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Novel Branched Polyamine-Modified Chlorin with a Photoinduced Antimicrobial Activity

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Abstract—Multiple drug resistance is one of the major threats to global health. One of the approaches to solving this problem is antimicrobial photodynamic therapy, however, currently used photosensitizers are not sufficiently effective against pathogens. Polycationic molecular constructs enhance the binding and penetration of photosensitizers into poorly permeable gram-negative bacteria. Such conjugates can be obtained by introducing polyethyleneimines into a photosensitizer molecule. In this study, we synthesized a branched tetraamine and introduced it into the pyrrole ring A of the natural chlorin molecule and assessed *in vitro* the photoinduced toxicity of the new photosensitizer against *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and *Escherichia coli* bacteria. Compared to its structural precursor, the obtained chlorin with a branched polyamine residue demonstrated an increased bactericidal effect when irradiated with light.

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INTRODUCTION

Multiple drug resistance of microorganisms is one of the serious threats to global health. The development of bacterial resistance to existing drugs and the shortage of structures that can potentially form the basis for new antibiotics have made the search for new approaches to combating antibiotic-resistant pathogens a priority. Among the new innovative methods is antimicrobial photodynamic therapy (APDT), which is equally effective against antibiotic-sensitive and antibiotic-resistant pathogens [1]. APDT uses photosensitizers (PSs) in combination with visible light of a certain wavelength for their excitation. The transfer of energy from a PS to molecular oxygen results in the formation of reactive oxygen species, including singlet oxygen as the main cytotoxic agent that kills bacteria by oxidizing their vital intracellular structures. The principal advantage of APDT is its ability to oxidatively destroy multiple structures within the target microbial cells, preventing them from developing resistance to subsequent cycles of photodynamic exposure. In addition, since the bactericidal effect of APDT is local, APDT does not exert a systemic destructive effect on the body's obligate microflora. Therefore, APDT simultaneously solves two main problems of modern antibiotic therapy: it overcomes the high drug resistance of pathogenic microorganisms and eliminates the possibility of systemic drug toxicity [2].

Previous studies have shown that gram-positive and gram-negative bacteria differ in their susceptibility to APDT. While neutral, anionic, and cationic PS molecules bind equally well to gram-positive bacteria, only cationic or neutral PSs bind to gram-negative bacteria. Several studies have demonstrated that the

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most effective photosensitizers for APDT contain positively charged groups [3].

A traditional method of synthesizing cationic PSs involves quaternization of nitrogen atoms in aliphatic amine residues located on the periphery of the tetrapyrrole macrocycle. As a rule, such alkylammonium derivatives can easily enter bacterial cells and, therefore, exhibit high photoinduced cytotoxicity. Several tetrapyrrole compounds (derivatives of porphyrins and their hydrogenated analogs containing alkylammonium substituents) have been described and their antimicrobial activity has been studied [4-6]. Another approach to developing cationic PSs for APDT is the synthesis of pyridyl-containing pigments and their subsequent quaternization with alkyl halides. This approach reduces the "dark" toxicity of PSs while maintaining their high photoinduced activity. Various tetrapyrrole macroheterocyclic compounds have been described, including derivatives of natural chlorophyll A containing one or more positively charged pyridinium groups at the macrocycle periphery [7-11]. Also, chlorin derivatives containing other heterocyclic fragments have been synthesized [12]. Our research group has previously produced chlorin and bacteriochlorin PSs modified with the residues of nicotinic and isonicotinic acids which exhibited a high photoinduced toxicity against gram-negative and gram-positive bacteria in biofilms, as well as in infected wound models *in vivo* [13-15].

Development of polycationic molecular constructs should facilitate internalization of PSs in gram-negative bacteria. One method of their production is introduction of various polyethyleneimines into a PS molecule. Hamblin et al. [16] described the synthesis of several chlorin e_6 derivatives containing fragments of both linear and branched polyethyleneimines [16]. The resulting cationic PSs, and particularly a derivative with a highly branched, high-molecular-weight amine, demonstrated a high antimicrobial activity against pathogenic microorganisms. However, despite their high biological activity, these conjugates remain difficult to synthesize and purify, which hinders their further use. The high-molecular-weight nature of the initial polyamines implies formation of a mixture of PS molecules with different molecular weights, which significantly limits the prospects of their clinical application. In this study, we introduced a low-molecular-weight branched polyamine into the chlorin molecule and investigated the photoinduced antimicrobial toxicity of the resulting derivate.

