Russian Journal of Bioorganic Chemistry, Vol. 30, No. 5, 2004, pp. 477–481. Translated from Bioorganicheskaya Khimiya, Vol. 30, No. 5, 2004, pp. 531–536.

Synthesis of Cationic Amphiphiles on the Basis of Deoxycholic Acid

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Received July 10, 2003; in final form, August 15, 2003

Abstract—Cationic derivatives of deoxycholic acid with *N*,*N*-dimethylenediamine, ε-aminocaproic acid, and pyridine as polar heads were synthesized. The cationic groups were linked to 3α- and 12α-hydroxy groups of the steroid moiety through ester or urethane bonds. Liposomal formulations of the compounds synthesized may be used for gene delivery in cells.

Key words: *cationic amphiphiles, deoxycholic acid, transfection*

INTRODUCTION

An interest in basically new technologies that allow the address delivery of new blocks of genetic information to defect cells for the subsequent expression increased in recent years. The contemporary medicine knows several hundreds of diseases that are directly connected with disturbances in gene functioning [1, 2]. Such defects could be repaired by the direct introduction into the cells of the corresponding organs and tissues of the genetic material that would specially be constructed and would provide the synthesis of missing protein. The gene therapy that is devoted to the introduction of DNA, mRNA, or oligonucleotides into organism with the aim to treat it is one of the directions of modern medicine [3, 4].

The delivery of genetic material in cell (transfection) is a necessary stage of genetic therapy. Various molecular constructs of viral and nonviral origin are used for its realization. One of the modern methods with a high therapeutic potential is the method of lipofection, which is based on the use of positively charged liposomes. It is promising, because liposomes are biodegradable and there is a minimal probability of the initiation of immune response or inflammation [5–7]. Metabolizable lipids with a minimal cytotoxicity are the most promising for solving applied problems, and the search for them is expedient to carry out among the modified natural lipids [8]. The compounds whose hydrophobic part is represented by the derivatives of steroid series are now rather attractive among the cationic lipids of various types. The steroid structure was found to significantly influence the DNA transfection efficiency [9, 10].

The further study of positively charged lipids, in particular, cholesterol and bile acid derivatives, could lead to the creation of efficient systems for the introduction in cell of various biologically active substances: nucleosides, oligo- and polynucleotides, hormones, proteins, and other natural and synthetic macromolecules with negatively charged moiety.

RESULTS AND DISCUSSION

We develop studies of the synthesis of cationic amphiphiles on the basis of cholesterol [3] and describe in this paper the synthesis of new representatives of the class of cationic lipids on the basis of deoxycholic acid (**I**). The use of polyfunctional deoxycholic acid as the hydrophobic part of cationic amphiphile allows the preparation of the compounds with several positively charged groups. Such an approach could affect the transfection efficiency, since the stability of liposome–DNA (genosome) complexes depend on the density of positive charge on the liposome surface [6, 7].

We synthesized cationic derivatives of deoxycholic acid with various modes of linkage of polar grouping to the steroid part of molecule. Octadecyl deoxycholate (**IIa**) and methyl deoxycholate (**IIb**) were used as starting compounds, which provided for the protection of carboxyl group, necessary in subsequent conversions, and helped change the total hydrophobicity of molecule, which seems to affect the transfection efficiency.

We synthesized cationic lipids (**Va**) and (**Vb**) (Scheme 1) in which nitrogen base is attached to the deoxycholate molecules with urethane bond, more stable in biological media, in order to reveal the relation between the structure and biological activity. The interaction of (**IIa**) and (**IIb**) with 1,1'-carbonyldiimidazole in dichloromethane catalyzed with triethylamine resulted in octadecyl and methyl 3α,12α-bis(1-imidazolylcarbonyloxy)-5β-cholan-24-oates (**IIIa**) and (**IIIb**) in 93–97% yields. These compounds were then treated with *N*,*N*-dimethylethylenediamine in dichlo-

