The chemical synthesis of phosphorothioate and phosphorodithioate analogues of lysophosphatidic acid (LPA) and cyclic phosphatidic acid (CPA)†

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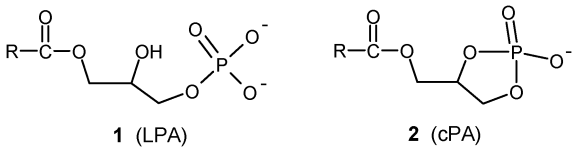
The chemical synthesis of new sulfur analogues of lysophospholipids has been described, including phosphorothioate/phosphorodithioate derivatives of lysophosphatidic acids (LPA) and phosphorothioate/phosphorodithioate derivatives of cyclic phosphatidic acids (cPA). For the preparation of LPA and cPA derivatives both oxathiaphospholane and dithiaphospholane approaches have been employed. Each lysophospholipid analogue has been synthesized as a series of five compounds, bearing five different fatty acid residues, both saturated (12 : 0, 14 : 0, 16 : 0, 18 : 0) and unsaturated (18 : 1), in the form of ammonium salts. The phosphorodithioate analogues of LPA were obtained as triethylammonium salts, however these were not stable and decomposed when transformed into the ammonium salt by ion exchange in aqueous methanol solution. The new sulfur analogues of LPA and cPA may share interesting biological properties of their parent compounds, and previously synthesized derivatives may behave as regulators of many metabolic processes and hopefully show new biological activity.

Introduction

Both LPA (1-acyl-*sn*-glycerol-3-phosphate) (**1**) and cPA (1-acyl-*sn*-glycerol-2,3-cyclic phosphate) (**2**) belong to the family of lysophospholipids,[1,2] containing one acyl group (fatty acid residue) and a phosphate moiety (either acyclic or cyclic) as depicted in Fig. 1.

Although LPA and cPA are minor lipid species present in human blood and other biological media, they are involved in numerous intracellular processes and are important intermediates in lipid biosynthesis.[3] From the structural point of view, LPA and cPA bear a polar phosphate head group and a single hydrophobic chain. In fact, both species are present in various biological fluids as a mixture of several compounds containing different fatty acid residues, either saturated (14 : 0, 16 : 0, 18 : 0) or unsaturated (16 : 1, 18 : 1, 18 : 2, 20 : 4).[4] Following the mass spectrometry measurements performed on human blood and its components, the highest abundance was found for the linoleoyl-(18 : 2)-derivative among the LPA compounds[4b] and for the oleoyl-(18 : 1)-derivative in the cPA series.[4d]



1 (LPA) **2** (cPA)

Fig. 1 Structures of natural LPA (**1**) and cPA (**2**). Substituents R–C(O)– refer to various fatty acid residues present in lysophospholipids.

LPA is produced extracellularly from lysophosphatidylcholine (LPC) through hydrolytic action of lysophospholipase D (lysoPLD), identical to autotaxin (ATX) or nucleotide pyrophosphatase/phosphodiesterase (NPP2),[4c] and degraded by lipid phosphate phosphatases (LPPs) to monoacyloglycerol (MAG).[5] Lysophosphatidylcholine is also a substrate for biosynthesis of cPA in blood.[2] This lipid is formed by intramolecular transphosphatidylation of LPC catalyzed by lysoPLD-like enzymes, although the mechanism of this enzyme, which directs the degradation of LPC to LPA or cPA, is still obscure.[6]

In spite of its simple structure, LPA is regarded as an important signalling molecule with a wide range of biological activities. For example, LPA elicits cell proliferation and survival, platelet aggregation, smooth-muscle contraction, neurite retraction and cytokine production.[5] Elevated levels of LPA promote cell migration and invasion and this compound in general plays an important role in cancer progression[7] and angiogenesis.[8] It has also been found that in experimental animals LPA promotes wound healing.[9]

Although physiological concentration of cPA in human blood is significantly (*ca.* 10 times) lower than that of LPA,[4d] its biological activity is also remarkable.[2] Despite structural similarity, some of its biological properties are different or even opposite to those of LPA.[2] For instance cPA, in contrast to LPA, inhibits cell proliferation[10] and tumor cell invasion,[11] thus showing anti-metastatic properties.

Both LPA and cPA mediate their cellular responses through the activation of specific lysophosphatidic acid receptors

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coupled to G proteins.[12] Thus, such receptors are being considered as potential drug targets. Taking into account that either agonists or antagonists of lysophosphatidic acid receptors may have therapeutic potential, the synthesis and structure–activity studies of numerous analogues of LPA and cPA were undertaken. These derivatives of lysophosphatidic acids are often described as "metabolically stabilized analogues" to stress the fact that labile structural elements of naturally occurring compounds are chemically stabilized.[13] Some of these compounds also act as inhibitors of enzymes involved in lysophosphatidic acid metabolism.[13]

The most frequently encountered modifications involve introducing fluorine atoms in different parts of LPA/cPA molecules, including their phosphonate and/or thiophosphonate analogues.[13,14] Typical modifications of LPA include the addition of an alkylated oxygen atom in position 2 of glycerol in order to prevent acyl migration.[15] cPA analogues are often synthesized as so-called "carba derivatives", containing substituted or unsubstituted phosphonate/thiophosphonate moieties.[16] In the LPA series, 1-oleoyl-2-*O*-methyl-*sn*-glycero-phosphorothioate (OMPT) was synthesized, as a metabolically stabilized analogue containing both an alkyl-protected 2-oxygen and a phosphorothioate function. This compound was prepared both in racemic and enantiomeric forms.[17] Racemic OMPT was found to be a potent agonist for the LPA_3 G-protein-coupled receptor.[17a] Further studies revealed, that (2*S*)-OMPT is 5- to 20-fold more active than (2*R*)-OMPT.[17b]

It should also be added that other thiolated acyl glycerol systems were recently described.[18] The new lipid analogues show interesting PLA2 activation properties[18a–c] and can be employed in constructing novel gene delivery vehicles.[18d]

Results and discussion

The interesting biological properties of LPA and cPA as well as their known analogues prompted us to undertake studies on the synthesis of new modifications of lysophosphatidic acids. We focused our attention on the derivatives of LPA and cPA, in which either one or two nonbridging phosphate oxygen atoms were substituted by sulfur to give phosphorothioate or phosphorodithioate analogues, respectively. From the previous studies performed on the synthesis of phosphorothioate and phosphorodithioate derivatives of nucleotides and oligonucleotides,[19] we expected that these lysophospholipid sulfur analogues should have similar physicochemical properties as natural lysophosphatidic acids, but should be more resistant towards hydrolytic enzymes.[20] In order to prevent possible acyl migration in LPA analogues, the oxygen atom in position 2 of glycerol was methylated. To ensure a representative range of lysophosphatidic acid analogues, each congener was prepared as a series of five different compounds, bearing the residues of the following fatty acids: (a) lauric (12 : 0), (b) myristic (14 : 0), (c) palmitic (16 : 0), (d) stearic (18 : 0), (e) oleic (18 : 1). Thus, sulfur LPA analogues were prepared as phosphorothioates (**3a–e**) and phosphorodithioates (**4a–e**). Similarly, sulfur cPA analogues were also obtained as phosphorothioates (**5a–e**) and phosphorodithioates (**6a–e**). All aforementioned compounds were previously unknown, except the phosphorothioate oleoyl analogue of LPA (**3e**), which is known as OMPT.[17]

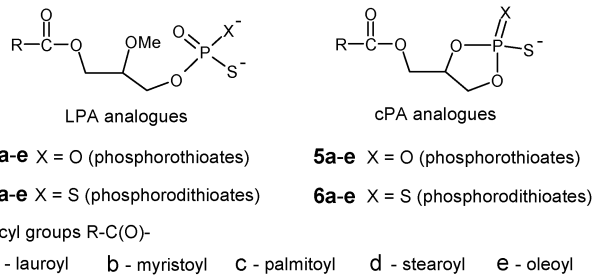


Fig. 2 Structures of the LPA and cPA sulfur analogues (phosphorothioates and phosphorodithioates) **3–6**.

