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Cationic Nitrophospoamphiphiles of Phosphonate and Amidophosphonate Types

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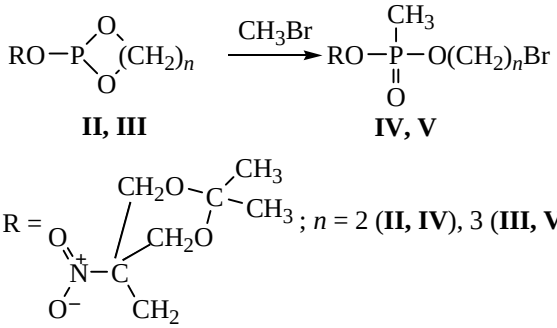
Abstract—Productive synthesis of cationic nitrophosphoamphiphilic lipids was developed basing on available tris(hydroxymethyl)nitromethane and cyclic reagents of trivalent phosphorus.

In recent years a promising branch of medicine is successfully developed treating the disturbances of gene functions, genetic therapy [1–3]. One of the key problems of this therapy is delivery into the target cells of the therapeutic gene (transfection) with the use of cationic liposomes [4–7]. Among the cationic lipids included into these liposomes are widely studied ammonium phosphorus-free lipids [8–12]. At the same time the positively charged ammonium (cationic) phospholipids are far less understood [8, 13–16], although the pioneering study of cationic phospholipides preparation by an example of glycerophosphonate analogs has been performed more than 25 years ago [17].

The present report concerns syntheses of new cationic lipid phosphonoamphiphiles proceeding from the acetone derivative of tris(hydroxymethyl)nitromethane (**I**). The choice of the trimethylolnitromethane core for the new lipid systems aimed at application in the genetic therapy was determined by the synthetic availability of the initial compound, its polarity, and also by the photosensitivity of its phospholipide derivatives. The latter quality may result in initiation of photochemical degradation reactions in the cell under light. These reactions may decrease the stability of the liposome membranes at fusion with endocytic vesicles and thus facilitate the transfection efficiency. A similar process was already studied in detail for a series of amphiphiles containing nitrobenzyl groups [18].

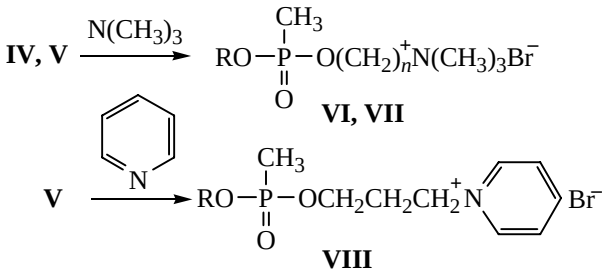
The first line of this investigation consisted in application of O,O-ethylene- (**II**) and trimethylene- (**III**) cyclophosphites of the acetone derivative of the nitro-oligool to the synthesis of methylphosphonate amphiphiles of ammonium type. To this end Arbuzov alkylation was performed on available alkenylphosphites **II** and **III** with bromomethane.



The reaction was carried out at 100–120°C. Bromoalkenylphosphonates **IV** and **V**, pale yellow oily compounds, were obtained in 40–65% yields. Phosphonates **IV** and **V** are stable compounds that can be stored for a long time under inert atmosphere in the dark at room temperature. The alkylation was monitored by ^{31}P NMR spectroscopy. ^{31}P NMR spectra of methylphosphonates **IV** and **V** consist of singlets at δ 31.47 and 30.82 ppm respectively. In the ^{1}H NMR spectra of these compounds characteristic groups from all protons were observed. The protons of PCH_3 group appear at δ 1.48 ppm as doublet due to the coupling of methyl group protons with the phosphorus nucleus, methylene protons of CH_2Br group give rise to triplets in the region 3.45–3.47 ppm, and the signals from protons of the dioxane fragment are observed as doublets at 3.39 and 4.35 ppm The other signals in the spectra of methylphosphonates **IV** and **V** are consistent with the assumed structures.

Bromoalkylenephosphonates **IV** and **V** were used in the synthesis of unconventional cationic phospho-amphiphiles as alkylating agents for aliphatic and heterocyclic nitrogen-containing bases of various types. We selected for alkylation the bases that formerly had been involved in the synthesis of phosphorus-free cationic

amphiphiles efficient in transfection [8, 19, 20]. Phosphonates **IV** and **V** were brought into reactions with trimethylamine and pyridine.



