Russian Journal of General Chemistry, Vol. 75, No. 7, 2005, pp. 1040–1044. Translated from Zhurnal Obshchei Khimii, Vol. 75, No. 7, 2005, pp. 1100–1105.

First Representatives of Nitrophospholipids

D. A. Predvoditelev, M. A. Malenkovskaya, S. V. Sipin, and E. E. Nifant'ev

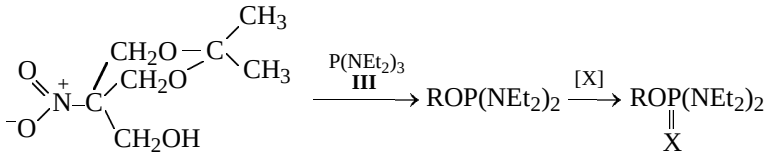
Moscow State Pedagogical University, Moscow, Russia

Received March 3, 2004

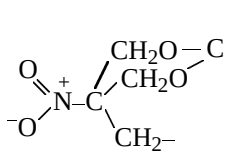
Abstract — General regularities of the phosphorylation of trimethylolnitromethane and its derivatives with amides and amidoesters of phosphorous acids were investigated. These procedures were found to hold promise for the design of amphiphilic phospholipids of a new type.

Great attention in modern lipidology is attracted to ampholite systems belonging to the type of cationic phospholipids [1–3]. It can be suggested that other classes of phospholipids containing polar fragments, too, present interest as bioregulators or structure-forming materials [4]. With this in mind we initiated research into design of a new class of phospholipids containing the polar nitro group in their structure.

In the present work we consider the syntheses of original phosphorus-containing systems on the basis of tris(hydroxymethyl)nitromethane (**I**), an available and convenient-in-operation reagent. First we investigated phosphorylation of an acetone derivative of tris(hydroxymethyl)nitromethane **II** with hexaethylprosphorous triamide (**III**), followed by oxidation, sulfurization, and selenization of intermediate phosphorous diamidoester **IV**.



II **IV** **V–VII** (1)

R =  ; X = O (**V**), S (**VI**), Se (**VII**).

The phosphorylation was carried out in dioxane at room temperature with simultaneous blowing out of the evolving diethylamine. The ^{31}P NMR spectrum of crude phosphorodiamidite **IV** contained a singlet at 136.68 ppm, characteristic of the simplest phosphorodiamidites.

Note that the phosphorylation of the simplest alcohols with aliphatic phosphorous triamides, catalyzed by amine hydrochlorides that "contaminate" the latter in the course of their synthesis by standard procedures, usually occurs at 70–120°C. For lower alcoholysis temperatures such catalysts as azoles, carboxylic and amidodithiocarbamic acids, etc. must be used [5]. Probably, the more active phosphorylation of acetal **II** is explained by the presence of a strongly electron-acceptor group in its structure.

Phosphorodiamidite **IV** was easily converted *in situ* into phosphate by mild oxidation with the urea–hydrogen peroxide adduct. Thio- and selenophosphates **VI** and **VII** were obtained by treatment of compound **IV** with sulfur and selenium, respectively. It is important that all the oxidation reactions studied occurred at room temperature within several hours, which prevented decomposition of terminal phosphorodiamidates **V**–**VII**.

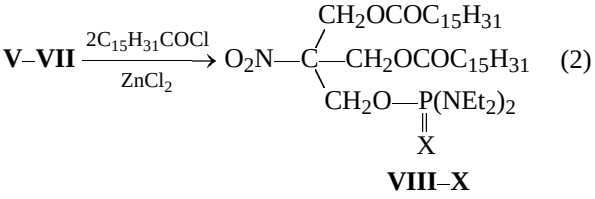
Phosphorodiamidates **V**–**VII** are oily substances stable on handling in the absence of air. Phosphoramidothioate **VI** is the most stable among them.

