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Synthesis of New Guanidine-Containing Amphiphiles and Their Pyrene Analog for Liposomal Delivery Systems and Visualization in Target Cells

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Abstract—New cationic amphiphiles with a guanidine head group have been synthesized starting from lipoamino acids with the goal of creating drug and genetic material delivery systems. Their analog containing a pyrene fragment has also been obtained as a potential agent for visualization of liposome transport in various cells.

Keywords: cationic amphiphiles, guanidines, diamines, pyrene, drug delivery systems.

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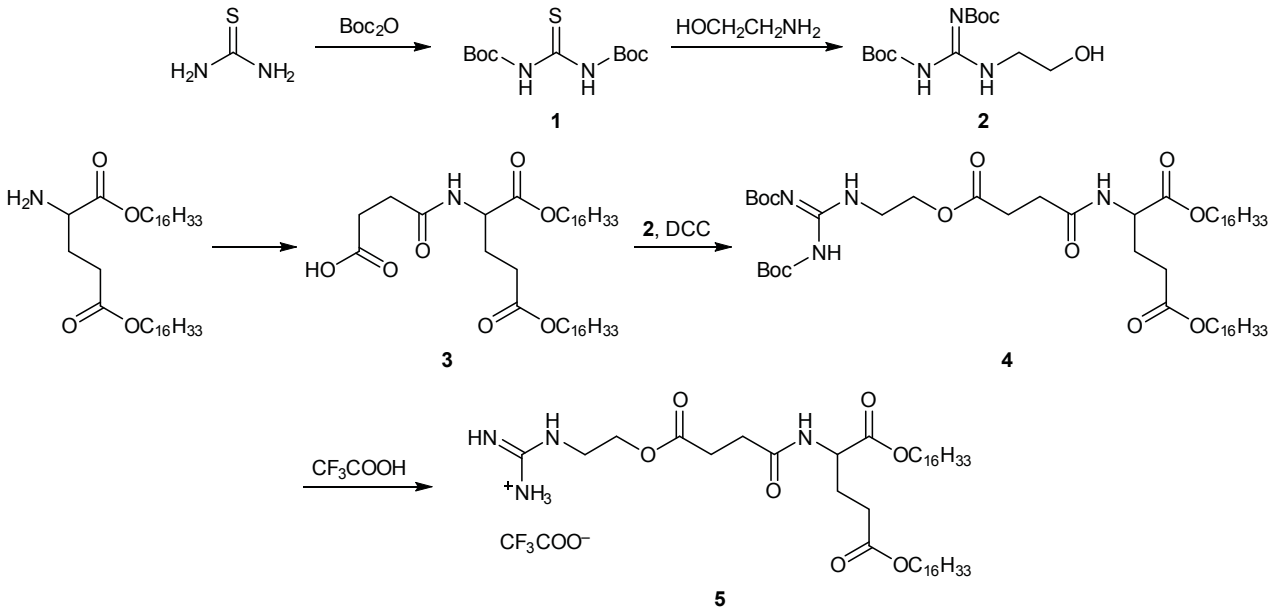
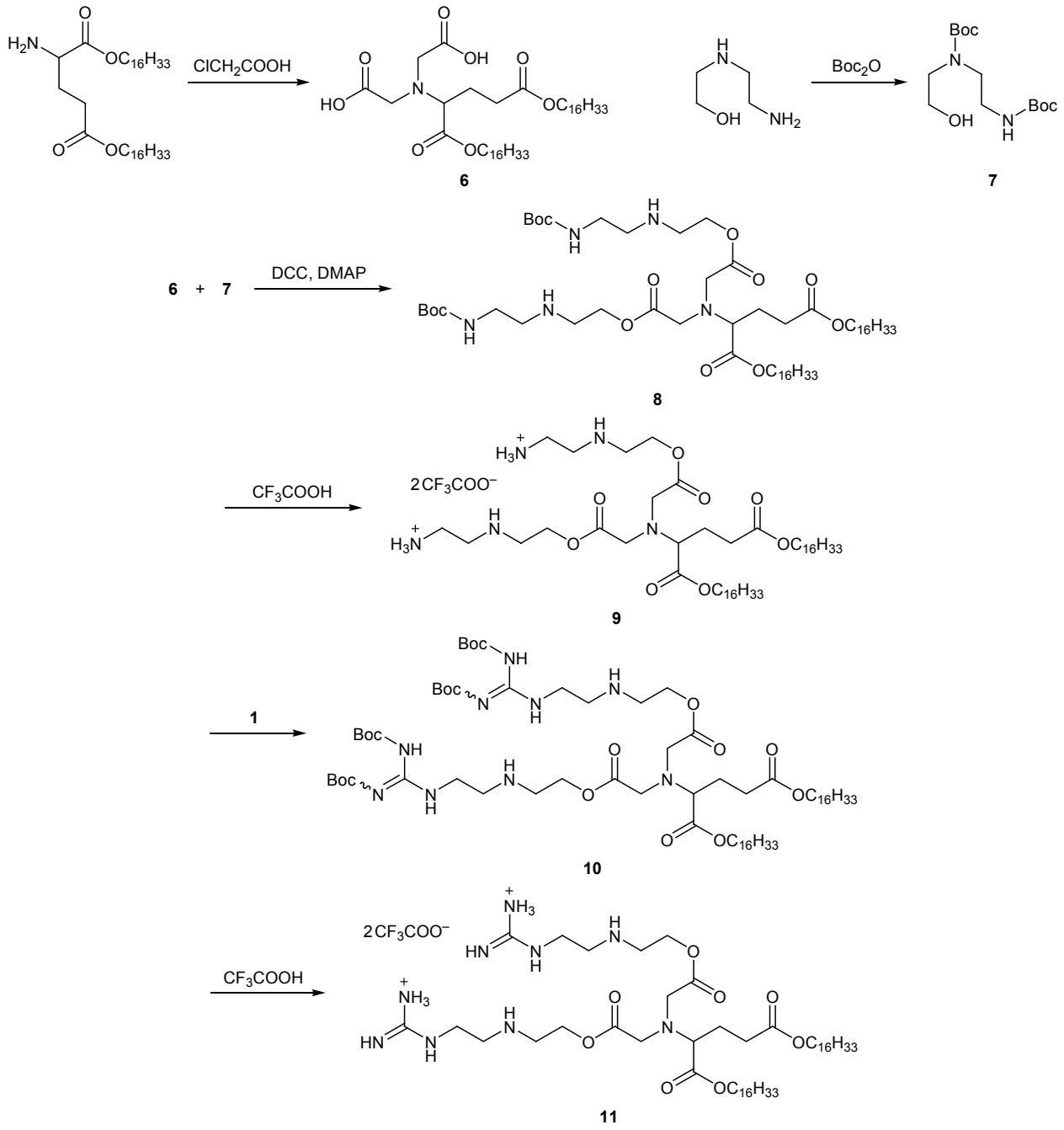
Cationic biocompatible non-viral vectors are widely used as delivery systems for drugs and genetic material; among these, cationic liposomes are considered safest [1]. The transfection efficiency and toxicity of cationic lipids are largely determined by their structure. Multivalent cationic amphiphiles possess a higher liposome surface charge and are more efficient in genetic material delivery to a cell nucleus than their singly charged analogs [2]. The use of natural polyamines such as spermidine and spermine enables interaction with DNA phosphate groups to form strong complexes with the transferred material [3]. Cationic peptide vectors are also extensively studied [4]; they are more advantageous than other non-viral systems due to their low toxicity and the ability to tightly pack and protect genetic material, as well as to recognize target-specific receptors on the cell membrane and deliver the package to the cell [5–8].

Scheme 1.



Scheme 2.

