeze-dried to give **16** (1.1221 g, 68%) as a white solid. IR ($CHCl_3$): 3350, 1728, 1679 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 0.88 (br t, 3H), 1.25 (br s, 18H), 1.47 (m, 2H), 3.24 (m, 2H), 4.32 (m, 1H), 4.40–4.60 (m, 2H), 4.66 (s, 2H), 6.87 (m, 1H), 6.90–7.33 (m, 5H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 14.0, 22.6, 26.8, 29.2, 29.3, 29.4, 29.5, 29.6, 31.9, 39.3, 65.1, 67.2, 70.7, 114.5, 122.0, 129.6, 157.6, 169.7. R_f ($CHCl_3$/EtOAc 1:1) 0.48. Anal. Calcd for $C_{23}H_{37}NO_5$: C, 67.78; H, 9.15; N, 3.44. Found: C, 67.66; H, 9.20; N, 3.17. MS MH^+ $C_{23}H_{37}NO_5H$ calcd: 408.2749, found: 408.2739. $[\alpha]_D^{20}$ −2.4 (*c* 0.81, $CHCl_3$/MeOH 4:1).

4.2.5.2.2. 3-(Dodecylamino)-3-oxo-2-[(tetrahydro-2′H-pyran-2′-yl)oxy]propyl-2″-phenoxyacetate. To a cloudy solution of **16** (0.6551 g, 1.6 mmol) in 5 mL of CH_2Cl_2 was added 3,4-dihydro-2*H*-pyran (0.6762 g, 8 mmol) and PPTS (80 mg, 0.32 mmol). The reaction mixture was stirred at room temperature for 2 h. The mixture was loaded directly on a silica gel column packed with $CHCl_3$ and eluted with $CHCl_3$/EtOAc (4:1). The fractions containing the product were combined, evaporated, the residue dissolved in benzene and freeze-dried to give the product (0.7701 g, 98%) as colorless oil. IR ($CHCl_3$): 3340, 2926, 2850, 1729, 1682 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 0.84 (br t, 3H), 1.22 (br s, 18H), 1.60 (m, 8H), 3.22 (m, 2H), 3.47 (m, 1H), 3.80 (m, 1H), 4.32 (m, 2H), 4.35–4.60 (m, 4H), 6.53 (m, 1H), 6.70–7.28 (m, 5H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 14.0, 18.9, 20.1, 22.5, 24.8, 24.9, 25.2, 25.3, 26.7, 29.0, 29.1, 29.3, 29.4, 29.5, 30.3, 30.5, 30.8, 31.7, 39.0, 62.4, 63.9, 65.0, 65.5, 74.8, 75.5, 98.8, 99.3, 114.4, 121.6, 129.35, 157.70, 168.75. R_f ($CHCl_3$/EtOAc 4:1) 0.49. Anal. Calcd for $C_{28}H_{45}NO_6$: C, 68.40; H, 9.23; N, 2.85. Found: C, 68.51; H, 9.35; N, 2.75. MS MNa^+ $C_{28}H_{45}NO_6Na$ calcd: 514.3145, found: 514.3144.

*4.2.5.2.3. Compound (**18**).* To a solution of 3-(dodecylamino)-3-oxo-2-[(tetrahydro-2′*H*-pyran-2′-yl)oxy]propyl-2″-phenoxyacetate (0.7502 g, 1.53 mmol) in 8 mL of $CHCl_3$/MeOH (5:3) cooled at 0 °C, was added *tert*-butylamine (0.8 mL, 7.6 mmol). The reaction mixture was stirred at 0 °C for 28 h and then the mixture was loaded directly on a silica gel column packed with $CHCl_3$ and eluted with a gradient of $CHCl_3$/EtOAc (4:1, followed by 2:1). The fractions containing the product were combined, evaporated, dissolved in benzene and freeze-dried to give **18** (0.4902 g, 92%) as white solid. IR ($CHCl_3$): 3350 br, 2854, 1677 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 0.84 (br t, 3H), 1.22 (br s, 18H), 1.54 (m, 6H), 1.82 (m, 2H), 3.22 (m, 2H), 3.49 (m, 1H), 3.70–3.80 (m, 2H), 4.17 (m, 1H), 4.52–4.63 (m, 1H), 6.57 (m, 1H), 6.92 (m, 1H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 14.0, 20.2, 20.6, 22.6, 24.8, 24.9, 26.7, 29.2, 29.2, 29.3, 29.4, 29.5, 30.8, 31.1, 31.8, 38.9, 62.6, 64.0, 64.2, 64.5, 77.3, 80.7, 100.2, 100.4, 169.9, 171.4. R_f ($CHCl_3$/EtOAc 5:2) 0.22. Anal. Calcd for $C_{20}H_{39}NO_4$: C, 67.19; H, 10.99; N, 3.92. Found: C, 67.39; H, 10.92; N, 3.79. MS MH^+ $C_{20}H_{39}NO_4H$ calcd: 358.2964, found: 358.2946.