The individuality and structure of the products were established by means of TLC and ^{1}H and ^{31}P NMR spectroscopy. The ^{31}P NMR spectra contained

1070-3632/05/7507-1040

singlets at δ_P 17.65, 79.63, and 82.22 ppm. The ^{1}H NMR spectra showed, along with signals of *gem*-dimethyl groups (δ 1.25 and 1.40 ppm) and methylene groups (δ 4.05 and 4.34 ppm) of the oligool, *N*-ethyl proton signals as characteristic triplet and quartet at 0.87 and 3.03 ppm, respectively.

Phosphoacetals **V**–**VII** were then subjected to direct acylation with palmitoyl chloride according to the procedure in [6].



(2)

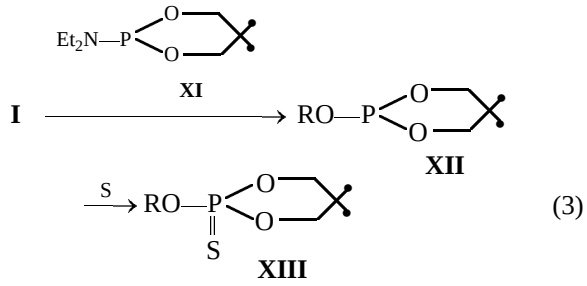
VIII–**X**

X = O (**VIII**), S (**IX**), Se (**X**).

The reaction was carried out at room temperature in chloroform in the presence of a catalytic amount of zinc chloride. Note that the reaction is complete within 1 h, unlike phosphorylation of glycerol phosphoacetals (up to 24 h).

Diacylphosphoramidates **VIII**–**X** were isolated in 50–70% yields by column chromatography (δ_P 19.27, 78.66, and 82.80 ppm, respectively). The ^{1}H NMR spectra of phosphatides **VIII**–**X** display characteristic multiplets in the range 0.89–2.34 ppm, assignable to the methyl and methylene protons of the fatty acid residues. The other signals in the ^{1}H NMR spectra are consistent with the proposed structures (see Experimental).

The nitro group in acetal **II** significantly decreases the temperature of its phosphorylation with a low-reactive cyclic amidoester **XI**.

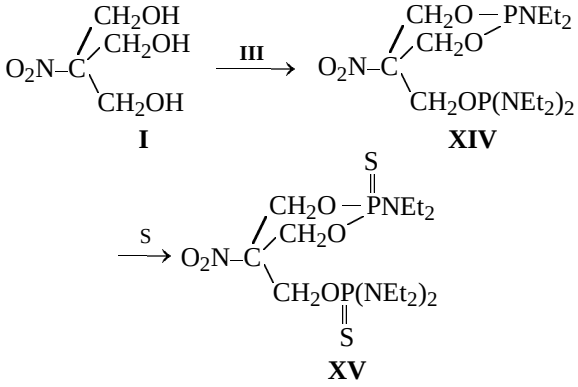


(3)

for R, see scheme (1).

The phosphorylation takes place in dioxane at 25°C, like with triamide **III**. Then phosphite **XII** was smoothly transformed *in situ* to phosphorothioate **XIII**. Its individuality and structure were proved by necessary physicochemical methods (see Experimental).

The fact that the electron-acceptor nitro group enhances the reactivity of triols was also observed in the reaction of unsaturated nitrotriol **I** with triamide **III**.

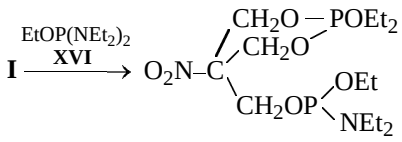


(4)

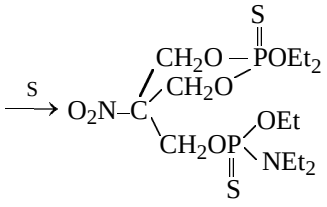
Like with acetal **III**, the reaction was carried out in dioxane at 25°C for 3–4 h at 1:1, 1:2, and 1:3 molar ratios of triol **I** and triamide **III**. Analysis of the reaction products was carried out by means of ^{31}P NMR and TLC. It was found that independent of the reagent ratio the process begins with formation of the phosphorinane ring, and which phosphorylation of the residual free hydroxy group takes place to give product **XIV**. We failed to stop this reaction on the first stage. Crude diphosphotriol **XIV** was oxidized with sulfur (25°C, 3 h) to form bis(phosphorothioate) **XV** that was isolated chromatographically in 65% yield. Note that the longer phosphorylation time leads to accumulation of unknown admixtures in the reaction mixture.

