covered starting material. The solution was cooled to room temperature and quenched by the addition of methanol (3 mL) and 10 drops of acetic acid. The solution was transferred to a 100-mL round-bottom flask and concentrated to a volume of 5 mL, diluted with ether, and filtered through a pad of Celite. The solvent was removed under reduced pressure, and the product was isolated by MPLC, collecting 8-mL fractions and eluting with hexanes (80 mL), 5% EtOAc/hexanes (80 mL), 10% EtOAc/hexanes (120 mL), and 20% EtOAc/hexanes (200 mL). The product was found in fractions 40–49 and concentrated to give 65 mg (82%) of a colorless oil: $[\alpha]_D$ −68.3° (*c* 32.5, $CHCl_3$); R_f 0.70 (toluene/dioxane/AcOH, 20:10:1 volume ratio); 300-MHz ^1H NMR ($CDCl_3$) δ 6.73 (ddd, *J* = 4.8 11.1, 15.7 Hz, 1 H), 6.57 (dd, *J* = 6.6, 15.6 Hz, 1 H), 6.14 (dd, *J* = 0.9, 15.6 Hz, 1 H), 5.74 (br d, *J* = 15.7 Hz, 1 H), 5.29 (ddq, *J* = 3.6, 11.1, 6.3 Hz, 1 H), 4.89 (ddq, *J* = 4.8, 6.6, 6.3 Hz, 1 H), 4.04 (ddd, *J* = 0.9, 6.6, 8.6 Hz, 1 H), 3.90 (ddd, *J* = 5.1, 5.8, 8.6 Hz, 1 H), 2.55 (dddd, *J* = 1.4, 3.6, 4.8, 12.8 Hz, 1 H), 2.29 (br dt, *J* = 12.8, 11.1 Hz, 1 H), 1.99 (ddd, *J* = 5.1, 6.6, 15.0 Hz, 1 H), 1.89 (ddd, *J* = 4.8, 5.8, 15.0 Hz, 1 H), 1.49 (s, 3 H), 1.43 (s, 3 H), 1.39 (d, *J* = 6.3 Hz, 3 H), 1.35 (d, *J* = 6.3 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 166.1, 165.4, 144.4, 142.1, 125.5, 125.4, 109.2, 79.0, 78.6, 69.1, 68.3, 40.9, 35.3, 27.2, 26.8, 20.5, 19.8; IR (neat) 2980, 2930, 1715, 1650, 1440, 1370, 1310, 1220, 1165, 970, 750; mass spectrum (FAB, *p*-nitrobenzyl alcohol), *m/e* (relative intensity) (M + 1) 325 (55.8), 309 (20.5), 289 (8.3), 279 (14.6), 267 (100e, 183 (55.5); exact mass calcd for $C_{17}H_{25}O_6$ 325.16511, found 325.16486.

Preparation of Colletodiol, (3*E*,6*R*,9*E*,11*R*,12*R*,14*R*)-11,12-Dihydroxy-6,14-dimethyl-1,7-dioxacyclotetradeca-3,9-diene-2,8-dione (7). To a stirring solution of **42** (63 mg, 0.19 mmol) in methanol (10 mL) was added Dowex 50W-8 H^+ resin (50 mg), and the reaction mixture was heated at reflux for a period of 36 h. The solution was cooled to room temperature, filtered, and concentrated. The product was purified by MPLC, collecting 7-mL fractions and eluting with 125-mL portions of 20% EtOAc/hexanes, 35% EtOAc/hexanes, 50% EtOAc/hexanes, and finally 75% EtOAc/hexanes. The product was found in fractions 50–59 and concentrated to give 41 mg (76%) of colletodiol, which was identical in every way with a natural sample: mp 165 °C (lit.[3] mp 164–167 °C); $[\alpha]_D$ +36.9° (*c* H), $CHCl_3$) (lit.[3] $[\alpha]_D$ +36° (*c* 1.0, $CHCl_3$)); R_f 0.37 (toluene/dioxane/AcOH, 20:10:1 volume ratio); 300-MHz ^1H NMR ($CDCl_3$) δ 6.74 (dd, *J* = 5.6, 15.7 Hz, 1 H), 6.72 (ddd, *J* = 4.9, 11.1, 15.6 Hz, 1 H), 6.14 (dd, *J* = 1.2, 15.7 Hz, 1 H), 5.73 (br d, *J* = 15.6 Hz, 1 H, 5.32 (ddq, *J* = 3.1, 11.1, 6.4 Hz, 1 H), 5.18 (ddq, *J* = 2.0, 4.5, 6.7 Hz, 1 H), 4.08 (ddd, *J* = 1.2, 5.6, 9.0 Hz, 1 H), 3.67 (ddd, *J* = 1.6, 5.9, 9.0 Hz, 1 H), 2.6 (br s, 2 H), 2.52 (dddd, *J* = 1.3, 3.1, 4.6, 12.6 Hz, 1 H), 2.24 (br dt, *J* = 12.6, 11.1 Hz, 1 H), 2.02 (ddd, *J* = 1.6, 4.5, 15.8, Hz, 1 H), 1.50 (ddd, *J* = 2.0, 5.0, 15.8 Hz, 1 H), 1.37 (d, *J* = 6.3 Hz, 3 H), 1.36 (d, *J* = 6.7 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 166.5, 165.1, 146.2, 144.1, 125.7, 123.9, 73.9, 71.8, 68.7, 67.9, 41.1, 36.2, 20.4, 18.1; IR ($CHCl_3$) 3240–3620 (br), 2980, 2950, 2860, 1715, 1655, 1445, 1350, 1315, 1260, 1170, 1105, 1055, 980; mass spectrum (CI, methane), *m/z* (relative intensity) (M + 1) 285 (3.4), 267 (6.3), 249 (5.9), 201 (4.0), 183 (13.7), 155 (11.0)e, 137 (16.0), 113 (100), 95 (14.8).

