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Tris(hydroxymethyl)nitromethane in Phospholipids Synthesis

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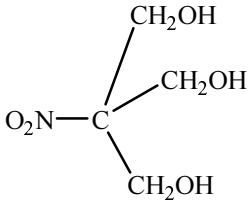
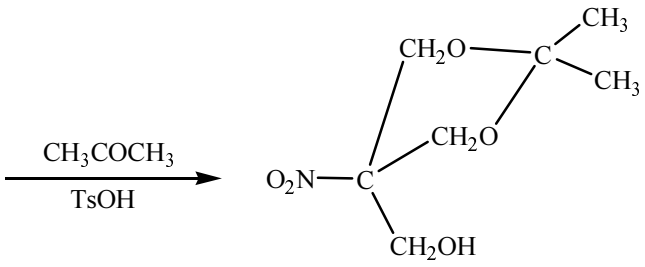
Abstract—The tris(hydroxymethyl)nitromethane acetone derivative was applied as an initial compound for phospholipid syntheses. This ketal was converted in succession into the corresponding O,O- and O,N-cyclophosphites used further for preparation by the methods of organophosphorus chemistry of the first representatives from the following phospholipid types: phosphocholines, phosphomethylcolamines, diol phospholipids, and also cationic phospholipids.

In extension of our studies on the synthesis of oligohydroxy phospholipids of nonglycerol type with the use of trivalent phosphorus compounds [1–3] we attempted to design previously unknown phospholipids proceeding from an available tris(hydroxymethyl)nitromethane acetone derivative (II).



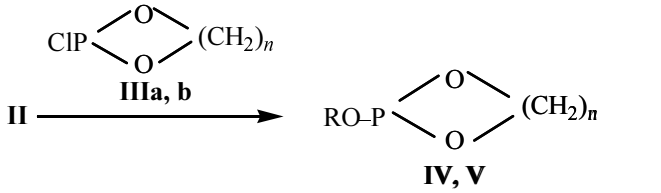
I



II

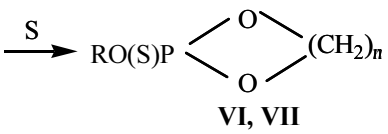
Ketal **II** applied to the synthesis of phospholipids was prepared by reaction of tris(hydroxymethyl)nitromethane (**I**) with acetone in the presence of catalytic amounts of anhydrous *p*-toluenesulfonic acid (TsOH). Compound **II** was isolated by column chromatography on silica gel in 52% yield.

The first stage of the work consisted in preparation from ketal **II** O,O-cyclophosphites and in the investigation of their transformations that might lead to the synthesis of phospholipids. Ketal **II** was phosphorylated with acyl chlorides of O,O-ethylene- (**IIIa**) and O,O-trimethylenephosphorous (**IIIb**) acids in the presence of triethylamine, and the formed phosphites **IV** and **V** were converted without isolation into the corresponding thionephosphates **VI** and **VII**.



IV, V

n = 2 (**IIIa, IV, VI**), 3 (**IIIb, V, VII**);



VI, VII

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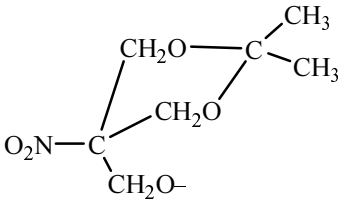
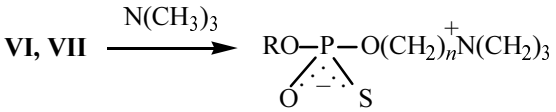


The phosphorylation was carried out in benzene at 0°C. In the ^{31}P NMR spectra of compounds **IV** and **V**

appeared singlets at δ_P 134.95 and 129.56 ppm respectively characteristic of cyclophosphites. The addition of sulfur to phosphites **IV** and **V** occurred in 59–75% yield.

In the ^{31}P NMR spectra of thionephosphates **VI** and **VII** the singlet peaks were observed at δ_P 82.89 and 60.89 ppm. In the ^1H NMR spectra the resonance from the protons of the *gem*-dimethyl groups appeared as singlets at δ 1.25 and 1.40 ppm, and the doublets from the protons of CH_2O groups at 4.05 and 4.34 ppm possessed a characteristic coupling constant of axial and equatorial protons of the dioxane fragment. In the spectrum of ethylenethionephosphate **VI** in the region of δ 4.43 ppm a multiplet was found belonging to the methylene protons of the phospholane ring, and in the spectrum of trimethylenethionephosphate **VII** the protons of the phosphorinane ring gave rise to characteristic multiplets at 1.90 and 4.40 ppm

O,O-Alkylenethionephosphates **VI** and **VII** were good alkylating agents for trimethylamine.



VIII, IX

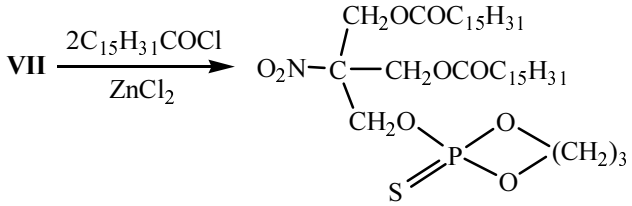
n = 2 (**VIII**) , 3 (**IX**).

Yields of specific thionephosphOcholines **VIII** and **IX** were 55 and 85% respectively.