$n = 2$ (**IV, VI**), 3 (**V, VII**).

Trimethylamine and pyridine were alkylated in the absence of solvent at 30–70°C within several hours. The yields of ammonium phosphonates **VI–VIII** reached 65–80%.

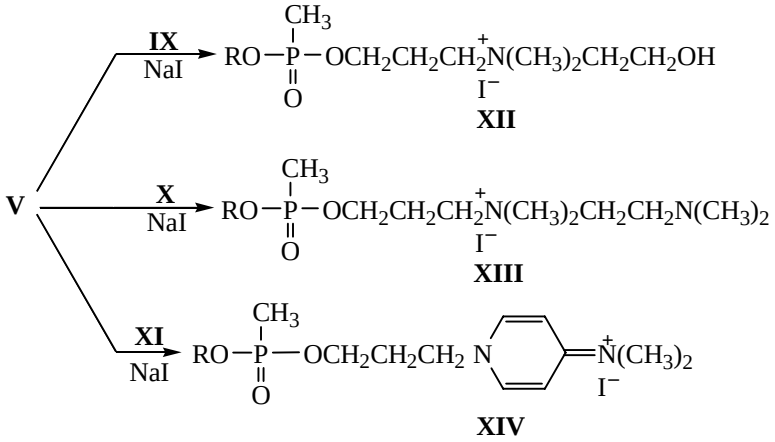
The homogeneity and structure of ammonium salts **VI–VIII** was proved by TLC, ^{31}P and ^{1}H NMR spectroscopy In the ^{1}H NMR spectra of phosphonates **VI** and **VII** appeared singlets at 3.3 ppm from protons of the $N^+(CH_3)_3$ group, and in the spectrum of phosphonate **VIII** signals were observed corresponding to α, β, and γ pyridinium protons. Besides in the spectra of phosphonates **VI–VIII** instead of triplets of the methylene protons of the CH_2Br moiety arose multiplets belonging to the protons of the CH_2N^+ group. The other peaks in the spectra of compounds **VI–VIII** virtually coincided with those in the spectra of initial bromoalkanes **IV** and **V**. Apart from alkylation of trimethylamine and pyridine we alkylated with bromophosphate **V** a series of the other nitrogen-containing compounds: dimethylethanolamine (**IX**), tetramethylethylenediamine (**X**), and dimethylaminopyridine (**XI**). The reaction between bromophosphonate **V** with bases **IX–XI** without solvent occurred at high temperature and afforded ammonium phosphoamphiphiles in low yield. Therefore we carried out alkylation of bases **IX–XI** with bromophosphonate **V** in DMSO solution in the presence of NaI at 60–100°C.

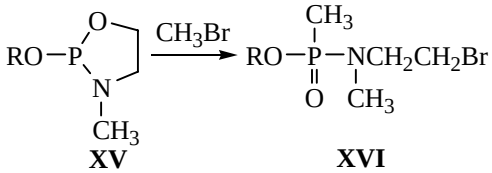
Note that the replacement of bromine under alkylation conditions by the more nucleophilic iodine made it possible to obtain ammonium salts **XII–XIV** in preparative yields (50–60%). Presumably the quaternization of base **XI** may occur both at the endocyclic anc exocyclic nitrogen atoms. It was previously shown on the phosphorus-free pyridinium amphophiles that the similar process involved the endocyclic nitrogen [19]. The ^{1}H NMR data of compound **IX** indicated that in the synthesis of compound **XIV** same as in the former case the alkylation proceeded at the nitrogen in the ring. In the ^{1}H NMR spectra of ammonium salts **XII–XIV** the proton signals from the main skeleton of the molecules are retained, and signals appear corresponding to the methyl protons of the groups $CH_2N^+(CH_3)_2$ in compounds **XII** and **XIII**, to methylene protons of the hydroxymethyl group in compoind **XII**, and to methyl protons of group $CH_2N(CH_3)_2$ in compound **XIII**. The signal at 6.47 ppm in the spectrum of compound **XIV** does not belong to the protons of the pyridinium ring (the resonance of the β-protons of the ring in compound **VIII** is located at 8.18 ppm) but corresponds to the aminovinyl proton in the fragment NCH=C<u>H</u>. Besides the peaks of the methyl groups from $=N^+(CH_3)_2$ were found at δ 2.97 ppm (Scheme 1).

