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Cationic Phospholipids on the Basis of Higher Fatty Alcohols

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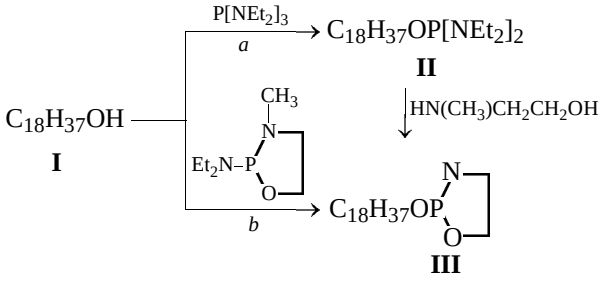
Abstract — A perspective synthetic approach to cationic amidodiester lipid constructions on the basis of cyclic phosphites derived from octadecan-1-ol is presented. The syntheses of phospholipids were carried out by oxidative decyclization of cyclic phosphoramidites with bromine.

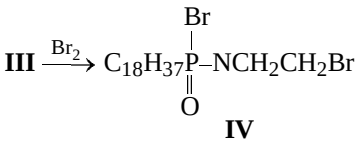
One of the important priorities of modern lipidology is synthesis of cationic ammonium analogs of natural phospholipids, as promising starting materials for genosome design and objects for other biomedical studies [1–3]. We have previously demonstrated the promise functionalyzed phosphorus acid halides hold for synthesis of cationic amidodiester lipids. Such key compounds have been prepared by oxidative decyclization with halogens (Br_2, Cl_2) of alkylene phosphoramidites derived from glycerol [4] or natural menthol [5]. It has therewith been established that phosphorous bromides prepared by the above reaction are the most convenient to use in the phospholipid synthesis [4]. Hence, the classical phosphorylation procedure widely used in phospholipid chemistry and based on the employment of traditional phosphorochloridates [6] has been successfully complemented by modern preparative techniques of organophosphorus chemistry [7].

The present work continues these studies and deals with the synthesis of first representatives, with the aid of phosphoramidobromidates, cationic amidodiester phospholipids containing an octadecyl radical. The synthesis of octadecyl phosphoramidobromidate **IV** was carried out in two stages. First we obtained alkoxyoxazaphospholane **III** by phosphorylation of octadecan-1-ol (**I**) with hexaethylphosphorous triamide followed by reaction of the phosphorodiamidite **II** formed with *N*-methylcolamine (procedure *a*) or by phosphorylation of octadecan-1-ol with 2-(diethylamino)-3-methyl-1,3,2-oxazaphospholane (procedure *b*).



Procedures *a* and *b* both were accomplished in dulute solutions in anhydrous dioxane or benzene at 80–85°C for 1 h with simultaneous distillation of the diethylamine fomed and solvent. The reaction progress was followed by ^{31}P NMR. Octadecyl phosphorodiamidite (**II**, δ_P 132.98 ppm, s) and cyclic phosphoramidite **III** (δ_P 136.70 ppm, s) were used in further syntheses without additional purification.

In the second stage, octadecyl phosphoramidite **III** was converted into octadecyl phosphoramidobromidate **IV** by oxidative decyclization with bromine under mild conditions (at 0°C).



Acid bromide **IV** withstands chromatographic purification on a silica gel column. Its yield per starting alcohol **I** achieves 55%. Note that the yields of com-

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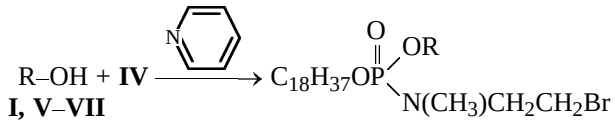
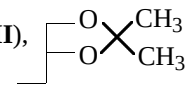
pound **IV** from phosphoramidite **III** prepared by procedures *a* and *b* are approximately the same, but procedure *b* is more convenient from the preparative viewpoint. Note that compound **IV** can be handled for several months in the darkness in the absence of air at room temperature.

