Stereospecific Synthesis of 2-Substituted Ether Phospholipids

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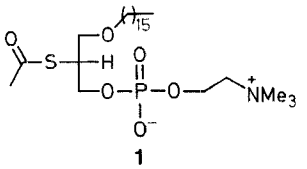
A new stereospecific synthesis of biologically active 2-substituted ether phospholipids is reported. The synthesis is based upon 1) using D-α,β-isopropylideneglycerol-γ-tosylate to provide the chiral center, 2) introducing the *sn*-2-thio function by nucleophilic sulfur displacement of the *p*-nitrobenzenesulfonyl-activated secondary glycerol function, and 3) elaborating the *sn*-3-phosphorylcholine moiety either by the β-bromoethyl phosphodichloridate-trimethylamine sequence, or via phosphorylation using 2-chloro-2-oxo-1,3,2-dioxaphospholane followed by nucleophilic ring opening of the phosphotriester with trimethylamine. Through the use of intermediates that became available from the sequence new *sn*-2-thioacyl and *sn*-2-thiomethyl ether phospholipids were prepared. The synthetic compounds include chromogenic substrates of phospholipase A_2 enzymes, a highly potent antihypertensive ether phospholipid and a structural analogue of antitumor active alkylphosphoglycerides. The synthetic methods developed have a great deal of flexibility providing convenient routes to a wide range of structurally variable ether phospholipids for physicochemical and enzymological studies.

Ether phospholipids belong to a class of highly potent biologically active phospholipid derivatives whose importance has recently been well demonstrated.[1-4] These compounds include platelet-activating,[1] antihypertensive,[2] and antitumor active[3] alkyl phosphoglycerides, as well as immunological response-modifiers.[4] Despite ongoing vigorous investigation of the biochemistry and cell-biology of ether phospholipids their physiological functioning as well as details of their mechanism of action remain to be elucidated. To achieve these goals and to understand the chemical basis of their biological action, structurally variable ether phospholipid compounds need to be prepared. Development of facile and efficient synthetic methods leading to the desired compounds, therefore, is an important prerequisite to addressing these problems, which are widely regarded as some of the most fundamental and timely issues of membrane chemistry today.[1-5]

As part of our research in this area, aimed at developing new synthetic methods for the preparation of phospholipids,[6-10] we recently focused on the synthesis of biologically active derivatives of platelet-activating factor.[7-9] Along these lines we have reported the synthesis of 2-thioPAF (**1**), an isosteric thioacetyl analogue of platelet-activating factor (PAF), **2**, that turned out to be a potent antihypertensive ether phospholipid.[9] In the present paper we describe the synthesis in detail, and demonstrate that the sequence can readily be extended through the use of some of the key intermediates to the preparation of other 2-substituted structurally modified phospholipid analogues. In addition, we now report a greatly improved phosphorylation method, which provides 1) more convenient and flexible elaboration of the polar headgroup of the molecule, 2) substantially improved yields, and 3) higher degree of purity of the final product.[11] The significance of this procedure becomes apparent if one considers that platelet-activating factor and its structural analogues exhibit high biological potency at concentration levels well below the picomolar range.[1,12]



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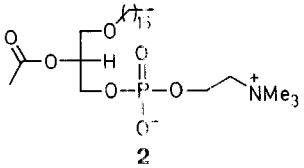

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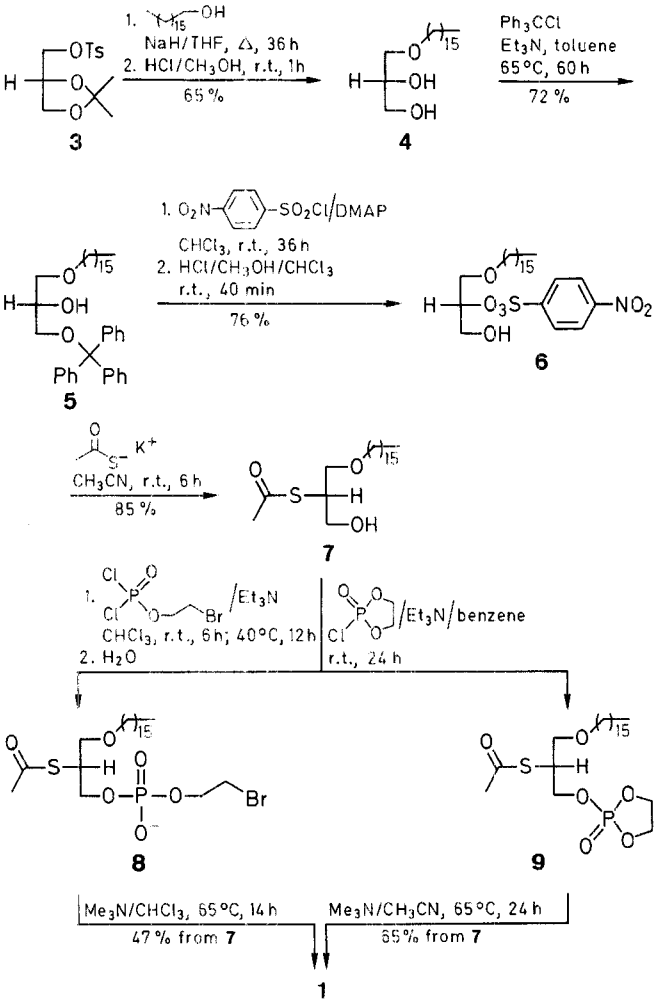
The structural design of compound **1** aimed at developing a chromogenic substrate suitable for detection and kinetic characterization of the enzyme responsible for the hydrolytic cleavage of the *sn*-2-function of PAF.[13] In addition, by developing a stereospecific synthesis of the compound **1** we sought to provide a general method allowing systematic variation of the structural components of the molecule **1**, in order to establish structure-