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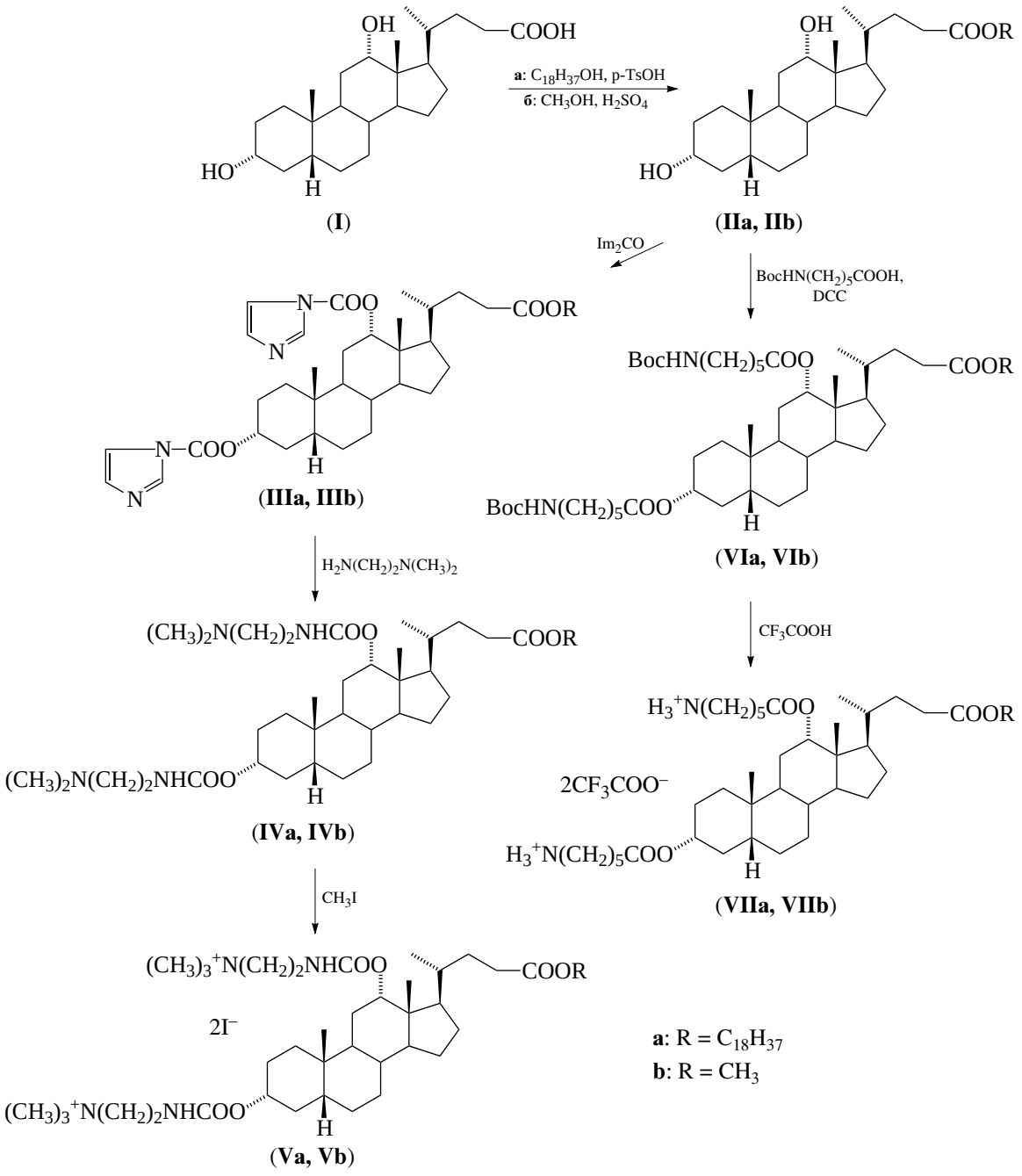
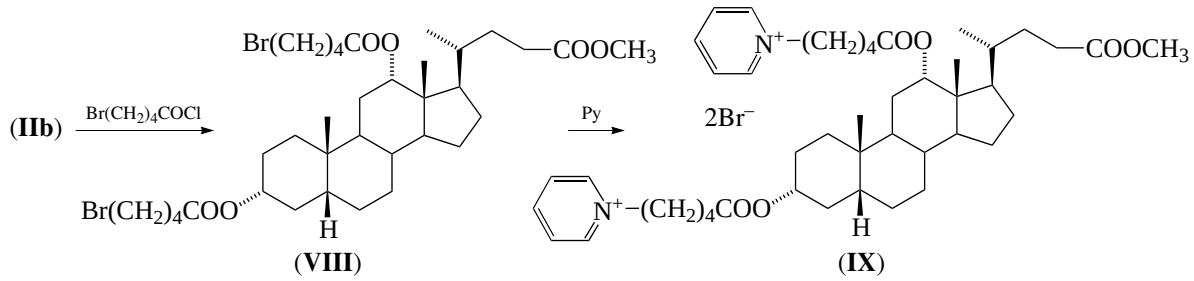


