CHEMISTRY AND TECHNOLOGY OF MEDICINAL COMPOUNDS AND BIOLOGICALLY ACTIVE SUBSTANCES

ХИМИЯ И ТЕХНОЛОГИЯ ЛЕКАРСТВЕННЫХ ПРЕПАРАТОВ И БИОЛОГИЧЕСКИ АКТИВНЫХ СОЕДИНЕНИЙ

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RESEARCH ARTICLE

Dedicated to Professor A.F. Mironov on the occasion of his 85th birthday

Amino acid derivatives of natural chlorins as a platform for the creation of targeted photosensitizers in oncology

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Objectives. *This study aims to obtain the amino acid derivatives of chlorophyll a and bacteriochlorophyll a for the targeted delivery of pigments to tumor foci. This will increase biocompatibility and, as a result, reduce toxic side effects. In addition to photodynamic efficiency, an additional cytotoxic effect is expected for the obtained conjugates of photosensitizers (PSs) with amino acids. This is owing to the participation of the latter in intracellular biochemical processes, including interaction with the components of the glutathione antioxidant system, leading to the vulnerability of tumor cells to oxidative stress.*

Methods. *In this work, we have implemented the optimization of the structure of a highly efficient infrared PS based on O-propyloxim-N-propoxybacteriopurpurinimide (DPBP), absorbing at 800 nm and showing photodynamic efficacy for the treatment of deep-seated and pigmented tumors, by introducing L-lysine, L-arginine, methionine sulfoximine (MSO), and buthionine sulfoximine (BSO) methyl esters. The structure of the obtained compounds was proved by mass spectrometry and nuclear magnetic resonance spectroscopy, and the photoinduced cytotoxicity was studied in vitro on the HeLa cell line.*

Results. *Conjugates of DPBP with amino acids and their derivatives, such as lysine, arginine, MSO, and BSO have been prepared. The chelating ability of DPBP conjugate with lysine was shown, and its Sn(IV) complex was obtained.*

Conclusions. *Biological testing of DPBP with MSO and BSO showed a 5–6-fold increase in photoinduced cytotoxicity compared to the parent DPBP PS. Additionally, a high internalization of pigments by tumor cells was found, and the dark cytotoxicity (in the absence of irradiation) of DPBP-MSO and DPBP-BSO increased fourfold compared to the initial DPBP compound. This can be explained by the participation of methionine derivatives in the biochemical processes of the tumor cell.*

Keywords: *chlorins, amino acids, bacteriochlorins, photosensitizers, photodynamic therapy, photo-induced cytotoxicity*

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НАУЧНАЯ СТАТЬЯ

К 85-летию со дня рождения профессора А.Ф. Миронова

Аминокислотные производные природных хлоринов как платформа для создания таргетных фотосенсибилизаторов в онкологии

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Цели. *Целью настоящей работы является получение аминокислотных производных хлорофилла a и бактериохлорофилла a для направленной доставки пигментов в опухолевые очаги, увеличения биосовместимости и, как следствие, уменьшения побочного токсического действия. Кроме фотодинамической эффективности для полученных конъюгатов фотосенсибилизаторов с аминокислотами ожидается дополнительный цитотоксический эффект, вызванный участием последних во внутриклеточных биохимических процессах, включая взаимодействие с компонентами глутатионовой антиоксидантной системы, приводящее к уязвимости опухолевых клеток к окислительному стрессу.*
Методы. *В настоящей работе реализована оптимизация структуры высокоэффективного ИК-фотосенсибилизатора на основе O-пропилоксим-N-пропоксибактериопурпуринимида (DPBP), поглощающего при 800 нм и показавшего фотодинамическую эффективность для лечения глубокозалегающих и пигментированных опухолей, путем введения на периферию макроцикла метиловых эфиров L-лизина, L-аргинина, метионинсульфоксимина (MSO) и бутионинсульфоксимина (BSO). Структура полученных соединений доказана методами масс-спектрометрии и ЯМР-спектроскопии, а фотоиндуцированная цитотоксичность исследована in vitro на линии клеток HeLa.*
Результаты. *Были получены конъюгаты O-пропилоксим-N-пропоксибактериопурпуринимида с аминокислотами и их производными, такими как, лизин, аргинин, метионинсульфоксимин и бутионинсульфоксимин. Показана хелатирующая способность конъюгата DPBP с лизином и получен его Sn(IV)-комплекс.*