MATERIALS AND METHODS

Equipment and materials. Chlorin e_6 trimethyl ester (**5**) was obtained from pheophorbide A methyl ester according to a previously described procedure [17]. Oxidation of chlorin e_6 trimethyl ester vinyl group to produce carboxyl derivative **6** was performed as described in [18]. Organic solvents were purified and prepared according to standard procedures. Kieselgel 40/60 silica gel and Kieselgel 60 silica gel (Merck, Germany) were used for column chromatography and preparative thin-layer chromatography (TLC), respectively. Analytical TLC was performed on Kieselgel 60 F_{245} plates (Merck). Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker DPX300 spectrometer in $CDCl_3$. Residual signals of ^1H nuclei were used to calibrate the scale. The experiments were performed according to standard Bruker procedures. High-resolution mass spectra were recorded on an Orbitrap Elite mass spectrometer (Thermo Scientific, USA). Absorption spectra were recorded on a Shimadzu UV1800 UV/VIS spectrophotometer in CH_2Cl_2. To measure the quantum yield of singlet oxygen generation, we used 5,6,11,12-tetraphenyltetracene as a trap and red light-emitting diode (LED) as a light source (λ = 660 ± 20 nm, Ps = 5 mW/cm^2) as described in [19]. For biological studies, LED-based irradiator (λ = 660 ± 10 nm, Ps = 10 mW/cm^2) was used as a source of red light.

Synthesis of polyamine 3. *tert*-Butyl (2-aminoethyl)carbamate (**2**) (1.246 g; 7.777 mmol) was dissolved in tetrahydrofuran (THF) (60 ml) under an argon atmosphere. Ethyl 3-bromo-2-(bromomethyl) propanoate (**1**) (532 mg; 1.942 mmol) in THF (18 ml) was added dropwise to the resulting solution over 6 min with vigorous stirring. The reaction mixture was stirred for 12 h at room temperature in an inert atmosphere, while the reaction was monitored by analytical TLC [Rf = 0.30; Hex (hexane) /EtOAc (ethyl acetate) as an eluent, 2/1, *v*/*v*]. Next, di-*tert*-butyl dicarbonate (1.693 g; 7.768 mmol) was added to the reaction mixture at 0°C. The mixture was stirred for another 12 h at room temperature under an inert atmosphere. The solvent was removed *in vacuo*. The desired product **3** was isolated by column chromatography (isocratic elution; Hex/EtOAc, 2/1, *v*/*v*). As a result, 128 mg of product **3** was obtained with a yield of 10.6%. ^1H NMR (300 MHz, $CDCl_3$, δ, ppm): 5.08 (2H, m, 2xNH); 4.14-4.07 (2H, q, O$\underline{CH_2}CH_3$); 3.35-3.09 (13H, m, CH, 2xCH$\underline{CH_2}$N, 2xN$\underline{CH_2}CH_2$NH, 2xNCH$_2\underline{CH_2}$NH); 1.44-1.41 (36H, m, 12xCH_3); 1.25-1.21 (3H, t, OCH$_2\underline{CH_3}$). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 173.27; 155.88-155.84; 129.39; 80.30; 79.04; 68.36; 60.88; 47.94; 44.79-44.75; 39.34; 30.79-27.68; 22.10; 14.03. ESI-HRMS *m/z* calculated $[M+H]^+$: 633.4075; found: 633.4050; calculated $[M+Na]^+$: 655.3894; found: 655.3865.

Synthesis of polyamine 4. Compound **3** (64 mg; 0.104 mmol) was dissolved in a mixture of THF (0.83 ml) and methanol (0.83 ml). Next, potassium hydroxide (113.7 mg; 2.026 mmol) dissolved in distilled

water (0.83 ml) was added to the resulting solution. The reaction mixture was stirred at room temperature for 4 h, while the reaction was monitored by analytical TLC [Rf = 0.15; eluent, EtOAc/Ethanol, 9/1, *v*/*v*]. After complete conversion of the starting reagent, the mixture was diluted with distilled water (5 ml) and citrate buffer (3 ml, pH 5). The product was isolated from the reaction mixture by extraction with ethyl acetate (50 ml). The organic layer was dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. As a result, 41 mg of compound **4** was obtained (yield, 65.1%). ESI-HRMS *m/z* calculated $[M+H]^+$: 605.3762; found: 605.3763; calculated $[M+Na]^+$: 627.3581; found: 627.3571.