Scheme 1.

romethane, which resulted in tertiary amines (**IVa**) and (**IVb**) in 62 and 66% yields, respectively. The interaction of (**IVa**) and (**IVb**) with methyl iodide and the subsequent chromatographic purification led to octadecyl and methyl 3α,12α-bis(*N*,*N*,*N*-trimethylammonioethylcarbamoyloxy)-5β-cholan-24-oate diiodides (**Va**) and (**Vb**) in 70 and 88% yields.

The synthesis of cationic lipids (**VIIa**) and (**VIIb**) containing NH_3^+ group in their polar head was achieved by the DCC-catalyzed acylation of starting esters (**IIa**) and (**IIb**) with *N*-Boc-ε-aminocaproic acid in dichloromethane to (**VIa**) and (**VIb**) (yield 58 and 64%, respectively) and subsequent removal of Boc protective group with trifluoroacetic acid in chloroform; octadecyl and methyl 3α,12α-bis(ε-ammoniocaproyloxy)-5β-cholan-24-oate bistrifluoroacetates (**VIIa**) and (**VIIb**) were obtained in 74 and 90% yields.

In addition to the compounds with aliphatic polar groups, we synthesized a cationic lipid (**IX**) with pyri-



Scheme 2.

dinium residue (Scheme 2). To this end, a spacer group was introduced into (**IIb**) by the reaction with 5-bromovaleric acid chloride. The reaction product (**VIII**) (81% yield) was heated in anhydrous pyridine, which resulted in cationic lipid (**IX**) in 90% yield.

The homogeneity and structure of the compounds synthesized were confirmed by ^{1}H NMR and mass spectra.

EXPERIMENTAL

We used in this work the following solvents and reagents: DMSO, *N*,*N*-dimethylethylenediamine, and ε-aminocaproic acid (Sigma, United States); carbonyldiimidazole (Acros, Belgium); and triethylamine (Vekton, Russia). ^{1}H NMR spectra were registered on a pulse Fourier-transform Bruker MSL-200 spectrometer (200.13 MHz) and Bruker MSL-300 spectrometer (300.13 MHz) in deuterochloroform with tetramethylsilane as an internal standard; chemical shifts are given in ppm and spin coupling constants, *J*, in Hz. Mass spectra were obtained on a time-of-flight Finnigan MAT 900XL-TRAP mass spectrometer (San Jose, CA, United States) with electrospray ionization (ESI MS). Silufol UV-254 (Chemapol, Czech Republic) was used for TLC; substance spot were detected on chromatograms by spraying with 10% phosphomolybdic acid solution followed by heating. The following solvent systems were used for TLC: (A) 10 : 1 chloroform–methanol, (B) 7 : 1 chloroform–methanol, (C) 5 : 1 chloroform–methanol, and (D) 65 : 25 : 4 chloroform–methanol–water. Silica gel L 40/100 μm (Chemapol, Czech Republic) was used for column chromatography.

Octadecyl deoxycholate (IIa). Deoxycholic acid (**I**) (0.70 g, 1.7 mmol) was fused together with octadecanol (0.72 g, 2.6 mmol) in the presence of *p*-toluenesulfonic acid (0.37 g, 2.1 mmol) for 2 h at 100°C. The residue was chromatographed on a column eluted with chloroform to give (**IIa**); yield 0.76 g (70%); R_f 0.77 (A); ^{1}H NMR: 0.65 (3 H, s, 18-CH_3), 0.84 [3 H, t, *J* 6.8, $(CH_2)_{16}C\underline{H}_3$), 0.87 (3 H, s, 19-$CH_3$), 0.90 (3 H, d, *J* 6.7, CH_3-21), 1.05–1.89 [58 H, steroid CH and CH_2 and $COOCH_2(C\underline{H}_2)_{16}CH_3$), 2.25 (1 H, ddd, *J* 7.0, 9.3, and 15.1, H23a), 2.38 (1 H, ddd, *J* 5.2, 9.8, and 15.1, H23b), 3.62 (1 H, m, H3), 3.98 (1 H, m, H12), and 4.06 (2 H, t, *J* 6.9, $COOCH_2$).

