Russian Journal of Organic Chemistry, Vol. 40, No. 9, 2004, pp. 1364–1370. Translated from Zhurnal Organicheskoi Khimii, Vol. 40, No. 9, 2004, pp. 1410–1417.

Synthesis of First Phospholipids from 2,2,6,6-Tetrakis(hydroxymethyl)cyclohexanol*

D. A. Predvoditelev[1], G. A. Savin[2], M. A. Malenkovskaya[1], and E. E. Nifant'ev[1]

[1] *Moscow State Pedagogical University, Nesvizhskii per. 3, Moscow, 119021 Russia*

[2] *Volgograd State Pedagogical University, Volgograd, Russia*

Received June 15, 2003

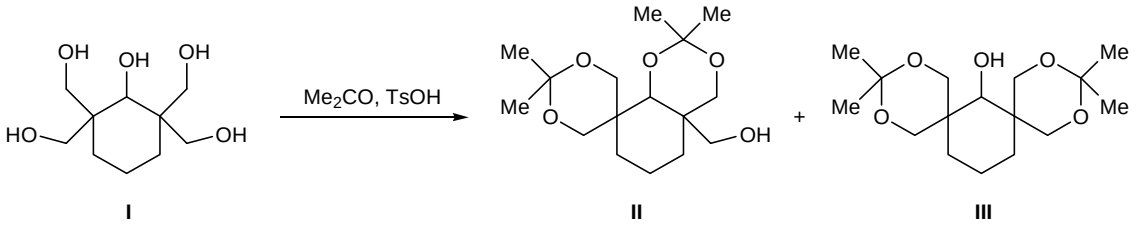
Abstract—A procedure has been developed for the synthesis of previously unknown thio phospholipids and cationic ammonium amidophosphorus lipids on the basis of 2,2,6,6-tetrakis(hydroxymethyl)cyclohexanol. Model phospholipids have been prepared using phosphorus-containing cyclic compounds.

Glycerophospholipids persistently attract attention of researchers [2, 3]. However, phospholipids based on aliphatic polyols have been studied poorly. Up to now, only a few examples of such compounds have been synthesized from aliphatic polyols, in particular from 1,2,4-butanetriol [4], 1,2,5-pentanetriol [5], tris- and tetrakis(hydroxymethyl)alkanes [6, 7], xylitol [8], and ribose [9]. Among the prepared phospholipids there were no compounds having structural fragments of aliphatic carbocyclic polyols. On the other hand, phospholipids whose molecules possess an additional rigidity factor should be characterized by a specific steric structure, and they may be important models for studies in various fields of physical and chemical biology and medicine.

The goal of the present work was to synthesize previously unknown thio phospholipids and cationic ammonium phosphoramide lipids having a 2,2,6,6-tetrakis(hydroxymethyl)cyclohexanol moiety as structural fragment. In the first step of our study, 2,2,6,6-tetrakis(hydroxymethyl)cyclohexanol (**I**) was brought into reaction with acetone to protect four hydroxy groups therein. As a result, two isomeric acetals **II** and **III** were obtained (Scheme 1). The reaction was performed in the presence of a catalytic amount of anhydrous *p*-toluenesulfonic acid with simultaneous removal of the liberated water by azeotropic distillation. Acetals **II** and **III** differed in the chromatographic mobility, and we succeeded in separating them by column chromatography on silica gel. Moreover, compound **III** was less soluble in organic solvents; therefore, products **II** and **III** can also be purified by recrystallization. The major product was compound **II** (yield 65%), while the yield of **III** was as low as 4.8%.

The structure of compounds **II** and **III** was confirmed by the ^{1}H NMR spectra. In the spectrum of bis-acetal **II**, protons of the geminal methyl groups appeared as singlets at δ 1.35 and 1.45 ppm, methylene protons of the cyclohexane fragment gave a multiplet at δ 1.39 ppm, and the singlet at δ 4.28 ppm was assigned to the 1-H proton. The unsymmetrical structure of compound **II** follows from the spectral pattern formed by signals from axial and equatorial protons in the 1,3-dioxane rings, which were located at δ 3.12 (d), 4.10 (d) and 3.74 (d), 3.95 ppm (d) with the coupling constants $^2J(ax–eq)$ = 11.9 and 10.99 Hz, respectively.

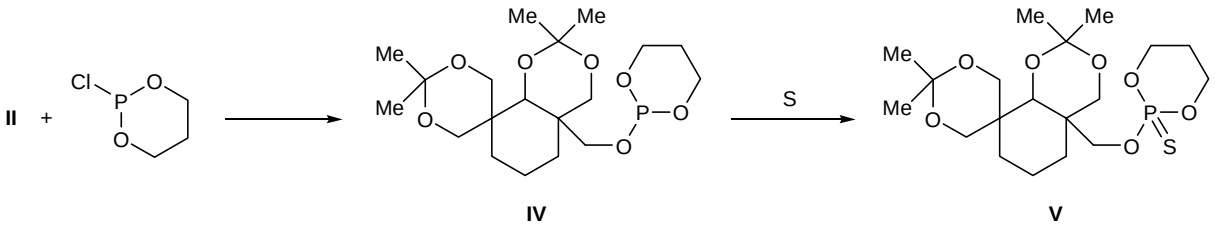
Scheme 1.