Synthesis of chlorin 7. 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) solution (71.75 mg; 0.189 mmol) and triethylamine (26.3 μl) in N,N-dimethylformamide (DMF) (5 ml) were added to derivative **6** (113 mg; 0.172 mmol). The reaction mixture was stirred under argon atmosphere at room temperature for 60 minutes, after which a solution of *Boc*-ethylenediamine 2 (30.3 mg; 0.189 mmol) in DMF (1 ml) was added to the mixture. After 15 min, the reaction mixture was evaporated under reduced pressure, and the residue was redissolved in CH_2Cl_2 (50 ml) and washed with water (2×100 ml). The organic layer was dried over Na_2SO_4 and evaporated *in vacuo*. The product was isolated by preparative TLC (CH_2Cl_2/MeOH (methanol), 20/1, *v*/*v*) resulting in 135 mg of compound **7** with a yield of 98%. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 9.80 (H, s, 10-H); 9.63 (H, s, 5-H); 8.81 (H, s, 20-H); 7.20 (H, m, 3^2-NH); 5.36-5.21 (2H, q, J = 19.6 Hz,15^1-CH_2^a, 15^1-CH_2^b); 5.21 (H, m, 3^5-NH); 4.53-4.40 (2H, m, 18-H, 17-H); 4.28 (3H, s, 13^1-$COOCH_3$); 3.87 (2H, m, 8^1-CH_2^a, 8^1-CH_2^b); 3.78 (3H, s, 15^2-$COOCH_3$); 3.65 (3H, s, 17^3-$COOCH_3$); 3.65-3.56 (2H, m, 3^3-CH_2); 3.65-3.56 (2H, m, 3^4-CH_2); 3.56 (3H, s, 12-CH_3); 3.22 (3H, s, 7-CH_3); 2.65-2.51 (2H, m, 17^2-CH_2^a , 17^2-CH_2^b); 2.28-2.16 (2H, m, 17^1-CH_2^a, 17^1-CH_2^b); 1.74 (3H, d, J = Hz, 18-CH_3); 1.71 (3H, t, J = Hz, 8^2-CH_3); 1.37 (9H, s, 3^6-C(CH_3)$_3$); -1.49 (H, I-NH); -1.80 (H, III-NH). ESI-HRMS *m/z* calculated $[M+H]^+$: 798.400; found: 798.400. UV/VIS (CH_2Cl_2) λ_{max}, nm (ε, M^{-1} cm^{-1}) 400 (121000); 501 (11220); 531 (4700); 614 (4560); 668 (43900).