Note that the trimethylamine alkylation with O,O-cyclothionephosphates of glycerol and other complex alcohols was extensily applied to the synthesis of phosphatidylcholine and its analogs [4–7]. It was established that the opening of dioxaphosphocyclanes occurred in the temperature range from 90 to 130°C. The trimethylamine alkylation with compounds **VI** and **VII** containing an electron-withdrawing nitro group occurred under considerably milder conditions (at 30–35°C for thionephosphate **VI** and at 50–60°C for thionephosphate **VII**).

In the ^{31}P NMR spectra of compounds **VIII** and **IX** singlet signals appeared in the regions characteristic of acyclic dialkylthionephosphates: δ_P 55.50 and 54.16 ppm, and in the ^1H NMR spectra were observed the signals from the methyl protons of ammonium group [δ 2.82 (**VIII**) and 3.10 ppm (**IX**)].

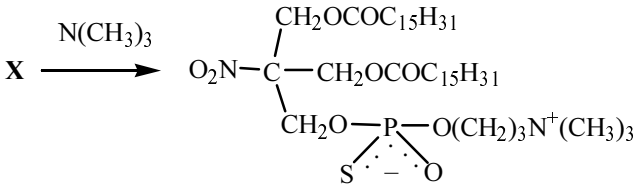
Compound **VII** was used in preparation of more complex phospholipids of kind **X** involving a direct catalytic acylation of acetals with carboxylic acids



X

chlorides [8].

The reaction was carried out in chloroform at cooling to 0°C in the presence of a catalytic amount of zinc chloride. Diacylthionephosphate **X** was isolated in a 60% yield by means of column chromatography (δ_P 61.56 ppm). In the ^1H NMR spectrum of phosphatide **X** were observed multiplets in the region 0.89–2.34 ppm belonging to methyl and methylene protons from the rests of the fatty acids. Methylene protons of the phosphorinane ring gave rise to broadened multiplets at δ 1.98, 4.02, and 4.40 ppm; therewith the axial and equatorial protons of these groups became nonequivalent due to the hindered rotation around the bond C–O–P caused by the presence



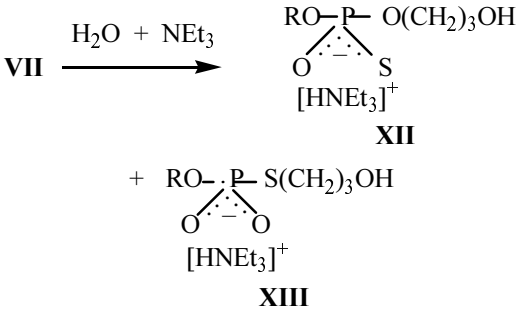
XI

in the molecule **X** of the bulky rests of fatty acids.

In order to go to phosphatide structures of betaine type we performed the alkylation of trimethylamine with cyclothionephosphate **X** to obtain colorless crystalline compound **XI** in a 48% yield. In the ^{31}P NMR spectrum of the latter a singlet signal was present at δ_P 54.98 ppm, and the ^1H NMR spectrum was in agreement with the assumed structure (see EXPERIMENTAL).

With the use of O,O-cyclothionephosphate **VII** we prepared diol phospholipids **XII** and **XIII**. To this end compound **VII** was hydrolyzed in an ampule in aqueous dioxane at 120°C in the presence of triethylamine at molar ratio 1:5. This reagents ratio was chosen in order to get the diol phospholipid derivatives as ammonium salts **XII** and **XIII**.

The analysis of the ^{31}P NMR spectrum of the reaction mixture revealed that alongside the signal at δ_P 57.14 ppm corresponding to thione derivative **XII** a signal at 16.31 ppm appeared characteristic of thiolphosphate **XIII** (intensity ratio 1:9). These data indicate that at the high-temperature hydrolysis of compound **VII** a thione-thiol

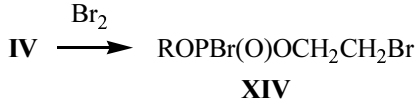


XII

XIII

isomerization occurred. A similar phenomenon was formerly observed during the alkylation of various trimethylenethionephosphates at a temperature higher than 100°C [1, 4]. In the ^{1}H NMR spectrum of compounds **XII** and **XIII** the proton signals from the main molecular skeleton are retained, and new signals appear corresponding to the protons of the ammonium group, and also characteristic signals of the β- and γ-methylene protons of the CH_2CH_2OH group. The multiplets of the α-methylene protons in the spectra of thione- (**XII**) and thiolphosphates (**XIII**) have essentially different chemical shifts: 4.42 ($POCH_2$) and 2.79 ppm ($PSCH_2$).

A special way of *O,O*-cyclophosphite **IV** application was its oxidative decyclization with bromine under mild conditions (at 0°C) resulting in the corresponding bromophosphate (**XIV**).

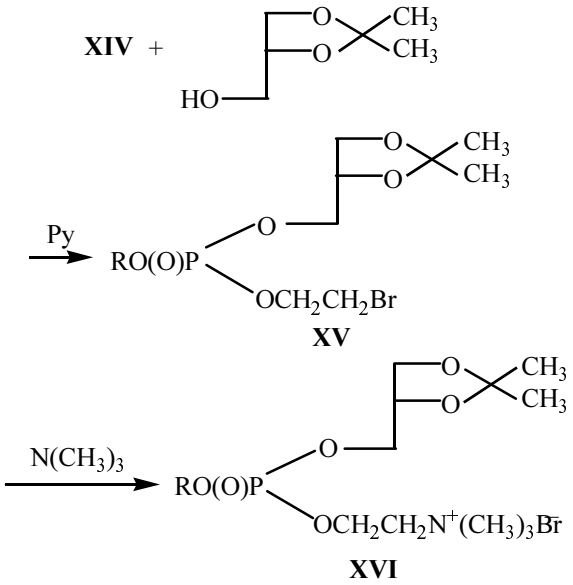


XIV

The yield of bromophosphate **XIV** isolated by column chromatography on silica gel attained 49%. Its ^{31}P NMR spectrum contained a singlet in the region δ_P –2.92 ppm, and in the ^{1}H NMR spectrum all signals were observed characteristic of all groups of protons (see EXPERIMENTAL).

