Synthesis of polycationic lipids based on cholesterol and spermine

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The synthesis of cholesterol-based cationic lipids in which the hydrophobic steroidal fragment is linked to a spermine residue as the hydrophilic domain by ester or carbamate linkage is described.

Key words: cholesterol, spermine, cationic lipids

In gene therapy, any curative action is achieved through introduction, into the organism, of DNA, mRNA, or oligonucleotides that reach the cells by special transport systems.[1] Cationic lipids and liposomes based on them are attractive systems for the delivery owing to their biodegradability and low probability of eliciting immune and inflammatory reactions.[2—5] A cationic lipid molecule is a combination of two structural domains, hydrophobic and hydrophilic ones, which are connected by linkers. Long-chain hydrocarbons, steroids, and diglycerides are used as the hydrophobic domains. The hydrophilic domain can contain one (monocationic lipids) or more (polycationic lipids) positively charged groups. Monocationic lipids are most often tertiary or quaternary derivatives of aliphatic or heterocyclic nitrogen bases. In polycationic lipids, natural or synthetic polyamines or amino acids are used as the hydrophilic domains.[3,6,7] The type of linking of the hydrophobic and hydrophilic domains determines the stability and toxicity of cationic amphiphiles in biological systems.[3,8] Stable lipids with ether linkage are more toxic to cells than acyl lipids, which are readily hydrolyzed in the cell by endogenous esterases. A carbamoyl linkage provides a more favorable compromise between the amphiphile stability and toxicity.

Natural polyamines, spermine and spermidine, play an important role in the vital activity of cells.[9] Polyamines can pack DNA to toroidal and rod-like structures[10] and the methylene fragments separating the nitrogen atoms play an important role in the interaction of polyamine with the DNA double helix.[11,12] The structure-functional studies have shown that lipophilic derivatives of polyamines condense DNA more efficiently than natural polyamines[13,14] and can be used as non-viral systems for the delivery of genetic material. Among lipophilic polyamines, spermine derivatives bind and condense DNA most efficiently[15,16] and, therefore, they better transport it to the cells. However, some researchers note that amphiphiles based on synthetic polyamines can deliver DNA to eukaryotic cells more efficiently than lipophilic derivatives of spermine.[17,18] On the basis of spermine, commercial products for transfection were developed.[19—22]

A number of approaches to the preparation of polycationic amphiphiles whose hydrophobic parts are bound to the polyamine by spacers of different length have been reported. Most of the methods include initial introduction of the spacer group to the polyamine matrix and subsequent linking of the obtained fragment to the activated hydrophobic component.[17,23—26] However, the overall yields of the target compounds in these syntheses are 14—25% (see Refs 17, 23, 27). A solid-state method was proposed[15,27] for the synthesis of cholesterol polycationic amphiphiles, which increased the yield to 87%.

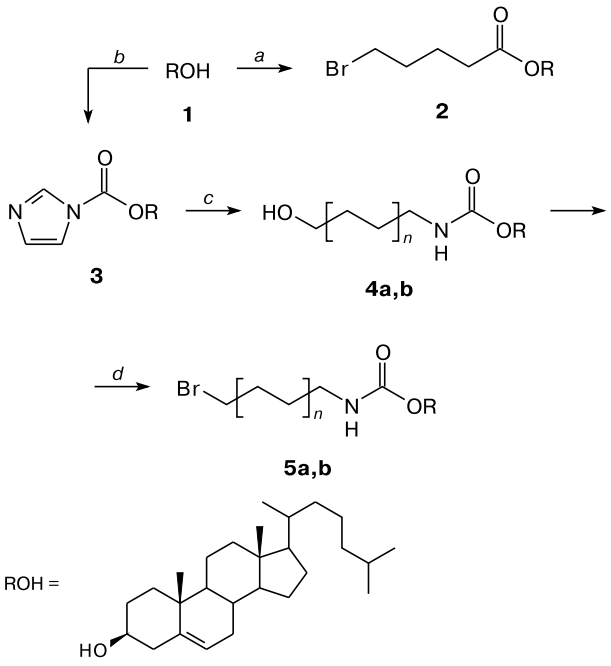
This communication presents a new method for the synthesis of unsymmetrical and symmetrical polycationic amphiphiles based on molecular cell components: cholesterol and spermine. The effect of the amphiphile structure on the cytotoxicity and DNA transfer properties was studied by varying the length of the spacer group separating spermine and cholesterol and the type of linkage (ester and carbamate linkages). Our synthetic scheme was based on the Fukuyama reaction: N-alkylation of 2-nitrobenzenesulfonamides derived from primary amines with alkyl halides and subsequent removal of 2-nitrobenzenesulfonyl group to give secondary amines.[28,29]

For implementing this approach, in the first stage we prepared bromo derivatives of cholesterol. Treatment of cholesterol (**1**) with an excess of 5-bromopentanoyl chloride gave cholesterol brominated derivative **2** with an ester bond (Scheme 1). The yield of bromide **2** was 96%. The structure of compound **2** was supported by ^{1}H NMR spectroscopy data, in particular, the presence of a triplet for the CH_2Br group at δ 3.40 and spin-spin coupling constant of 6.7 Hz.

Compounds with carbamoyl group were obtained by treatment of cholesterol (**1**) with *N*,*N*′-carbonyldiimid-

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 1, pp. 254—261, January, 2010.

Scheme 1



n = 1 (**a**), 2 (**b**)

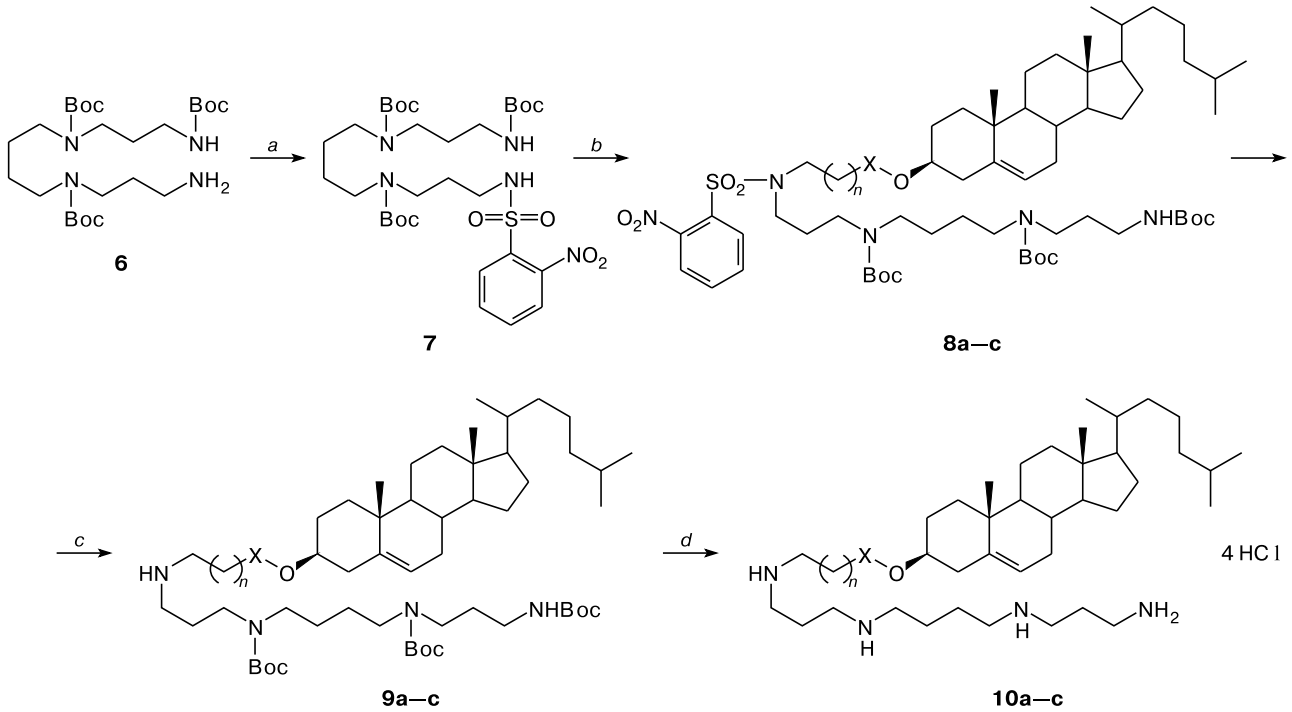
Reagents and conditions: (*a*) $Br(CH_2)_4COCl$, Py, 24 °C; (*b*) CDI, Et_3N, 40 °C; (*c*) $HO(CH_2)_mNH_2$, m = 4, 6, 40 °C; (*d*) CBr_4, Ph_3P, 24 °C.

azole (CDI) to give imidazolide **3** (see Scheme 1), which was then made to react with amino alcohols to give hydroxy derivatives **4a** and **4b**. The hydroxy group in alcohols **4a**,**b** was replaced by bromine by treatment of **4a**,**b** with CBr_4 in the presence of Ph_3P; this gave bromides **5a**,**b** in 72 and 75% yields (over three steps).

The reaction of Boc-protected derivative **6**, obtained from spermine by regioselective protection/deprotection,[24] with 2-nitrobenzenesulfonyl chloride yielded derivative **7**, which was isolated in 88% yield by column chromatography on silica gel (Scheme 2). The structure of compound **7** was confirmed by NMR spectra exhibiting proton (δ_H 7.59—8.11) and carbon (δ_C 125.25—148.29) signals of the aromatic ring.