Metyl deoxycholate (IIb). Conc. H_2SO_4 (0.09 ml) was added to a solution of sodium deoxycholate (0.50 g, 1.2 mmol) in methanol (6 ml). The reaction mixture was refluxed for 3 h, neutralized with methanolic solution of KOH to pH 6, and evaporated. The residue was dissolved in chloroform; the solution was washed with water (2 × 15 ml), dried with Na_2SO_4, and evaporated. The residue was chromatographed on a column eluted with chloroform. The yield of (**IIb**) was 0.450 g (91%); R_f 0.68 (C); ^{1}H NMR: 0.65 (3 H, s, 18-CH_3), 0.86 (3 H, s, 19-CH_3), 0.90 (3 H, d, *J* 6.9, CH_3-21), 0.99–1.89 (24 H, steroid CH and CH_2), 2.22 (1 H, ddd, *J* 6.8, 9.0, and 15.4, H23a), 2.35 (1 H, ddd, *J* 5.1, 9.8, and 15.4, H23b), 3.59 (1 H, m, H3), 3.63 (3 H, s, OCH_3), and 3.92 (1 H, m, H12).

Octadecyl 3α,12α-bis(1-imidazolylcarbonyloxy)-5β-cholan-24-oate (IIIa). A solution of (**IIa**) (0.300 g, 0.5 mmol), 1,1'-carbonyldiimidazol (0.300 g, 1.8 mmol), and triethylamine (0.19 ml) in dichloromethane (2 ml) was stirred for 3 h at 40°C and evaporated. The residue was dissolved in chloroform (15 ml), washed with 3% HCl (2 × 5 ml) and water, dried with Na_2SO_4, and chromatographed on a column eluted with 30 : 1 chloroform–methanol to give (**IIIa**); yield 0.360 g (93%); R_f 0.81 (B); ^{1}H NMR: 0.79 (3 H, s, 18-CH_3), 0.84 (3 H, t, *J* 6.7, CH_3-21), 0.87 [3 H, t, *J* 6.8, $(CH_2)_{16}C\underline{H}_3$), 0.90 (3 H, s, 19-$CH_3$), 1.15–2.00 [58 H, steroid CH and CH_2 and $COOCH_2(C\underline{H}_2)_{16}CH_3$], 2.15 (1 H, ddd, *J* 7.3, 9.0, and 15.4, H23a), 2.27 (1 H, ddd, *J* 5.5, 9.8, and 15.4, H23b), 4.00 (2 H, t, *J* 6.9, $COOCH_2$), 4.85 (1 H, m, H3), 5.32 (1 H, m, H12), 7.05–7.12 (2 H, m, Im), 7.32–7.47 (2 H, m, Im), and 8.05–8.19 (1 H, m, Im).

Methyl 3α,12α-bis(1-imidazolylcarbonyloxy)-5β-cholan-24-oate (IIIb). A solution of (**IIb**) (0.300 g, 0.7 mmol), 1,1'-carbonyldiimidazol (0.479 g, 2.9 mmol), and triethylamine (0.2 ml) in dichloromethane (2 ml) was stirred for 5 h at 40°C and evaporated. The residue was dissolved in chloroform (2 ml), washed with 3% HCl (2 × 5 ml) and water, dried with Na_2SO_4, and chromatographed on a column eluted with 30 : 1 chloroform–methanol to give (**IIIb**); yield 0.428 g

(97%); mp 55–56°C; R_f 0.56 (A); ^{1}H NMR: 0.80 (3 H, s, 18-CH_3), 0.84 (3 H, d, *J* 6.7, 21-CH_3), 0.95 (3 H, s, 19-CH_3), 1.01–1.99 (26 H, steroid CH and CH_2), 2.22 (1 H, ddd, *J* 6.9, 9.0, and 15.4, H23a), 2.35 (1 H, ddd, *J* 5.3, 9.8, and 15.4, H23b), 3.63 (1 H, s, OCH_3), 4.83 (1 H, m, H3), 5.35 (1 H, m, H12), 7.01–7.12 (2 H, m, Im), 7.32–7.46 (2 H, m Im), and 8.05–8.19 (1 H, m, Im).