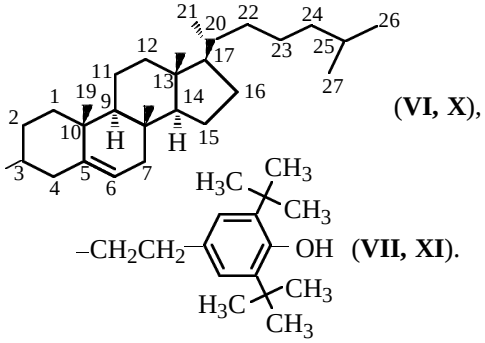
The ^{31}P NMR spectrum of phosphoramidobromidate **IV** contains a singlet at δ_P 5.18 ppm. The ^{1}H NMR spectrum of this compound displays signals characteristic of all the proton groups. Hence, protons of the $PNCH_3$ group appear as a doublet signal at δ 2.75 ppm ($^3J_{PH}$ 8.24 Hz) due to coupling of methyl protons with phosphorus, and methylene protons of the $PNCH_2CH_2Br$ group give a complex multiplet at 3.40 ppm. α-Methylene protons of the octadecyl radical give a doublet of triplets at 4.10 ppm as the result of coupling with phosphorus and β-methylene protons. β-Methylene protons of this radical appear as a multiplet at 1.71 ppm. The other signals in the spectrum of compound **IV** correspond to the presented structure (see Experimental).

Octadecyl phosphoramidobromidate **IV** was used for phosphorylation of alcohols of the lipid series. Its reaction with octadecan-1-ol (**I**) gave symmetrical phosphoramidate **VIII**, whereas reactions with glycerol derivative **V** and natural cholesterol[1] (**VI**) gave unsymmetrical phosphoramidates **IX** and **X**. In addition, compound **IV** was used to introduce lipid fragments in ionol derivative **VIII**, a well known antioxidant widely used in membrane studies [9, 10].



VIII–**XI**

R = $C_{18}H_{37}$ (**I, VIII**),  (**V, IX**),



(**VI, X**),

(**VII, XI**).

[1] The numbering of carbon atoms in the cholesterol radical corresponds to that accepted in cholesterol chemistry [8].

The reactions of phosphoramidobromidate **IV** with 1,2-*O*-isopropylideneglycerol (**V**) and cholesterol (**VI**) were carried out in anhydrous benzene in the presence of pyridine for 12 h, and with octadecan-1-ol and 2,6-di-*tert*-butyl-4-(3-hydroxypropyl)phenol (**VII**), heating at 40°C for 2 h was needed. After purification on a silica gel column, the yields of phospholipids **VIII**–**IX** were 61–62 (from **IX** and **X** and 50–51% (from **VIII** and **XI**). Note that octadecyl phosphoramidobromidate **IV** is more reactive than menthyl phosphoramidobromidate [5]. The latter reacts with glycerol and cholesterol derivatives under more rigid conditions (60–80°C, 12–30 h).

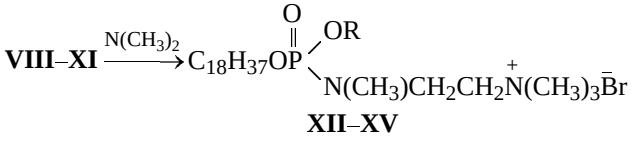
The individuality and structure of octadecyl phosphoramidates **VIII**–**XI** were proved by physicochemical methods. The ^{31}H NMR spectra of compounds **VIII** and **XI** contain singlets (δ_P 9.01 and 8.99 ppm, respectively) and those of compounds **IX** and **X**, broadened singlets (δ_P 9.62 and 8.51 ppm, respectively), since the latter compounds exist as mixtures of diastereomers.

The ^{1}H NMR spectra of compounds **VIII**–**XI** contain all expected proton signals. Hence, in the ^{1}H NMR spectrum of symmetrical amidodiester **VIII**, the signals of the $C_{18}H_{37}O$ group doubled integral intensity compared with the respective signals in the spectrum of acid bromide **IV**. The ^{1}H NMR spectrum of glycerol derivative **IX** shows singlets at δ 1.35 and 1.42 ppm due to methyl protons of the acetonyl protective group. The multiplet of the methine proton on the β-carbon atom of the glycerol residue is observed at 3.41 ppm. The ^{1}H NMR spectra of cholesterol phospholipid[2] **X** and ionol derivative **XI**, too, are fully consistent with the presented structures (see Experimental).

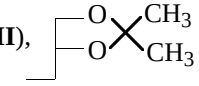
In the final stage of the work, β-bromoethylphosphoramidates **VIII**–**XI** were converted into cationic lipids by treatment with trimethylamine.