4.3. General procedures for phosphorylation of the 1,2-disubstituted glyceric acid derivatives

*4.3.1. 3-(Dodecyloxy)-2-[(10′-(7″-mercapto-4″methylcoumarin-7yl)decanoyl)oxy]-3-(oxopropyl)-phosphocholine (**19**).* To a solution of **13** (0.2802 g, 0.46 mmol) in 40 mL freshly distilled benzene partially submerged in an ice-bath was added 2-chloro-2-oxo-1,2,3-dioxaphospholane (130 μL, 1.4 mmol) followed by a solution of triethylamine (80 μL, 0.57 mmol) in 10 mL benzene, added drop-wise. Eight hours later more phosphorylating agent was added (150 μL, 1.6 mmol) and the reaction mixture was stirred at room temperature overnight. The precipitate that formed was filtered, and the filtrate was evaporated to give a white waxy residue. This residue was dispersed in 25 mL of anhydrous acetonitrile, and the dispersion was transferred to pressure bottle and cooled to −10 °C. To this mixture was added excess trimethylamine (4 mL), the

pressure bottle was sealed, heated to 65 °C, and kept for 24 h. Cooling to room temperature and later in an ice-bath led to formation of a white precipitate. This precipitate was filtered, washed with 3×20 mL cold acetonitrile, and chromatographed on silica gel with a solution of $CHCl_3$/MeOH/H_2O (65:25:4). The combined CH_3CN phase was evaporated to give an oily residue, which was dissolved in the mixture of $CHCl_3$/MeOH/H_2O (65:25:4) and purified on a separate silica gel column packed with $CHCl_3$/MeOH (4:1) and eluted with $CHCl_3$/MeOH/H_2O (65:25:4). The fractions containing the product were collected, evaporated, dispersed in benzene, and freeze-dried to give **19** as a white solid (in a combined yield of 0.1695 g, 48%). IR ($CHCl_3$): 3330, 1731br, 1632, 1206 cm^{-1}; ^1H NMR ($CDCl_3$+CD_3OD, 200 MHz) δ 0.79 (br t, 3H), 1.18 (br s, 26H), 1.58 (m, 8H), 2.21 (t, 2H, J=7.2 Hz), 2.35 (s, 3H), 2.91 (t, 2H, J=6.8 Hz), 3.15 (br s, 9H), 4.07 (m, 4H), 4.25–4.38 (m, 4H), 4.87 (m, 1H), 6.15 (s, 1H), 7.08–7.27 (m, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 8.6, 14.1, 18.5, 22.6, 24.7, 25.8, 28.5, 28.7, 28.8, 29.0, 29.2, 29.3, 29.5, 29.6, 31.8, 32.1, 33.9, 45.7, 54.4, 59.8, 64.5, 65.6, 66.0, 72.2, 113.6, 116.8, 122.8, 124.5, 143.7, 152.3, 153.8, 160.6, 169.2, 173.1. ^{31}P NMR ($CDCl_3$, 160 MHz, pyrophosphate ref. ext.) δ −2.91. R_f ($CHCl_3$/MeOH/H_2O 65:25:4) 0.45. Anal. Calcd for $C_{40}H_{66}NO_{10}PS\cdot 2H_2O$: C, 58.59; H, 8.60; N, 1.71. Found: C, 58.91; H, 8.47; N, 1.94. MS MH^+ $C_{40}H_{66}NO_{10}PSH$ calcd: 784.4223, found: 784.4206. $[\alpha]_D^{20}$ −6.4 (*c* 1.09, $CHCl_3$/MeOH 4:1).