Finally the synthetic procedure developed above was applied to preparation of methylamidophosphonic amphiphiles of the cationic type. As initial compound for this synthesis *N,O*-ethylenamidophosphite **XV** was chosen.

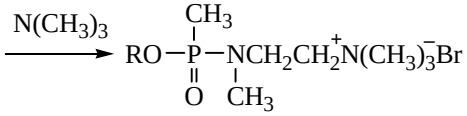
To prepare the target amphiphiles **XVI** and **XVII** first ethylenamidophosphite **XV** was alkylated with bromo-

Scheme 1.





XV **XVI**



XVII

methane (60°C, 8 h). Then amidophosphonate **XVI** isolated by column chromatography on silica gel was transformed by treatment with trimetylamine into ammonium phospholipid **XVII** (40°C, 6 h). Phospholipid **XVII** precipitated from the benzene solution as a colorless powder (yield 38%, δ_P 36.08 ppm). In the ^{1}H NMR spectra of phosphonates **XVI** and **XVII** the signals were observed characteristic of all proton groups. The protons of the methyl group attached to nitrogen gave rise to a doublet in the region 2.68–2.80 ppm. In the spectrum of compound **XVI** the triplet of the methylene protons of CH_2Br group was observed at δ 3.43 ppm. The spectrum of N-choline phosphonate **XVII** contained a singlet at δ 3.49 ppm corresponding to the protons of ammonium group, and a multiplet from the protons of CH_2N^+ gtoup at δ 4.03 ppm.

Thus this investigation resulted in the synthesis of a series of unconventional ammonium nitrophosphoamphiphiles of phosphonate and amidophosphonate type containing both aliphatic and heterocyclic nitrogen bases. All compounds are models of lipid systems promising for practical application.

EXPERIMENTAL

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

For adsorption chromatography on a column of 15 mm diameter was applied silica gel L 100–250 μm, R_f values were measured by TLC on Silufol UV-254 plates using as eluents mixtures benzene–dioxane, 3:1 (A), hexane–dioxane, 3:1 (B), chloroform–methanol–water, 65:25:4 (C). Melting points were measured in sealed capillaries at heating rate 1 deg/min.

2,2-(*O*,*O*-Isopropylidenedioxymethyl)-2-nitroethanol (**I**) was obtained by procedure [21], 2-chloro-1,3,2-dioxaphospholane by method [22], 2-chloro-1,3,2-dioxaphosphorinane as described in [23], and 2-*N*-diethyl-3-*N*-methyl-1,3,2-oxaazaphospholane as in [24]. The compounds used had constants in agreement with the published data.

***O*-[2,2-(*O*,*O*-Isopropylidenedioxymethyl)-2-nitroethyl]-*O*-(2-bromoethyl)methylphosphonate (IV).** A sealed ampule with a solution of ethylenephosphite **II** prepared by procedure [21] from 0.5 g (2.6 mmol) of compound **I**, 0.3 g (2.6 mmol) of *O*,*O*-ethylenechlorophosphite, 0.3 g (2.6 mmol) of triethylamine, and 2.47 g (26 mmol) of bromomethane in 20 ml of anhydrous benzene was heated at 100°C for 15 h. The solvent was removed in a vacuum, and phosphonate **IV** was isolated on a column packed with silica gel (20 g) and filled with hexane. Compound **IV** was eluted with 50 ml of a mixture hexane–dioxane, 3:1. The solvents were removed in a vacuum, and the residue was heated for 2 h at 40°C (1 mm Hg). Yield 0.39 g (40%), n_D^{20} 1.5102, R_f 0.55 (A), 0.27 (B). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.35 s, 1.43 s [6H, $C(CH_3)_2$], 1.49 d (3H, PCH_3, $^2J_{PH}$ 17.60 Hz), 3.47 t (2H, CH_2Br), 3.98 d ($2H_e$) and 4.32 d ($2H_a$) (CH_2OC, $^2J_{H_aH_e}$ 12.3 Hz), 4.29 m (2H, $POCH_2$), 4.43 d (2H, CH_2OP, $^3J_{HP}$ 6.6 Hz). ^{31}P NMR spectrum (chloroform), δ, ppm: 31.47 s. Found, %: C 32.12; H 5.21; P 8.44. $C_{10}H_{19}BrNO_7P$. Calculated, %: C 31.93; H 5.09; P 8.23. *M* 376.15.