The structures of synthesized sulfur analogues of LPA (**3a–e**, **4a–e**) and cPA (**5a–e**, **6a–e**) are shown in Fig. 2.

Needless to say, the pleiotropic biological/therapeutic activity of LPA and cPA and their hitherto known analogues kindled hopes that the phosphorothioate/phosphorodithioate derivatives **3–6** may have interesting biological properties as well. It should also be added, that for preliminary biological studies all phosphorothioate/phosphorodithioate compounds **3–6** were synthesized in a racemic form.

The synthesis of 1-acylglycerols

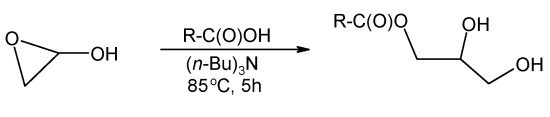
The starting point for the synthesis of the sulfur analogues of both LPA and cPA were 1-acylglycerols, which were prepared according to the procedure described by Lok *et al.*[21] Thus, racemic glycidol (**7**) was incubated for 5 h at *ca.* 85 °C with equimolar amounts of corresponding fatty acids in the presence of a catalytic quantity of tributylamine (Scheme 1).

Crude 1-acylglycerols (**8a–e**) were purified by crystallization from diethyl ether–diisopropyl ether (1 : 1, v/v) to give products in 42–68% isolated yield. The purity of **8** was checked by TLC, and their identity confirmed by comparison of melting points with literature data and by ^{1}H NMR. The details are given in the experimental section.

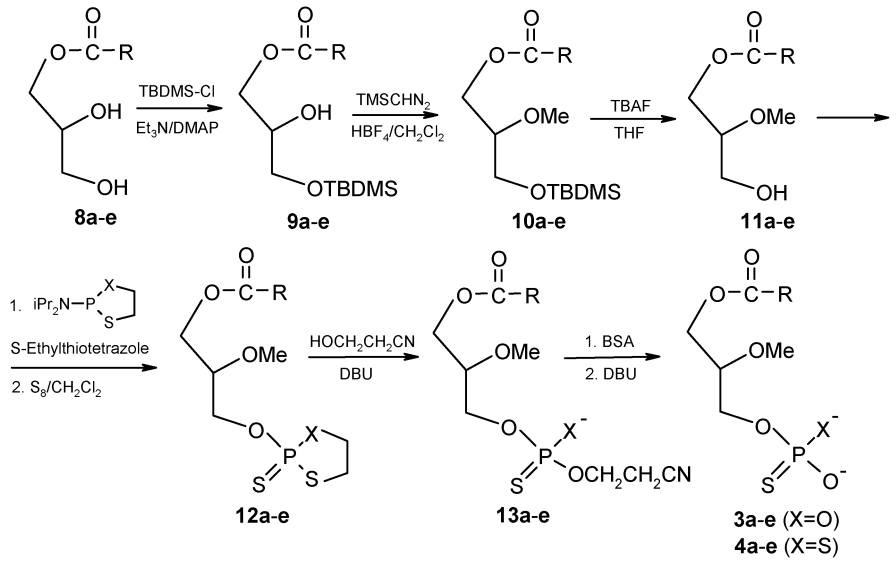
The synthesis of sulfur analogues of LPA

The first step in the functionalization of 1-acylglycerols (**8a–e**) was the selective protection of the primary hydroxyl function in the presence of a secondary one. For this purpose a *tert*-butyldimethylsilyl group (TBDMS) was chosen, which has been successfully employed for selective protection of primary hydroxyls in the synthesis of prostaglandins,[22] nucleosides[23] and modified lipids.[24] The TBDMS group was introduced in 42–78% yield by reacting each of **8a–e** with *tert*-butyldimethylsilyl chloride and triethylamine in the presence of catalytic amounts of 4-dimethylaminopyridine (DMAP) (see Scheme 2).

The resulting 3-*O*-silyl ethers **9a–e** were further methylated at the 2-hydroxyl group with trimethylsilyldiazomethane



Scheme 1 The synthesis of the 1-monoacylglycerols (**8a–e**). The fatty acid residues R–C(O)– correspond to those shown in Fig. 2.



Scheme 2 The synthesis of phosphorothioate (**3a–e**) and phosphorodithioate (**4a–e**) analogues of LPA. The fatty acid residues R–C(O)– correspond to those shown in Fig. 2.

($TMSCHN_2$) in the presence of 40% aqueous fluoroboric acid (HBF_4).[25] Fortunately, the acidic procedure did not affect the TBDMS group present in the molecule,[24] and fully protected lipids **10a–e** were isolated in 48–92% yield. The TBDMS group was removed by a standard method, employing tetra(*n*-butyl)ammonium fluoride (TBAF) in THF solution[22–24] to give 3-*OH* compounds **11a–e** in 64–92% yield. For the introduction of sulfur-containing phosphate groups, oxathiaphospholane and dithiaphospholane approaches were used, previously developed by us for the synthesis of modified nucleotides.[19g,k]

LPA phosphorothioates (3a–e)

Phosphorothioate derivatives of lysophospholipids (**3a–e**) were synthesized according to the oxathiaphospholane approach, and successfully employed by us for the preparation of nucleoside phosphorothioates.[19k] Thus, the 3-*OH* lipids **11a–e** were reacted with 2-*N*,*N*-diisopropylamino-1,3,2-oxathiaphospholane in the presence of *S*-ethylthiotetrazole, and then with elemental sulfur, to give 3-*O*-(2-thio-1,3,2-oxathiaphospholane) lipid derivatives **12a–e** (X = O) in 42–65.5% yield (two signals for the stereoisomers were observed in the ^{31}P NMR spectrum at δ *ca.* 105 ppm). Such a chemical shift was described earlier as a characteristic of 2-alkoxy-2-thio-1,3,2-oxathiaphospholane derivatives.[19a] Compounds **12a–e** (X = O) were treated with an excess of 3-hydroxypropionitrile in the presence of 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU) to result, after elimination of episulfide, in the formation of 3-*O*-(*O*-2-cyanoethyl)-phosphorothioates **13a–e** (X = O) in 61–88% yield (two signals were observed for the stereoisomers in the ^{31}P NMR spectrum at δ *ca.* 58 ppm, characteristic of phosphorothioate *O*,*O*-diesters). The *O*-2-cyanoethyl groups were removed according to the procedure described by Sekine *et al.*[26] by the action of DBU on **13a–e** (X = O), after its silylation with *N*,*O*-bis(trimethylsilyl)acetamide (BSA). The final 1-acyloxy-2-*O*-methyl-*sn*-glycerol-3-*O*-phosphorothioates **3a–e** were isolated as ammonium salts in 40–48% yield, after ion–exchange on a Amberlyst®-NH_4^+ resin in 90% aqueous methanol solution. It should be added that ammonium salts were found to be more stable than independently prepared sodium salts of **3a–e** (not shown). Physicochemical data of the synthesized racemic 1-acyloxy-2-*O*-methyl-*sn*-glycerol-3-*O*-phosphorothioates (ammonium salts) **3a–e** are listed in Table 1. The compounds **3a–e** were found to be soluble in alcohols (methanol, ethanol) and in water. They were isolated in the form of white solids, and were stable when stored at −20 °C.