*4.3.2. 3-(Dodecylamino)-2-[(10′-(7″-mercapto-4″-methylcoumarin-7″yl)decanoyl)oxy]-3-(oxopropyl)-phosphocholine (**20**) (route I).* To a suspension of **17** in 25 mL freshly distilled benzene, partially submerged in an ice-water bath was added 2-chloro-2-oxo-1,2,3-dioxaphospholane (70 μL, 0.57 mmol) followed by a solution of triethylamine (82 μL, 0.60 mmol) in 5 mL benzene drop-wise. Six hours later, more phosphorylating agent was added (30 μL, 0.32 mmol) to this mixture. The reaction mixture was stirred at room temperature for 22 h, then the mixture was filtered and the solvent was evaporated to give a white residue. This residue was dispersed in 25 mL MeCN, the dispersion was transferred to pressure bottle and cooled to −10 °C, followed by addition of trimethylamine (2 mL). The pressure bottle was sealed and heated to 65 °C for 48 h. Cooling to room temperature and then with an ice-bath yielded a white precipitate, and the acetonitrile phase was evaporated to give an oily residue. The precipitate and the oily residue were purified on separate silica gel columns, packed with $CHCl_3$/MeOH (4:1), and eluted with $CHCl_3$/MeOH/H_2O (65:25:4). Both columns yielded identical product **20** as a white solid (overall 0.211 g, 54%). IR ($CHCl_3$): 3340br, 1728, 1656, 1210 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 0.83 (br t, 3H), 1.20 (br s, 32H), 1.52 (m, 2H), 2.38 (s, 3H), 2.94 (t, 2H, J=7.4 Hz), 3.10 (br s, 9H), 3.20–3.35 (m, 6H), 3.72 (m, 2H), 4.22 (m, 2H), 5.25 (m, 1H), 6.17 (s, 1H), 7.08–7.12 (m, 2H), 7.41 (d, 1H, J=8 Hz). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 8.8, 14.3, 19.8, 22.8, 24.9, 27.1, 28.8, 29.0, 29.3, 29.5, 29.6, 29.7, 29.8, 29.9, 32.1, 34.1, 39.6, 46.0, 54.5, 59.8, 64.7, 65.1, 73.2, 113.8, 117.0, 123.0, 124.7, 144.0, 152.6, 154.0, 160.9, 167.7, 173.9, 173.7. ^{31}P NMR ($CDCl_3$, 160 MHz, pyrophosphate ref. ext.) δ −1.4 br. R_f ($CHCl_3$/MeOH/H_2O 65:25:4) 0.45. Anal. Calcd for $C_{40}H_{67}N_2O_9PS\cdot CHCl_3$: C, 54.57; H, 7.60; N, 3.10. Found: C, 54.84; H, 7.41; N, 3.93. MS MH^+ $C_{40}H_{67}N_2O_9PSH$ calcd: 783.4378, found: 783.4331. $[\alpha]_D^{20}$ −4.7° (c 1.11, $CHCl_3$/MeOH 4:1).