Scheme A

function correlations delineating the role of each individual substituent in determining the physicochemical and biological properties of PAF. Our synthetic strategy, outlined in Scheme A is based on the use of D-α,β-isopropylideneglycerol-γ-tosylate (**3**) as chiral precursor. In contrast to the corresponding alcohol (α,β-isopropylideneglycerol), which has been reported to undergo self-catalyzed racemization on storage,[14] compound **3** provides a stereochemically stable starting material for the synthesis of the target compound **1**. The choice of the D-enantiomer is dictated by the recognition that introduction of the sulfur atom via nucleophilic displacement at the activated hydroxyl function occurs with inversion of configuration of the chiral *sn*-2-position.[15] Thus, in order to obtain the incipient L-enantiomer phospholipid, the thioacetyl group must be elaborated from the corresponding precursor of opposite stereochemistry.

Alkylation of tosylate **3** with hexadecanol/sodium hydride in tetrahydrofuran produced the corresponding 1-hexadecyl ether. Subsequent acid-catalyzed cleavage of the acetonide moiety using anhydrous methanolic hydrogen chloride gave 1-hexadecyl glycerol **(4)** (65% yield from **3**). The primary alcohol function of **4** was converted to the corresponding trityl ether **5** by allowing **4** to react with triphenylmethyl chloride in toluene in the presence of triethylamine (72%). Activation of the secondary hydroxyl group was carried out using *p*-nitrobenzenesulfonyl chloride with 4-(dimethylamino)pyridine (DMAP) in anhydrous chloroform at room temperature (84%). The use of *p*-nitrobenzenesulfonate as leaving group for thioacetate displacement proved to be highly efficient, yet because of severe steric hindrance at the *sn*-2-position the triphenylmethyl group had first to be removed for allowing the reaction to take place. Acid-catalyzed cleavage of the *sn*-3-trityl ether in chloroform/methanol solution with hydrogen chloride gas yielded the alcohol **6** as a white crystalline solid (mp 54 °C) in nearly quantitative yield.

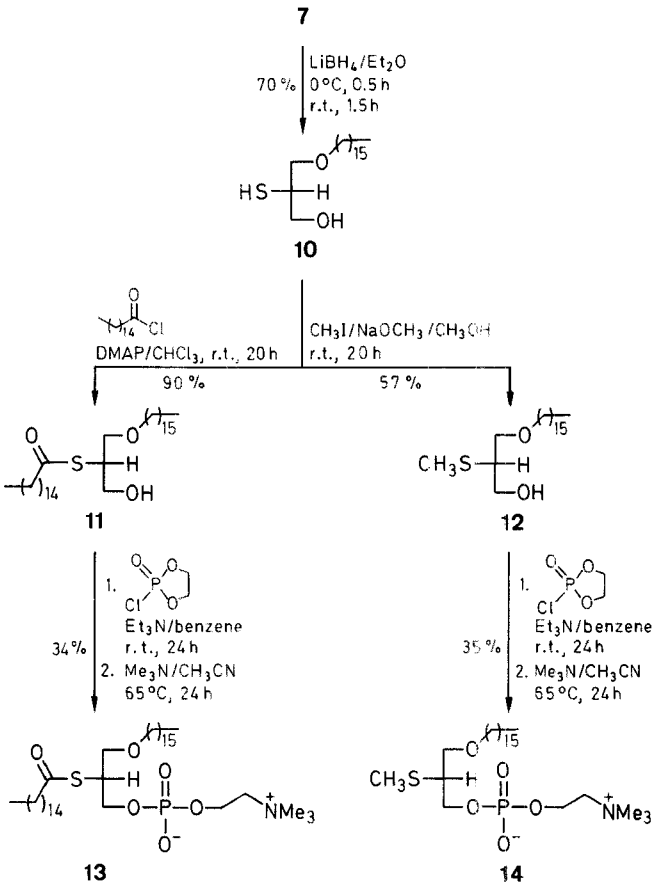
Reaction between *p*-nitrobenzenesulfonate **6** and potassium thioacetate in anhydrous acetonitrile proceeds readily at room temperature to yield the corresponding thioacetyl compound **7**. Isolation and purification of **7**, however, is greatly complicated due to the presence of the neighboring hydroxyl group. Specifically, compound **7** is susceptible to facile acid- and base-catalyzed $S \rightarrow O$ acyl migration,[16] such that chromatography on either silica gel or alumina leads to mixtures of *O*-acyl and *S*-acyl products. Gel-filtration, on the other hand, using Sephadex LH-20 with chloroform/methanol (1:1) allows isolation of the alcohol **7** as a sulfhydryl-negative[17] product in the form of a strongly hygroscopic low-melting solid (85%).