According to the ^{31}P NMR data, phosphite **XIV** and bis(phosphorothioate) **XV** are mixtures of two geometric isomers of phosphorinanes with equatorial and axial of the amide groups on phosphorus. Hence, the ^{31}P NMR spectrum of phosphite **XIV** shows two groups of signals from the *exo*- and *endo*-phosphite fragments: a singlet at δ_P 135.8 ppm and two singlets at δ_P 144.2 and 145.3 ppm. The respective signals of the phosphorothioate fragments of compound **XV** appear as a singlet at δ_P 74.9 ppm and two singlets at δ_P 74.94 and 79.9 ppm. The intensity ratio of the first signal and of the sum of the two other ones is 1:1. The stereoisomers of phosphorodithioate **XV** have different chromatographic mobility in several systems. In the ^{1}H NMR spectrum of cyclic phosphorothioates **XV**, there are two types of signals related to the axial and equatorial methylene protons of the phosphocycles with axial and equatorial location of the amide group, as well as two well-defined multiplet signals of the methylene protons of the amide groups at the phosphocycle.

Ethyl tetraethylphosphorodiamidite (**XVI**), too, phosphorylates unsubstituted triol **I** in mild conditions.



XVII



(5)

XVIII

It occured that, unlike phosphorylation with triamide **III**, the process begins with formation of a dialkyl phosphoramidite (δ_P 147.24 ppm) and then to a cyclic triester (δ_P 122.40 ppm). Similarly to the case of formation of phosphorinane **XV**, we failed to stop this process on the first stage.

Further on crude diphosphotriol **XVII** was subjected to sulfurization into bis(phosphorothioate) **XVIII** that was isolated by column chromatography on silica gel in 60% yield (δ_P 76.52 and 62.17 ppm, 1:1 ratio). The ^{1}H NMR spectrum of compound **XVIII** contained signals of all characteristic groups of the proposed structure. Hence, the OEt and NEt_2 methyl proton signals were observed at 0.85 and 1.07 ppm, while the methylene protons of the same groups give signals at 3.16 and 4.20 ppm. Furthermore, the spectrum displays multiplets of the axial and equatorial protons of the cyclic fragment of the molecule at 4.56 and 4.96 ppm with a characteristic coupling constant ($^3J_{HP}$).

Hence, we showed that phosphamide amphiphiles derived from oligools containing an electron-acceptor group, such as NO_2, can be prepared in high yields by means of reaction with alphatic phospho(III)amides at room temperature, which opens up the way to use of such systems.

EXPERIMENTAL

The ^{1}H NMR spectra were measured on a Bruker WM-250 spectrometer (250 MHz) against internal HMDS. The proton signals were assigned on the basis of double resonance data. The ^{31}P–{^{1}H} NMR spectra were obtained on a Bruker WR-80SY spectrometer (32.4 MHz) against external 85% phosphoric acid. All syntheses including trivalent phosphorus derivatives were carried out under dry argon.

Adsorption chromatography was carried out on a 10-mm column of silica gel L 100–250 μm. Thin-layer chromatography was performed on Silufol UV-254 plates in 3:1 hexane–dioxane (A), 3:1 benzene–dioxane (B), and benzene (C).

Hexaethylphosphorous triamide (**III**) was prepared as described in [7], and 2-(diethylamino)-5,5-dimethyl-1,3,2-dioxaphosphorinane, as described in [8]. Ethyl tetraethylphosphorodiamidite was prepared by the procedure in [9]. Physicochemical characteristics of the compounds used agreed with published data.

