Russian Journal of Organic Chemistry, Vol. 38, No. 11, 2002, pp. 1615–1619. Translated from Zhurnal Organicheskoi Khimii, Vol. 38, No. 11, 2002, pp. 1671–1675.

Synthesis of Diesteramide Phospholipids of Cationic Type

D. A. Predvoditelev, G. V. Kosarev, S. V. Suvorkin, and E. E. Nifant'ev

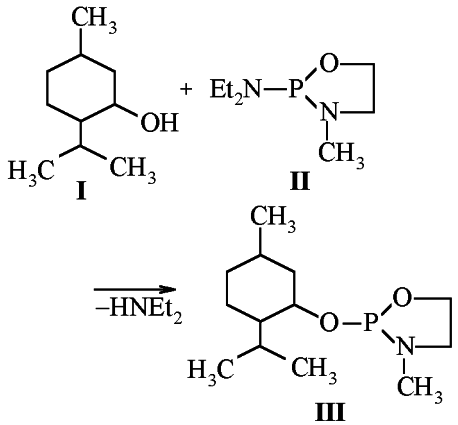
Moscow State Pedagogical University, Moscow, 119021 Russia

Received January 8, 2002

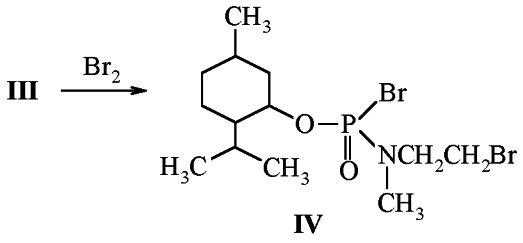
Abstract—A promising approach to synthesis of diesteramide lipid structures of cationic type proceeding from menthol cycloamidophosphites was considered. The latter easily undergo an oxidative decyclization when treated with bromine. The bromoamidophosphates thus obtained readily react with glycerol and cholesterol derivatives providing models of lipo(P → N)cholines of cationic type which are among the most important substances in the modern phospholipid chemistry.

We have demonstrated formerly that the use of bromides instead of chlorides of the acids of pentavalent phosphorus is promising for creating the phosphorus site in the molecules of glycerophospholipids and various phosphoramide analogs thereof. Therewith we have established that the synthesis of bromophosphates containing in their molecules hydrophilic fragments of widespread phospholipids can be accomplished by bromination of cyclic esters and amides of alkylene phosphites and amidophosphites [1]. Thus the common for the phospholipid chemistry phosphorylation method based on the use of traditional chlorophosphates [2] was well supplemented with the modern preparative achievements of the organophosphorus chemistry [3, 4].

Here we report on the synthesis of cationic phospholipids based on glycerol and cholesterol derivatives belonging to the class of diesteramidophosphates. We selected a menthyl bromoamidophosphate (**IV**) as a particular phosphorylating agent. This bromide was prepared in two stages. In the first stage *l*-menthol, an important natural biologically active substance, was brought into reaction with cycloamidophosphite diethylamide (**II**) to obtain previously unknown menthyl 2-hydroxyoxaazaphospholane (**III**). The menthol phosphorylation was previously performed only with acyclic amidophosphites [5–7].



The homogeneity and structure of menthyl cycloamidophosphate **III** were proved by a number of physicochemical methods, among them ^{1}H and ^{31}P NMR spectroscopy. The ^{31}P NMR spectrum of compound **III** consists of two singlets at δ_P 138.49 and 138.89 ppm due to the existence of the compound as a mixture of two diastereomers. In the ^{1}H NMR spectrum of cycloamidophosphate **III** appear the proton signals from all groups of the assumed structure with the expected integral intensity: two doublet signals (δ 0.71 and 0.84 ppm, $^{3}J_{HH}$ 6.8 Hz) belonging to the methyl protons of menthyl moiety, a multiplet in the region 1.14–2.04 ppm ($CH_3C\underline{H}$, $OCHC\underline{H}CH_2$, $CH_3C\underline{H}CH_3$ from menthyl), a doublet at δ 2.59 ppm corresponding to the methyl protons from the $PNCH_3$ group, multiplets at δ 2.94 and 4.18 ppm from methylene protons of the phospholane ring, and a multiplet at δ 3.62 ppm from the methine proton of the POCH group. In the second stage menthol cycloamidophosphate **III** was transformed into menthyl bromoamidophosphate **IV**.