Phosphorylation of the alcohol **7** could be accomplished either with 2-bromoethyl phosphorodichloridate or using 2-chloro-2-oxo-1,3,2-dioxaphospholane. When the latter reagent was used higher yields and fewer phosphate-positive by-products were obtained. However, because of the high reactivity of the cyclic phosphorylating agent the alcohol **7** had to be thoroughly dried before the reaction. This could be readily achieved by freeze-drying the compound **7** from benzene. The anhydrous solid obtained as a white powder was then phosphorylated and the phosphotriester intermediate ring-opened with trimethylamine in dry acetonitrile at 65 °C to give the target phospholipid **1** as a crystalline solid (65%).

In the course of developing the stereospecific scheme for the preparation of phosphodiester **1** a number of intermediates became available providing useful precursors for the synthesis of other biologically active phospholipids. Thus, reductive cleavage of thioacetate **7** by lithium borohydride in ether affords the corresponding alcohol-thiol **10**, which can be specifically functionalized at sulfur to give two new types of phospholipid analogues.

Acylation of **10** by palmitoyl chloride leads to 1-*O*-hexadecyl-2-*S*-palmitoyl-2-deoxy-*sn*-glycerol **(11)**, an immediate precursor of phospholipid **13** (Scheme **B**). The latter is a chromogenic substrate of the enzyme phospholipase A_2 that catalyzes the formation of lysoPAF as part of the *de novo* biosynthesis of platelet-activating factor.[18] Alkylation of **10**, on the other hand, using methyl iodide in methanol/sodium methoxide yields the corresponding *sn*-2-methyl thioether **12** that subsequently can be phosphorylated at the *sn*-3-position to give the 2-thio analogue **14** of 1-hexadecyl-2-methyl-*sn*-glycero-3-phosphocholine,[19] one of the most potent antileukemic ether phospholipids.[3] Using related alkylating agents under similar reaction conditions should provide a series of new synthetic phospholipids to establish structure–activity relationships with respect to the antitumor action of these compounds.

In addition to developing a facile and efficient method for the preparation of *sn*-2-sulfur substituted ether phospholipids a number of useful synthetic strategies have emerged from the sequences. The first one concerns the stereospecific introduction of the thio function both for thioester and thioester development. Specifically, selection of *p*-nitrobenzenesulfonate as the activated hydroxyl derivative ensures rapid and nearly quantitative displacement by thioacetate, with complete stereospecificity. The inversion of chirality at the *sn*-2-position[15] is evidenced by complete enzymatic hydrolysis of the phospholipid product **1** and the S_N2 mechanism is strongly supported by the observation that in the presence of the bulky *sn*-3-triphenylmethyl group no reaction at all has occurred. The fact that the thioacetate displacement proceeds without any epoxide formation, elimination or acyl migration clearly demonstrates that the acetyl group not only prevents oxidation of the thiolate nucleophile but also greatly reduces the basicity of the sulfur anion under the reaction conditions.



Scheme **B**

The second principal contribution of general synthetic significance involves the use of lithium borohydride rather than lithium aluminum hydride for the reductive cleavage of the thioester function (Scheme **B**). Replacement of the aluminum compound by lithium borohydride greatly improves the procedure for isolation and purification of the product **10**. Specifically, the use of borohydride reducing agent circumvents a rather tedious filtration, which is greatly complicated by complexation between aluminum and the chelating β-hydroxy mercapto function of the reduction product. The modified proce-

dure gives much better yields as well, most likely due to decreasing the exposure of the rapidly oxidizing thiol compound **10**.

It should also be pointed out in this context that the anhydrous conditions used for phosphorylation of alcohols **7** and **11** with 2-chloro-2-oxo-1,3,2-dioxaphospholane might be applicable for the synthesis of phospholipid derivatives that contain reactive functional groups susceptible to hydrolysis and/or acyl migration. Using the cyclic phosphochloridate for two-step introduction of the polar headgroup eliminates the need for employing aqueous or acidic treatment generally required for phosphorylations relying on phosphodichloridates or using the acid-labile phosphotriester method.[14]

We have been able to obtain evidence indicating that compound **1** is a potent antihypertensive phospholipid.[20] In addition, both compounds **1** and **13** are completely hydrolyzed by the enzyme phospholipase A_2. Using bee-venom phospholipase A_2 we have determined the stereochemical purity of each phospholipid: **1** gave an average of 97 ± 5%, and **13** was found to be 98 ± 5% on exhaustive hydrolysis monitored both titrimetrically and by spectrophotometric determination of the liberated sulfhydryl, using 5,5′-dithiobis(2-nitrobenzoic acid).[17] These results clearly indicate that the synthetic sulfur analogues should prove to be useful mechanistic probes for kinetic studies of the corresponding ether phospholipid-specific phospholipase A_2 enzymes.[18]

In conclusion, the syntheses that we have described provide facile and efficient routes to a wide range of 2-substituted ether phospholipids. The strength of the methods developed lies in their simplicity and flexibility, and they are likely to become applicable for the synthesis of additional types of phospholipid derivatives. Work toward this goal is underway in our laboratory.

