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Design, Synthesis, and Evaluation of Water-Soluble Phospholipid Analogues as Inhibitors of Phospholipase C from *Bacillus cereus*

Christopher L. Franklin, Hui Li, and Stephen F. Martin*

Department of Chemistry and Biochemistry, The University of Texas at Austin, Austin, Texas 78712

sfmartin@mail.utexas.edu

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The rate of hydrolysis of natural phospholipids by the phosphatidylcholine-preferring phospholipase C from *Bacillus cereus* (PLC_{Bc}) follows the order phosphatidylcholine > phosphatidylethanolamine ≫ phosphatidyl-L-serine. To probe the structural basis for this substrate specificity, a series of water-soluble, nonhydrolyzable substrate analogues were needed so their complexes with the enzyme could be studied via X-ray crystallography and isothermal titration calorimetry (ITC). Accordingly the water-soluble dithiophospholipids **2**−**10** having choline, ethanolamine, and L-serine headgroups were synthesized, and the inhibitory activity of each was determined in an assay using 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine (C6PC) as the monomeric substrate. The 1,2-dibutanoyl dithiophosphocholine **2** was a weak inhibitor, whereas the related 1,2-dipentanoyl dithiophosphocholine **3** and the ethylene glycol dithiophosphocholines **4** and **5** were moderate inhibitors. The 1,2-*ω*-hydroxydiacyl dithiophosphocholines **6** and **7** were potent inhibitors, while the related compound **8**, which had shorter acyl side chains, was a weak inhibitor. The dithiophosphoethanolamine **9** was a modest inhibitor, whereas the dithiophospho-L-serine **10** was a somewhat weaker inhibitor. Overall, the phospholipid analogues had increasing K_i values according to the order **2** ≪ **10** < **3** < **4** ≈ **5** ≈ **8** < **9** ≪ **6** ≪ **7** and increasing solubility according to the sequence **5** ≈ **7** < **4** ≈ **6** ≈ **9** < **3** < **10** < **8** < **2**.

Introduction

The phosphatidylcholine-preferring phospholipase C from *Bacillus cereus* (PLC_{Bc}) (EC 3.1.4.3) is a 28.6 kDa monomeric metalloenzyme that contains three zinc atoms in its active site and catalyzes the hydrolyses of phosphatidylcholines (PC), phosphatidylethanolamines (PE), and phosphatidyl-L-serines (PS) to produce diacylglycerol (DAG) and an alkyl phosphate corresponding to the phosphorylated headgroup (Scheme 1).[1] In mammalian systems, DAG has been shown to activate protein kinase C as a key step in signal transduction.[2] However, because no mammalian PLC that hydrolyzes PC has yet been isolated and characterized, there has been an interest in studying the bacterial enzyme as a potential model for a mammalian enzyme. The development of a system for overexpressing PLC_{Bc} in *E. coli*[3] has enabled a detailed series of investigations to probe the mechanism and substrate specificity of this enzyme.[3−6]

Although the mechanistic aspects of the PLC_{Bc}-catalyzed hydrolysis of phospholipids are now reasonably well understood, little is known about the origin of its preferential hydrolysis of phospholipids according to the order PC > PE > PS. For example, the relative rates of hydrolyses of the synthetic phospholipids 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine (C6PC), 1,2-dihexanoyl-*sn*-glycero-3-phosphoethanolamine (C6PE), and 1,2-dihexanoyl-*sn*-glycero-3-phospho-L-serine (C6PS) follow the ratio of 10:7:1 (k_{cat}/K_M).[1,7] Hence, we initiated a program directed toward developing a better understanding of the structural basis for the substrate specificity of PLC_{Bc}.[6,8] Initial work in this area revealed that substrate specificity could be modulated by replacing the residues Glu4, Tyr56, and Phe66 that comprise the putative headgroup binding site in wild-type PLC_{Bc}, and mutants having dramatically altered specificity profiles were identified. To determine how these mutations affected function and substrate specificity, it is necessary to undertake studies of complexes of wild-type and selected mutants with nonhydrolyzable substrate analogues using the tools of isothermal titration calorimetry (ITC) and X-ray crystallography.

ITC is a powerful technique that may be used to measure the thermodynamic quantities ΔG, ΔS, and ΔH associated with the binding of ligands to proteins. However, to determine these parameters accurately, a specific combination of ligand affinity and solubility are required.[9] For example, a compound having a K_i of 1 mM must be soluble at a concentration of about 2 M. The

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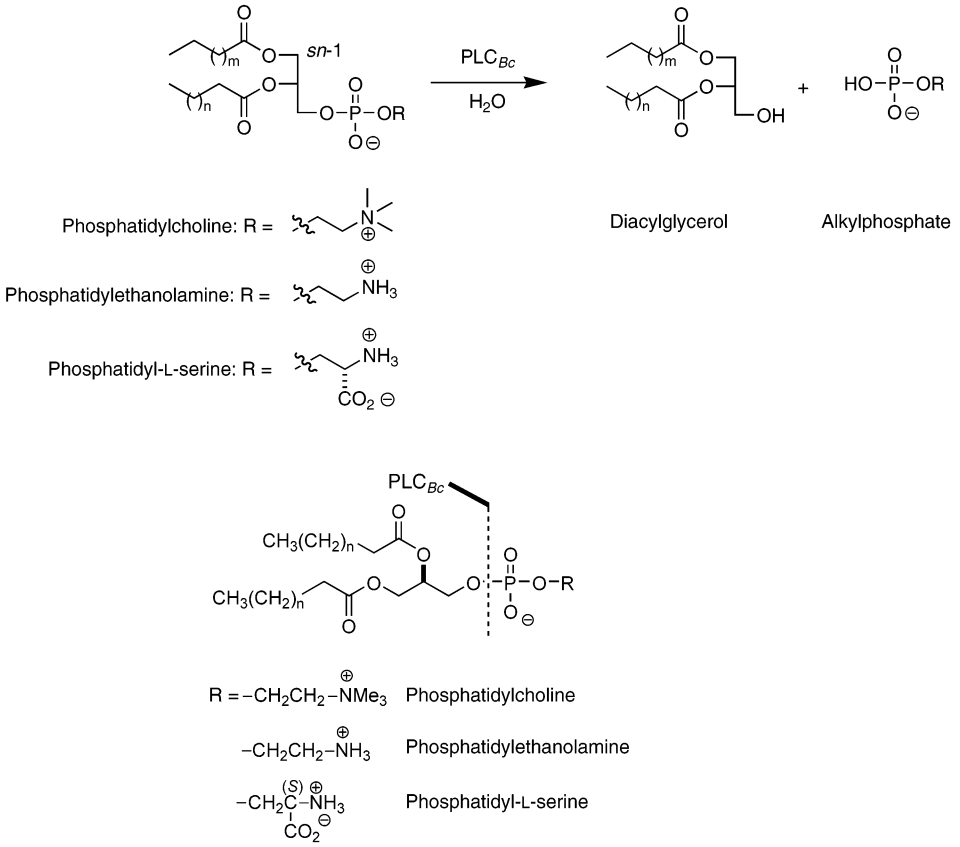
SCHEME 1



requirements for X-ray crystallography are less strict, and we have obtained structures of wild-type PLC$_{Bc}$ and its Asp55Asn mutant complexed with a phosphonate inhibitor having a $K_i = 1.15$ mM at 0.5 M in 45% aqueous $(NH_4)_2SO_4$.[10,11] Phospholipid analogues having solubilities of at least 1 mM and K_i values below about 100 μM would be needed to be able to use both techniques effectively. We now describe the syntheses of a number of water-soluble phospholipids having choline, ethanolamine, and L-serine headgroups; the potencies of these compounds as inhibitors of PLC$_{Bc}$ were also determined.

Design of Inhibitors. We previously discovered that replacing both of the nonbridging oxygen atoms of the phosphate group of water-soluble phosphatidylcholines with sulfur atoms provided dithiophosphate analogues that were potent inhibitors of PLC$_{Bc}$. For example, the dithiophosphatidylcholine derivative **1** exhibited a K_i of 32 μM.[12] The solubility of **1** in water (0.2 mM) was, however, too low to be of use for the planned ITC and crystallographic studies.[13] Preparing phospholipid analogues of **1** having greater solubility in water should simply be a matter of decreasing the overall hydrophobicity of the molecule. The most direct strategy toward this objective would entail modifying the side chains, and several tactics were explored.

The phosphatidylcholine derivatives **2** and **3** were selected as the first targets because decreasing the length of acyl side chains of phospholipids was known to increase their water solubility. However, shortening the acyl side chains on phospholipid substrates has been shown to increase their K_M values,[14,15] so we were concerned that analogues of **1** with shorter side chains might be less potent inhibitors. Consequently, we considered two alternatives that would allow us to maintain longer side chain(s) while increasing solubility.

Derivatives of **1** bearing only one acyl side chain would be expected to be more soluble than those having two side chains. Since phospholipid derivatives bearing a single acyl chain on an ethylene glycol backbone were known to be substrates for PLC$_{Bc}$,[16] we reasoned that the ethylene glycols **4** and **5** might then be inhibitors of PLC$_{Bc}$; these compounds were thus selected as objects for study. To preserve both acyl groups on a glycerol framework, it would be necessary to introduce hydrophilic groups onto the side chains to increase solubility. Hence, compounds **6** and **7** having hydroxyl groups at the terminus of each acyl side chain having eight or more carbon atoms were identified as targets.