***O*-[2,2-(*O*,*O*-Isopropylidenedioxymethyl)-2-nitroethyl]-*O*-(2-bromopropyl)methylphosphonate (V).** A sealed ampule with a solution of *O*,*O*-trimethylenephosphite **III** prepared by procedure [21] from 0.6 g (3.12 mmol) of compound **I**, 0.48 g (3.12 mmol) of *O*,*O*-trimethylenechlorophosphite, 0.36 g (3.12 mmol) triethylamine, and 3.04 g (32 mmol) of bromomethane in 20 ml of anhydrous benzene was heated at 120°C for 20 h. The solvent was removed in a vacuum, and phosphonate **V** was isolated on a column packed with silica gel (30 g) and filled with hexane. Compound **V** was eluted with 50 ml of a mixture hexane–dioxane, 3:1. The solvents were removed in a vacuum, and the residue was heated for 2 h at 40°C (1 mm Hg). Yield 0.79 g (65%), n_D^{20} 1.5058, R_f 0.60 (A), 0.30 (B). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.38 s, 1.40 s [6H, $C(CH_3)_2$], 1.48 d (3H, PCH_3, $^2J_{PH}$ 17.59 Hz), 2.12 m (2H, $OCH_2C\underline{H}_2CH_2$), 3.45 t (2H, CH_2Br), 4.00 d ($2H_e$) and 4.35 d ($2H_a$) (CH_2OC, $^2J_{H_aH_e}$

12.65 Hz), 4.12 m (2H, $POCH_2$), 4.45 d (2H, CH_2OP, $^3J_{HP}$ 5.5 Hz). ^{31}P NMR spectrum (chloroform), δ, ppm: 30.82 s. Found, %: C 34.01; H 5.72; P 8.12. $C_{11}H_{21}BrNO_7P$. Calculated, %: C 33.86; H 5.43; P 7.94. *M* 390.18.

***O*-[2,2-(*O*,*O*-Isopropylidenedioxymethyl)-2-nitroethyl]-*O*-(2-trimethylammonioethyl)methylphosphonate bromide (VI).** A sealed ampule with a solution of 0.2 g (0.5 mmol) of bromoderivative **IV** and 0.16 g (2.6 mmol) trimethylamine in 10 ml of anhydrous benzene was heated at 35–40°C for 12 h. The precipitate was washed with benzene (2×2 ml), acetone (2×2 ml), and kept for 2 h at 40°C (1 mm Hg). Yield 0.14 g (65%), mp 140–142°C (started to melt at 90°C), R_f 0.60 (C). ^1H NMR spectrum ($CDCl_3$ –CD_3OD, 99:1), δ, ppm: 1.34 s, 1.37 s [6H, $C(CH_3)_2$], 1.51 d (3H, PCH_3, $^2J_{PH}$ 17.40 Hz), 3.28 C (9H, N^+Me_3), 3.52 br.m (2H, $POCH_2C\underline{H}_2N^+$), 3.84 br.m (2H, $POC\underline{H}_2CH_2N^+$), 4.04 d ($2H_e$) and 4.49 d ($2H_a$) ($CH_2OC$, $^2J_{H_aH_e}$ 12.51 Hz), 4.59 m (2H, CH_2 OP, $^3J_{HP}$ 5.6 Hz). ^{31}P NMR spectrum (chloroform–methanol, 99:1), δ, ppm: 31.63 s. Found, %: C 38.01; H 5.62; P 7.32. $C_{13}H_{25}BrN_2O_7P$. Calculated, %: C 38.20; H 5.83; P 7.17. *M* 432.24.