Выводы. *Биологические испытания DPBP с метионинсульфоксимином и бутионинсульфоксимином показали 5–6 кратное увеличение фотоиндуцированной цитотоксичности по сравнению с исходным фотосенсибилизатором DPBP. При этом обнаружена высокая интернализация пигментов опухолевыми клетками, а темновая цитотоксичность (при отсутствии облучения) DPBP-MSO и DPBP-BSO увеличилась в 4 раза по сравнению с исходным соединением DPBP, что может быть объяснено участием производных метионина в биохимических процессах опухолевой клетки.*

Ключевые слова: *хлорины, аминокислоты, бактериохлорины, фотосенсибилизаторы, фотодинамическая терапия, фотоиндуцированная цитотоксичность*

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INTRODUCTION

The addition of amino acids into photosensitizer (PS) molecules, on the one hand, increases the hydrophilicity of pigments, and on the other hand, improves the selectivity of their accumulation in tumor tissues, since amino acids are involved in numerous intracellular processes, including the accelerated proliferation of tumor cells [1, 2]. In addition, some amino acids, including non-proteinogenic ones, can affect various processes inside the cell, weakening its defenses, for example, against oxidative stress. Due to the structural features of some amino acids, they can be used as complexing agents for metal cations and organometallic complexes, which have their antitumor activity [3].

In clinical practice, derivatives of natural chlorins with amino acids are already being used, and their efficiency for the therapy of tumors of various origins has been proven [4, 5]. The relay race of such studies was continued at the N.A. Preobrazhensky Department of Chemistry and Technology of Biologically Active Compounds, Medical and Organic Chemistry in the group of Professor M.A. Grin and Professor A.F. Mironov, where bacteriochlorin derivatives with methyl esters of L-lysine, L-arginine, methionine sulfoximine (MSO), and buthionine sulfoximine (BSO), as well as the structural isomers of some of the earlier mentioned conjugates were first obtained. The publication of this work is timed to the anniversary of the outstanding scientist working in the field of creating new PSs, and the creator of the first Russian drug, Photogem, Professor A.F. Mironov.

The development of new highly specific anticancer drugs is an urgent task for medical and pharmaceutical chemistry [6]. First, the need to develop targeted anticancer drugs is dictated by numerous side effects and the general systemic toxicity of chemotherapeutic drugs used in clinical oncology [7].

The strategy of attaching fragments of other biomolecules to the leader molecule, such as amino acids [1, 2], peptides [8, 9], and carbohydrates [10–12], is employed for the targeted delivery of drugs to tumor foci; biocompatibility is increased, and thus, toxic side effects are reduced.

Of particular interest is the addition of amino acids, because the hydrophilicity and bioavailability of anticancer drugs increase, and their side effects decrease simultaneously. Several endogenous carriers of amino acids are concentrated in tumor cells and contribute to the internalization of the amino acid derivatives of various pharmacologically active substances [13]. For example, the histidine transport system in the body consists of 50% of the SLC15A4 (PHT1) transporter protein, 30% of other amino acid transporters, and only 20% of all histidine is delivered to cells using passive transport and other transport systems. The main carrier protein, PHT1, transports histidine and oligopeptides from lysosomes to the cytosol of eukaryotic cells. It is known to overexpress the arginine transporters, CAT-1, in rectal cancer cells. An in vitro study has shown that disabling CAT-1 in tumor cells induces apoptosis and death, and the carrier protein itself is a unique molecular biomarker and therapeutic target in tumor cells. Studies show that the intracellular transport of certain amino acids is a common feature of most neoplastic cells [14]. A classic example of the use of amino acids in the chemical design of alkylating agents is the creation of the antitumor drug, Melphalan (Fig. 1) (trade name Sarcolysin), which is a derivative of bis-β-chloroethylamine and the amino acid, L-phenylalanine. Melphalan is less toxic than the original chlormethine (embichinum). The resistance of tumor cells to melphalan also develops more slowly than that to chlormethine [15], which is primarily due to the presence of an amino acid residue in the preparation structure [16].

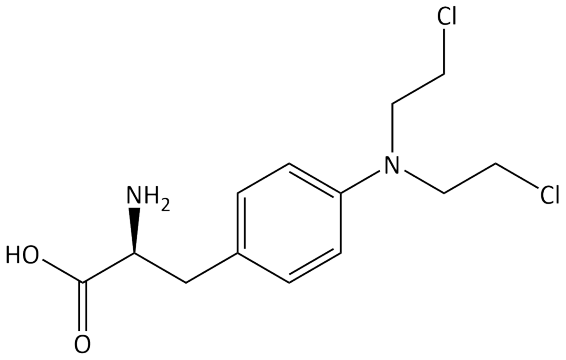


Fig. 1. Structure of Melphalan.