Inasmuch as one of the long-range goals of this project was to correlate enzyme structure with specificity, a structurally related series of phospholipids, including the

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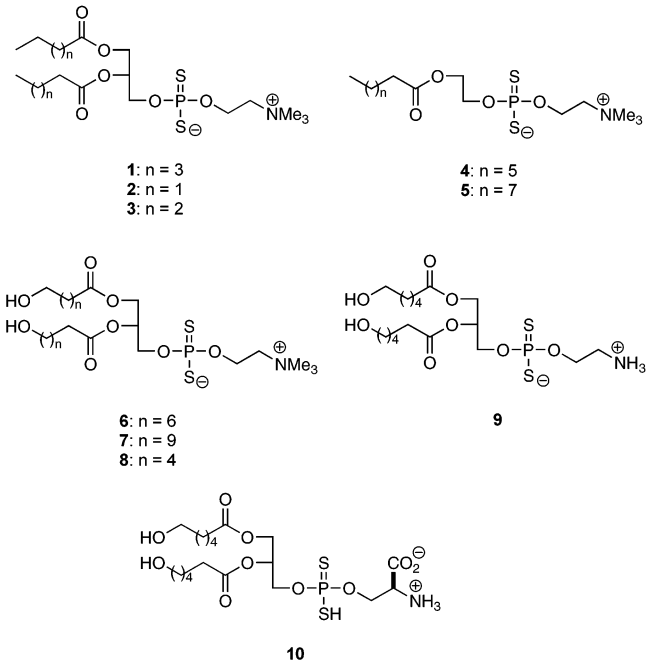
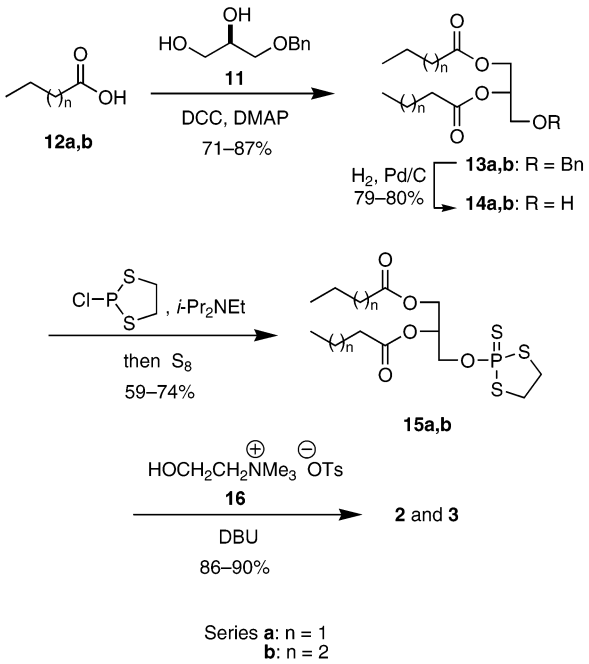


phosphatidylcholine **8**, the phosphatidylethanolamine **9**, and the phosphatidyl-L-serine **10**, were chosen as targets for investigation. On the basis of the crystal structures of complexes of PLC_{Bc} and its D55N mutant with non-hydrolyzable substrate analogues,[10,11] we anticipated that the terminal hydroxyl groups on **6**−**10** should be exposed to solvent in the lipid−enzyme complex. Hence, their presence would not be expected to affect binding, but it would be necessary to verify this prediction.

Results and Discussion

Synthesis of Phospholipid Analogues. The syntheses of **2**−**10** were achieved employing an approach that featured a phosphite coupling reaction that had been developed in our laboratories.[17−20] In the event, diol **11**[21,22] was acylated with either butanoic acid (**12a**) or pentanoic acid (**12b**) in the presence of dicyclohexylcarbodiimide (DCC) and 4-*N*,*N*-(dimethylamino)pyridine (DMAP) to give the diesters **13a** and **13b**, respectively (Scheme 2). Subsequent deprotection of these intermediates by catalytic hydrogenolysis gave the alcohols **14a**,**b**,[23] which could not be stored because of their propensity to undergo 1,2-acyl migrations to give a 1,3-diacyl glycerol. Rather, **14a** and **14b** were immediately treated with 2-chloro-[1,3,2]-dithiaphospholane[24] and then elemental sulfur to give the corresponding thiophosphorodithiolanes **15a** and **15b** in good yield. Reaction of **15a** and **15b** with choline tosylate (**16**) in the presence of 1,8-diazabicyclo[5.4.0]-

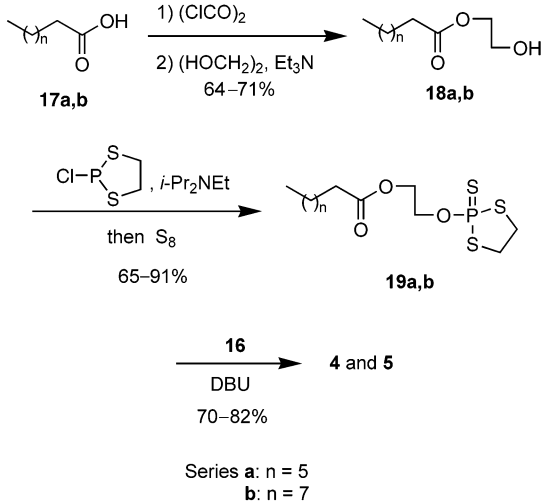
SCHEME 2



undec-7-ene (DBU) gave the phosphatidylcholines **2** and **3**, respectively, in excellent yields.

The ethylene glycol-derived phospholipid analogues **4** and **5** were synthesized by a similar sequence of reactions. Namely, the acid chlorides produced from **17a** and **17b** were treated with an excess (3 equiv) of ethylene glycol to give the known monoesters **18a**[25] and **18b**,[26] respectively (Scheme 3). Compounds **18a** and **18b** were then converted into **4** and **5**, respectively, in good overall yields via their corresponding thiophosphorodithiolanes **19a** and **19b** as described for the preparation of **2** and **3**.

SCHEME 3



The synthesis of the dithiophospholipids **6**−**10** required the design of a protocol for introducing a hydroxyl group on the terminus of the acyl side chain, and two different

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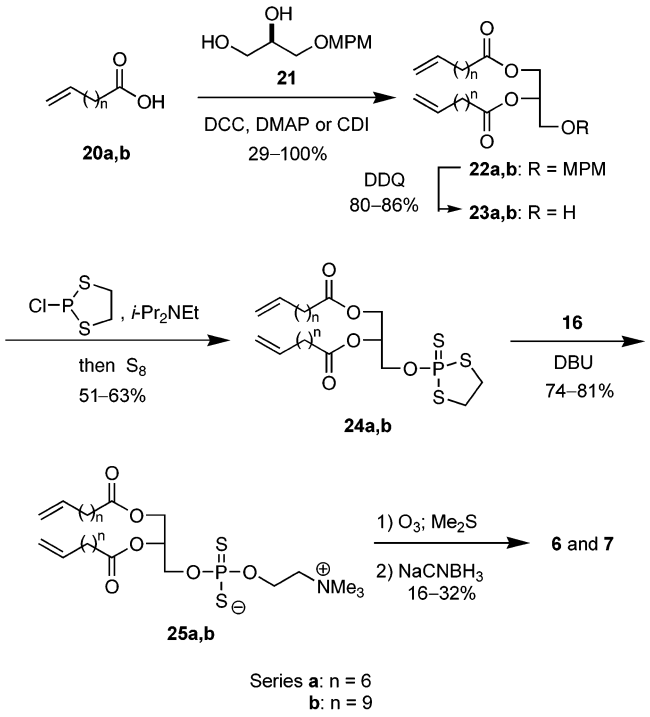
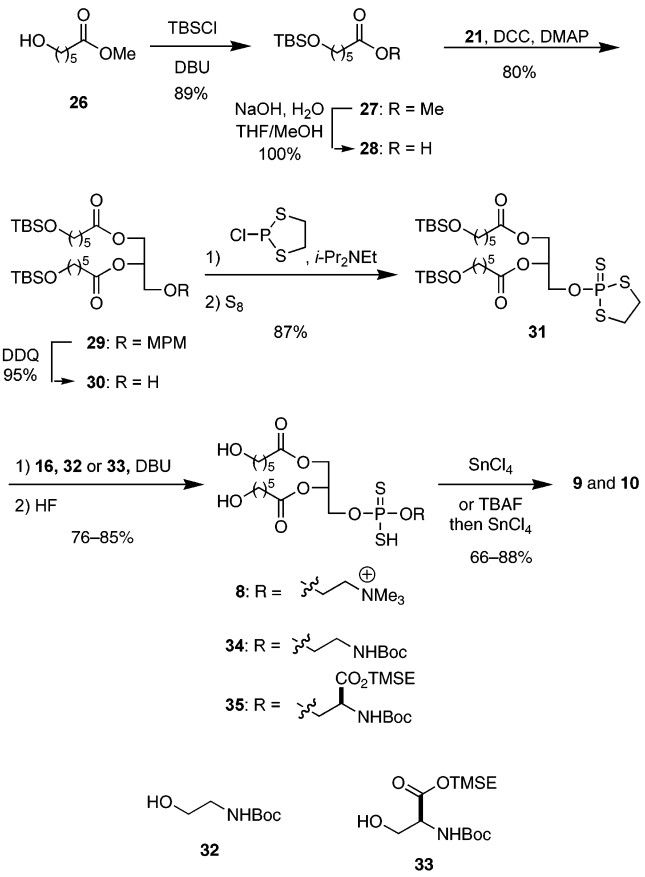
SCHEME 4



SCHEME 5



strategies were explored. In the first of these, a terminal alkene was used as the latent hydroxyl function, whereas in the second a protected hydroxyl group was present from the onset of the synthetic sequence. We envisioned a priori that the former strategy might be superior because the overall synthesis of the target molecules would be shorter, and hence perhaps more efficient, because protection/deprotection steps would be avoided.

Having ω-unsaturated fatty acids as glycerol side chains required a protecting group for the *sn*-3 hydroxyl group that could be removed in the presence of olefins. We previously found that the MPM-protected glycerol **21** was well suited to this task.[18] Consequently, **21** was diacylated with carboxylic acid **20a** in the presence of DCC and DMAP to give **22a** in essentially quantitative yield (Scheme 4). The synthesis of **22b** by acylation of **21** was, however, problematic as the DCC/DMAP-mediated coupling of **21** and **20b** as well as the reaction of **21** with dodecenyl chloride gave only trace amounts of the desired diester **21b**. Surprisingly, the major product of these reactions was that of monoacylation at the *sn*-1 position. We eventually found that reaction of **20b** with **21** in the presence of *N*,*N*′-carbonyldiimidazole (CDI) gave **22b**, albeit in 29% yield after some experimentation. Removal of the MPM protecting group from **22a** and **22b** with dichlorodicyanobenzoquinone (DDQ) proceeded smoothly to deliver the alcohols **23a** and **23b**,[27] which were subjected immediately to our dithiophosphite coupling procedure as before to give the thiophosphorodithiolanes **24a** and **24b**, respectively. Subsequent reaction of **24a** and **24b** with choline tosylate (**16**) and DBU then provided the unsaturated phosphatidylcholine derivatives **25a** and **25b**, respectively, in good overall yields. Ozonolysis of the double bonds in both **25a** and **25b** followed by reduction of the intermediate aldehyde gave the dithiophosphocholine derivatives **6** and **7**, respectively, albeit in low yields. Subsequent experiments suggested that the low yields of **6** and **7** might have arisen from concomitant *S*-oxidation of the dithiophosphate moiety during ozonolysis (vide infra), although this possibility was not verified experimentally.