***O*-[2,2-(*O*,*O*-Isopropylidenedioxymethyl)-2-nitroethyl]-*O*-(3-trimethylammoniopropyl)-methylphosphonate bromide (VII)** prepared in the same way as phosphonate **VI** from 0.25 g (0.64 mmol) of bromoderivative **V** and 0.19 g (3.2 mmol) of trimethylamine in 15 ml of anhydrous benzene in 10 h at 50°C. Yield 0.24 g (85%), mp 160–162°C (started to melt at 100°C), R_f 0.63 (C). ^1H NMR spectrum ($CDCl_3$–CD_3OD, 99:1), δ, ppm: 1.38 s, 1.42 s [6H, $C(CH_3)_2$], 1.54 d (3H, PCH_3, $^2J_{PH}$ 17.60 Hz), 2.18 m (2H, $OCH_2C\underline{H}_2CH_2N^+$), 3.34 s (9H, N^+Me_3), 3.62 br.m (2H, $POCH_2CH_2C\underline{H}_2N^+$), 4.07 br.m (2H, $POC\underline{H}_2CH_2CH_2N^+$), 4.11 d ($2H_e$) and 4.45 d ($2H_a$) ($CH_2OC$, $^2J_{H_aH_e}$ 12.65 Hz), 4.55 m (2H, CH_2 OP, $^3J_{HP}$ 5.72 Hz). ^{31}P NMR spectrum (chloroform–methanol, 99:1), δ, ppm: 31.03 s. Found, %: C 37.93; H 6.32; P 7.19. $C_{14}H_{27}BrN_2O_7P$. Calculated, %: C 37.68; H 6.10; P 6.94. *M* 446.27.

O-[2,2-(O,O-Isopropylidenedioxymethyl)-2-nitroethyl]-O-(3-pyridinopropyl)methylphosphonate bromide (VIII). In 2 ml of anhydrous pyridine was heated at 70°C for 12 h 0.2 g (0.5 mmol) of bromoderivative **V**. The solvent was removed in a vacuum, and the residue was washed in succession with benzene (1×1 ml), hexane (1×1 ml), ether (1×1ml), and kept for 2 h at 40°C (1 mm Hg). Yield 0.17 g (80%), n_D^{20} 1.5455, R_f 0.45 (B). ^1H NMR spectrum (CD_3SOCD_3), δ, ppm: 1.27 s, 1.44 s [6H, $C(CH_3)_2$], 1.50 d (3H, PCH_3, $^2J_{PH}$ 17.03 Hz), 2.30 m (2H, $OCH_2C\underline{H}_2CH_2N^+$), 3.78 d ($2H_e$) and 4.35 d ($2H_a$) ($CH_2OC$, $^2J_{H_aH_e}$ 13.20 Hz), 4.03 m (2H, $POC\underline{H}_2CH_2CH_2N^+$, $^3J_{PH}$ 7.14 Hz), 4.38 m (2H, CH_2OP, $^3J_{HP}$ 6.04 Hz), 4.75 m (2H, $POCH_2CH_2C\underline{H}_2N^+$, $^3J_{HH}$ 7.15 Hz), 8.18 m (2H), 8.58 m (1H), 9.15 m (2H) ($C_5H_5N^+$). ^{31}P NMR spectrum (chloroform–methanol, 99:1), δ, ppm: 31.88 s. Found, %: C 36.29; H 6.32; P 7.38. $C_{13}H_{26}BrN_2O_7P$. Calculated, %: C 36.04; H 6.05; P 7.15. *M* 433.25.