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Registry No. **7**, 21142-67-6; **14**, 116809-48-4; **15**, 116809-49-5; **16**, 6884-01-1; **16** (2,3-diol), 3162-96-7; **17**, 101155-76-4; **17** (iodide), 116809-47-3; **20**, 101155-81-1; **21**, 72212-13-6; **22**, 94498-98-3; **22** (triol), 116907-46-1; α-**22** (triol), 116907-47-2; **24**, 24915-95-5; (*S*)-**24**, 56816-01-4; **25**, 116809-41-7; *epi*-**25**, 116809-42-8; **26**, 116809-44-0; **27a**, 116809-45-1; **27b**, 116809-46-2; **28**, 32443-51-9; **29a**, 94499-07-7; **29b**, 94595-41-2; **30**, 116907-49-4; **32**, 116809-52-0; **33**, 110410-39-4; **35**, 116809-43-9; **36**, 116809-50-8; **36** (diastereomer), 116907-48-3; **37**, 116809-51-9; **38**, 3095-95-2; **39**, 116840-66-5; **40**, 116809-53-1; **41**, 116908-88-4; **42**, 91273-95-9; $Ph_3P{=}CHCOOEt$, 1099-45-2; $CH_2{=}CHCH_2OTBS$, 85807-85-8.

Synthesis of Sulfur-Substituted Phospholipid Analogues as Mechanistic Probes of Phospholipase A_2 Catalysis

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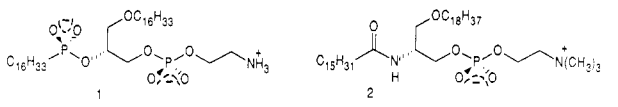
Phospholipid analogues with sulfur-containing functional groups that replace the ester at the 2-position of the glycerol backbone have been prepared. Thiophosphonate analogues in which one of the nonbridging phosphonate oxygens is replaced by sulfur were synthesized by a newly developed route. Both phosphorus stereoisomers were prepared. The synthesis involves the reaction of phosphoroamidites with hydrogen sulfide in the presence of tetrazole to give thiophosphites, which react with terminal olefins in the presence of a radical initiator to give thiophosphonates. A phospholipid analogue in which the ester at the 2-position was replaced with a thioamide was found to readily cyclize to the thiazoline. To circumvent this problem, amide and thioamide analogues were prepared that contain a phosphonate group linked to carbon 3 of the glycerol backbone in place of the phosphate group.

Introduction

Recently we reported that a phospholipid analogue **1** containing a phosphonate in place of the ester at the 2-position of the glycerol backbone was a tight-binding inhibitor of phospholipase A_2.[1] Compound **1** binds some 2000-fold tighter to the enzyme than the analogous ester substrate. Others have reported that amide analogous such as **2** are also good inhibitors, binding some 40-fold tighter than the substrate.[2]



1 2

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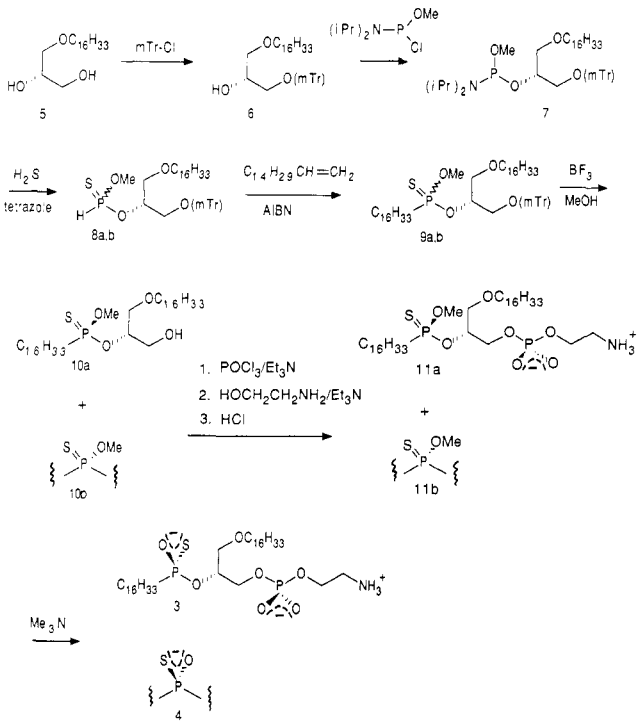
0022-3263/89/1954-0906$01.50/0

Scheme I. Synthesis of Thiophosphonate Phospholipid Analogues 3 and 4 (See Text for Details)



Phospholipase A_2 requires calcium for activity.[3] Two roles for the metal have been proposed on the basis of the high-resolution X-ray structure.[4] Firstly, calcium may be involved in binding to the phosphate group of the substrate. Tsai and colleagues using phospholipids substituted with sulfur in the phosphate group have provided direct evidence for a phosphate–calcium coordination.[5] Secondly, calcium may serve a catalytic role by coordinating to the enzyme-susceptible ester carbonyl group and the derived tetrahedral intermediate. It is tempting to speculate that the phosphonate group of **1** and the amide carbonyl group of **2** are bound to the metal in the active site of phospholipase A_2. One approach to testing this idea is to prepare and study derivatives of **1** and **2** containing sulfur. The obvious choices are thiophosphonates and thioamides. For the former compounds, sulfur substitution in the nonbridging phosphonate oxygens creats a chiral center at phosphorous, and the availability of both isomers would be desirable.

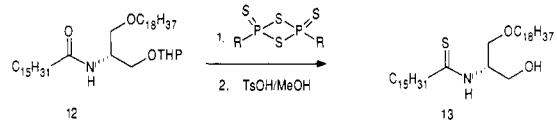
Attempted synthesis of thiophosphonate phospholipid analogues by conventional methods failed because of solubility problems. A new method of synthesis was developed that should be generally useful and is described below. The biochemical studies with these compounds will be published elsewhere.