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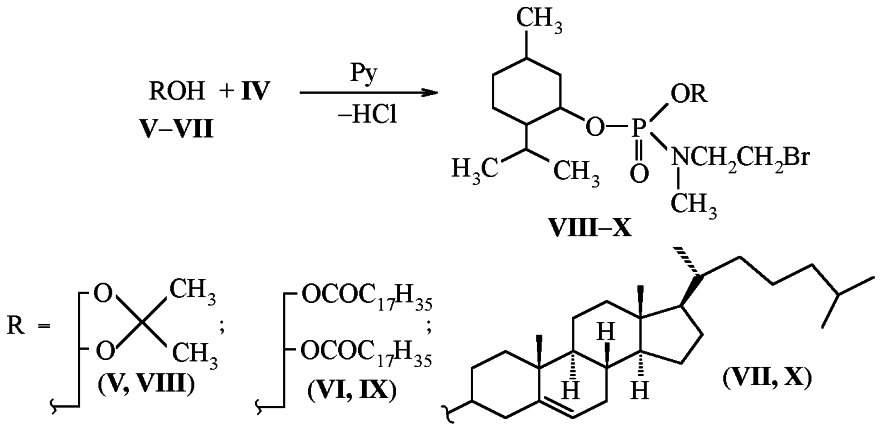


The oxidative bromination was carried out in chloroform under mild conditions (at –20°C). As shown by TLC and ^{1}H and ^{31}P NMR data bromide **IV** formed in quantitative yield. The freshly prepared bromophosphate **IV** was not subjected to further purification and was directly used after evaporation of solvent as a phosphorylating agent in reaction with 1,2-*O*-isopropylideneglycerol (**V**), 1,2-distearoyl glycerol (**VI**), and natural cholesterol **VII**.

The reaction of 1,2-*O*-isopropylideneglycerol (**V**) with bromoamidophosphate **IV** was carried out in benzene in the presence of pyridine at 60°C for 12 h. The reaction with 1,2-distearoyl glycerol (**VI**) and cholesterol (**VII**) required heating of the reaction mixture to 80°C for 30 h (here dioxane was used as solvent). After the purification by column chromatography on silica gel the yields of amidophosphate phospholipids **VIII–X** amounted respectively to 63, 58, and 49%.

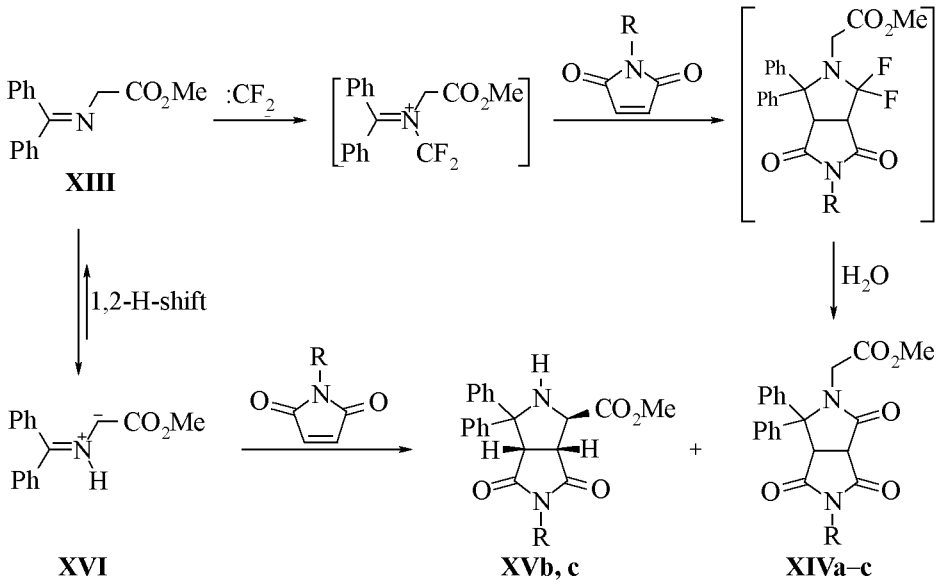
The homogeneity and structure of menthyl amidophosphates **VIII–X** were confirmed by physicochemical methods. In the ^{31}P NMR spectrum of phospholipids **VIII–X** a broad singlet at δ_P 9–10 ppm indicated that the obtained menthyl amidophosphates contained mixtures of diastereomers. We failed to register separate signals of the diastereomers in the ^{31}P NMR spectra due to insufficient resolution ability of our spectrometer.

