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2-Hydroxymethyl-2-propyl-1,3-propanediol in the Synthesis of Phospholipids

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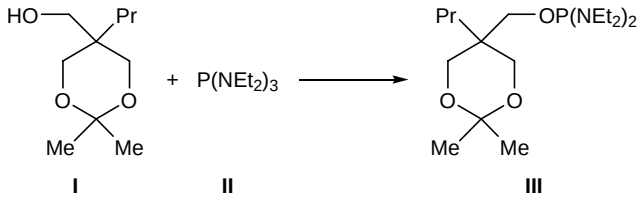
Abstract—New representatives of nonglycerol polyol phospholipids were synthesized from 2-hydroxymethyl-2-propyl-1,3-propanediol through intermediate phosphoramidites and cyclic phosphites obtained from isopropylidene derivative of the title triol. Treatment of these intermediate products with sulfur or selenium afforded the corresponding thio- and selenophosphates which were converted into lipids by direct acylation with higher carboxylic acid chlorides.

Phospholipids in which the alcohol fragment is not glycerol but other polyols are very promising for use in biochemical and biophysical studies. Therefore, development of methods for synthesizing such compounds is an important problem [2, 3]. Phospholipids modified at the alcoholic fragment were previously synthesized on the basis of 2-hydroxymethyl-2-methyl-1,3-propanediol (metriol) and 1,2,4-butanetriol [4], D-mannite and D-glucite [5], 1,2,5-pentanetriol [6], and isomeric 1,2,3-cyclopentanetriols [7–12]. The products were successfully used in studies on membrane models [13, 14] and catalytic activity of some phospholipases [4, 8, 15]. However, the above syntheses were performed with the use of phosphorus(V) compounds as phosphorylating agents, and the yields of the target products were not high.

New methods of synthesis of nonglycerol polyol phospholipids involve phosphorus(III) compounds as phosphorylating agents [16, 17], which make it possible to obtain not only phospholipids but also their sulfur- and selenium-containing analogs. The latter attract interest as models for various biophysical studies using NMR spectroscopy. Some advances in the synthesis of lipids by the phosphite technique have been reported [18–25].

In continuation of our previous studies in this line, the present communication reports on the synthesis of previously unknown phospholipids on the basis of 2-hydroxymethyl-2-propyl-1,3-propanediol. As initial compound we used readily accessible 5-hydroxymethyl-2,2-dimethyl-5-propyl-1,3-dioxane (**I**) which was subjected to phosphorylation with hexaethylphosphorous triamide (**II**) to obtain compound **III** (Scheme 1). In order to raise the yield of the target product, 3 equiv of triamide **II** was used, and excess reagent was distilled off when the reaction was complete. The formation of phosphorodiamidite **III** was monitored by ^{31}P NMR spectroscopy: a singlet at δ_P 134 ppm appeared in the spectrum of the reaction mixture.

Scheme 1.



I **II** **III**

Compound **III** was converted (without isolation and purification) to the corresponding thio- and selenophosphates **IV** and **V** by treatment with sulfur or selenium, respectively (Scheme 2). The transformation of phosphorodiamidite **III** into phosphorus(V) compounds **IV** and **V** was monitored by TLC and ^{31}P NMR spectroscopy. The ^{31}P NMR spectra of the reaction mixtures contained singlets at δ_P 79 (**IV**) and 81 ppm (**V**). In addition, the ^{31}P NMR spectrum of selenium derivative **V** contained satellites due to ^{31}P–^{77}Se coupling with a constant $^1J_{P,Se}$ = 850 Hz. Compounds **IV** and **V** were isolated by column chromatography, and their yield attained 68%. The structure of **IV** and **V** was proved by the data of elemental analysis and NMR spectroscopy. Unlike initial acetal **I**, amidophosphates **IV** and **V** showed in the ^{1}H NMR spectra signals from protons of the N-ethyl groups (δ 1.11 and 3.10 ppm)

* For short communication, see [1].

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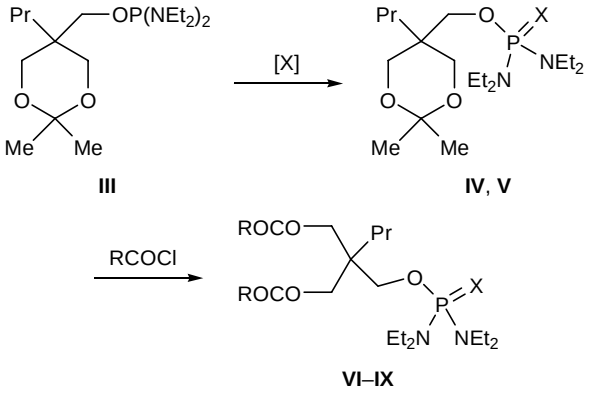
Scheme 2.