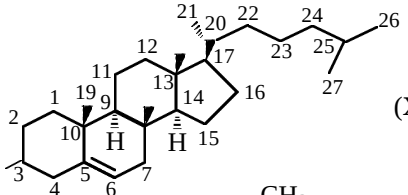
The reactions with trimethylamine were carried out at 70–80°C for 4–8 h. The yields of ammonium derivatives **XII**–**XV** were 63–71%. The ^{31}P NMR spectra of compounds **XII**–**XV** are quite similar to the spectra of compounds **VIII**–**XI**. In ^{1}H NMR spectra of **XII**–**XV**, the multiplet of methylene protons of the $PN(CH_3)CH_2CH_2$ group is shifted downfield relative to the respective signal of **VIII**–**XI**, and a singlet at 3.55 ppm from methyl protons on the cationic center $N^+(CH_3)_3$ appears.

[2] Analysis of the ^{1}H NMR spectra of cholesterol phospholipids **X** and **XIV** was carried out with account for the detailed assignment of cholesterol proton signals given in [8, 11, 12].

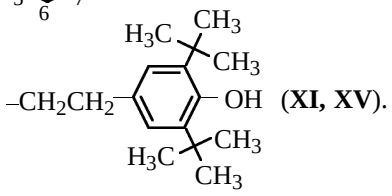


VIII–XI → **XII–XV**

R = $C_{18}H_{37}$ (**VIII, XII**),  (**IX, XIII**),



(**X, XIV**),



(**XI, XV**).

To conclude, oxidative decyclization of cyclic phosphoramidites with bromine allows synthesis of phosphorus acid bromides of complex structure under mild conditions and in high yields. It is significant that using of such bromides as phosphorylating agents in the chemistry of natural compounds is a convenient preparative method for creating a phosphorus entity and deserves wide synthetic use.

EXPERIMENTAL

The ^{1}H NMR spectra were obtained on a Bruker WM-250 instrument (250 MHz), internal reference HMDS; signal assignment was performed on the basis of double resonance spectra. The ^{31}P–{^{1}H} NMR spectra were measured on a Bruker WP-80SY spectrometer (32.4 MHz), external reference 85% phosphoric acid.

Absorption chromatography was performed on a column 10 mm in diameter, packed with silica gel L (100–250 μm). Thin-layer chromatography was performed on Silufol UV-254 plates in benzene (A), benzene–dioxane, 3:1 (B), hexane–dioxane, 3:1 (C), and chloroform–methanol–water, 64:25:4 (D). The melting points were determined in a sealed capillary, heating rate 1 deg min^{-1}.

2-(Diethylamino)-3-methyl-1,3,2-oxazaphospholane was prepared according to [13]. The constants of this compound were coincident with published data.

Octadecyl *N*-(2-bromoethyl)-*N*-methylphosphoramidobromidate (IV). *a*. A mixture of 2 g of alcohol **I** and 3.7 g of hexaethylphosphorous triamide in 3 ml of anhydrous dioxane was heated for 2 h at 80°C with distillation of the evolving diethylamine and dioxane. The formation of phosphorodiamidite **II** was controlled by ^{31}P NMR (δ_P 132.98 ppm, s). Further compound **II** was subjected to a vacuum (1 mm Hg) at 130– 150°C for 2 h, mixed with 0.55 g of *N*-methylethanolamine and 5 ml of anhydrous dioxane, and the mixture was heated at 75–85°C. A solution of cyclic phosphoramidite **III** (δ_P 136.70 ppm, s) in 5 ml of anhydrous benzene was treated at –5°C with a solution of 1.18 g of bromine in 5 ml of the same solvent, which was added dropwise with vigorous stirring. After 10 min, the solvent was removed in a vacuum, and product **IV** was isolated on a column of silica gel (10 g), filled with hexane. The product was eluted with 30 ml of hexane–dioxane, 10:1. The solvents were removed in a vacuum, and the residue was kept for 2 h at 40°C (1 mm Hg), yield 2.2 g (55%), mp 70–72°C, R_f 0.85 (A) and 0.65 (B). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 0.88 t [3H, C*H*$_3$(CH$_2$)$_{15}$CH$_2$·CH$_2$O, $^3J_{HH}$ 6.76 Hz], 1.26 s [30H, CH$_3$(C*H*$_2$)$_{15}$CH$_2$·CH$_2$O], 1.71 m [2H, CH$_3$(CH$_2$)$_{15}$C*H*$_2$CH$_2$O], 2.75 d (3H, PNCH$_3$, $^3J_{PH}$ 8.24 Hz), 3.40 m (4H, NCH$_2$·CH$_2$Br), 4.10 d.t [2H, CH$_3$(CH$_2$)$_{15}$CH$_2$C*H*$_2$O]. ^{31}P NMR spectrum ($CHCl_3$), δ_P, ppm: 5.16 s. Found, %: C 47.32; H 8.30; P 5.97. $C_{21}H_{44}Br_2NO_2P$. Calculated, %: C 47.29; H 8.32; P 5.81.

