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# Synthesis of Cationic Cholesterol Derivatives with Fragments of Aliphatic Nitrogen-Containing Bases

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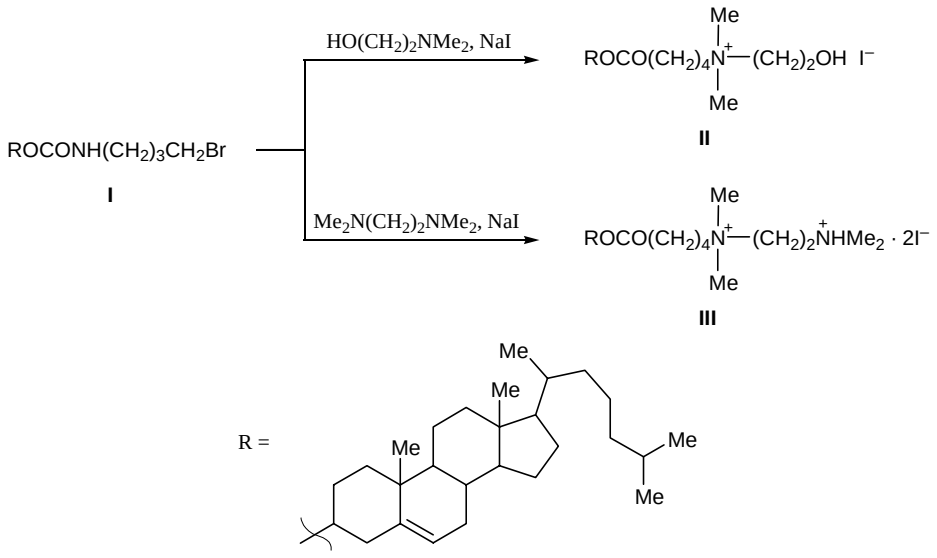
**Abstract**—Cationic cholesterol derivatives were synthesized, in which fragments of aliphatic amines (2-dimethylaminoethanol, *N*,*N*-dimethylethylenediamine, and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine) are attached to the cholesterol backbone either directly or through a spacer ester or carbamate moiety. The products may be used as efficient agents for liposome-mediated transfection.

In the recent time, new representatives of cationic amphipiles of the lipid nature are extensively studied with a view to use them in gene therapy [1, 2]. Increased interest in these compounds is explained by their high biological activity. The latest transfection methods utilize positively charged liposomes as mediators. At present, nucleic acid transfer with the aid of cationic liposomes is a well developed and widely used procedure both *in vitro* and *in vivo* due to its simplicity and accessibility [3].

From the applied viewpoint, the most interesting are liposomes containing metabolizable lipids with minimal cytotoxicity. Cationic amphiphiles having a hydrophobic cholesterol residue are known to be widely used for transfection of eucariotic cells (especially nerve cells) with a high efficiency [4, 5]. Commercial preparations were developed on the basis of cholesterol derivatives for immunotherapy of tumors [6, 7].

In continuation of our studies on the synthesis of cholesterol-based cationic amphiphiles [8], the present communication reports on new representatives of cationic lipids differing by the mode of linking of the polar group to the cholesterol fragment, the nature of nitrogen-containing base, and the number of positively charged groups. The target compounds were synthesized by reactions of cholest-5-en-3β-yl 5-bromovalerate (**I**) with 2-dimethylaminoethanol, *N*,*N*-di-

**Scheme 1.**

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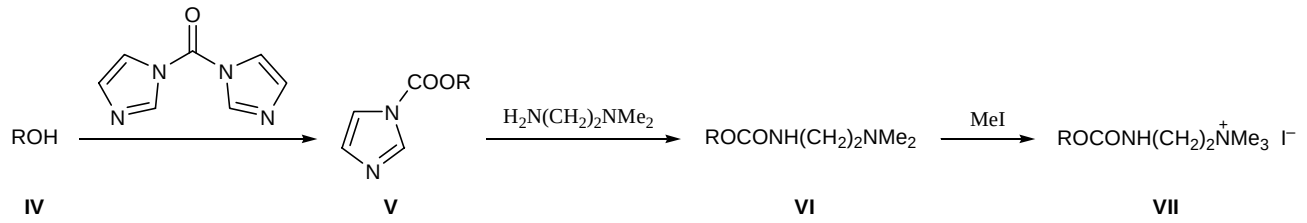
**Scheme 2.**