IV, X = S; **V**, X = Se; **VI**, **VII**, R = $C_{13}H_{27}$; **VIII**, **IX**, R = $C_{17}H_{35}$.

and a doublet signal from the CH_2OP protons at δ 3.97 ppm with a typical spin–spin coupling constant $^3J_{HP}$ of 4.9 Hz.

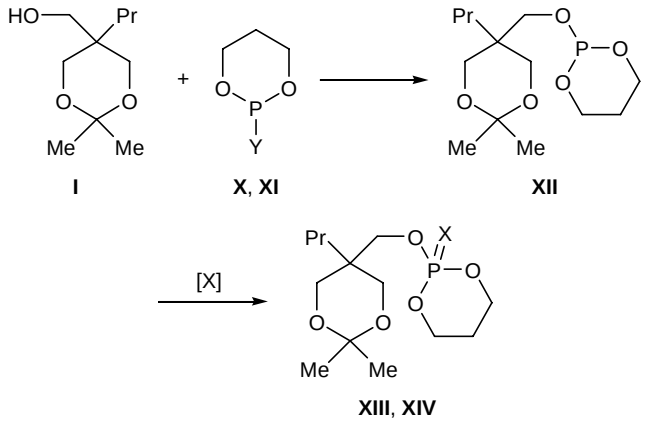
Compounds **IV** and **V** were subjected to direct acylation with myristoyl and stearoyl chlorides (Scheme 2) according to the procedure reported in [26]. The resulting diacyl derivatives **VI**–**IX** were isolated in up to 52% yield by column chromatography on silica gel. It should be noted that the ^{31}P NMR spectra of acylated compounds **VI**–**IX** almost did not differ from the spectra of initial compounds **IV** and **V**. In the ^1H NMR spectra of **VI**–**IX** we observed resonance signals at δ 0.85–2.31 ppm from fatty acid residues instead of those belonging to protons of the geminal methyl groups in initial compounds **IV** and **V**.

We also performed phosphorylation of alcohol **I** with cyclic phosphorous acid derivatives **X** and **XI** (Scheme 3). The phosphorylation conditions were different, depending on the reagent used. The reaction with 2-diethylamino-1,3,2-dioxaphosphinane (**X**) was carried out without a solvent, the liberated diethylamine being distilled off under slightly reduced pressure. The phosphorylation of **I** with 2-chloro-1,3,2-dioxaphosphinane (**XI**) was carried out in anhydrous diethyl ether in the presence of triethylamine as hydrogen chloride acceptor. Both syntheses were characterized by similar efficiencies, but the use of reagent **X** seems to be preferred, for in this case no aggressive hydrogen chloride is formed.

In the ^{31}P NMR spectra of the reaction mixtures we observed a singlet at δ_P 130 ppm which is typical of phosphite **XII**. Compound **XII** was converted (without isolation and purification) into the corresponding thio- and selenophosphates **XIII** and **XIV** (Scheme 3) which were isolated in up to 70% yield by column chromatography on silica gel. The assumed structure of products **XIII** and **XIV** is confirmed by the presence in their ^1H NMR spectra of signals from protons in the phosphinane ring at δ 0.62–1.38 and 3.74 ppm. The ^{31}P NMR spectra of **XIII** and **XIV** contained signals at δ_P 63 and 68 ppm, respectively; the latter was characterized by satellites due to coupling with ^{77}Se, $^1J_{P,Se}$ = 990 Hz.