Acyl bromide **XIV** is less stable than previously known lipid acyl bromides [9, 10]. It can be stored after isolation in an individual state in the dark at exclusion of air no longer than 48 h, then it turns into a dark substance insoluble in common solvents. Compound **XIV** immediately after isolation was used to phosphorylate 1,2-*O*-isopropylideneglycerol and to obtain unsymmetrical trialkylphosphate **XV** that finally was treated with trimethylamine to afford a sample of a cationic phospholipid **XVI** [11–13].

After isolation by column chromatography on silica gel compounds **XV** and **XVI** were obtained in 45–50%



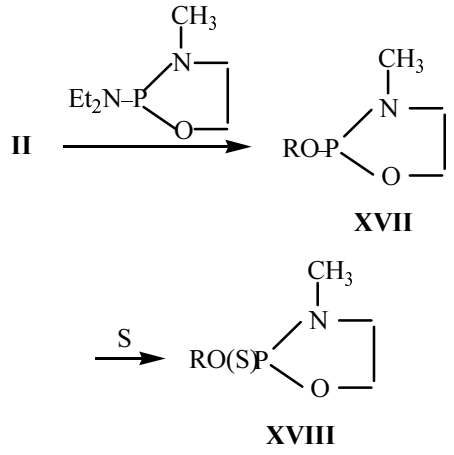
XV

XVI

yield. The structure of phosphates **XV** and **XVI** was proved by physicochemical methods.

The final stage of the study consisted in application to the phospholipid syntheses of O,N-phosphocyclic derivatives of tris(hydroxymethyl)nitromethane ketal (**II**). Compound **XVII** was obtained by ketal **II** phosphorylation with 2-diethylamido-3-methyl-1,3,2-oxaazaphospholane.

The reaction was carried out in a dilute dioxane solution at 80°C with monitoring by ^{31}P NMR spectroscopy (singlet, δ_P 138.05 ppm). Compound **XVII** was not isolated in an individual state but was converted into amidothionephosphate **XVIII** whose yield after chromatography on a column packed with silica gel attained 55%. In the ^{31}P NMR spectrum a singlet appeared at δ_P 84.90 ppm



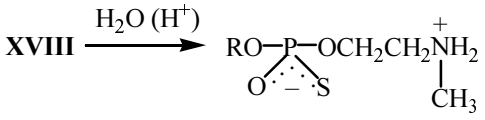
XVII

XVIII

The ^{1}H NMR spectrum of compound **XVIII** contained at δ 2.68 ppm a doublet from the methyl protons of the NCH_3 group ($^2J_{PH}$ 12.20 Hz), and multiplets from protons of $PNCH_2$ and $POCH_2$ groups at 3.32 and 4.25 ppm respectively. The chemical shifts of the other proton signals slightly deviated from those in the spectra of cyclothionephosphates **VI** and **VII** (see EXPERIMENTAL).

Compound **XVIII** was used to prepare phospholipid **XIX** with an N-methylcolamine fragment. The synthesis involved an acid hydrolysis of amidothionephosphate **XVIII** in aqueous dioxane (pH 5).

The chromatographically pure phosphobetaine **XIX** was obtained in a 80% yield. Its ^{31}P NMR spectrum contained a signal in the thionephosphate region (δ_P 57.30 ppm). In the ^{1}H NMR spectrum of this compound in contrast to the spectrum of cyclic amidophosphate **XVIII** instead of a doublet of methyl protons from the $PNCH_3$ group (δ 2.68 ppm) appeared a singlet from N^+CH_3 (δ 2.74 ppm) and proton signals belonging to CH_2N^+ and N^+H_2 groups at 3.21 and 4.90 ppm respectively. The other signals in the ^{1}H NMR spectrum of thionephosphate **XIX** were in agreement with the assumed structure (see EXPERIMENTAL).



XIX

EXPERIMENTAL

^{1}H NMR spectra were registered on spectrometer Bruker WM-250 (250 MHz), chemical shifts were measured with respect to HMDS used as internal reference. The assignment of signals was performed basing on the double resonance spectra. ^{31}P-{^{1}H} NMR spectra were obtained on spectrometer Bruker WP-80SY at operating frequency 32.4 MHz, external reference 85% phosphoric acid.

Adsorption chromatography was carried out on a column of diameter 10 mm charged with silica gel L 100–250 μm; R_f values were determined by TLC on Silufol UV-254 plates, eluents: hexane–dioxane, 3:1 (A), benzene–dioxane, 3:1 (B), chloroform–methanol–water, 65:25:4 (C).

Melting points were measured in sealed capillary at heating rate 1 deg/min.

2-Chloro-1,3,2-dioxaphospholane (**IIIa**) was prepared as described before [14], 2-chloro-1,3,2-dioxaphosphorinane (**IIIb**) was obtained as in [15], 2-diethylamino-3-methyl-1,3,2-oxaazaphospholane was synthesized by procedure [16]. The physical constants of compounds obtained were in agreement with the published data.