Synthesis of chlorin 8. Compound **7** (47 mg; 0.059 mmol) was dissolved in freshly prepared 10% CF_3COOH in CH_2Cl_2; the reaction mixture was stirred under argon atmosphere for 30 min, then diluted with CH_2Cl_2 (50 ml) and washed with 5% Na_2CO_3 solution (2×100 ml). The organic layer was dried over Na_2SO_4 and evaporated *in vacuo*. The residue was redissolved in DMF (1.5 ml), and the resulting solution was added to a solution of compound **4** (41 mg; 0.068 mmol) with HBTU (28 mg; 0.074 mmol) and triethylamine (TEA) (7.6 mg; 0.075 mmol) in DMF (2 ml). The reaction mixture was stirred under argon atmosphere for 16 h. After complete conversion of compound **4**, the reaction mixture was evaporated *in vacuo*; the residue was redissolved in CH_2Cl_2 and washed with distilled water (4×100 ml). The organic layer was dried over Na_2SO_4 and evaporated *in vacuo*. The target compound **8** was purified by preparative TLC in CH_2Cl_2/MeOH (45/1, *v*/*v*). Yield, 13 mg; 17.1%. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 9.40 (H, s, 10-H); 9.36 (H, s, 5-H); 8.79 (H, s, 20-H); 6.97 (H, m, 3^2-NH); 6.56 (H, m, 3^5-NH); 5.44-5.27 (2H, q, J = 18.7 Hz, 15^1-CH_2^a, 15^1-CH_2^b); 4.50-4.43 (2H, m, 18-H, 17-H); 4.29 (3H, s, 13^1-$COOCH_3$); 3.81 (3H, s,15^2-$COOCH_3$); 3.67 (3H, s, 17^3-$COOCH_3$); 3.45 (3H, s, 12-CH_3); 3.31 (3H, s, 7-CH_3); 2.64-2.50 (2H, m, 17^2-CH_2^a, 17^2-CH_2^b); 2.26-2.15 (2H, m, 17^1-CH_2^a, 17^1-CH_2^b); 1.79 (3H, d, J = 7.8 Hz, 18-CH_3); 1.79 (3H, t, J = 8.6 Hz, 8^2-CH_3); 1.44 (36H, s, Boc). ESI-HRMS *m/z* calculated $[M+H]^+$: 1284.7006; found: 1284.7059. UV/VIS (CH_2Cl_2) λ_{max}, nm (ε, M^{-1} cm^{-1}) 401 (122000); 501 (11240); 530 (4700); 612 (4500); 667 (43840).

Synthesis of conjugate 9. Compound **8** (10 mg; 0.0078 mmol) was dissolved in freshly prepared 50% CF_3COOH in CH_2Cl_2, and the reaction mixture was stirred under argon atmosphere for 60 min. The reaction progress was monitored by analytical TLC in the CH_2Cl_2/MeOH mixture (20 : 1, *v*/*v*). The reaction mixture was diluted with CH_2Cl_2 (50 ml) and washed with 10% Na_2CO_3 solution (2×100 ml). The organic layer was dried over Na_2SO_4 and evaporated *in vacuo* to obtain product **9** (5 mg; yield, 72.5%). ESI-HRMS *m/z* calculated $[M+H]^+$: 884.4909; found: 884.4898. UV/VIS (CH_2Cl_2) λ_{max}, nm (ε, M^{-1} cm^{-1}): 401 (121300); 501 (11250); 534 (4730); 613 (4540); 668 (43900).

Photoinduced toxicity assay. To study the antimicrobial activity, the following reference strains of gram-positive and gram-negative bacteria were used: *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, and *Escherichia coli* ATCC 25922. The initial concentration of the microbial suspension in the experiments was 3×10^3 CFU/ml. Cells in the positive control groups were kept under identical conditions and exposed to light but without preincubation with the PS. Cells in the negative control groups were neither exposed to light nor incubated with the PS. Microbial suspension was added to the wells of a microplate (150 μl per well), and solutions of compounds **5** and **9** were added so that the final PS concentration reached 0.44, 1.32, and 3.96 μM, respectively . The cells were incubated with PSs for 30 min and then irradiated with red light (λ = 660 nm; P_s = 10 mW/cm^2) until a light dose of 10 J/cm^2 was achieved. After irradiation, the microplates were incubated for 24 h at 37°C. To determine microbial survival rate, bacterial cells were seeded on a dense agar medium with a 2-μL bacteriological loop. After 24-h incubation, the number of grown colonies was determined. The experiments were carried out in triplicate.