(2,2-Dimethyl-2-nitro-1,3-dioxan-5-yl) tetraethylphosphorodiamidate (V). A solution of 0.3 g of compound **II** and 0.4 g of hexaethylphosphorous triamide (molar ratio 1:1) in 2 ml of anhydrous dioxane was stirred for 6 h at 25°C. The evolving diethylamine was blown off. The formation of phosphorodiamidite **IV** was controlled by ^{31}P NMR spectroscopy (δ_P 136.68 ppm, br.s, in dioxane). After that 0.15 g of the urea–hydrogen peroxide adduct was added to the reaction mixture at room temperature, and the reaction mixture was kept at this temperature for 1 h. Excess adduct was filtered off, and dioxane was removed in a vacuum. Phosphorodiamidate **V** was purified on a column of silica gel (5 g), filled with benzene. The target compound was eluted with 30 ml of a 5:1 benzene–dioxane mixture. The solvents were removed in a vacuum, and the residue was kept for 2 h at 40°C (1 mm Hg). Yield 0.24 g (40%), n_D^{20} 1.4705, R_f 0.24 (A), 0.34 (B). ^{1}H NMR spectrum (C_6D_6), δ, ppm: 0.89 t (12H, NCH_2CH_3, $^3J_{HH}$ 9.32 Hz). 1.15 s, 1.19 s, 1.20 s, 1.23 s [6H, $C(CH_3)_2$], 2.76 q (8H, NCH_2CH_3), 3.93 d, 3.95 d ($2H_e$) and 4.40 d, 4.46 d ($2H_a$) (CH_2OC, $^2J(H_aH_e$ 12.81 Hz), 4.24 d (2H, CH_2OP, $^3J_{HP}$ 4.57 Hz). ^{31}P NMR spectrum (benzene), δ_P, ppm: 17.65 br.s. Found, %: C 47.41; H 8.39; P 8.32. $C_{15}H_{32}N_3O_6P$. Calculated, %: C 47.23; H 8.46; P 8.12. *M* 381.41.

(2,2-Dimethyl-2-nitro-1,3-dioxan-5-yl) tetraethylphosphorodiamidothioate (VI) was prepared analogously to compound **V** from 0.3 g of compound **II**, 0.4 g of compound **III**, and 0.06 g of sulfur. The reaction was carried out at room temperature for 1.5 h. Excess sulfur was filtered off, and dioxane was removed in a vacuum. Compound **VI** was purified on a column of silica gel (5 g), filled with hexane, eluent 10:1 hexane–dioxane mixture (25 ml). The solvent was removed in a vacuum, and the residue was kept for 2 h at 40°C (1 mm Hg). Yield 0.45 g (70%), n_D^{20} 1.4935, R_f 0.65 (A), 0.85 (B). ^{1}H NMR spectrum (C_6D_6), δ, ppm: 1.06 t (12H, NCH_2CH_3, $^3J_{HH}$ 7.15 Hz), 1.39 s, 1.41 s [6H, $C(CH_3)_2$], 3.03 q (8H, NCH_2CH_3), 4.03 d ($2H_e$) and 4.43 d ($2H_a$) (CH_2OC,

$^2J(H_aH_e)$ 12.65 Hz), 4.25 d, 2H, CH_2OP, $^3J_{HP}$ 5.5 Hz). ^{31}P NMR spectrum (benzene), δ_P, ppm: 79.63 br.s. Found, %: C 45.55; H 8.33; P 7.52. $C_{15}H_{32}N_3O_5PS$. Calculated, %: C 45,32; H 8.12; P 7.79. *M* 397.47.