In the ^{1}H NMR spectrum of menthyl amidophosphates **VIII–X** appear the proton signals from all groups of the assumed structures with the corresponding integral intensities. In the ^{1}H NMR spectrum of the glycerol derivative **VIII** are observed doublet signals at 0.75 and 0.84 ppm from methyl groups in menthyl moiety of the molecule, a multiplet signal in the 0.91–2.24 ppm region (CH_2CH_2, $CHCH_2CH$, CH_3CH, $OCHCHCH_2$, CH_3CHCH_3 of menthyl group), singlets at δ 1.27 and 1.35 from the methyl protons of isopropylidene group, a doublet at 2.65 ppm ($^3J_{PH}$ 9.59 Hz) belonging to $PNCH_3$ group, a multiplet at 3.30–3.36 ppm from methylene protons in the $PNCH_2CH_2Br$ group, a complex multiplet in the 3.62–4.08 ppm region from methylene and methine protons of the (CH_2CHCH_2OPOCH) group, and also a multiplet at 4.24 ppm of the methine proton attached to the β-carbon of the glycerol skeleton. The proton spectra of phosphoglycerol derivative **IX** and cholesterol phospholipid* **X** were fully consistent with the assumed structures (see EXPERIMENTAL).

In the final stage of the study the β-bromoethyleneamidophosphates **VIII–X** were converted into phospholipids with cationic groups by treatment with trimethylamine.

The reaction with trimethylamine was accomplished at 80°C within 16 h and furnished the target products **XI–XIII** in respective yields 73, 75, and 69%. The ^{31}P NMR spectra of the cationic amidophosphate lipid analogs **XI–XIII** obtained were virtually identic to those of menthyl amidophosphates **VIII–X**. Unlike the ^{1}H NMR spectra of the latter in the spectra of cationic phospholipids **XI–XIII** occurred the downfield shift of methylene protons multiplet belonging to the group $PN(CH_3)CH_2CH_2$, and the appearance of a new singlet at 3.5 ppm corresponding to the methyl protons of the cationic center.

* In the analysis of ^{1}H NMR spectra of the cholesterol-containing phospholipids **X, XIII** we took into account the detailed description and assignment of the spectral signals of protons in cholesterol published in [8].



It should be noted in conclusion that the application of bromophosphates instead of chlorophosphates as phosphorylating agents in lipid synthesis is a convenient preparative method for creating the phosphorus site and provides a possibility to obtain under relatively mild conditions complex phosphorus derivatives of lipids, in particular those containing in the structure fragments of important biologically active natural compounds.

EXPERIMENTAL

^{1}H NMR spectra of compounds **III, IV, VIII–XIII** were recorded on spectrometer Bruker WM-250 (250 MHz) from solutions in $CDCl_3$, internal reference TMS; the proton signals were assigned with the help of double resonance spectra. NMR spectra $^{31}P^-\{^1H\}$ of compounds **III, IV, VIII–XIII** were registered on spectrometer Bruker WP-80 SY from solutions in $CHCl_3$ at 32.4 MHz with an external standard (85% phosphoric acid).

For column chromatography was used a column of 15 mm diameter packed with silica gel L 100–250 μ; the R_f values were measured by TLC on Silufol UV-254 plates using as eluents benzene–dioxane, 3:1 (A), hexane–dioxane, 3:1 (B), chloroform–methanol, 3:1 (C), and methanol–water, 4:1 (D). Vizualizing of spots was done by iodine vapor and calcination at 250–300°C. The melting points were determined in sealed capillaries heating at a rate 1 deg min^{-1}.

All the syntheses with the use of trivalent phosphorus compounds were carried out under atmosphere of dry argon. The solvents applied were dried by standard procedures.

2-Diethylamino-3-methyl-1,3,2-oxaazaphospholane (**II**) was prepared by method [9] and possessed constants identical to those published.

