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Synthesis of Cationic Cholesterol Derivatives with Succinyl Spacer Group*

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Abstract—Syntheses of *N*,*N*,*N*-trimethyl[2-(3β-cholesteryloxy)syccinyloxyethyl]ammonium iodide, *N*,*N*-dimethyl-*N*-2-hydroxyethyl[2-(3β-cholesteryloxy)syccinyloxyethyl]ammonium iodide, and *N*-[(3β-cholesteryloxy)syccinyl]piperazine were performed. The compounds synthesized in a liposomal form may be used for delivery of genetical material into cells.

An extensive research has been carried out within the last decade on new representatives from the class of cationic amphiphiles of lipid character aiming at elucidation of the relation between their structure and their activity in biological systems [1, 2]. This class compounds attract attention because they may be used in the composition of cationic liposomes as delivery agents of genetic material to eukaryotic cells (transfection), and as antitumor and antiviral agents [3, 4].

The molecule of a cationic amphiphile may be regarded as a combination of several structural units (domains) linked to each other with a specific type of chemical bond. Three main structural domains may be quoted in the molecule of cationic lipids: hydrophobic part, positively charged group, and a moiety connecting them (spacer). The variation of these components provides versatile structures of cationic lipid amphiphiles.

Cholesterol is one among the commonly used domains for preparation of various types of cationic amphiphiles. It is known that cholesterol stabilizes the lipid bilayer, is nontoxic, and a number of cationic cholesterol derivatives have turned out to be efficient in transfection [5].

We formerly synthesized cholesterol-containing cationic amphiphiles with heterocyclic bases where the rest of 5-bromovaleric acid served as the connecting link [6].

Here we report on preparation of cationic cholesterol derivatives with nitrogen-containing aliphatic and heterocyclic bases linked to cholesterol with a succinyl group.

The reaction of cholesterol with succinic anhydride in the chloroform—DMSO mixture in the presence of pyridine and 4-*N*,*N*-dimethylaminopyridine (110°C, 3 h) afforded in 97% yield 3β-cholesteryl succinate (**I**). The latter was converted into acyl chloride **II** by treatment with thionyl chloride, and the acyl chloride without isolation was reacted with *N*,*N*-dimethylaminoethanol. The reaction furnished *N*,*N*-dimethyl-[2-(3β-cholesteryloxy)syccinyloxyethyl]amine (**III**) in 89% yield. By heating the latter with methyl iodide in DMSO (65°C, 6 h) we prepared *N*,*N*,*N*-trimethyl-[2-(3β-cholesteryloxy)syccinyloxyethyl]ammonium iodide (**IV**) in 32% yield. The reaction of the tertiary amine **III** with 2-bromoethanol was carried out in the presence of sodium iodide in acetone (65°C, 3 h) to give *N*,*N*-dimethyl-*N*-2-hydroxyethyl[2-(3β-cholesteryloxy)syccinyloxyethyl]ammonium iodide (**V**) in 57% yield (see the scheme).

Besides the cholesterol derivatives containing tertiary ammonium bases of the aliphatic series **IV, V**, we prepared also *N*-[(3β-cholesteryloxy)syccinyl]-piperazine (**VI**) from 3β-cholesteryl syccinate (**I**) and piperazine in 27% yield. We did not perform quaternization of compound **VI** because the primary, secondary, and tertiary amines with p*K* within physiological pH range were known to be capable of protonation [7]. The structure and homogeneity of all

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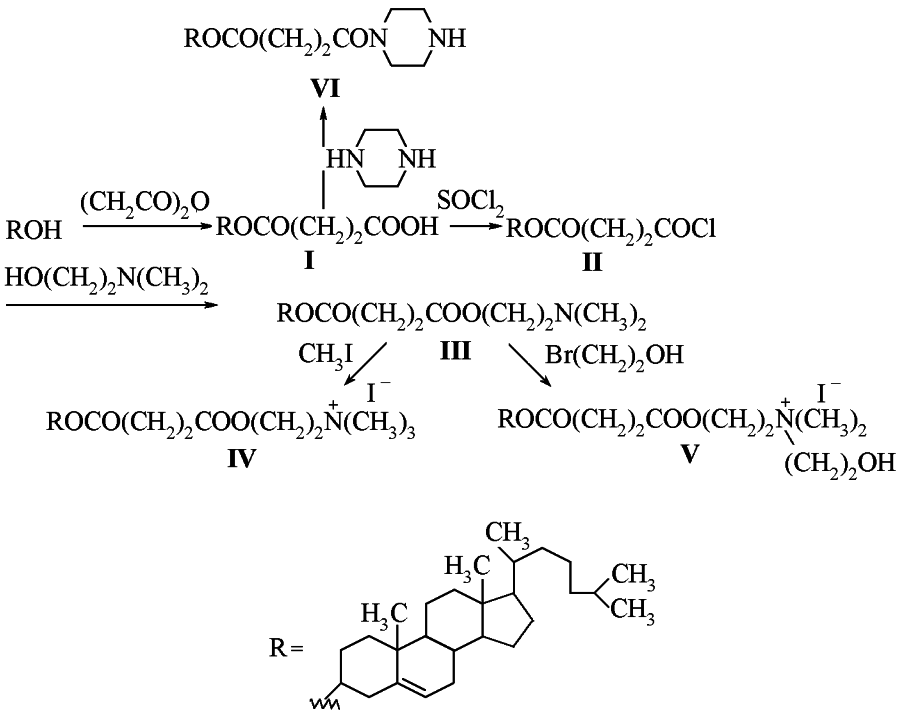
Scheme.