methylethylenediamine, and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine. The reaction of **I** with 2-dimethylaminoethanol was performed in DMSO in the presence of NaI. The use of sodium iodide instead of sodium bromide allowed us to shorten the reaction time and reduce the temperature. As a result, we obtained 4-(cholest-5-en-3β-yloxycarbonyl)butyl(2-hydroxyethyl)dimethylammonium iodide **II** in 56% yield (Scheme 1). The reaction of ester **I** with *N*,*N*,*N*′,*N*′-tetramethylethylenediamine in DMSO in the presence of NaI afforded 83% of 4-(cholest-5-en-3β-yloxycarbonyl)butyldimethyl(2-dimethylammonioethyl)-ammonium diiodide **III**. The molecule of cationic amphiphile **III** contains a quaternary ammonium group and a protonated tertiary nitrogen atom, which is confirmed by the $^1$H NMR and mass spectrometric data. In the $^1$H NMR spectrum of **III** we observed signals from the $C\mathbf{H}_2N^+CH_2$ and $C\mathbf{H}_2NH^+(CH_3)_2$ groups (δ 3.68–3.81 ppm), indicating the presence of two cationic centers. The latter factor could essentially affect its transfection efficiency, for the stability of complexes between cationic liposomes and DNA depends on the charge density on the liposome surface.

In cationic cholesterol derivatives **II** and **III**, the charged "head" is attached to the steroid fragment through a spacer ester group. With the goal of elucidating relations between the structure and biological activity we synthesized cationic lipid **VII** (Scheme 2). The ammonium residue in molecule **VII** is linked to the cholesterol fragment through a carbamate moiety which is more stable under biological conditions. The reaction of cholesterol with *N*,*N*′-carbonyldiimidazole in methylene chloride in the presence of triethylamine gave 73% of cholest-5-en-3β-yl imidazole-1-carboxylate **V**. The latter was brought into reaction with *N*,*N*-dimethylethylenediamine in methylene chloride to obtain cholest-5-en-3β-yl 2-dimethylaminoethylcarbamate (**VI**) in 89% yield. Amine **VI** was then treated with methyl iodide, and chromatographic separation of the reaction mixture afforded 78% of 2-(cholest-5-en-3β-yloxycarbonylamino)ethyltrimethylammonium iodide (**VII**). The structure of the products was confirmed by the $^1$H NMR and mass spectra.

## EXPERIMENTAL

The following reagents and solvents were used: cholesterol, DMSO, 2-dimethylaminoethanol, *N*,*N*-dimethylethylenediamine, and 5-bromovaleric acid from Fluka (Switzerland), 6-aminocapric acid from Sigma (USA), sodium iodide from Merck (Germany), triethylamine from Vekton (Russia), *N*,*N*,*N*′,*N*′-tetramethylethylenediamine from Fluka (Germany), and 1,1'-carbonyldiimidazole from Acros (Belgium). The solvents used as reaction media were distilled over $P_2O_5$. The $^1$H NMR spectra were recorded on a Bruker MSL-200 Fourier spectrometer (200 MHz) in chloroform-*d*; the chemical shifts were measured relative to trimethylsilane as internal reference. The mass spectra were obtained on a Finnigan MAT 900XL-TRAP time-of-flight mass spectrometer (San Jose, CA, USA) with electrospray ionization. Silufol UV-254 plates (Chemapol, Czechia) were used for thin-layer chromatography; spots were visualized by treatment with a 10% solution of phosphomolybdic acid, followed by calcination; solvent systems: chloroform–methanol, 6:1 (A), chloroform–methanol–water, 65:25:4 (B), chloroform–methanol, 7:1 (C). Column chromatography was performed on silica gel L 40/100 μm (Chemapol, Czechia).