The ammonium phosphorothioates (**3a–e**) and all intermediate compounds (**9a–e**; **10a–e**; **11a–e**; **12a–e**, X = O; **13a–e**, X = O) were isolated by silica gel flash chromatography (FC). Their purity and identity was confirmed by TLC, $^1H/^{31}P$ NMR and MALDI TOF MS. The details are given in the experimental section.

LPA phosphorodithioates (4a–e)

For the synthesis of the phosphorodithioate derivatives of LPA (**4a–e**) the dithiaphospholane method was employed, which was previously introduced by us for the preparation of nucleoside phosphorodithioates.[19g] The starting materials were again 3-*OH* lipids (**11a–e**), which were reacted with 2-*N*,*N*-diisopropylamino-1,3,2-dithiaphospholane in the presence of *S*-ethylthiotetrazole, and then with elemental sulfur, to give 3-(2-thio-1,3,2-dithiaphospholane) lipid derivatives **12a–e** (X = S) in 45–51% yield, with a ^{31}P NMR δ *ca.* 123 ppm. Such a chemical shift value was earlier described as characteristic for this class of compounds.[19b,g] The dithiaphospholane ring was cleaved by the action of an excess of 3-hydroxypropionitrile in the presence of DBU to give, after elimination of episulfide, 3-*O*-(*O*-2-cyanoethyl)-phosphorodithioates **13a–e** (X = S). The observed ^{31}P NMR chemical shifts for **13a–e** (*ca.* 115 ppm) were characteristic for phosphorodithioate *O*,*O*-diesters,[19g] however the products isolated by column chromatography were found to contain *ca.* 30% of 3-hydroxypropionitrile (^1H NMR examination). This impurity

Table 1 Physicochemical characteristics of 1-acyloxy-2-*O*-methyl-*sn*-glycerol-3-phosphorothioates (ammonium salts) **3a–e**

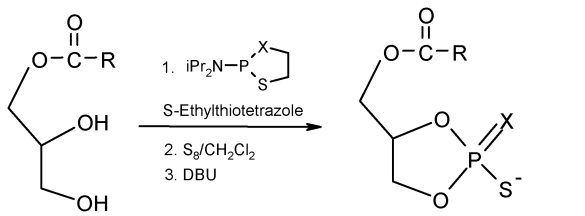
Sulfur modified lysophospholipid	δ ^{31}P NMR (CD_3OD) [ppm]	Molecular weight [Da]	
		Calculated[a]	Measured[b]
Lauroyloxy-thio-LPA **3a**	54.40	384.46	383.2
Myristoyloxy-thio-LPA **3b**	53.25	412.51	410.8
Palmitoyloxy-thio-LPA **3c**	54.65	440.56	438.9
Stearoyloxy-thio-LPA **3d**	54.65	468.62	467.0
Oleoyloxy-thio-LPA **3e**	53.04	466.60	465.3

[a] Acidic form. [b] MALDI TOF MS (*m*/*z*, $M^+ - H$ ions).

was removed by prolonged (48 h) maintaining of samples under high vacuum and finally **13a–e** (X = S) were obtained in 53–61% yield. The removal of the *O*-2-cyanothyl group from **13a–e** (X = S) was performed by the BSA/DBU procedure.[26] Analysis of the ^{31}P NMR spectra of the crude products of reaction showed that their major components had chemical shifts close to those expected for **4a–e**. For the crude products ^{31}P NMR chemical shifts of *ca.* 101 ppm ($CDCl_3$) were observed, which were similar to those observed for the nucleoside monoester phosphorodithioates (88.6–90.6 ppm, Et_3NH^+ salts, D_2O solution).[19g,27] However, attempts to isolate the product by silica gel column chromatography with "normal" $CHCl_3$–MeOH elution resulted in its complete decomposition. Purification of the product could be performed when 1% of triethylamine was added to the eluting system. The increased stability of nucleoside phosphorodithioates at basic conditions, with the strong tendency to decomposition at lower or even neutral pH, was observed earlier by us and other authors.[19g,27] After silica gel column chromatography with $CHCl_3$–MeOH elution in the presence of 1% of Et_3N, single products were isolated which were identified by ^{31}P NMR (δ *ca.* 91 ppm, $CDCl_3$), ^{1}H NMR and MALDI TOF MS as desired phosphorodithioates **4a–e**. Unfortunately, the decomposition of **4a–e** occurred when freshly prepared triethylammonium salts were passed through the Amberlyst®-NH_4^+ resin in 90% aqueous methanol solution in order to obtain ammonium salts suitable for biological testing. The decomposition products could not be identified either by NMR or by MS. These observations suggest that the phosphorodithioate derivatives of LPA (**4a–e**) can be obtained by using the procedure outlined in Scheme 2 and the products can be purified by silica gel chromatography with basic protection. However, the compounds decompose during ion exchange and therefore can not be used for biological testing.

The synthesis of sulfur analogues of cPA

The racemic 1-acylglycerols (**8a–e**), described above as precursors of modified LPA, were also employed by us for the chemical synthesis of sulfur analogues of cPA. On the basis of previous experiences we decided to employ oxathiaphospholane- and/or dithiaphospholane derivatives of **8a–e** as convenient intermediates for the transfer of sulfur atoms into lysophospholipid molecules. In fact, the only example of using this type of compounds for the synthesis of cyclic organophosphorus derivatives was described by Baraniak and Stec,[28] who obtained a six-membered ring adenosine cyclic (3′–5′)-phosphorodithioate by the reaction of an appropriately



Scheme 3 The synthesis of phosphorothioate (**5a–e**) and phosphorodithioate (**6a–e**) analogues of cPA. The fatty acid residues R–C(O)– correspond to those shown in Fig. 2.

protected adenosine 5′-*O*-(2-thio-1,3,2-dithiaphospholane) with potassium *tert*-butoxide in DMF solution. The general procedure for our synthesis of sulfur analogues of cPA is outlined in Scheme 3.

The synthesis of cPA phosphorothioates (5a–e)

Phosphorothioate derivatives of cyclophospholipids (**5a–e**) were synthesized by reacting each of **8a–e** with 2-*N*,*N*-diisopropylamino-1,3,2-oxathiaphospholane in the presence of *S*-ethylthiotetrazole, and then with elemental sulfur, in CH_2Cl_2 solution. The crude product was further reacted without isolation with an excess of DBU and was found to be a major component, the ^{31}P NMR spectrum ($CDCl_3$) shows two closely located peaks in the 71.5–74.5 ppm range, in *ca.* 1 : 1 ratio. Such ^{31}P NMR chemical shift values were close to those described for nucleoside cyclic 2′,3′-*O*,*O*-phosphorothioates (74.7–76.6 ppm in D_2O),[29] thus suggesting, for **5a–e**, a five-membered phosphorothioate structure. It can be assumed, that the reaction of **8** with the oxathiaphospholane derivative occurred predominantly at the more reactive primary oxygen atom, and the addition of strongly basic DBU induced the nucleophilic attack of the other oxygen atom leading to the formation of the five-membered ring phosphorothioate. The presence of the two NMR signals for each product can be attributed to the presence of two centres of chirality, one at the C2–carbon and the other at the phosphorus. Each product **5a–e** is then a mixture of four stereoisomers constituting two racemates. Compounds having isomeric relationship showed different ^{31}P NMR chemical shifts and slightly different chromatographic mobility. We were able to separate them by silica gel column chromatography into faster-moving (FAST) and slower-moving (SLOW) isomers. The purified and separated isomers of each of **5a–e** were isolated as