Melting points were determined on a Mel-Temp apparatus and are uncorrected. IR spectra were recorded on a Beckman AccuLab 2 spectrophotometer. ^{1}H-NMR (internal TMS) were taken on an IBM NR-80 instrument. Optical rotations were determined either on a Perkin Elmer 241 MC or Rudolph AutoPol III polarimeter. D-α,β-Isopropylidene glycerol-γ-tosylate, hexadecanol, 4-(dimethylamino) pyridine, triphenylchloromethane, 4-nitrobenzenesulfonyl chloride, potassium thioacetate were obtained from Fluka Chemical Corporation. Anhydrous Me_3N (Kodak) was used as received. CH_3CN (Burdick and Jackson) and Et_3N (Fluka) were dried over activated Linde 4Å molecular sieves (Fluka). Benzene (Burdick and Jackson) and toluene (Burdick and Jackson) were kept on sodium wire, $CHCl_3$ (Mallinckrodt) was distilled from P_2O_5, and THF was distilled from sodium/benzophenone just prior to use. 2-Bromoethyl phosphorodichloridate[21] and 2-chloro-2-oxo-1,3,2-dioxaphospholane[22] were prepared by literature procedures. Column chromatography was carried out either with silica gel 60 (70–230 mesh ASTM, E. M. Laboratories) or with neutral alumina (Serva). The silica gel support was constantly kept at 120 °C and was cooled to room temperature only prior to use in a dessicator over P_2O_5. TLC was carried out on Whatman MK6F plates. The phospholipids were visualized by molybdic acid spray[23] and by charring (50% aq. H_2SO_4). Trityl compounds were visualized by UV light, and all other compounds were detected by iodine vapor. The thiols were additionally visualized by dipping into methanolic solution of 5,5′-dithiobis(2-nitrobenzoic acid).[17] Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN, and by Desert Analytics, Tucson, AZ.

1-Hexadecyl-D-glycerol (4):
To a suspension of NaH (44 mmol, 1.76 g of 60% dispersion in mineral oil, washed with petroleum ether (3 × 40 mL) in an atmosphere of N_2) in dry THF (25 mL) cooled to 0 °C is added dropwise hexadecanol (9.68 g, 40 mmol) in THF (100 mL). The mixture is stirred at room temperature for 15 min and heated at 60 °C for 1.5 h. The suspension is then cooled to 0 °C, and a solution of **3** (11.44 g, 40 mmol) in THF (100 mL) is added dropwise. The resulting mixture is heated at reflux for 36 h, diluted with water (100 mL) and most of the solvents are removed *in vacuo*. The residue is then extracted with ether (3 × 100 mL) and the combined ethereal extract is washed with brine, and dried ($MgSO_4$). Removal of the solvent affords an oil (12.0 g), which contained mostly 2,3-isopropylidene-D-hexadecyl glycerol. This is used as obtained for acid-catalyzed deprotection of the diol function, as follows:

Dry HCl gas is bubbled through dry CH_3OH (150 mL) at a gentle rate for 45 min. To this is added the oil obtained by alkylation in a solution of $CHCl_3$ (40 mL). The resulting solution is stirred at room temperature for 1 h. The solvents are then removed *in vacuo*, and the resulting semi-solid is dried *in vacuo* over KOH pellets for 4 h. From this crude product, 1-hexadecyl-D-glycerol **(4)** is crystallized using $CHCl_3$/petroleum ether (bp 30–60 °C) affording **4** as a solid; yield: 8.2 g (65% from **3**); mp 65 °C; $[\alpha]_D^{23} = -1.44°$ (c = 1.32, $CH_3OH/CHCl_3$, 1:4) (Lit.[26] mp 65.6 °C).

1-Hexadecyl-3-triphenylmethyl-D-glycerol (5):
A suspension of **4** (4.9 g, 15.5 mmol) in dry toluene (70 mL) containing Et_3N (2.12 g, 21 mmol) is heated at 65 °C with triphenylchloromethane (5.3 g, 19 mmol) for a period of 60 h. The $Et_3N \cdot HCl$ is filtered off and washed with dry benzene (20 mL). Evaporation of the solvent from the filtrate yields a brown oil, which is purified by chromatography on neutral alumina (90 g) with $CHCl_3$/petroleum ether (3:1). Fractions containing the product are combined, concentrated, and the residue is diluted with petroleum ether (bp 30–60 °C, 100 mL) to precipitate triphenylmethyl carbinol, which is then filtered off. The product is finally recrystallized from petroleum ether (bp 30–60 °C) at −20 °C, to give trityl ether **5**; yield: 6.2 g (72%); mp 49 °C; $[\alpha]_D^{23} = -3.22°$ (c = 1.55, $CH_3OH/CHCl_3$, 1:4) (Lit.[27] mp 49 °C).