Results and Discussion

The synthesis of the desired thiophosphonates **3** and **4** is diagrammed in Scheme I. Preparation of the starting material, 1-hexadecyl-*sn*-glycerol **5**, is accomplished by using standard procedures, starting from 1,2-isopropylidene-*sn*-glycerol.[6] The enantiomeric purity of **5** was determined to be greater than 99% by reaction with 2(*S*)-methoxy-2-phenylacetyl chloride (*O*-methyl mandelyl chloride) followed by high-resolution NMR analysis of the diester.[7] Experiments in which the *O*-methyl mandelate esters derived from both enantiomers of 1-alkylglycerol were used showed that the diastereomers were easily distinguished in the NMR spectrum. This method of analysis is much more sensitive than polarimetry and should be generally useful for the determination of enantiomeric excess with synthetic phospholipid intermediates.

Diol **5** was selectively monomethoxytritylated to give **6**, which was the starting point for the incorporation of the thiophosphonate group. The first step was the preparation of thiophosphites **8a,b** as a mixture of diastereomers in high yield (97%) by first reaction of **6** with *N,N*-diisopropylmethylphosphonamidic chloride to give phosphoroamidite **7** followed by reaction with the hydrogen sulfide in the presence of tetrazole. No reaction with hydrogen sulfide was observed in the absence of tetrazole. Thiophosphites can normally be converted into thiophosphonates by a reaction in which the thiophosphite is first deprotonated with base followed by alkylation with an alkyl halide. This reaction failed in the present case since the anion derived from **8a,b** was insoluble in a variety of solvents. This problem was overcome by forming the carbon–phosphorous bond in a radical reaction between thiophosphites **8a,b** and a terminal olefin.[8] Thus **8a,b** could be dissolved in 1-hexadecene at 100 °C in the presence of the radical initiator 2,2′-azobis[2-methylpropionitrile] to give thiophosphonates **9a,b** in excellent yield (97%). Detritylation with BF_3/MeOH afforded alcohols **10a,b** which could be easily separated by chromatography on silica gel. The incorporation of the phosphoryl ethanolamine group was accomplished by using standard methods.[9] Finally, thiophosphonate methyl esters **11a,b** were demethylated with trimethylamine in toluene to furnish the desired phospholipid analogues **3** and **4**. The absolute stereochemistry at phosphorous for these compounds has not been assigned at this time.

Attempts to prepare phospholipid analogues in which the ester at the 2-position is replaced by a thioamide were unsuccessful. Treatment of the tetrahydropyranyl ether **12** (THP) of known chiral amide[10] with Lawesson's reagent[11] and deprotection produced thioamide **13**. It was not



possible to incorporate the phosphocholine group. Treatment of alcohol **13** with 2-chloro-2-oxo-1,3-dioxaphospholane[12] gave only thiazoline **15** presumably as the result of an intramolecular S-alkylation from the intermediate phosphate triester **14**. It was possible to trans-

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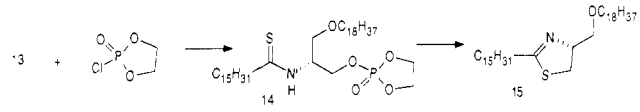
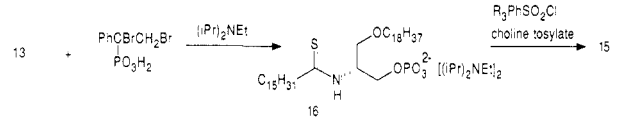


form **13** into phosphate monoester **16** by treatment with (1-phenyl-1,2-dibromomethyl)phosphonic acid as a source of metaphosphate[13] under basic conditions; however, attempted coupling of **16** with choline using 2,4,6-triisopropylbenzenesulfonyl chloride[14] resulted in the formation of thiazoline **15**. From these studies, it was apparent that the desired thioamide would not be stable.



In order to prevent the cyclization of the thioamide to the thiazoline, phosphonates **17** and **18** were prepared as outlined in Scheme II. Amino alcohol **19** was reacted with *p*-toluenesulfonyl chloride to give tosylamino tosylate **20**. Reaction of **20** with an excess amount of the lithium salt of diethyl methylphosphonate formed diethyl phosphonate **22** via the aziridine **21**. The aziridine could be isolated, if desired, by treating **20** with less base. Diethyl ester **22** was hydrolyzed by heating in aqueous hydrochloric acid and the phosphonic acid **23** obtained was reacted with choline tosylate in the presence of trichloroacetonitrile[15] to give phosphonyl choline **24**. The tosyl group proved difficult to remove by normal reductive reactions. Na/NH_3, Na/naphthalene, and Red-Al (bis(2-methoxyethoxy)aluminum hydride) (Aldrich) reductions were inaccessible due to solubility problems, while amalgams, such as Al(Hg) and Na(Hg), gave no reaction. The tosyl group was successfully removed by photolysis in methanol/ether[16] to give amine **25**. Compound **25** was coupled with either hexadecanoyl chloride or methyl dithiohexadecanoate[17] to give **17** and **18**, respectively.

Experimental Section

General Methods. ^1H NMR spectra were obtained at 300 and 500 MHz. ^{31}P NMR were recorded at 121 MHz with trimethyl phosphate as a reference standard (assigned to 2.0 ppm). Coupling constants for resolved multiplets are reported in hertz. Thin-layer chromatography was performed on silica gel plates (0.25 mm, Merck), and the following detection methods were used: AMA, dipped into a solution of 5% ammonium molybdate, 4.2% H_2SO_4, and 0.6% sodium arsenate and heated on a hot plate; N, sprayed with 1% ninhydrin in ethanol and heated in an oven; PAA, dipped into a solution containing *p*-anisaldehyde (37 mL), 95% ethanol (1.35 L), concentrated H_2SO_4 (50 mL), and acetic acid (15 mL) and heated on a hot plate. Flash chromatography was performed with silica gel (230–400 mesh, Merck) with use of the following solvents: chloroform (C), ether (E), ethyl acetate (EA), methanol (M), methylene chloride (MC), low-boiling petroleum ether (PE), and water (W). Ether and THF were dried by distillation from Na/benzophenone under Ar. Methylene chloride and chloroform were dried by distillation from P_2O_5 under Ar. Accurate mass liquid secondary ion mass spectra (LSIMS) were obtained at the University of California, San Francisco, or at the University of Washington mass spectrometry facilities. Except where noted, the purity of all titled compounds was established to be at least 95% by TLC and ^1H NMR analyses.