(2,2-Dimethyl-2-nitro-1,3-dioxan-5-yl) tetraethylphosphorodiamidoselenothioate (VII) was prepared similarly to compound **V** from 0.3 g of compound **II**, 0.4 g of compound **III**, and 0.15 g of selenium. The reaction was carried out at room temperature for 3 h. Excess selenium was filtered off, and dioxane was removed in a vacuum. Compound **VII** was purified on a column of silica gel (5 g), filled with hexane, eluent hexane (20 ml). The solvent was removed in a vacuum, and the residue was kept for 2 h at 40°C (1 mm Hg). Yield 0.53 g (75%), n_D^{20} 1.5110, R_f 0.7 (A), 0.9 (B). ^1H NMR spectrum (C_6D_6), δ, ppm: 0.91 t (12H, NCH_2CH_3, $^3J_{HH}$ 7.14 Hz), 1.12 s, 1.15 s [6H, $C(CH_3)_2$], 2.91 q (8H, NCH_2CH_3), 3.80 d ($2H_e$) and 4.33 d ($2H_a$) (CH_2OC, $^2J(H_aH_e)$ 11.65 Hz), 4.29 d (2H, CH_2OP, $^3J_{HP}$ 3.85 Hz). ^{31}P NMR spectrum (benzene), δ_P, ppm: 82.22 br.s and two satellites (J_{PSe} 869.16 Hz). Found, %: C 40.71, H 7.38; P 6.72. $C_{15}H_{32}N_3O_5PSe$. Calculated, %: C 40.54; H 7.26; P 6.97. *M* 444.41.

2-Nitro-3-(palmitoyloxy)-2-(palmitoyloxymethyl)propyl tetraethylphosphorodiamidate (VIII). To a solution of 1.5 g of phosphorodiamidate **V** in 3 ml of chloroform, 0.008 g of $ZnCl_2 \cdot 1.5H_2O$, and 0.22 g of palmitoyl chloride were added. The reaction mixture was kept at this temperature for 1 h. Chloroform was removed in a vacuum. Compound **VIII** was purified on a column of silica gel (10 g) filled with hexane, eluent 3 : 1 hexane–dioxane (20 ml). The solvents were removed in a vacuum, and the residue was kept for 2 h at 40°C (1 mm Hg). Yield 0.16 g (50%), amorphous material, mp 28–29°C, R_f 0.43(A), 0.55 (B). ^1H NMR spectrum ($CDCl_3$), δ, ppm: 0.88 t (6H, CH_3CH_2, $^3J_{HH}$ 6.7 Hz), 1.08 t (12H, NCH_2CH_3), 1.26 m [48H, $CH_3(CH_2)_{12}$], 1.60 m [4H, $CH_2CH_2 \cdot C(O)$], 2.33 m [4H, $CH_2CH_2C(O)$]. 2.99 m (8H, NCH_2CH_3), 4.04 d (2H, CH_2OP, $^3J_{HP}$ 5.48 Hz), 4.65 s [4H, $CH_2OC(O)$]. ^{31}P NMR spectrum (chloroform), δ_P, ppm: 19.27 br.s. Found, %: C 67.51; H 11.58; P 3.71. $C_{44}H_{88}N_3O_6P$. Calculated, %: C 67.22; H 11.28; P 3.94. *M* 786.14.

2-Nitro-3-(palmitoyloxy)-2-(palmitoyloxymethyl)propyl tetraethylphosphorodiamidothioate (IX) was obtained analogously to compound **VIII** from 0.1 g of phosphorodiamidothioate **VI**, 0.005 g of $ZnCl_2 \cdot 1.5H_2O$) and 0.15 g of palmitoyl chloride for 1.5 h. Compound **IX** was isolated on a column of silica gel (5 g) filled with hexane, eluent 5 : 1 hexane–dioxane (15 ml). The solvents were removed in a vacuum, and the residue was kept for 2 h at 40°C (1 mm Hg). Yield 0.15 g (68%), amorphous material, mp 31–32°C, R_f 0.65 (A), 0.80 (B). ^1H NMR spectrum is analogous to that of compound **VIII**. ^{31}P NMR spectrum (chloroform), δ_P, ppm: 78.66 br.s. Found, %: C 65.63; H 11.28; P 3.61. $C_{44}H_{88}N_3O_5PS$. Calculated, %: C 65.87; H 11.06; P 3.86. *M* 802.21.