1-Hexadecyl-2-(4-nitrobenzenesulfonyl)-D-glycerol (6):
To a solution of compound **5** (4.2 g, 7.53 mmol) in dry $CHCl_3$ (100 mL) are added 4-(dimethylamino)pyridine (1.22 g, 10 mmol) and 4-nitrobenzenesulfonyl chloride (2.1 g, 9.46 mmol). The mixture is then stirred at room temperature for 36 h. Water (30 mL) is added, followed by stirring for 10 min. The product is then extracted with $CHCl_3$ (3 × 50 mL), the combined $CHCl_3$ extract is washed with brine, and dried ($MgSO_4$). The solvent is evaporated *in vacuo* and the residue is chromatographed on freshly activated silica gel to give the 4-nitrobenzenesulfonate of **5**; yield: 4.7 g (84%). The product is triturated with petroleum ether (bp 30–60 °C), and filtered to provide an analytically pure sample; mp 84–85 °C; $[\alpha]_D^{23} = -1.36°$ (c = 1.32, $CH_3OH/CHCl_3$, 1:4).

$C_{44}H_{57}NO_7S$	calc.	C 71.03	H 7.72	N 1.88	S 4.31
(744.0)	found	71.16	7.48	1.81	4.54

IR (Nujol): ν = 1340, 1175 cm^{-1} (OSO_2).

^{1}H-NMR ($CDCl_3$): δ = 0.88 (br t, 3 H, CH_3); 1.25 [s, 28 H, $(CH_2)_{14}$]; 3.21–3.61 (m, 6 H, CH_2O); 4.78 (m, 1 H, CH); 7.27 (s, 15 H, Ph_3); 8.01–8.30 (dd, 4 H_{arom}).

A solution of the 4-nitrobenzenesulfonate ester of **5** (3.6 g, 4.84 mmol) in $CHCl_3$ (30 mL) is added to a mixture of $CHCl_3/CH_3OH$ (100 mL, 1:1) through which dry HCl gas has been bubbled for 40 min. The resulting solution is stirred at room temperature for 1 h. The solvents are then removed and the residue is dried over KOH pellets for 2 h *in vacuo*. The product is then chromatographed on activated silica gel using $CHCl_3$ as eluent to afford **6** as a white solid; yield: 2.2 g (76% from **5**); mp 54 °C; $[\alpha]_D^{23} = -4.58°$ (c = 1.55, $CH_3OH/CHCl_3$, 1:4).

$C_{25}H_{43}NO_7S$	calc.	C 59.85	H 8.64	N 2.79	S 6.39
(501.7)	found	60.16	8.55	2.87	6.82

IR (Nujol): ν = 3400 cm^{-1} (OH).

^{1}H-NMR ($CDCl_3$): δ = 0.88 (br t, 3 H, CH_3); 1.26 [s, 28 H, $(CH_2)_{14}$]; 3.25–3.87 (m, 6 H, CH_2O); 4.75 (m, 1 H, CH); 8.08–8.44 (dd, 4 H_{arom}).

1-Hexadecyl-2-thioacetyl-L-glycerol (7):
A suspension of 4-nitrobenzenesulfonate **6** (1.8 g, 3.6 mmol) in dry CH_3CN (35 mL) is stirred with KSAc (0.5 g, 4.39 mmol) at room temperature for a period of 6 h. The precipitated salt is filtered off and the CH_3CN is evaporated *in vacuo*. To the residue $CHCl_3$ (50 mL) is added and the additional precipitate is filtered. The solvent is then removed from the filtrate and the resulting brown oil is passed through a Sephadex LH-20 column (50 g) with $CHCl_3/CH_3OH$ (1:1). The fractions containing the product are combined and concentrated to give thioacetate **7** as a light brown semi-solid; yield: 1.15 g (85%); R_f 0.21 ($CHCl_3$). Treatment of **7** with 5,5′-dithiobis(2-nitrobenzoic acid) does not give any yellow color[17] indicating that no $S \rightarrow O$ migration of acetyl

group has occurred during the purification procedure. The compound **7** is then freeze-dried from benzene and used directly for the next reaction.

IR (Nujol): ν = 3400; 1090 (OH); 1680 cm^{-1} (CO of thioester).

^{1}H-NMR ($CDCl_3$): δ = 0.88 (br t, 3 H, CH_3); 1.25 [s, 28 H, $(CH_2)_{14}$]; 2.34 (s, 3 H, $SCOCH_3$); 3.40–3.82 (m, 7 H, $CH_2OCH_2CHCH_2$).