(2,2-Dimethyl-2-nitro-1,3-dioxan-5-yl)methanol (II). In 20 g (344 mmol) of anhydrous acetone was thoroughly stirred for 12 h 2 g (13.2 mmol) of 2-hydroxymethyl-2-nitro-1,3-propanediol (**I**) in the presence of 0.05 g (0.25 mmol) of *p*-toluenesulfonic acid. Then the mixture was neutralized with sodium carbonate till pH 7.0, the precipitate was filtered off, and the solvent was removed in a vacuum. Ketal **II** was dissolved in chloroform, applied to a column packed with silica gel, and eluted with a mixture benzene–dioxane, 3:1. The solvents were removed in a vacuum, the residue was washed with ethyl ether (2×5 ml) and maintained for 1 h at 40°C and 1 mm Hg. Yield 1.3 g (52%), mp 134–135°C, R_f 0.32 (A), 0.65 (A). ^{1}H NMR spectrum (CD_3SOCD_3), δ, ppm: 1.25 s and 1.40 s (6H) [$C(CH_3)_2$], 3.72 d (2H, $C\underline{H}_2OH$, $^3J_{HH}$ 5.49 Hz), 4.05 d (2H$_e$) and 4.34 d (2H$_a$) (CH_2OC, $^2J_{a,e}$ 13.12 Hz), 5.48 t (1H, OH, $^3J_{HH}$ 5.49 Hz). Found, %: C 44.11; H 7.01. $C_7H_{13}NO_5$. Calculated, %: C 43.97; H 6.85. *M* 191.18.

2-[(2,2-Dimethyl-5-nitro-1,3-dioxan-5-yl)-methoxy]-2-thioxo-1,3,2-dioxaphospholane (VI). To a solution of a mixture of 0.5 g (2.6 mmol) of compound **II** and 0.3 g (2.6 mmol) of triethylamine in 10 ml of anhydrous benzene was added dropwise while stirring at cooling to 0°C 0.3 g (2.6 mmol) of 2-chlorodioxaphospholane (**IIIa**) in 5 ml of the same solvent. The reaction mixture was kept at this temperature for 1 h, then it was warmed to the room temperature. Phospholane **IV** formation was monitored by ^{31}P NMR spectroscopy [^{31}P NMR spectrum (benzene), δ, ppm: 134.95 s]. The triethylamine hydrochloride was filtered off, to the filtrate 0.1 g (2.9 mmol) of sulfur was added, and the mixture was heated to 80°C for 4 h. The excess sulfur was filtered off, and benzene was removed in a vacuum. Thionephosphate **VI** was purified by chromatography on a column packed with silica gel (10 g) and filled with benzene. Compound **VI** was eluted with 35 ml of benzene, the solvent was removed in a vacuum, the residue was maintained for 2 h at 40°C and 1 mm Hg. Yield 0.5 g (59%), mp 105–106°C, R_f 0.3 (A), 0.85 (B). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.43 s, 1.44 s [6H, $C(CH_3)_2$], 4.05 d (2H$_e$) and 4.38 d (2H$_a$) (CH_2OC, $^2J_{a,e}$ 12.8 Hz), 4.43 m (4H, OCH_2CH_2O), 4.56 d (2H, CH_2OP, $^3J_{HP}$

9.14 Hz). ^{31}P NMR spectrum (chloroform), δ, ppm: 82.89 s. Found, %: C 34.71; H 5.39; P 10.12. $C_9H_{16}NO_7PS$. Calculated, %: C 34.50; H 5.15; P 9.89. *M* 313.27.

2-[(2,2-Dimethyl-5-nitro-1,3-dioxan-5-yl)-methoxy]-2-thioxo-1,3,2-dioxaphosphorinane (VII) was obtained in the same way as compound **VI** from 0.5 g (2.6 mmol) of compound **II**, 0.4 g (2.6 mmol) of 2-chloro-1,3,2-dioxaphosphorinane (**IIIb**), 0.2 g (2.6 mmol) of triethylamine, and 0.1 g (2.8 mmol) of sulfur in 20 ml of anhydrous benzene. Phosphorinane **V** formation was monitored by ^{31}P NMR spectroscopy (^{31}P NMR spectrum, δ, ppm: 129.56 s). The treatment with sulfur was carried out at 80–85°C for 3 h. Reaction product **VII** was purified by chromatography on a column packed with silica gel (15g) and filled with benzene. Compound **VII** was eluted with 40 ml of benzene–dioxane mixture, 10:1. The solvent was removed in a vacuum, the residue was maintained for 2 h at 40°C and 1 mm Hg. Yield 0.64 g (75%), mp 98–100°C, R_f 0.35 (A), 0.90 (B). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.42 s, 1.43 s [6H, $C(CH_3)_2$], 1.9 m (2H, $OCH_2C\underline{H}_2CH_2O$), 4.08 d (2H$_e$) and 4.47 d (2H$_a$) ($CH_2OC$, $^2J_{a,e}$ 11.60 Hz), 4.32 m (2H$_e$) and 4.42 m (2H$_a$) ($OC\underline{H}_2CHC\underline{H}_2O$, $^3J_{HP}$ 10.45 Hz), 4.50 m (2H, CH_2OP, $^3J_{HP}$ 8.98 Hz). ^{31}P NMR spectrum (chloroform), δ, ppm: 60.89 s. Found, %: C 36.59; H 5.34; P 9.71. $C_{10}H_{18}NO_7PS$. Calculated, %: C 36.70; H 5.54; P 9.46. *M* 327.29.