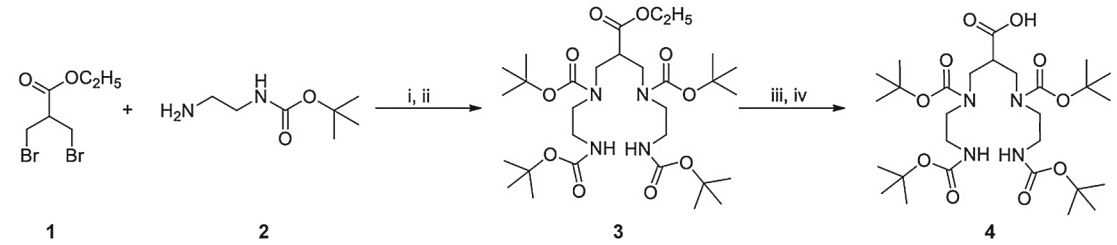
RESULTS AND DISCUSSION

As the initial PS molecule, we selected chlorin e_6 trimethyl ether, which is a derivative of natural chlorophyll A. Drugs based on this class of compounds have proven to be effective as PSs in photodynamic therapy and are actively used in clinical practice [20-24]. Using the information on how the position of a substituent affects the biological activity [25-27], we introduced the polyamine residue into pyrrole A of the chlorin macrocycle. To synthesize the chlorin conjugate with the polyamine, we acquired a branched tetraamine and chemically modified the vinyl group of the chlorin macrocycle.

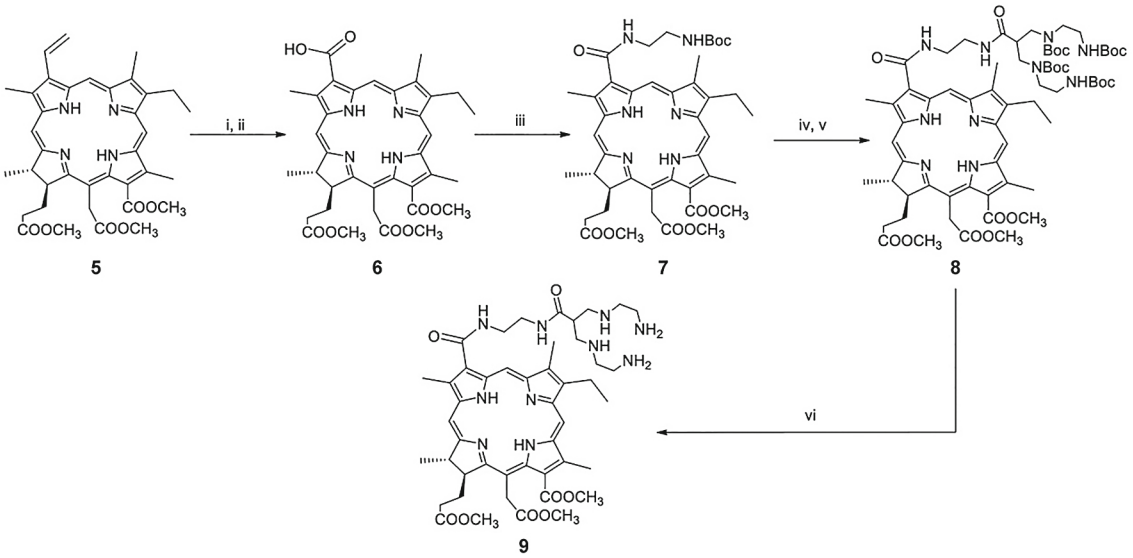
As a polyamine for conjugation with chlorin, we selected symmetrical *N*,*N*′-bis(2-aminoethyl)-1,3-propanediamine containing a carboxyl group in position 2. The synthesis of this compound from ethylenediamine and 3-bromo-2-(bromomethyl)propanoic acid was described in earlier papers [28], but we opted for an alternative method in order to simplify purification of the target compound, increase its purity, and reduce formation of transalkylation by-products.

The starting compound for the polyamine synthesis was ethyl 3-bromo-2-(bromomethyl)propanoate (**1**) obtained from diethyl malonate according to a previously described method [29, 30]. As a result of 1-[(tert-butoxycarbonyl)amino]-2-aminoethane (**2**) alkylation with dibromo derivative **1** and subsequent treatment of the reaction mixture with di-*tert*-butyl dicarbonate, branched polyamine **3** was obtained (Scheme 1). To attach the latter to chlorin, the ester group was hydrolyzed under alkaline conditions to obtain derivative **4**. The structure of the resulting tetraamine was confirmed by high-performance liquid chromatography coupled with mass spectrometry (HPLC/MS). A molecular ion corresponding to this structure was found in the high-resolution mass spectrum of compound **4**.