2-Nitro-3-(palmitoyloxy)-2-(palmitoyloxymethyl)propyl tetraethylphosphorodiamidoselenoate (X) was obtained analogously to compound **VIII** from 0.2 g of phosphorodiamidoselenoate **VII**, 0.01 g of $ZnCl_2 \cdot 1.5H_2O$, and 0.25 g of palmitoyl chloride for 1 h. Compound **X** was isolated on a column of silica gel (10 g) filled with hexane, eluent 3 : 1 hexane–dioxane (20 ml). The solvents were removed in a vacuum, and the residue was kept in a vacuum for 2 h at 40°C (1 mm Hg). Yield 0.25 g (70%), mp 36–37°C, R_f 0.60 (A), 0.75 (B). ^1H NMR spectrum is similar to that of compound **VIII**. ^{31}P NMR spectrum (chloroform), δ_P, ppm: 82.80 br.s and two satellites, J_{PSe} 790.63 Hz. Found, %: C 62.51; H 10.23; P 3.81. $C_{44}H_{88}N_3O_5PSe$. Calculated, %: C 62.23; H 10.45; P 3.65. *M* 849.14.

5,5-Dimethyl-2-[2-nitro-3-(palmitoyloxy)-2-(palmitoyloxymethyl)propoxy]-1,3,2λ^5-dioxaphosphorinane 2-sulfide (XIII) was obtained analogously to compound **V** from 0.3 g of compound **II**, 0.3 g of 2-(diethylamino)-5,5-dimethyl-1,3,2-dioxaphosphorinane (**XI**), and 0.06 g of sulfur. Cyclic phosphite **XII** was formed at 25°C for 30 h. ^{31}P NMR spectrum (dioxane), δ_P, ppm: 122.02 br.s. Addition of sulfur was carried out at 25°C for 5 h. Excess sulfur was filtered off, and dioxane was removed in a vacuum. Compound **XIII** was purified on a column of silica gel, (10 g) filled with hexane, eluent 15 : 1 hexane–dioxane (25 ml). The solvents were removed in a vacuum, and the residue was kept for 2 h at 40°C (1 mm Hg). Yield 0.28 g (50%), mp 148–149°C, R_f 0.35 (A), 0.80 (B). ^1H NMR spectrum (C_6D_6), δ, ppm: 0.86 s, 1.25 s [6H, $OCH_2C(CH_3)_2CH_2O$], 1.39 s, 1.43 s [6H, $C(CH_3)_2$], 3.90 m [4H, $CH_2C(CH_3)_2CH_2O$], 4.04 ($2H_e$), and 4.44 d ($2H_a$) (CH_2OC, $^2J(H_aH_e)$ 13.16 Hz), 4.48 d (2H, CH_2OP, $^3J_{HP}$ 6.58 Hz). ^{31}P NMR spectrum (benzene), δ_P, ppm: 59.22 br.s. Found, %: C 40.34; H 6.41; P 8.59. $C_{12}H_{22}NO_7PS$. Calculated, %: C 40.56; H 6.24; P 8.72. *M* 344.35.

5-[Bis(diethylamino)phosphinothioyloxymethyl]-2-(diethylamino)-5-nitro-1,3,2λ^5-dioxaphosphorinane 2-sulfide (XV). A solution of 0.3 g of tris(hydroxymethyl)nitromethane (**I**) and 0.33 g of compound **III** (molar ratio 1 : 2) in 3 ml of anhydrous dioxane was stirred for 4 h at 25°C. The evolving diethylamine was blown off. The formation of compound

XV was controlled by ^{31}P NMR spectroscopy, δ_P, ppm: 144.21 br.s; 145.36 br.s; 135.83 br.s (1 : 1 : 1). After that the reaction mixture was treated with 0.14 g of sulfur and kept at room temperature for 3 h. Excess sulfur was filtered off, and dioxane was removed in a vacuum. Compound **XV** was purified on a column of silica gel (10 g) filled with benzene, eluent benzene (25 ml). The solvent was removed in a vacuum, and the residue was kept for 2 h at 40°C (1 mm Hg). Yield 0.64 g (65%), n_D^{20} 1.5210, R_f 0.5 (B). ^1H NMR spectrum (C_6D_6), δ, ppm: 1.08 t (15H, NCH_2CH_3, $^3J_{HH}$ 7.02 Hz), 3.05 m and 3.33 m (12H, NCH_2CH_3), 4.13 d (2H, CH_2OPN, $^3J_{HP}$ 6.1 Hz), 4.41 m, 4.58 m ($2H_e$) and 4.84 m, 4.99 m ($2H_a$) (CH_2OP, $J(H_eH_e)$ 5.19 Hz). ^{31}P NMR spectrum (benzene), δ_P, ppm: 73.72 s and 79.88 s (1 : 1). Found, %: C 39.44; H 7.71; P 12.49, $C_{16}H_{36}N_4O_5P_2S_2$. Calculated, %: C 39.17; H 7.40; P 12.63. *M* 490.56.