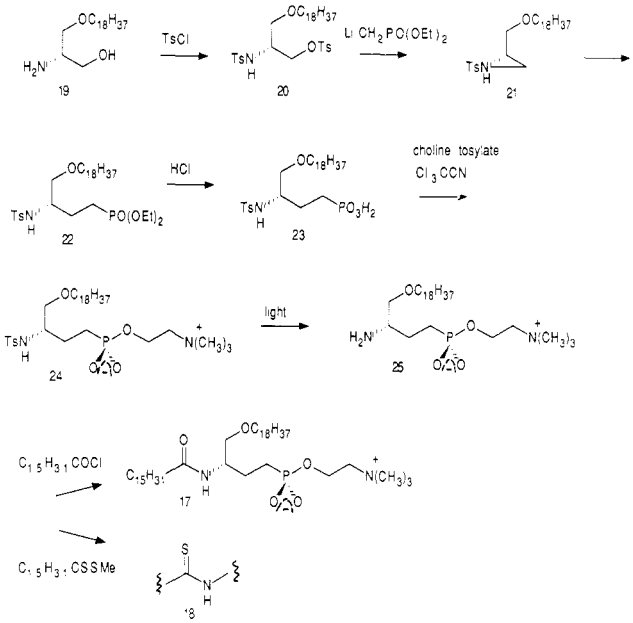
1-Hexadecyl-*sn*-glycerol (5). 1,2-Isopropylidene-*sn*-glycerol were prepared from D-mannitol[6a] and alkylated with hexadecyl bromide followed by deprotection[6b] to give 3-hexadecyl-*sn*-glycerol. Inversion of the configuration at carbon 2 was accomplished as described[6c] to produce **5**. The enantiomeric purity of **5** was determined by reaction with (*S*)-*O*-methylmandelyl chloride as follows. (*S*)-*O*-Methylmandelic acid[7] (100 mg) was treated with $SOCl_2$ (0.5 mL) in dry benzene (3 mL) and refluxed in an oil bath (85–90 °C) for 1.5–2 h. The solution was concentrated to dryness *in vacuo*. A solution of **5** (32 mg), 4-(dimethylamino)pyridine (2 mg), and pyridine (60 mg) in dry CH_2Cl_2 (2 mL) was added, and the reaction was stirred overnight at room temperature. The solvent was removed in vacuo, and the residue was dissolved in ether and washed with 5% $NaHCO_3$, 0.1 N HCl, and water. The organic layer was dried ($MgSO_4$) and concentrated in vacuo. NMR analysis ($CDCl_3$, 500 MHz) for **5** δ 4.53, 4.76 (PhC*H*OMeCOOR); for *ent*-**5** δ 4.64, 4.71.

1-Hexadecyl-3-(methoxytrityl)-*sn*-glycerol (6). Compound **5** was treated with methoxytrityl chloride as described[6b] to give **6** after purification on silica.

1-Hexadecyl-2-(*N*,*N*-diisopropylmethylphosphonamidyl)-3-(methoxytrityl)-*sn*-glycerol (7) and Thiophosphites (8a,b). Compound **6** (3.3 g, 5.6 mmol) and triethylamine (1.15 mL, 8.4 mmol) were dissolved in dry CH_2Cl_2 (22 mL) under Ar. With stirring, *N*,*N*-diisopropylmethylphosphonamidic chloride (1.22 g, 6.2 mmol, Aldrich) was added via a syringe. After 10 min at room temperature, a TLC analysis of the reaction mixture (10% EA in PE) showed the absence of starting material. Ethyl acetate (15–20 mL) was added, the triethylamine hydrochloride was removed by filtration, and the filtrate was dried down for 3–4 h in vacuo. To the residue was added dry CH_2Cl_2 (30 mL), and tetrazole (0.49 g, 7 mmol, Aldrich). Hydrogen sulfide was slowly bubbled into the solution for 1.5 h, and the mixture was stirred overnight at room temperature. Most of the hydrogen sulfide was removed by warming the solution to 40 °C in a fume hood, a solution of 25% ethyl acetate in petroleum ether was added, and the solid that precipitated was removed by filtration. The filtrate was concentrated to dryness in vacuo and purified on silica (first with 100 mL of PE followed by 8% EA in PE) to give **8a,b** (3.7 g, 97%) as a viscous liquid: TLC (R_f 0.64, 10% EA in PE, AMA); NMR ($CDCl_3$, 300 MHz) δ 0.89 (t, J = 6.5, 3 H), 1.29 (m, 26 H), 1.51 (m, 2 H), 3.28 (m, 2 H), 3.21–3.65 (m, 4 H), 3.72 (dd, J = 15.0, 13.0, 3 H), 3.80 (s, 3 H), 4.88 (br s, 1 H), 6.73 (d, J = 7.5, 2 H), 7.18–7.35 (m, 10 H), 7.45 (d, J = 8.0, 2 H), 7.87 (dd, 1 H, J = 665, 7.0, PH).