Ethylenediamine was used as a linker to introduce polyamine **4** into chlorin molecule (Scheme 2). The amidation of chlorin **6** with 1-[(tert-butoxycarbonyl)amino]-2-aminoethane **2** was carried out using HBTU to obtain the target compound **7** with a high yield. The transformations of substituents in pyrrole A were accompanied by noticeable changes in the



Scheme 1. Reagents and conditions: (i) TEA, THF, 24 h; (ii) Boc_2O, 1 h, 5°C then 12 h, 20°C; (iii) KOH, H_2O/THF/MeOH, 4 h, 20°C; (iv) H_2O, citrate buffer (pH 5), 20°C.



Scheme 2. Reagents and conditions: (i) OsO_4, THF, $NaIO_4/H_2O$, Ar, 0.75 h, 20°C; (ii) THF, NH_2SO_2OH, dimethyl sulfoxide (DMSO), $NaClO_2/H_2O$, Ar 0.5 h, 20°C; (iii) HBTU, TEA, DMF, Ar, then **2**, 0.3 h, 20°C; (iv) 10% CF_3COOH/CH_2Cl_2, Ar, 0.5 h, 20°C; (v) DMF, **4**, HBTU, TEA, Ar, 12 h, 20°C; (vi) 50% CF_3COOH/CH_2Cl_2, Ar, 0.5 h, 20°C, then Na_2CO_3/H_2O.

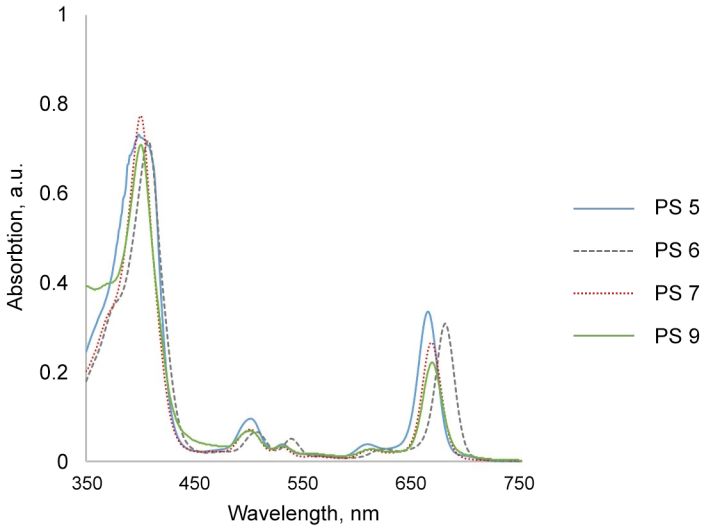


Fig. 1. UV/VIS spectra of compounds **5**-**7** and conjugate **9** in CH_2Cl_2.

spectral properties of chlorin e_6 derivatives. Thus, oxidation of the chlorin vinyl group to a formyl group was accompanied by a bathochromic shift by 30 nm (up to 693 nm) of the maximum in the electronic absorption spectrum. Subsequent oxidation to carboxylic acid **6** was accompanied by a hypsochromic shift of the absorption maximum by 10 nm, and then by another shift by 15 nm during amidation (Scheme 1).

After removal of the protecting Boc-group in compound **7**, it was coupled with the tetraamine moiety **4** using HBTU to produce conjugate **8**. The structure of this compound was confirmed by NMR spectroscopy. The ^{1}H NMR spectrum of the conjugate contained signals from the polyamine moiety (protons of CH, CH_2-groups in the region of 3.4-3.1 ppm; Boc – 1.45 ppm) and amide bonds (6.98 and 6.57 ppm), as well as a set of signals characteristic of the chlorin macrocycle. The shift of the signal of the *meso*-proton of chlorin in position 5 of the macrocycle to a stronger field also indicated successful conjugation. A molecular ion corresponding to the calculated value was detected in the high-resolution mass spectrum of compound **8**.

To remove the protecting Boc-groups, compound **8** was treated with a 50% solution of trifluoroacetic acid in dichloromethane, followed by washing the reaction mixture with a sodium carbonate solution and isolation of chlorin **9** as a free amine. A signal corresponding to the calculated value for the divalent ion was detected in the high-resolution mass spectrum of compound **9**. Therefore, we have successfully introduced a branched tetraamine fragment into pyrrole A of chlorin e_6 trimethyl ester.