***O*-[(2,2-Dimethyl-5-nitro-1,3-dioxan-5-yl)-methyl]thionephosphocholine (VIII).** A sealed ampule containing a solution of 0.2 g (0.64 mmol) of thionephosphate **VI** and 0.11 g (1.9 mmol) of trimethylamine in 5 ml of anhydrous benzene was maintained at 30–35°C for 80 h. The solvent was removed in a vacuum, the oily residue was washed with benzene (2×5 ml), hexane (2×5 ml), and dried for 2 h at 40°C and 1 mm Hg. Yield 0.13 g (55%), mp 145–147°C (became moist at 99°C), R_f 0.00 (B), 0.45 (D). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.25 s, 1.41 s [6H, $C(CH_3)_2$], 2.82 br.s [9H, $N^+(CH_3)_3$], 3.74 m (2H, CH_2N^+), 3.98 d (2H$_\varepsilon$) and 4.38 d (2H$_a$) (CH_2OC, $^2J_{a,\varepsilon}$ 12.65 Hz), 4.06 m (2H, $POC\underline{H}_2CH_2N^+$, $^3J_{HP}$ 11.68 Hz), 4.40 d (2H, CH_2OP, $^3J_{HP}$ 10.03 Hz). ^{31}P NMR spectrum (chloroform), δ, ppm: 55.50 s. Found, %: C 38.59; H 6.93; P 8.61. $C_{12}H_{25}N_2O_7PS$. Calculated, %: C 38.70; H 6.78; P 8.32. *M* 372.39.

***O*-[(2,2-Dimethyl-5-nitro-1,3-dioxan-5-yl)-methyl]thionephosphohomocholine (IX)** was obtained in the same way as phosphocholine **VIII** from 0.3 g (0.9 mmol) of cyclothionephosphate **VII** and 0.16 g (2.7 mmol) of trimethylamine in 5 ml of anhydrous benzene at 55–60°C in 72 h. Yield 0.3 g (85%), mp 182–183°C (became moist at 165°C), R_f 0.00 (B), 0.15 (C), 0.5 (D). ^{1}H NMR spectrum ($CDCl_3$–CD_3OD, 99:1), δ, ppm: 1.32 s, 1.39 s [6H, $C(CH_3)_2$], 2.02 m (2H, $POCH_2C\underline{H}_2CH_2N^+$), 3.10 br.s [9H, $N^+(CH_3)_3$], 3.48 br.m (2H, CH_2N^+), 3.93 m (2H, $OPOC\underline{H}_2CH_2CH_2N^+$, $^3J_{HP}$ 11.00 Hz), 4.07 d (2H$_\varepsilon$) and 4.47 d (2H$_a$) (CH_2OC, $^2J_{a,\varepsilon}$ 13.43 Hz), 4.40 d (2H, CH_2OP, $^3J_{HP}$ 13.73 Hz). ^{31}P NMR spectrum (chloroform–methanol, 99:1), δ, ppm: 54.16 s. Found, %: C 40.63; H 7.32; P 8.39. $C_{13}H_{27}N_2O_7PS$. Calculated, %: C 40.41; H 7.04; P 8.02. *M* 386.41.

2-[(2-Nitro-3-palmitoyloxy-2-palmitoyloxymethyl)propoxy]-2-thioxo-1,3,2-dioxaphosphorinane (X). To a solution of 0.1 g (0.3 mmol) of thionephosphate **VII** in 5 ml of chloroform at 0°C was added 0.008 g (0.08 mmol) of $ZnCl_2 \cdot 1.5H_2O$ and 0.17 g (0.6 mmol) of palmitoyl chloride. The reaction mixture was kept for 1 h at 0°C, then for 24 h at 20–25°C. Chloroform was removed in a vacuum. Compound **X** was subjected to chromatography on a column packed with silica gel (15 g) and filled with hexane, elution with 20 ml of hexane–dioxane mixture, 3:1. The solvent was removed in a vacuum, and the residue was kept for 2 h at 40°C and 1 mm Hg, yield of amorphous compound 0.14 g (60%), mp 58–59°C, R_f 0.45 (A), 0.85 (B). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 0.89 t (6H, $C\underline{H}_3CH_2$, $^3J_{HH}$ 6.7 Hz), 1.26 m [48H, $CH_3(C\underline{H}_2)_{12}$], 1.61 m [4H, $C\underline{H}_2CH_2C(O)$], 1.98 br.m (2H, $OCH_2C\underline{H}_2CH_2O$), 2.34 m [4H, $CH_2C\underline{H}_2C(O)$], 4.02 br.m (2H$_\varepsilon$) and 4.40 br.m (2H$_a$) ($OC\underline{H}_2CH_2C\underline{H}_2O$), 4.04 d (2H, $C\underline{H}_2OP$, $OCH_2CH_2CH_2O$, $^3J_{HP}$ 12.51 Hz), 4.60 C [4H, $CH_2OC(O)$]. ^{31}P NMR spectrum (chloroform), δ, ppm: 61.56 s. Found, %: C 61.56; H 10.01; P 4.31. $C_{39}H_{74}NO_9PS$. Calculated, %: C 61.31; H 9.76; P 4.05. *M* 764.023.