b. A mixture of 1.7 g of octadecan-1-ol (**I**) and 1 g of 2-(diethylamino)-3-methyl-1,3,2-oxazaphospholane in 3 ml of anhydrous benzene was heatedfor 1.5 h at 80–85°C with distillation of the evolving diethylamine and benzene. The formation of cyclic phosphoramidite **III** was controlled by ^{31}P NMR (δ_P 136.61 ppm, s). Compound **III** was converted into acid bromide **IV** as described above using 0.99 g of bromine in 3 ml of anhydrous benzene. ^{31}P NMR spectrum, δ_P, ppm: 5.17 s. Yield 2.17 g (65.5 %). The chromatographic mobility, melting point, and ^{1}H and ^{31}P NMR spectra of this sample were analogous to those of the sample obtained by procedure *a*.

Dioctadecyl *N*-(2-bromoethyl)-*N*-methylphosphoramidate (VIII). To a solution of 0.86 g of acid bromide **IV** in 5 ml of anhydrous benzene, a solution of 0.45 g of octadecan-1-ol and 0.13 g of freshly distilled pyridine in 3 ml of anhydrous benzene was added dropwise with stirring and cooling to 0°C. The reaction mixture was heated at 40°C for 1.5 h. Pyridine hydrobromide was filtered off, the solvent was removed in a vacuum, and compound **VIII** was isolated on a column of silica gel (10 g), filled with hexane. The product was eluted with 50 ml of hexane. The solvent was removed in a vacuum, and the residue the residue was kept for 2 h at 40°C (1 mm Hg),

yield 0.57 g (50%), mp 45–46°C, R_f 0.85 (A) and 0.60 (B). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 0.88 t [6H, C*H*$_3$(CH$_2$)$_{15}$CH$_2$CH$_2$O, $^3J_{HH}$ 7.01 Hz], 1.25 s [60H, CH$_3$(C*H*$_2$)$_{16}$CH$_2$CH$_2$O], 1.65 m [4H, CH$_3$(CH$_3$)$_{15}$·C*H*$_2$CH$_2$O], 2.71 d (3H, PNCH$_3$, $^3J_{PH}$ 9.50 Hz), 3.43 m [2H, N(CH$_3$)C*H*$_2$], 3.64 t (2H, CH$_2$Br, $^3J_{HH}$ 6.68 Hz), 3.98 d.t [4H, CH$_3$(CH$_2$)$_{15}$CH$_2$C*H*$_2$O, $^3J_{HH}$ 6.58 Hz, $^3J_{PH}$ 11.58 Hz]. ^{31}P NMR spectrum ($CDCl_3$), δ_P, ppm: 9.01 s. Found, %: C 66.21; H 11.70; P 4.29. $C_{39}H_{81}BrNO_3P$. Calculated, %: C 66.07; H 11.52; P 4.37.