The key step of the synthesis of unsymmetrical polycationic lipids **10a—c** was the Fukuyama N-alkylation of substituted sulfonamide **7** with cholesterol brominated derivatives **2**, **5a**,**b**. Compounds **8a—c** were isolated in 86—94% yields and characterized by mass spectrometry and ^1H and ^{13}C NMR spectroscopy data. The ^1H NMR spectra show the set of proton signals for the polyamine and cholesterol components. In the final step, the amino groups in lipoconjugates **8a—c** were deprotected. First, the 2-nitrobenzenesulfonyl group was removed by treatment with benzenethiol in the presence of K_2CO_3. The removal of the *tert*-butoxycarbonyl protection was accomplished by treating compounds **9a—c** with 4 *M* HCl in

Scheme 2



X = CO (**a**), NHCO (**b**, **c**); n = 3 (**a**, **b**), 5 (**c**)

Reagents and conditions: (*a*) 2-$NO_2C_6H_4SO_2Cl$, Et_3N, 24 °C; (*b*) **2** or **5a**,**b**, Cs_2CO_3, DMF, 60 °C; (*c*) PhSH, K_2CO_3, 24 °C; (*d*) 4 *M* HCl—dioxane, 24 °C.

dioxane for 2 h. The unsymmetrical lipophilic polyamines **10a—c** were obtained in 89, 86, and 79% yields (over two steps), respectively. The structures of the products were confirmed by data from NMR spectroscopy and mass spectrometry.

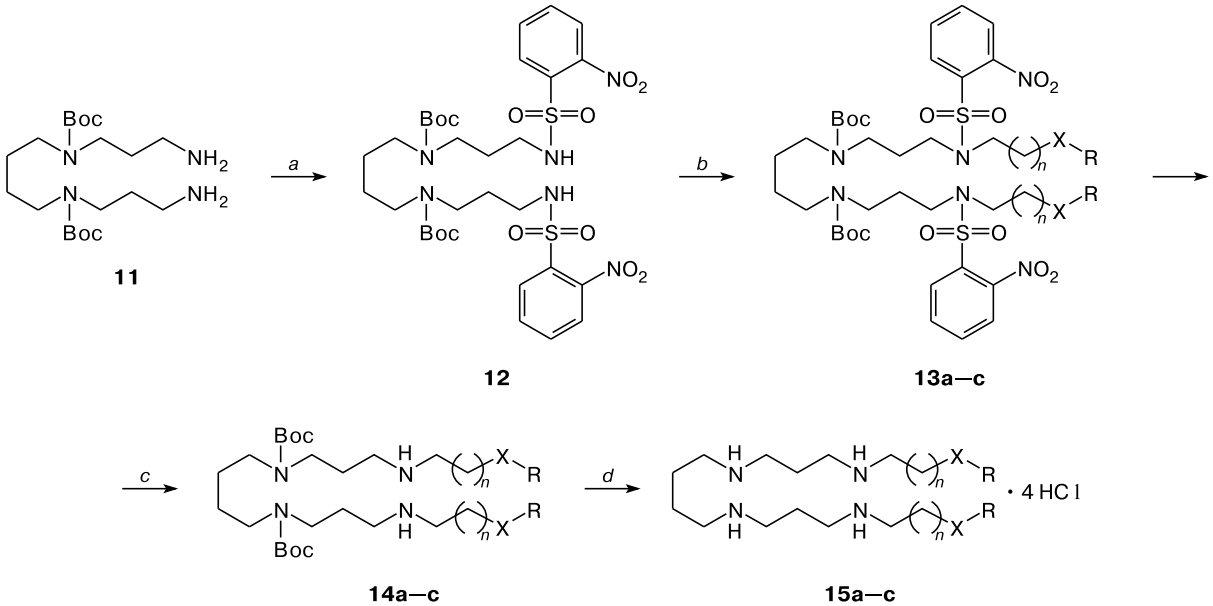
In order to find new transfection agents, we synthesized symmetrical cationic polyamines with different spacer lengths between the hydrophobic and polyamine parts and different types of spacer binding to the hydrophobic domain (Scheme 3).

Di-Boc-protected spermine **11** was prepared using a known approach.[30] Spermine was treated with a twofold excess of ethyl trifluoroacetate to protect the primary amino groups. The secondary amino groups were protected by reaction with di-*tert*-butyl dicarbonate to afford a fully protected spermine derivative. The removal of the trifluoroacetyl groups on treatment with NaOH furnished partially protected polyamine **11** containing two free primary amino groups. N-Sulfonylation of this product with an excess of 2-nitrobenzenesulfonyl chloride in the presence of Et_3N gave diamide **12**, which was isolated in 63% yield. The structure of compound **12** was confirmed by NMR spectroscopy. The signals of aromatic protons (δ_H 7.59—8.11) appearing in the ^1H NMR spectrum and a downfield shift of the methylene proton signals of the CH_2NH group (δ_H 2.81—3.15 for **11**, δ_H 3.10—3.38 for **12**) attest to the presence of benzenesulfonyl groups. The condensation of cholesterol brominated derivatives **2**, **5a**,**b** with sulfonamide **12** was carried out according to the Fukuyama reaction, the yields of derivatives **13a—c** were 51—60%. The final step of the synthesis was deprotection of amino groups as in the case of compounds **10a—c**. First, the 2-nitrobenzenesulfonyl (compounds **14a—c**) and then *tert*-butoxycarbonyl protective groups were removed to give the target symmetric lipopolyamines **15a—c** in 54, 76, and 67% yields over two steps.

Cationic lipids **15a—c** can be referred to the class of gemini surfactants, *i.e.*, compounds in which two hydrophobic domains are symmetrically linked through rigid or flexible spacers with a hydrophilic cationic domain. They have a very high surface activity, which makes them interesting for biological and biomedical studies. During the last several years, about 250 gemini-surfactants have been synthesized most of which showed medium or high level of transfection activity in standard tests *in vitro*.[31]

Thus, we prepared unsymmetrical and symmetrical polycationic amphiphiles based on cholesterol and spermine differing in the mode of attachment of the spacer to the hydrophobic domain (ester and carbamate bonds) and in the spacer length (4 and 6 methylene units).

Scheme 3



Experimental

Distilled solvents, Russian (Khimmed) and foreign (Merck, Fluka, Aldrich, Acros) commercial reagents were used. CH_2Cl_2 and Et_3N were refluxed with CaH_2 and distilled prior to the reaction, DMF was kept over calcined molecular sieves 4 Å. N^1,N^4,N^9-Tri-*tert*-butoxycarbonyl-1,12-diamino-4,9-diazadodecane (**6**) and N^4,N^9-di-*tert*-butoxycarbonyl-1,12-diamino-4,9-diazadodecane (**11**) were prepared by known procedures.[24,30]

Thin-layer chromatography was carried out on Kieselgel 60 F_{254} plates (Merck) in solvent systems petroleum ether—EtOAc, 4 : 1 (A); $CHCl_3$—MeOH, 40 : 1 (B); 36 : 1 (C); 25 : 1 (D); 20 : 1 (E); 13 : 1 (F); $CHCl_3$—MeOH—Pr^iNH_2, 4 : 1 : 2 (G), 3 : 1 : 2 (H). The compounds were detected by treatment with chlorine followed by visualization using a benzidine solution,[32] the Dragendorff reagent,[32] phosphomolybdic acid—cerium(IV) sulfate system followed by heating,[33] or UV light (254 nm). Column chromatography was carried out on silica gel Kieselgel 60 (0.040—0.063 mm, Merck). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance DPX-300 pulse Fourier transform spectrometer (Germany) in $CDCl_3$ unless otherwise stated (internal $SiMe_4$). Mass spectra were run on a Bruker Ultraflex time-of-flight mass spectrometer (Germany) with laser desorption ionization using 2,5-dihydroxybenzoic and cyanohydroxycinnamic acids as the matrices. For compounds **9a**,**c**, **10a**—**c**, and **15b**,**c**, the mass spectra were measured on an Agilent 1100 GC/MS spectrometer (Agilent Technologies, USA) by chemical ionization under atmospheric pressure. IR spectra were recorded on a Shimadzu IR-435 spectrometer (Japan). The melting points were determined on a Boetius hot stage (Germany). Compounds **10a**—**c** и **15a**—**c** decompose without melting above 270 °C.