Thiophosphonates 9a,b. Compounds **8a,b** (1.51 g, 2.2 mmol) and 1-hexadecene (1.48 g, 6.6 mmol, Aldrich) were added to a 4-mL dry, heavy-walled reaction vial. The vial was tightly capped and heated in an oil bath at 100 °C. 2,2′-Azobis[2-methylpropionitrile] (90 mg, Chemalog) was quickly added, and the vial was heated

Scheme II. Synthesis of Amide and Thioamide Phospholipid Analogues 17 and 18 (See Text for Details)



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at 105 °C for 100 min. The mixture was applied directly to a silica column followed by elution first with PE then with 2.5% EA in PE to give the product as a clear oil (1.95 g, 97%): TLC (R_f 0.70, 10% EA in PE, AMA); NMR ($CDCl_3$, 500 MHz) δ 0.90 (t, J = 6.5, 6 H), 1.10–1.42 (m, 52 H), 1.59 (br s, 2 H), 1.68 (br s, 2 H), 1.90–2.02 (m, 2 H), 3.19–3.20 (m, 2 H), 3.35–3.48 (m, 2 H), 3.55 (d, J = 13.5, 1.5 H), 3.58–3.66 (m, 2 H), 3.70 (d, J = 13.5, 1.5 H), 3.80 (s, 3 H), 4.89 (br s, 1 H), 6.74 (d, J = 8.5, 2 H), 7.19–7.50 (m, 12 H). Anal. Calcd for $C_{56}H_{91}O_5PS$ (907.41): C, 74.13; H, 10.11. Found: C, 74.57; H, 10.30.

Thiophosphonates 10a and 10b. Compounds **9a,b** (1.95 g, 2.1 mmol) were dissolved in dry MC (25 mL). The solution was cooled to 0 °C, and BF_3–methanol (1.02 mL, 14% BF_3 in methanol, Sigma) was added. After 20 min, TLC showed the absence of starting material. The solution was washed twice with ice-cold water, and the organic layer was dried over $MgSO_4$. The mixture of diastereomers **10a,b** were separated on silica (first with 200 mL of PE and then with 3 L of 5% EA in PE and last with 500 mL of 10% EA in PE) to give **10a** (174 mg, 13%) and **10b** (200 mg, 15%) as white waxes. A substantial amount of mixed product was recovered from the column and saved for future use. For **10a**: TLC (R_f 0.37, 10% EA in PE, AMA); NMR ($CDCl_3$, 500 MHz) δ 0.89 (t, J = 6.6, 6 H), 1.30 (m, 50 H), 1.37 (m, 2 H), 1.58 (m, 2 H), 1.65 (m, 2 H), 2.03 (m, 2 H), 3.46 (m, 2 H), 3.61 (m, 2 H), 3.74 (d, J = 14.0, 3 H), 3.79 (m, 2 H), 4.78 (br s, 1 H); ^{31}P NMR ($CDCl_3$, 121 MHz) δ 102.83; LSIMS (positive ion mode) 635 (M + 1). Anal. Calcd for $C_{36}H_{75}O_4PS$ (635.06): C, 68.09; H, 11.90. Found: C, 68.23; H, 12.02. For **10b**: TLC (R_f 0.22, 10% EA in PE, AMA); NMR ($CDCl_3$, 500 MHz) similar to **10a** except there are small differences in chemical shifts and coupling patterns; ^{31}P NMR ($CDCl_3$, 121 MHz) δ 103.18; LSIMS (positive ion mode) 635 (M + 1).

Phosphatidylethanolamine Analogues 11a and 11b. A solution of **10a** (174 mg, 0.27 mmol) and triethylamine (43 μL, 0.3 mmol) in dry CH_2Cl_2 (2 mL) was added to freshly distilled $POCl_3$ (26 μL, 0.27 mmol) in a 10-mL flask at 0 °C under Ar via syringe over 15 min. The solution was stirred at room temperature for 1.5 h then at 40 °C for about 20 min. A solution of 2-aminoethanol (20 mg, 0.33 mmol) and triethylamine (130 μL, 0.9 mmol) in dry CH_2Cl_2 (0.5 mL) was added, and stirring was continued for 2 h. The precipitated solid was removed by filtration, and the filtrate was concentrated to dryness in vacuo. The residue was treated with 2-propanol (4 mL), chloroform (4 mL), and 1 N HCl (0.5 mL) with stirring at room temperature for 1.5 h. The solution was dried in vacuo, and the crude material was purified on silica (first with 10% M in C then with 25% M in C) to give the product as a white powder (90 mg, 43%) after lyophilization from benzene containing a few drops of chloroform and methanol: TLC (R_f 0.44, 25% M in C, AMA, or N); NMR ($CDCl_3$, 500 MHz) δ 0.89 (t, J = 7.0, 6 H), 1.3 (m, 50 H), 1.39 (s, 2 H), 1.64 (br s, 2 H), 1.75 (br s, 2 H), 1.98 (m, 2 H), 3.20 (br s, 2 H), 3.46 (m, 2 H), 3.64 (m, 2 H), 3.72 (d, J = 13.8, 3 H), 3.96 (br s, 2 H), 4.15 (br s, 2 H), 4.80 (br s, 1 H), 8.3–8.75 (br s, 3 H); ^{31}P NMR ($CDCl_3$, 121 MHz) δ 0.0, 101.75; LSIMS (positive ion mode) 758 (M + 1). Anal. Calcd for $C_{38}H_{81}O_7NP_2S$ (758.13): C, 60.20; H, 10.77; N, 1.84. Found: C, 58.69; H, 10.91; N, 1.81. The same procedure was followed to prepare **11b**: TLC (R_f 0.30, 25% M in C, AMA, or N); ^{31}P NMR ($CDCl_3$, 121 MHz) δ −1.0, 102.93; LSIMS (positive ion mode) 758 (M + 1).