1,2-*O*-Isopropylideneglycerol octadecyl *N*-(2-bromoethyl)-*N*-methylphosphoramidate (IX) was obtained analogously to compound **VIII** from 0.96 g of acid bromide **IV**, 0.24 g of 1,2-*O*-isopropylideneglycerol, and 0.15 g of pyridine in 10 ml of anhydrous benzene at room temperarure for 10 h. Compound **IX** was isolated pure on a column of silica gel (10 g), filled with hexane. The product was eluted with 80 ml of hexane–dioxane, 3 : 1. The solvents were removed in a vacuum, and the residue was kept in a vacuum for 2 h at 40°C (1 mm), yield 0.51 g (61%), mp 35–37°C, R_f 0.48 (A) and 0.40 (B). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 0.86 t [3H, C*H*$_3$(CH$_3$)$_{15}$CH$_2$·CH$_2$O, $^3J_{HH}$ 6.39 Hz], 1.24 s [30 H, CH$_3$(C*H*$_2$)$_{15}$·CH$_2$CH$_2$O], 1.35 s (3H), 1.42 s (3H) [C(CH$_3$)$_2$], 1.66 m [2H, CH$_3$(CH$_2$)$_{15}$C*H*$_2$CH$_2$O], 2.72 d (3H, PNCH$_3$, $^3J_{PH}$ 9.77 Hz), 3.38–3.48 m (4H, NCH$_2$·CH$_2$Br), 3.82 m (2H, C*H*$_2$CHCH$_2$OP), 3.96 m (2H, CH$_2$CHC*H*$_2$OP), 4.07 d.t [2H, CH$_3$(CH$_2$)$_{15}$CH$_2$C*H*$_2$O, $^3J_{HH}$ 6.51 Hz, $^3J_{PH}$ 11.15 Hz], 4.31(1H, CH$_2$C*H*·CH$_2$OP, $^3J_{HH}$ 5.50 Hz). ^{31}P NMR spectrum ($CHCl_3$), δ_P, ppm: 9.62 br.s. Found, %: C 58.43; H 9.96; P 5.52. $C_{27}H_{55}NO_3P$. Calculated, %: C 58.68; H 10.03; P 5.61.

Cholesteryl octadecyl *N*-(2-bromoethyl)-*N*-methylphosphoramidate (X) was prepared analogously to compound **VIII** from 0.65 g of acid bromide **IV**, 0.47 g of cholesterol (**VI**), and 0.1 g of pyridine in 10 ml of anhydrous benzene at room temperature for 12 h. Compound **X** was isolated pure on a column of silica gel (15 g), filled with hexane. The product was eluted with 100 ml of hexane–dioxane, 1 : 1. The solvent was removed in a vacuum, and the residue was kept for 2 h at 40°C (1 mm Hg), yield 0.63 g (62%), mp 32–33°C, R_f 0.75 (A) and 0.55 (B). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 0.68–2.42 ppm (cholesteryl H),[3] 0.85 t [3H, C*H*$_3$(CH$_2$)$_{15}$·CH$_2$CH$_2$O, $^3J_{HH}$ 6.62 Hz], 1.26 s [30H, CH$_3$(C*H*$_2$)$_{15}$·CH$_2$CH$_2$O], 1.65 m [2H, CH$_3$(CH$_2$)$_{15}$C*H*$_2$CH$_2$O], 2.71 d (3H, PNCH$_3$, $^3J_{PH}$ 10.01 Hz), 3.40–3.43 m (4H, NCH$_2$CH$_2$Br), 3.94 d.t [2H, CH$_3$(CH$_2$)$_{15}$CH$_2$C*H*$_2$O, $^3J_{HH}$ 6.58 Hz, $^3J_{PH}$ 12.01 Hz], 4.15 m (1H, cholesteryl OPC^{3}H), 5.38 d (1H, cholesteryl C^{6}H, $^3J_{HH}$ 3.20 Hz). ^{31}P NMR spectrum ($CHCl_3$), δ_P, ppm: 8.51 br.s. Found, %: C 68.83; H 10.81; P 3.91. $C_{48}H_{89}BrNO_3P$. Calculated, %: C 68.70; H 10.89; P 3.69.