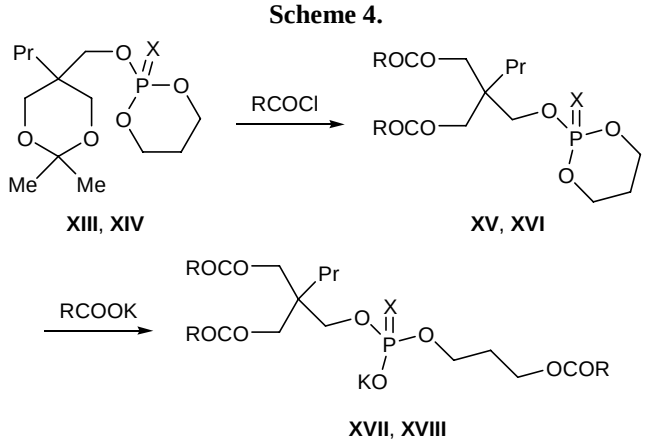
Phosphates **XIII** and **XIV** were subjected to direct acylation with stearoyl chloride according to the procedure described in [26] (Scheme 4). The resulting distearoyl derivatives **XV** and **XVI** were isolated in up to 52% yield by column chromatography on silica gel. Their structure was confirmed by the NMR spectra and elemental analyses. Finally, cyclic phosphates **XV** and **XVI** were treated with potassium stearate [26] to obtain potassium salts **XVII** and **XVIII**, respectively (yield 52%; Scheme 4), which were purified by recrystallization from acetone. In the ^1H NMR spectra of

Scheme 3.



X, Y = NEt_2; **XI**, Y = Cl; **XIII**, X = S; **XIV**, X = Se.

Scheme 4.



R = $C_{17}H_{35}$; **XV**, **XVII**, X = S; **XVI**, **XVIII**, X = Se.

salts **XVII** and **XVIII** we observed increased intensity of signals from protons of the fatty acid residues, while signals from the phosphinane ring protons disappeared. The chemical shifts of the phosphorus atoms in salts **XVII** and **XVIII** were equal to 56 and 59 ppm, respectively. In the ^{31}P NMR spectrum of **XVIII**, the P–Se coupling constant decreased to 780 Hz due to formation of ambident ion [27].

Thus we have synthesized previously unknown analogs of phosphatide acids on the basis of 2-hydroxymethyl-2-propyl-1,3-propanediol. Specific structure of the obtained lipids makes them promising as models for enzymatic, membrane, and other biophysical studies.

EXPERIMENTAL

The ^{1}H NMR spectra of compounds **IV**–**IX** and **XIII**–**XVIII** were recorded from 1 M solutions in chloroform-*d* on a Bruker AH-400 spectrometer at 400 MHz); the signals were assigned using double resonance techiques. The ^{31}P NMR spectra of **III**–**IX** and **XII**–**XVIII** were recorded on a Bruker WP-80SY instrument (32.4 MHz) using benzene as solvent and 85% phosphoric acid as external reference. Silica gel L (100/250 μm) was used for column chromatography. TLC analysis was performed using Silufol UV-254 plates [benzene–dioxane, 3:1 (A); benzene–dioxane, 10:1 (B); hexane–dioxane, 3:1 (C)]; phosphorus-containing substances were detected by treatment with Molybdenum Blue [28], and sulfur-containing substances, by treatment with a 1% aqueous solution of silver nitrate [29] and by calcination. All syntheses with phosphorus(III) compounds were performed under dry argon. The progress of reactions was monitored by TLC and NMR spectroscopy.

2,2-Dimethyl-5-propyl-1,3-dioxan-5-ylmethyl *N,N,N′,N′*-tetraethylphosphorodiamidite (III). A mixture of 2.82 g (15 mmol) of acetal **I** and 11.1 g (45 mmol) of hexaethylphosphorous triamide (**II**) was heated for 2 h at 90–100°C with simultaneous removal of diethylamine under a residual pressure of 380 mm. Excess triamide **II** was distilled off, and the residue (compound **III**) was brought into further syntheses without preliminary purification. R_f 0.4 (A). ^{31}P NMR spectrum (C_6H_6): δ_P 134.0 ppm, s.

***O*-(2,2-Dimethyl-5-propyl-1,3-dioxan-5-ylmethyl) *N,N,N′,N′*-tetraethylphosphorodiamidothioate (IV).** Elemental sulfur, 0.23 g (7 mmol), was added to a solution of 2.56 g (7 mmol) of crude product **III** in 20 ml of benzene, and the mixture was left overnight at 18–20°C. The solvent was distilled off, and compound **IV** was isolated by column chromatography on silica gel using benzene as eluent. Yield 1.78 g (64.3%), n_D^{20} = 1.4850, R_f 0.55 (B). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 0.87 t (3H, C**H**$_3$CH$_2$CH$_2$C), 1.11 t (12H, C**H**$_3$CH$_2$N), 1.24 m (4H, CH$_3$C**H**$_2$C**H**$_2$C), 1.34 s and 1.39 s (6H, CH$_3$C), 3.10 q (8H, CH$_3$C**H**$_2$N, $^3J_{HP}$ = 12.2 Hz), 3.64 s (4H, CCH$_2$OC), 3.97 d (2H, CH$_2$OP, $^3J_{HP}$ = 4.9 Hz). ^{31}P NMR spectrum (C_6H_6): δ_P 79.0 ppm, s. Found, %: C 54.68; H 10.01; N 7.12; P 7.84. $C_{18}H_{39}N_2O_3PS$. Calculated, %: C 54.79; H 9.96; N 7.10; P 7.86.