At this juncture, we needed to prepare water-soluble analogues of phospholipids having ethanolamine and L-serine headgroups. Preliminary solubility studies and enzyme assays suggested that analogues having two hydroxy-substituted side chains as **6** and **7** would likely be preferred, so we embarked upon the syntheses of **9** and **10**. To have a series of closely analogous phospholipids for comparisons, it would then also be necessary to prepare **8**. Initial efforts to prepare **8**−**10** by modifying the strategy outlined in Scheme 4 were unsuccessful because ozonolysis of the terminal carbon−carbon double bond in the side chains was unavoidably accompanied by oxidation of the dithiophosphate moiety. Examination of the ^{31}P NMR of the reaction mixtures suggested that both monothiophosphate and phosphate products were being formed. To obviate this difficulty, another approach to **8**−**10** was devised in which protected hydroxy acids were employed for the side chains.In the event, the hydroxyl group of **26** was first protected as a silyl ether, and the ester was then saponified to give the protected hydroxy acid **28** (Scheme 5).[28] Diacylation of the protected

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TABLE 1. Results of Kinetic and Solubility Studies on Phospholipid Analogues

phospholipid	K_i (mM)[a]	solubility (mM)[b]
2	37	50
3	1.2	7
4	0.51	3
5	0.42	1
6	0.018	3
7	0.0054	1
8	1.0	37
9	0.18	3
10	5.9	17

[a] Accuracy in the value of the K_is is ±25%. [b] Accuracy in the values of the solubilities is ±10%.

glycerol **21** with **28** and subsequent removal of the MPM protecting group with DDQ gave **30**, which was converted into the thiophosphorodithiolane **31** in the usual manner by dithiophosphite coupling and sulfurization. Reaction of **31** with **16**, **32**, or **33** in the presence of DBU followed by removal of the silyl protecting groups from the side chains using hydrofluoric acid provided the requisite dithiophosphatidylcholine **8** and the partially protected ethanolamine and serine analogues **34** and **35**, respectively. N-Deprotection of **34** was effected simply with $SnCl_4$, whereas removal of the serine protecting groups from **35** required sequential treatment with tetrabutylammonium fluoride (TBAF) and $SnCl_4$. We had originally envisioned that the acid of serine might be better protected as its *tert*-butyl ester because we thought that both the *tert*-butyl ester and the Boc group could be removed in a single operation. However, cleavage of the *tert*-butyl ester from a compound related to **35** required more forcing conditions than were necessary to remove the Boc group, and the deprotection step was not clean. The evidence suggested that a *tert*-butyl cation was being trapped by the dithiophosphate ester, even in the presence of cation scavengers such as thioanisole and sodium thiosulfate.

Enzyme Assays. The potencies of the phospholipid analogues **2**−**10** as inhibitors of PLC_{Bc} were determined using a colorimetric Pi quantitation assay developed in this lab using C6PC as the substrate (see Experimental Section).[7] All of the synthetic analogues were found to be inhibitors. We attempted to determine the critical micelle concentration (CMC) of each phospholipid analogue in water using a dye inclusion assay,[29] but each compound precipitated prior to forming micelles. The concentration at which precipitation occurred was then taken as the solubility. The K_i and solubility of each analogue are shown in Table 1.

Examination of the results in Table 1 reveals some useful trends between inhibitor structure and potency. As expected, the short-chain phosphatidylcholine analogues **2** and **3** were sufficiently soluble in water. However, having respective K_i values of 37 and 1.2 mM, they were considerably weaker inhibitors of PLC_{Bc} than **1**, which had a K_i of 32 μM.[12] Decreasing the length of the acyl side chains in this series of inhibitors thus results in significant decreases in their K_i values. This effect parallels the observation for the K_Ms of the corresponding 1,2-diacyl-*sn*-glycero-3-phosphocholine substrates, which have been determined to be 21, 11, and 2.4 mM, respectively.[15]

The ethylene glycol-derived phosphocholines **4** and **5** were both good inhibitors, exhibiting similar K_is and solubilities. Hence, as the presence of a diacylglycerol subunit is not mandatory for substrates of PLC_{Bc},[16] it is also not an essential structural requirement for inhibitors of the enzyme.

A noticeable increase in inhibitory activity was observed upon extending the length of each of the acyl side chains from six to eight carbon atoms in the series of dihydroxy phosphatidylcholine analogues **6**−**8**; longer chain lengths appeared to have little beneficial effect on potency. In exploratory work, we found that there was a large drop in K_i for derivatives of **6**−**8** bearing fewer than six carbon atoms in the side chains. Because these findings parallel those obtained for **1**−**3**, there appears to be a correlation between side chain length and K_i of inhibitors of PLC_{Bc} that is similar to the trend observed between side chain length and K_M for substrates of PLC_{Bc}.[14,15] Finally, the ethanolamine-derived and L-serine-derived inhibitors **9** and **10**, respectively, which are congeners of **8**, are also good inhibitors.

Conclusion

A series of phospholipid analogues were prepared and evaluated for their water solubility and inhibition of PLC_{Bc}. Water solubility was enhanced by shortening the acyl side chains, reducing the number of acyl side chains, and introducing hydroxyl groups on the termini of the acyl side chains. The key structural feature that conferred inhibitory activity on the compounds was the replacement of the two nonbridging oxygen atoms of the phosphodiester group with sulfur atoms. Within a given series, compounds having shorter acyl side chains and higher solubilities were typically poorer inhibitors, a trend reminiscent of that observed for substrates of PLC_{Bc}. As a consequence of these studies, we have identified a number of new phospholipid derivatives having different headgroups that can be used in future energetic and structural studies to correlate structure with function and specificity of wild-type PLC_{Bc} and selected mutants thereof that exhibit altered specificity profiles. In this context, it is notable that compound **3** has recently been used as a ligand in a crystallographic study of the D55N mutant of PLC.[11] The results of these and related studies will be reported in due course.

Experimental Section

General. Unless otherwise noted, all solvents and reagents were reagent grade and used without purification. Acetonitrile (MeCN), methanol (MeOH), and tetrahydrofuran (THF) were passed over molecular sieves prior to use.[30] Dichloromethane (CH_2Cl_2) was distilled from CaH_2 and stored over 4 Å molecular sieves under argon. *N*,*N*′-Diisopropylethylamine (Hünig's Base) was distilled from ninhydrin and stored over 4 Å molecular sieves under argon. 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) was distilled from barium oxide and stored over 4 Å molecular sieves under argon. 4-*N*,*N*-(Dimethylamino)-pyridine (DMAP) was recrystallized from toluene. Triethylamine (Et_3N) was distilled from CaH_2 prior to use. Reactions involving air- or moisture-sensitive reagents or intermediates were performed under an inert atmosphere of nitrogen or

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argon in glassware that had been oven or flame dried. Reaction temperatures are reported as the temperature of the bath surrounding the vessel. Flash chromatography was performed according to the Still protocol with ICN silica gel 60.[31] Melting points are uncorrected. ^{1}H, ^{13}C, and ^{31}P NMR spectra were obtained as solutions in $CDCl_3$ unless otherwise indicated, and chemical shifts are reported in parts per million (ppm, δ) downfield from internal standard Me_4Si (TMS) for ^{1}H and ^{13}C and from external standard 85% phosphoric acid for ^{31}P. Coupling constants are reported in hertz (Hz). Spectral splitting patterns are designated as: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; comp, complex multiplet; and br, broad. Infrared spectra were recorded either as neat thin films on sodium chloride plates or as solutions in an appropriate solvent as indicated and are reported in wavenumbers (cm^{-1}). The IUPAC system for nomenclature of phosphorus-containing compounds of biochemical importance and lipids is used. The numbering of the glycerol backbone, including glycerol derivatives, follows the *sn* numbering system.[32] Percent yields are given for compounds that were ≥95% pure as judged by NMR.

General Procedure for Preparing Diesters 13a,b, 22a, and 29. A solution of DCC (30 mmol) in CH_2Cl_2 (30 mL) was added dropwise (30 min) to a solution of **11** or **21** (10 mmol), DMAP (10 mmol), and the appropriate acid (25 mmol) in CH_2Cl_2 (30 mL) at room temperature. The solution was stirred under argon at room temperature for 22 h. The solid was removed by filtration through Celite, and the filtrate was concentrated under reduced pressure. The residual oil was purified by flash chromatography eluting with the solvent system indicated.

3-Benzyl-1,2-dibutanoyl-*sn*-glycerol (13a). Obtained as a colorless oil in 79% yield after purification by flash chromatography eluting with hexanes/EtOAc (10:1): ^{1}H NMR (300 MHz) δ 7.34−7.21 (comp, 5 H), 5.23 (app dtd, J = 6.3, 5.1, 3.9 Hz, 1 H), 4.52 (d, J_{AB} = 12.0 Hz, 1 H), 4.49 (d, J_{AB} = 12.0 Hz, 1 H), 4.33 (app dd, J = 12.0, 3.9 Hz, 1 H), 4.17 (app dd, J = 12.0, 6.3 Hz, 1 H), 3.56 (app d, J = 5.1 Hz, 2 H), 2.28 (t, J = 7.5 Hz, 2 H), 2.23 (t, J = 7.5 Hz, 2 H), 1.61 (sept, J = 7.5 Hz, 4 H), 0.92 (t, J = 7.5 Hz, 3 H), 0.90 (t, J = 7.5 Hz, 3 H); ^{13}C NMR (75 MHz) δ 172.9, 172.6, 137.5, 128.2, 127.5, 127.4, 73.1, 69.8, 68.0, 62.4, 35.9, 35.7, 18.2, 18.1, 13.4, 13.3; IR (neat) ν 2968, 2122, 1731, 1457 cm^{-1}; mass spectrum (CI) m/z 323.1863 [$C_{18}H_{27}O_5$ (M + 1) requires 323.1858], 251, 235 (base), 215, 181.