3-Methyl-2-(3-menthyloxy)-1,3,2-oxaazaphospholane (III). A solution of a mixture of 1.55 g of l-menthol (**I**) and 1.52 g of cycloamidophosphite (**II**) in 3 ml of benzene were kept for 4 h at 80°C distilling off the forming diethylamine. Menthyl cycloamidophosphite (**III**) was isolated as individual compound by vacuum distillation at 1–10^{-4} mm Hg and bath temperature 82°C. Yield 2.25 g (87%), n_D^{20} 1.4822, R_f (elution system): 0.42 (A), 0.38 (B), 0.83 (C). ^{1}H NMR spectrum, δ, ppm: 0.71 d (3H), 0.84 d [6H, CH_3 (menthyl), $^3J_{HH}$ 6.8 Hz], 1.14–2.04 m [9H, C$\underline{H}_2$, $(CH_3)_2C\underline{H}$ (menthyl)], 2.59 d (3H, $PNCH_3$, $^3J_{PH}$ 10.4 Hz), 2.94 m (2H, $NC\underline{H}_2CH_2O$), 3.62 m [1H, POCH (menthyl)], 4.18 m (2H, $NCH_2C\underline{H}_2O$). ^{31}P NMR spectrum, δ, ppm: 138.49 s, 138.89 s. Found, %: C 59.98; H 10.12; P 11.87. $C_{13}H_{26}NO_2P$. Calculated, %: C 60.21; H 10.11; P 11.94.

***O*-(3-Menthyl)-[*N*-(2-bromoethyl-*N*-methyl]-amidobromophosphate (IV).** To a solution of 2-menthylphospholane **III** in 10 ml of chloroform at –30°C while vigorous stirring was added dropwise a solution of 1.23 g of bromine in 5 ml of chloroform. In 10 min the solvent was removed in a vacuum, and the compound **IV** obtained was kept at 1 mm Hg and 40°C for 4 h. Yield 3.23 g (100%), n_D^{20} 1.4924, R_f (elution system): 0.84 (A), 0.67 (B), 0.94 (C). ^{1}H NMR spectrum, δ, ppm: 0.74 d (3H), 0.85 d [6H,

CH_3 (menthyl), $^3J_{HH}$ 6.4 Hz], 1.25–2.04 m [9H, CH_2 and CH (menthyl)], 2.62 d (3H, $PNCH_3$, $^3J_{PH}$ 9.39 Hz), 3.35 m (4H, NCH_2CH_2Br), 3.96 m [1H, POCH (menthyl)]. ^{31}P NMR spectrum, δ, ppm: 2.99 s, 3.67 s. Found, %: C 37.31; H 6.20; P 7.44. $C_{13}H_{26}Br_2NO_2P$. Calculated, %: C 37.25; H 6.25; P 7.39.

***O*-(1,2-*O*-Isopropylidene-3-glycerol)-*O*-(3-menthyl)-[*N*-(2-bromoethyl-*N*-methyl]amidophosphate (VIII).** To a solution of 0.84 g of bromide **IV** in 4 ml of benzene was added dropwise at stirring a solution of a mixture of 0.26 g of 1,2-)-isopropylideneglycerol (**V**) and 0.16 g of freshly distilled pyridine in 1 ml of benzene. The reaction mixture was heated to 60°C for 12 h. The separated precipitate of pyridine hydrobromide was filtered off, the solvent was removed in a vacuum. Glycerophosphate **VIII** was isolated as an individual compound by chromatography on a column charged with silica gel (10 g) and filled with benzene. A mixture of benzene–dioxane, 7:1 (250 ml) was used as eluent. The solvents were removed in a vacuum, and the compound obtained was kept at 1 mm Hg and 40°C for 3 h. Yield 0.59 g (63%), n_D^{20} 1.4782, R_f (elution system): 0.47 (A), 0.32 (B), 0.87 (C). ^1H NMR spectrum, δ, ppm: 0.75 d (3H), 0.84 d [6H, CH_3 (menthyl), $^3J_{HH}$ 6.7 Hz], 0.91–2.24 m [9H, CH_2 and CH (menthyl)], 1.27 s (3H), 1.35 s [3H, $C(CH_3)_2$], 2.65 d (3H, $PNCH_3$, $^3J_{PH}$ 9.59 Hz), 3.30–3.36 m (4H, NCH_2CH_2Br), 3.62–4.08 m (5H, $C\underline{H}_2CHC\underline{H}_2OPOC\underline{H}$), 4.24 m (1H, $CH_2C\underline{H}CH_2OP$, $^3J_{HH}$ 5.6 Hz). ^{31}P NMR spectrum, δ, ppm: 9.15 br.s. Found, %: C 48.33; H 7.87; P 6.52. $C_{19}H_{37}BrNO_5P$. Calculated, %: C 48.51; H 7.93; P 6.58.