***O*-(2,2-Dimethyl-5-propyl-1,3-dioxan-5-ylmethyl) *N,N,N′,N′*-tetraethylphosphorodiamidoselenoate (V)** was synthesized in a similar way from 2.56 g (7 mmol) of crude compound **III** and 0.55 g (7 mmol) of selenium. Yield 2.10 g (68%), n_D^{20} = 1.5008, R_f 0.55 (B). The ^{1}H NMR spectrum of compound **V** was similar to that of **IV**. ^{31}P NMR spectrum (C_6H_6): δ_P 81.0 ppm, s, $^1J_{P,Se}$ = 850 Hz (satellites). Found, %: C 48.83; H 8.91; N 6.38; P 6.98. $C_{18}H_{39}N_2O_3PSe$. Calculated, %: C 48.97; H 8.90; N 6.34; P 7.02.

***O*-[2,2-Bis(myristoyloxymethyl)pentyl] *N,N,N′,N′*-tetraethylphosphorodiamidothioate (VI).** Compound **IV**, 0.89 g (2 mmol), and myristoyl chloride, 1 g (2 mmol), were dissolved in 20 ml of dry chloroform, 5 mg of anhydrous zinc chloride was added, the mixture was kept for 8 h at 18–20°C and filtered, the solvent was distilled off from the filtrate, and the residue was subjected to column chromatography on silica gel using benzene as eluent. Yield 0.81 g (51.9%), oily liquid, R_f 0.7 (C). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 0.85 t [6H, C**H**$_3$(CH$_2$)$_{10}$], 0.89 t [3H, C**H**$_3$(CH$_2$)$_2$C], 1.13 t (12H, C**H**$_3$CH$_2$N), 1.24 m [44H, CH$_3$(C**H**$_2$)$_2$C, CH$_3$(C**H**$_2$)$_{10}$], 1.61 m (4H, OCOCH$_2$C**H**$_2$), 2.31 t (4H, OCOC**H**$_2$CH$_2$), 3.09 m (8H, CH$_3$C**H**$_2$N, $^3J_{HP}$ = 12.4 Hz), 3.68 s (4H, CCH$_2$OCO), 4.01 d (2H, CH$_2$OP, $^3J_{HP}$ = 4.92 Hz). ^{31}P NMR spectrum (C_6H_6): δ_P 79.1 ppm, s. Found, %: C 66.51; H 11.21; N 3.63; P 3.96. $C_{43}H_{87}N_2O_3PS$. Calculated, %: C 66.62; H 11.31; N 3.61; P 4.00.

***O*-[2,2-Bis(myristoyloxymethyl)pentyl] *N,N,N′,N′*-tetraethylphosphorodiamidoselenoate (VII)** was synthesized in a similar way from 1.12 g (3 mmol) of compound **V** and 1.48 g (6 mmol) of myristoyl chloride in the presence of 5 mg of anhydrous zinc chloride. Yield 1.28 g (52%), oily liquid, R_f 0.7 (C). The ^{1}H NMR spectrum of **VII** was similar

to that of **VI**. ^{31}P NMR spectrum (C_6H_6): δ_P 80.7 ppm, s. Found, %: C 62.69; H 10.68; N 3.44; P 3.72. $C_{43}H_{87}N_2O_3PSe$. Calculated, %: C 62.82; H 10.67; N 3.41; P 3.77.

***O*-[2,2-Bis(stearoyloxymethyl)pentyl] *N,N,N′,N′*-tetraethylphosphorodiamidothioate (VIII)** was synthesized in a similar way from 0.89 g (2 mmol) of compound **IV** and 1.21 g (4 mmol) of stearoyl chloride in the presence of 5 mg of anhydrous zinc chloride. Yield 0.92 g (51.7%), mp 48–49°C, R_f 0.7 (C). The ^{1}H NMR spectrum of **VIII** was similar to that of **VI**. ^{31}P NMR spectrum (C_6H_6): δ_P 79.1 ppm, s. Found, %: C 68.89; H 11.59; N 3.19; P 3.42. $C_{51}H_{103}N_2O_3PS$. Calculated, %: C 69.03; H 11.70; N 3.16; P 3.49.