(5-Bromopentanoyl)cholesterol (2). Pyridine (3 mL) and then a solution of 5-bromopentanoyl chloride (2.25 g, 11.3 mmol) in CH_2Cl_2 (2.5 mL) were added to a cooled (0 °C) solution of cholesterol (**1**) (2.18 g, 5.6 mmol) in anhydrous CH_2Cl_2 (15 mL). The reaction mixture was stirred for 1 h at 24 °C, washed with 10% HCl (3×5 mL) and water (5 mL), and dried with Na_2SO_4, and the solvent was evaporated. The residue was chromatographed on a column with silica gel using a $CHCl_3$—MeOH mixture (40 : 1) as the eluent to give compound **2** as white crystals (2.955 g, 96%), R_f 0.48 (A), m.p. 112—114 °C. Found (%): C, 70.30; H, 10.00. $C_{32}H_{53}BrO_2$. Calculated (%): C, 69.92; H, 9.72; Br, 14.54; O, 5.82. IR, ν/cm^{-1}: 2830, 1736, 1460, 1370, 1240, 670. ^{1}H NMR, δ: 0.68 (s, 3 H, C(13)Me); 0.79, 0.80 (both d, 3 H each, C(25)Me, J = 6.5 Hz); 0.86 (d, 3 H, C(20)Me, J = 6.5 Hz); 1.02 (s, 3 H, C(10)Me); 0.88—1.65 (m, 23 H, Chol, OC(O)CH$_2$(C$\underline{H}_2$)$_2$); 1.68—2.00 (m, 7 H, Chol); 2.13—2.34 (m, 2 H, H_2C(4)), 3.06—3.19 (m, 2 H, OC(O)CH$_2$); 5.27—5.34 (m, 1 H, H(6)).

(Cholest-5-en-3β-yl) imidazole-1-carboxylate (3). *N*,*N*′-Carbonyldiimidazole (1.678 g, 10.35 mmol) and triethylamine (2 mL, 3.74 mmol) were added to a solution of cholesterol (**1**) (4.00 g, 10.35 mmol) in anhydrous CH_2Cl_2 (20 mL). The reaction mixture was stirred under reflux for 16 h, washed with 10% HCl (3×5 mL) and water (5 mL), and dried with Na_2SO_4, and the solvent was evaporated to give compound **3** as white crystals (4.904 g, 99%), R_f 0.7 (B), m.p. 124—126 °C. Found (%): C, 76.26; H, 10.40; N, 5.56. 2 $C_{35}H_{61}NO_3 \cdot H_2O$. Calculated (%): C, 76.03; H, 10.08; N, 5.72; O, 8.17. IR, ν/cm^{-1}: 2840, 1730, 1640, 1520, 1460, 1380. ^{1}H NMR, δ: 0.61 (s, 3 H, C(13)Me); 0.79 (d, 3 H, C(25)Me, J = 6.5 Hz); 0.80 (d, 3 H, C(25)Me, J = 6.5 Hz); 0.84 (d, 3 H, C(20)Me, J = 6.5 Hz); 0.99 (s, 3 H, C(10)Me); 0.90—1.58 (m, 19 H, Chol); 1.58—2.02 (m, 7 H, Chol); 2.38—2.47 (m, 2 H, H_2C(4)); 4.69—4.83 (m, 1 H, H(3)); 5.34—5.40 (m, 1 H, H(6)); 7.00, 7.36, 8.09 (all s, 1 H each, Im). ^{13}C NMR, δ: 11.88, 18.88, 19.35, 21.21, 22.63, 22.87, 23.98, 24.44, 27.81, 28.19, 28.38, 31.98, 35.96, 36.33, 36.71, 36.92, 38.03, 39.67, 39.83, 42.47, 50.11, 56.27, 56.81, 79.20, 117.44, 123.95, 130.05, 137.14, 138.74, 148.05.

(Cholest-5-en-3β-yl) *N*-(4-hydroxybutyl)carbamate (4a). 4-Aminobutan-1-ol (0.287 g, 2.78 mmol) was added to a solution of compound **3** (1.00 g, 2.08 mmol) in anhydrous CH_2Cl_2 (10 mL). The reaction mixture was stirred under reflux for 18 h, washed with 3% HCl (8 mL) and water (5×5 mL), and the organic solvent was evaporated. The residue was chromatographed on a column with silica gel using a $CHCl_3$—MeOH mixture (30 : 1) as the eluent to give compound **4a** as white crystals (0.895 g, 87%), R_f 0.45 (E), m.p. 154—156 °C. MS, m/z (I_{rel} (%)): 524.314 [M + Na]$^+$ (100). Calculated for $C_{32}H_{56}NO_3$: 501.418 [M]$^+$. ^{1}H NMR, δ: 0.61 (s, 3 H, C(13)Me); 0.79 (d, 3 H, C(25)Me, J = 6.5 Hz); 0.80 (d, 3 H, C(25)Me, J = 6.5 Hz); 0.85 (d, 3 H, C(20)Me, J = 6.5 Hz); 0.94 (s, 3 H, C(10)Me); 0.98—1.57 (m, 23 H, Chol, NHCH$_2$(C$\underline{H}_2$)$_2$); 1.68—2.02 (m, 7 H, Chol); 2.14—2.38 (m, 2 H, H_2C(4)); 3.05—3.20 (m, 2 H, CH$_2$N); 3.59 (t, 2 H, C$\underline{H}_2$OH, J = 5.9 Hz); 4.36—4.48 (m, 1 H, H(3)); 4.48—4.62 (m, 1 H, NH); 5.27—5.35 (m, 1 H, H(6)). ^{13}C NMR, δ: 12.10, 18.99, 19.56, 21.14, 22.79, 23.07, 23.93, 24.38, 26.54, 28.12, 28.26, 29.64, 31.96, 35.91, 36.27, 36.65, 37.06, 38.65, 39.61, 39.82, 40.70, 42.40, 49.94, 56.21, 56.77, 62.10, 74.57, 122.63, 139.89, 156.72.

(Cholest-5-en-3β-yl) *N*-(6-hydroxyhexyl)carbamate (4b) was prepared as compound **4a** from compound **3** (1.00 g, 2.08 mmol) and 6-aminohexan-1-ol (0.366 g, 3.12 mmol). Chromatography on silica gel using $CHCl_3$—MeOH (50 : 1) gave compound **4b** as white crystals (0.992 g, 90%), R_f 0.48 (E), m.p. 186—188 °C. Found (%): C, 76.96; H, 11.22; N, 2.57. $C_{35}H_{61}NO_3$. Calculated (%): C, 77.29; H, 11.30; N, 2.58. ^{1}H NMR, δ: 0.61 (s, 3 H, C(13)Me); 0.79 (d, 3 H, C(25)Me, J = 6.5 Hz); 0.80 (d, 3 H, C(25)Me, J = 6.5 Hz); 0.85 (d, 3 H, C(20)Me, J = 6.5 Hz); 0.94 (s, 3 H, C(10)Me); 0.98—1.57 (m, 27 H, Chol, NHCH$_2$(C$\underline{H}_2$)$_4$); 1.68—2.00 (m, 7 H, Chol); 2.13—2.35 (m, 2 H, H_2C(4)); 3.03—3.16 (m, 2 H, CH$_2$N); 3.57 (t, 2 H, C$\underline{H}_2$OH, J = 6.4 Hz); 4.34—4.49 (m, 1 H, H(3)); 5.27—5.34 (m, 1 H, H(6)). ^{13}C NMR, δ: 12.03, 18.89, 19.51, 21.21, 22.73, 22.99, 24.00, 24.46, 25.48, 26.57, 28.19, 28.35, 28.41, 30.19 , 32.06, 35.97, 36.36, 36.73, 37.17, 38.76, 39.69, 39.91, 40.87, 42.49, 50.18, 56.30, 56.86, 62.90, 74.39, 122.64, 140.03, 156.40.

(Cholest-5-en-3β-yl) *N*-(4-bromobutyl)carbamate (5a). Triphenylphosphine (0.793 g, 3.02 mmol) was added to a cooled (0 °C) solution of compound **4a** (0.843 g, 1.68 mmol) in anhydrous CH_2Cl_2 (10 mL). Then CBr_4 (1.0 g, 3.02 mmol) was added in portions and the mixture was stirred for 1 h at 24 °C. Methanol (5 mL) was added and the solvent was evaporated. The residue was chromatographed on a column with silica gel with $CHCl_3$ as the eluent to give compound **5a** as white crystals (0.400 g, 84%), R_f 0.8 (E), m.p. 92—94 °C. Found (%): C, 68.10; H, 9.90; N, 2.48. $C_{32}H_{54}BrNO_2$. Calculated (%): C, 68.06; H, 9.64; N, 2.48. ^{1}H NMR, δ: 0.61 (s, 3 H, C(13)Me); 0.79 (d, 3 H, C(25)Me, J = 6.5 Hz); 0.80 (d, 3 H, C(25)Me, J = 6.5 Hz); 0.85 (d, 3 H,

C(20)Me, J = 6.5 Hz); 0.94 (s, 3 H, C(10)Me); 0.98—1.57 (m, 23 H, Chol, $NHCH_2(C\underline{H}_2)_2$); 1.70—2.00 (m, 7 H, Chol); 2.15—2.38 (m, 2 H, $H_2C(4)$); 3.05—3.16 (m, 2 H, CH_2N); 3.35 (t, 2 H, CH_2Br, J = 6.5 Hz); 4.36—4.48 (m, 1 H, H(3)); 4.48—4.62 (m, 1 H, NH); 5.27—5.34 (m, 1 H, H(6)). ^{13}C NMR, δ: 12.17, 18.74, 19.37, 21.20, 22.87, 23.14, 24.00, 24.44, 28.18, 28.33, 28.90, 30.00, 32.02, 33.43, 35.97, 36.33, 36.71, 37.12, 38.72, 39.67, 39.88, 40.12, 42.46, 50.02, 56.27, 56.83, 74.48, 122.69, 139.93, 156.34.