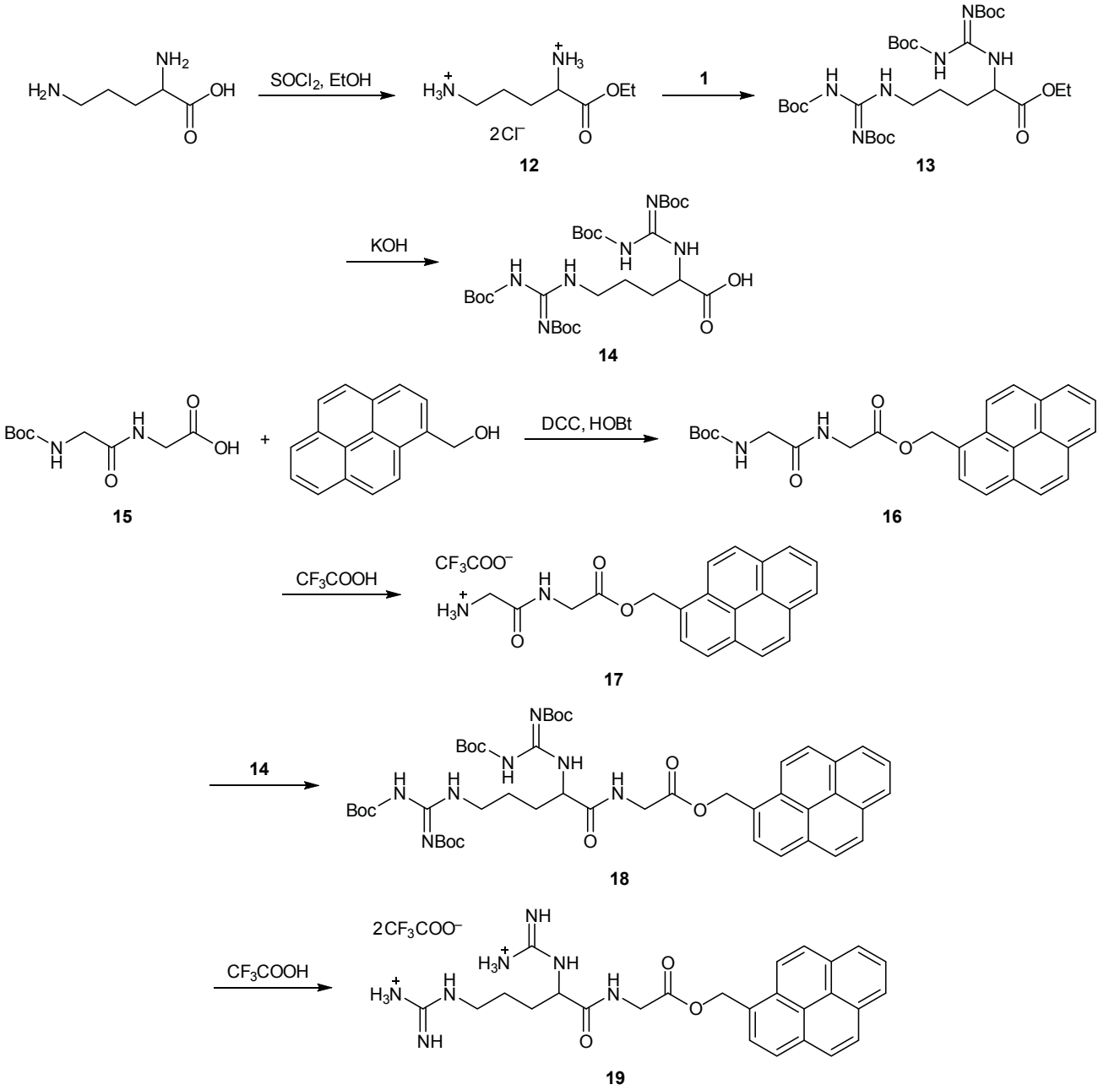


Sen and Chaudhuri [9] described the properties of guanidine-based cationic amphiphiles with hydrocarbon fragments [9]. Taking into account the ability of a guanidinium group to bind to biological counterions and involve them in transport through plasma membranes, the design of guanidinium-containing systems for delivery of biologically active compounds to cells is a challenging problem [10].