I **II** **III**

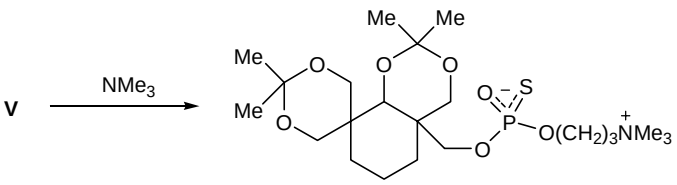
* For short communication, see [1].

Scheme 2.



IV **V**

Scheme 3.



VI

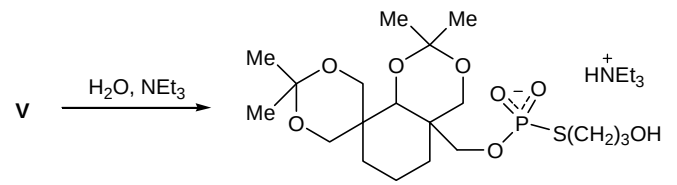
The hydroxy proton in **II** resonated at δ 3.87 ppm. The ^{1}H NMR spectrum of isomeric bis-acetal **III** showed a different pattern from protons of the CH_2O groups in the symmetrically arranged dioxane rings. These protons gave rise to doublets at δ 3.34 and 3.91 ppm with a characteristic coupling constant between the axial and equatorial protons. The singlet from the 1-H proton neighboring to the hydroxy group was displaced downfield (δ 4.56 ppm). Insofar as the yield of compound **III** did not exceed 5%, further syntheses of phospholipids were performed with the use of bis-acetal **II**.

Phosphorylation of bis-acetone derivative **II** with 2-chloro-1,3,2-dioxaphosphinane at 0°C in the presence of triethylamine afforded the corresponding phosphite **IV**. Its accumulation was monitored by ^{31}P NMR spectroscopy (δ_P 130 ppm). Phosphite **IV** was converted (without isolation) into thiophosphate **V** which was isolated in 60% yield by recrystallization from hexane (Scheme 2). The ^{31}P NMR spectrum of compound **V** contained a singlet at δ_P 62.06 ppm, and its ^{1}H NMR spectrum was consistent with the assumed structure. Protons in the dioxaphosphinane ring appeared in the ^{1}H NMR spectrum as multiplets at δ 2.17 and 4.08 ppm.

Thiophosphate **V** was used to obtain compound **VI** as a model of terminal choline thio phospholipid. For this purpose, compound **V** was treated with trimethylamine (Scheme 3). The yield of product **VI** was 82%. It showed in the ^{31}P NMR spectrum a singlet typical of acyclic *O*,*O*-dialkyl thiophosphates (δ_P 56.62 ppm). The ^{1}H NMR spectrum of **VI** contained a singlet from the N-methyl protons at δ 3.31 ppm, multiplets from β- and γ-methylene protons of the $CH_2CH_2N^+$ group at δ 2.27 and 3.34 ppm, and signals from the other protons, whose position was almost the same as in the spectrum of the initial compound (see Experimental).

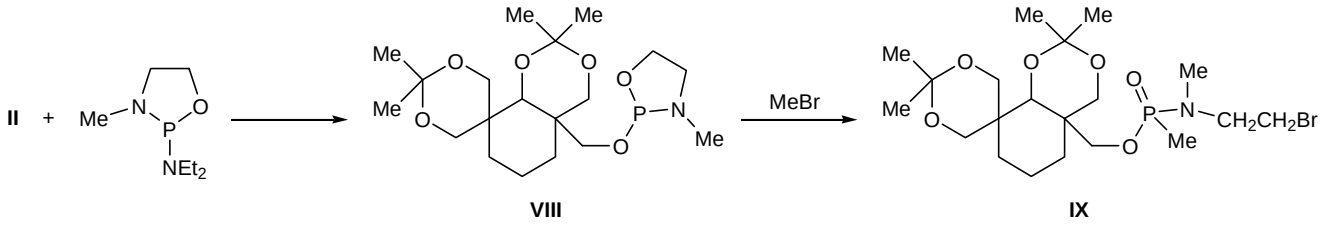
Apart from phosphocholine derivative **VI**, thiophosphate **V** was used to synthesize a model diol phospholipid (**VII**). The synthesis was effected by hydrolysis of **V** in dioxane in the presence of 5 equiv of triethylamine (Scheme 4). Excess triethylamine was taken with a view to obtain product **VII** as the corresponding ammonium salt. It should be noted that our previous attempts to carry out the hydrolysis in boiling dioxane were unsuccessful. Salt **VII** was isolated from the dioxane solution by precipitation with hexane (yield 45%). In the ^{31}P NMR spectrum of the product we observed a broadened signal in the region typical of thiolic thiophosphates (δ_P 14.55 ppm). This indicates

Scheme 4.

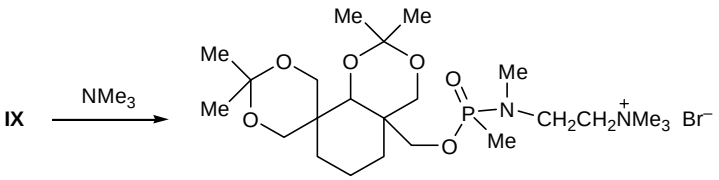


VII

Scheme 5.