(Cholest-5-en-3β-yl) *N*-(6-bromohexyl)carbamate (5b) was prepared in the same way as compound **5a** from compound **4b** (0.585 g, 1.1 mmol), Ph_3P (0.579 g, 2.2 mmol), and CBr_4 (0.945 g, 2.89 mmol). Compound **5b** was obtained as white crystals (0.548 g, 84%), R_f 0.85 (E), m.p. 106—108 °C. Found (%): C, 69.24; H, 10.15; N, 2.31. $C_{35}H_{60}BrNO_2$. Calculated (%): C, 69.28; H, 9.97; N, 2.31. ^1H NMR, δ: 0.61 (s, 3 H, C(13)Me); 0.79 (d, 3 H, C(25)Me, J = 6.5 Hz); 0.80 (d, 3 H, C(25)Me, J = 6.5 Hz); 0.85 (d, 3 H, C(20)Me, J = 6.5 Hz); 0.94 (s, 3 H, C(10)Me); 0.98—1.57 (m, 27 H, Chol, $NHCH_2(C\underline{H}_2)_4$); 1.68—1.98 (m, 7 H, Chol); 2.12—2.34 (m, 2 H, $H_2C(4)$); 3.03—3.15 (m, 2 H, CH_2N); 3.34 (t, 2 H, CH_2Br, J = 6.8 Hz); 4.35—4.48 (m, 1 H, H(3)); 4.48—4.62 (m, 1 H, NH); 5.28—5.33 (m, 1 H, H(6)). ^{13}C NMR, δ: 12.04, 18.90, 19.52, 21.23, 22.74, 23.00, 24.01, 24.47, 26.08, 27.99, 28.19, 28.37, 28.42, 30.08, 32.07, 32.81, 33.91, 35.98, 36.37, 36.75, 37.18, 38.76, 39.70, 39.92, 40.94, 42.50, 50.20, 56.32, 56.87, 74.40, 122.66, 140.03, 156.35.

4,9,N^{12}-Tri(*tert*-butoxycarbonyl)-N^1-(2-nitrophenylsulfonyl)-1,12-diamino-4,9-diazadodecane (7). Molecular sieves 4 Å (1.0 g), Et_3N (0.239 mL, 1.716 mmol), and 2-nitrobenzenesulfonyl chloride (0.228 g, 1.02 mmol) were added to a cooled (0 °C) solution of compound **6** (0.431 g, 0.858 mmol) in anhydrous CH_2Cl_2 (5 mL). The reaction mixture was stirred for 1 h at 24 °C. The molecular sieves were filtered off and washed with CH_2Cl_2, and the solvent was removed *in vacuo*. The residue was chromatographed on a column with silica gel using a $CHCl_3$—MeOH mixture (50 : 1) as the eluent to give compound **7** as a crystallizing oil (0.507 g, 88%), R_f 0.40 (D). MS, m/z (I_{rel} (%)): 710.341 $[M + Na]^+$ (100). Calculated for $C_{31}H_{53}N_5O_{10}S$: 687.351 $[M]^+$. ^1H NMR, δ: 1.27 (br.s, 31 H, 3 $C(CH_3)_3$ and $NCH_2(C\underline{H}_2)_2CH_2N$); 1.52—1.71 (m, 4 H, 2 $NCH_2C\underline{H}_2CH_2N$); 2.92—3.11 (m, 8 H, 4 NCH_2); 3.11—3.30 (m, 4 H, 2 $NHC\underline{H}_2$); 7.59—7.71 (m, 2 H); 7.71—8.89 (m, 1 H); 8.00—8.11 (m, 1 H, C_6H_4). ^{13}C NMR (δ: 25.96, 28.54, 28.59, 28.93, 37.91, 41.03, 43.78, 44.23, 46.82, 47.05, 79.16, 79.74, 80.02, 125.25, 130.93, 132.68, 133.51, 148.29, 156.17.

9,14,N^{17}-Tri(*tert*-butoxycarbonyl)-5-(2-nitrophenylsulfonyl)-1-[(cholest-5-en-3β-yl)oxycarbonyl]-17-amino-5,9,14-triazaheptadecane (8a). Cesium carbonate (0.083 g, 0.254 mmol) and bromide **2** (0.168 g, 0.306 mmol) were added successively to a solution of compound **7** (0.175 g, 0.254 mmol) in anhydrous DMF (3 mL). The reaction mixture was stirred for 1 h at 60 °C. The precipitate was filtered off through Celite® 545 and washed with CH_2Cl_2. After removal of the solvents *in vacuo*, the residue was chromatographed on a column with silica gel using a $CHCl_3$—MeOH mixture (100 : 1) as the eluent to give compound **8a** as a crystallizing oil (0.253 g, 86%), R_f 0.38 (C). MS, m/z (I_{rel} (%)): 1178.741 $[M + Na]^+$ (100). Calculated for $C_{63}H_{105}N_5O_{12}S$: 1155.748 $[M]^+$. ^1H NMR, δ: 0.61 (s, 3 H, C(13)Me); 0.79 (d, 3 H, C(25)Me, J = 6.5 Hz); 0.80 (d, 3 H, C(25)Me, J = 6.5 Hz); 0.83 (d, 3 H, C(20)Me, J = 6.5 Hz); 0.94 (s, 3 H, C(10)Me), 0.90—2.00 (m, 38 H, Chol, 2 $CH_2(C\underline{H}_2)_2CH_2$, 2 $NCH_2C\underline{H}_2CH_2N$); 1.36 (br.s, 18 H) and 1.39 (br.s, 9 H, 3 $C(CH_3)_3$); 2.11—2.33 (m, 4 H, $H_2C(4)$, $OC(O)CH_2$); 2.95—3.12 (m, 8 H, 4 NCH_2); 3.12—3.30 (m, 6 H, $C\underline{H}_2NH$, 2 NCH_2); 4.43—4.59 (m, 1 H, H(3)); 5.01—5.26 (m, 1 H, NH); 5.26—5.34 (m, 1 H, H(6)); 7.50—7.59 (m, 1 H); 7.59—7.70 (m, 2 H) and 7.87—8.00 (m, 1 H, C_6H_4). ^{13}C NMR, δ: 11.94, 18.80, 19.39, 21.11, 22.03, 22.64, 22.89, 23.90, 24.36, 25.61, 25.79, 27.87, 28.07, 28.29, 28.53, 31.94, 31.98, 34.00, 35.86, 36.26, 36.68, 37.07, 37.67, 38.22, 39.60, 39.82, 42.40, 43.94, 44.54, 45.19, 46.77, 47.07, 50.12, 56.23, 56.78, 74.02, 79.58, 122.73, 124.24, 130.80, 131.73, 133.50, 133.58 139.71, 148.13, 155.50, 156.10, 172.51.

9,14,N^{17}-Tri(*tert*-butoxycarbonyl)-5-(2-nitrophenylsulfonyl)-N^1-[(cholest-5-en-3β-yl)oxycarbonyl]-1,17-diamino-5,9,14-triazaheptadecane (8b) was prepared in the same way as compound **8a** from compound **7** (0.098 g, 0.142 mmol) and bromide **5a** (0.096 g, 0.170 mmol) in the presence of Cs_2CO_3 (0.046 g, 0.142 mmol) to give compound **8b** as a crystallizing oil (0.157 g, 94%), R_f 0.4 (B). MS, m/z (I_{rel} (%)): 1193.762 $[M + Na]^+$ (100). Calculated for $C_{63}H_{106}N_6O_{12}S$: 1170.759 $[M]^+$. ^1H NMR, δ: 0.61 (s, 3 H, C(13)Me); 0.79 (d, 3 H, C(25)Me, J = 6.5 Hz); 0.80 (d, 3 H, C(25)Me, J = 6.5 Hz); 0.84 (d, 3 H, C(20)Me, J = 6.5 Hz); 0.94 (s, 3 H, C(10)Me); 0.90—2.00 (m, 38 H, Chol, 2 $CH_2(C\underline{H}_2)_2CH_2$, 2 $NCH_2C\underline{H}_2CH_2N$); 1.36 (br.s, 18 H) and 1.39 (br.s, 9 H, 3 $C(C\underline{H}_3)_3$); 2.11—2.33 (m, 2 H, $H_2C(4)$); 2.91—3.12 (m, 10 H, $C\underline{H}_2NH$, 4 NCH_2); 3.12—3.30 (m, 6 H, $C\underline{H}_2NH$, 2 NCH_2); 4.32—4.47 (m, 1 H, H(3)); 4.55—4.96 (m, 1 H, NH); 4.96—5.25 (m, 1 H, NH); 5.25—5.33 (m, 1 H, H(6)); 7.50—7.59 (m, 1 H); 7.59—7.70 (m, 2 H) and 7.87—8.00 (m, 1 H, C_6H_4). ^{13}C NMR, δ: 11.97, 18.84, 19.44, 21.17, 22.67, 22.92, 23.95, 24.40, 25.61, 26.01, 27.20, 27.57, 28.11, 28.30, 28.58, 32.01, 35.90, 36.31, 36.54, 36.69, 37.13, 37.80, 38.70, 39.63, 39.87, 40.36, 42.44, 44.16, 44.70, 45.57, 46.70, 50.15, 56.27, 56.82, 74.36, 79.66, 122.56, 124.27, 130.83, 131.77, 133.48, 133.61, 139.98, 148.18, 155.56, 156.16, 156.35.