***O*-[2,2-Bis(stearoyloxymethyl)pentyl] *N,N,N′,N′*-tetraethylphosphorodiamidoselenoate (IX)** was synthesized in a similar way from 1.12 g (3 mmol) of compound **V** and 1.82 g (6 mmol) of stearoyl chloride in the presence of 5 mg of anhydrous zinc chloride. Yield 1.42 g (50.8%), mp 29–30°C, R_f 0.7 (C). The ^{1}H NMR spectrum of **IX** was similar to that of **VI**. ^{31}P NMR spectrum (C_6H_6): δ_P 80.7 ppm, s, $^1J_{P,Se}$ = 851 Hz (satellites). Found, %: C 65.43; H 11.09; N 3.06; P 3.29. $C_{51}H_{103}N_2O_3PSe$. Calculated, %: C 65.56; H 11.11; N 3.00; P 3.32.

2-(2,2-Dimethyl-5-propyl-1,3-dioxan-5-ylmethoxy)-1,3,2-dioxaphosphinane (XII). *a*. A mixture of 2.82 g (15 mmol) of compound **I** and 2.66 g (15 mmol) of phosphoramidite **X** was heated for 2 h at 90–100°C, the liberated diethylamine being distilled off under a residual pressure of 380 mm. Product **XII** was brought into further syntheses without preliminary purification. R_f 0.7 (A). ^{31}P NMR spectrum (C_6H_6): δ_P 130.0 ppm, s.

b. Compound **XI**, 2.11 g (15 mmol), was added dropwise under vigorous stirring to a solution of 2.82 g (15 mmol) of acetal **I** and 1.52 g (15 mmol) of triethylamine in 20 ml of anhydrous diethyl ether, maintained at –10°C. The precipitate was filtered off, the solvent was distilled off from the filtrate, and the residue (compound **XII**) was used in further syntheses without preliminary purification. R_f 0.7 (A). ^{31}P NMR spectrum (C_6H_6): δ_P 130.0 ppm, s.

2-(2,2-Dimethyl-5-propyl-1,3-dioxan-5-ylmethoxy)-1,3,2λ^5-dioxaphosphinane 2-sulfide (XIII). Elemental sulfur, 0.16 g (5 mmol), was added to a solution of 1.46 g (5 mmol) of crude phosphite **XII** in 20 ml of dry benzene, and the mixture was left to stand overnight at 18–20°C. The solvent was distilled off, and the residue was subjected to column chromatography on silica gel using benzene as eluent. Yield 1.05 g (64.8 %), n_D^{20} = 1.4998, R_f 0.4 (C). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 0.62 m and 1.38 m (2H, $POCH_2C\mathbf{H}_2CH_2O$), 0.89 t [3H, $C\mathbf{H}_3(CH_2)_2C$], 1.20 m [4H, $CH_3(C\mathbf{H}_2)_2C$], 1.37 s, 1.42 s (6H, CH_3C), 3.65 s (4H, CCH_2OC), 3.74 m (4H, $POCH_2CH_2C\mathbf{H}_2O$), 4.3 d (2H, CH_2OP, $^3J_{HP}$ = 4.86 Hz). ^{31}P NMR spectrum (C_6H_6): δ_P 63.0 ppm, s. Found, %: C 48.10; H 7.74; P 9.54. $C_{13}H_{25}O_5PS$. Calculated, %: C 48.14; H 7.77; P 9.56.

2-(2,2-Dimethyl-5-propyl-1,3-dioxan-5-ylmethoxy)-1,3,2λ^5-dioxaphosphinane 2-selenide (XIV) was synthesized in a similar way from 1.46 g (5 mmol) of crude compound **XII** and 0.39 g (5 mmol) of selenium. Yield 1.3 g (70%), n_D^{20} = 1.5165, R_f 0.4 (C). The ^{1}H NMR spectrum of **XIV** was similar to that of **XIII**. ^{31}P NMR spectrum (C_6H_6): δ_P 68.0 ppm, s, $^1J_{P,Se}$ = 990 Hz (satellites). Found, %: C 42.10; H 6.83; P 8.30. $C_{13}H_{25}O_5PSe$. Calculated, %: C 42.05; H 6.79; P 8.35.