compounds synthesized were confirmed by TLC, ^{1}H NMR and mass spectra.

EXPERIMENTAL

In the study were used distilled solvents and reagents: cholesterol, succinic anhydride, DMSO, and also piperazine (Sigma Chemical, Germany), 2-bromoethanol (Aldrich). ^{1}H NMR spectra were registered on spectrometer Bruker MSL-200 (200 MHz) operating in a pulse mode with Fourier transform, solvent $CDCl_3$, internal reference TMS. Mass spectra were measured on a time-of-flight mass spectrometer Vision 2000 with laser-desorption ionization on a matrix. IR spectra were recorded on spectrophotometer Shimadzu UR-435 from mulls in mineral oil. The melting points were measured on a Boetius device and were listed without correction. TLC was carried out on Silufol UV-254 plates (Chemapol, Czechia), development in iodine vapor or by calcination. The following solvent systems were used for TLC: chloroform–methanol, 7:1 (A), 5:1 (B), 3:1 (C). In column chromatography silica gel L100/250 μ (Chemapol, Czechia) was used.

3β-Cholesteryl hydrosuccinate (I). To a solution of 1.9 g (5 mmol) of cholesterol, 1.5 g (10 mmol) of succinic anhydride, 0.10 g (1 mmol) of 4-*N*,*N*-dimethylaminopyridine in 30 ml of chloroform and 3 ml of DMSO 10 ml of pyridine was added, and the mixture was heated to 110°C for 3 h. The reaction mixture was diluted with 20 ml of chloroform, washed with water (2 × 20 ml), dried with Na_2SO_4, and evaporated. The residue was recrystallized from methanol–acetonitrile mixture (1:1). Yield 1.89 g (97%), R_f 0.71 (Å), mp 168–170°C. IR spectrum (cm^{-1}): 2830, 1730, 1700, 1460, 1380, 1180, 1020. ^{1}H NMR spectrum (ppm): 0.68 s (3H, CH_3), 0.86 d (3H, CH_3 *J* 6.8 Hz), 0.89 d (3H, CH_3 *J* 6.7 Hz,), 1.01 s (3H, CH_3), 1.03–1.61 m (21H, cholesteryl), 1.81–2.03 m (5H, cholesteryl), 2.30 br.d (2H, $OCHCH_2C{=}CH$, *J* 9 Hz), 2.61 m (4H, $OCOCH_2CH_2OCO$), 4.60 m (1H, OCOCH), 5.35 m (1H, C=CH). Mass spectrum, *m/z 486.2* [M^+]. $C_{33}H_{50}O_4$. Calculated *M* 486.7.

***N*,*N*-Dimethyl[2-(3β-cholesteryloxy)syccinyloxyethyl]amine (III).** A solution of 0.75 g (1.8 mmol) of cholesteryl hydrosuccinate (I) in 8 ml of chloroform was kept for 48 h at room temperature with 0.5 ml (20% excess) of thionyl chloride. On completion of the reaction the solution was evaporated. Acyl chloride **II** dissolved in 6 ml of chloroform with addition of 0.15 ml of pyridine was added dropwise to 0.2 ml of *N*,*N*-dimethylaminoethanol (50% excess), and the mixture was left standing at room temperature for 24 h. Afterwards the reaction mixture was diluted with 25 ml of chloroform, washed with

water (2 × 25 ml), and dried with Na_2SO_4. The solvent was evaporated, the residue was subjected to chromatography (eluent chloroform–methanol, 25 : 1). Yield 0.74 g (89%), R_f 0.64 (B), mp 42–43°C. ^{1}H NMR spectrum, δ, ppm: 0.68 s (3H, CH_3), 0.86 d (3H, CH_3, *J* 6.8 Hz), 0.89 d (3H, CH_3, *J* 6.7 Hz), 1.01 s (3H, CH_3), 1.03–1.61 m (21H, cholesteryl), 1.81–2.03 m (5H, cholesteryl), 2.20–2.30 m (8H, $OCHCH_2C{=}CH$, $2NCH_3$), 2.66 m (4H, $OCOCH_2CH_2OCO$), 4.56 m (1H, OCOCH), 5.31 m (1H, C=CH). Mass spectrum, *m/z*: 558.4 M^+. $C_{35}H_{59}NO_4$. Calculated: *M* 557.8.