*4.3.3. 3-(Dodecylamino)-3-oxo-2-[(tetrahydro-2H-pyran-2-yl)oxy] propyl phosphocholine (**21**).* To a solution of **18** (0.4462 g, 1.25 mmol) in 15 mL freshly distilled benzene, partially submerged in an ice-bath, was added 2-chloro-2-oxo-1,2,3-dioxaphospholane (0.17 mL, 1.86 mmol) followed by a solution of triethylamine (0.26 mL, 1.86 mmol). After addition of NEt_3, the ice-bath was removed and the mixture was stirred for 8 h at room temperature. The mixture was filtered and the residue collected was washed with benzene. The solvent was evaporated and the oily residue obtained was dissolved in 25 mL of CH_3CN, the solution was transferred to pressure bottle and frozen in a dry ice-bath at −10 °C. To this solid was added excess trimethylamine (5 mL), and then the pressure bottle was sealed, heated to 65 °C, and kept for 60 h. After 60 h, the pressure bottle was cooled to room temperature, and then in an ice-bath. A white precipitate formed on the walls of pressure bottle. The solvent was evaporated, and the residue in combination with the precipitate was dissolved in $CHCl_3$/MeOH/H_2O (65:25:4) and loaded on a silica gel column packed with the same solvent, and eluted with $CHCl_3$/MeOH/aq NH_3 (1:9:1). The fractions containing the product were collected, evaporated, dissolved in benzene, and freeze-dried to give **21** (0.4372 g, 67%) as white solid. IR ($CHCl_3$): 3312, 2852, 1672 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 0.86 (br t, 3H), 1.24 (br s, 18H), 1.52 (m, 6H), 1.77 (m, 2H), 3.10 (m, 2H), 3.36 (br s, 10H), 3.79 (m, 2H), 3.95–4.15 (m, 2H), 4.28 (m, 4H), 4.81 (m, 1H), 7.00 (m, 1H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 14.9, 17.3, 20.1, 22.6, 25.1, 27.4, 29.3, 29.6, 30.6, 31.8, 39.2, 52.9, 53.4, 55.8, 59.7, 63.5, 67.2, 74.9, 100.0, 170.1. ^{31}P NMR ($CDCl_3$, 160 MHz, pyrophosphate ref. ext.) δ −0.62 R_f ($CHCl_3$/MeOH/H_2O 65:25:4) 0.25. Anal. Calcd for $C_{25}H_{51}N_2O_7P\cdot H_2O$: C, 55.54; H, 9.88; N, 5.18. Found: C, 55.18; H, 9.83; N, 5.14. MS MH^+ $C_{25}H_{51}N_2O_7PH^+$ calcd: 523.3512, found: 523.3503.

*4.3.4. 3-[(12′-(7″-Mercapto-4″-methylcoumarin-7″yl))dodecyloxy]-3-oxo-2-[(tetrahydro-2H-pyran-2-yl)oxy]propyl phosphocholine (**23**).* To a solution of **14** (0.9651 g, 1.76 mmol) in 30 mL benzene in an ice-bath were added 2-chloro-2-oxo-1,2,3-dioxaphospholane (0.32 mL, 3.5 mmol) followed by triethylamine (0.5 mL, 3.5 mmol) drop-wise. After the addition of NEt_3, the ice-bath was removed and the reaction mixture was left stirring at room temperature for 4 h. The mixture was filtered and the crystalline precipitate of triethylamine hydrochloride was removed. The solvent was evaporated and the oily residue was dissolved in 45 mL of anhydrous acetonitrile, the resulting solution was transferred to a pressure bottle and frozen to −10 °C. To this frozen solution was added excess trimethylamine (5 mL), the pressure bottle was then sealed and heated to 65 °C for 24 h. After that the mixture was cooled to room temperature, and then kept at 7 °C overnight, when a white precipitate formed. The precipitate was separated from the acetonitrile solution, which was then evaporated to give an oily residue. Both samples were purified by silica gel chromatography using separate columns, packed with $CHCl_3$/MeOH (4:1) and eluted with In both cases, with $CHCl_3$/MeOH/H_2O (65:25:4) the fractions corresponding to the product were isolated, evaporated, dispersed in benzene, and freeze-dried to give **23** as a white solid (overall yield: 0.9217 g 74%, from precipitate 0.7201 g, and from the MeCN phase 0.2016 g). IR ($CHCl_3$): 3350, 2852, 1732, 1621, 1207 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.27 (br s, 16H), 1.66 (m, 10H), 2.39 (s, 3H), 2.97 (t, 2H, J=7.2 Hz), 3.36 (br s, 9H), 3.80 (m, 6H), 4.10 (t, 2H, J=6.8 Hz), 4.31 (m, 3H), 4.80 (m, 1H), 6.19 (s, 1H), 7.12–7.43 (m, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 18.5, 19.1, 25.2, 25.8, 25.9, 28.5, 28.6, 28.9, 29.1, 29.3, 29.5, 32.1, 54.3, 59.4, 62.3, 65.2, 66.2, 98.9, 113.7, 116.9, 122.9, 124.5, 143.8, 152.2, 153.8, 160.6, 170.7. ^{31}P NMR ($CDCl_3$, 160 MHz, pyrophosphate external reference) δ −1.71 and −1.07. R_f ($CHCl_3$/MeOH/H_2O 65:25:4) 0.31. Anal. Calcd for $C_{35}H_{56}NO_{10}PS\cdot 2H_2O\cdot CHCl_3$: C, 49.74; H, 7.07; N, 1.61. Found: C, 49.77; H, 7.07; N, 1.52. MS MNa^+ $C_{35}H_{56}NO_{10}PSNa$ calcd: 736.3260, found: 736.3239.