Scheme 6.



that the process is accompanied by the thione–thiol isomerization. Analogous isomerizations were observed previously in the synthesis of other thio phospholipids [10, 11]. The ^{1}H NMR spectrum of **VII** retained signals from protons in the tricyclic moiety, and new signals appeared due to protons in the trialkylammonium cation. Also, signals from the β- and γ-methylene protons in the CH_2CH_2OH group were present. An important feature is the position of signal from protons of the methylene group attached to sulfur. This signal is displaced upfield relative to the corresponding signal in the spectra of related phosphoryl compounds ($\Delta\delta$ = 1.6 ppm). The downfield signal at δ 9.55 ppm was assigned to the hydroxy proton in the $HOCH_2CH_2CH_2$ group.

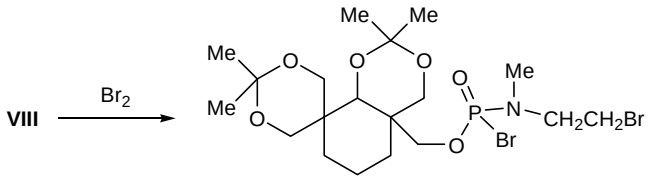
Another cyclic phosphorus-containing compound obtained from bis-acetal **II** was oxazaphospholidine derivative **VIII**. It was synthesized by phosphorylation of **II** with 2-diethylamino-3-methyl-1,3,2-oxazaphospholidine in a dilute solution in anhydrous benzene at 80–85°C (reaction time 1 h) with simultaneous removal of the liberated diethylamine and the solvent (Scheme 5). The progress of the reaction was monitored by ^{31}P NMR spectroscopy. Cyclic phosphoramidite **VIII** thus obtained (δ_P 136.63 ppm, s) was brought into further syntheses of lipids without additional purification. First of all, we performed alkylation of **VIII** with methyl bromide according to Arbuzov in order to obtain methylphosphonamidate lipid model **IX**. The yield of methylphosphonate **IX** attained 55%, calculated on the initial bis-acetal **II**. The ^{31}P–{^{1}H} NMR spectrum of **IX** contained a singlet at δ_P 32.98 ppm. In the ^{1}H NMR spectrum of **IX**, signals from protons of all structural fragments were present. The PCH_3 signal appeared as a doublet at δ 1.46 ppm due to coupling with the phosphorus atom ($^2J_{PH}$ = 16.59 Hz).

Phosphonate **IX** was used to synthesize a cationic lipid having a methylphosphonamidate moiety. Quaternization of triethylamine with compound **IX** occurred in 5 h at 90°C (Scheme 6). The progress of the reaction was monitored by thin-layer chromatography. Chromatographically pure ammonium salt **X** was isolated as a solid amorphous substance (yield 60%). In the ^{1}H NMR spectrum of **X** we observed a singlet from protons of the $(CH_3)_3N^+$ group at δ 3.47 ppm, signal from the CH_2Br moiety disappeared, and a multiplet appeared due to methylene protons of the CH_2N^+ group. The other signals in the spectrum of **X** were almost the same as in the spectrum of initial phosphonate **IX**.

Cyclic phosphoramidite **VIII** was also converted into bromoamidophosphate **XI** via oxidative decyclization with bromine under mild conditions (0°C) (Scheme 7). Like other lipid bromoamidophosphates [12, 13], compound **XI** did not change during isolation by column chromatography on silica gel (yield 55%). It can be stored without appreciable decomposition for several months at room temperature in the dark with protection from air. Pure bromophosphate **XI** showed in the ^{31}P NMR spectrum a singlet at δ_P 5.13 ppm. In the ^{1}H NMR spectrum of **XI**, signal from the $PNCH_3$ protons (δ 2.71 ppm) was split into a doublet due to coupling with phosphorus ($^3J_{HP}$ = 9.45 Hz), and methylene protons of the NCH_2CH_2Br fragment gave an unresolved multiplet at δ 3.42 ppm. The other

signals in the spectrum were consistent with the assumed structure (see Experimental).

Scheme 7.



XI

Using bromo derivative **XI**, we performed phosphorylation of natural cholesterol. Amidophosphate **XII** thus formed was treated with trimethylamine to obtain compound **XIII** which can be regarded as a model of a cationic cholesterol phospholipid (Scheme 8). The reaction of **XI** with cholesterol was carried out in anhydrous benzene in the presence of pyridine at room temperature (reaction time 18 h). Phosphate **XII** was isolated in 53% yield by column chromatography on silica gel. Its structure was proved by spectral data. In the ^{31}P NMR spectrum of phosphate **XII** (which was a mixture of diastereoisomers), a broadened singlet was present at δ_P 8.95 ppm. The ^{1}H NMR spectra of cholesterol phospholipids **XII** and **XIII** were analyzed with account taken of the data in [11, 14], where detailed assignment of all signals from the cholesterol fragment was given. In the spectrum of **XII**, the 3-H proton of the cholesterol moiety gave a multiplet at δ 4.43 ppm, while the 6-H signal was a doublet at δ 5.38 ppm due to coupling with 7-H (see Experimental).

The reaction of amidophosphate **XII** with trimethylamine occurred at 60°C in 8 h, and the yield of ammonium salt **XIII** was 50%. The ^{31}P NMR spectrum of **XIII** was almost similar to the spectrum of **XII**. Unlike the latter, signal from the $NCH_2C\mathbf{H}_2$ protons in the ^{1}H NMR spectrum of **XIII** was located in a weaker field, and the singlet at δ 3.49 ppm was assigned to the methyl protons in the cationic center [$(CH_3)_3N^+$].