***O*-[2,2-(*O*,*O*-Isopropylidenedioxymethyl)-2-nitroethyl]-*O*-(3-*N*,*N*-dimethyl-*N*-hydroxyethyl-ammoniopropyl)methylphosphonate iodide (XII)**. A mixture of 0.15 g (0.4 mmol) of bromoderivative **V**, 0.06 g (0.7 mmol) of freshly distilled *N*,*N*-dimethylethanolamine, 0.17 g (1.2 mmol) of sodium iodode was dissolved in 3 ml of anhydrous DMSO and heated at 90–100°C for 8 h. To the reaction mixture 15 ml of chloroform was added, the solution was washed with water (2×15 ml), and dried over Na_2SO_4. Target product **XII** was precipitated from the chloroform solution with 10 ml of hexane, washed with ether, and dried in a vacuum for 2 h at 40°C (1 mm Hg). Yield 0.11 g (50%), n_D^{20} 1.5473, R_f 0.41 (C). ^1H NMR spectrum ($CDCl_3$), δ, ppm: 1.23 s, 1.38 s [6H, $C(CH_3)_2$], 1.53 d (3H, PCH_3, $^2J_{PH}$ 17.05 Hz), 2.03 m (2H, $OCH_2C\underline{H}_2CH_2N^+$), 2.59 s (6H, N^+Me_2), 3.04 m (2H, CH_2N^+), 3.76 m (2H, $POC\underline{H}_2CH_2CH_2N^+$), 3.97 d ($2H_e$) and 4.41 d ($2H_a$) ($CH_2OC$, $^2J_{H_aH_e}$ 5.12 Hz), 4.04 m (2H, $N^+C\underline{H}_2CH_2OH$), 4.15 m (2H, $C\underline{H}_2$ OH), 4.50 m (2H, CH_2OP, $^3J_{HP}$ 12.79 Hz), 6.90 m (1H, OH). ^{31}P NMR spectrum (chloroform), δ, ppm: 31.35 s. Found, %: C 34.51; H 6.35; P 6.11. $C_{15}H_{32}IN_2O_8P$. Calculated, %: C 34.23; H 6.13; P 5.89. *M* 526.32.

***O*-[2,2-(*O*,*O*-Isopropylidenedioxymethyl)-2-nitroethyl]-*O*-[3-*N*,*N*-dimethyl-*N*-(2-dimethylamino-ethyl)ammoniopropyl]methylphosphonate iodide (XIII)** was obtained similarly to compound **XII** from 0.2 g (0.5 mmol) of bromoderivative **V**, 0.23 g (1.5 mmol) of sodium iodide, and 0.058 g (0.5 mmol) of freshly distilled *N*,*N*,*N*',*N*'-tetramethylethylenediamine **X** in 3 ml of anhydrous DMSO at 90–95°C in 10 h. Yield 0.18 g (61%), 1.5549, R_f 0.52 (C). ^1H NMR spectrum ($CDCl_3$), δ, ppm: 1.18 s, 1.33 s [6H, $C(CH_3)_2$], 1.55 d (3H, PCH_3, $^2J_{PH}$ 16.39 Hz), 2.18 m (2H, $OCH_2C\underline{H}_2CH_2N^+$), 2.35 s (6H, NMe_2), 2.55 s (6H, N^+Me_2), 3.68–3.78 m (4H, $N^+CH_2CH_2N$), 3.97 d ($2H_e$) and 4.32 d ($2H_a$) (CH_2OC, $^2J_{H_aH_e}$ 4.89 Hz), 4.43–4.45 m (6H, CH_2OPOCH_2, CH_2N^+, $^3J_{HP}$ 10.68 Hz). ^{31}P NMR spectrum (chloroform), δ, ppm:

30.98 s. Found, %: C 36.93; H 6.98; P 5.39. $C_{17}H_{37}IN_3O_7P$. Calculated, %: C 36.70; H 6.70; P 5.57. *M* 556.38

***O*-[2,2-(*O*,*O*-Isopropylidenedioxymethyl)-2-nitroethyl]-*O*-(3-*p*-dimethylammonio-*N*- pyridinopropyl)methylphosphonate iodide (XIV)** was obtained similarly to compound **XII** from 0.3 g (0.8 mmol) bromoderivative **V**, 1.2 g (8 mmol) of sodium iodide, and 0.28 g (2.3 mmol) of *N*,*N*-dimethylaminopyridine **XI** in 5 ml of anhydrous DMSO at 70–80°C in 6 h. To the reaction mixture 20 ml of chloroform was added, the solution was washed with water (2×30 ml), and dried over Na_2SO_4. The solvent was removed in a vacuum, and the residue was washed in succession with benzene, hexane, and ether, and kept in a vacuum for 2 h at 40°C (1 mm Hg). Yield 0.22 g (50%), mp 102–105°C, R_f 0.51 (C). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.37 s, 1.40 s [6H, $C(CH_3)_2$], 1.52 d (3H, PCH_3, $^2J_{PH}$ 16.97 Hz), 2.10 m (2H, $OCH_2C\underline{H}_2CH_2$), 2.97 s [6H, $N^+(CH_3)_2$], 3.61 t (2H, CH_2N^+), 3.95 d (2H$_e$) and 4.46 d (2H$_a$) (CH_2OC, $^2J_{H_aH_e}$ 13.74 Hz), 4.01 m (2H, $POC\underline{H}_2CH_2CH_2$, $^3J_{HP}$ 5.5 Hz), 4.36 m (2H, CH_2OP, $^3J_{HP}$ 13.2 Hz), 6.47 br.s (2H, NCH=C$\underline{H}$), 8.18 br.s (2H, NC$\underline{H}$=CH). ^{31}P NMR spectrum (chloroform), δ, ppm: 31.56 s. Found, %: C 38.49; H 5.38; P 5.83. $C_{18}H_{31}IN_3O_7P$. Calculated, %: C 38.65; H 5.59; P 5.54. *M* 559.34.