***N,N,N*-trimethyl[2-(3β-cholesteryloxy)syccinyloxyethyl]ammonium iodide (IV).** A solution of 0.080 g (0.1 mmol) of tertiary amine **III** in 3 ml of DMSO was heated for 6 h to 65°C with 0.3 ml (0.4 mmol) of methyl iodide. The reaction mixture was diluted with 30 ml of chloroform, washed with water (2 × 10 ml), and dried with Na_2SO_4. The solvent was evaporated, the residue was subjected to chromatography (eluent chloroform–methanol, 25 : 1). Yield 0.031 g (32%), R_f 0.34 (Å). ^{1}H NMR spectrum, δ, ppm: 0.68 s (3H, CH_3), 0.86 d (3H, CH_3, *J* 6.8 Hz), 0.89 d (3H, CH_3, *J* 6.7 Hz), 1.01 s (3H, CH_3), 1.03–1.61 m (21H, cholesteryl), 1.81–2.03 m (5H, cholesteryl), 2.30 m (2H, $OCHCH_2C{=}CH$), 2.66 m (4H, $OCOCH_2CH_2OCO$), 3.45 s [9H, $^+N(CH_3)_3$], 3.49 m (2H, OCOCH2CH2N$^+$), 3.92 m (2H, CH_2N^+), 4.58 m (1H, OCOCH), 5.35 m (1H, C=CH). Mass spectrum, *m/z*: 572.1 $[M–I]^+$. $C_{37}H_{62}INO_4$. Calculated: *M* 572.8.

***N,N*-Dimethyl-*N*-2-hydroxyethyl[2-(3β-cholesteryloxy)syccinyloxyethyl]ammonium iodide (V).** A solution of 0.180 g (0.25 mmol) of tertiary amine **III** in 9 ml of acetone was heated to 65°C for 3 h with 0.2 ml (0.6 mmol) of 2-bromoethanol and 0.60 g of sodium iodide. The residue after removal of the solvent was subjected to chromatography eluting in succession with chloroform and chloroform–methanol mixture (25 : 1). Yield 0.135 g (57%), R_f 0.52 (C). ^{1}H NMR spectrum, δ, ppm: 0.68 s (3H, CH_3), 0.86 d (3H, CH_3, *J* 6.8 Hz), 0.89 d (3H, CH_3, *J* 6.7 Hz), 1.01 s (3H, CH_3), 1.03–1.61 m (21H, cholesteryl), 1.81–2.03 m (5H, cholesteryl), 2.30 m (2H, $OCHCH_2C{=}CH$), 2.66 m (4H, $OCOCH_2CH_2OCO$), 3.35 s [6H, $N^+(CH_3)_2$], 3.78 m [2H, $CH_2N^+(CH_3)_2$], 4.00–4.34 m [4H, $HOCH_2CH_2N^+(CH_3)_2$], 4.58 m (1H, OCOCH), 5.35 m (1H, C=CH). Mass spectrum, *m/z*: 602.5 $[M–I]^+$. $C_{37}H_{64}IINO_5$. Calculated: 602.9.

***N*-[(3β-Cholesteryloxy)syccinyl]piperazine (VI).** A solution of 0.45 g (1 mmol) of cholesteryl hydrosuccinate (**I**), 0.18 g (2 mmol) of piperazine, 1 g of dicyclohexylcarbodiimide in a mixture of 11 ml acetonitrile and 8 ml of chloroform was heated to 35°C for 2 h. The residue of dicyclohexylurea was filtered off and washed with acetonitrile. The solution was evaporated, the residue was subjected to chromatography (eluent dichloromethane–methanol, 30 : 1). Yield 0.130 g (27%), R_f 0.43 (C). ^{1}H NMR spectrum, δ, ppm: 0.68 s (3H, CH_3), 0.86 d (3H, CH_3, *J* 6.8 Hz), 0.89 d (3H, CH_3, *J* 6.8 Hz), 1.01 s (3H, CH_3), 1.03– 1.61 m (21H, cholesteryl), 1.81–2.03 m (5H, cholesteryl), 2.25 br.d (2H, $OCHCH_2C{=}CH$, *J* 9 Hz), 2.58 br.s (4H, $OCOCH_2CH_2OCO$), 2.81 m (4H, $2CH_2NH$), 3.49 m (4H, $2CH_2NCO$), 4.55 m (1H, OCOCH), 5.30 m (1H, C=CH). Mass spectrum, *m/z*: 577.3 $[M+Na]^+$.

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