4.4. General procedure for hydrolytic cleavage of the tetrahydropyranyl protecting group to produce the lysophospholipid analogues

*4.4.1. 3-(Dodecylamino)-2-hydroxy-3-oxopropyl phosphocholine (**22**).* To a cloudy solution of **21** (0.3521 g, 0.67 mmol) in 25 mL 1,4-dioxane was added 0.15 mL 12 M aq HCl. The reaction mixture was

stirred at room temperature for 2 h. At the end of the reaction 30 mL dioxane was added, and then the reaction 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 packed with $CHCl_3$ and eluted with $CHCl_3$/MeOH/H_2O (65:25:4). The fractions containing the product were collected, evaporated, dispersed in benzene, and freeze-dried to give **22** (0.2762 g, 94%) as white solid. IR ($CHCl_3$): 3352, 1675 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 0.86 (br t, 3H), 1.24 (br s, 16H), 1.52 (m, 2H), 3.33 (br s, 13H), 3.72 (m, 3H), 4.21 (m, 4H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 14.0, 22.6, 27.1, 29.3, 29.5, 29.7, 31.9, 39.2, 54.2, 59.7, 65.9, 68.2, 71.8, 171.6. ^{31}P NMR ($CDCl_3$, 160 MHz, pyrophosphate ref. ext.) δ −1.01. R_f ($CHCl_3$/MeOH/H_2O 65:25:4) 0.25. Anal. Calcd for $C_{20}H_{43}N_2O_6P\cdot 1.5H_2O$: C, 51.60; H, 9.96; N, 6.02. Found: C, 51.49; H, 9.99; N, 6.06. MS MH^+ $C_{20}H_{43}N_2O_6PH$ calcd: 439.2937, found: 439.2927. $[\alpha]_D^{20}$ −5.4 (*c* 1.12, $CHCl_3$/MeOH 4:1).

*4.4.2. 2-Hydroxy-3-[(12′-(7″-mercapto-4″-methylcoumarin))dodecyloxy]-3-oxopropyl phosphocholine (**24**).* To a solution of **23** (0.1202 g, 0.17 mmol) in 2 mL of $CHCl_3$ was added 50 μL of 12 M aq HCl. The reaction mixture was stirred at room temperature for 2 h. At the end of the reaction to this solution was added 100 mL benzene, and the mixture was freeze-dried to give a white residue. This residue was dissolved in $CHCl_3$/MeOH/H_2O (65:25:4) and purified on a silica gel column packed with $CHCl_3$/MeOH (4:1) and eluted with $CHCl_3$/MeOH/H_2O (65:25:4). The fractions containing the product were isolated, evaporated, dispersed in benzene, and freeze-dried to give **24** (62 mg, 57%) as white solid. IR ($CHCl_3$): 3330, 1737 br, 1615, 1205 cm^{-1}; ^1H NMR ($CDCl_3$+CD_3OD, 200 MHz) δ 1.28 (br s, 16H), 1.69 (m, 6H), 2.64 (s, 3H), 2.98 (t, 2H, *J*=7.2 Hz), 3.24 (br s, 9H), 3.36 (s, 1H), 3.66 (br s, 2H), 3.68 (m, 2H), 4.35 (m, 1H), 6.23 (s, 1H), 7.18–7.56 (m, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 18.4, 25.6, 28.5, 28.7, 29.0, 29.3, 29.5, 30.5, 32.0, 54.2, 59.2, 66.3, 113.3, 113.6, 116.1, 122.9, 124.5, 143.9, 152.8, 153.6, 161.2. ^{31}P NMR ($CDCl_3$, 160 MHz, pyrophosphate external reference) δ −1.16. R_f ($CHCl_3$/MeOH/H_2O 65:25:4) 0.31. Anal. Calcd for $C_{30}H_{48}NO_9PS\cdot 5/2H_2O$: C, 53.40; H, 7.92; N, 2.08. Found: C, 53.32; H, 7.56; N, 1.71. $[\alpha]_D^{20}$ −3.7 (*c* 0.94, $CHCl_3$/MeOH 4:1).