A similar strategy for the inclusion of amino acids in the composition of targeted drugs is implemented for PSs. The main cytotoxic agent in photodynamic therapy (PDT) is singlet oxygen ($^1O_2$); the action of which is not specific, since reactive oxygen species interact and destroy various biomolecules, such as lipids, proteins, and nucleic acids. The lifetime of the biochemically active singlet oxygen form in cells is 250 ns [17]. The diffusion of the $^1O_2$ molecule calculated based on this value is in good agreement with the experimental data on the radius of action of $^1O_2$ in cells and tissues, which does not exceed 50 nm [18]. Since the size of human tissue cells is relatively large (about 100 μm), $^1O_2$ is consumed close to the place of its formation. In this regard, the effectiveness of the cytotoxic action of a PS directly depends on its location in the tumor cell. This is confirmed by the fact that no direct correlation was found between the quantum yield of $^1O_2$ and PS cytotoxicity.

The accumulation of our scientific group's work with PSs of the chlorin and bacteriochlorin series shows that, most often, the intracellular accumulation of pigments occurs nonspecifically (diffusely or granularly in the cytoplasm) [19, 20] and does not affect the vital cell compartments, which together with a small radius of action of $^1O_2$ limits the effectiveness of PSs.

Currently, the priorities of scientists who create anticancer drugs have shifted toward the development of subcellular-oriented drugs [21], in particular, mitochondria-targeted drugs [22]. Damage to the mitochondria, which are the energy depots of the cell, disrupts protein biosynthesis and can lead to the launch of the tumor apoptosis [23].

It is known that molecules containing positively charged groups have membranotropic properties with respect to mitochondria [24]. One of the known cationic domains in mitochondria-oriented preparations is a guanidine residue [25], which, due to a delocalized positive charge, binds to carboxyl and phosphate groups on membranes, providing an efficient transmembrane transfer of PSs.

Amino acids have been repeatedly used to modify PSs based on porphyrins and chlorins. The purpose of this modification was to increase the hydrophilicity of pigments, as well as mitochondrial targeting of drugs. An example of the successful use of amino acids to increase the hydrophilicity of pigments is the enzymatic transesterification of chlorophyll a with various amino acids, peptides, and proteins, implemented in an Israeli scientific group. The use of the enzyme, chlorophyllase, as a catalyst for transesterification, allowed the authors to obtain esters of chlorophyll *a* under mild conditions; for example, with methyl ester of serine (chlorophyllideserine) (Fig. 2). The latter showed a 100-fold increase in photocytotoxicity in the culture of M2R melanoma cells in comparison with the official drug, Photosan [26].

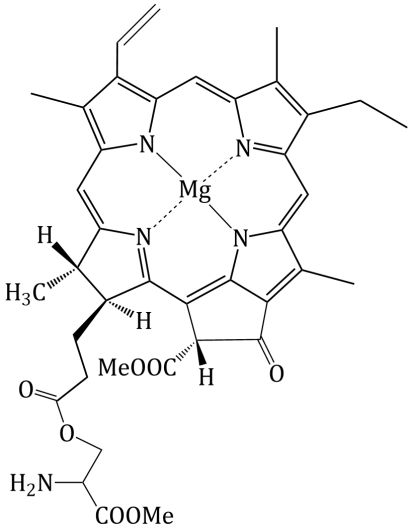


Fig. 2. Structure of chlorophyllideserine.

In *in vivo* experiments, water-soluble chlorophyllideserine was removed from normal tissue within 72 h and predominantly accumulated in the tumor, with low skin toxicity. This PS exhibited high photodynamic activity on M2R melanoma in mice [27].

The group of Professor K. Smith carried out the chemical synthesis of various amino acid derivatives of chlorin e_6. The leader compound obtained in the course of the study is mono L-aspartylchlorin e_6 (Fig. 3). This drug, called Talaporfin, belongs to the second-generation PS and is used in the treatment of various forms of cancer [4, 5]. In its pharmacodynamic parameters, the inhibition of tumor growth, increase in life expectancy, and rate of elimination from the body, the drug is superior to the drug, Photofrin, which is widely used in cancer PDT.

The scheme for the synthesis of Talaporfin includes the preparation of methylpheophorbide *a*, opening of the exocycle in the latter with sodium methoxide, and esterification of the product with

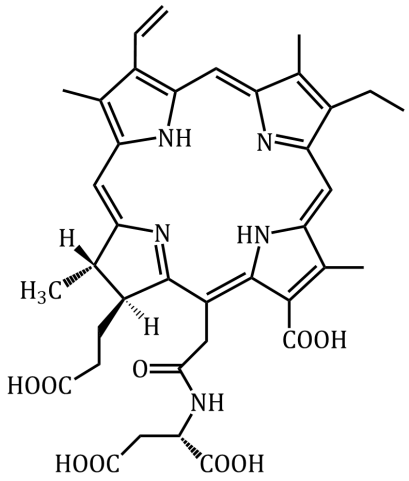


Fig. 3. Structure of Talaporfin.

diazomethane to obtain the trimethyl ester of chlorin e_6. Alkaline hydrolysis of ester groups and the interaction of chlorin e_6 with dimethyl aspartic acid after the removal of the protective groups results in mono L-aspartylchlorin e_6 [28].