3-Benzyl-1,2-dipentanoyl-*sn*-glycerol (13b). Obtained as a colorless oil in 71% yield after purification by flash chromatography eluting with hexanes/EtOAc (10:1): ^{1}H NMR (300 MHz) δ 7.34−7.21 (comp, 5 H), 5.22 (app dtd, J = 6.5, 5.1, 3.9 Hz, 1 H), 4.52 (d, J_{AB} = 12.2 Hz, 1 H), 4.49 (d, J_{AB} = 12.2 Hz, 1 H), 4.32 (app dd, J = 11.9, 3.9 Hz, 1 H), 4.16 (app dd, J = 11.9, 6.5 Hz, 1 H), 3.56 (app d, J = 5.1 Hz, 2 H), 2.29 (t, J = 7.5 Hz, 2 H), 2.25 (t, J = 7.5 Hz, 2 H), 1.63−1.50 (comp, 4 H), 1.38−1.24 (comp, 4 H), 0.88 (t, J = 7.5 Hz, 3 H), 0.87 (t, J = 7.5 Hz, 3 H); ^{13}C NMR (75 MHz) δ 173.2, 172.9, 137.6, 128.3, 127.6, 127.5, 73.1, 69.8, 68.1, 62.5, 33.9, 33.6, 26.9, 26.8, 22.1, 22.0, 13.5; IR (neat) ν 2955, 1743, 1457 cm^{-1}; mass spectrum (CI) m/z 351.2174 [$C_{20}H_{31}O_5$ (M + 1) requires 351.2171], 265, 249 (base), 243.

1,2-Di-(8′-nonenoyl)-3-(*p*-methoxybenzyl)-*sn*-glycerol (22a). Obtained as a colorless oil in 100% yield after purification by flash chromatography eluting with hexanes/EtOAc (1:1): ^{1}H NMR (300 MHz) δ 7.21 (d, J = 8.7 Hz, 2 H), 6.85 (d, J = 8.7 Hz, 2 H), 5.80 (ddt, J = 17.0, 10.2, 6.7 Hz, 2 H), 5.22 (app dtd, J = 6.4, 5.1, 3.9 Hz, 1 H), 5.00−4.89 (comp, 4 H), 4.48 (d, J_{AB} = 11.4 Hz, 1 H), 4.45 (d, J_{AB} = 11.4 Hz, 1 H), 4.33 (app dd, J = 11.7, 3.9 Hz, 1 H), 4.17 (app dd, J = 11.7, 6.4 Hz, 1 H), 3.78 (s, 3 H), 3.53 (app d, J = 5.1 Hz, 2 H), 2.29 (t, J = 7.5 Hz, 2 H), 2.25 (t, J = 7.5 Hz, 2 H), 2.06−1.97 (comp, 4 H), 1.65−1.51 (comp, 4 H) 1.40−1.20 (comp, 14 H); ^{13}C NMR (75 MHz) δ 181.7, 138.9, 129.7, 129.3, 114.3, 113.8, 72.9, 70.0, 67.9, 62.7, 55.2, 34.1, 33.7, 28.9, 28.7, 24.8; IR (neat) ν 2929, 2856, 1741, 1514, 1249, 1172, 1104, 910 cm^{-1}; mass spectrum (CI) m/z 488.3139 [$C_{29}H_{44}O_6$ (M + 1) requires 488.3138], 363, 238, 225 (base).

1,2-Di-(11′-dodecenoyl)-3-(*p*-methoxybenzyl)-*sn*-glycerol (22b). A solution of CDI (245 mg, 1.5 mmol) in CH_2Cl_2 (1.5 mL) was added dropwise to a solution of **21** (107 mg, 0.5 mmol) and **20b** (200 mg, 1.0 mmol) in CH_2Cl_2 (2.0 mL) at room temperature. The solution was stirred at room temperature for 1 h. The solid was removed by filtration through Celite, and the filtrate was concentrated under reduced pressure. The oil was purified by flash chromatography eluting with hexanes/EtOAc (7:3) to give 85 mg (29%) of **22b** as a colorless oil: ^{1}H NMR (300 MHz) δ 7.21 (d, J = 8.7 Hz, 2 H), 6.85 (d, J = 8.7 Hz, 2 H), 5.79 (ddt, J = 17.2, 10.4, 6.7 Hz, 2 H), 5.24−5.17 (m, 1 H), 5.01−4.88 (comp, 4 H), 4.46 (d, J_{AB} = 11.7 Hz, 1 H), 4.43 (d, J_{AB} = 11.7 Hz, 1 H), 4.31 (app dd, J = 11.7, 3.9 Hz, 1 H), 4.15 (app dd, J = 11.7, 6.3 Hz, 1 H), 3.78 (s, 3 H), 3.53 (app d, J = 5.1 Hz, 2 H), 2.29 (t, J = 7.7 Hz, 2 H), 2.25 (t, J = 7.7 Hz, 2 H), 2.05−1.98 (comp, 4 H), 1.65−1.50 (comp, 4 H) 1.40−1.20 (comp, 24 H); ^{13}C NMR (75 MHz) δ 173.4, 173.1, 159.3, 139.2, 129.7, 129.3, 114.1, 113.8, 72.9, 70.0, 67.9, 62.7, 55.2, 34.3, 34.1, 33.8, 29.4, 29.4, 29.2, 29.1, 29.1, 28.9, 24.9, 24.8; IR ν 2925, 2853, 1740, 1513, 1248 cm^{-1}; mass spectrum (CI) m/z 573.4138 [$C_{35}H_{57}O_6$ (M + 1) requires 573.4155], 573, 249, 199 (base).

1,2-Di-(6′-*t*-butyldimethylsilyloxyhexanoyl)-3-(*p*-methoxybenzyl)-*sn*-glycerol (29). Obtained as a colorless oil in 80% yield after purification by flash chromatography eluting with hexanes/EtOAc (8:1): ^{1}H NMR (400 MHz) δ 7.21 (d, J = 8.7 Hz, 2 H), 6.85 (d, J = 8.7 Hz, 2 H), 5.19 (app pent, J = 5.1 Hz, 1 H), 4.46 (d, J_{AB} = 12.0 Hz, 1 H), 4.42 (d, J_{AB} = 12.0 Hz, 1 H), 4.30 (dd, J = 12.0, 3.8 Hz, 1 H), 4.14 (dd, J = 12.0, 6.5 Hz, 1 H), 3.78 (s, 3 H), 3.57 (t, J = 6.5 Hz, 4 H), 3.53 (app d, J = 5.1 Hz, 2 H), 2.30 (t, J = 7.5 Hz, 2 H), 2.26 (t, J = 7.5 Hz, 2 H), 1.65−1.55 (comp, 4 H), 1.53−1.46 (comp, 4 H), 1.37−1.30 (comp, 4 H), 0.86 (s, 18 H), 0.02 (s, 12 H); ^{13}C NMR (62 MHz) δ 173.2, 172.9, 159.3, 129.8, 129.3, 113.8, 72.9, 70.0, 67.8, 62.9, 62.7, 55.2, 34.3, 34.0, 32.4, 25.9, 25.4, 24.7, 24.6, 18.3, −5.3; IR (neat) ν 2934, 1740, 1613, 1513 cm^{-1}; mass spectrum (CI) m/z 669.4190 [$C_{35}H_{65}O_8Si_2$ (M + 1) requires 669.4218], 669, 653, 611 (base), 591, 531.

2-Hydroxyethyl Octanoate (18a). Oxalyl chloride (333 μL, 3.8 mmol) was added to a solution of octanoic acid (500 mg, 3.5 mmol) in CH_2Cl_2 (35 mL), and the solution was stirred at 0 °C for 1 h. The ice bath was then removed and the solution stirred for 1 h at room temperature. The solvent was removed under reduced pressure, and the resulting pale yellow oil was dissolved in CH_2Cl_2 (35 mL). The solution was cooled to 0 °C, and ethylene glycol (580 μL, 10.4 mmol) and Et_3N (967 μL, 6.9 mmol) were added. The solution was stirred for 1 h at 0 °C and for 16 h at room temperature. The solution was washed with saturated aqueous $NaHCO_3$ (1 × 50 mL), and the aqueous layer was extracted with CH_2Cl_2 (2 × 25 mL). The organic layers were combined, dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The oil was purified by flash chromatography eluting with hexanes/EtOAc (1:1) to give 418 mg (64%) of **18a** as a colorless oil whose characteristics (^{1}H NMR, ^{13}C NMR, IR, HRMS) are consistent with those reported.[33]

2-Hydroxyethyl Decanoate (18b). A solution of decanoyl chloride (2 mL, 9.6 mmol) in CH_2Cl_2 (10 mL) was added dropwise to a solution of ethylene glycol (1.6 mL, 28.9 mmol) and Et_3N (2.7 mL, 19.3 mmol) in CH_2Cl_2 (13 mL) at 0 °C. The solution was stirred for 1 h at 0 °C and then for 16 h at room temperature. The solution was washed with saturated aqueous $NaHCO_3$ (1 × 75 mL), and the aqueous layer was extracted with CH_2Cl_2 (3 × 25 mL). The organic layers were combined,

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dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The oil was purified by flash chromatography eluting with hexanes/EtOAc (1:1) to provide 1.48 g (71%) of **18b** as a pale yellow oil: ^{1}H NMR (300 MHz) δ 4.19−4.16 (m, 2 H), 3.81−3.77 (m, 2 H), 2.31 (t, J = 7.5 Hz, 2 H), 2.09 (s, 1 H), 1.66−1.54 (m, 2 H), 1.34−1.18 (comp, 12 H), 0.86−0.82 (m, 3 H); ^{13}C NMR (75 MHz) δ 175.0, 66.6, 62.0, 34.9, 32.6, 30.1, 30.0, 29.9, 25.7, 23.4, 14.9; IR (neat) ν 3425, 2926, 2858, 1745, 1455, 1171, 740 cm^{-1}; mass spectrum (CI) m/z 217.1801 [$C_{12}H_{25}O_3$ (M + 1) requires 217.1804], 217 (base), 155.

General Procedure for Preparing Alcohols 14a,b from Ethers 13a,b. A solution of **13a**,**b** (1.6 mmol) in 10% AcOH/EtOH containing 10% Pd/C (0.16 mmol) was stirred under H_2 (1 atm) at room temperature for 4 days. The catalyst was removed by filtration through Celite, and the filter cake was washed with EtOAc (20 mL). The filtrate and washings were combined, washed with $NaHCO_3$ (2 × 20 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The oil was purified by flash chromatography eluting with the solvent system indicated.