Phosphatidylethanolamine Analogues 3 and 4. Compound **11a** (86 mg, 0.11 mmol) and toluene (0.5 mL) were added to a 10-mL flask, which was cooled to about −20 °C. The flask was charged with about 1 mL of trimethylamine. The flask was tightly stoppered and stirred in an oil bath at 60 °C for 23 h. The solution was concentrated in vacuo, and the residue was purified on silica (first with 50 mL of 10% M in C followed by 200 mL of 25% M in C and 300 mL of 50% M in C) to give the pure product as a white powder (52 mg, 62%) after lyophilization from benzene: TLC (R_f 0.14, 25% M in C, AMA, or N); NMR (10% CD_3OD in $CDCl_3$, 500 MHz) δ 0.89 (t, J = 7.0, 6 H, methyls), 1.25–1.45 (m, 52 H, $CH_3(CH_2)_{13}$), 1.58 (br s, 2 H, OCH_2CH_2), 1.70 (br s, 2 H, CH_2CH_2PS), 1.85 (br s, 2 H, CH_2PS), 3.05 (br s, 2 H, CH_2N), 3.45 (s, 2 H, $OCH_2(CH_2)_{14}$), 3.55 (br s, 2 H, $CH_2OC_{16}H_{33}$), 3.9–4.2 (br s, 4 H, CH_2OPOCH_2), 4.5 (br s, 1 H, CHOPS); ^{31}P NMR (10% CD_3OD in $CDCl_3$, 121 MHz) δ −2.5 (br s), 80.5 (br s); LSIMS (positive ion mode) 744 (M + 1). The same procedure was used to prepare **4**: TLC (R_f 0.64, 25% NH_4OH in ethanol, N); ^{31}P NMR (10% CD_3OD in $CDCl_3$, 121 MHz) 2.0–4.0, 83.0–86.0; LSIMS (positive ion mode) 744 (M + 1).

1-Octadecyl-2-deoxy-2-(hexadecanoylamino)-3-(tetrahydropyranyl)-*sn*-glycerol (12). 1-Octadecyl-2-deoxy-2-(hexadecanoylamino)-*sn*-glycerol[10] (1.24 g, 2.13 mmol) was suspended in dry chloroform (10 mL) and warmed to 35 °C in order to dissolve the alcohol. Dihydropyran (0.359 g, 4.27 mmol) and pyridinium tosylate (0.037 g, 0.15 mmol) were added successively, and the reaction mixture was stirred for 3 h at room temperature. The solution was diluted with chloroform, and the organic layer was washed with 1 N HCl, 5% $NaHCO_3$, and brine. Drying ($MgSO_4$), concentration, and purification on silica (40% E in PE) yielded the product (1.16 g, 82%) as a white wax: TLC (R_f 0.27, 50% E in PE, AMA); NMR ($CDCl_3$, 300 MHz) δ 0.88 (t, J = 6.5, 6 H), 1.12–1.40 (m, 54 H), 1.45–1.85 (m, 10 H), 2.18 (t, J = 7.0, 2 H), 3.39–3.57 (m, 5 H), 3.62–3.73 (m, 1 H), 3.78–3.89 (m, 2 H), 4.19 (m, 0.5 H), 4.27 (m, 0.5 H), 4.56 (m, 0.5 H), 4.61 (m, 0.5 H), 5.82 (d, 0.5 H), 6.04 (d, 0.5 H).

1-Octadecyl-2-deoxy-2-(thiohexadecanoylamino)-*sn*-glycerol (13). To a solution of amide **12** (1.03 g, 1.55 mmol) in dry THF (20 mL) under Ar was added Lawesson's reagent (0.376 g, 0.931 mmol, Aldrich). The mixture was refluxed for 45 min, and then methanol (10 mL) and *p*-toluenesulfonic acid (0.027 g, 0.157 mmol) were added, and the mixture was stirred 4 h at room temperature. The solvents were evaporated in vacuo, and the residue was dissolved in ether and washed with 1 N HCl, 5% $NaHCO_3$, and brine. Concentration and purification on silica (100% ether) afforded thioamdie **13** (0.509 g, 55%) as a white wax: TLC (R_f 0.44, 100% C, AMA); NMR ($CDCl_3$, 300 MHz) δ 0.88 (t, J = 6.5, 6 H), 1.20–1.36 (m, 54 H), 1.56 (m, 2 H), 1.78 (m, 2 H), 2.69 (t, J = 7.5, 2 H), 3.38–3.51 (m, 2 H), 3.72–3.86 (m, 3 H), 4.01 (dd, J = 12.0, 3.0, 1 H), 4.73 (m, 1 H), 7.89 (d, J = 9.0, 1 H); IR ($CHCl_3$) 2950, 1530, 1480 cm^{-1}.

2-Hexadecyl-4-[(octadecyloxy)methyl]-2-thiazoline (15). To a mixture of **13** (127 mg, 0.213 mmol) in dry chloroform (10 mL) at 4 °C was added triethylamine (30 μL, 0.215 mmol). To this was added 2-chloro-2-oxo-1,3,2-dioxaphospholane[12] (30.6 mg, 0.215 mmol) in chloroform (0.3 mL) in one portion. The mixture was stirred at room temperature for 24 h. The triethylamine hydrochloride was filtered, and the filtrate was concentrated in vacuo. Purification on silica (20% E in PE) gave **15** as the major product: TLC (R_f 0.36, 25% E in PE, AMA); NMR ($CDCl_3$, 300 MHz) δ 0.89 (t, J = 7.0, 6 H), 1.20–1.45 (m, 54 H), 1.60 (m, 4 H), 2.50 (t, J = 7.5, 2 H), 3.23 (dd, J = 11.0, 7.0, 1 H), 3.32–3.54 (m, 4 H), 3.68 (dd, J = 9.5, 4.0, 1 H), 4.64 (m, 1 H); IR ($CHCl_3$) 2950, 1702, 1615, 1460, 1100 cm^{-1}.

Bis(diisopropylethylammonium) 1-Octadecyl-2-deoxy-2-(thiohexadecanoylamino)-*sn*-glycero-3-phosphate (16). Solid (1-phenyl-1,2-dibromoethyl)phosphonic acid[13] (35.1 mg, 0.102 mmol) was added to a solution of thioamide **13** (55.3 mg, 0.093 mmol) in dry chloroform (2 mL). The mixture was vigorously stirred at room temperature, and diisopropylethylamine amine (35.5 μL, 0.204 mmol, Aldrich) was added in one portion. The reaction was stirred overnight, and then the chloroform was removed in vacuo at 25 °C. The solid residue was suspended in ether, cooled to 0 °C, and filtered to afford the product, which contained trialkylammonium bromide: NMR ($CDCl_3/CD_3OD$, 500 MHz) δ 0.88 (t, J = 7.0, 6 H), 1.12–1.35 (m, 54 H), 1.55 (m, 2 H), 1.78 (m, 2 H), 2.66 (t, J = 7.5, 2 H), 3.48 (m, 2 H), 3.67 (m, 2 H), 4.10 (m, 2 H), 4.83 (m, 1 H).