Studying the photophysical properties of the obtained compound showed that the introduction of the tetraamine residue into pyrrole A led to an insignificant bathochromic shift of the long-wave absorption maximum from 664 nm (original chlorin e_6 trimethyl ester) to 669 nm (Fig. 1). The quantum yield of the singlet oxygen generation was measured using 5,6,11,12-tetraphenyltetracene as a trap according to a previously described method [19]. Modification of position 3 led to a slight decrease in this parameter (Table 1). The lack of obvious differences in the photophysical parameters of PS molecules **5** and **9** allowed us to evaluate the effect of the polyamine fragment on the compound internalization into pathogenic bacterial cells *in vitro*.

The photoinduced antimicrobial cytotoxicity of the obtained chlorin **9** was assessed against a number of gram-positive and gram-negative bacteria (*S. aureus*, *E. coli*, *E. faecalis*, *P. aeruginosa*) (Fig. 2) using original chlorin e_6 trimethyl ester (**5**) for comparison. The water-soluble forms of the PSs were obtained as emulsions using Kolliphor ELP surfactant according to the method previously described by our research group [31]. Bacterial cells were incubated for 30 min with the PS solutions at the concentrations of 0.44, 1.32, and 3.96 μM. To assess the photocytotoxic effect, the cells were irradiated with red light (λ = 660 ± 10 nm, P_s = 10 mW/cm^2) until a light dose of 10 J/cm^2 was achieved. Bacterial cells that were irradiated in the absence of PS were used as a control group.

Table 1. Photophysical parameters of the obtained conjugate **9** and original chlorin e6 trimethyl ester (**5**)

Compound	Absorption maximum, λ, nm	Singlet oxygen quantum yield
5	664	0.55 ± 0.01
9	669	0.49 ± 0.01