1,2-Dibutanoyl-*sn*-glycerol (14a). Obtained as a colorless oil in 80% yield after purification by flash chromatography eluting with hexanes/EtOAc (7:3) whose characteristics (^{1}H NMR, IR, LRMS, HRMS) were consistent with those published.[34]

1,2-Dipentanoyl-*sn*-glycerol (14b). Obtained as a colorless oil in 69% yield after purification by flash chromatography eluting with hexanes/EtOAc (7:3): ^{1}H NMR (300 MHz) δ 5.01 (app pent, J = 5.1 Hz, 1 H), 4.26 (app dd, J = 12.0, 4.2 Hz, 1 H), 4.13 (app dd, J = 12.0, 6.0 Hz, 1 H), 3.64 (app d, J = 5.1 Hz, 2 H), 2.73 (br s, 1 H), 2.27 (t, J = 7.2 Hz, 2 H), 2.24 (t, J = 7.2 Hz, 2 H), 1.58−1.47 (comp, 4 H), 1.27 (ddqd, J = 18.4, 14.9, 7.2, 2.7 Hz, 4 H), 0.84 (t, J = 7.2 Hz, 3 H), 0.83 (t, J = 7.2 Hz, 3 H); ^{13}C NMR (75 MHz) δ 173.7, 173.4, 71.9, 62.1, 61.1, 33.8, 33.6, 26.8, 26.8, 22.0, 13.5; IR (neat) ν 3478, 2955, 1731, 1258, 1171, 953 cm^{-1}; mass spectrum (CI) m/z 261.1694 [$C_{13}H_{25}O_5$ (M + 1) requires 261.1702], 243, 199, 187, 159 (base).

General Procedure for Preparing Alcohols 23a,b and 30 from Ethers 22a,b and 29. DDQ (1.3 mmol) was added to a solution of **22a**,**b** or **29** (1.0 mmol) in CH_2Cl_2/H_2O (20:1, 5 mL), and the mixture was stirred for 2.5 h at room temperature. The solids were removed by filtration, and $NaHCO_3$ (1 × 5 mL) was added. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (2 × 5 mL). The organic layers were combined, washed with $NaHCO_3$ (1 × 5 mL) and brine (1 × 5 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The oil was purified by flash chromatography eluting with the solvent system indicated.

1,2-Di-(8′-nonenoyl)-*sn*-glycerol (23a). Obtained as a colorless oil in 80% yield after purification by flash chromatography eluting with hexanes/EtOAc (7:3): ^{1}H NMR (300 MHz) δ 5.80 (ddt, J = 17.1, 10.4, 6.6 Hz, 2 H), 5.09 (app pent, J = 5.0 Hz 1 H), 5.03−4.91 (comp, 4 H), 4.33 (app dd, J = 11.7, 4.4 Hz 1 H), 4.23 (app dd, J = 11.7, 5.7 Hz 1 H), 3.76−3.69 (m, 2 H), 2.35 (t, J = 7.2 Hz, 2 H), 2.33 (t, J = 7.2 Hz, 2 H), 2.11 (br s, 1 H), 2.04 (q, J = 6.9 Hz, 4 H), 1.70−1.56 (comp, 4 H), 1.45−1.24 (comp, 12 H); ^{13}C NMR (75 MHz) δ 173.7, 173.4, 138.9, 114.3, 72.1, 62.0, 61.5, 34.2, 34.0, 33.7, 28.9, 28.9, 28.6, 24.8, 24.8; IR ($CDCl_3$) ν 3468, 2923, 2861, 1739, 1639, 1165, 910 cm^{-1}; mass spectrum (CI) m/z 369.2631 [$C_{21}H_{37}O_5$ (M + 1) requires 369.2641], 351 (base), 213, 137.

1,2-Di-(11′-dodecenoyl)-*sn*-glycerol (23b). Obtained as a colorless oil in 86% yield after purification by flash chromatography eluting with hexanes/EtOAc (7:3): ^{1}H NMR (300 MHz) δ 5.78 (ddt, J = 17.1, 10.2, 6.6 Hz, 2 H), 5.06 (app pent, J = 4.8 Hz, 1 H), 5.00−4.88 (comp, 4 H), 4.30 (app dd, J = 12.0, 4.8 Hz, 1 H), 4.21 (app dd, J = 12.0, 5.7 Hz, 1 H), 3.70 (app d, J = 4.8 Hz, 2 H), 2.32 (t, J = 7.4 Hz, 2 H), 2.30 (t, J = 7.4 Hz, 2 H), 2.05−1.98 (comp, 4 H), 1.65−1.53 (comp, 4 H) 1.40−1.21 (comp, 24 H); ^{13}C NMR (75 MHz) δ 173.8, 139.2, 114.1, 72.1, 62.0, 61.5, 34.3, 34.1, 29.4, 29.4, 29.2, 29.1, 28.9, 24.9, 24.9; IR (CH_2Cl_2) ν 3440, 2923, 2852, 2358, 1741, 1460, 1260, 1164, 908 cm^{-1}; mass spectrum (CI) m/z 453.3572 [$C_{27}H_{49}O_5$ (M + 1) requires 453.3580], 453, 435, 255 (base).

1,2-Di-(6′-*t*-butyldimethylsilyloxyhexanoyl)-*sn*-glycerol (30). Obtained as a colorless oil in 95% yield after purification by flash chromatography eluting with hexanes/EtOAc (5:1 to 4:1): ^{1}H NMR (300 MHz) δ 5.05 (app pent, J = 5.1 Hz, 1 H), 4.29 (dd, J = 11.8, 4.6 Hz, 1 H), 4.20 (dd, J = 11.8, 5.6 Hz, 1 H), 3.70 (app d, J = 5.1 Hz, 2 H), 3.58 (t, J = 6.4 Hz, 2 H), 3.57 (t, J = 6.4 Hz, 2 H), 2.33 (t, J = 7.3 Hz, 2 H), 2.31 (t, J = 7.3 Hz, 2 H), 2.20−1.96 (br s, 1 H), 1.68−1.50 (comp, 4 H), 1.50−1.45 (comp, 4 H), 1.39−1.30 (comp, 4 H), 0.86 (s, 18 H), 0.02 (s, 12 H); ^{13}C NMR (75 MHz) δ 173.9, 173.5, 72.3, 63.2, 62.2, 61.7, 34.5, 32.7, 32.6, 26.2, 25.6, 25.6, 25.0, 24.9, 18.6. −5.1; IR (CH_2Cl_2) ν 3468, 2930, 2858, 1742, 1099, 836, 775 cm^{-1}; mass spectrum (CI) m/z 529.3646 [$C_{27}H_{57}O_7Si_2$ (M + 1) requires 529.3643], 529 (base), 491, 417, 303, 247, 229.

General Procedure for Converting Alcohols 14a,b, 18a,b, 23a,b, and 30 to Thiophosphorodithiolanes 15a,b, 19a,b, 24a,b, and 31. 2-Chloro-[1,3,2]-dithiaphospholane (1.0 mmol) was added dropwise to a solution of the appropriate alcohol (1.0 mmol) and Hünig's base (1.1 mmol) in MeCN (10 mL) at −42 °C. The solution was stirred at −42 °C for 2 h, whereupon the ice bath was removed and the solution stirred for 1 h at room temperature. A solution of sulfur (3.0 mmol) in CS_2 (3.0 mL) was added, and the solution was stirred vigorously overnight at room temperature. The solution was concentrated under reduced pressure, and the residue was dissolved in acetone (10 mL). The sulfur was removed by filtration through a plug of glass wool, and the filtrate was concentrated under reduced pressure. The oil was purified by flash chromatography eluting with the solvent system indicated.

2-*O*-(1′,2′-Dibutanoyl-*sn*-glycero)-2-thioxo-2λ^5-[1,3,2]-dithiaphospholane (15a). Obtained as a yellow oil in 74% yield after purification by flash chromatography eluting with hexanes/EtOAc (7:3): ^{1}H NMR (300 MHz) δ 5.25 (app pent, J = 5.0 Hz, 1 H), 4.33−4.13 (comp, 4H), 3.70−3.55 (comp, 4 H), 2.31 (t, J = 7.2 Hz, 2 H), 2.28 (t, J = 7.2 Hz, 2 H), 1.63 (ddqd, J = 19.2, 14.7, 7.2, 4.3 Hz, 4 H), 0.93 (t, J = 7.2 Hz, 3 H), 0.92 (t, J = 7.2 Hz, 3 H); ^{13}C NMR (75 MHz) δ 173.1, 172.6, 69.2 (d, J_{CP} = 8.8 Hz), 65.5 (d, J_{CP} = 8.8 Hz), 61.7, 60.4, 41.5 (d, J_{CP} = 5.5 Hz), 36.0, 35.9, 21.0, 18.3, 14.2, 13.6; ^{31}P NMR (121 MHz) δ 124.1; IR (neat) ν 3459, 2962, 1731, 1171, 692 cm^{-1}; mass spectrum (CI) m/z 387.0524 [$C_{13}H_{24}O_5PS_3$ (M + 1) requires 387.0524], 371, 299, 215 (base).

2-*O*-(1′,2′-Dipentanoyl-*sn*-glycero)-2-thioxo-2λ^5-[1,3,2]-dithiaphospholane (15b). Obtained as a colorless oil in 59% yield after purification by flash chromatography eluting with hexanes/EtOAc (7:3): ^{1}H NMR (300 MHz) δ 5.26 (app pent, J = 5.0 Hz, 1 H), 4.33−4.11 (comp, 4H), 3.70−3.57 (comp, 4 H), 2.34 (t, J = 7.2 Hz, 2 H), 2.31 (t, J = 7.2 Hz, 2 H), 1.65−1.54 (comp, 4 H), 1.33 (ddqd, J = 15.3, 18.0, 7.2, 2.9 Hz, 4 H), 0.90 (t, J = 7.2 Hz, 6 H); ^{13}C NMR (75 MHz) δ 173.1, 172.7, 69.1 (d, J_{CP} = 8.8 Hz), 65.4 (d, J_{CP} = 8.8 Hz), 61.6, 41.4 (d, J_{CP} = 6.6 Hz), 33.8, 33.6, 26.8, 22.1, 22.0, 13.6; ^{31}P NMR (121 MHz) δ 124.0; IR (neat) ν 2955, 1737, 1463, 1165 cm^{-1}; mass spectrum (CI) m/z 415.0820 [$C_{15}H_{28}O_5PS_3$ (M + 1) requires 415.0837], 399, 313, 243 (base).