Octadecyl 3α,12α-bis(*N*,*N*-dimethylaminoethylcarbamoyloxy)-5β-cholan-24-oate (IVa). A solution of (**IIIa**) (0.4 g, 0.5 mmol) and *N*,*N*-dimethylethylenediamine (0.2 ml) in dichloromethane (3 ml) was stirred for 24 h at 15–20°C, diluted with dichloromethane (15 ml), washed with 5% KOH (2 × 8 ml) and water (2 × 15 ml), dried with Na_2SO_4, and evaporated. The residue was chromatographed on a column successively eluted with 50 : 1, 25 : 1, and 10 : 1 chloroform–methanol mixtures to give (**IVa**); yield 0.267 g (62%); R_f 0.33 (D); MS (*m*/*z*): 876.2 $[M + H]^+$; ^{1}H NMR: 0.68 (3 H, s, 18-CH_3), 0.81–2.0 (67 H, m, 19-CH_3, 21-CH_3, $COOCH_2(C\underline{H}_2)_{16}C\underline{H}_3$, and steroid CH and CH_2), 2.07–2.38 (2 H, m, H23), 2.58 [12 H, s, 2 $N(CH_3)_2$], 2.75–2.88 [4 H, m, $2C\underline{H}_2N(CH_3)_2$), 3.40–3.52 (4 H, m, $2NHC\underline{H}_2$), 4.05 (2 H, t, *J* 6.9, $COOCH_2$), 4.6 (1 H, m, H3), 4.95 (1 H, m, H12), 5.85 (1 H, m), and 6.01 (1 H, m, 2 NH).

Methyl 3α,12α-bis(*N*,*N*-dimethylaminoethylcarbamoyloxy)-5β-cholan-24-oate (IVb). A solution of (**IIIb**) (0.400 g, 0.67 mmol) and *N*,*N*-dimethylethylenediamine (0.23 ml, 2.0 mmol) in dichloromethane (2.5 ml) was stirred for 8 h at 25°C, diluted with dichloromethane (6 ml), washed with 5% KOH (2 × 8 ml), dried with Na_2SO_4, and evaporated. The residue was chromatographed on a column successively eluted with 30 : 1, 10 : 1 and 5 : 1 chloroform–methanol mixtures to give (**IVb**); yield 0.280 g (66%); R_f 0.28 (C); MS (*m*/*z*): 634.9 $[M + H]^+$; ^{1}H NMR: 0.65 (3 H, s, 18-CH_3), 0.84 (3 H, d, *J* 6.7, 21-CH_3), 0.88 (3 H, s, 19-CH*J* 6.7, CH_3-21), 0.88), 0.92–1.99 (26 H, m, steroid CH and CH_2), 2.20 [6 H, s, $N(CH_3)_2$], 2.24 [6 H, s, $N(CH_3)_2$], 2.30–2.48 [6 H, m, 23-CH_2 and $2C\underline{H}_2N(CH_3)_2$], 3.18–3.30 (4 H, m, $2NHC\underline{H}_2$), 3.64 (3 H, s, OCH_3), 4.55 (1 H, m, H3), 4.92 (1 H, m, H12), and 5.05–5.20 (1 H, m, NH).

Octadecyl 3α,12α-bis(*N*,*N*,*N*-trimethylammonioethylcarbamoyloxy)-5β-cholan-24-oate diiodide (Va). Methyl iodide (0.12 ml) was added to a solution of tertiary amine (**IVa**) (0.065 g (0.07 mmol) in DMSO (2 ml); the reaction mixture was stirred for 8 h at 70°C, diluted with chloroform (15 ml), washed with water (2 × 15 ml), dried with Na_2SO_4, and evaporated. The residue was chromatographed on a column successively eluted with10 : 1 and 5 : 1 chloroform–methanol mixtures to give quaternary iodide (**Va**); yield 0.060 g (70%); R_f 0.32 (D); ^{1}H NMR: 0.65 (3 H, s, 18-CH_3), 0.68 (3 H, d, *J* 6.7, 21-CH_3), 0.85 [3 H, t, *J* 6.8, $(CH_2)_{16}C\underline{H}_3$], 0.89 (3 H, s, 19-$CH_3$), 0.92–1.95 [56 H, m, $COOCH_2(C\underline{H}_2)_{16}CH_3$ and steroid CH and CH_2], 2.14–2.42 (2 H, m, H23), 3.44 [18 H, s, $2N^+(CH_3)_3$], 3.65–3.89 [8 H, m, $2C\underline{H}_2N(CH_3)_2$ and 2 $2NHC\underline{H}_2$], 4.01 (2 H, t, *J* 6.9, $COOCH_2$), 4.54 (1 H, m, H3), 4.92 (1 H, m, H12), 6.09 (1 H, m, NH), and 6.44 (1 H, m, NH).