Table 2 Physicochemical characteristics of 1-acyloxy-*sn*-glycerol-2,3-cyclic phosphorothioates (ammonium salts) **5a–e**

Sulfur modified cyclophospholipid	Yield [%]	Separated isomers	δ ^{31}P NMR (CD_3OD) [ppm]	Molecular weight [Da]	
				Calculated[a]	Measured[b]
Lauroyloxy-thio-cPA **5a**	66	FAST	72.85	352.42	351.6
	—	SLOW	72.67	—	351.4
Myristoyloxy-thio-cPA **5b**	68	FAST	72.71	380.47	379.3
	—	SLOW	72.50	—	380.0
Palmitoyloxy-thio-cPA **5c**	70	FAST	73.65	408.52	407.7
	—	SLOW	73.40	—	407.0
Stearoyloxy-thio-cPA **5d**	58	FAST	72.83	436.58	435.4
	—	SLOW	72.64	—	435.6
Oleoyloxy-thio-cPA **5e**	59	FAST	74.86	434.56	433.2
	—	SLOW	73.95	—	433.2

[a] Acidic form. [b] MALDI TOF MS (m/z, $M^+ - H$ ions).

ammonium salts after ion–exchange on Amberlyst®-NH_4^+ resin, which were found to be more stable than the independently prepared sodium salts (not shown). Their purity and identity was confirmed by TLC, $^1H/^{31}P$ NMR and MALDI TOF MS. The yields and physicochemical characteristics of the synthesized racemic 1-acyloxy-*sn*-glycerol-2,3-cyclic phosphorothioates (ammonium salts) **5a–e** are listed in Table 2.

The details are given in the experimental section. The compounds **5a–e** were found to be soluble in alcohols (methanol, ethanol) and in water. They were isolated in the form of white solids, and were stable when stored at −20 °C.

The synthesis of cPA phosphorodithioates (6a–e)

The synthesis of phosphorodithioate analogues of cPA (**6a–e**) was performed in an analogous manner to the corresponding phosphorothioate derivatives (Scheme 3). Thus, 1-acylglycerols (**8a–e**) were reacted with 2-*N*,*N*-diisopropylamino-1,3,2-dithiaphospholane in the presence of *S*-ethylthiotetrazole, and then with elemental sulfur. Further reaction of the crude product with DBU resulted in formation of a mixture, showing a signal in the ^{31}P NMR spectrum (in $CDCl_3$) at *ca.* 130 ppm as the major component. Similar values of ^{31}P NMR chemical shifts (136.9–138.2 ppm in D_2O) were reported for nucleoside cyclic 2′,3′-*O*,*O*-phosphorodithioates,[30] suggesting the five-membered phosphorodithioate structure for **6a–e**. The reaction of **8** with the dithiaphospholane derivative most probably occurred at the more reactive primary oxygen atom. Further reaction with strongly basic DBU induced cyclization of the intermediate 3-*O*-(2-thio-1,3,2-dithiaphospholane) compound. The chromatographically purified products **6a–e** were isolated as ammonium salts after ion-exchange on Amberlyst®-NH_4^+ resin, which appeared to be more stable than the independently prepared sodium salts (not shown). Their purity and identity was confirmed by TLC, $^1H/^{31}P$ NMR and MALDI TOF MS. The yields and physicochemical data of prepared racemic 1-acyloxy-*sn*-glycerol-2,3-cyclic phosphorodithioates (ammonium salts) **6a–e** are listed in Table 3.

The details are given in the experimental section. The compounds **6a–e** were found to be soluble in alcohols (methanol, ethanol) and in water. They were isolated in the form of white solids, and were stable when stored at −20 °C.

Conclusions

Chemically modified lysophospholipids and cyclophospholipids may share interesting biological properties of their parent natural compounds, and hitherto synthesized derivatives may be regulators of many metabolic processes and can therefore be regarded as potential therapeutics. In this paper the chemical synthesis of compounds of this series was described, including phosphorothioate/phosphorodithioate analogues of LPA and phosphorothioate/phosphorodithioate analogues of cPA. Oxathiaphospholane and dithiaphospholane derivatives were employed as reactive intermediates. Each analogue was synthesized as a series of five compounds, bearing five different fatty acid residues both saturated (12 : 0, 14 : 0, 16 : 0, 18 : 0) and unsaturated (18 : 1). For preliminary biological studies, all compounds were prepared as racemates. Unfortunately, phosphorodithioate analogues of LPA were not stable and could be isolated only as triethylammonium salts by column chromatography under basic conditions.

Due to the presence of the additional centre of chirality at phosphorus, phosphorothioate analogues of cPA were

Table 3 Physicochemical characteristics of the 1-acyloxy-*sn*-glycerol-2,3-cyclic phosphorodithioates (ammonium salts) **6a–e**

Sulfur modified cyclophospholipid	Yield [%]	δ ^{31}P NMR (CD_3OD) [ppm]	Molecular weight [Da]	
			Calculated[a]	Measured)[b]
Lauroyloxy-dithio-cPA **6a**	45	133.93	368.49	366.9
Myristoyloxy-dithio-cPA **6b**	40	133.89	396.54	395.0
Palmitoyloxy-dithio-cPA **6c**	47	132.46	424.59	423.5
Stearoyloxy-dithio-cPA **6d**	41	132.50	452.65	451.0
Oleoyloxy-dithio-cPA **6e**	42	132.40	450.63	449.0

[a] Acidic form. [b] MALDI TOF MS (m/z, $M^+ - H$ ions).

obtained in the form of two stereoisomers, which were separated by silica gel column chromatography.

All synthesized compounds will be tested under *in vitro* conditions as potential factors influencing wound healing. The biological testing of synthesized sulfur analogues of LPA and cPA would also involve determination of their stability towards lysophospholipid phosphatases at cell culture conditions. In preliminary experiments we were able to show that both phosphate (unmodified) and phosphorothioate oleoyl cPA were stable after 48 h incubation at 37 °C at 100 μM concentration in thermally inactivated bovine fetal serum or in human serum (TLC control).

Experimental

General procedures

The purity of all products and intermediates was controlled by TLC, performed on Kieselgel $60F_{254}$ alufoil plates (Merck) with iodine or phosphoromolybdic acid detection. Flash chromatography (FC) was run on silica gel 230–400 mesh (Merck) and followed by TLC. ^{1}H and ^{31}P NMR spectra were recorded on a Bruker DPX 250 spectrometer (250.13 MHz for ^{1}H) and referenced to Me_4Si (internal) or 85% H_3PO_4 (external standard). ^{31}P NMR spectra were obtained with standard proton decoupling. MALDI TOF mass spectra were recorded on a Voyager-Elite spectrometer in negative ($M^+ - H$) mode (dihydroxybenzoic acid matrix, nitrogen laser at 337 nm with 310 μJ energy, spectra acquired at *ca.* 10% above the threshold level). The experimental procedures will be described in detail for palmitoyl derivatives. The syntheses with other fatty acid residues were performed in an analogous way.

Materials

Unless otherwise noted, reagents, solvents and other materials were obtained from commercial sources (Aldrich, Fluka). 2-*N*,*N*-Diisopropylamino-1,3,2-oxathiaphospholane[19a] and 2-*N*,*N*-diisopropylamino-1,3,2-dithiaphospholane[19b] were synthesized as described by us earlier.