1-Octadecyl-2-deoxy-2-[(*p*-tolylsulfonyl)amino]-3-(*p*-tolylsulfonyl)-*sn*-glycerol (20). Amino alcohol **19**[10] (1.56 g, 4.55 mmol) was suspended in dry pyridine (25 mL) and cooled to 0 °C. Tosyl chloride (2.60 g, 13.64 mmol) dissolved in pyridine (5 mL) was added dropwise, and the resulting orange solution was stirred overnight at room temperature. The pyridine was removed in vacuo, and the residue was dissolved in ether. The pyridinium hydrochloride was filtered, and the filtrate was washed twice with 1 N HCl, twice with 5% $NaHCO_3$, and twice with brine. The organic layer was dried ($MgSO_4$) and concentrated in vacuo followed by purification of the residue on silica (30% E in PE) to afford **20** (2.29 g, 77%) as a white powder after lyophilization from benzene: TLC (R_f 0.35, 50% E in PE, AMA); NMR ($CDCl_3$, 300 MHz) δ 0.93 (t, J = 6.5, 3 H), 1.20–1.48 (m, 32 H), 2.51 (s, 3 H), 2.55 (s, 3 H), 3.26 (m, 3 H), 3.43 (dd, J = 10.0, 4.0, 1 H),

3.57 (m, 1 H), 3.96 (dd, J = 11.0, 5.0, 1 H), 4.08 (dd, 1 H), 4.95 (d, J = 8.0, 1 H), 7.33 (d, J = 8.0, 2 H), 7.40 (d, 2 H), 7.75 (d, J = 7.0, 2 H), 7.79 (d, J = 7.0, 2 H); $[\alpha]_D$ +16.30° (c 2.08, chloroform).

Diethyl [4-(Octadecyloxy)-3(*S*)-[(*p*-tolylsulfonyl)-amino]but-1-yl]phosphonate (22). Diethyl methylphosphonate (1.94 mL, 13.3 mmol, Aldrich) was dissolved in dry THF (7 mL) under Ar and cooled to −78 °C. The n-butyllithium (6.2 mL of a solution in hexane, 13.3 mmol) was added dropwise, and the resulting light yellow solution was stirred for 30 min at −78 °C. Compound **21** (2.16 g, 3.32 mmol) dissolved in THF (5 mL) was transferred with a double-ended needle into the solution and stirred for 3 h at −20 °C. The reaction was quenched with 1 N HCl, the water layer was extracted three times with ether, and the combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. Purification of the residue on silica (100% C to 1% M in C) gave pure **22** (1.76 g, 84%) as a white powder after lyophilization from benzene: TLC, (R_f 0.12, 100% E, AMA); NMR ($CDCl_3$, 500 MHz) δ 0.90 (t, J = 7.5, 3 H), 1.18–1.26 (m, 30 H), 1.30 (t, J = 7.0, 6 H), 1.45 (t, 2 H), 1.67 (m, 1 H), 1.80 (m, 3 H), 2.44 (s, 3 H), 3.10 (dd, J = 10.0, 5.0, 1 H), 3.18 (dd, J = 10.0, 6.5, 1 H), 3.26 (m, 2 H), 3.35 (m, 1 H), 4.06 (m, 4 H), 5.21 (d, J = 5.2, 1 H), 7.30 (d, J = 7.0, 2 H), 7.76 (d, J = 7.0, 2 H); $[\alpha]_D$ −1.62° (c 2.16, chloroform).

[4-(Octadecyloxy)-3(*S*)-[(*p*-tolylsulfonyl)amino]but-1-yl]phosphonic Acid (23). Diethyl phosphonate **22** (1.29 g, 2.04 mmol), suspended in 7.5 N HCl (40 mL), was stirred vigorously at 100 °C overnight. The mixture was cooled, diluted with water, and extracted three times with 10% methanol/chloroform. The combined organic layers were washed with water and evaporated in vacuo. Lyophilization from benzene furnished phosphonic acid **23** (1.02 g, 87%) as a white powder: TLC (R_f 0.22, 65/30/5 C/M/W, AMA); NMR ($CDCl_3$, 500 MHz) δ 0.88 (t, J = 7.0, 3 H), 1.15–1.31 (m, 30 H), 1.38 (m, 2 H), 1.75–1.94 (m, 4 H), 2.41 (s, 3 H), 3.13 (m, 2 H), 3.23 (m, 2 H), 3.39 (m, 1 H), 5.35 (br s, 1 H), 7.28 (d, J = 7.0, 2 H), 7.76 (d, J = 7.0, 2 H).

[4-(Octadecyloxy)-3(*S*)-[(*p*-tolylsulfonyl)amino]but-1-yl]phosphocholine (24). The solid choline tosylate[18] (2.87 g, 10.44 mmol) and **23** (1.0 g, 1.74 mmol) were dried on a pump overnight. Then anhydrous pyridine (35 mL) was added, and the mixture was warmed to 50 °C. The trichloroacetonitrile (6.28 mL, 62.63, mmol, Aldrich) was added dropwise, and the solution was stirred at 50 °C for 48 h. After the pyridine was evaporated in vacuo, the residue was dissolved in 50% methanol/chloroform and passed through a mixed-bed ion-exchange column (Rexyn I-300, Fisher) and eluted with the same solvent. The eluate was concentrated to dryness in vacuo and then purified further on silica (65/25/4 C/M/W) to afford pure **24** (0.91 g, 79%) as a white powder: TLC (R_f 0.11, 65/30/5 C/M/W, AMA); NMR ($CDCl_3/CD_3OD$, 300 MHz) δ 0.88 (t, J = 7.0, 3 H), 1.20–1.34 (m, 30 H), 1.37 (m, 2 H), 1.52–1.88 (m, 4 H), 2.42 (s, 3 H), 3.09–3.32 (m, 5 H), 3.23 (s, 9 H), 3.59 (m, 2 H), 4.26 (m, 2 H), 7.30 (d, J = 7.0, 2 H), 7.76 (d, J = 7.0, 2 H).