In order to visualize the delivery process mediated by guanidinium-based carriers, it is reasonable to use fluorophores such as pyrene derivatives. Liposomes formed by amphiphiles containing a pyrenylmethanol fragment as a hydrophobic moiety and mono-, di-, or polyamine head groups showed a strong ability to inhibit tumor cell proliferation and the possibility of simultaneously visualizing their penetration into target cells [11].

Therefore, search for drug delivery agents among synthetic cationic amphiphiles with guanidine-based head groups and their fluorescent analogs is important

Scheme 3.



for a deeper insight into their biodistribution processes and intracellular localization, as well as for the design of most efficient delivery systems.

The goal of the present work was to synthesize new cationic amphiphiles **5** and **11** with polar guanidine head groups, as well as a pyrene-containing guanidine tripeptide (OrnGlyGly) **19**, as subjects for further study of their intracellular localization and functional activity of polar head groups in target cells.

The synthesis of compound **5** is outlined in Scheme 1. The addition of Boc-protected thiourea **1** [12] to 2-aminoethanol in the presence of mercury chloride as catalyst [13] gave protected guanidine **2**. L-Glutamic acid dihexadecyl ester was modified with a succinic acid residue [14], and the resulting succinamic acid derivative **3** was esterified with alcohol **2** in the presence of *N*,*N*′-dicyclohexylcarbodiimide (DCC) and *N*-hydroxysuccinimide (NHS) as carboxyl group activators. The subsequent deprotection of **4** by treatment with trifluoroacetic acid afforded target compound **5** in 60% yield. The structure of **5** was confirmed by ^{1}H NMR and mass spectra.

Scheme 2 illustrates the synthesis of amphiphile **11**. The hydrophobic moiety of **11** was built up from L-glutamic acid dihexadecyl ester which was treated with chloroacetic acid to obtain dicarboxylic acid **6**. Reaction of the latter with Boc-protected *N*-(2-aminoethyl)-2-aminoethanol **7** [15], followed by deprotection, guanidination with thiourea **1**, and final deprotection, produced compound **11**. The MALDI mass spectrum of **11** contained the molecular ion peak with *m*/*z* 969.794 $[M]^+$. Molecule **11** features increased number of cationic groups due to branching in the head group and the presence of ethylenediamine fragments.

Pyrene-containing fluorophore **19** was synthesized by a block method (Scheme 3). Bis-guanidine L-ornithine derivative **14** was obtained by reaction of L-ornithine ethyl ester dihydrochloride (**12**) with Boc-protected thiourea **1** and subsequent alkaline hydrolysis of the ester group. The IR spectrum of **14** showed absorption bands typical of a free carboxy group. Intermediate block **17** was synthesized by addition of pyren-1-ylmethanol to commercially available Boc-Gly-Gly (**15**) in the presence of DCC and 1-hydroxybenzotriazole (HOBt), followed by deprotection. The ^{1}H NMR spectrum of **17** contain no signal assignable to *tert*-butoxycarbonyl group, but signals of the CH_2 groups of the Gly-Gly fragment [δ 3.45 and 3.90 ppm, s (2H each)] and pyrene residue (δ 7.75–8.50 ppm) were present. The amidation of carboxylic acid **14** with amino acid **17** in the presence of DCC, NHS, and 4-dimethylaminopyridine (DMAP) and removal of the Boc protecting groups from **18** afforded conjugate **19**. The structures of **14**, **17**, and **19** were confirmed by ^{1}H NMR, IR, and mass spectra. The MALDI mass spectrum of **19** displayed the molecular ion peak with *m*/*z* 546.285 $[M]^+$.