O-[(2-Nitro-3-palmitoyloxy-2-palmitoyloxymethyl)propyl]thionephosphohomocholine (XI) was obtained in the same way as phosphocholine **VIII** from 0.1 g (0.13 mmol) of cyclothionephosphate **X** and 0.03 g (0.52 mmol) of trimethylamine in 5 ml of anhydrous benzene at 70–75°C in 10 h. Yield 0.04 g (35%), mp 190–192°C (became moist at 150°C), R_f 0.35 (C). ^{1}H NMR spectrum NMR spectrum ($CDCl_3$), δ, ppm: 0.88 t (6H, $C\underline{H}_3CH_2$, $^3J_{HH}$ 6.74 Hz), 1.26 m [48H, $CH_3(C\underline{H}_2)_{12}$], 1.60 m [4H, $C\underline{H}_2CH_2C(O)$], 2.14 br.m (2H, $POCH_2C\underline{H}_2CH_2N^+$), 2.34 m [4H, $CH_2C\underline{H}_2C(O)$], 3.17 br.s [9H, $N^+(CH_3)_3$], 4.04 d (2H, CH_2OP, $^3J_{HP}$ 12.20 Hz), 4.07 m (2H, $POCH_2CH_2C\underline{H}_2N^+$), 4.12 m (2H,

OPOC$\underline{H}_2$CH$_2$CH$_2$N$^+$, $^3J_{HP}$ 12.21 Hz), 4.58 C [4H, CH$_2$OC(O)]. ^{31}P NMR spectrum (chloroform), δ, ppm: 54.98 s. Found, %: C 61.39; H 10.31; P 3.59. $C_{42}H_{83}N_2O_9PS$. Calculated, %: C 61.28; H 10.16; P 3.76. *M* 823.14.

Triethylammonium *O*-[(2,2-dimethyl-5-nitro-1,3-dioxan-5-yl)methyl]-*O*-(3-hydroxypropyl)-thionephosphate (XII) and triethylammonium *O*-[(2,2-dimethyl-5-nitro-1,3-dioxan-5-yl)methyl]-S-(3-hydroxypropyl)thiolphosphate (XIII). A sealed ampule containing a solution of 0.1 g (0.3 mmol) of cyclic thionephosphate **VII**, 0.01 g (0.6 mmol) of water, and 0.15 g (1.5 mmol) of triethylamine in 2 ml of dioxane (pH > 10) was heated to 120°C for 5 h. Dioxane and excess triethylamine were removed in a vacuum, the oily residue was dissolved in 1 ml of dioxane and twice reprecipitated with hexane, washed with ethyl ether (2×5 ml), and dried for 2 h at 50°C and 1 mm Hg. Yield 0.1 g (43%), mp 164–165°C, R_f 0.00 (B), 0.48 (C). ^{1}H NMR spectrum (CDCl$_3$–CD$_3$OD, 99:1), δ, ppm: 1.31 t (9H, NCH$_2$C$\underline{H}_3$, $^3J_{HH}$ 7.29 Hz), 1.33 s, 1.36 s [6H, C(CH$_3$)$_2$], 2.00 br.m (2H, POCH$_2$C$\underline{H}_2$CH$_2$OH), 2.79 br.m (2H, PSC$\underline{H}_2$CH$_2$CH$_2$OH), 3.08 q (6H, NC$\underline{H}_2$CH$_3$, $^3J_{PH}$ 7.14 Hz), 3.30 d (2H$_e$) and 4.47 d (2H$_a$) (CH$_2$OC, $^2J_{a,e}$ 12.65), 3.95–4.42 br.m (6H, C$\underline{H}_2$OPOC$\underline{H}_2$CH$_2$C$\underline{H}_2$OH). ^{31}P NMR spectrum (chloroform–methanol, 99:1), δ, ppm: 57.14 s (**XII**) and 16.31 s (**XIII**) (intensity ratio 1:9). Found, %: C 42.98; H 7.78; P 7.01. $C_{16}H_{35}N_2O_8PS$. Calculated, %: C 43.04; H 7.90; P 6.94. *M* 446.50.

***O*-[(2,2-Dimethyl-5-nitro-1,3-dioxan-5-yl)-methyl]-O-(2-bromomethyl)bromophosphate (XIV).** To a solution of cyclophosphite **IV** (δ$_P$, ppm: 134.89 s) prepared from 0.4 g (2.08 mmol) of compound **II**, 0.24 g (2.08 mmol) of 2-chlorodioxaphospholane (**IIIa**), and 0.24 g (2.08 mmol) of triethylamine in 15 ml of anhydrous benzene at –5°C while vigorous stirring was added dropwise a solution of 0.33 g (2.08 mmol) of bromine in 3 ml of the same solvent. In 10 min the solvent was removed in a vacuum, and compound **XIV** obtained was purified on a column packed with silica gel (10 g) and filled with benzene. Acyl bromide **XIV** was eluted with 25 ml of benzene–dioxane mixture, 3:1. The solvent was removed in a vacuum, and the residue was kept for 2 h at 40°C and 1 mm Hg. Yield 0.45 g (49%), R_f 0.25 (A), 0.75 (B). ^{1}H NMR spectrum (CDCl$_3$), δ, ppm: 1.42 s and 1.44 C [6H, C(CH$_3$)$_2$], 3.52 m (2H, OCH$_2$C$\underline{H}_2$Br), 4.03 d (2H$_e$) and 4.38 d (2H$_a$) (CH$_2$OC, $^2J_{a,e}$ 12.79 Hz), 4.30 m (2H, OC$\underline{H}_2$CH$_2$Br), 4.54 d (2H, CH$_2$OP, $^3J_{HP}$ 5.43 Hz). ^{31}P NMR spectrum (chloroform), δ, ppm: –2.92 s. Found, %: C 24.79; H 3.93; P 7.31. $C_9H_{16}Br_2NO_7P$. Calculated, %: C 24.51; H 3.66; P 7.02. *M* 441.03.