11,16,N^{19}-Tri(*tert*-butoxycarbonyl)-7-(2-nitrophenylsulfonyl)-N^1-[(cholest-5-en-3β-yl)oxycarbonyl]-1,19-diamino-7,11,16-triazanonadecane (8c) was prepared in the same way as compound **8a** from compound **7** (0.203 g, 0.302 mmol) and bromide **5b** (0.215 g, 0.362 mmol) in the presence of Cs_2CO_3 (0.098 g, 0.302 mmol). Compound **8c** was obtained as a crystallizing oil (0.382 g, 88%), R_f 0.45 (C). MS, m/z (I_{rel} (%)): 1221.424 $[M + Na]^+$(100). Calculated for $C_{65}H_{110}N_6O_{12}S$: 1198.790 $[M]^+$. ^1H NMR, δ: 0.61 (s, 3 H, C(13)Me); 0.79 (d, 3 H, C(25)Me, J = 6.5 Hz); 0.80 (d, 3 H, C(25)Me, J = 6.5 Hz); 0.84 (d, 3 H, C(20)Me, J = 6.5 Hz); 0.94 (s, 3 H, C(10)Me); 0.90—2.00 (m, 42 H, Chol protons, $NHCH_2(C\underline{H}_2)_2CH_2N$, 2 $NCH_2C\underline{H}_2CH_2N$, $CH_2(C\underline{H}_2)_4CH_2$); 1.37 (br.s, 18 H) and 1.39 (br.s, 9 H, 3 $C(C\underline{H}_3)_3$); 2.11—2.35 (m, 2 H, $H_2C(4)$); 2.92—3.13 (m, 10 H, $C\underline{H}_2NH$, 4 NCH_2); 3.13—3.33 (m, 6 H, $C\underline{H}_2NH$, 2 NCH_2); 4.33—4.47 (m, 1 H, H(3)); 4.50—4.85 (m, 1 H, NH); 4.96—5.25 (m, 1 H, NH); 5.26—5.35 (m, 1 H, H(6)); 7.51—7.59 (m, 1 H); 7.59—7.70 (m, 2 H) and 7.87—8.00 (m, 1 H, C_6H_4). ^{13}C NMR, δ: 12.00, 18.87, 19.48, 21.20, 22.70, 23.00, 23.98, 24.43, 25.91, 26.31, 26.37, 27.60, 28.09, 28.14, 28.36, 28.60, 30.30, 32.05, 35.93, 36.34, 36.72, 37.16, 37.81, 38.74, 39.66, 39.90, 40.88, 42.47, 44.13, 44.67, 45.28, 46.82, 47.48, 50.19, 56.30, 56.85, 74.36, 79.65, 122.57, 124.29, 130.87, 131.73, 133.57, 133.68, 140.03, 148.20, 155.59, 156.18, 156.33.

9,14,N^{17}-Tri(*tert*-butoxycarbonyl)-1-[(cholest-5-en-3β-yl)-oxycarbonyl]-17-amino-5,9,14-triazaheptadecane (9a). Potassium carbonate (0.072 g, 0.520 mmol) and then PhSH (0.13 mL, 1.310 mmol) were added with stirring to a solution of compound **8a** (0.150 g, 0.130 mmol) in DMF (3 mL). After 1 h, the reaction mixture was filtered through Celite® 545, the precipitate was washed with MeOH, and the solvent was evaporated *in vacuo*. Chromatography on silica gel ($CHCl_3$—MeOH—25% aq. NH_3, 25 : 1 : 0.1) gave compound **9a** as a yellowish crystallizing oil (0.079 g, 83%), R_f 0.33 (F). MS, *m/z* (I_{rel} (%)): 971.7 $[M + H]^+$ (100). Calculated for $C_{57}H_{102}N_4O_8$: 970.7 $[M]^+$. ^{1}H NMR, δ: 0.61 (s, 3 H, C(13)Me); 0.79 (d, 3 H, C(25)Me, J = 6.5 Hz); 0.80 (d, 3 H, C(25)Me, J = 6.5 Hz); 0.84 (d, 3 H, C(20)Me, J = 6.5 Hz); 0.94 (s, 3 H, C(10)Me); 0.90—2.00 (m, 38 H, Chol, 2 $CH_2(C\underline{H}_2)_2CH_2$, 2 $NCH_2C\underline{H}_2CH_2N$); 1.36 (br.s, 18 H) and 1.39 (br. s, 9 H, 3 $C(CH_3)_3$); 2.15—2.33 (m, 4 H, $OC(O)CH_2$, $H_2C(4)$); 2.60—2.87 (m, 4 H, $C\underline{H}_2NHC\underline{H}_2$); 2.96—3.35 (m, 10 H, $C\underline{H}_2NH$, 4 NCH_2); 4.44—4.59 (m, 1 H, H(3)); 5.01—5.26 (m, 1 H, NHBoc); 5.26—5.33 (m, 1 H, H(6)). ^{13}C NMR, δ: 11.94, 18.81, 19.40, 21.12, 22.29, 22.66, 22.91, 23.91, 24.37, 25.82, 27.88, 28.09, 28.31, 28.54, 31.94, 31.99, 34.02, 35.87, 36.27, 36.68, 37.07, 37.53, 38.22, 39.60, 39.82, 42.39, 43.42, 43.99, 44.35, 45.45, 46.88, 47.25, 50.12, 56.22, 56.78, 74.12, 79.70, 122.74, 139.71, 156.14, 172.53.

9,14,N^{17}-Tri(*tert*-butoxycarbonyl)-N^1-[(cholest-5-en-3β-yl)oxycarbonyl]-1,17-diamino-5,9,14-triazaheptadecane (9b) was prepared in the same way as compound **9a** from **8b** (0.130 g, 0.110 mmol), K_2CO_3 (0.050 g, 0.360 mmol), and PhSH (0.1 mL, 1.10 mmol). Compound **9b** was obtained as a yellowish amorphous solid (0.107 g, 99%). R_f 0.35 (E). MS, *m/z* (I_{rel} (%)): 986.796 $[M + H]^+$ (100). Calculated for $C_{57}H_{103}N_5O_8$: 985.781 $[M]^+$. ^{1}H NMR, δ: 0.61 (s, 3 H, C(13)Me); 0.79 (d, 3 H, C(25)Me, J = 6.5 Hz); 0.80 (d, 3 H, C(25)Me, J = 6.5 Hz); 0.84 (d, 3 H, C(20)Me, J = 6.5 Hz); 0.94 (s, 3 H, C(10)Me); 0.90—2.00 (m, 38 H, Chol, 2 $CH_2(C\underline{H}_2)_2CH_2$, 2 $NCH_2C\underline{H}_2CH_2N$); 1.36 (br.s, 18 H, 2 $C(CH_3)_3$); 1.39 (br.s, 9 H, $C(C\underline{H}_3)_3$); 2.11—2.33 (m, 2 H, $H_2C(4)$); 2.53—2.82 (m, 4 H, $C\underline{H}_2NHC\underline{H}_2$); 2.95—3.33 (m, 12 H, 2 $C\underline{H}_2NH$, 4 NCH_2); 4.32—4.47 (m, 1 H, H(3)); 4.55—4.96 (m, 1 H, NH); 4.96—5.25 (m, 1 H, NH); 5.25—5.33 (m, 1 H, H(6)). ^{13}C NMR, δ: 11.92, 18.77, 19.40, 21.10, 22.62, 22.88, 23.88, 24.34, 26.01, 27.40, 28.05, 28.25, 28.28, 28.51, 31.93, 35.84, 36.23, 36.61, 37.06, 37.58, 38.66, 39.57, 39.79, 40.29, 42.36, 43.73, 44.21, 46.56, 46.84, 50.07, 56.19, 56.75, 74.21, 79.67, 122.46, 139.94, 156.15, 156.41.

11,16,N^{19}-Tri-(*tert*-butoxycarbonyl)-N^1-[(cholest-5-en-3β-yl)oxycarbonyl]-1,19-diamino-7,11,16-triazanonadecane (9c) was prepared in the same way as compound **9a** from **8c** (0.106 g, 0.088 mmol), K_2CO_3 (0.053 g, 0.380 mmol), and PhSH (0.1 mL, 1.10 mmol).Compound **9c** was obtained as a yellowish amorphous solid (0.079 g, 89%), R_f 0.37 (F). MS, *m/z* (I_{rel} (%)): 1014.7 $[M + H]^+$ (100). Calculated for $C_{59}H_{107}N_5O_8$: 1013.8 $[M]^+$. ^{1}H NMR, δ: 0.61 (s, 3 H, C(13)Me); 0.79 (d, 3 H, C(25)Me, J = 6.5 Hz); 0.80 (d, 3 H, C(25)Me, J = 6.5 Hz); 0.84 (d, 3 H, C(20)Me, J = 6.5 Hz); 0.94 (s, 3 H, C(10)Me); 0.90—2.00 (m, 42 H, Chol, $NHCH_2(C\underline{H}_2)_2CH_2N$, 2 $NCH_2C\underline{H}_2CH_2N$, $CH_2(C\underline{H}_2)_4CH_2$); 1.37 (br.s, 18 H) and 1.39 (br. s, 9 H, 3 $C(C\underline{H}_3)_3$); 2.11—2.35 (m, 2 H, $H_2C(4)$); 2.47—2.76 (m, 4 H, $C\underline{H}_2NHC\underline{H}_2$); 2.93—3.30 (m, 12 H, 2 $C\underline{H}_2NH$, 4 NCH_2); 4.32—4.52 (m, 1 H, H(3)); 4.50—4.85 (m, 1 H, NH); 4.96—5.25 (m, 1 H, NH); 5.26—5.35 (m, 1 H, H(6)). ^{13}C NMR, δ: 11.95, 18.81, 19.43, 21.13, 22.66, 22.92, 23.92, 24.38, 25.59, 25.96, 26.31, 26.37, 27.60, 28.09, 28.29, 28.32, 28.54, 29.93, 31.97, 35.89, 36.27, 36.65, 37.10, 37.55, 38.69, 39.60, 39.83, 40.79, 42.40, 43.73, 44.20, 44.30, 45.83, 45.90, 46.47, 46.60, 46.84, 47.08, 50.10, 56.22, 56.78, 74.22, 79.56, 122.50, 139.97, 156.13, 156.18, 156.33.