Thus, we have proposed synthetic approaches to new guanidine-based cationic amphiphiles and their pyrene-containing analog starting from lipoamino acids and diamines. The synthesized compounds are planned to be used as drug and genetic material delivery agents, as well as for visualization of intracellular localization of cationic liposomes and lipoplexes derived therefrom.

EXPERIMENTAL

Commercially available L-ornithine (Acros Organics), 2-chloroacetic acid (Sigma Aldrich), L-aspartic acid (L-Asp, Sigma Aldrich), thiourea (Sigma Aldrich), 2-aminoethanol (Acros Organics), di-*tert*-butyl dicarbonate (Boc_2O, Sigma Aldrich), *N*,*N*′-dicyclohexylcarbodiimide (DCC, Sigma Aldrich), and trifluoroacetic acid (TFA, Biochem) were used without further purification.

The ^{1}H NMR spectra were recorded on a Bruker WM-400 spectrometer (Germany) at 400 MHz using chloroform-*d* as solvent and hexamethyldisiloxane as internal standard. The IR spectra were measured on a Bruker Equinox 55 spectrometer (Germany) with Fourier transform. The mass spectra (MALDI) were obtained with a Finnigan MAT Vision 2000 time-of-flight mass spectrometer using 2,5-dihydroxybenzoic acid as matrix. Elemental analysis was performed using a Thermo Finnigan Flash EA 1112 Series CHNS analyzer. Sorbfil plates (Krasnodar, Russia) were used for thin-layer chromatography with the following solvent systems as eluents: chloroform–methanol, 9 : 1 (A), 20 : 1 (B); toluene–ethyl acetate, 5 : 1 (C); toluene–chloroform–butan-2-one–propan-2-ol, 10 : 6 : 3 : 1 (D). Spots were visualized by heating in open flame (spirit lamp); compounds with carbon–carbon multiple bonds were detected by treatment with a 10% solution of potassium permanganate, and those with free amino groups, with a 5% ninhidrin solution, followed by heating to 50–80°C. Column chromatography was performed on silica gel (0.060–0.200 mm, 60 Å; Acros Organics, Belgium).

***N*,*N*″-Bis(*tert*-butoxycarbonyl)-*N*′-(2-hydroxyethyl)guanidine (2).** Thiourea **1**, 0.50 g (1.81 mmol), was added to a solution of 0.11 g (1.81 mmol) of 2-aminoethanol in THF, and the mixture was stirred for 10 h at room temperature. The product was purified by recrystallization from methanol. Yield 0.05 g (86%), R_f 0.90 (D). ^{1}H NMR spectrum, δ, ppm: 1.42 s (18H, *t*-Bu), 3.54–3.62 m (2H, CH_2), 3.78 t (2H, CH_2), 4.36 t (1H, OH), 5.40–5.48 m (1H, CH), 8.70 d (1H, NH), 11.41 d (1H, NH).

***N*′-{2-[(4-{[1,5-Bis(hexadecyloxy)-1,5-dioxopentan-2-yl]amino}-4-oxobutanoyl)oxy]ethyl}guanidinium trifluoroacetate (5).** Compound **3** [14], 0.07 g (0.10 mmol), was dissolved in methylene chloride, 30 mg (0.15 mmol) of DCC and 16 mg (0.11 mmol) of NHS were added, and the mixture was stirred for 1 h at 0°C. The precipitate was filtered off, 0.03 g (0.20 mmol) of compound **2** was added to the filtrate, and the mixture was stirred for 24 h at room temperature. The product was isolated by preparative thin-layer chromatography on silica gel using solvent system C as eluent and was deprotected by treatment with trifluoroacetic acid in methylene chloride for 3 h. The solvent and volatile compounds were removed under reduced pressure. Yield 14.00 mg (60%), amorphous material, R_f 0.20 (B). ^{1}H NMR spectrum, δ,