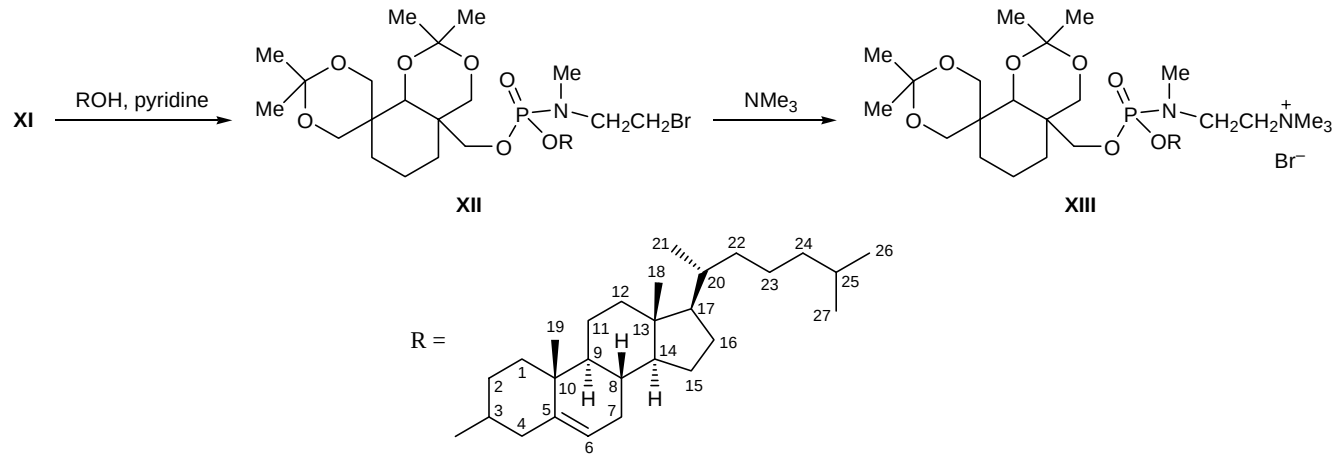
Thus we have synthesized a number of new phospholipid systems, including cationic ones, which contain a carbocyclic polyol fragment. It is important that ammonium phosphorus-free lipids on the basis of glycerol and other related compounds [15–17] are now extensively studied from the viewpoint of their possible use in gene therapy for delivery of gene materials into a cell.

EXPERIMENTAL

The ^{1}H NMR spectra were recorded on a Bruker WM-250 spectrometer (250 MHz); the chemical shifts were measured relative to HMDS as internal reference; signals were assigned on the basis of the double resonance spectra. The ^{31}P–{^{1}H} NMR spectra were obtained on a Bruker WP-80SY instrument (32.4 MHz) using 85% phosphoric acid as external reference. The products were isolated and purified by chromatography using a 10-mm (i.d.) column charged with silica gel L (100–250 μm); R_f values were determined by TLC on Silufol UV-254 plates using benzene–dioxane (3:1) (A), hexane–dioxane (3:1) (B), chloroform–methanol–water (65:25:4 (C), and chloroform–dioxane (20:1) (D) as eluents. The melting points were determined in sealed capillaries on heating at a rate of 1 deg/min.

2-Chloro-1,3,2-dioxaphosphinane was synthesized by the procedure reported in [18], and 2-diethylamino-3-methyl-1,3,2-oxazaphospholidine was prepared as described in [19].

Scheme 8.



XII **XIII**

(2,2,2',2'-Tetramethyldihydro-4*H*,5*H*-spiro-[1,3-benzodioxine-8,5'-[1,3]dioxan]-4a(8a*H*)-yl)-methanol (II) and 3,3,11,11-tetramethyl-2,4,10,12-tetraoxadispiro[5.1.5.3]hexadecan-7-ol (III). A round-bottom flask equipped with a Dean–Stark trap and a reflux condenser was charged with 11 g (25 mmol) of 2,2,6,6-tetrakis(hydroxymethyl)cyclohexanol (**I**) and 50.8 g (874 mmol) of acetone, and 250 ml of anhydrous benzene and 0.1 g (0.5 mmol) of *p*-toluenesulfonic acid were added. The mixture was heated under reflux until water no longer separated. The mixture was neutralized with sodium carbonate and filtered, the filtrate was evaporated, and the residue was recrystallized from heptane (2×100 ml) to obtain bis-acetal **II** in 56% yield. The mother liquors were combined and evaporated, the residue was dissolved in chloroform, and the solution was applied to a column charged with silica gel. The column was eluted with hexane–dioxane (20:1) to isolate compounds **II** and **III**. The eluate was evaporated under reduced pressure, and the residue was kept for 2 h at 40°C (1 mm). Yield of **II** from the mother liquor 1.32 g (8.8%); mp 114–115°C (from heptane); R_f 0.38 (B), 0.45 (D). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.34 s, 1.35 s, 1.41 s, and 1.45 s (3H each, CH_3); 1.39 m (6H, $CH_2CH_2CH_2$); 2.6 br.s (1H, OH); 3.12 d (2H, 4'-H$_{eq}$, 6'-H$_{eq}$) and 4.10 d (2H, 4'-H$_{ax}$, 6'-H$_{ax}$) ($^2J_{ax,eq}$ = 11.9 Hz); 3.87 s (2H, C**H**$_2$OH); 3.74 d (1H, 4-H$_{eq}$) and 3.95 d (1H, 4-H$_{ax}$) ($^2J_{ax,eq}$ = 10.99 Hz); 4.28 s (1H, CH). Found, %: C 64.08; H 9.36. $C_{16}H_{28}O_5$. Calculated, %: C 63.97; H 9.40. *M* 300.38.