1-Hexadecyl-2-thioacetyl-2-deoxyglycerophosphocholine (2-ThioPAF) (1):

(i) *By Phosphorylation of 1-Hexadecyl-2-thioacetyl-sn-glycerol* **7** *with 2-Bromoethyl Phosphorodichloridate:* To a mixture of Et_3N (0.55 g, 5.5 mmol) and 2-bromoethyl phosphorodichloridate (0.6 g, 2.48 mmol) in dry $CHCl_3$ (20 mL) is added alcohol **7** (0.56 g, 1.5 mmol) dropwise at 0 °C. The resulting solution is stirred at room temperature for 6 h and at 40 °C for 12 h. It is then cooled to 0 °C and 0.1 M aq. KCl solution (10 mL) is added. The mixture is subsequently stirred at room temperature for another 1 h. To this solution CH_3OH (10 mL) is added and the pH is adjusted to 3 by adding conc. HCl. It is then extracted with $CHCl_3$ (3 × 30 mL), the organic layer is dried ($MgSO_4$) and concentrated to afford bromoethyl phospholipid **8**; yield: 0.76 g (90%). This product is dried over P_2O_5 *in vacuo* and used for the next reaction directly.

^{1}H-NMR ($CDCl_3$): δ = 0.88 (br t, 3 H, CH_3); 1.25 [s, 28 H, $(CH_2)_{14}$]; 2.36 (s, 3 H, $SCOCH_3$); 3.43–4.40 (m, 11 H, $CH_2OCH_2CHCH_2OPO_3CH_2CH_2Br$).

A solution of compound **8** (0.76 g, 1.35 mmol) in dry $CHCl_3$ (25 mL) is transferred into a pressure-bottle, cooled in a dry-ice bath, and dry Me_3N (1.5 mL) is added. The bottle is then sealed and heated at 65 °C for 14 h. The mixture is cooled to room temperature and CH_3OH is added to dissolve any precipitate. The solvents are evaporated and the crude residue is chromatographed on activated silica gel ($CHCl_3$/CH_3OH/H_2O, 65:24:5) to afford 2-ThioPAF **(1)**, yield: 0.375 g (47%); $[\alpha]_D^{23}$ –6.07° (c = 1.07, CH_3OH/$CHCl_3$, 1:4); R_f 0.35 ($CHCl_3$/CH_3OH/H_2O, 65:25:4).

$C_{26}H_{54}NO_6PS \cdot H_2O$	calc.	C 55.99	H 10.12	N 2.51	P 5.55	S 5.75
(557.8)	found	55.82	10.11	2.69	5.33	5.45

IR (Nujol): ν = 1680 (CO of thioester), 1065 cm^{-1} (C–O–C).

^{1}H-NMR ($CDCl_3$): δ = 0.88 (br t, 3 H, CH_3); 1.26 [s, 28 H, $(CH_2)_{14}$]; 2.34 (s, 3 H, $SCOCH_3$); 3.45 (s, 9 H, $-NMe_3$); 3.45–4.35 (m, 11 H, $CH_2OCH_2CHCH_2OPO_3CH_2CH_2$).

(ii) *By Phosphorylation of* **7** *with 2-Chloro-2-oxo-1,3,2-dioxaphospholane:*

To a cooled solution (0 °C) of 1-hexadecyl-2-thioacetyl glycerol (**7**; 1.1 g, 2.94 mmol, freeze-dried from dry benzene) in dry benzene (60 mL), Et_3N (0.32 g, 3.17 mmol) is added, followed by 2-chloro-2-oxo-1,3,2-dioxaphospholane (0.44 g, 3.1 mmol) in dry benzene (10 mL). The mixture is stirred at 0 °C for 15 min and then at room temperature for 24 h. The crystalline $Et_3N \cdot HCl$ that precipitates is filtered off, and the solvent is evaporated *in vacuo* to give 2-(1-hexadecyl-2-thioacetyl-*sn*-glycero)-2-oxo-1,3,2-dioxaphospholane as a phosphate-positive white semi-solid (1.3 g, 92%).

^{1}H-NMR ($CDCl_3$): δ = 0.88 (br t, 3 H, CH_3); 1.26 [s, 28 H, $(CH_2)_{14}$]; 2.35 (s, 3 H, $SCOCH_3$); 3.55–3.63 (m, 7 H, $CH_2OCH_2CHCH_2O$); 4.20–4.49 [m, 4 H, $PO_2(OCH_2)_2$].

This compound should not be stored but used as soon as possible for the next reaction without further treatment.

The phosphate triester is transferred into a pressure bottle with dry CH_3CN (30 mL), and the pressure bottle is cooled in a dry-ice bath. To this dry Me_3N (2 mL) is added, and the mixture is heated on an oil bath at 65 °C for 24 h. Cooling and subsequent filtration yield 1.02 g (65%) crude phospholipid. A 0.8 g sample of this product is chromatographed on activated silica gel (12 g) with $CHCl_3$/CH_3OH/H_2O (65:25:4). The fractions containing the pure product are combined and most of the solvents removed under N_2 at room temperature. The residue is then suspended in dry benzene (60 mL) and freeze-dried to afford analytically pure 2-ThioPAF **(1)** as a white solid; yield: 0.55 g (65%). (Analytical data for this compound are described above).