ppm: 0.90 t (6H, CH_3), 1.30 s [52H, $(CH_2)_{13}$], 1.54–1.70 m (4H, β-CH_2), 1.98–2.40 m (2H, β-CH_2, Glu), 2.50 t (2H, δ-CH_2, Glu), 2.55–2.65 m (4H, CH_2, Suc), 2.80 t (2H, NHC**H**$_2$$CH_2$), 3.44–3.56 m (4H, OCH_2), 3.92–4.18 m (4H, α-CH_2), 4.48–4.52 m (1H, α-H, Glu), 6.44 s (3H, NH). Found, %: C 67.58; H 10.79; N 7.21. $C_{44}H_{84}N_4O_7$. Calculated, %: C 67.65; H 10.84; N 7.17.

9-[1,5-Bis(hexadecyloxy)-1,5-dioxopentan-2-yl]-7,11-dioxo-6,12-dioxa-3,9,15-triazaheptadecane-1,17-diaminium bis(trifluoroacetate) (9). Diacid **6**, 0.50 g (0.70 mmol), was dissolved in methylene chloride, 0.18 g (0.90 mmol) of DCC and 12 mg (0.10 mmol) of DMAP were added, and the mixture was stirred for 1 h at 0°C. The precipitate was filtered off, 0.85 g (2.81 mmol) of **7** was added to the filtrate, and the mixture was stirred for 24 h at room temperature. The product was isolated by column chromatography and was deprotected by treatment with trifluoroacetic acid in methylene chloride for 3 h. The solvent was removed under reduced pressure. Yield 0.15 g (31%), R_f 0.15 (C). ^{1}H NMR spectrum, δ, ppm: 0.81 t (6H, CH_3), 1.21 s [52H, $(CH_2)_{13}$], 1.47–1.64 m (4H, β-CH_2), 1.68 s (4H, NCH_2CO), 1.79–2.01 m (2H, β-CH_2, Glu), 1.96 t (4H, NH_2), 2.03–2.40 m (2H, δ-CH_2, Glu), 2.46 t (1H, CH, Glu), 3.20–3.44 m (2H, α-CH_2), 3.56–3.74 m (4H, C**H**$_2$NH), 4.02 t (4H, CH_2O), 4.20–4.30 m (8H, NHC**H**$_2$C**H**$_2$$NH_3$), 4.31 t (4H, C**H**$_2$NH), 4.84–5.36 m (2H, NH).

***N′*^{1},*N′*^{2}-{9-[1,5-Bis(hexadecyloxy)-1,5-dioxopentan-2-yl]-7,11-dioxo-6,12-dioxa-3,9,15-triazaheptadecane-1,17-diyl}diguanidium bis(trifluoroacetate) (11).** Compound **9**, 0.10 g (0.11 mmol), was dissolved in THF, 0.063 g (0.22 mmol) of **1**, 0.074 g (0.27 mmol) of $HgCl_2$, and a catalytic amount of triethylamine were added, and the mixture was stirred for 10 h at room temperature. The product was isolated by recrystallization from methanol. Protecting groups were removed by treatment with trifluoroacetic acid in methylene chloride for 3 h. Yield 0.14 g (56%), amorphous powder, R_f 0.70 (A). Mass spectrum: *m*/*z* 969.784 $[M]^+$. Found, %: C 63.19; H 10.79; N 12.87. $C_{44}H_{84}N_4O_7$. Calculated, %: C 63.12; H 10.70; N 12.99. *M* 969.793.