2-[2,2-Bis(stearoyloxymethyl)pentyloxy]-1,3,2λ^5-dioxaphosphinane 2-sulfide (XV) was synthesized as described above for compound **VI** from 0.65 g (2 mmol) of thiophosphate **XIII** and 1.21 g (4 mmol) of stearoyl chloroide in the presence of 5 mg of anhydrous zinc chloride in 20 ml of dry chloroform. Yield 0.85 g (52%), mp 52–53°C, R_f 0.7 (C). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 0.86 t [6H, $C\mathbf{H}_3(CH_2)_{14}$], 0.90 t [3H, $C\mathbf{H}_3(CH_2)_2C$], 1.24 m [60H, $CH_3(C\mathbf{H}_2)_{14}$, $CH_3(C\mathbf{H}_2)_2C$], 1.61 m (4H, $OCOCH_2C\mathbf{H}_2$), 1.78 m, 2.22 m (2H, $POCH_2C\mathbf{H}_2CH_2O$), 2.32 t (4H, $OCOC\mathbf{H}_2CH_2$), 3.68 s (4H, CCH_2OC), 3.74 m (4H, $POCH_2CH_2C\mathbf{H}_2O$), 4.35 d (2H, CH_2OP, $^3J_{HP}$ = 5.36 Hz). ^{31}P NMR spectrum (C_6H_6): δ_P 62.0 ppm, s. Found, %: C 67.56; H 10.96; P 3.76. $C_{46}H_{89}O_7PS$. Calculated, %: C 67.60; H 10.89; P 3.79.

2-[2,2-Bis(stearoyloxymethyl)-1-pentyloxy]-1,3,2λ^5-dioxaphosphinane 2-selenide (XVI) was synthesized in a similar way from 0.74 g (2 mmol) of selenophosphate **XIV** and 1.21 g (4 mmol) of stearoyl chloride in the presence of 5 mg of anhydrous zinc chloride in 20 ml of dry chloroform. Yield 0.86 g (50%), mp 63–64°C, R_f 0.7 (C). The ^{1}H NMR spectrum of **XVI** was similar to that of **XV**. ^{31}P NMR spectrum (C_6H_6): δ_P 68.0 ppm, s, $^1J_{P,Se}$ = 990 Hz (satellites). Found, %: C 69.79; H 10.41; P 3.53. $C_{46}H_{89}O_7PSe$. Calculated, %: C 69.93; H 10.38; P 3.59.

Potassium *O*-[2,2-bis(stearoyloxymethyl)pentyl] *O*-[3-(stearoyloxy)propyl thiophosphate (XVII). A mixture of 0.82 g (1 mmol) of thiophosphate **XV**

and 0.32 g (1 mmol) of potassium stearate was heated for 12 h at 120°C in a sealed ampule. The mixture was diluted with chloroform and filtered, the solvent was distilled off from the filtrate, and the residue was recrystallized from acetone (3×10 ml). Yield 0.59 g (51.6%), mp 60–61°C, R_f 0.0 (A). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 0.85 t [9H, C**H**$_3$(CH$_2$)$_{14}$], 0.89 t [3H, C**H**$_3$(CH$_2$)$_2$C], 1.22 m [88H, CH$_3$(C**H**$_2$)$_{14}$, CH$_3$(C**H**$_2$)$_2$C], 1.60 m (6H, OCOCH$_2$C**H**$_2$), 1.92 m (6H, POCH$_2$C**H**$_2$CH$_2$OC), 2.31 t (6H, OCOC**H**$_2$CH$_2$), 3.92 m (4H, CH$_2$OP), 4.16 m (6H, OCOCH$_2$C, OCOC**H**$_2$CH$_2$CH$_2$OP). ^{31}P NMR spectrum (C_6H_6): δ_P 56.0 ppm, s. Found, %: C 67.32; H 11.01; P 2.69. $C_{64}H_{124}KO_9PS$. Calculated, %: C 67.44; H 10.97; P 2.72.

Potassium *O*-[2,2-bis(stearoyloxymethyl)pentyl] *O*-[3-(stearoyloxy)propyl] selenophosphate (XVIII) was synthesized in a similar way from 0.86 g (1 mmol) of compound **XVI** and 0.32 g (1 mmol) of potassium stearate. Yield 0.62 g (52%), mp 66–68°C, R_f 0.0 (A). The ^{1}H NMR spectrum of **XVIII** was similar to that of **XVII**. ^{31}P NMR spectrum (C_6H_6): δ_P 59.0 ppm, s, $^1J_{P,Se}$ = 780 Hz (satellites). Found, %: C 64.59; H 10.51; P 2.57. $C_{64}H_{124}KO_9PSe$. Calculated, %: C 64.77; H 10.53; P 2.61.

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