The synthesis of racemic 1-acylglycerols (8a–e) (general procedure)[21]

Into a mixture of corresponding fatty acid (52 mmol) and tributylamine (0.2 g) was added (dropwise over 20 min, with stirring at 85 °C) 4 g (54 mmol) of freshly distilled racemic glycidol. The mixture was maintained at 85 °C for 5 h, cooled, and the crude product crystallized and recrystallized from a diisopropyl ether–diethyl ether mixture (*ca.* 1 : 1, v/v).

1-Lauroylglycerol (8a). Yield 61% (white crystalline solid). Mp 62–63 °C (lit.,[31] mp 62 °C). ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* 6.8 Hz), 1.26 (br s, 16H), 1.63 (m, 2H), 2.10 (br s, 1H), 2.35 (t, 2H, *J* 7.6 Hz), 2.52 (br s, 1H), 3.65 (m, 2H), 3.93 (m, 1H), 4.20 (m, 2H).

1-Myristoylglycerol (8b). Yield 62% (white crystalline solid). Mp 68–69 °C (lit.,[31] mp 69 °C). ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* 6.8 Hz), 1.26 (br s, 20H), 1.63 (m, 2H), 2.12 (br s, 1H), 2.35 (t, 2H, *J* 7.8 Hz), 2.56 (br s, 1H), 3.65 (m, 2H), 3.94 (m, 1H), 4.18 (m, 2H).

1-Palmitoylglycerol (8c). Yield 68% (white crystalline solid). Mp 76–77 °C (lit.,[21] mp 76 °C). ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* 6.8 Hz), 1.26 (br s, 24H), 1.63 (m, 2H), 2.06 (br s, 1H), 2.38 (t, 2H, *J* 7.5 Hz), 2.60 (br s, 1H), 3.65 (m, 2H), 3.94 (m, 1H), 4.18 (m, 2H).

1-Stearoylglycerol (8d). Yield 62% (white crystalline solid). Mp 80–81 °C (lit.,[21] mp 80–81 °C). ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* 6.8 Hz), 1.25 (br s, 28H), 1.63 (m, 2H), 2.10 (br s, 1H), 2.35 (t, 2H, *J* 7.3 Hz), 2.50 (br s, 1H), 3.65 (m, 2H), 3.93 (m, 1H), 4.18 (m, 2H).

1-Oleoylglycerol (8e). Yield 42% (white crystalline solid). Mp 35 °C (lit.,[21] mp 34–35 °C). ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* 6.8 Hz), 1.30 (m, 20H), 1.60 (br s, 1H), 1.63 (m, 2H), 2.02 (m, 4H), 2.18 (br s, 1H), 2.35 (t, 2H, *J* 7.8 Hz), 2.49 (br s, 1H), 3.71 (m, 2H), 3.93 (m, 1H), 4.18 (m, 2H), 5.35 (m, 2H).

The synthesis of sulfur analogues of LPA

The synthesis of sulfur analogues of LPA (phosphorothioates and phosphorodithioates) was performed starting from racemic 1-acylglycerols (**8a–e**). Here, the syntheses starting from the palmitoyl derivative (**8c**) will be described in detail. The analogues containing other fatty acid residues were prepared in exactly the same way and identified by NMR spectroscopy and MALDI TOF MS.

The synthesis of LPA phosphorothioates (3a–e)

1-*O*-Palmitoyl-3-*O*-*tert*-butyldimethylsilyl-*sn*-glycerol (9c). A solution of 1.0 g (6.6 mmol) of TBDMS-Cl in anhydrous methylene chloride (2.5 cm^3) was added dropwise at room temperature into a solution containing 2.1 g (6.3 mmol) of **8c**, 0.67 g (6.6 mmol) of Et_3N and 38 mg (0.31 mmol) of DMAP. Stirring at room temperature was continued for 17 h, and the resulting solution was diluted with chloroform (50 cm^3) and washed with water (50 cm^3). The organic layer was dried with anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by flash column chromatography (FC) on silica gel, using chloroform with gradually increasing content of methanol (from 0 to 2.5%, v/v) as eluent, yielding **9c** (1.19 g, 42%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 0.066 (s, 6H), 0.88 (t, 3H, *J* 6.8 Hz), 0.89 (s, 9H), 1.22 (br s, 24H), 1.59 (m, 2H), 2.30 (t, 2H, *J* 7.6 Hz), 2.43 (br s, 1H), 3.61 (m, 2H), 3.84 (m, 1H), 4.10 (m, 2H).

1-*O*-Palmitoyl-2-*O*-methyl-3-*O*-*tert*-butyldimethylsilyl-*sn*-glycerol (10c). $TMSCHN_2$ (2.7 cm^3 of 2 M hexane solution) was added at 0 °C to a vigorously stirred mixture of **9c** (1.19 g, 2.7 mmol) in dichloromethane (20 cm^3) and 40% aqueous HBF_4 (0.4 cm^3). Stirring at 0 °C was continued and after 30 min three portions of 2 M $TMSCHN_2$ solution (1.35 cm^3 each) were added at 20 min intervals. After addition of last portion of $TMSCHN_2$, stirring was continued at 0 °C for 30 min and at room temperature for 30 min. Then, 2 cm^3 of 10% $NaHCO_3$ solution was added with stirring and the layers were separated. The organic layer was dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by silica gel FC using chloroform with gradually increasing content of methanol (from 0 to 2.5%, v/v) as eluent. The methylated

product **10c** (0.78 g, 60.5%) was isolated as a colourless oil. ^{1}H NMR ($CDCl_3$) δ 0.068 (s, 6H), 0.88 (t, 3H, J 6.8 Hz), 0.90 (s, 9H), 1.26 (br s, 24H), 1.63 (m, 2H), 2.33 (t, 2H, J 7.8 Hz), 3.42 (m, 1H), 3.43 (s, 3H), 3.69 (m, 2H), 4.19 (m, 2H).

1-*O*-Palmitoyl-2-*O*-methyl-*sn*-glycerol (11c). TBAF (2.27 g, 9.6 mmol) was added to a solution of **10c** (0.78 g, 1.63 mmol) in anhydrous THF (25 cm^3) and the mixture was stirred at room temperature for 2 h. The solvent was evaporated and the residue was purified by silica gel FC with chloroform–methanol gradient (from 1 to 2% MeOH, v/v) as eluent. The product **11c** (0.35 g, 64%) was isolated as a white solid. ^{1}H NMR ($CDCl_3$) δ 0.85 (t, 3H, J 6.9 Hz), 1.21 (br s, 24H), 1.57 (m, 2H), 2.28 (t, 2H, J 7,7 Hz), 2.87 (br s, 1H), 3.43 (s, 3H), 3.45 (m, 1H), 3.60 (m, 2H), 4.19 (m, 2H).