(2*S*)-2,5-Bis{[*N*,*N′*-bis(*tert*-butoxycarbonyl)carbamimidoyl]amino}pentanoic acid (14). A solution of 1.00 g (3.60 mmol) of **1** in 3 mL of DMF was added at room temperature to a solution of 0.3 g (1.78 mmol) of ester **12** in 3 mL of DMF. The solvent was removed under reduced pressure, and compound **13** was isolated by column chromatography using solvent system D as eluent. The product was dissolved in 20 mL of methanol, and potassium hydroxide was added at room temperature until pH 12. The mixture was stirred for 3 h at room temperature, KU-9 (H^+) exchanger was added, and the mixture was stirred for 3 h more and filtered. Yield of **14** 0.210 g (78%), R_f 0.25 (A). IR spectrum (film), ν, cm^{-1}: 3353 (OH), 3103 (NH), 2929 (CH_3), 1740 (C=O), 1251 (C–O–C), 1171 (C–N). ^{1}H NMR spectrum, δ, ppm: 1.50 s (36H, *t*-Bu), 1.62–1.90 m (4H, β,γ-CH_2), 3.26–3.56 m (2H, δ-CH_2), 3.80 s (1H, CH), 7.80 q (2H, NH).

2-Oxo-2-{[2-oxo-2-(pyren-1-ylmethoxy)ethyl]-amino}ethan-1-aminium trifluoroacetate (17). Compound **15**, 0.15 g (0.60 mmol), was dissolved in anhydrous methylene chloride, 0.23 g (1.14 mmol) of DCC and 0.135 g (1.14 mmol) of HOBt in DMF were added, and the mixture was stirred for 2 h at 0°C. The precipitate of dicyclohexylurea was filtered off, 0.14 g (0.60 mmol) of pyren-1-ylmethanol was added to the filtrate, and the mixture was stirred for 24 h at room temperature. The product was purified by column chromatography using solvent system D as eluent. Intermediate **16** thus obtained was dissolved in 50 mL of chloroform, a solution of 0.20 g (1.68 mmol) of 50% trifluoroacetic acid in chloroform was added dropwise, and the mixture was stirred for 1 h on a magnetic stirrer until a white solid separated. The solvent and excess trifluoroacetic acid were removed under reduced pressure. Yield 0.13 g (48%), R_f 0.50 (A). ^{1}H NMR spectrum, δ, ppm: 3.45 s (2H, CH_2), 3.90 s (2H, CH_2), 4.91 s (2H, CH_2O), 5.22–5.58 m (2H, NH_2), 7.75–8.50 m (9H, H_{arom}).

***N′*^{1},*N′*^{2}-((4*S*)-5-Oxo-5-{[2-oxo-2-(pyren-1-ylmethoxy)ethyl]amino}pentane-1,4-diyl)diguanidinium bis(trifluoroacetate) (19).** Compound **14**, 0.21 g (0.34 mmol), was dissolved in THF, 0.14 g (0.68 mmol) of DCC, 78 mg (0.68 mmol) of NHS, and 83 mg (0.68 mmol) of DMAP were added, and the mixture was stirred for 24 h at 0°C. The precipitate of dicyclohexylurea was filtered off, a solution of 0.13 g (0.34 mmol) of **17** in 100 mL of THF was added to the filtrate, and the mixture was stirred for 48 h at 25°C. Intermediate product **18** was isolated by column chromatography using solvent system D as eluent. It was dissolved in 50 mL of chloroform, a solution of 0.20 g (1.68 mmol) of 30% trifluoroacetic acid in chloroform was added dropwise, and the mixture was stirred for 1 h on a magnetic stirrer until a white solid separated. The solvent was removed under reduced pressure. Yield 0.05 g (55%), white powder, R_f 0.51 (A). ^{1}H NMR spectrum, δ, ppm: 1.70–2.00 m (4H,

β-CH_2, γ-CH_2, Orn), 3.37–3.43 m (2H, δ-CH_2, Orn), 3.42–3.48 m (2H, CH_2), 3.50–3.70 m (2H, CH_2), 4.30 s (1H, CH), 5.48 s (2H, CH_2O), 7.75–8.50 m (9H, H_{arom}). Mass spectrum: *m*/*z* 546.285 $[M]^+$. Found, %: C 61.59; H 6.31; N 20.77. $C_{28}H_{34}N_8O_4$. Calculated, %: C 61.52; H 6.27; N 20.50. *M* 546.270.