17-Amino-1-[(cholest-5-en-3β-yl)oxycarbonyl]-5,9,14-triazaheptadecane tetrahydrochloride (10a). A 4 *M* solution of HCl in dioxane (4 mL) was added to a solution of compound **9a** (0.164 g, 0.169 mmol) in 4 mL of MeOH, and the mixture was stirred for 2 h at 24 °C. The solvents were removed *in vacuo*, the residue was recrystallized from a $CHCl_3$—EtOH mixture (1 : 1) to give compound **10a** as beige-colored crystals (0.136 g, 99%), R_f 0.35 (G). MS, *m/z* (I_{rel} (%)): 671.5 $[M - 4\ HCl + H]^+$ (100). Calculated for $C_{42}H_{82}Cl_4N_4O_2$: 670.61 $[M - 4\ HCl]^+$. ^{1}H NMR (D_2O), δ: 0.61 (s, 3 H, C(13)Me); 0.77 (d, 3 H, C(25)Me, J = 6.5 Hz); 0.79 (d, 3 H, C(25)Me, J = 6.5 Hz); 0.84 (d, 3 H, C(20)Me, J = 6.5 Hz); 0.94 (s, 3 H, C(10)Me); 0.90—2.00 (m, 38 H, Chol, $NHCH_2(C\underline{H}_2)_2CH_2N$, 2 $NCH_2C\underline{H}_2CH_2N$, $CH_2(C\underline{H}_2)_2CH_2$); 2.11—2.32 (m, 2 H, $H_2C(4)$); 2.90—3.15 (m, 16 H, $OC(O)CH_2$, 7 CH_2N); 4.20—4.40 (m, 1 H, H(3)); 5.20—5.35 (m, 1 H, H(6)).

1,17-Diamino-N^1-[(cholest-5-en-3β-yl)oxycarbonyl]-5,9,14-triazaheptadecane tetrahydrochloride (10b) was prepared in the same way as compound **10a** from **9b** (0.120 g, 0.122 mmol). Compound **10b** was obtained as beige-colored crystals (0.098 g, 97%), R_f 0.38 (G). MS, *m/z* (I_{rel} (%)): 686.6 $[M - 4\ HCl + H]^+$ (100). Calculated for $C_{42}H_{83}Cl_4N_5O_2$: 685.63 $[M - 4\ HCl]^+$. ^{1}H NMR (D_2O), δ: 0.60 (s, 3 H, C(13)Me); 0.76 (d, 3 H, C(25)Me, J = 6.5 Hz); 0.79 (d, 3 H, C(25)Me, J = 6.5 Hz); 0.85 (d, 3 H, C(20)Me, J = 6.5 Hz); 0.95 (s, 3 H, C(10)Me); 0.90—2.00 (m, 38 H, Chol, $NHCH_2(C\underline{H}_2)_2CH_2N$, 2 $NCH_2C\underline{H}_2CH_2N$, $CH_2(C\underline{H}_2)_2CH_2$); 2.10—2.33 (m, 2 H, $H_2C(4)$); 2.91—3.15 (m, 16 H, 8 CH_2N); 4.13—4.39 (m, 1 H, H(3)); 5.17—5.38 (m, 1 H, H(6)).

1,19-Diamino-N^1-[(cholest-5-en-3β-yl)oxycarbonyl]-7,11,16-triazanonadecane tetrahydrochloride (10c) was prepared in the same way as compound **10a** from **9c** (0.144 g, 0.142 mmol). Compound **10c** was obtained as white crystals (0.116 g, 95%), R_f 0.40 (G). MS, *m/z* (I_{rel} (%)): 714.6 $[M - 4\ HCl + H]^+$(100). Calculated for $C_{44}H_{87}Cl_4N_5O_2$: 713.65 $[M - 4\ HCl]^+$. ^{1}H NMR (D_2O), δ: 0.61 (s, 3 H, C(13)Me); 0.77 (d, 3 H, C(25)Me, J = 6.5 Hz); 0.79 (d, 3 H, C(25)Me, J = 6.5 Hz); 0.85 (d, 3 H, C(20)Me, J = 6.5 Hz); 0.94 (s, 3 H, C(10)Me); 0.91—2.05 (m, 42 H, Chol, $NHCH_2(C\underline{H}_2)_2CH_2N$, 2 $NCH_2C\underline{H}_2CH_2N$, $CH_2(C\underline{H}_2)_4CH_2$); 2.12—2.35 (m, 2 H, $H_2C(4)$); 2.91—3.14 (m, 16 H, 8 $C\underline{H}_2N$); 4.13—4.40 (m, 1 H, H(3)); 5.17—5.38 (m, 1 H, H(6)).

4,9-Di(*tert*-butoxycarbonyl)-1,12-bis(2-nitrophenylsulfonylamino)-4,9-diazadodecane (12). Molecular sieves 4 Å (0.5 g), Et_3N (1.0 mL, 6.72 mmol) and 2-nitrobenzenesulfonyl chloride (0.893 g, 4.03 mmol) were added successively to a cooled (0 °C) solution of compound **11** (0.676 g, 1.68 mmol) in anhydrous CH_2Cl_2 (5 mL). The reaction mixture was stirred for 5 h at 24 °C. The molecular sieves were filtered off and washed with CH_2Cl_2, and the solvents were removed *in vacuo*. The residue was chromatographed on a column with silica gel ($CHCl_3$ → $CHCl_3$—MeOH, 50 : 1) to give compound **12** as a yellowish crystallizing oil (0.818 g, 63%), R_f 0.35 (D). ^{1}H NMR, δ: 1.35 (br.s, 22 H, 2 $C(C\underline{H}_3)_3$, $CH_2(C\underline{H}_2)_2CH_2$); 1.48—1.70 (m, 4 H, 2 $CH_2C\underline{H}_2CH_2$); 2.86—3.10 (m, 8 H, 4 NCH_2); 3.10—3.38

(m, 4 H, 2 NCH_2); 7.50—7.75 (m, 4 H); 7.95—8.05 (m, 2 H) and 8.05—8.20 (m, 2 H, 2 C_6H_4). ^{13}C NMR, δ: 25.42, 25.83, 28.38, 28.86, 29.22, 40.66, 43.32, 44.25, 46.46, 46.90, 79.84, 124.47, 130.62, 132.41, 135.69, 148.02, 148.18, 155.42, 156.38.

9,14-Di(*tert*-butoxycarbonyl)-5,18-bis(2-nitrophenylsulfonyl)-1,22-di[(cholest-5-en-3β-yl)oxycarbonyl]-5,9,14,18-tetraazadocosane (13a). Cesium carbonate (0.084 g, 0.259 mmol) and bromide **2** (0.300 g, 0.546 mmol) were added successively to a solution of compound **12** (0.200 g, 0.259 mmol) in anhydrous DMF (7 mL). The reaction mixture was stirred for 1 h at 60 °C. The precipitate was filtered off through Celite® 545 and washed with CH_2Cl_2. After removal of the solvents *in vacuo*, the residue was chromatographed on a column with silica gel using a $CHCl_3$—MeOH—25% aq. NH_3 mixture (60 : 2 : 0.1 → → 60 : 4 : 0.1) as the eluent to give compound **13a** as a light-yellow crystallizing oil (0.240 g, 61%). R_f 0.38 (B). ^1H NMR, δ: 0.61 (s, 6 H, 2 C(13)Me); 0.79 (d, 6 H, 2 C(25)Me, J = 6.5 Hz); 0.80 (d, 6 H, 2 C(25)Me, J = 6.5 Hz); 0.83 (d, 6 H, 2 C(20)Me, J = 6.5 Hz); 0.94 (s, 6 H, 2 C(10)Me); 0.90—2.07 (m, 68 H, Chol, 3 $CH_2(C\underline{H}_2)_2CH_2$, 2 $NCH_2C\underline{H}_2CH_2N$); 1.36 (br.s, 18 H, 2 $C(CH_3)_3$); 2.20—2.38 (m, 8 H, 2 $H_2C(4)$; 2 $OC(O)CH_2$); 3.00—3.17 (m, 8 H, 4 NCH_2); 3.17—3.31 (m, 8 H, 4 NCH_2); 4.33—4.49 (m, 2 H, 2 H(3)); 5.25—5.34 (m, 2 H, 2 H(6)); 7.49—7.60 (m, 2 H); 7.58—7.69 (m, 4 H) and 7.88—7.98 (m, 2 H, 2 C_6H_4). ^{13}C NMR, δ: 11.95, 18.64, 19.21, 20.96, 21.88, 22.46, 22.69, 23.75, 24.19, 25.58, 25.74, 27.30, 27.46, 27.72, 27.89, 28.11, 28.37, 31.81, 31.82, 33.84, 35.68, 36.12, 36.52, 36.93, 38.07, 39.44, 39.68, 42.25, 44.48, 45.07, 46.99, 47.02, 50.01, 56.12, 56.64, 73.85, 79.37, 122.2, 124.06, 130.60, 131.54, 133.38, 139.58, 147.00, 155.32, 172.30.