1-*O*-Palmitoyl-2-*O*-methyl-*sn*-glycerol-3-*O*-(2-thio-1,3,2-oxathiaphospholane) (12c, X = O). 2-*N*,*N*-Diisopropylamino-1,3,2-oxathiaphospholane[19a] (0.36 g, 1.04 mmol) was added dropwise, with stirring at room temperature, to a solution of **11c** (0.35 g, 1.04 mmol) and *S*-ethylthiotetrazole (ChemGenes) (0.135 g, 1.04 mmol) in anhydrous CH_2Cl_2 (8 cm^3). After stirring for 2 h at room temperature, 100 mg of dry elemental sulfur was added and stirring was continued overnight. The reaction mixture was filtered, the solvent was evaporated and the residue was purified by silica gel FC with a chloroform–methanol gradient (from 1 to 2% MeOH, v/v) as eluent. The product **12c** (X = O) (0.29 g, 59%) was isolated as a pale-yellow oil. ^{1}H NMR ($CDCl_3$) δ 0.86 (t, 3H, J 6.8 Hz), 1.23 (br s, 24H), 1.60 (m, 2H), 2.23 (t, 2H, J 7.6 Hz), 3.446 (s, 1.5H), 3.449 (s, 1.5H), 3.51 (m, 2H), 3.63 (m, 1H), 4.09–4.28 (m, 4H), 4.34–4.59 (m, 2H); ^{31}P NMR ($CDCl_3$) δ 105.31 (s), 105.42 (s), the peaks were in 1 : 1 ratio.

1-*O*-Palmitoyl-2-*O*-methyl-*sn*-glycerol-3-*O*-(*O*-2-cyanoethyl)-phosphorothioate (13c, X = O). DBU (88.5 mg, 0.58 mmol) was added dropwise, at room temperature, into a stirred solution of **12c** (X = O) (0.28 g, 0.58 mmol) and 3-hydroxypropionitrile (0.21 g, 2.9 mmol) in anhydrous dichloromethane (8 cm^3). The solution was stirred for 1 h, evaporated, and the residue purified by silica gel FC with a chloroform–methanol gradient (from 8 to 33% MeOH, v/v) as eluent to give **13c**, X = O (0.216 g, 75%) as a colourless oil. ^{1}H NMR ($CDCl_3$) δ 0.81 (t, 3H, J 6.9 Hz), 1.19 (br s, 24H), 1.55 (m, 2H), 2.26 (t, 2H, J 7.7 Hz), 2.71 (t, 2H, J 6.0 Hz), 3.37 (s, 3H), 3.40 (m, 2H), 3.58 (m, 1H), 3.83 (m, 2H), 4.11 (m, 2H); ^{31}P NMR ($CDCl_3$) δ 58.01 (s), 58.08 (s), the peaks were in 1 : 1 ratio.

1-*O*-Palmitoyl-2-*O*-methyl-*sn*-glycerol-3-*O*-phosphorothioate (3c). Into a solution of **13c**, X = O (0.216 g, 0.44 mmol) in anhydrous dichloromethane (10 cm^3) was added BSA (1.42 g, 6.9 mmol) followed by DBU (0.26 g, 1.7 mmol). After 2 h at room temperature the solution was evaporated and the residue was purified by silica gel FC with a chloroform–methanol gradient (from 10 to 100% MeOH, v/v) as eluent. Fractions containing phosphorothioate monoester (^{31}P NMR ($CDCl_3$) δ 44.61) were combined, evaporated and subjected to ion–exchange on Amberlyst®-NH_4^+ resin in 90% aqueous methanol solution, yielding **3c** in the form of ammonium salt (0.12 g, 63%) as a white solid. ^{1}H NMR (CD_3OD) δ 0.81 (t, 3H, J 6.6 Hz), 1.20 (br s, 24H), 1.53 (m, 2H), 2.26 (t, 2H, J 7.4 Hz), 3.37 (s, 3H), 3.58 (m, 1H), 3.92 (m, 2H), 4.14 (m, 2H), 4.80 (br s, 8H); ^{31}P NMR (CD_3OD) δ 54.65; MALDI TOF MS ($M^+ - H$) m/z 438.9 (calculated MW 440.56 Da for the protonated form).

1-*O*-Lauroyl-2-*O*-methyl-*sn*-glycerol-3-*O*-phosphorothioate (3a). Prepared exactly as for **3c**. White solid (ammonium salt). ^{1}H NMR (CD_3OD) δ 0.88 (t, 3H, J 6.9 Hz), 1.25 (br s, 16H), 1.60 (m, 2H), 2.33 (t, 2H, J 7.8 Hz), 3.46 (s, 3H), 3.68 (m, 1H), 3.98 (m, 2H), 4.22 (m, 2H), 5.01 (br s, 8H); ^{31}P NMR (CD_3OD) δ 54.40; MALDI TOF MS ($M^+ - H$) m/z 438.9 (calculated MW 440.56 Da for the protonated form).

1-*O*-Myristoyl-2-*O*-methyl-*sn*-glycerol-3-*O*-phosphorothioate (3b). Prepared exactly as **3c**. White solid (ammonium salt). ^{1}H NMR (CD_3OD) δ 0.90 (t, 3H, J 6.8 Hz), 1.30 (br s, 20H), 1.61 (m, 2H), 2.35 (t, 2H, J 7.7 Hz), 3.46 (s, 3H), 3.68 (m, 1H), 4.00 (m, 2H), 4.23 (m, 2H), 4.89 (br s, 8H); ^{31}P NMR (CD_3OD) δ 53.25; MALDI TOF MS ($M^+ - H$) m/z 410.8 (calculated MW 412.51 Da for the protonated form).

1-*O*-Stearoyl-2-*O*-methyl-*sn*-glycerol-3-*O*-phosphorothioate (3d). Prepared exactly as **3c**. White solid (ammonium salt). ^{1}H NMR (CD_3OD) δ 0.81 (t, 3H, J 6.8 Hz), 1.20 (br s, 28H), 1.52 (m, 2H), 2.26 (t, 2H, J 7.8 Hz), 3.37 (s, 3H), 3.60 (m, 1H), 3.92 (m, 2H), 4.13 (m, 2H), 4.80 (br s, 8H); ^{31}P NMR (CD_3OD) δ 54.65; MALDI TOF MS ($M^+ - H$) m/z 467.0 (calculated MW 468.62 Da for the protonated form).

1-*O*-Oleoyl-2-*O*-methyl-*sn*-glycerol-3-*O*-phosphorothioate (3e). Prepared exactly as **3c**. White solid (ammonium salt). ^{1}H NMR (CD_3OD) δ 0.81 (t, 3H, J 6.8 Hz), 1.30 (m, 20H), 1.61 (m, 2H), 2.02 (m, 4H), 2.35 (t, 2H, J 7.7 Hz), 3.45 (s, 3H), 3.68 (m, 1H), 3.99 (m, 2H), 4.21 (m, 2H), 4.98 (br s, 8H), 5.33 (m, 2H); ^{31}P NMR (CD_3OD) δ 53.04; MALDI TOF MS ($M^+ - H$) m/z 465.3 (calculated MW 466.60 Da for the protonated form). Lit.[17b] for (2*R*)-OMPT: ^{31}P NMR (CD_3OD) δ 52.76; MALDI TOF MS ($M^+ + Na$), m/z 489.

The synthesis of LPA phosphorodithioates (4a–e)

1-*O*-Palmitoyl-2-*O*-methyl-*sn*-glycerol-3-*O*-(2-thio-1,3,2-dithiaphospholane) (12c, X = S). 2-*N*,*N*-Diisopropylamino-1,3,2-dithiaphospholane[19b] (315 mg, 0.91 mmol) was added dropwise, with stirring at room temperature, to a solution of **11c** (320 mg, 0.91 mmol) and *S*-ethylthiotetrazole (ChemGenes) (120 mg, 0.91 mmol) in anhydrous CH_2Cl_2 (7 cm^3). After stirring for 2 h at room temperature, 100 mg of dry elemental sulfur was added and stirring was continued overnight. The reaction mixture was filtered, the solvent was evaporated and the residue was purified by silica gel FC with a chloroform–methanol gradient (from 1 to 2% MeOH, v/v) as eluent. The product **12c** (X = S) (205 mg, 45%) was isolated as a pale-yellow oil. ^{1}H NMR ($CDCl_3$) δ 0.85 (t, 3H J 6.7 Hz), 1.23 (br s, 24H), 1.61 (m, 2H), 2.32 (t, 2H, J 7.8 Hz), 3.46 (s, 3H), 3.59 (m, 2H), 3.53–3.73 (m, 3H), 4.06–4.30 (m, 4H); ^{31}P NMR ($CDCl_3$) δ 123.50; MALDI TOF MS (positive mode) m/z 521.9 ($M^+ + Na$) (calculated MW 498.74).