Yield of compound **III** 0.72 g (4.8%); mp 92–93°C; R_f 0.48 (B), 0.6 (D). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.34 s, 1.35 s, 1.41 s, and 1.45 s (3H each, CH_3); 1.39 m (6H, $CH_2CH_2CH_2$); 2.6 br.s (1H, OH); 3.34 d (4H, H$_{eq}$) and 3.91 d (4H, H$_{ax}$) ($^2J_{ax,eq}$ = 11.6 Hz); 4.56 s (1H, C**H**OH). Found, %: C 64.02; H 9.40. $C_{16}H_{28}O_5$. Calculated, %: C 63.97; H 9.40. *M* 300.38.

2-[(2,2,2',2'-Tetramethyldihydro-4*H*,5*H*-spiro-[1,3-benzodioxine-8,5'-[1,3]dioxan]-4a(8a*H*)-yl)-methoxy]-1,3,2λ^5-dioxaphosphinane 2-sulfide (V). A solution of 0.93 g (6.7 mmol) of 2-chloro-1,3,2-dioxaphosphinane in 10 ml of anhydrous benzene was added dropwise with stirring and cooling (0°C) to a solution of 2 g (6.7 mmol) of compound **II** and 0.67 g (6.7 mmol) of triethylamine in 20 ml of anhydrous benzene. The mixture was stirred for 1 h at 0°C and was allowed to warm up to room temperature. The formation of phosphite **IV** was monitored by ^{31}P NMR spectroscopy: δ_P 130 ppm, s. Triethylamine hydrochloride was filtered off, 0.23 g (7.3 mmol) of sulfur was added to the filtrate, and the mixture was heated to 60–70°C and was kept for 3 h at that temperature. Excess sulfur was filtered off, and the solvent was removed from the filtrate under reduced pressure. Thiophosphate **V** was purified by recrystallization from hexane. The product was dired for 2 h at 40°C (1 mm). Yield 1.75 g (60%), mp 136–137°C, R_f 0.25 (A). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.34 s, 1.35 s, 1.41 s, and 1.45 s (3H each, CH_3); 1.39 m (6H, $CH_2CH_2CH_2$); 2.17 m (2H, $OCH_2C\mathbf{H}_2CH_2O$); 3.12 d (2H, 4'-H$_{eq}$, 6'-H$_{eq}$) and 4.10 d (2H, 4'-H$_{ax}$, 6'-H$_{ax}$) ($^2J_{ax,eq}$ = 11.85 Hz); 3.74 d (1H, 4-H$_{eq}$) and 3.95 d (1H, 4-H$_{ax}$) ($^2J_{ax,eq}$ = 10.88 Hz); 4.08 m (4H, $OC\mathbf{H}_2CH_2C\mathbf{H}_2O$); 4.28 s (1H, CH). ^{31}P NMR spectrum (chloroform): δ_P 62.06 ppm, s. Found, %: C 52.55; H 7.36; P 7.32. $C_{19}H_{33}O_7PS$. Calculated, %: C 52.28; H 7.62; P 7.10. *M* 436.491.

***O*-3-(Trimethylammonio)propyl *O*-(2,2,2',2'-tetramethyldihydro-4*H*,5*H*-spiro[1,3-benzodioxine-8,5'-[1,3]dioxan]-4a(8a*H*)-yl)methyl thiophosphate (VI).** An ampule was charged with a solution of 0.2 g (0.45 mmol) of thiophosphate **V** and 0.18 g (3 mmol) of trimethylamine in 5 ml of anhydrous benzene. The ampule was sealed and heated for 20 h at 80–90°C. The solvent was removed by decanting, and the residue was washed with anhydrous benzene (2×5 ml) and dried for 2 h at 40°C under reduced pressure (1 mm). Yield 0.18 g (82%); mp 244–246°C; R_f 0.00 (A), 0.45 (C). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.33 s, 1.34 s, 1.36 s, and 1.39 s (3H each, CCH_3); 1.54 m (6H, $CH_2CH_2CH_2$); 2.27 m (2H, $OCH_2C\mathbf{H}_2$-CH_2O); 3.31 br.s (9H, Me_3N^+); 3.34 br.m (2H, CH_2N^+); 3.81 m (6H, OCH_2); 4.10 m (4H, CH_2OP, $^3J_{PH}$ = 11.9 Hz); 4.40 br.s (1H, CHO). ^{31}P NMR spectrum (chloroform): δ_P 56.62 ppm, s. Found, %: C 55.05; H 8.39; P 6.62. $C_{22}H_{42}O_7PS$. Calculated, %: C 54.86; H 8.79; P 6.43. *M* 481.601.