1-Hexadecyl-2-thio-L-glycerol (10):

A solution of compound **7** (1.15 g, 3.07 mmol) in dry ether (50 mL) is cooled to 0 °C. To this is added $LiBH_4$ (0.075 g, 3.4 mmol) in one portion. The resulting solution is stirred at 0 °C for 30 min and then at room temperature for 1.5 h. Excess borohydride is then decomposed by adding 0.5 N HCl (20 mL) and stirring for 15 min. The product is extracted with ether (3 × 50 mL), the combined organic extract is washed with brine and dried ($MgSO_4$). Evaporation of the solvent affords 0.7 g (70%) alcohol-thiol **10** as a low-melting solid.

IR (Nujol): ν = 3420 (OH); 1095 cm^{-1} (C–O).

^{1}H-NMR ($CDCl_3$): δ = 0.87 (br t, 3 H, CH_3); 1.25 [s, 28 H, $(CH_2)_{14}$]; 2.34 (br s, SH, OH, exchangeable with D_2O); 3.37–3.78 (m, 7 H, $CH_2OCH_2CHCH_2O$). This product is used without further treatment, as soon as possible for the next step.

1-Hexadecyl-2-*S*-palmitoyl-L-glycerol (11):

A solution of **10** (0.7 g, 2.1 mmol), palmitoyl chloride (0.48 g, 1.75 mmol) and 4-(dimethylamino)pyridine (0.3 g, 2.46 mmol) in dry $CHCl_3$ (30 mL) is stirred at room temperature under N_2 for 20 h. The solvent is evaporated *in vacuo*, and the residue passed through a Sephadex LH-20 column (50 g) using $CHCl_3$/CH_3OH (1:1) as eluent. Although the product obtained (0.9 g, 90%) is sometimes found to contain a small amount of the diacyl compound,[24] it cannot be further purified due to rapid migration of the acyl group; R_f 0.26 ($CHCl_3$).

IR (Nujol): ν = 3410 (OH), 1680 cm^{-1} (CO of thioester).

^{1}H-NMR ($CDCl_3$): δ = 0.88 (br t, 6 H, $2CH_3$); 1.25 [s, 54 H, $(CH_2)_{27}$]; 2.23–2.53 (m, 2 H, $SCOCH_2$); 3.45–4.26 (m, 7 H, $CH_2OCH_2CHCH_2O$). This product is freeze-dried from dry benzene and used for the next step directly.

1-Hexadecyl-2-*S*-methyl-L-glycerol (12):

A suspension of **10** (0.75 g, 2.26 mmol) in dry CH_3OH (20 mL) is stirred with CH_3ONa (0.216 g, 4 mmol) and CH_3I (0.25 g, 1.76 mmol) in N_2 atmosphere at room temperature for 20 h. CH_3OH is then evaporated *in vacuo*, the crude product is suspended in water (30 mL) and extracted with $CHCl_3$ (3 × 30 mL). The organic layer is washed with brine, dried ($MgSO_4$), and concentrated. The residue is then chromatographed on silica gel with $CHCl_3$ giving thioether **12** as a low-melting solid; yield: 0.35 (57%); R_f 0.30 ($CHCl_3$); $[\alpha]_D^{23}$ –7.86° (c = 1.17, CH_3OH/$CHCl_3$, 1:4).

^{1}H-NMR ($CDCl_3$): δ = 0.88 (br t, 3 H, CH_3); 1.25 [s, 28 H, $(CH_2)_{14}$]; 2.13 (s, 3 H, SCH_3); 3.37–3.75 (m, 8 H, $CH_2OCH_2CHCH_2OH$).

The compound is freeze-dried from dry benzene before being used for phosphorylation. For analysis the acetate is prepared by treatment of the alcohol **12** with acetyl chloride in the presence of 4-(dimethylamino)pyridine. The resulting oil is chromatographed on silica gel with $CHCl_3$ to yield the analytically pure acetate ester of **12**.

$C_{22}H_{44}SO_3$	calc.	C 67.99	H 11.41	S 8.25
(388.6)	found	68.27	11.34	8.36

1-Hexadecyl-2-*S*-palmitoyl-2-deoxyglycerophosphocholine (13):

To a cooled (0 °C) suspension of alcohol **11** (0.72 g, 1.26 mmol) in dry benzene (50 mL) containing Et_3N (0.16 g, 1.6 mmol) is added 2-chloro-2-oxo-1,3,2-dioxaphospholane (0.2 g, 1.4 mmol) in dry benzene (5 mL) and the mixture is stirred at room temperature for 24 h. The $Et_3N \cdot HCl$ that precipitates is filtered off, and the solvent is evaporated *in vacuo* to give the phosphate triester (0.78 g). This product is suspended in dry CH_3CN (25 mL), transferred to a pressure bottle and cooled in a dry-ice bath. To this solution dry Me_3N (1.5 mL) is added. The mixture in the pressure bottle is heated at 65 °C for a period of 24 h. Cooling and subsequent filtration affords 0.6 g (65%) of **13** as an off-white solid. This product is chromatographed on freshly activated silica gel (14 g) with $CHCl_3$/CH_3OH/H_2O (65:25:4). The solvent from the combined pure fractions is evaporated under nitrogen at room temperature, the residue is suspended in dry benzene (60 mL) and freeze-dried to afford **13** as a white solid; yield: 0.31 g (34% due to incomplete recovery from silica gel[25]); $[\alpha]_D^{23}$ –3.90° (c = 1.23, CH_3OH/$CHCl_3$, 1:4); R_f 0.30 ($CHCl_3$/CH_3OH/H_2O (65:25:4).