9,14-Di(*tert*-butoxycarbonyl)-5,18-bis(2-nitrophenylsulfonyl)-1,22-di[(cholest-5-en-3β-yl)oxycarbonylamino]-5,9,14,18-tetraazadocosane (13b) was prepared in the same way as compound **13a** from **12** (0.290 g, 0.375 mmol) and **5a** (0.509 g, 0.901 mmol) in the presence of Cs_2CO_3 (0.244 g, 0.750 mmol). Chromatography ($CHCl_3$—MeOH, 100 : 0.5 → 100 : 1) gave compound **13b** as a light-yellow crystallizing oil (0.390 g, 60%), R_f 0.38 (C). ^1H NMR, δ: 0.61 (s, 6 H, 2 C(13)Me); 0.79 (d, 6 H, 2 C(25)Me, J = 6.5 Hz); 0.80 (d, 6 H, 2 C(25)Me, J = = 6.5 Hz); 0.83 (d, 6 H, 2 C(20)Me, J = 6.5 Hz); 0.94 (s, 6 H, 2 C(10)Me); 0.90—2.00 (m, 68 H, Chol, 3 $CH_2(C\underline{H}_2)_2CH_2$, 2 $NCH_2C\underline{H}_2CH_2N$); 1.36 (br.s, 18 H, 2 $C(CH_3)_3$); 2.11—2.38 (m, 4 H, 2 $H_2C(4)$); 2.96—3.14 (m, 12 H, 2 $C\underline{H}_2NH$, 4 NCH_2); 3.14—3.29 (m, 8 H, 4 NCH_2); 4.33—4.49 (m, 2 H, 2 H(3)); 4.58—4.75 (m, 2 H, 2 NH); 5.26—5.33 (m, 2 H, 2 H(6)); 7.51—7.58 (m, 2 H); 7.58—7.69 (m, 4 H) and 7.88—7.98 (m, 2 H, 2 C_6H_4). ^{13}C NMR, δ: 11.95, 18.81, 19.43, 21.13, 22.66, 22.92, 23.92, 24.37, 25.52, 26.02, 27.13, 27.54, 28.09, 28.27, 28.32, 28.56, 31.96, 31.98, 35.88, 36.27, 36.64, 37.08, 38.68, 39.60, 39.81, 40.30, 42.39, 44.71, 45.20, 45.67, 47.28, 47.64, 50.09, 56.22, 56.77, 74.27, 79.62, 122.53, 124.25, 130.72, 131.81, 133.36, 133.65, 139.93, 148.11, 155.53, 156.33.

11,16-Di(*tert*-butoxycarbonyl)-7,20-bis(2-nitrophenylsulfonyl)-1,26-di[(cholest-5-en-3β-yl)oxycarbonylamino]-7,11,16,20-tetraazahexacosane (13c) was prepared in the same way as compound **13a** from **12** (0.200 g, 0.259 mmol) and **5b** (0.338 g, 0.569 mmol) in the presence of Cs_2CO_3 (0.084 g, 0.259 mmol). Chromatography ($CHCl_3$—MeOH, 100 : 0.5 → 100 : 1) afforded compound **13c** as a light-yellow crystallizing oil (0.262 g, 56%), R_f 0.40 (C). ^1H NMR, δ: 0.61 (s, 6 H, 2 C(13)Me); 0.79 (d, 6 H, 2 C(25)Me, J = 6.5 Hz); 0.80 (d, 6 H, 2 C(25)Me, J = 6.5 Hz); 0.83 (d, 6 H, 2 C(20)Me, J = 6.5 Hz); 0.94 (s, 6 H, 2 C(10)Me); 0.90—2.00 (m, 76 H, Chol, $NCH_2(C\underline{H}_2)_2CH_2N$, 2 $NCH_2C\underline{H}_2CH_2N$, 2 $CH_2(C\underline{H}_2)_4CH_2$); 1.37 (br.s, 18 H, 2 $C(CH_3)_3$); 2.11—2.37 (m, 4 H, 2 $H_2C(4)$); 2.96—3.14 (m, 12 H, 2 $C\underline{H}_2NH$, 4 NCH_2); 3.14—3.30 (m, 8 H, 4 NCH_2); 4.33—4.50 (m, 2 H, 2 H(3)); 4.55—4.78 (m, 2 H, 2 NH); 5.26—5.32 (m, 2 H, 2 H(6)); 7.51—7.58 (m, 2 H); 7.59—7.68 (m, 4 H) and 7.88—7.97 (m, 2 H, 2 C_6H_4). ^{13}C NMR, δ: 11.97, 18.83, 19.45, 21.14, 22.68, 22.94, 23.93, 24.40, 25.68, 26.25, 26.38, 27.50, 28.03, 28.11, 28.30, 28.34, 28.57, 29.97, 31.97, 35.90, 36.28, 36.66, 37.10, 38.70, 39.61, 39.83, 40.79, 42.41, 44.65, 45.14, 47.20, 50.10, 56.22, 56.78, 74.25, 79.61, 122.53, 124.26, 130.77, 131.78, 133.54, 133.62, 139.98, 148.11, 155.54, 156.29.

9,14-Di(*tert*-butoxycarbonyl)-1,22-di[(cholest-5-en-3β-yl)oxycarbonyl]-5,9,14,18-tetraazadocosane (14a). Potassium carbonate (0.035 g, 0.252 mmol) and then PhSH (0.13 mL, 1.13 mmol) were added with stirring to a solution of compound **13a** (0.215 g, 0.126 mmol) in DMF (2 mL). After 1 h, the reaction mixture was filtered through Celite® 545, the precipitate was washed with MeOH, and the solvent was evaporated *in vacuo*. The residue was chromatographed on a column with silica gel using a $CHCl_3$—MeOH—25% aq. NH_3 mixture (100 : 10 : 1) as the eluent to give compound **14a** as a yellow crystallizing oil (0.100 g, 60%). R_f 0.32 (E). MS, m/z (I_{rel} (%)): 1339.642 $[M]^+$ (100). Calculated for $C_{84}H_{146}N_4O_8$: 1339.114 $[M]^+$. ^1H NMR, δ: 0.61 (s, 6 H, 2 C(13)Me); 0.79 (d, 6 H, 2 C(25)Me, J = 6.5 Hz); 0.80 (d, 6 H, 2 C(25)Me, J = 6.5 Hz); 0.83 (d, 6 H, 2 C(20)Me, J = 6.5 Hz); 0.94 (s, 6 H, 2 C(10)Me); 0.90—2.03 (m, 68 H, Chol, 3 $CH_2(C\underline{H}_2)_2CH_2$, 2 $NCH_2C\underline{H}_2CH_2N$); 1.37 (br.s, 18 H, 2 $C(CH_3)_3$); 2.30—2.35 (m, 8 H, 2 $H_2C(4)$, 2 $OC(O)CH_2$); 2.50—2.70 (m, 8 H, 2 $C\underline{H}_2NHC\underline{H}_2$); 3.00—3.30 (m, 8 H, 4 NCH_2); 4.40—4.50 (m, 2 H, 2 H(3)); 5.25—5.33 (m, 2 H, 2 H(6)). ^{13}C NMR, δ: 12.03, 18.91, 19.47, 21.24, 22.71, 22.94, 24.03, 24.46, 26.07, 28.03, 28.16, 28.38, 28.66, 29.20, 32.10, 34.60, 35.95, 36.40, 36.80, 37.21, 38.37, 39.72, 39.97, 42.54, 45.03, 47.03, 49.55, 50.30, 56.41, 56.93, 74.04, 79.59, 122.76, 139.90, 155.97, 172.99.