Trimethylammonium *S*-3-hydroxypropyl *O*-(2,2,2',2'-tetramethyldihydro-4*H*,5*H*-spiro[1,3-benzodioxine-8,5'-[1,3]dioxan]-4a(8a*H*)-yl)methyl thiophosphate (VII). An ampule was charged with a solution of 0.1 g (0.23 mmol) of thiophosphate **V**, 0.01 g (0.5 mmol) of water, and 0.2 g (1.9 mmol) of triethylamine in 3 ml of dioxane (pH > 10). The ampule was sealed and heated for 5 h at 110–120°C. The solvent and excess triethylamine were removed under reduced pressure, the oily residue was dissolved in 1.5 ml of dioxane, and the product was precipitated two times with hexane, filtered off, washed with diethyl ether, and dried for 3 h at 50°C under reduced pressure (1 mm). Yield 0.06 g (45%); mp 220–222°C; R_f 0.00 (A), 0.5 (C). ^{1}H NMR spectrum ($CDCl_3$), δ,

ppm: 1.31 t (9H, $NCH_2C\mathbf{H}_3$, $^3J_{HH}$ = 7.36 Hz), 1.37 br.m [18H, CCH_3, $CH_2CH_2CH_2$], 1.99 m (2H, $SCH_2C\mathbf{H}_2CH_2OH$), 2.79 t (2H, $SC\mathbf{H}_2CH_2CH_2OH$, $^3J_{PH}$ = 7.15 Hz), 2.80 q (6H, $NC\mathbf{H}_2CH_3$, $^3J_{PH}$ = 7.12 Hz), 3.24 d (2H, 4'-H_{eq}, 6'-H_{eq}) and 3.97 d (2H, 4'-H_{ax}, 6'-H_{ax}) ($^2J_{ax,eq}$ = 12.10 Hz), 3.40 d (1H, 4-H_{eq}) and 3.89 d (1H, 4-H_{ax}) ($^2J_{ax,eq}$ = 11.99 Hz), 3.74 m (2H, CH_2OP, $^3J_{PH}$ = 11.55 Hz), 3.87 s (1H, CH), 3.89 m (2H, $C\mathbf{H}_2OH$), 4.07 br. s (1H, HN^+), 9.55 br.s (1H, OH). ^{31}P NMR spectrum (dioxane): δ_P 14.55 ppm, s. Found, %: C 55.38; H 8.56; P 5.63. $C_{26}H_{50}NO_8PS$. Calculated, %: C 55.00; H 8.88; P 5.45. *M* 567.709.

(2,2,2',2'-Tetramethyldihydro-4*H*,5*H*-spiro[1,3-benzodioxine-8,5'-[1,3]dioxan]-4a(8a*H*)-yl)methyl *N*-(2-bromoethyl)-*N*,*P*-dimethylphosphonamidate (IX). A mixture of 0.5 g (1.66 mmol) of compound **II** and 0.3 g (1.66 mmol) of 2-diethylamino-3-methyl-1,3,2-oxazaphospholidine in 2 ml of anhydrous benzene was heated for 1.5 h at 80–85°C with simultaneous removal of the solvent and liberated diethylamine. The formation of phosphoramidite **VIII** was monitored by ^{31}P NMR spectroscopy: δ_P 136.63 ppm, s. A solution of 1 g of methyl bromide in 10 ml of anhydrous benzene was added, the mixture was placed in an ampule, and the ampule was sealed and heated for 10 h at 90°C. The solvent was removed under reduced pressure, and phosphonamidate **IX** was isolated by chromatography on a column charged with silica gel (8 g) and filled with hexane. The column was eluted with 20 ml of a 1:1 hexane–dioxane mixture. The eluate was evaporated under reduced pressure, and the residue was dried for 2 h at 40°C (1 mm). Yield of **IX** 0.46 g (55%); R_f 0.40 (A), 0.29 (B). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.36 s (6H) and 1.41 s (6H) (CCH_3), 1.37 m (6H, $CH_2CH_2CH_2$), 1.46 d (3H, PCH_3, $^2J_{PH}$ = 16.59 Hz), 2.68 d (3H, NCH_3, $^3J_{PH}$ = 9.45 Hz), 3.28 d (2H, 4'-H_{eq}, 6'-H_{eq}) and 4.02 d (2H, 4'-H_{ax}, 6'-H_{ax}) ($^2J_{ax,eq}$ = 11.9 Hz), 3.36 d (1H, 4-H_{eq}) and 3.82 d (1H, 4-H_{ax}) ($^2J_{ax,eq}$ = 11.34 Hz), 3.40–3.46 m (4H, $NC\mathbf{H}_2CH_2Br$), 3.71 s (1H, CH), 3.95 m (2H, CH_2OP, $^3J_{PH}$ = 10.64 Hz). ^{31}P NMR spectrum (chloroform): δ_P 32.98 ppm, s. Found, %: C 48.39; H 7.69; P 6.50. $C_{20}H_{37}BrNO_6P$. Calculated, %: C 48.19; H 7.48; P 6.21. *M* 498.399.