4.5. General procedure for acylation of the 2-hydroxy group of the lysophospholipid analogues

*4.5.1. .Compound (**20**) (route II).* To a solution of (**22**) (0.2497 g, 0.57 mmol) in 15 mL of $CHCl_3$ were added 10-(7′-mercapto-4′-methyl-7′-yl)decanoic acid (0.4129 g, 1.14 mmol), DCC (0.2352 g, 1.14 mmol), DMAP (0.1393 g, 1.14 mmol), and 0.5 g of glass beads.[21] The mixture was sonicated for 5 h at 25 °C. The glass beads and the DCC–urea were filtered off, and the filtrate was evaporated. The residue was dissolved in $CHCl_3$/MeOH (4:1) and purified on silica gel column packed with $CHCl_3$, and eluted first with $CHCl_3$/MeOH (4:1), to remove the impurities, and then with $CHCl_3$/MeOH/H_2O (65:25:4) to elute the product. The fractions containing the product were combined, evaporated, dispersed in benzene, and freeze-dried to give **20** (0.2767 g, 0.35 mmol, 62%) as white solid (analytical data are described above, at the synthesis of compound **20** in *route I*).

*4.5.2. (2-[(12′-[(9″H-Fluoren-9-yl)methoxycarbonyl]amino]dodecanoyl)oxy)-3-(12-((7-mercapto-4-methylcoumarin-7-yl)dodecyl)oxy)-3-oxopropyl phosphocholine (**25**).* To a solution of **24** (0.4995 g, 0.8 mmol) in 25 mL $CHCl_3$ were added 12-(9-fluorenylmethoxy carbonyl)-*N*-aminododecanoic acid (0.9987 g, 2.3 mmol), DCC (0.6621 g, 3.2 mmol), DMAP (0.4012 g, 3.2 mmol), and 1 g of glass beads,[21] and the mixture was sonicated for 5 h at 25 °C. The glass beads and the DCC/urea were filtered off, the filtrate was evaporated, and the residue obtained was dissolved in $CHCl_3$/MeOH (4:1) and purified on silica gel column packed with $CHCl_3$ and eluted first with $CHCl_3$/MeOH (4:1) to remove the impurities, and then with $CHCl_3$/MeOH/H_2O (65:25:4) to elute the product. The fractions containing the product were combined, evaporated, dispersed in benzene, and freeze-dried to give **25** (0.5020 g, 60%) as white solid. IR (Nujol): 3335, 1730 br, 1690, 1608, 1207 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.23 (br s, 30H), 1.62 (m, 8H), 2.34 (br s, 5H), 2.93 (t, 2H, *J*=7.2 Hz), 3.16 (br m, 2H), 3.35 (br s, 9H), 3.80 (m, 2H), 4.06–4.12 (m, 2H), 4.25–4.40 (m, 5H), 4.35 (m, 2H), 5.09 (m, 1H), 5.20 (m, 1H), 6.15 (s, 1H), 7.07–7.42 (m, 7H), 7.58 (d, 2H, *J*=7.3 Hz), 7.75 (d, 2H, *J*=7.3 Hz). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 18.4, 24.6, 25.6, 26.7, 28.4, 28.5, 28.8, 29.0, 29.2, 29.4, 29.9, 32.0, 33.8, 41.0, 47.2, 54.3, 59.3, 64.1, 65.7, 66.4, 72.2, 113.5, 116.8, 119.8, 122.8, 124.4, 124.9, 126.9, 127.5, 141.1, 143.9, 152.2, 153.7, 156.3, 160.6, 168.1, 173.0. ^{31}P NMR ($CDCl_3$, 160 MHz, pyrophosphate reference) δ −1.07. R_f ($CHCl_3$/MeOH/H_2O 65:25:4) 0.44. Anal. Calcd for $C_{57}H_{81}N_2O_{12}PS\cdot 0.5H_2O$: C, 64.69; H, 7.81; N, 2.65. Found: C, 64.75; H, 7.62; N, 3.16. MS MH^+ $C_{57}H_{81}N_2O_{12}PSH$ calcd: 1049.5326, found: 1049.5314. $[\alpha]_D^{20}$ −2.4 (*c* 0.78, $CHCl_3$/MeOH 4:1).