***O*-(1,2-Distearoyl-3-glycerol)-*O*-(3-menthyl)-[*N*-(2-bromoethyl-*N*-methyl]amidophosphate (IX).** Similarly to the synthesis of glyceroamidophosphate **VIII** from 0.42 g of bromide **IV**, 0.62 g of distearoylglycerol (**VI**), and 0.08 g of pyridine in 10 ml of anhydrous dioxane by heating to 80°C for 26 h was obtained compound **IX**. Glycerophosphate **IX** was isolated as an individual compound by chromatography on a column charged with silica gel (10 g) and filled with benzene. A mixture of benzene–dioxane, 8:1 (250 ml) was used as eluent. The solvents were removed in a vacuum, and the compound obtained was kept at 1 mm Hg and 40°C for 3 h. Yield 0.54 g (58%), mp 64–67°C, R_f (elution system): 0.71 (A), 0.59 (B), 0.94 (C). ^1H NMR spectrum, δ, ppm: 0.85 m [9H, CH_3 (menthyl) and 6H, $CH_2C\underline{H}_3$ (distearoylglycerol)], 1.05–2.29 m [9H, CH_2 and CH (menthyl)], 1.26 m [56H, $CH_3(C\underline{H}_2)_{14}$], 1.64 m [4H, $C\underline{H}_2CH_2C(O)$], 2.02 m [4H, $CH_2C\underline{H}_2C(O)$], 2.75 d (3H, $PNCH_3$, $^3J_{PH}$ 9.89 Hz), 3.37–3.58 m (4H, NCH_2CH_2Br), 4.10 m [1H, POCH, (menthyl)], 4.18 m (2H, $C\underline{H}_2CHCH_2OP$), 4.37 m (2H, $CH_2CHC\underline{H}_2OP$), 5.30 m (1H, $CH_2C\underline{H}CH_2OP$). ^{31}P NMR spectrum, δ, ppm: 10.28 br.s. Found, %: C 64.40; H 10.71; P 3.25. $C_{52}H_{101}BrNO_7P$. Calculated, % : C 64.84; H 10.57; P 3.22.

***O*-(3-Menthyl)-*O*-(3-cholesteryl)-[*N*-(2-bromoethyl-*N*-methyl]amidophosphate (X).** Likewise from 0.54 g of bromide **IV**,).5 g of cholesterol (**VII**), and 0.1 g of pyridine in 10 ml of anhydrous dioxane by heating to 80°C for 26 h was obtained compound **X**. Cholesteryl phosphate **X** was isolated as an individual compound by chromatography on a column charged with silica gel (10 g) and filled with benzene. A mixture of benzene–dioxane, 8:1 (250 ml) was used as eluent. The solvents were removed in a vacuum, and the oily compound obtained was kept at 1 mm Hg and 40°C for 3 h. Yield 0.46 g (48%), R_f (elution system): 0.69 (A), 0.46 (B), 0.84 (C). ^1H NMR spectrum, δ, ppm: 0.68–2.47 (H, cholesterol)*, 0.79–2.50 m [9H, CH_2 and CH (menthyl)], 2.68 d (3H, $PNCH_3$, $^3J_{PH}$ 9.52 Hz), 3.40–3.47 m (4H, $NC\underline{H}_2C\underline{H}_2Br$), 4.11–4.32 m [2H, POCH (menthyl) and C^3H (cholesterol)], 5.38 m [1H, HC^6 (cholesterol)]. ^{31}P NMR spectrum, δ, ppm: 9.81 br.s. Found, %: C 66.12; H 9.82; P 4.21. $C_{40}H_{71}BrNO_3P$. Calculated, %: C 66.28; H 9.87; P 4.27.