2-[Methyl[(2,2,2',2'-tetramethyldihydro-4*H*,5*H*-spiro[1,3-benzodioxine-8,5'-[1,3]dioxan]-4a(8a*H*)-yl)methoxy]phosphinoyl(methyl)amino]ethyl-(trimethyl)ammonium bromide (X). An ampule was charged with a solution of 0.4 g (0.8 mmol) of phosphonamidate **IX** and 0.15 g (2.4 mmol) of trimethylamine in 5 ml of anhydrous benzene. The ampule was sealed and heated for 5 h at 90°C. The solvent was removed under reduced pressure, and the residue was washed with anhydrous benzene (2×2 ml). Product **X** was additionally purified by reprecipitation from acetone with hexane and was dried for 3 h at 40°C (1 mm). Yield 0.27 g (60%), mp 189–190°C (wets at 105°C), R_f 0.43 (C). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm:1.23 s (6H) and 1.40 s (6H) (CCH_3), 1.35 m (6H, $CH_2CH_2CH_2$), 1.45 d (3H, PCH_3, $^2J_{PH}$ = 16.18 Hz), 2.77 d (3H, $PNCH_3$, $^3J_{PH}$ = 10.07 Hz), 3.25 m (3H, 4-H_{eq}, 4'-H_{eq}, 6'-H_{eq}) and 3.87 m (3H, 4-H_{ax}, 4'-H_{ax}, 6'-H_{ax}), 3.47 s (9H, Me_3N^+), 3.58 br.m (2H, $NC\mathbf{H}_2CH_2$), 3.68 br.m (2H, CH_2N^+), 4.00 m (2H, CH_2OP), 4.13 s (1H, CH). ^{31}P NMR spectrum (chloroform): δ_P 34.03 ppm, s. Found, %: C 49.89; H 8.61; P 5.79. $C_{23}H_{46}BrN_2O_6P$. Calculated, %: C 49.55; H 8.32; P 5.56. *M* 557.511.

(2,2,2',2'-Tetramethyldihydro-4*H*,5*H*-spiro[1,3-benzodioxine-8,5'-[1,3]dioxan]-4a(8a*H*)-yl)methyl *N*-(2-bromoethyl)-*N*-methylamidobromophosphate (XI). A solution of 0.2 g (1.33 mmol) of bromine in 3 ml of anhydrous benzene was added dropwise under vigorous stirring and cooling to –5°C to a solution of compound **VIII** (δ_P 136.63 ppm, s), prepared from 0.4 g (1.33 mmol) of bis-acetal **II** and 0.2 g (1.33 mmol) of 2-diethylamino-3-methyl-1,3,2-oxazaphospholidine in 5 ml of anhydrous benzene. After 10 min, the solvent was removed under reduced pressure, and the product was isolated by chromatography on a column charged with silica gel (10 g) and filled with hexane. The column was eluted with 25 ml of hexane–dioxane (5:1). The eluate was evaporated under reduced pressure, and the residue was dried for 2 h at 40°C (1 mm). Yield 0.83 g (53%); mp 29–30°C; R_f 0.75 (A), 0.55 (B). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.36 m (6H, $CH_2CH_2CH_2$), 1.40 s (6H) and 1.45 s (6H) (CCH_3), 2.71 d (3H, $PNCH_3$, $^3J_{PH}$ = 9.47 Hz), 3.14 d (2H, 4'-H_{eq}, 6'-H_{eq}) and 4.11 d (2H, 4'-H_{ax}, 6'-H_{ax}) ($^2J_{ax,eq}$ = 11.9 Hz), 3.42 m (4H, NCH_2CH_2Br), 3.76 d (1H, 4-H_{eq}) and 3.96 d (1H, 4-H_{ax}) ($^2J_{ax,eq}$ = 9.83 Hz), 3.79 m (2H, CH_2OP, $^3J_{PH}$ = 12.21 Hz), 4.30 s (1H, CH). ^{31}P NMR spectrum (chloroform): δ_P: 5.13 ppm, s. Found, %: C 39.54; H 6.59; P 5.80. $C_{18}H_{34}Br_2NO_6P$. Calculated, %: C 39.22; H 6.22; P 5.62. *M* 551.263.

Cholesteryl (2,2,2',2'-tetramethyldihydro-4*H*,5*H*-spiro[1,3-benzodioxine-8,5'-[1,3]dioxan]-4a(8a*H*)-yl)methyl *N*-(2-bromoethyl)-*N*-methylamidophosphate (XII). A solution of 0.2 g (0.54 mmol) of cholesterol and 0.1 g (0.54 mmol) of freshly distilled pyridine in 2 ml of anhydrous benzene was added

dropwise under stirring and cooling to 0°C to a solution of 0.3 g (0.54 mmol) of amidobromophosphate **XI** in 3 ml of anhydrous benzene. The mixture was stirred for 18 h at room temperature, the precipitate of pyridine hydrobromide was filtered off, the solvent was removed from the filtrate under reduced pressure, and the residue was applied to a column charged with silica gel (8 g) and filled with hexane. Product **XII** was isolated by elution with 50 ml of a 1:1 hexane–dioxane mixture. The eluate was evaporated under reduced pressure, and the residue was dried for 2 h at 40°C (1 mm). Yield 0.25 g (53%); mp 33–34°C; R_f 0.71 (A), 0.55 (B). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 0.68–2.42 (cholesterol), 1.37 s and 1.46 s (6H each, 2-CH_3, 2'-CH_3), 1.41 m (6H, $CH_2CH_2CH_2$), 3.15 d (3H, $PNCH_3$, $^3J_{PH}$ = 11.90 Hz), 3.37 d (2H, 4'-H_{eq}, 6'-H_{eq}) and 4.11 d (2H, 4'-H_{ax}, 6'-H_{ax}) ($^2J_{ax,eq}$ = 9.59 Hz), 3.77 m (4H, NCH_2CH_2Br), 3.81–3.91 m (4H, C^4H_2, CH_2OP), 4.32 s (1H, CH), 4.43 m (1H, 3''-H, cholesterol), 5.38 d (1H, 6''-H, cholesterol, $^3J_{HH}$ = 3.01 Hz). ^{31}P NMR spectrum (chloroform): δ_P 8.95 ppm, br.s. Found, %: C 63.84; H 9.39; P 3.84. $C_{46}H_{79}BrNO_7P$. Calculated, %: C 63.58; H 9.16; P 3.56. *M* 868.989.