*4.5.3. 2-((12-(7-(Diethylamino)-2-oxo-2H-chromene-3-carboxamido)dodecanoyl)oxy)-3-((12-((4-methyl-2-oxo-2H-chromen-7-yl)thio)dodecyl)oxy)-3-oxopropyl phosphocholine (**26**).* To a solution of **25** (0.1309 g, 0.125 mmol) in 5 mL $CHCl_3$ was added DBU (0.1021 g, 0.66 mmol), and the reaction mixture was stirred at room temperature for 1 h, when cleavage of the FMOC protecting group was completed. To this solution was then added *p*-nitrophenyl ester of 7-*N*,*N*-diethylaminocoumarin-3-carboxylate (0.095 g, 0.25 mmol) and DMAP (30 mg, 0.25 mmol). The reaction was stopped after 4 h, when the negative ninhydrin test indicated that acylation of the chain-terminal amino group reached completion. The mixture was loaded directly on a silica gel column packed with $CHCl_3$ and eluted with $CHCl_3$/MeOH (4:1) to remove the impurities, and then with $CHCl_3$/MeOH/H_2O (65:25:4). The fractions containing the product were combined, evaporated, dispersed in benzene, and freeze-dried to give **26** (87.8 mg, 66%) as yellow solid. IR ($CHCl_3$): 3331, 1732 br, 1598, 1208 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.25 (br s, 36H), 1.61 (m, 8H), 2.39 (br s, 5H), 2.97 (t, 2H, *J*=7.2 Hz), 3.36 (br m, 16H), 3.80 (m, 2H), 4.10 (m, 2H), 4.20–4.35 (m, 4H), 5.22 (m, 1H), 6.18 (s, 1H), 6.46 (s, 1H), 6.65 (d, 1H, *J*=6.8 Hz), 7.09–7.47 (m, 5H), 8.76 (s, 1H), 8.78 (m, 1H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 12.4, 18.5, 24.7, 25.7, 27.0, 28.4, 28.6, 28.8, 29.1, 29.2, 29.3, 29.5, 32.1, 33.8, 39.6, 45.0, 54.5, 59.3, 64.2, 65.7, 66.5, 72.1, 96.5, 108.3, 109.9, 110.4, 113.6, 116.8, 122.8, 124.5, 131.0, 143.8, 147.9, 152.3, 153.8, 157.5, 160.7, 162.7, 168.2, 173.0. ^{31}P NMR ($CDCl_3$, 160 MHz, pyrophosphate ref. ext.) δ −1.11. R_f ($CHCl_3$/MeOH/H_2O 65:25:4) 0.45. Anal. Calcd for $C_{56}H_{86}N_3O_{13}PS\cdot 2H_2O$: C, 60.68; H, 8.18; N, 3.79. Found: C, 60.83; H, 7.83; N, 3.88. MS $[M-H]^+$ $C_{56}H_{86}N_3O_{13}PS-H^+$ calcd: 1070.5540, found: 1070.5573. $[\alpha]_D^{20}$ −3.7 (*c* 0.81, $CHCl_3$/MeOH 4:1).