***O*-(1,2-*O*-Isopropylidene-3-glycerol)-*O*-(3-menthyl)-[*N*-methyl-*N*-(2-trimethylammonioethyl)]-amidophosphate bromide (XI).** A sealed ampule with solution of 0.5 g of amidophosphate **VIII** and 0.63 g of trimethylamine in 2 ml of anhydrous benzene was heated to 80°C for 6 h. The solvent was evaporated in a vacuum, and the residue was dissolved in chloroform and reprecipitated with ethyl ether. The thick oily substance was washed with ether (2 × 2 ml) and was kept in a vacuum ($1–10^{-4}$ mm Hg) over P_2O_5 at 40°C for 3 h. Yield 0.41 g (73%). R_f (elution system): 0.24 (C), 0.61 (D). ^1H NMR spectrum, δ, ppm: 0.72 d (3H), 0.83 d [6H, CH_3 (menthyl), $^3J_{HH}$ 6.8 Hz], 1.06–2.28 m [9H, CH_2 and CH (menthyl)], 1.24 s (3H), 1.32 s [3H, $[C(CH_3)_2]$, 2.64 d (3H, $PNCH_3$, $^3J_{PH}$ 9.94 Hz), 3.55 s [9H, $N^+(CH_3)_3$], 3.76 m [4H, $PN(CH_3)C\underline{H}_2C\underline{H}_2$], 3.82–4.08 m (5H, $C\underline{H}_2CHC\underline{H}_2OPOC\underline{H}$), 4.31 mm(1H,

* Here and hereinafter the region of δ 0.67–2.47 ppm characteristic of protons attached to atoms $C^{1,2}$, $C^{4,5}$ and C^{7-27} of the phosphocholesterol part of the molecule [8] will be designated as (H, cholesterol).

$CH_2C\underline{H}CH_2OP$, $^3J_{HH}$ 5.6 Hz). ^{31}P NMR spectrum, δ, ppm: 9.84 br.s. Found, %: C 49.98; H 8.67; P 5.88. $C_{22}H_{46}BrN_2O_5P$. Calculated, %: C 49.90; H 8.76; P 5.85.

***O*-(1,2-Distearoyl-3-glycerol)-*O*-(3-menthyl)[*N*-methyl-*N*-(2-trimethylammonioethyl)]amidophosphate bromide (XII)** was prepared in a similar way as compound **XI** from 0.3 g of amidophosphate **IX** and 0.17 g of trimethylamine at 80°C within 16 h. Yield 0.24 g (75%), mp 142–147°C. R_f (elution system): 0.09 (A), 0.27 (C). ^1H NMR spectrum, δ, ppm: 0.78 m [9H, CH_3 (menthyl) and 6H, $CH_2C\underline{H}_3$ (distearoylglycerol)], 0.89–2.32 m [9H, CH_2 and CH (menthyl)], 1.29 m [56H, $CH_3(C\underline{H}_2)_{14}$], 1.68 · [4H, $C\underline{H}_2CH_2C(O)$], 1.98 m [4H, $CH_2C\underline{H}_2C(O)$], 2.77 d (3H, $PNCH_3$), 3.16 s [9H, $N^+(CH_3)_3$], 3.28 m (2H, $PNCH_2C\underline{H}_2$), 3.53 m (2H, $PNC\underline{H}_2CH_2$), 4.10 m [1H, POCH (menthyl)], 4.14 m (2H, $C\underline{H}_2CHCH_2OP$), 4.32 m (2H, $CH_2CHC\underline{H}_2OP$), 5.14 m (1H, $CH_2C\underline{H}CH_2OP$). ^{31}P NMR spectrum, δ, ppm: 10.03 br.s. Found, %: C 64.52; H 10.84; P 3.05. $C_{55}H_{110}BrN_2O_7P$. Calculated, %: C 64.61; H 10.84; P 3.03.

***O*-(3-Menthyl)-*O*-(3-cholesteryl)-[*N*-methyl-*N*-(2-trimethylammonioethyl)]amidophosphate bromide (XIII).** Likewise from 0.2 g of amidophosphate X and 0.16 g of trimethylamine at 80°C within 16 h was prepared compound **XIII**. Yield 0.15 g (69%), mp 136–138°C. R_f (elution system): 0.04 (A), 0.21 (C). ^1H NMR spectrum, δ, ppm: 0.68–2.47 (H, cholesterol), 0.73–2.31 m [9H, CH_2 and CH (menthyl)], 2.62 d (3H, $PNCH_3$, $^3J_{PH}$ 9.52 Hz), 3.07 s [9H, $N^+(CH_3)_3$], 3.36 m (2H, $PNCH_2C\underline{H}_2$), 3.68 m (2H, $PNC\underline{H}_2CH_2$), 3.80–4.16 m [2H, POCH (menthyl) and C^3H (cholesterol)], 5.21 m [1H, HC^6 (cholesterol)]. ^{31}P NMR spectrum, δ, ppm: 9.96 br.s. Found, %: C 65.73; H 10.22; P 3.91. $C_{43}H_{80}BrN_2O_3P$. Calculated, %: C 65.88; H 10.29; P 3.95.

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