1-*O*-Palmitoyl-2-*O*-methyl-*sn*-glycerol-3-*O*-(*O*-2-cyanoethyl)-phosphorodithioate (13c, X = S). DBU (62.6 mg, 0.41 mmol)

was added dropwise, at room temperature, into a stirred solution of **12c** (X = S) (205 mg, 0.41 mmol) and 3-hydroxypropionitrile (146 mg, 2.0 mmol) in anhydrous dichloromethane (5 cm^3). The solution was stirred for 2 h, evaporated, and the residue purified by silica gel FC with chloroform–methanol gradient (from 8 to 33% MeOH, v/v) as eluent to give the product as a colourless oil. Preliminary ^{1}H NMR investigation ($CDCl_3$) revealed the presence of *ca.* 30% of 3-hydroxypropionitrile in the sample (two triplets, δ 2.51 and 3.72, J 6.2 Hz). The sample was kept for 48 h at high vacuum (*ca.* 0.001 mmHg) at room temperature in order to remove impurity. This treatment gave 0.16 g (59%) of **13c** (X = S) in the form of a colourless oil. ^{1}H NMR ($CDCl_3$) δ 0.82 (t, 3H J 6.8 Hz), 1.19 (br s, 24H), 1.54 (m, 2H), 2.28 (t, 2H, J 7.7 Hz), 3.41 (s, 3H), 3.42–3.67 (m, 5H), 3.57–4.26 (m, 4H); ^{31}P NMR ($CDCl_3$) δ 114.99; MALDI TOF MS ($M^+ - H$) m/z 508.0 (calculated MW 509.70 Da for the protonated form).

1-*O*-Palmitoyl-2-*O*-methyl-*sn*-glycerol-3-*O*-phosphorodithioate (4c). (**A**) Into a solution of **13c** (X = S) (160 mg, 0.24 mmol) in anhydrous dichloromethane (7 cm^3) was added BSA (770 mg, 3.74 mmol) followed by DBU (670 mg, 4.64 mmol). After 31 h at room temperature the solution was evaporated and the residue was examined by ^{31}P NMR (in $CDCl_3$) showing the presence of signal at 101.58 ppm as the major component. The crude product was chromatographed on silica gel column with a chloroform–methanol gradient (from 8 to 33% MeOH, v/v) as eluent. Unfortunately, the ^{31}P NMR analysis of the products eluted from the column ($CDCl_3$) showed a mixture of compounds with chemical shifts in 41–88 ppm range as a result of decomposition of a product **4c**.

(**B**) The reaction of **13c** (X = S) with DBU/BSA was repeated exactly as in (**A**). The crude product was chromatographed as above, with 1% addition of triethylamine to the eluting solvent. The fraction with a mobility similar to that of corresponding sulfur analogue was collected and examined by ^{31}P NMR, showing the single product with a chemical shift 91.14 ppm ($CDCl_3$), that was identified as triethylammonium salt of **4c** (pale-yellow oil, 92 mg, 58%). ^{1}H NMR ($CDCl_3$) δ 0.79 (t, 3H, J 6.7 Hz), 1.16 (br s, 24H), 1.26 (t, 18H, J 7.3 Hz), 1.52 (m, 2H), 2.23 (t, 2H, J 7.6 Hz), 3.07 (q, 12H, J 7.3 Hz), 3.36 (s, 3H), 3.60 (m, 1H), 3.91–4.07 (m, 3H), 4.19–4.27 (m, 1H); ^{31}P NMR ($CDCl_3$) δ 91.15; MALDI TOF MS ($M^+ - H$) m/z 455.0 (calculated MW 456.64 Da for the protonated form).

(**C**) The triethylammonium salt of **4c** (60 mg) was dissolved in 90% aqueous methanol (20 cm^3) and passed through a column filled with Amberlyst®-NH_4^+ resin (20 cm^3). The column was washed with 90% aqueous methanol (60 cm^3) and the solvent was evaporated. Unfortunately, ^{31}P NMR examination of the residue (in CD_3OD) revealed the presence of several decomposition products in the 68–76 ppm range. The attempts to identify the products of decomposition of **4c** were unsuccessful.

The synthesis of cPA phosphorothioates (5a–e)

In this paragraph the synthesis starting from palmitoyl derivative (**8c**) will be described in detail. The analogues containing other fatty acid residues were prepared in exactly the same way.

1-*O*-Palmitoyl-*sn*-glycerol-2,3-cyclic phosphorothioate (5c). 2-*N*,*N*-Diisopropylamino-1,3,2-oxathiaphospholane[19a] (0.31 g, 1.5 mmol) was added dropwise, with stirring at room temperature, to a solution of **11c** (0.50 g, 1.5 mmol) and *S*-ethylthiotetrazole (ChemGenes) (0.195 g, 1.5 mmol) in anhydrous CH_2Cl_2 (10 cm^3). After stirring for 2 h at room temperature, 100 mg of dry elemental sulfur was added and stirring was continued overnight. Then, DBU (0.52 g, 3.2 mmol) was added with stirring and the reaction mixture was kept for 2 h at room temperature. The resulting solution was filtered and evaporated. The ^{31}P NMR spectrum of the residue (in $CDCl_3$) showed the presence of two major peaks at δ 71.51 and 74.42 ppm. The crude product was carefully chromatographed on silica gel with a chloroform–methanol gradient (from 3 to 17% MeOH, v/v) as eluent. This procedure allowed for complete separation of the stereoisomers of **5c,** having different chromatographic mobility and therefore named FAST and SLOW. The stereoisomers were separately subjected to ion–exchange on Amberlyst®-NH_4^+ resin in 90% aqueous methanol solution giving FAST **5c** and SLOW **5c** in the form of ammonium salts as white solids in a total yield of 0.41 g (70%). ^{1}H NMR (CD_3OD) FAST δ 0.82 (t, 3H, J 6.7 Hz), 1.26 (br s, 24H), 1.61 (m, 2H), 2.36 (t, 2H, J 7.7 Hz), 3.97 (m, 1H), 4.15–4.35 (m, 3H), 4.57 (m, 1H); ^{31}P NMR (CD_3OD) FAST δ 72.89, SLOW δ 72.65; MALDI TOF MS ($M^+ - H$) FAST m/z 407.7, SLOW m/z 407.0 (calculated MW 408.52 Da for the protonated form).

1-*O*-Lauroyl-*sn*-glycerol-2,3-cyclic phosphorothioate (5a). Prepared exactly as for **5c** in the form of two separated stereoisomers in 66% total yield. White solids (ammonium salts). ^{1}H NMR (CD_3OD) FAST δ 0.89 (t, 3H, J 6.9 Hz), 1.28 (br s, 16H), 1.62 (m, 2H), 2.37 (t, 2H, J 7.8 Hz), 3.98 (m, 1H), 4.15–4.35 (m, 3H), 4.58 (m, 1H); ^{31}P NMR (CD_3OD) FAST δ 72.85, SLOW δ 72.67; MALDI TOF MS ($M^+ - H$) FAST m/z 351.6, SLOW m/z 351.4 (calculated MW 352.42 Da for the protonated form).