9,14-Di(*tert*-butoxycarbonyl)-1,22-di[(cholest-5-en-3β-yl)oxycarbonylamino]-5,9,14,18-tetraazadocosane (14b) was prepared in the same way as compound **14a** from **13b** (0.200 g, 0.115 mmol), K_2CO_3 (0.048 g, 0.345 mmol), and PhSH (0.14 mL, 1.15 mmol). Chromatography ($CHCl_3$—MeOH—25% aq. NH_3, 120 : 7.6 : 1) gave compound **14b** as a light-yellow crystallizing oil (0.121 g, 77%). R_f 0.30 (E). MS, m/z (I_{rel} (%)): 1370.339 $[M + H]^+$ (100). Calculated for $C_{84}H_{148}N_6O_8$: 1369.136 $[M]^+$. ^1H NMR, δ: 0.61 (s, 6 H, 2 C(13)Me); 0.79 (d, 6 H, 2 C(25)Me, J = 6.5 Hz); 0.80 (d, 6 H, 2 C(25)Me, J = 6.5 Hz); 0.83 (d, 6 H, 2 C(20)Me, J = 6.5 Hz); 0.94 (s, 6 H, 2 C(10)Me); 0.90—2.00 (m, 68 H, Chol, 3 $CH_2(C\underline{H}_2)_2CH_2$, 2 $NCH_2C\underline{H}_2CH_2N$); 1.37 (br.s, 18 H, 2 $C(CH_3)_3$); 2.10—2.35 (m, 4 H, 2 $H_2C(4)$); 2.47—2.62 (m, 8 H, 2 $C\underline{H}_2NHC\underline{H}_2$); 2.98—3.28 (m, 12 H, 2 $C\underline{H}_2NH$, 4 NCH_2); 4.34—4.49 (m, 2 H, 2 H(3)); 4.58—4.70 (m, 2 H, 2 NH); 4.92—5.11 (m, 2 H, 2 NH); 5.25—5.33 (m, 2 H, 2 H(6)). ^{13}C NMR, δ: 12.00, 18.86, 19.49, 21.19, 22.70, 22.96, 23.97, 24.43, 25.86, 26.14, 27.13, 27.97, 28.15, 28.37, 28.62, 32.02, 35.94, 36.33, 36.70, 37.15, 38.76, 39.66, 39.88, 40.86, 42.45, 44.45, 45.17, 46.81, 47.41, 50.15, 56.28, 56.83, 74.25, 79.54, 122.55, 140.02, 155.53, 156.38.

11,16-Di(*tert*-butoxycarbonyl)-1,26-di(cholest-5-en-3β-yloxycarbonylamino)-7,11,16,20-tetraazahexacosane (14c) was prepared in the same way as compound **14a** from **13c** (0.330 g, 0.184 mmol), K_2CO_3 (0.076 g, 0.552 mmol), and PhSH (0.19 mL, 1.83 mmol). Compound **14c** was obtained as a light-yellow crystallizing oil (0.185 g, 71%), R_f 0.33 (F). MS, *m/z* (I_{rel} (%)): 1426.238 $[M + H]^+$ (100). Calculated for $C_{88}H_{156}N_6O_8$: 1425.198 $[M]^+$. ^{1}H NMR, δ: 0.61 (s, 6 H, 2 C(13)Me); 0.79 (d, 6 H, 2 C(25)Me, *J* = 6.5 Hz); 0.80 (d, 6 H, 2 C(25)Me, *J* = 6.5 Hz); 0.83 (d, 6 H, 2 C(20)Me, *J* = 6.5 Hz); 0.94 (s, 6 H, 2 C(10)Me); 0.90—2.00 (m, 76 H, Chol, $NCH_2(C\underline{H}_2)_2CH_2N$, 2 $NCH_2C\underline{H}_2CH_2N$, 2 $CH_2(C\underline{H}_2)_4CH_2$); 1.37 (br.s, 18 H, 2 $C(CH_3)_3$); 2.12—2.35 (m, 4 H, 2 $H_2C(4)$); 2.47—2.61 (m, 8 H, 2 $C\underline{H}_2NHC\underline{H}_2$); 2.99—3.27 (m, 12 H, 2 $C\underline{H}_2NH$, 4 NCH_2); 4.34—4.49 (m, 2 H, 2 H(3)); 4.57—4.70 (m, 2 H, 2 NH); 5.26—5.33 (m, 2 H, 2 H(6)). ^{13}C NMR, δ: 11.97, 18.83, 19.45, 21.14, 22.68, 22.94, 23.93, 24.40, 25.68, 26.25, 26.38, 27.50, 28.03, 28.11, 28.30, 28.34, 28.57, 29.97, 31.97, 35.90, 36.28, 36.66, 37.10, 38.70, 39.61, 39.83, 40.79, 42.41, 44.65, 45.14, 47.20, 50.10, 56.22, 56.78, 74.25, 79.61, 122.53, 139.98, 155.54, 156.29.

1,22-Di[(cholest-5-en-3β-yl)oxycarbonyl]-5,9,14,18-tetraazacosane tetrahydrochloride (15a). A 4 *M* solution of HCl in dioxane (4 mL) was added to a solution of compound **14a** (0.100 g, 0.075 mmol) in MeOH (3 mL) and the mixture was stirred for 2 h at 24 °C. The solvent was removed *in vacuo* to give compound **15a** as beige-colored crystals (79 mg, 82%), R_f 0.40 (G). MS, *m/z* (I_{rel} (%)): 1139.784 $[M - 4\ HCl]^+$ (100). Calculated for $C_{74}H_{134}Cl_4N_4O_4$: 1139.009 $[M - 4\ HCl]^+$. ^{1}H NMR (DMSO-d_6 — $CDCl_3$, 1 : 3), δ: 0.61 (s, 6 H, 2 C(13)Me); 0.77 (d, 6 H, 2 C(25)Me, *J* = 6.5 Hz); 0.79 (d, 6 H, 2 C(25)Me, *J* = 6.5 Hz); 0.84 (d, 6 H, 2 C(20)Me, *J* = 6.5 Hz); 0.94 (s, 6 H, 2 C(10)Me); 0.90—2.03 (m, 68 H, Chol, $NHCH_2(C\underline{H}_2)_2CH_2N$, 2 $NCH_2C\underline{H}_2CH_2N$, 2 $CH_2(C\underline{H}_2)_2CH_2$); 2.15—2.25 (m, 8 H, 2 $H_2C(4)$, 2 $OC(O)CH_2$); 2.90—3.15 (m, 16 H, 8 $C\underline{H}_2N$); 4.20—4.40 (m, 2 H, 2 H(3)); 5.20—5.35 (m, 2 H, 2 H(6)).

1,22-Di[(cholest-5-en-3β-yl)oxycarbonylamino]-5,9,14,18-tetraazadocosane tetrahydrochloride (15b) was prepared in the same way as compound **15a** from **14b** (0.086 g, 0.063 mmol). Compound **15b** was obtained as beige-colored crystals (82 mg, 99%), R_f 0.40 (G). MS, *m/z* (I_{rel} (%)): 1170.1 $[M - 4\ HCl + H]^+$ (100). Calculated for $C_{74}H_{136}Cl_4N_6O_4$: 1169.13 $[M - 4\ HCl]^+$. ^{1}H NMR (CD_3OD—$CDCl_3$, 1 : 4), δ: 0.61 (s, 6 H, 2 C(13)Me); 0.77 (d, 6 H, 2 C(25)Me, *J* = 6.5 Hz); 0.79 (d, 6 H, 2 C(25)Me, *J* = 6.5 Hz); 0.83 (d, 6 H, 2 C(20)Me, *J* = 6.5 Hz); 0.94 (s, 6 H, 2 C(10)Me); 0.90—2.04 (m, 68 H, Chol, $NHCH_2(C\underline{H}_2)_2CH_2NH$, 2 $NCH_2C\underline{H}_2CH_2N$, 2 $CH_2(C\underline{H}_2)_4CH_2$); 2.11—2.34 (m, 4 H, 2 $H_2C(4)$); 2.41—2.60 (m, 8 H, 2 $C\underline{H}_2NHC\underline{H}_2$); 3.00—3.28 (m, 12 H, 2 $C\underline{H}_2NH$, 4 NCH_2); 4.33—4.49 (m, 2 H, 2 H(3)); 5.26—5.33 (m, 2 H, 2 H(6)).

1,26-Di(cholest-5-en-3β-yloxycarbonylamino)-7,11,16,20-tetraazahexacosane tetrahydrochloride (15c) was prepared in the same way as compound **15a** from **14c** (0.160 g, 0.112 mmol). Compound **15c** was obtained as beige-colored crystals (146 mg, 95%), R_f 0.45 (G). MS, *m/z* (I_{rel} (%)): 1226.3 $[M - 4\ HCl + H]^+$ (100). Calculated for $C_{78}H_{144}Cl_4N_6O_4$: 1225.13 $[M - 4\ HCl]^+$. ^{1}H NMR (DMSO-d_6—$CDCl_3$, 1 : 3), δ: 0.61 (s, 6 H, 2 C(13)Me); 0.79 (d, 6 H, 2 C(25)Me, *J* = 6.5 Hz); 0.80 (d, 6 H, 2 C(25)Me, *J* = 6.5 Hz); 0.83 (d, 6 H, 2 C(20)Me, *J* = 6.5 Hz); 0.94 (s, 6 H, 2 C(10)Me), 0.91—2.04 (m, 76 H, Chol, $NHCH_2(C\underline{H}_2)_2CH_2NH$, 2 $NCH_2C\underline{H}_2CH_2N$, 2 $CH_2(C\underline{H}_2)_4CH_2$); 2.12—2.35 (m, 4 H, 2 $H_2C(4)$); 2.40—2.61 (m, 8 H, 2 $C\underline{H}_2NHC\underline{H}_2$); 2.99—3.27 (m, 12 H, 2 $C\underline{H}_2NH$, 4 NCH_2); 4.34—4.49 (m, 2 H, 2 H(3)); 4.57—4.70 (m, 2 H, 2 NH); 5.26—5.33 (m, 2 H, 2 H(6)).

This work was supported by the Russian Foundation for Basic Research (Project No. 07-03-00632a) within a Federal target program (contract No. 02.512.11.2200).

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Received May 15, 2009;
in revised form October 26, 2009