***O*-[(2,2-Dimethyl-5-nitro-1,3-dioxan-5-yl)-methyl]-*O*-(1,2-*O*-isopropylidene-3-glycero)-*O*-(2-bromoethyl)phosphate (XV).** To a solution of 0.1 g (0.23 mmol) of acyl bromide **XIV** in ml of anhydrous benzene at stirring while cooling to 0°C was added dropwise a solution of a mixture of 0.03 g (0.23 mmol) of 1,2-*O*-isopropylideneglycerol and 0.02 g (0.23 mmol) of freshly distilled pyridine in 2 ml of the same solvent. The reaction mixture was heated to 40°C for 3 h. Pyridine hydrobromide was filtered off, the solvent was removed in a vacuum, and compound **XV** was isolated on a column charged with silica gel (10 g)and filled with hexane. Compound **XV** was eluted with 40 ml of hexane–dioxane mixture, 3:1. The solvent was removed in a vacuum, and the residue was kept for 2 h at 40°C and 1 mm Hg. Yield 0.06 g (50%) (viscous oily substance), R_f 0.40 (A), 0.48 (B). ^{1}H NMR spectrum (CDCl$_3$), δ, ppm: 1.42 s and 1.44 s [12H, C(CH$_3$)$_2$], 3.49 m (2H, OCH$_2$C$\underline{H}_2$Br), 3.61 m (2H$_e$) and 4.07 m (2H$_a$) (2H, C$\underline{H}_2$CHCH$_2$OP), 4.02 d (2H$_e$) and 4.37 d (2H$_a$) (CH$_2$OC, $^2J_{a,e}$ 12.21 Hz), 4.29 m (4H, OC$\underline{H}_2$CH$_2$Br, CH$_2$CHC$\underline{H}_2$OP), 4.53 d (2H, CH$_2$OP, $^3J_{HP}$ 5.80 Hz), 4.68 m (1H, CH$_2$C$\underline{H}$CH$_2$OP). ^{31}P NMR spectrum (chloroform), δ, ppm: –1.09 s. Found, %: C 36.83; H 5.71; P 6.51. $C_{15}H_{27}BrNO_{10}P$. Calculated, %: C 36.60; H 5.53; P 6.29. *M* 492.27.

2-{O-[(2,2-Dimethyl-5-nitro-1,3-dioxan-5-yl)-methyl-O-(1,2-O-isopropylidene-3-glycero)-phosphoryl]ethyl}trimethylammonium bromide (XVI) was obtained in the same way as compound **VIII** from 0.1 g (0.20 mmol) of phosphate **XV** and 0.1 g (0.80 mmol) of trimethylamine in 3 ml of anhydrous benzene in 5 h at 55°C. The solvent was removed in a vacuum, the residue was washed with anhydrous benzene (2×2 ml). The product was additionally purified by recrystallization ftom acetone at cooling to 5°C. Then the compound obtained was maintained for 3 h at 40°C and 1 mm Hg. Yield 0.05 g (45%), mp 153–154°C (became moist at 90°C), R_f 0.75 (C). ^{1}H NMR spectrum (CDCl$_3$), δ, ppm: 1.37 s and 1.44 s (12H) [C(CH$_3$)$_2$], 2.82 br.s [9H, N$^+$(CH$_3$)$_3$], 3.58 d (2H$_e$) and 3.95 d (2H$_a$) (CH$_2$OC, $^2J_{a,e}$ 11.55 Hz), 3.68 m (2H, CH$_2$N$^+$), 3.79 m (2H, C$\underline{H}_2$CHCH$_2$OP), 4.24 m (4H, CH$_2$CHC$\underline{H}_2$OP, OC$\underline{H}_2$CH$_2$N$^+$), 4.42 d (2H, CH$_2$OP, $^3J_{HP}$ 7.70 Hz), 4.52 m (1H, CH$_2$C$\underline{H}$CH$_2$OP). ^{31}P NMR spectrum (chloroform), δ, ppm: –1.12 s. Found, %: C 39.00; H 6.39; P 5.81. $C_{18}H_{36}BrN_2O_{10}P$. Calculated, %: C 39.21; H 6.58; P 5.62. *M* 551.38.