$C_{40}H_{82}NO_6PS \cdot 2.5H_2O$	calc.	C 61.50	H 11.22	N 1.79	P 3.97	S 4.10
(781.2)	found	61.46	11.15	1.61	4.21	4.47

^{1}H-NMR ($CDCl_3$): δ = 0.88 (br t, 6 H, $2CH_3$); 1.25 [s, 54 H, $(CH_2)_{27}$]; 2.43–2.53 (m, 2 H, $SCOCH_2$); 3.40 (s, 9 H, NMe_3); 3.65–4.33 (m, 11 H, $CH_2OCH_2CHCH_2OPO_3CH_2CH_2N$).

1-Hexadecyl-2-*S*-methyl-2-deoxyglycerophosphocholine (14):

Alcohol **13** is phosphorylated with 2-chloro-2-oxo-1,3,2-dioxaphospholane employing the same experimental conditions as described for compound **1** to obtain the cyclic triester.

^{1}H-NMR ($CDCl_3$): δ = 0.88 (br t, 3 H, CH_3); 1.26 [s, 28 H, $(CH_2)_{14}$]; 2.17 (s, 3 H, SCH_3); 3.38–3.66 (m, 7 H, $CH_2OCH_2CHCH_2O$); 4.24–4.37 [m, 4 H, $PO_2(OCH_2)_2$].

This product is then similarly subjected to ring-opening with dry Me_3N and finally chromatographed on activated silica gel with $CHCl_3$/CH_3OH/H_2O (65:25:4) to give phospholipid **14** as a white hygroscopic solid; crude yield: 66%; isolated yield from alcohol **13**: 35%;[25] $[\alpha]_D^{23} + 4.43°$ ($c = 1.15$, $CH_3OH/CHCl_3$, 1:4); R_f 0.17 ($CHCl_3$/CH_3OH/H_2O, 65:25:4).

$C_{25}H_{54}NO_5PS \cdot 2H_2O$ (547.8) calc. C 54.82 H 10.67 N 2.56 P 5.65 S 5.85
found 55.15 10.96 2.86 5.56 5.84

^{1}H-NMR ($CDCl_3$): $\delta = 0.89$ (br t, 3H, CH_3); 1.26 [s, 28H, $(CH_2)_{14}$]; 2.15 (s, 3H, SCH_3); 3.37 (s, 9H, NME_3); 3.64–4.30 (m, 11H, $CH_2OCH_2CHCH_2OPO_3CH_2CH_2$).

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1988

- Chen, Q.-Y., He, Y.-B. *Synthesis* **1988**, 896. On page 897 the amount of $Pd(PPh_3)_2Cl_2$ in the general procedure for Arenes **3** should be 0.075 mmol.

1989

- Bhaha, S.K., Hajdu, J. *Synthesis* **1989**, 16. Throughout the paper thioacetyl should be replaced by acetylthio. Hence **7** is named 2-*S*-acetyl-1-*O*-hexadecyl-L-2-thioglycerol.

- Burger, A., Hetru, C., Luu, B. *Synthesis* **1989**, 93. On page 94 the formulae of the Horner–Emmons reagent used is:

$$(EtO)_2\overset{\overset{O}{\|}}{P}CCl_2Li$$

and the correct name in the experimental section p. 96 is: diethyl dichloromethylphosphonate.

- Schinzer, D. *Synthesis* **1989**, 179. On page 180 compound **14b** is (2*R*,3*RS*,4*SR*)-3-hydroxy-2,4,6-trimethyl-5-heptenoyltriethylsilane.

- Cristau, H.J., Fonte, M., Torreilles, E. *Synthesis* **1989**, 301. On page 301 compound **7** is 2-(2-benzylaminoethoxy)-1-[(2-methyl-1,3-dioxolan-2-yl)methyl]ethyltriphenylphosphonium iodide.

- Zhou, W.-S., Zhou, Y.-P., Jiang, B. *Synthesis* **1989**, 426. On page 427 compound **8** is (22*E*,24*R*)-3α,5-cyclo-5α-ergosta-7,22-dien-6-one and **9** is (22*E*,24*R*)-3α5-cyclo-5α-ergost-22-en-6-one.

- Stuart, J.G., Nicholas, K.M. *Synthesis* **1989**, 454. In the title abstract and text propargyl nitriles should read propargyl cyanides.

- Schick, H., Eichhorn, I., *Synthesis* **1989**, 477. On page 481 the final entry to Table 4 should read:
$CH_2CH{=}CH-(CH_2)_3CO_2Me$