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CONFLICT OF INTERESTS

The authors declare the absence of conflict of interests.

REFERENCES

1. Ozpolat, B., Sood, A.K., and Lopez-Berestein, G., *Adv. Drug Delivery Rev*., 2014, vol. 66, p. 110. https://doi.org/10.1016/j.addr.2013.12.008
2. Martin, B., Sinlos, M., Aissaoui, A., Oudrhiri, N., Hauchecorne, M., Vigneron, J.-P., Lehn, J.-M., and Lehn, P., *Curr. Pharm. Des*., 2005, vol. 11, p. 375. https://doi.org/10.2174/1381612053382133
3. Leal, C., Ewert, K., Shirazi, S., Bouxsein, N., and Safinya, C., *Langmuir*, 2011, vol. 27, p. 7691. https://doi.org/10.1021/la200679x
4. Hoyer, J. and Neundorf, I., *Acc. Chem. Res*., 2012, vol. 45, p. 1048. https://doi.org/10.1021/ar2002304
5. Martin, M. and Rice, K., *AAPS J*., 2007, vol. 9, p. E18. https://doi.org/10.1208/aapsj0901003
6. Koloskova, O.O., Gileva, A.M., Drozdova, M.G., Grechihina, M.V., Suzina, N.E., Budanova, U.A., Sebyakin, Yu.L., Kudlay, D.A., Shilovskiy, I.P., Sapozhnikov, A.M., Kovalenko, E.I., Markvicheva, E.A., and Khaitov, M.R., *Colloids Surf., B*, 2018, vol. 167, p. 328. https://doi.org/10.1016/j.colsurfb.2018.04.003
7. Koloskova, O.O., Nikonova, A.A., Budanova, U.A., Shilovskiy, I.P., Kofiadi, I.A., Ivanov, A.V., Smirnova, O.A., Zverev, V.V., Sebyakin, Yu.L., Andreev, S.M., and Khaitov, M.R., *Eur. J. Pharm. Biopharm*., 2016, vol. 102, p. 159. https://doi.org/10.1016/j.ejpb.2016.03.014
8. Zhang, X.-X., La Manna, C.M., Kohman, R.E., McIntosh, T.J., Han, X., and Grinstaff, M.W., *Soft Matter*, 2013, vol. 9, p. 4472. https://doi.org/10.1039/C3SM27633C
9. Sen, J. and Chaudhuri, A., *J. Med. Chem*., 2005, vol. 48, p. 812. https://doi.org/10.1021/jm049417w
10. Wexselblatt, E., Esko, J., and Tor, Y., *J. Org. Chem*., 2014, vol. 79, p. 6766. https://doi.org/10.1021/jo501101s
11. Sheng, R., An, F., Wang, Z., and Li, M., *RSC Adv*., 2015, vol. 5, p. 12338. https://doi.org/10.1039/C4RA06879C
12. Exposito, A., Fernandez-Suarez, M., Iglesias, T., Munoz, L., and Riguera, R., *J. Org. Chem*., 2001, vol. 66, p. 4206. https://doi.org/10.1021/jo010076t
13. Katritzky, A.R. and Rogovoy, B.V., *Arkivoc*, 2005, part (iv), p. 49. https://doi.org/10.3998/ark.5550190.0006.406
14. Koloskova, O.O., Borodin, Yu.G., Budanova, U.A., and Sebyakin, Yu.L., *Biofarm. Zh*., 2010, vol. 2, no. 6, p. 16.
15. Marusova (Soloveva), V.V., Zagitova, R.I., Budanova, U.A., and Sebyakin, Y.L., *Moscow Univ. Chem. Bull. (Engl. Transl.)*, 2018, vol. 73, p. 74. https://doi.org/10.3103/S0027131418020098