2-[Cholesteryloxy[(2,2,2',2'-tetramethyldihydro-4*H*,5*H*-spiro[1,3-benzodioxine-8,5'-[1,3]dioxan]-4a(8a*H*)-yl)methoxy]phosphinoyl(methyl)amino]-ethyl(trimethyl)ammonium bromide (XIII). An ampule was charged with a solution of 0.4 g (0.46 mmol) of amidophosphate **XII** and 0.1 g (1.38 mmol) of trimethylamine in 4 ml of anhydrous benzene. The ampule was sealed and heated for 8 h at 60°C. The precipitate of salt **XIII** was filtered off, washed in succession with benzene (2×5 ml), acetone (2×5 ml), and diethyl ether (2×5 ml), and dried for 2 h at 40°C (1 mm). Yield 0.2 g (50%), mp 210–212°C (wets at 175°C), R_f 0.6 (C). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 0.66–2.40 (cholesterol), 1.36 s and 1.44 s (6H each, 2-CH_3, 2'-CH_3), 1.39 m (6H, $CH_2CH_2CH_2$), 2.74 d (3H, $PNCH_3$, $^3J_{PH}$ 9.77 Hz), 3.15 d (2H, 4'-H_{eq}, 6'-H_{eq}) and 4.10 d (2H, 4'-H_{ax}, 6'-H_{ax}) ($^2J_{ax,eq}$ = 11.50 Hz), 3.39 m (2H, $NC\mathbf{H}_2CH_2N^+$), 3.49 s [9H, $(CH_3)_3N^+$], 3.55 m (2H, $NCH_2C\mathbf{H}_2N^+$), 3.78–3.91 m (4H, C^4H_2, CH_2OP, $^3J_{PH}$ = 11.59 Hz), 4.01 m (1H, 3''-H, cholesterol), 4.27 s (1H, CH), 5.36 br.d (1H, 6''-H, cholesterol). ^{31}P NMR spectrum (chloroform): δ_P 9.08 ppm, br.s. Found, %: C 63.74; H 9.39; P 3.63. $C_{49}H_{88}BrN_2O_7P$. Calculated, %: C 63.41; H 9.56; P 3.34. *M* 928.099.

This study was performed under financial support by the Russian Foundation for Basic Research (project no. 01-03-32459).

REFERENCES

1. Savin, G.A., Predvoditelev, D.A., and Nifant'ev, E.E., *Russ. J. Org. Chem*., 2002, vol. 38, p. 1550.
2. Eibl, H., *Chem. Phys. Lipids*, 1980, vol. 26, p. 405.
3. Stepanov, A. and Shvets, V.I., *Chem. Phys. Lipids*, 1986, vol. 41, p. 1.
4. Bonsen, P.P.M., Burbach-Westerhuis, G.J., De Haas, G.H., and Van Deenen, L.L.M., *Biochim. Biophys. Acta*, 1972, no. 3, p. 199.
5. Bonsen, P.P.M., De Haas, G.H., Pieterson, W.A., and Van Deenen, L.L.M., *Biochim. Biophys. Acta*, 1972, vol. 270, p. 364.
6. Bezuglov, V.V., Molotkovskii, Yul.G., and Bergel'-son, L.D., *Zh. Org. Khim*., 1974, vol. 10, p. 1398.
7. Predvoditelev, D.A. and Nifant'ev, E.E., *Russ. J. Org. Chem*., 1995, vol. 31, p. 1559.
8. Nifant'ev, E.E., Predvoditelev, D.A., Malenkov-skaya, M.A., and Bel'skii, V.K., *Russ. J. Gen. Chem*., 2000, vol. 70, p. 1917.
9. Hird, G.S., McIntosh, T.Y., and Grinstaff, M.W., *J. Am. Chem. Soc*., 2000, vol. 122, p. 8097.
10. Nifantyev, E.E., Predvoditelev, D.A., Rasadkina, E.N., and Bekker, A.R., *Phosphorus Sulfur*, 1987, vol. 34, p. 109.
11. Malenkovskaya, M.A., Predvoditelev, D.A., Bel'-skii, V.K., and Nifant'ev, E.E., *Russ. J. Gen. Chem*., 2003, vol. 73, p. 1870.
12. Predvoditelev, D.A., Suvorkin, S.V., and Nifant'-ev, E.E., *Russ. J. Gen. Chem*., 2001, vol. 71, p. 873.
13. Predvoditelev, D.A., Malenkovskaya, M.A., and Nifan-t'ev, E.E., *Russ. J. Gen. Chem*., 2003, vol. 73, p. 1530.
14. Facke, T. and Borger, S., *Tetrahedron*, 1995, vol. 51, p. 3521.
15. Miller, A.D., *Angew. Chem*., 1998, vol. 37, p. 1768.
16. Maslov, M.A., Sycheva, E.V., Morozova, N.G., and Serebrennikova, G.A., *Izv. Ross. Akad. Nauk, Ser. Khim*., 2000, no. 3, p. 385.
17. Zelenin, A.V., *Vestn. Ross. Akad. Nauk*, 2001, vol. 71, p. 1440.
18. Gefter, E.L., *Zh. Obshch. Khim*., 1956, vol. 26, p. 1440.
19. Mukaiyama, T. and Kodaira, Y., *Bull. Chem. Soc. Jpn*., 1966, vol. 39, p. 1297.