*4.5.4. 3-((12-((4-Methyl-2-oxo-2H-chromen-7-yl)thio)dodecyl)oxy)-2-(12′-((1″-pyrrolidinyloxy-3″-carboxy)dodecyl)oxy)-3-oxopropyl phosphocholine (**27**).* Yield: 52%. IR ($CHCl_3$): 3349, 1739br, 1210m cm^{-1}; R_f ($CHCl_3$/MeOH/H_2O 65:25:4) 0.50. Anal. Calcd for $C_{51}H_{85}N_3O_{12}PS\cdot 3/2H_2O$: C, 59.92; H, 8.68; N, 4.11. Found: C, 60.09; H, 8.69; N, 3.82. MS MH^+ $C_{51}H_{85}N_3O_{12}PSH$ calcd: 995.5664, found: 995.5715. $[\alpha]_D^{20}$ −3.9 (*c* 0.92, $CHCl_3$/MeOH 4:1).

*4.5.5. 2-((12-(Ferrocenyl)carboxamidododecyl)oxy)-2-oxo-3-((12-((4-methyl-2-oxo-2H-chromen-7-yl)thio)dodecyl)oxy)-3-oxopropyl phosphocholine (**28**).* Yield: 58%. IR ($CHCl_3$): 3350, 1735br, 1650, 1540m, 1208 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.24 (br s, 30H), 1.58 (m, 8H), 2.37 (br s, 5H), 2.94 (t, 2H, *J*=7.2 Hz), 3.34 (br m, 10H), 3.80 (m, 2H), 4.05–4.16 (br s, 12H), 4.28 (br s, 4H), 4.69 (br s, 2H), 5.22 (m, 1H), 6.17 (br s, 1H), 7.04–7.55 (m, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 15.0, 18.5, 24.7, 25.8 26.9, 28.4, 28.6, 28.8, 29.0, 29.4, 29.9,

32.0, 33.7, 38.4, 54.4, 59.4, 65.8, 68.0, 69.6, 70.2, 72.0, 113.5, 116.8, 122.8, 124.5, 143.8, 152.3, 153.8, 160.6, 168.1, 168.7, 170.0, 173.0. ^{31}P NMR ($CDCl_3$, 160 MHz, pyrophosphate ref. ext.): no signal was observed, a broad area around zero. R_f ($CHCl_3$/MeOH/H_2O 65:25:4) 0.57. Anal. Calcd for $C_{53}H_{79}FeN_2O_{11}PS\cdot 2H_2O$: C, 59.21; H, 7.78; N, 2.61. Found: C, 59.28; H, 7.70; N, 2.25. MS MH^+ $C_{53}H_{79}FeN_2O_{11}PSH$ calcd: 1039.4569, found: 1039.4593. $[\alpha]_D^{20}$ −4.4° (*c* 0.95, $CHCl_3$/MeOH 4:1).

4.6. Enzymatic hydrolysis of the phospholipids 19, 20, 26–28

In a typical experiment to a sample of phosphocholine (2.5 mg, 2.5 μmol) was added to a solution of 4.1 mL Tris buffer (0.05 M, pH 8.50), containing 0.1 mL Triton X-100 and 50 mM $CaCl_2$. The mixture was vortexed, followed by incubation of the resulting dispersion at 40 °C for 10 min in a constant temperature water bath. To the optically clear dispersion that resulted was added bee-venom phospholipase A_2 (8 μg in 45 μL buffer) to initiate the reaction. The reaction mixture was kept at 40 °C, and formation of the products was analyzed by thin layer chromatography, ($CHCl_3$/MeOH/H_2O, 65:25:4). The compounds were visualized by UV-absorption, fluorescence, iodine adsorption, and molybdic acid spray. TLC analysis showed complete hydrolysis for each substrate in the series of the synthetic phospholipids. Identity of the products was confirmed with authentic samples of the lysophospholipids and the labeled fatty acids.

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