O-[(2,2-Dimethyl-5-nitro-1,3-dioxan-5-yl)-methyl]-3-methyl-2-thioxo-1,3,2-oxaazaphospholane (XVIII). A mixture of 0.5 g (2.6 mmol) of compound **II** and 0.42 g (2.6 mmol) of 2-diethylamino-3-methyl-1,3,2-oxaazaphospholane in 2 ml of anhydrous benzene was heated at 80–85°C for 1.5 h while distilling off eliminating diethylamine and benzene. Cyclophosphite **XVII** formation was monitored by ^{31}P NMR spectroscopy (δ, ppm: 138.05 s). To the obtained solution of cycloamidophosphite **XVII** was added 0.1 g (2.8 mmol) of sulfur, and the mixture was heated at 85°C for 4 h. Excess sulfur was filtered off, benzene was removed in a vacuum. Thionephosphate **XVII** was purified by chromatography on a column packed with silica gel (10 g) and filled with benzene. Compound **XVIII** was eluted with 30 ml of benzene–dioxane mixture, 1:1. The solvent was removed in a vacuum, the residue was maintained for 2 h at 40°C and 1 mm Hg. Yield 0.47 g (55%), mp 89–90°C, R_f 0.25 (A), 0.80 (B). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.37 s and 1.44 s (6H) [$C(CH_3)_2$], 2.68 d (3H, $PNCH_3$, $^3J_{PH}$ 12.20 Hz), 3.32 m (2H, $OCH_2C\underline{H}_2N$), 4.02 d (2H$_\varepsilon$) and 4.36 d (2H$_a$) (CH_2OC, $^2J_{a,\varepsilon}$ 12.80 Hz), 4.25 m (2H, $OC\underline{H}_2CH_2N$), 4.50 d (2H, CH_2OP, $^3J_{HP}$ 9.08 Hz). ^{31}P NMR spectrum (chloroform), δ, ppm: 84.90 C. Found, %: C 36.69; H 5.73; P 9.61. $C_{10}H_{19}N_2O_6PS$. Calculated, %: C 36.81; H 5.87; P 9.49. *M* 326.31.

***O*-[(2,2-Dimethyl-5-nitro-1,3-dioxan-5-yl)methyl]-*O*-[(2-*N*-methylammonio)-ethyl]-thionephosphate (XIX).** To a solution of 0.1 g (0.31 mmol) of cyclic thionephosphate **XVIII** in 3 ml of dioxane was added 0.005 g (0.28 mmol) of water (pH 5), and the reaction mixture was heated for 2 h at 90°C. The solvent was removed in a vacuum, the oily residue was dissolved in 1 ml of dioxane, twice reprecipitated with hexane (2×2 ml), and dried for 2 h at 50°C and 1 mm Hg. Yield 0.09 g (80%), mp 175–177°C (became moist at 155°C), R_f 0.00 (B), 0.75 (C). ^{1}H NMR spectrum (CD_3OD–saturated solution of KOH, 99.9:0.1), δ, ppm: 1.37 s and 1.48 s (6H) [$C(CH_3)_2$], 2.74 d (3H, NCH_3), 3.21 t (2H, CH_2N^+, $^4J_{HH}$ 4.88 Hz), 3.98 d (2H$_e$) and 4.29 d (2H$_a$) (CH_2OC, $^2J_{a,e}$ 13.12 Hz), 4.51 m (4H, CH_2OPOCH_2), 4.9 br.s (2H, N^+H_2). ^{31}P NMR spectrum (methanol– saturated solution of KOH, 99.9:0.1), δ, ppm: 57.30 s. Found, %: C 34.59; H 5.95; P 8.75. $C_{10}H_{21}N_2O_7PS$. Calculated, %: C 34.88; H 6.15; P 9.00. *M* 344.33.

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REFERENCES

1. Predvoditelev, D.A. and Nifant'ev, E.E., *Usp. Khim.,* 1997, vol. 66, p. 47.
2. Predvoditelev, D.A., Kosarev, G.V., Suvorkin, S.V., and Nifant'ev, E.E., *Zh. Org. Khim.* , 2002, vol. 38, p. 1671.
3. Predvoditelev, D.A., Malenkovskaya, M.A., and Nifant'ev, E.E., *Zh. Obshch. Khim.* , 2003, vol. 73, p. 1620.
4. Predvoditelev, D.A. and Nifant'ev, E.E., *Zh. Org. Khim.*, 1995, vol. 31, p. 1761.
5. Malenkovskaya, M.A., Predvoditelev, D.A., Bel'skii, V.K., and Nifant'ev, E.E., *Zh. Obshch. Khim.* , 2003, vol. 73, p. 1976.
6. Burchardt, A., Takahashi, T., Takenchi, J., and Haufe, G., *J. Org. Chem.,* 2001, vol. 66, p. 2078.
7. Shin, J., Qualls, M.M., Boomer, J.A., Babarge, J., and Thompson, D.N., *J. Am. Chem. Soc.,* 2001, vol. 123, p. 508.
8. Nifantyev, E.E., Predvoditelev, D.A., Malenkovskaya, M.A., Bekker, A.R., and Meshkov, C.V., *Phosphorus Sulfur and Silicon*, 1994, vol. 89, 199.
9. Predvoditelev, D.A., Cuvorkin, S.V., and Nifant'ev, E.E., *Zh. Obshch. Khim.* , 2001, vol. 71, p. 930.
10. Predvoditelev, D.A., Kosarev, G.V., and Nifant'ev, E.E., *Zh. Obshch. Khim.* , 2003, vol. 73, p. 1611.
11. Li, S. and Huang, L., *Gene Ther*., 2000, vol. 7, p. 31.
12. Maslov, M.A., Morozova, N.G., and Serebrennikova, G.A., *Izv. Akad.Nauk, Ser.Khim.,* 2002, vol. 10, p. 1778.
13. Sokolova, T.V., Klesareva, Yu.S., and Serebrennikova, G.A., *Zh. Org. Khim.* , 2004, vol. 40, p. 365.
14. Gefter, E.L., *Zh. Obshch. Khim.* , 1956, vol. 26, p. 1440.
15. Zwierak, A., *Canad. J. Chem.,* 1967, vol. 45, p. 2501.
16. Lucas, H.J., Mitchel, F.N., and Scally, C.N., *J. Am. Chem. Soc*., 1950, vol. 72, p. 5491.