2-*O*-(2′-Hydroxyethyl Octanoate)-2-thioxo-2λ^5-[1,3,2]-dithiaphospholane (19a). Obtained as a yellow oil in 91% yield after purification by flash chromatography eluting with hexanes/EtOAc (7:3): ^{1}H NMR (300 MHz) δ 4.32−4.23 (comp, 4 H), 3.71−3.55 (comp, 4 H), 2.31 (t, J = 7.5 Hz, 2 H), 1.66−1.54 (comp, 2 H), 1.34−1.20 (comp, 8 H), 0.88−0.80 (comp, 3 H); ^{13}C NMR (75 MHz) δ 173.5, 65.7 (d, J_{CP} = 8.8 Hz), 62.5 (d, J_{CP} = 8.8 Hz), 41.4, 34.1, 31.6, 29.0, 28.9, 24.8, 22.6, 14.0; ^{31}P NMR (121 MHz) δ 123.3; IR (neat) ν 3416, 2937, 2253, 1737,

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1637, 904, 730 cm^{-1}; mass spectrum (CI) *m/z* 343.0617 [$C_{12}H_{24}O_3PS_3$ (M + 1) requires 343.0625], 343 (base), 326, 298.

2-*O*-(2′-Hydroxyethyl Decanoate)-2-thioxo-2λ^5-[1,3,2]-dithiaphospholane (19b). Obtained as a dark yellow oil in 65% yield after purification by flash chromatography eluting with hexanes/EtOAc (7:3): ^{1}H NMR (300 MHz) δ 4.32−4.24 (comp, 4 H), 3.73−3.55 (comp, 4 H), 2.31 (t, J = 7.5 Hz, 2 H), 1.66−1.53 (comp, 2 H), 1.35−1.18 (comp, 12 H), 0.88−0.80 (comp, 3 H); ^{13}C NMR (75 MHz) δ 173.5, 65.7 (d, J_{CP} = 8.8 Hz), 62.5 (d, J_{CP} = 8.8 Hz), 41.4, 34.1, 31.8, 29.3, 29.2, 29.1, 24.8, 22.6, 14.1; ^{31}P NMR (121 MHz) δ 123.3; IR (neat) ν 2931, 2856, 1743, 1457, 1165, 730, 692 cm^{-1}; mass spectrum (CI) *m/z* 371.0923 [$C_{14}H_{28}O_3PS_3$ (M + 1) requires 371.0938], 371, 199 (base).

2-*O*-[1′,2′-Di-(8″-nonenoyl)-*sn*-glycero]-2-thioxo-2λ^5-[1,3,2]-dithiaphospholane (24a). Obtained as a yellow oil in 63% yield after purification by flash chromatography eluting with hexanes/EtOAc (7:3): ^{1}H NMR (300 MHz) δ 5.80 (ddt, J = 17.1, 10.3, 6.7 Hz, 2 H), 5.28 (app pent, J = 5.0 Hz, 1 H), 5.03−4.91 (comp, 4 H), 4.33 (app dd, J = 11.9, 4.7 Hz, 1 H), 4.26 (app dd, J = 7.2, 4.7 Hz, 1 H), 4.19 (app dd, J = 11.1, 5.0 Hz, 1 H), 4.18 (app dd, J = 11.9, 5.9 Hz, 1 H), 3.72−3.60 (comp, 4 H), 2.35 (t, J = 7.2 Hz, 2 H), 2.33 (t, J = 7.2, 2 H), 2.08−2.01 (comp, 4 H), 1.69−1.56 (comp 4 H), 1.45−1.25 (comp 12 H); ^{13}C NMR (75 MHz) δ 173.2, 172.8, 138.9, 114.3, 69.2 (d, J_{CP} = 8.2 Hz), 65.5 (d, J_{CP} = 8.2 Hz), 61.7, 41.5 (d, J_{CP} = 5.5 Hz), 34.2, 34.0, 33.7, 28.9, 28.9, 28.7, 28.7, 24.8; ^{31}P NMR (121 MHz) δ 124.5; IR ($CHCl_3$) ν 2927, 2854, 2360, 1740, 1639, 1160, 1018 cm^{-1}; mass spectrum (CI) *m/z* 523.1778 [$C_{23}H_{40}O_5PS_3$ (M + 1) requires 523.1776], 523 (base), 419, 351.

2-*O*-[1′,2′-Di-(11″-dodecenoyl)-*sn*-glycero]-2-thioxo-2λ^5-[1,3,2]dithiaphospholane (24b). Obtained as a yellow oil in 51% yield after purification by flash chromatography eluting with hexanes/EtOAc (7:3): ^{1}H NMR (300 MHz) δ 5.79 (ddt, J = 17.1, 10.2, 6.6 Hz, 2 H), 5.26 (app pent, J = 5.1 Hz, 1 H), 5.01−4.87 (comp, 4 H), 4.31 (app dd, J = 11.7, 4.5 Hz, 1 H), 4.24 (app dd, J = 6.6, 4.5 Hz, 1 H), 4.17 (app dd, J = 10.8, 5.1 Hz, 1 H), 4.16 (app dd, J = 11.7, 5.9 Hz, 1 H), 3.70−3.57 (comp, 4 H), 2.33 (t, J = 7.2 Hz, 2 H), 2.30 (t, J = 7.2 Hz, 2 H), 2.05−1.98 (comp, 4 H), 1.65−1.53 (comp, 4 H) 1.40−1.22 (comp, 24 H); ^{13}C NMR (125 MHz) δ 173.3, 172.8, 139.2, 114.1, 69.2 (J_{CP} = 9 Hz), 65.5 (J_{CP} = 8.2 Hz), 61.7, 41.5 (J_{CP} = 9 Hz), 34.2, 34.1, 33.8, 29.4, 29.4, 29.3, 29.2, 29.1, 29.1, 28.9, 24.9; ^{31}P NMR (121 MHz) δ 124.5; IR ($CDCl_3$) ν 2926, 2854, 1739, 1162, 1018, 911, 733, 699 cm^{-1}; mass spectrum (CI) *m/z* 607.2728 [$C_{29}H_{52}O_5PS_3$ (M + 1) requires 607.2715], 607, 437, 435 (base), 273.

2-*O*-[1′,2′-Di-(6″-*t*-butyldimethylsilyloxyhexanoyl)-*sn*-glycero]-2-thioxo-2λ^5-[1,3,2]dithiaphospholane (31). Obtained as a pale yellow oil in 87% yield after purification by flash chromatography eluting with hexanes/EtOAc (6:1 to 3:1): ^{1}H NMR (400 MHz) δ 5.22 (app pent, J = 5.1 Hz, 1 H), 4.30−4.22 (comp, 2 H), 4.17 (dd, J = 10.9, 5.1 Hz, 1 H), 4.13 (dd, J = 11.6, 5.8 Hz, 1 H), 3.69−3.54 (comp, 8 H), 2.31 (t, J = 8.2 Hz, 2 H), 2.29 (t, J = 8.2 Hz, 2 H), 1.64−1.55 (comp, 4 H), 1.52−1.45 (comp, 4 H), 1.37−1.29 (comp, 4 H), 0.84 (s, 18 H), −0.01 (s, 12 H); ^{13}C NMR (75 MHz) δ 173.0, 172.6, 69.1 (d, J_{CP} = 12.6 Hz), 65.3 (d, J_{CP} = 12.6 Hz), 62.8, 61.6, 41.4 (d, J_{CP} = 4.6 Hz), 41.3, 34.0, 33.9, 32.3, 25.8, 25.2, 24.5, 18.2, −5.4; ^{31}P NMR (121 MHz) δ 124.5; IR (neat) ν 2931, 1740, 1461 cm^{-1}; mass spectrum (CI) *m/z* 703.2785 [$C_{29}H_{60}O_7Si_2PS_3$ (M + 1) requires 703.2777], 703 (base), 687, 645, 531, 457, 256.

General Procedure for Converting Thiophosphorodithiolanes 15a,b, 19a,b, and 24a,b into Dithiophosphatidylcholines 2−5 and 25a,b. DBU (1.0 mmol) was added to a solution of the appropriate thiophosphorodithiolane (1.0 mmol) and choline *p*-toluenesulfonate (**16**) (1.0 mmol) in MeCN (10 mL), and the resulting solution was stirred at room temperature for 15 min. The solution was concentrated under reduced pressure, and the residue was purified by flash chromatography eluting with the solvent system indicated.

1,2-Dibutanoyl-*sn*-glycero-3-dithiophosphocholine (2). Obtained as a yellow glass in 90% yield after purification by flash chromatography eluting with acetone/$CHCl_3$/H_2O (67:32:1): ^{1}H NMR (300 MHz) δ 5.31−5.24 (m, 1 H), 4.52 (comp, 2 H), 4.38 (app dd, J = 12.0, 3.3 Hz, 1 H), 4.19−4.10 (comp, 3 H), 3.96 (comp, 2 H), 3.43 (s, 9 H), 2.30 (t, J = 7.5 Hz, 2 H), 2.28 (t, J = 7.5 Hz, 2 H), 1.62 (ddqd, J = 18.9, 14.7, 7.5, 3.9 Hz, 4 H), 0.94 (t, J = 7.5, Hz, 3 H), 0.93 (t, J = 7.5, Hz, 3 H); ^{13}C NMR (75 MHz) δ 173.4, 173.0, 70.1, 66.2, 63.5, 62.9, 59.4, 55.0, 36.1, 35.9, 18.4, 18.3, 13.6, 13.6; ^{31}P NMR (121 MHz) δ 116.9; IR ($CHCl_3$) ν 3434, 2962, 2359, 1731 cm^{-1}; mass spectrum (CI) *m/z* 430.1484 [$C_{16}H_{33}NO_6PS_2$ (M + 1) requires 430.1487], 382, 283, 243, 215 (base).