Methyl 3α,12α-bis(*N*,*N*,*N*-trimethylammonioethylcarbamoyloxy)-5β-cholan-24-oate diiodide (Vb). Methyl iodide (0.08 ml, 0.5 mmol) was added to a solution of tertiary amine (**IVb**) (0.035 g (0.05 mmol) in methylethylketone (1 ml); the reaction mixture was stirred for 4 h at 60°C and evaporated. The residue was chromatographed on a column successively eluted with10 : 1 and 5 : 1 chloroform–methanol mixtures to give quaternary iodide (**Vb**); yield 0.045 g (88%); R_f 0.23 (D); MS, *m*/*z*: 649.8 $[M - CH_3]^+$; ^{1}H NMR: 0.69 (3 H, s, 18-CH_3), 0.81 (3 H, d, *J* 6.7, 21-CH_3), 0.88 (3 H, s, 19-CH_3), 0.92–2.00 (24 H, m, steroid CH and CH_2), 2.10–2.35 (2 H, m, H23), 3.48 [18 H, s, $2N^+(CH_3)_3$], 3.61 (3 H, s, OCH_3), 3.68–3.90 [8 H, m, $2NHCH_2CH_2N$), 4.52 (1 H, m, H3), 4.92 (1 H, m, H12), 6.01 (1 H, m, NH), and 6.70 (1 H, m, NH).

Octadecyl 3α,12α-bis(*N*-*tert*-butyloxycarbonylamidocapronoyloxy)-5β-cholan-24-oate (VIa). Octadecyl deoxycholate (**IIa**) (0.100 g, 0.2 mmol) was added to a solution of *N*-Boc-aminocaproic acid (0.143 g, 0.6 mmol), DCC (0.250 g, 1.2 mmol), and a catalytic amount of DMAP in dichloromethane (2 ml). The reaction mixture was stirred for 3 h at 15–20°C and evaporated. The residue was treated with ether, and the dicyclohexylurea precipitate was filtered off. The filtrate was evaporated and chromatographed on a column eluted with 60 : 1 dichloromethane–methanol mixture. The yield of (**VIa**) was 0.134 g (58%); R_f 0.39 (A); MS, *m*/*z*: 1073.9 $[M]^+$; ^{1}H NMR: 0.70 (3 H, s, 18-CH_3), 0.78 (3 H, d, *J* 6.7, CH_3-21), 0.86 [3 H, t, *J* 6.8, $(CH_2)_{16}C\underline{H}_3$), 0.88 (3 H, s, 19-$CH_3$), 1.01–2.0 [88 H, m, $COOCH_2(C\underline{H}_2)_{16}CH_3$, steroid CH and CH_2, and $2NHCH_2(C\underline{H}_2)_3CH_2CO$], 2.05–2.40 (6 H, m, $2OOCCH_2$ and H23), 3.05–3.15 (4 H, m, $2C\underline{H}_2NH$), 4.05 (2 H, t, *J* 6.9, $COOCH_2$), 4.72 (1 H, m, H3), and 5.01 (1 H, m, H12).