**4-(Cholest-5-en-3β-yloxycarbonyl)butyl(2-hydroxyethyl)dimethylammonium iodide (II).** A solution of 0.15 g (0.3 mmol) of ester **I**, 0.2 g (0.9 mmol) of NaI, and 0.04 ml of 2-dimethylaminoethanol (50% excess) in 3 ml of DMSO was heated for 3 h at 90°C. The mixture was cooled, 20 ml of chloroform was added, and the mixture was washed with water (2×15 ml), dried over $Na_2SO_4$, and evaporated. The residue was subjected to column chromatography using chloroform–methanol (20:1) as eluent. Yield 0.103 g (56%), $R_f$ 0.57 (A). $^1$H NMR spectrum, δ, ppm: 0.68 s (3H, 18-$CH_3$), 0.86 d (6H, 26-$CH_3$, 27-$CH_3$, $J$ = 6.8 Hz), 0.92 d (3H, 21-$CH_3$, $J$ = 6.7 Hz), 1.02 s (3H, 19-$CH_3$), 1.10–2.05 m [30H, 26H in cholesterol, $N^+CH_2(C\mathbf{H}_2)_2$], 2.28 m (2H, 4-$CH_2$), 2.39 t (2H, $C\mathbf{H}_2COO$, $J$ = 6.8 Hz), 3.35 s [6H, $N^+(C\mathbf{H}_3)_2$], 3.58–3.72 m (4H, $C\mathbf{H}_2OH$, $C\mathbf{H}_2N^+CH_2$), 4.15 m (2H,

$N^+CH_2CH_2OH$), 4.5 m (1H, 3-H), 5.35 m (1H, 6-H). Mass spectrum: *m/z* 558.3 $[M - I]^+$. $C_{36}H_{64}INO_3$. Calculated: 558.8 $[M - I]^+$.

**4-(Cholest-5-en-3β-yloxycarbonyl)butyldimethyl(2-dimethylammonioethyl)ammonium diiodide (III).** A solution of 0.15 g (0.3 mmol) of ester **I**, 0.2 g (0.9 mmol) of NaI, and 0.06 ml (0.6 mmol) of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine in 3 ml of DMSO was heated for 10 h at 110°C. The mixture was cooled, diluted with 20 ml of chloroform, washed with water (2×15 ml), dried over $Na_2SO_4$, and evaporated. The residue was purified by column chromatography using chloroform–methanol (20:1) as eluent. Yield 0.150 g (83%), $R_f$ 0.62 (B). $^1$H NMR spectrum, δ, ppm: 0.68 s (3H, 18-$CH_3$), 0.86 d (6H, 26-$CH_3$, 27-$CH_3$, $J$ = 6.8 Hz), 0.92 d (3H, 21-$CH_3$, $J$ = 6.7 Hz), 1.02 s (3H, 19-$CH_3$), 1.10–2.05 m [30H, 26H in cholesterol, $N^+CH_2(CH_2)_2$], 2.28 d (2H, 4-$H_2$, $J$ = 7.0 Hz), 2.39 t (2H, $CH_2COO$, $J$ = 6.8 Hz), 2.91 s [6H, $N^+(CH_3)_2$], 3.41 s [6H, $N^+(CH_3)_2$], 3.68–3.81 m [4H, $CH_2N^+CH_2$, $CH_2NH^+(CH_3)_2$], 4.19 m (2H, $CH_2N^+CH_2$), 4.53 m (1H, 3-CH), 5.35 m (1H, 6-CH). Mass spectrum: *m/z* 585.1 $[M - I]^+$. $C_{39}H_{73}IN_2O_2$. Calculated: 585.8 $[M - I]^+$.