5-[(Diethylamino)ethoxyphosphinothioyloxymethyl]-2-ethoxy-5-nitro-1,3,2λ^5-dioxaphosphorinane 2-sulfide (XVIII) was obtained analogously to compound **XV** from 0.3 g of compound **I**, 0.88 g of phosphorodiamidite **XVI** (molar ratio 1: 2), and 0.14 g of sulfur. The formation of compound **XVII** was complete within 5 h [^{31}P NMR spectrum (dioxane), δ_P, ppm: 122.40 br.s, 147.24 br.s (1 : 1)]. Sulfurization was performed at room temperature for 3.5 h. Excess sulfur was filtered off, and dioxane was removed in a vacuum. Compound **XVIII** was purified on a column of silica gel (10 g) filled with benzene, eluent benzene (25 ml). The solvent was removed in a vacuum, and the residue was kept for 2 h at 40°C (1 mm Hg). Yield 0.52 g (60%), n_D^{20} 1.5035, R_f 0.35 (A), 0.77 (B). ^1H NMR spectrum (C_6D_6), δ, ppm: 0.85 t (6H, OCH_2CH_3), 1.07 t (6H, NCH_2CH_3, $^3J_{HH}$ 6.95 Hz), 3.16 q (4H, NCH_2CH_3), 3.96 m (2H, CH_2OPN), 4.20 m (4H, $POCH_2CH_2$), 4.56 m ($2H_e$) and 4.96 m ($2H_a$) (CH_2OP, $^2J(H_a\ H_e)$ 7.68 Hz). ^{31}P NMR spectrum (benzene), δ_P, ppm: 62.17 br.s and 76.52 br.s (1 : 1). Found, %: C 30.34; H 6.31; P 14.48. $C_{12}H_{26}N_2O_7P_2S_2$. Calculated, %: C 30.02; H 6.01; P 14.19. *M* 436.43.

ACKNOWLEDGMENTS

The work was financially supported by the Russian Foundation for Basic Research (project no. 01-03-32 459).

REFERENCES

1. Zhadanov, R.I., Pododed, O.V., and Vlassov, V.V., *Bioelectrochemistry,* 2002, vol. 58, p. 53.
2. Maslov, M.A., Sychova, E.V., Morozova, N.G., and Serebrennikova, G.A., *Izv. Ross. Akad. Nauk, Ser. Khim.,* 2003, no. 3, p. 385.
3. Predvoditelev, D.A., Savin, G.A., Malenkovskaya, M.A., and Nifant'ev, E.E., *Zh. Org. Khim.,* 2004, vol. 40, no. 9, p. 1410.
4. Predvoditelev, D.A., Kosarev, G.V., and Nifant'ev, E.E., *Zh. Obshch. Khim.,* 2003, vol. 73, no. 10, p. 1611.
5. Nifantyev, E.E., Gratchev, M.K., and Burmistrov, S.Yu., *Chem. Rev.,* 2000, vol. 100, no. 10, p. 3755.
6. Nifant'ev, E.E. and Predvoditelev, D.A., *Usp. Khim.,* 1997, vol. 66, p. 47.
7. Zwierzak, A., *Bull Acad. Pol. Sci., Ser. Sci. Chim.,* 1965, vol. 13, no. 6, p. 609.
8. Zwierzak, A., *Can. J. Chem.,* 1967, vol. 21, no. 45, p. 2501.
9. Michaelis, A., *Chem. Ber.,* 1872, vol. 5, p. 4.