3-(2,6-Di-*tert*-butyl-4-hydroxyphenyl)propyl octadecyl *N*-(2-bromoethyl)-*N*-methylphosphoramidate (XI) was prepared analogously to compound **VIII** from 0.45 g of acid bromide **IV**, 0.27 g of 2,6-di-*tert*-butyl-4-(3-hydroxypropyl)phenol (**VII**), and 0.1 g of pyridine in 15 ml of anhydrous benzene at 40°C for 2 h. Compound **XI** was isolated pure on a column of silica gel (10 g), filled with hexane. The product was eluted with 80 ml of hexane–dioxane, 3 : 1. The solvent was removed in a vacuum, and the residue was kept for 2 h at 40°C (1 mm Hg), yield 0.37 g (51%), n_D^{20} 1.4980, R_f 0.88 (A) and 0.53 (B). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 0.88 t [3H, C*H*$_3$·(CH$_2$)$_{15}$CH$_2$CH$_2$O, $^3J_{HH}$ 6.58 Hz], 1.26 s [30H, CH$_3$(C*H*$_2$)$_{15}$CH$_2$CH$_2$O], 1.43 s [18H, (CH$_3$)$_3$C], 1.68 m [2H, CH$_3$(CH$_2$)$_{15}$C*H*$_2$CH$_2$O], 1.97 m (2H, OCH$_2$C*H*$_2$CH$_2$), 2.62 t (2H, C*H*$_2$C$_7$H$_5$, $^3J_{HH}$ 8 Hz), 2.72 d (3H, PNCH$_3$, $^3J_{PH}$ 9.34 Hz), 3.42–3.54 m (4H, NCH$_2$CH$_2$Br), 3.90–4.10 m [4H, OC*H*$_2$CH$_2$CH$_3$, CH$_3$(CH$_2$)$_{15}$CH$_2$CH$_2$O), 5.10 s (1H, OH), 6.97 s (2H, C$_6$H$_2$). ^{31}P NMR spectrum ($CHCl_3$), δ_P, ppm: 8.99 s. Found, %: C 63.72; H 9.59; P 4.41. $C_{38}H_{68}BrNO_4P$. Calculated, %: C 63.84; H 9.73; P 4.33.

Dioctadecyl *N*-(2-bromoethyl)-*N*-methylphosphoramidate bromomethylate (XII). A sealed ampule with a solution of 0.4 g phosphoramidate **VIII** and 0.5 g of trimethylamine in 4 ml of anhydrous benzene was heated for 5 h at 60°C. The precipitate that formed 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 Hg). Yield 0.31 g (70%), mp 192–193°C (gets wet at 110°C), R_f 0.66 g (B). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 0.87 t [6H, C*H*$_3$(CH$_2$)$_{15}$CH$_2$CH$_2$O], 1.24 s [60 H, CH$_3$(C*H*$_2$)$_{15}$CH$_2$CH$_2$O], 2.71 d (3H, PNCH$_3$, $^3J_{PH}$ 9.87 Hz), 3.47 m [2H, NC*H*$_2$CH$_2$N$^+$], 3.51 s [9H, N$^+$(CH$_3$)$_3$], 3.71 m (2H, NCH$_2$C*H*$_2$N$^+$), 3.93 m (4H, CH$_3$(CH$_2$)$_{15}$CH$_2$C*H*$_2$O]. ^{31}P NMR spectrum ($CHCl_3$), δ_P, ppm: 9.15 s. Found, %: C 64.63; H 10.44; P 4.01. $C_{42}H_{90}BrN_2O_3P$. Calculated, %: C 64.50; H 10.31; P 3.96.

1,2-*O*-Isopropylideneglycerol octadecyl *N*-(2-bromoethyl)-*N*-methylphosphoramidate bromomethylate (XIII) was obtained analogously to bromomethylate **XII** from 0.5 g of phosphoramidate **IX**

[3] Here and hereinafter, the range δ_P 0.68–2.42 ppm characteristic of the protons on the C^{1,2}, C^4,C^5, and C$^{7-27}$ atoms of the phosphocholesterol moiety [8] is labeled "cholesteryl H."

and 0.65 g of trimethylamine in 3 ml of anhydrous benzene for 5 h at 80°C. The solvent was removed in a vacuum, and the residue was washed with anhydrous benzene (2 × 2 ml). Additional purification of bromomethylate **XIII** was carried out by crystallization from anhydrous acetone at 5°C (acetone-insoluble admixtures were preliminarily removed by centrifugation at 50°C). The product was kept for 3 h at 40°C (1 mm Hg), yield **XIII** 0.42 g (71%), mp 183–185°C (gets wet at 85°C), R_f 0.61 (B). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 0.87 t [3H, $CH_3(CH_2)_{15}CH_2CH_2O$, $^3J_{HH}$ 6.59 Hz], 1.25 s [30H, $CH_3(CH_2)_{15}CH_2CH_2O$], 1.35 s and 1.42 s {2x3H, $[C(CH_3)]_3$}, 1.65 m [2H, $CH_3(CH_2)_{15}CH_2CH_2O$], 2.62 d (3H, $PNCH_3$, $^3J_{PH}$ 9.90 Hz), 3.46 m (2H, $NCH_2CH_2N^+$), 3.55 s [9H, $N^+(CH_3)_3$], 3.68 m (2H, $NCH_2CH_2N^+$), 3.79 m (2H, CH_2CHCH_2OP), 3.96 m [2H, $CH_3(CH_2)_{15}CH_2CH_2O$], 4.06 m (2H, CH_3CHCH_2OP), 4.29 m (1H, $CH_2CH \cdot CH_2OP$). ^{31}P NMR spectrum ($CHCl_3$), δ_P, ppm: 9.53 br.s. Found, %: C 55.81; H 9.89; P 4.69. $C_{30}H_{64}BrN_2O_5P$. Calculated, %: C 55.97; H 10.02; P 4.81.