1,2-Dipentanoyl-*sn*-glycero-3-dithiophosphocholine (3). Obtained as a yellow glass in 86% yield after purification by flash chromatography eluting with acetone/$CHCl_3$/H_2O (67:32:1): ^{1}H NMR (300 MHz) δ 5.30−5.20 (m, 1 H), 4.57−4.46 (comp, 2 H), 4.35 (app dd, J = 11.9, 3.2 Hz, 1 H), 4.17−4.07 (comp, 3 H), 3.99−3.91 (comp, 2 H), 3.41 (s, 9 H), 2.30 (t, J = 7.5 Hz, 2 H), 2.27 (t, J = 7.5 Hz, 2 H), 1.62−1.49 (comp, 4 H), 1.31 (ddqd, J = 18.9, 14.7, 7.5, 3.9 Hz, 4 H), 0.88 (t, J = 7.5, Hz, 3 H), 0.88 (t, J = 7.5, Hz, 3 H); ^{13}C NMR (75 MHz) δ 173.4, 173.0, 70.1, 66.2, 63.5, 62.9, 59.4, 55.0, 36.1, 35.9, 18.4, 18.3, 13.6, 13.6; ^{31}P NMR (121 MHz) δ 116.9; IR ($CHCl_3$) ν 3434, 2962, 2359, 1731 cm^{-1}; mass spectrum (CI) *m/z* 458.1800 [$C_{18}H_{37}NO_6PS_2$ (M + 1) requires 458.1800], 458, 296, 243 (base).

Ethylene Glycol 2-Octanoyl-1-dithiophosphocholine (4). Obtained as a white foam in 82% yield after purification by flash chromatography eluting with acetone/$CHCl_3$/H_2O (67:32:1): ^{1}H NMR (300 MHz) δ 4.56−4.46 (m, 2 H), 4.30−4.12 (comp, 4 H) 4.00−3.90 (m, 2 H), 3.42 (s, 9 H), 2.28 (t, J = 7.7 Hz, 2 H), 1.62−1.50 (m, 2 H), 1.30−1.16 (comp, 8 H), 0.88−0.78 (m, 3 H); ^{13}C NMR (75 MHz) δ 173.8, 63.4, 55.1, 34.2, 31.6, 29.1, 28.9, 24.9, 22.6, 14.1; ^{31}P NMR (121 MHz) δ 116.3; IR (CH_2Cl_2) ν 3422, 2265, 1731, 1644, 910, 723 cm^{-1}; mass spectrum (CI) *m/z* 386.1583 [$C_{15}H_{33}NO_4PS_2$ (M + 1) requires 386.1589], 386 (base), 228, 207.

Ethylene Glycol 2-Decanoyl-1-dithiophosphocholine (5). Obtained as a white foam in 70% yield after purification by flash chromatography eluting with acetone/$CHCl_3$/H_2O (67:32:1): ^{1}H NMR (300 MHz) δ 4.56−4.46 (comp, 2 H), 4.30−4.14 (comp, 4 H) 4.00−3.92 (comp, 2 H), 3.43 (s, 9 H), 2.28 (t, J = 7.5 Hz, 2 H), 1.62−1.50 (m, 2 H), 1.31−1.17 (comp, 12 H), 0.88−0.81 (m, 3 H); ^{13}C NMR (75 MHz) δ 173.6, 63.2 (d, J_{CP} = 8.8 Hz), 59.3, 54.9, 34.1, 31.7, 29.3, 29.2, 29.1, 29.0, 24.7, 22.5, 14.0; ^{31}P NMR (121 MHz) δ 116.1; IR (CH_2Cl_2) ν 3422, 2931, 2856, 2259, 2216, 1731, 1669 cm^{-1}; mass spectrum (CI) *m/z* 414.1894 [$C_{17}H_{37}NO_4PS_2$ (M + 1) requires 414.1902], 414 (base), 304.

1,2-Di-(8′-nonenoyl)-*sn*-glycero-3-dithiophosphocholine (25a). Obtained as a yellow foam in 74% yield after partial purification by flash chromatography eluting with acetone/$CHCl_3$/H_2O (67:32:1) and used directly in the next step: ^{31}P NMR (121 MHz) δ 117.5.

1,2-Di-(11′-dodecenoyl)-*sn*-glycero-3-dithiophosphocholine (25b). Obtained as a pale yellow foam in 81% yield after purification by flash chromatography eluting with acetone/$CHCl_3$/H_2O (67:32:1): ^{1}H NMR (300 MHz) δ 5.79 (ddt, J = 17.1, 10.5, 6.7 Hz, 2 H), 5.30−5.22 (m, 1 H), 5.01−4.87 (comp, 4 H), 4.60−4.50 (comp, 2 H), 4.40−4.33 (m, 1 H), 4.17−4.09 (comp, 3 H), 3.98−3.91 (comp, 2 H), 3.41 (s, 9 H), 2.29 (t, J = 7.4 Hz, 2 H), 2.27 (t, J = 7.4 Hz, 2 H), 2.06−1.97 (comp, 4 H), 1.65−1.50 (comp, 4 H) 1.40−1.21 (comp, 24 H); ^{13}C NMR (125 MHz) δ 173.6, 173.2, 139.2, 114.1, 70.2 (d, J_{CP} = 8.8 Hz), 66.4 (d, J_{CP} = 8.8 Hz), 63.6, 63.0, 59.4, 55.2, 49.2, 34.4, 34.2, 33.9, 33.8, 29.4, 29.3, 29.3, 29.1, 28.9, 25.6, 24.9, 24.9; ^{31}P NMR (121 MHz) δ 118.0; IR ($CHCl_3$) ν 2924, 2852, 1728, 1466, 1094, 954, 908, 690 cm^{-1}; mass spectrum (CI) *m/z* 650.3659 [$C_{32}H_{61}NO_6PS_2$ (M + 1) requires 650.3678], 435, 284 (base), 225.

General Procedure for Converting Dithiophosphatidylcholines 25a,b into Dithiophosphatidylcholines 6 and 7. Ozone gas was bubbled into a solution of **25a**,**b** (0.4 mmol)

in CH_2Cl_2/MeOH (5:1, 8.0 mL) at −78 °C until the solution turned blue. Air was then bubbled into the solution until the solution became colorless. Me_2S (63 μL, 0.82 mmol) was added to the solution, and the mixture was stirred at room temperature for 30 min. The solution was concentrated under reduced pressure. A solution of MeOH·HCl (pH 4, 8.0 mL) and $NaCNBH_3$ (54 mg, 0.82 mmol) were added, and the solution was stirred at room temperature overnight. The solution was concentrated under reduced pressure, and the residue was purified by flash chromatography over neutral alumina eluting with the solvent system indicated.

1,2-Di-(8′-hydroxyoctanoyl)-*sn*-glycero-3-dithiophosphocholine (6). Obtained as a white foam in 16% yield after purification by flash chromatography eluting with $CHCl_3$/MeOH/H_2O (25:13:2): ^{1}H NMR (500 MHz, DMSO-d_6) δ 5.09−5.00 (m, 1 H), 4.31−4.25 (m, 2 H), 4.11−3.96 (comp, 3 H), 3.73−3.69 (comp, 2 H), 3.56 (s, 1 H), 3.51−3.48 (m, 2 H), 3.35 (s, 2 H), 3.18 (s, 9 H), 2.29−2.23 (comp, 4 H), 1.55−1.41 (comp, 8 H), 1.30−1.18 (comp, 10 H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 172.6, 172.3, 103.9, 70.6, 70.5, 65.5, 62.4, 58.2, 53.1, 52.3, 51.1, 33.5, 33.4, 33.3, 33.2, 32.0, 28.5, 28.3, 28.1, 28.0, 24.3, 24.3, 24.2, 23.9; ^{31}P NMR (121 MHz) δ 117.4; IR ν 3396, 2939, 2859, 1738, 1650, 1465, 1366, 1241, 1090, 969 cm^{-1}; mass spectrum (CI) *m*/*z* 574.2665 [$C_{24}H_{49}NO_8PS_2$ (M + 1) requires 574.2637], 360, 202 (base).

1,2-Di-(11′-hydroxyundecanoyl)-*sn*-glycero-3-dithiophosphocholine (7). Obtained as a white foam in 32% yield after purification by flash chromatography eluting with $CHCl_3$/MeOH/H_2O (25:13:2): ^{1}H NMR (300 MHz, CD_3OD) δ 5.28−5.19 (m, 1 H), 4.43−3.98 (comp, 6 H), 3.68−3.63 (comp, 4 H), 3.68 (s, 2 H), 3.67−3.63 (m, 2 H), 3.30 (s, 2 H), 3.23 (s, 9 H), 2.29−2.23 (comp, 4 H), 1.55−1.41 (comp, 8 H), 1.30−1.18 (comp, 22 H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 172.6, 172.3, 103.9, 70.5 (d, J_{CP} = 8.8 Hz), 65.4, 62.4 (d, J_{CP} = 8.8 Hz), 58.3 (d, J_{CP} = 8.8 Hz), 53.1, 52.8, 52.2, 51.1, 33.5, 33.4, 33.2, 32.0, 28.9, 28.8, 28.8, 28.7, 28.6, 28.6, 28.4, 28.4, 24.4, 24.4, 24.4, 24.0; ^{31}P NMR (121 MHz) δ 117.9; IR ν 3424, 2926, 2854, 1737, 1664, 1456, 1366, 1236, 762 cm^{-1}; mass spectrum (CI) *m*/*z* 658.3515 [$C_{30}H_{61}NO_8PS_2$ (M + 1) requires 658.3576], 443, 292 (base).

General Procedure for Converting Thiophosphorodithiolane 31 into 8, 34, and 35. DBU (1.0 mmol) was added to a solution of **31** (1.0 mmol) and **16**, **32**, or **33** (1.0 mmol) in MeCN (10 mL), and the solution was stirred under nitrogen at room temperature for 45 min. The solution was concentrated under reduced pressure, and the residue was purified by flash chromatography eluting with acetone/$CHCl_3$ (3:2). The diester (0.11 mmol) thus obtained was dissolved in CH_3CN (2.5 mL) in a plastic bottle, and 2.8 M HF (0.42 mL) was added. The mixture was stirred at room temperature for 45 min, and brine (5 mL) was added. The mixture was extracted with $CHCl_3$ (2 × 5 mL). The combined organic phases were dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography eluting with the solvent system indicated.