Methyl 3α,12α-bis(*N*-*tert*-butyloxycarbonylamidocapronoyloxy)-5β-cholan-24-oate (VIb). A solution of methyl deoxycholate (**IIb**) (0.250 g, 0.6 mmol), *N*-Boc-aminocaproic acid (0.355 g (1.5 mmol), DCC (0.475 g, 2.3 mmol) and a catalytic amount of DMAP in dichloromethane (3 ml) was stirred for 23 h at 25°C and evaporated. The residue was treated with ether, and dicyclohexylurea was filtered off. The filtrate was evaporated and chromatographed on a column successively eluted with chloroform and 30 : 1 chloroform–methanol mixture. Ester (**VIb**) was obtained; yield 0.312 g (64%); R_f 0.85 (B); MS, *m*/*z*: 874.8 $[M + K]^+$; ^{1}H NMR: 0.70 (3 H, s, 18-CH_3), 0.78 (3 H, d, *J* 6.7, 21-CH_3), 0.88

[3 H, s, 19-CH(3 H, c, CH_3-19), 0.92–2.01), 0.92–2.01 [56 H, m, 26 steroid CH and CH_2, 2($C\underline{H}_2$)$_3CH_2COO$, and 2C(CH_3)$_3$], 2.15–2.38 (6 H, m, H23 and 2$OOCCH_2$), 3.01–3.18 (4 H, m, 2$NHC\underline{H}_2$), 3.62 (3 H, c, OCH_3), 4.63 (1 H, m, H3), and 5.08 (1 H, m, H12).

Octadecyl 3α,12α-bis(ε-ammmoniocapronoyloxy)-5β-cholan-24-oate bistrifluoroacetate (VIIa). A solution of (**VIa**) (0.050 g) and trifluoroacetic acid (0.08 ml) in chloroform (0.5 ml) was stirred for 2 h at 40°C and evaporated. The residue was chromatographed on a column eluted with 30 : 1 chloroform–methanol mixture to get (**VIIa**); yield 0.035 g (90%); R_f 0.42 (D); MS, *m*/*z*: 873.9 [M – CF_3COO]$^+$; ^{1}H NMR: 0.71 (3 H, s, 18-CH_3), 0.77 (3 H, d, J 6.7, 21-CH_3), 0.86 [3 H, t, J 6.8, (CH_2)$_{16}C\underline{H}_3$], 0.88 (3 H, s, 19-CH_3), 1.01–2.51 [70 H, m, $COOCH_2$($C\underline{H}_2$)$_{16}CH_3$, steroid CH and CH_2, and 2 2$HNCH_2$($C\underline{H}_2$)$_3CH_2CO$], 2.02–2.41 (6 H, m, 2$OOCCH_2$ and H23), 2.81–3.00 (4 H, m, 2CH_2N^+), 4.05 (2 H, t, J 6.9, $COOCH_2$), 4.72 (1 H, m, H3), 5.1 (1 H, m, H12), and 7.85 (6 H, br s, 2N^+H_3).

Methyl 3α,12α-bis(ε-ammmoniocapronoyloxy)-5β-cholan-24-oate bistrifluoroacetate (VIIb). A solution of (**VIb**) (0.170 g, 0.2 mmol) and trifluoroacetic acid (0.2 ml, 2.4 mmol) in chloroform (2 ml) was stirred for 2 h at 50°Cand evaporated. The residue was chromatographed on a column successively eluted with chloroform and 30 : 1 and 10 : 1 chloroform–methanol mixtures to get (**VIIb**); yield 0.124 g (74%); R_f 0.36 (B); MS, *m*/*z*: 636.1 [M]$^+$; ^{1}H NMR: 0.68 (3 H, s, 18-CH_3), 0.76 (3 H, d, J 6.7, CH_3-21), 0.88 (3 H, s, 19-CH_3), 0.92–2.02 [38 H, m, 26 steroid CH and CH_2 and 2$HNCH_2$($C\underline{H}_2$)$_3CH_2CO$], 2.15–2.38 (6 H, m, 2$OOCCH_2$ and H23), 2.70–2.79 (4 H, m, 2CH_2N^+), 3.62 (3 H, s, OCH(3 H, c, OCH_3), 4.61 (1 H, m, H3), 5.00 (1 H, m, H12), and 7.98 (6 H, m, 2N^+H_3).