[3(*S*)-Amino-4-(octadecyloxy)but-1-yl]phosphocholine (25). Compound **24** (0.503 g, 0.76 mmol) was dissolved in 12.5% methanol in ether (320 mL). The methanol was first degassed by bubbling Ar through it for 1 h. The solution was irradiated with a mercury lamp (550 W) for 1 h. The solvent was evaporated in vacuo, and the residue was purified on a ion-exchange column (Biorad AG50W-X4). Crude amine **25** was loaded on a prepared column in the H^+ form in chloroform/methanol/water (5/5/1) and was washed with 12 column volumes of the same solvent to remove neutral and negatively charged impurities. The desired amine was eluted with chloroform/methanol/concentrated ammonium hydroxide (5/5/1), and the fractions containing the product were concentrated to give amine **25** (0.167 g, 43%) as a light yellow powder: TLC (R_f 0.13, C/M/concentrated NH_4OH, 1/9/1, AMA); NMR ($CDCl_3/CD_3OD$, 300 MHz) δ 0.88 (t, J = 7.0, 3 H), 1.22–1.38 (m, 30 H), 1.50–1.80 (m, 6 H), 3.13 (m, 1 H), 3.22 (s, 9 H), 3.33 (m, 1 H), 3.46 (m, 3 H), 3.62 (m, 2 H), 4.25 (m, 2 H).

[4-(Octadecyloxy)-3(*S*)-(hexadecanoylamino)but-1-yl]-phosphocholine (17). Amine **25** (0.115 g, 0.227 mmol) was suspended in pyridine (3 mL) at 60 °C, and hexadecanoyl chloride (0.156 g, 0.568 mmol, Aldrich) was added dropwise. The resulting orange solution was stirred overnight at the same temperature. The pyridine was removed in vacuo, and the residue was dissolved in 50% methanol/chloroform. The solution was passed through a Rexyn I-300 column and was eluted with the same solvent. The eluate was evaporated to dryness, and the residue was purified on silica (C/M/W, 65/25/4) to afford the amide **17** (0.051 g, 30%) as a white powder: TLC (R_f 0.23, C/M/W, 65/30/5, AMA); NMR ($CDCl_3/CD_3OD$, 300 MHz) δ 0.87 (t, J = 7.0, 6 H), 1.15–1.34 (m, 54 H), 1.44–1.87 (m, 8 H), 2.16 (t, J = 7.0, 2 H), 3.23 (s, 9 H), 3.29–3.45 (m, 4 H), 3.58 (m, 2 H), 3.96 (m, 1 H), 4.22 (m, 2 H); $[\alpha]_D$ +45.9° (c 0.85, 20% M in C); LSIMS (positive ion mode) calcd for $C_{43}H_{89}N_2O_5$ (P + 1) 744.6514, obsd 744.650.

Methyl Dithiohexadecanoate. The cited procedure[17] was modified as follows. THF (175 mL) and diisopropylamine (2.94 mL, 21 mmol) were added to a dry flask under Ar and cooled to −50 °C. n-Butyllithium (10 mL, 2.1 M in hexane) was added followed by hexadecanoic acid (2.56 g, 10 mmol) and HMPA (1.8 mL, 10 mmol) in THF (25 mL) while the temperature was maintained at −50 °C. Dianion formation was completed by heating the solution to 35 °C for 30 min. The solution was cooled to −30 °C, and carbon disulfide (0.66 mL, 11 mmol) was added and allowed to react for 10 min. The solution was cooled to −50 °C, and iodomethane (0.62 mL, 10 mmol) was added. The temperature was maintained at −50 °C for 30 min. The mixture was acidified with 1 N HCl at −50 °C with a rapid increase in temperature. The water layer was separated and extracted three times with petroleum ether. The combined organic layers were washed with 1 N HCl and then water and dried over Na_2SO_4, and the solvent was removed in vacuo at 40 °C. The heating caused the spontaneous decarboxylation of the methyl 2-carboxydithiohexadecanoate. The crude product was purified on silica (100% PE) to give the product as a yellow oil.

[4-(Octadecyloxy)-3(*S*)-(thiohexadecanoylamino)but-1-yl]phosphocholine (18). To a suspension of amine **25** (74 mg, 0.146 mmol) in dry pyridine (1.5 mL) under Ar at 70 °C was added methyl dithiohexadecanoate (132 mg, 0.437 mmol). The reaction mixture was stirred overnight at 70 °C, and then the pyridine was removed in vacuo. The residue was dissolved in 10% methanol/chloroform and passed through a column of Reyxn I-300 being eluted with the same solvent. Concentration and purification on silica (C/M/W, 65/25/4) furnishes thioamide **18** (55 mg, 50%), which contained a small amount of amide **17**. The thioamide was purified by normal-phase HPLC (C/M/W, 65/25/4, retention time 7.8 min). All evaporation of solvent were done at 25 °C in order to avoid decomposition of the thioamide to the amide: TLC (R_f 0.30, C/M/W, 65/30/5, AMA); NMR ($CDCl_3/CD_3OD$, 300 MHz) δ 0.89 (t, J = 7.0, 6 H, methyls), 1.18–1.48 (m, 54 H, $(CH_2)_{15}$ and $(CH_2)_{12}$), 1.56 (m, 2 H, CH_2CH_2CS), 1.69–2.05 (m, 6 H, OCH_2CH_2 and CH_2CH_2P), 2.68 (br m, 2 H, CH_2CS), 3.28 (s, 9 H, NMe_3), 3.35–3.60 (m, 4 H, CH_2OCH_2), 3.78 (m, 2 H, CH_2NMe_3), 4.42 (m, 2 H, CH_2OPO), 4.73 (m, 1 H, CH_4NH); LSIMS (positive ion mode) 761 (M + 1).

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