1-*O*-Myristoyl-*sn*-glycerol-2,3-cyclic phosphorothioate (5b). Prepared exactly as for **5c** in the form of two separated stereoisomers in 59% total yield. White solids (ammonium salts). ^{1}H NMR (CD_3OD) FAST δ 0.80 (t, 3H, J 6.8 Hz), 1.20 (br s, 20H), 1.52 (m, 2H), 2.26 (t, 2H, J 7.9 Hz), 3.88 (m, 1H), 4.05–4.24 (m, 3H), 4.49 (m, 1H); ^{31}P NMR (CD_3OD) FAST δ 72.71, SLOW δ 72.50; MALDI TOF MS ($M^+ - H$) FAST m/z 379.3, SLOW m/z 380.0 (calculated MW 380.47 Da for the protonated form).

1-*O*-Stearoyl-*sn*-glycerol-2,3-cyclic phosphorothioate (5d). Prepared exactly as for **5c** in the form of two separated stereoisomers in 58% total yield. White solids (ammonium salts). ^{1}H NMR (CD_3OD) FAST δ 0.87 (t, 3H, J 6.7 Hz), 1.24 (br s, 20H), 1.60 (m, 2H), 2.34 (t, 2H, J 7.5 Hz), 3.98 (m, 1H), 4.16–4.36 (m, 3H), 4.56 (m, 1H); ^{31}P NMR (CD_3OD) FAST δ 72.83, SLOW δ 72.64; MALDI TOF MS ($M^+ - H$) FAST m/z 435.4, SLOW m/z 435.6 (calculated MW 436.58 Da for the protonated form).

1-*O*-Oleoyl-*sn*-glycerol-2,3-cyclic phosphorothioate (5e). Prepared exactly as for **5c** in the form of two separated stereoisomers in 59% total yield. White solids (ammonium salts). ^{1}H NMR (CD_3OD) FAST δ 0.83 (t, 3H, J 6.7 Hz), 1.24 (m, 20H), 1.55 (m, 2H), 1.97 (m, 4H), 2.31 (t, 2H, J 7.8 Hz), 4.04 (m, 1H), 4.20-4.39 (m, 3H), 4.59 (m, 1H); ^{31}P NMR (CD_3OD) FAST δ 74.86, SLOW δ 73.95; MALDI TOF MS ($M^+ - H$) FAST m/z 433.2, SLOW m/z 433.2 (calculated MW 434.56 Da for the protonated form).

The synthesis of cPA phosphorodithioates (6a–e)

In this paragraph the synthesis starting from palmitoyl derivative (**8c**) will be described in detail. The analogues containing other fatty acid residues were prepared in exactly the same way.

1-*O*-Palmitoyl-*sn*-glycerol-2,3-cyclic phosphorodithioate (6c). 2-*N*,*N*-Diisopropylamino-1,3,2-dithiaphospholane[19b] (330 mg, 1.5 mmol) was added dropwise, with stirring at room temperature, to a solution of **11c** (500 mg, 1.5 mmol) and *S*-ethylthiotetrazole (ChemGenes) (195 mg, 1.5 mmol) in anhydrous CH_2Cl_2 (10 cm^3). After stirring for 2 h at room temperature, 100 mg of dry elemental sulfur was added and stirring was continued overnight. Then, DBU (0.52 g, 3.2 mmol) was added with stirring and the reaction mixture was kept for 3 h at room temperature. The resulting solution was filtered and evaporated. The ^{31}P NMR spectrum of the residue (in $CDCl_3$) showed the presence of a major peak at δ 130.2 ppm. The crude product was chromatographed by silica gel FC with a chloroform–methanol gradient (from 3 to 20% MeOH, v/v) as eluent. Fractions containing cyclic phosphorodithioate were combined, evaporated and subjected to ion-exchange on Amberlyst®-NH_4^+ resin in 90% aqueous methanol solution, yielding **6c** in the form of ammonium salt (300 mg, 47%) as white solid. ^{1}H NMR (CD_3OD) δ 0.88 (t, 3H, J 6.9 Hz), 1.27 (br s, 24H), 1.62 (m, 2H), 2.37 (t, 2H, J 7.7 Hz), 4.01 (m, 1H), 4.16–4.37 (m, 3H), 4.60 (m, 1H); ^{31}P NMR (CD_3OD) δ 132.46; MALDI TOF MS ($M^+ - H$) m/z 423.5 (calculated MW 424.59 Da for the protonated form).

1-*O*-Lauroyl-*sn*-glycerol-2,3-cyclic phosphorodithioate (6a). Prepared exactly as for **6c** in 45% yield as a white solid (ammonium salt). ^{1}H NMR (CD_3OD) δ 0.89 (t, 3H, J 6.8 Hz), 1.26 (br s, 16H), 1.61 (m, 2H), 2.37 (t, 2H, J 7.6 Hz), 4.01 (m, 1H), 4.16-4.38 (m, 3H), 4.59 (m, 1H); ^{31}P NMR (CD_3OD) δ 133.93; MALDI TOF MS ($M^+ - H$) m/z 366.9 (calculated MW 368.49 Da for the protonated form).

1-*O*-Myristoyl-*sn*-glycerol-2,3-cyclic phosphorodithioate (6b). Prepared exactly as for **6c** in 40% yield as a white solid (ammonium salt). ^{1}H NMR (CD_3OD) δ 0.88 (t, 3H, J 6.9 Hz), 1.31 (br s, 20H), 1.61 (m, 2H), 2.36 (t, 2H, J 7.5 Hz), 4.02 (m, 1H), 4.16–4.39 (m, 3H), 4.61 (m, 1H); ^{31}P NMR (CD_3OD) δ 133.88; MALDI TOF MS ($M^+ - H$) m/z 395.0 (calculated MW 396.54 Da for the protonated form).

1-*O*-Stearoyl-*sn*-glycerol-2,3-cyclic phosphorodithioate (6d). Prepared exactly as for **6c** in 41% yield as a white solid (ammonium salt). ^{1}H NMR (CD_3OD) δ 0.86 (t, 3H, J 7.1 Hz), 1.24 (br s, 28H), 1.59 (m, 2H), 2.37 (t, 2H, J 7.3 Hz), 3.98 (m, 1H), 4.13–4.39 (m, 3H), 4.56 (m, 1H); ^{31}P NMR (CD_3OD) δ 132.50; MALDI TOF MS ($M^+ - H$) m/z 451.0 (calculated MW 452.65 Da for the protonated form).

1-*O*-Oleoyl-*sn*-glycerol-2,3-cyclic phosphorodithioate (6e). Prepared exactly as for **6c** in 42% yield as a white solid (ammonium salt). ^{1}H NMR (CD_3OD) δ 0.88 (t, 3H, J 6.8 Hz), 1.30 (m, 20H), 1.56 (m, 2H), 2.01 (m, 4H), 2.36 (t, 2H, J 8.0 Hz), 4.01 (m, 1H), 4.16–4.38 (m, 3H), 4.59 (m, 1H), 5.32 (m, 2H); ^{31}P NMR (CD_3OD) δ 132.40; MALDI TOF MS ($M^+ - H$) m/z 449.0 (calculated MW 450.63 Da for the protonated form).

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