***O*-[2,2-(*O*,*O*-Isopropylidenedioxymethyl)-2-nitroethyl]-(*N*-methyl-*N*-2-bromoethylamido)-methylphosphonate (XVI).** A sealed ampule with a solution of amidophosphate **XV** obtained by procedure [21] from 0.5 g (3.12 mmol) of compound **I**, 0.42 g 2.6 mmol) of *N*-diethylamino-3-methyl-1,3,2-oxaazaphospholane, and 2.47 g (2.6 mmol) of bromomethane in 20 ml of anhydrous benzene was heated at 60°C for 8 h. The solvent was removed in a vacuum, and phosphonate **XVI** was isolated on a column packed with silica gel (20 g) and filled with hexane. Compound **XVI** was eluted with 35 ml of a mixture hexane–dioxane, 1:1. The solvents were removed in a vacuum, and the residue was heated for 2 h at 40°C (1 mm Hg). Yield 0.46 g (45%), n_D^{20} 1.5093, R_f 0.55 (A), 0.35 (B). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.25 s, 1.32 s [6H, $C(CH_3)_2$], 1.45 d (3H, PCH_3, $^2J_{PH}$ 16.49 Hz), 2.68 d (3H, $PNCH_3$, $^3J_{PH}$ 8.8 Hz), 3.43 t (2H, CH_2Br), 3.69 m (2H, $PNC\underline{H}_2CH_2$), 3.78 d (2H$_e$) and 4.96 d (2H$_a$) (CH_2OC, $^2J_{H_aH_e}$ 9.9 Hz), 4.12 d (2H, CH_2OP, $^3J_{HP}$ 6.6 Hz). ^{31}P NMR spectrum (chloroform), δ, ppm: 34.30 s. Found, %: C 34.23; H 5.98; P 8.15. $C_{11}H_{22}BrN_2O_6P$. Calculated, %: C 33.94; H 5.70; P 7.96. *M* 389.20.

***O*-[2,2-(*O*,*O*-Isopropylidenedioxymethyl)-2-nitroethyl]-(*N*-methyl-*N*-2-trimethylammonio-ethylamido)methylphosphonate bromide (XVII)** was obtained analogously to phosphonate **VI** from 0.2 g (0.51 mmol) of compound **XVI** and 0.14 g (2.5 mmol) trimethylamine in 15 ml of anhydrous benzene in 6 h at 40°C. Yield 0.09 g (38%), mp 151–152°C (started to melt at 95°C), R_f 0.55 (C). ^{1}H NMR spectrum ($CDCl_3$ – CD_3OD, 99:1), δ, ppm: 1.26 s, 1.38 s [6H, $C(CH_3)_2$], 1.45 d (3H, PCH_3, $^2J_{PH}$ 15.94 Hz), 2.80 d (3H, $PNCH_3$, $^3J_{PH}$ 9.9 Hz), 3.49 C (9H, N^+Me_3), 3.56 br.m (2H, $PNC\underline{H}_2CH_2N^+$), 3.79 d (2H$_e$) and 3.94 d (2H$_a$) ($CH_2OC$, $^2J_{H_aH_e}$ 6.6 Hz), 4.03 br.m (2H, $PNCH_2C\underline{H}_2N^+$), 4.10 m (2H, CH_2 OP). ^{31}P NMR spectrum (chloroform–methanol, 99:1), δ, ppm: 36.08 s. Found, %: C 37.79; H 7.13; P 7.23. $C_{14}H_{31}BrN_3O_6P$. Calculated, %: C 37.51; H 6.97; P 6.91. *M* 448.30.

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