Cholesteryl octadecyl *N*-(2-bromoethyl)-*N*-methylphosphoramidate bromomethylate XIV was prepared analogously to bromomethylate **XII** from 0.45 g of compound **X** and 0.55 g of trimethylamine in 3 ml of anhydrous benzene for 4 h at 60°C, yield 0.34 g (69%), mp 230–232°C (gets wet at 180°C), R_f 0.65 (B). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 0.66–2.45 ppm (cholesteryl H), 0.86 t [3H, $CH_3(CH_2)_{15} \cdot CH_2CH_2O$, $^3J_{HH}$ 6.60 Hz], 1.23 s [30H, $CH_3(CH_2)_{15} \cdot CH_2CH_2O$], 1.62 m [2H, $CH_3(CH_2)_{15}CH_2CH_2O$], 2.74 d (3H, $PNCH_3$, $^3J_{PH}$ 9.76 Hz), 3.46 m (2H, $NCH_2CH_2N^+$), 3.51 s [9H, $N^+(CH_3)_3$], 3.91 m [3H, $CH_3(CH_2)_{15}CH_2CH_2O$, cholesteryl OPC^3H], 4.03 m (2H, $NCH_2CH_2N^+$), 5.36 d (1H, cholesteryl C^6H, $^3J_{HH}$ 4.40 Hz). ^{31}P NMR spectrum ($CHCl_3$), δ_P, ppm: 9.15 br.s. Found, %: C 66.53; H 10.69; P 3.45. $C_{51}H_{98}BrN_2O_3P$. Calculated, %: C 66.71; H 10.76; P 3.37.

3-(2,6-Di-*tert*-butyl-4-hydroxyphenyl)propyl octadecyl *N*-(2-bromoethyl)-*N*-methylphosphoramidate bromomethylate (XV) was obtained analogously to bromomethylate **XII** from 0.37 g of compound **XI** and 0.48 g of trimethylamine in 3 ml of anhydrous benzene for 8 h at 80°C, yield 0.25 g (63%), mp 140–142°C (gets wet at 98°C), R_f 0.59 (B). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 0.87 t [3H, $CH_3 \cdot (CH_2)_{15}CH_2CH_2O$, $^3J_{HH}$ 6.65 Hz], 1.24 s [30H, $CH_3(CH_2)_{15}CH_2CH_2O$], 1.42 s [18H, $(CH_3)_3C$], 1.60 m [2H, $CH_3(CH_2)_{15}CH_2CH_2O$], 1.96 m (2H, $OCH_2CH_2CH_2$), 2.67 t (2H, $CH_2C_6H_5$, $^3J_{HH}$ 7.95 Hz), 2.77 d (3H, $PNCH_3$, $^3J_{PH}$ 10.07 Hz), 3.44 s [9H, $N^+(CH_3)_3$], 3.48 m (2H, $NCH_2CH_3N^+$), 3.65 m (2H, $NCH_2CH_2N^+$), 3.83–4.00 m [4H, $OCH_2CH_2CH_2$, $CH_3(CH_2)_{15}CH_2CH_2O$), 5.54 s (1H, OH), 6.95 s (2H, C_6H_2). ^{31}P NMR spectrum ($CHCl_3$), δ_P, ppm: 9.01 s. Found, %: C 63.54; H 9.93; P 4.28. $C_{41}H_{78}BrN_2O_4P$. Calculated, %: C 63.62; H 10.16; P 4.00.

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