Methyl 3α,12α-bis(5-bromopentanoyloxy)-5β-cholan-24-oate (VIII). Pyridine (0.1 ml) and 5-bromoisovaleric acid chloride (0.780 g (3.9 mmol) were added to a stirred solution of (**IIb**) (0.220 g, 0.5 mmol) in chloroform (2 ml) at 0°C. The reaction mixture was kept for 1 h at 25°C and evaporated. The residue was chromatographed on a column eluted with chloroform to give (**VIII**); yield 0.322 g (81%); R_f 0.86 (B); MS, *m*/*z*: 754.9 [M + Na]; ^{1}H NMR: 0.73 (3 H, s, 18-CH_3), 0.81 (3 H, d, J 6.7, CH_3-21), 0.91 (3 H, s, 19-CH(3 H, c, CH_3-19), 1.05–2.01 [32 H, m, steroid CH and CH_2 and 2($C\underline{H}_2$)$_2CH_2Br$], 2.18–2.45 (6 H, m, 2 $OOCCH_2$ and H23), 3.44 (4 H, q, J 6.4, 2CH_2Br), 3.67 (3 H, c, OCH_3), 4.75 (1 H, m, H3), and 5.10 (1 H, m, H12).

Methyl 3α,12α-bis[5-(pyridinio)pentanoyloxy]-5β-cholan-24-oate dibromide (IX). A solution of (**VIII**) (0.110 g, 0.15 mmol) in pyridine (2 ml) was heated for 6 h at 60°C and evaporated. The residue was chromatographed on a column successively eluted with 30 : 1 and 6 : 1 chloroform–methanol mixtures to give (**IX**); yield 0.131 g (90%); R_f 0.37 (B); MS, *m*/*z*: 765.1 [M + Na]$^+$; ^{1}H NMR: 0.69 (3 H, s, 18-CH_3), 0.74 (3 H, d, J 6.7, CH_3-21), 0.87 (3 H, s, 19-CH_3), 1.05–2.65 [38 H, m, steroid CH and CH_2, 2$COOCH_2$($C\underline{H}_2$)$_3CH_2N^+$, and H23], 3.63 (3 H, s, OCH_3), 4.75 (1 H, m, H3), 4.98–5.12 (5 H, m, 2CH_2N^+ and H12), 8.03–8.19 (4 H, m), 8.40–8.55 (2 H, m), and 9.45–9.66 (4 H, m, 2$C_5H_5N^+$).

ACKNOWLEDGMENTS

This work was supported by the Russian Foundation for Basic Research, project nos. 01-03-33234, 00-15-866, and 03-03-32482, and the grants: Scientific Research of High Schools on the Priority Directions of Science and Technology, subsection Medicinal and Biologically Active Substances no. 203.05.04.005, and the President's Grant for the Support of Leading Scientific Schools of Russia no. NSh-2013.2003.3.

REFERENCES

1. Balasubramaniam, R., Bennet, M., Aberle, A., Malone, J., Nantz, M., and Malone, R., *Gene Therapy*, 1996, vol. 3, pp. 163–172.
2. Zelenin, A.V., *Vestnik RAN*, 2001, vol. 71, pp. 387–404.
3. Konstantinova, T.V., Klykov, V.N., and Serebrennikova, G.A., *Bioorg. Khim.*, 2001, vol. 27, pp. 453–456.
4. Maslov, M.A., Sycheva, E.V., Morozova, N.G., and Serebrennikova, G.A., *Izv. Ross. Acad. Nauk, Ser. Khim.*, 2000, no. 2, pp. 385–400.
5. Fujiwara, T., Hasegawa, S., Hirashima, N., Nakanishi, M., and Ohwada, T., *Biochim. Biophys. Acta*, 2000, vol. 1468, pp. 396–402.
6. Yoshimura, T., Hasegawa, S., Hirashima, N., Nakanishi, M., and Ohwada, T., *Bioorg. Med. Chem. Lett.*, 2001, vol. 11, pp. 2897–2901.
7. Ren, T., Zhang, G., Liu, D., and Liu, F., *Bioorg. Med. Chem. Lett.*, 2000, vol. 10, pp. 891–894.
8. Okayama, R., *FEBS Lett.*, 1997, vol. 408, pp. 232–234.
9. Fujiwara, T., Hirashima, N., Hasegawa, S., Nakanishi, M., and Ohwada, T., *Bioorg. Med. Chem.*, 2001, vol. 9, pp. 1013–1024.
10. Takakura, Y., Nishikawa, M., Yamashita, F., and Hashida, M., *Eur. J. Pharm. Sci.*, 2001, vol. 13, pp. 71–76.