**Cholest-5-en-3β-yl imidazole-1-carboxylate (V).** A solution of 0.2 g (0.5 mmol) of cholesterol (**IV**), 0.13 g (0.8 mmol) of 1,1'-carbonyldiimidazole, and 0.13 ml (1.3 mmol) of triethylamine in 4 ml of methylene chloride was stirred for 3 h at 40°C. The mixture was evaporated, the residue was dissolved in 2 ml of chloroform, and the solution was washed with hydrochloric acid (2× 5 ml), dried over $Na_2SO_4$, and evaporated. The residue was purified by column chromatography using chloroform–methanol (30:1) as eluent. Yield 0.28 g (73%), $R_f$ 0.89 (C). $^1$H NMR spectrum, δ, ppm: 0.69 s (3H, $CH_3$), 0.86 d (6H, 26-$CH_3$, 27-$CH_3$, $J$ = 6.8 Hz), 0.92 d (3H, 21-$CH_3$, $J$ = 6.7 Hz), 1.10 s (3H, 19-$CH_3$), 1.11–2.06 m (26H in cholesterol), 2.50 d (2H, 4-$H_2$, $J$ = 6.7 Hz), 4.82 m (1H, 3-CH), 5.42 m (1H, 6-H), 7.05 s (1H, 5'-H), 7.41 s (1H, 4'-H), 8.11 s (1H, 2'-H).

**Cholest-5-en-3β-yl 2-dimethylaminoethylcarbamate (VI).** A solution of 0.2 g (0.4 mmol) of compound **V** and 0.070 g (0.8 mmol) of *N*,*N*-dimethylethylenediamine in 4 ml of methylene chloride was stirred for 18 h at 25°C. The mixture was cooled, diluted with 6 ml of chloroform, washed with a 5% solution of potassium hydroxide (2×8 ml), dried over $Na_2SO_4$, and evaporated. The residue was purified by column chromatography using in succession chloroform and chloroform–methanol (30:1) as eluent. Yield 0.188 g (89%), $R_f$ 0.58 (C). $^1$H NMR spectrum, δ, ppm: 0.68 s (3H, 18-$CH_3$), 0.86 d (6H, 26-$CH_3$, 27-$CH_3$ $J$ = 6.8 Hz), 0.92 d (3H, 21-$CH_3$, $J$ = 6.7 Hz), 1.02 s (3H, 19-$CH_3$), 1.02–2.01 m (26H in cholesterol), 2.31 m (2H, 4-$CH_2$), 2.81 s [6H, $N(CH_3)_2$], 3.2 t [2H, $CH_2N(CH_3)_2$, $J$ = 7.2 Hz)], 3.63 m (2H, $NHCH_2$), 4.45 m (1H, 3-H), 5.35 m (1H, 6-H). Mass spectrum: *m/z* 501.1 $[M + H]^+$. $C_{32}H_{56}N_2O_2$. Calculated: 500.6 $[M]^+$.

**2-(Cholest-5-en-3β-yloxycarbonylamino)ethyltrimethylammonium iodide (VII).** Methyl iodide, 0.15 ml (1 mmol), was added to a solution of 0.05 g (0.09 mmol) of tertiary amine **VI** in 2 ml of methylene chloride, and the mixture was stirred for 4 h at 40°C and evaporated. The residue was subjected to column chromatography using chloroform–methanol (25:1 to 10:1) as eluent. Yield 0.041 g (78%), $R_f$ 0.43 (C). $^1$H NMR spectrum, δ, ppm**:** 0.68 s (3H, 18-$CH_3$), 0.86 d (6H, 26-$CH_3$, 27-$CH_3$, $J$ = 6.8 Hz), 0.92 d (3H, 21-$CH_3$, $J$ = 6.7 Hz), 1.02 s (3H, 19-$CH_3$), 1.02–2.01 m (26H in chlolesterol), 2.29 d (2H, 4-$CH_2$, $J$ = 6.5 Hz), 3.42 s [9H, $N^+(CH_3)_3$], 3.8 m [4H, $NH(CH_2)_2N^+(CH_3)_2$], 4.45 m (1H, 3-CH), 5.35 m (1H, 6-CH), 6.08 m (1H, NH). Mass spectrum: *m/z* 515.4 $[M - I]^+$. $C_{33}H_{59}IN_2O_2$. Calculated: 515.6 $[M - I]^+$.

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