1,2-Di-(6′-hydroxyhexanoyl)-*sn*-glycero-3-dithiophosphocholine (8). Obtained as a colorless glass in 95% yield after purification by flash chromatography eluting with MeOH/$CHCl_3$ (1:1); ^{1}H NMR (300 MHz, CD_3OD) δ 5.32−5.24 (m, 1 H), 4.51−4.42 (comp, 2 H), 4.42 (dd, *J* = 12.0, 3.6 Hz, 1 H), 4.23 (dd, *J* = 12.0, 6.7 Hz, 1 H), 4.20−4.08 (comp, 2 H), 3.75−3.70 (comp, 2 H), 3.57 (t, *J* = 6.5 Hz, 2 H), 3.57 (t, *J* = 6.5 Hz, 2 H), 3.29 (s, 9 H), 2.43−2.34 (comp, 4 H), 1.72−1.50 (comp, 8 H), 1.50−1.36 (comp, 4 H); ^{13}C NMR (75 MHz, CD_3OD) δ 174.9, 174.6, 71.8 (d, *J* = 9.2 Hz), 67.2, 64.8 (d, *J* = 5.9 Hz), 63.7, 62.7, 60.6 (d, *J* = 5.0 Hz), 54.9, 35.1, 34.9, 33.3, 26.4, 25.8; ^{31}P NMR (121 MHz, CD_3OD) δ 117.8; IR (MeOH) ν 3396, 2932, 2860, 1730, 1043, 960, 690 cm^{-1}; mass spectrum (CI) *m*/*z* 518.2032 [$C_{20}H_{41}NO_8PS_2$ (M + 1) requires 518.2011], 362, 303 (base), 189.

1,2-Di-(6′-hydroxyhexanoyl)-*sn*-glycero-3-dithiophospho-(*N*-*t*-butoxycarbonyl)ethanolamine (34). Obtained as a colorless oil in 85% yield after purification by flash chromatography eluting with acetone/$CHCl_3$ (3:2): ^{1}H NMR (300 MHz, acetone-d_6) δ 6.27−6.16 (br s, 1 H), 5.25 (app dtd, *J* = 6.4, 5.1, 3.6 Hz, 1 H), 4.41 (dd, *J* = 11.8, 3.6 Hz, 1 H), 4.22 (dd, *J* = 11.8, 6.4 Hz, 1 H), 4.08 (dd, *J* = 8.6, 5.1 Hz, 2 H), 3.98 (dt, *J* = 10.2, 4.1 Hz, 2 H), 3.70−3.60 (comp, 2 H), 3.60−3.50 (comp, 4 H), 3.38−3.26 (comp, 2 H), 2.40−2.32 (comp, 4 H), 1.70−1.48 (comp, 8 H), 1.48−1.38 (comp, 13 H); ^{13}C NMR (75 MHz, acetone-d_6) δ 173.5, 173.3, 157.0, 78.8, 71.3 (d, J_{CP} = 9.3 Hz), 64.9, 63.7 (d, J_{CP} = 6.0 Hz), 63.4, 62.2, 41.7, 34.7, 34.5, 33.2, 28.6, 26.0, 25.4. 25.4; ^{31}P NMR (121 MHz, acetone-d_6) δ 118.2; IR (CH_2Cl_2) ν 3400, 2964, 2936, 1737, 1694, 1171, 1036, 686 cm^{-1}; mass spectrum (CI$^-$) *m*/*z* 574.1915 [$C_{22}H_{41}NO_{10}PS_2$ (M − 1) requires 574.1910], 574 (base), 473, 433, 291.

1,2-Di-(6′-hydroxyhexanoyl)-*sn*-glycero-3-dithiophospho-(*N*-*t*-butoxycarbonyl)-L-serine Trimethylsilylethyl Ester (35). Obtained as a colorless oil in 76% yield after purification by flash chromatography eluting with acetone/$CHCl_3$ (3:2): ^{1}H NMR (300 MHz, acetone-d_6) δ 6.44 (d, *J* = 7.4 Hz, 1 H), 5.24−5.17 (m, 1 H), 4.38 (dd, *J* = 12.0, 3.6 Hz, 1 H), 4.23−4.12 (comp, 6 H), 4.04 (dd, *J* = 8.7, 5.4 Hz, 2 H), 3.60−3.48 (comp, 6 H), 2.40−2.28 (comp, 4 H), 1.70−1.30 (comp, 21 H), 1.10−1.08 (m, 2 H), 0.05 (s, 9 H); ^{13}C NMR (75 MHz, acetone-d_6) δ 173.5, 173.3, 171.2, 156.4, 79.2, 71.2 (d, J_{CP} = 9.3 Hz), 65.2 (d, J_{CP} = 6.6 Hz), 63.9, 63.7, 63.4, 62.2, 55.5 (d, J_{CP} = 7.7 Hz), 34.7, 34.5, 33.3, 28.5, 26.1, 26.0, 25.5, 25.4, 17.9, −1.5; ^{31}P NMR (121 MHz, acetone-d_6) δ 119.4; IR (CH_2Cl_2) ν 3410, 2932, 1731, 1250, 694 cm^{-1}; mass spectrum (CI) *m*/*z* 718. 2520 [$C_{28}H_{53}NO_{12}PSiS_2$ (M − 1) requires 718.2516], 686, 390, 277 (base).

1,2-Di-(6′-hydroxyhexanoyl)-*sn*-glycero-3-dithiophosphoethanolamine (9). A solution of $SnCl_4$ in heptane (1 N) (155 μL, 0.34 mmol) was added dropwise to a solution of **34** (18 mg, 0.031 mmol) in CH_3CN (2.5 mL) at 0 °C. The solution was stirred at room temperature for 45 min, and the solvent was removed under reduced pressure. The residue was purified by flash chromatography eluting first with acetone/$CHCl_3$ (3:2) and then with $CHCl_3$/MeOH/H_2O (5:1:0.1) to give 13 mg (88%) of **9** as a colorless glass: ^{1}H NMR (300 MHz, CD_3OD) δ 5.28−5.20 (m, 1 H), 4.40 (dd, *J* = 11.8, 3.6 Hz, 1 H), 4.23−4.05 (comp, 5 H), 3.55 (t, *J* = 6.5 Hz, 2 H), 3.54 (t, *J* = 6.5 Hz, 2 H), 3.23−3.18 (m, 2 H), 2.40−2.31 (comp, 4 H), 1.70−1.48 (comp, 8 H), 1.48−1.34 (comp, 4 H); ^{13}C NMR (75 MHz, CD_3OD) δ 174.9, 174.6, 71.7, 65.0, 63.7, 63.1, 62.7, 41.3, 35.2, 34.9, 33.2, 26.4, 26.4, 25.8; ^{31}P NMR (121 MHz, CD_3OD) δ 122.0; IR (MeOH) ν 3369, 2934, 1732, 1009, 689 cm^{-1}; mass spectrum (FAB) *m*/*z* 476.1553 [$C_{17}H_{35}NO_8PS_2$ (M + 1) requires 476.1542], 277, 242 (base), 185.

1,2-Di-(6′-hydroxyhexanoyl)-*sn*-glycero-3-dithiophospho-L-serine (10). A solution of **35** (45 mg, 0.063 mmol) in THF (3 mL) containing 1 N TBAF in THF (187 μL, 0.187 mmol) was stirred for 90 min. The solvent was removed under reduced pressure, and the residue was purified by flash chromatography eluting with acetone/$CHCl_3$ (3:1) to yield 34 mg (88%) of diol as a colorless oil contaminated with a small amount of TBAF: ^{31}P NMR (121 MHz, CD_3OD) δ 119.3; mass spectrum (CI$^-$) *m*/*z* 618.1793 [$C_{23}H_{41}NO_{12}PS_2$ (M − 1) requires 618.1808], 524, 409 (base), 204.

A solution of $SnCl_4$ in heptane (1 N) (310 μL, 0.31 mmol) was added to a solution of the diol (34 mg, 0.055 mmol) thus obtained in CH_3CN (5 mL) at 0 °C. The mixture was stirred at room temperature for 1 h, and the solvent was removed under reduced pressure. The residue was purified by flash chromatography eluting first with acetone/$CHCl_3$ (3:1) and then with $CHCl_3$/MeOH/H_2O (2:2:0.1) to give 21.6 mg (75%) of **10** as a colorless glass: ^{1}H NMR (300 MHz, CD_3OD) δ 5.27−5.20 (m, 1 H), 4.52−3.97 (comp, 7 H), 3.55 (t, *J* = 6.4 Hz, 2 H), 3.54 (t, *J* = 6.4 Hz, 2 H), 2.40−2.32 (comp, 4 H), 1.70−1.48 (comp, 8 H), 1.47−1.31 (comp, 4 H); ^{13}C NMR (75 MHz, CD_3OD) δ 175.0, 174.7, 169.4, 71.8 (d, J_{CP} = 9.3 Hz), 64.9 (d, J_{CP} = 6.0 Hz), 64.1 (d, J_{CP} = 6.0 Hz), 63.7, 62.7, 62.7, 54.4 (d, J_{CP} = 8.8 Hz), 35.1, 34.9, 33.3, 26.4, 26.4, 25.8; ^{31}P NMR (121 MHz,

CD_3OD) δ 117.9; IR (MeOH) ν 3341, 2936, 1730, 1025, 689 cm^{-1}; mass spectrum (FAB-) *m/z* 519.1357 [$C_{18}H_{37}NO_7P$ (M − 1) requires 519.1362], 431, 245, 205, 183 (base).

Inorganic Phosphate Quantitation Assay. The activity constants, V_{max}/K_M, of PLC_{Bc} were determined using a sensitive, enzyme-coupled assay that is based on the quantitation of Pi.[7] In summary, the phosphorylated headgroup produced by the PLC-catalyzed hydrolysis of phospholipids was treated with alkaline phosphatase to liberate Pi, which formed a complex with ammonium molybdate. The complex was then reduced to a blue molybdenum state with ascorbic acid to yield a blue solution with a λ_{max} at 700 nm. The initial velocity versus substrate concentration data were obtained in duplicate in the presence and absence of inhibitor at substrate concentrations well below their CMCs in the assay buffer. The activity constants, V_{max}/K_M, were obtained from the initial slopes of the initial velocity versus substrate concentration curves at low substrate concentrations, and the K_i for each inhibitor was determined from these data using eq 1.

$$\nu = V_{max}[S]/\alpha K_M + [S] \quad \text{where } \alpha = 1 + [I]/K_i \qquad (1)$$

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Supporting Information Available: Copies of ^1H NMR spectra of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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