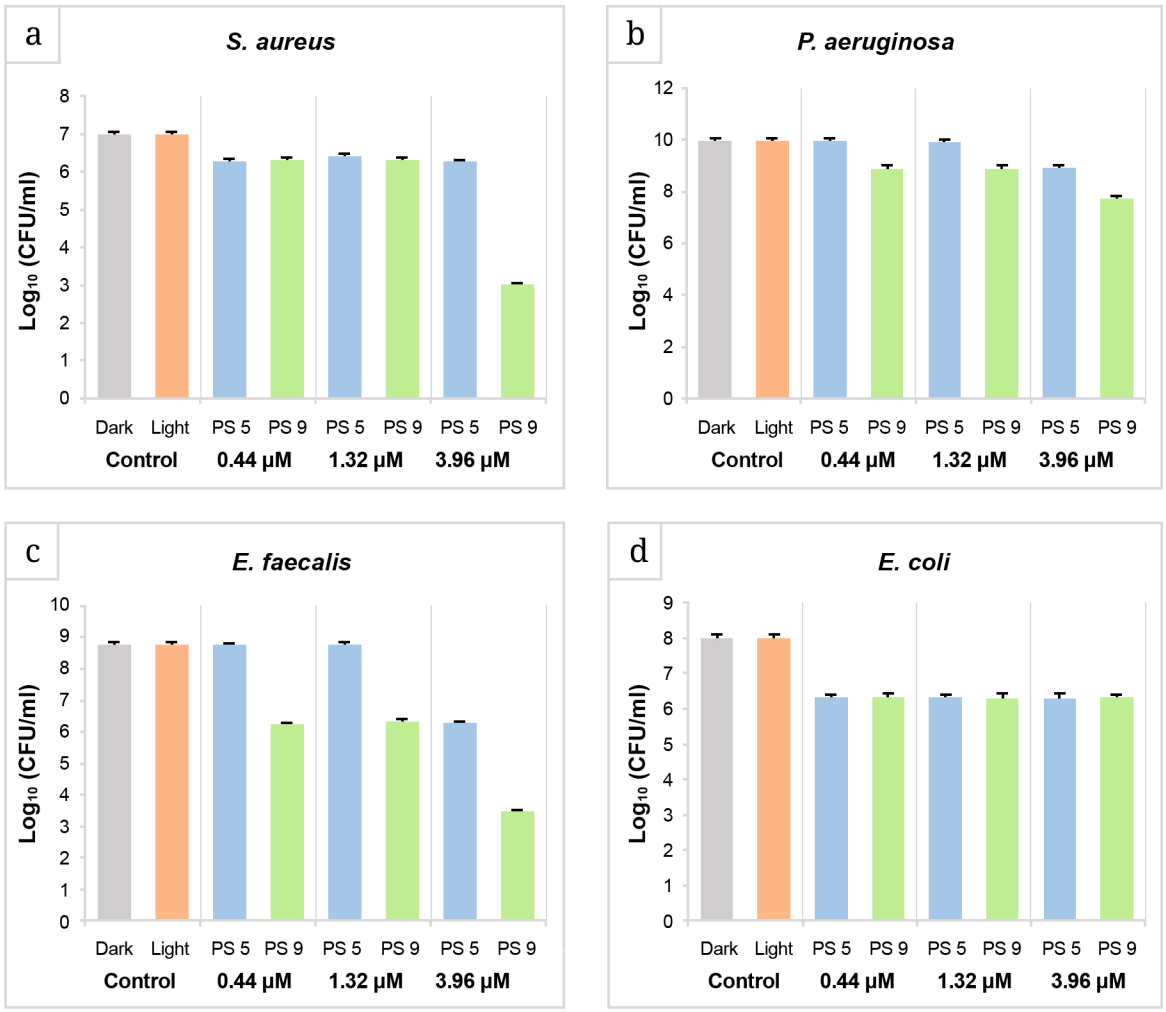


Fig. 2. Photoinactivation of gram-positive *S. aureus* and *E. faecalis* (a, c) and gram-negative *P. aeruginosa* and *E. coli* (b, d) using photosensitizers **5** and **9**.

No suppression of bacterial growth was detected upon irradiation with red light in the absence of PS, while exposed to light of cells that had been preincubated with compounds **5** or **9** resulted in the concentration-dependent inhibition of bacterial growth. Tetraamine-modified chlorin **9** exhibited greater photoinduced cytotoxicity than the original compound **5** (Fig. 2), which was evidenced by the fact that the compound **9** at a concentration of 3.96 μM caused stable photoinactivation of gram-positive bacteria *S. aureus* and *E. faecalis*. Virtually no photoinactivation of the gram-negative bacterium *P. aeruginosa* was found for PS **5** at the concentrations of 0.44 μM and 1.32 μM, whereas the photosensitizing effect of PS **9** on this strain was an order of magnitude greater over the entire range of the studied concentrations. The bacteriostatic effect of compounds **5** and **9** toward light-irradiated *E. coli* was similar. It should be noted that in the studied concentration range, the efficacy of both compounds against gram-negative bacteria was lower than against gram-positive ones. This is consistent with previous studies, in which inactivation of gram-negative bacteria required higher PS concentrations and higher light doses [16, 32]. Nevertheless, our findings suggest that the introduction of low-molecular-weight branched tetraamine into the chlorin molecule increases its affinity to bacterial cell wall.

CONCLUSION

In this study, we synthesized a new PS containing a branched polyamine residue in the pyrrole A of chlorin macrocycle and examined its photoinduced antimicrobial activity. The introduction of a polyamine structural fragment into the chlorin molecule facilitated its internalization into pathogenic cells and, therefore, consequently enhanced the photoinduced cytotoxicity of the resulting compound.

The properties of the photosensitizer can presumably be improved by quaternization of polyamine nitrogen atoms to produce alkylammonium salts. We plan to test this hypothesis in future studies.

Abbreviations. APDT, antimicrobial photodynamic therapy; Boc, *tert*-butoxycarbonyl; DMF, N,N-dimethylformamide; ESI, electrospray ionization; HBTU, 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HRMS, high-resolution mass spectrometry; NMR, nuclear magnetic resonance; TLC, thin-layer chromatography.

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Contributions. N.V.S., M.A.G., and Yu.L.V. developed the concept and supervised the study; G.M.G., E.A.S., M.A.S., D.A.M., and V.V.S. performed the experiments; N.V.S., M.A.S., and M.A.G. discussed the results; N.V.S. wrote the draft of the article; N.V.K. and D.A.M edited the manuscript.

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Ethics approval and consent to participate. This work does not contain any studies involving human and animal subjects.

Conflict of interest. The authors of this work declare that they have no conflicts of interest.

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