The amidation of chlorin e_6 can take place at one of three carboxyl groups; therefore, it was believed for a long time that aspartic acid was attached at the 17^3-position. However, later, the structure of mono L-aspartylchlorin e_6, which is the pharmaceutical substance of the drug, was finally clarified using regioselective chemical synthesis [29].

The presence of the guanidine group alone or in the composition of arginine attached to the chlorin-series PS, Verteporfin (Vizudin drug), ensures the accumulation of the latter in the mitochondria of tumor cells. This fact was discovered during the study of the intracellular distribution of the modified drug by fluorescence microscopy on the line of human carcinoma cells resistant to the action of cisplatin [30]. Other amino acids, including lysine, serine, and tyrosine, orient 5-(4-carboxyphenyl)-10,15,20-tris(3-methoxyphenyl) porphyrin into the lysosomes of HeLa tumor cells [31].

The ability of the guanidine group to be protonated in physiological fluids ensures the participation of arginine in many metabolic processes. Therefore, the inclusion of the latter in the structure of drugs, including PSs, largely determines their pharmacokinetic and pharmacodynamic properties. There is a research project in which the derivatives of zinc phthalocyanine and arginine with a free and esterified carboxyl group of amino acids were obtained. The two drugs were compared by different biological tests, and it was shown that both PSs differ insignificantly in photophysical parameters, while the results of biological tests revealed significant differences in their properties. For the conjugate of the Zn-complex of phthalocyanine with ethyl arginine ether, a twofold increase in the accumulation in tumor cells and a fivefold increase in photoinduced cytotoxicity were found [32].

In addition to antitumor PDT, antimicrobial PDT is currently being actively developed. The tropism of PSs to gram-negative bacteria is significantly increased in the presence of positively charged groups in their structure. The inclusion of lysine into the tetraphenylporphyrin molecule enhances the antimicrobial activity of the latter against antibiotic-resistant strains of *Staphylococcus aureus*, *E. coli*, and *P. Aeruginosa*, with tetra-substituted tetraphenylporphyrin being the most promising [33].

The well-known ability of amino acids to chelate metal cations due to the presence of carboxyl, amino, thiol, hydroxyl, and other groups in their molecules is actively used in the creation of the metal complexes of porphyrins and related compounds containing a metal at the periphery of the macrocycle.

Another important intracellular target for targeted therapy is the thiol-containing tripeptide—glutathione. It is a key molecule that plays an important role in the regulation of redox status, drug detoxification, and cell protection from damage by free radicals, peroxides, and toxins [34]. Currently, active research is being carried out on therapeutic agents acting on the glutathione antioxidant system. Some of them are already at the stage of *in vitro* and *in vivo* preclinical trials. [35]. Sulfur-containing amino acids—cysteine, methionine, and its derivatives: MSO and BSO—are targeted molecules that target components of the antioxidant system of tumor cells, making the latter vulnerable to photodynamic effects.

All of the above motivated us to research the synthesis and biological properties of the conjugates of natural chlorins with amino acids to obtain targeted PSs with optimal amphiphilicity, improved bioavailability, and high photodynamic efficiency.

MATERIALS AND METHODS

All solvents used in this work were prepared according to standard procedures. Column chromatography was performed on silica gel 60 (0.040–0.063 mm, *Merck*, Germany). For preparative thin-layer chromatography (TLC), glass plates with silica gel 60 (*Merck*) were used; for analytical TLC, aluminum plates with Kieselgel 60 F_{245} silica gel (*Merck*) were used.

Electronic absorption spectra were obtained on a Shimadzu UV1800 UV/VIS spectrophotometer (*Shimadzu*, Japan) in 10 mm thick cuvettes; dichloromethane was used as a solvent. Nuclear magnetic resonance (NMR) ^{1}H spectra were recorded

using a Bruker DPX 300 spectrometer (*Bruker Daltonics*, Germany) in $CDCl_3$.

Mass spectra were obtained using a Bruker Ultraflex TOF/TOF spectrometer (*Bruker Daltonics*) by the MALDI method with 2,5-dihydroxybenzoic acid as a matrix.

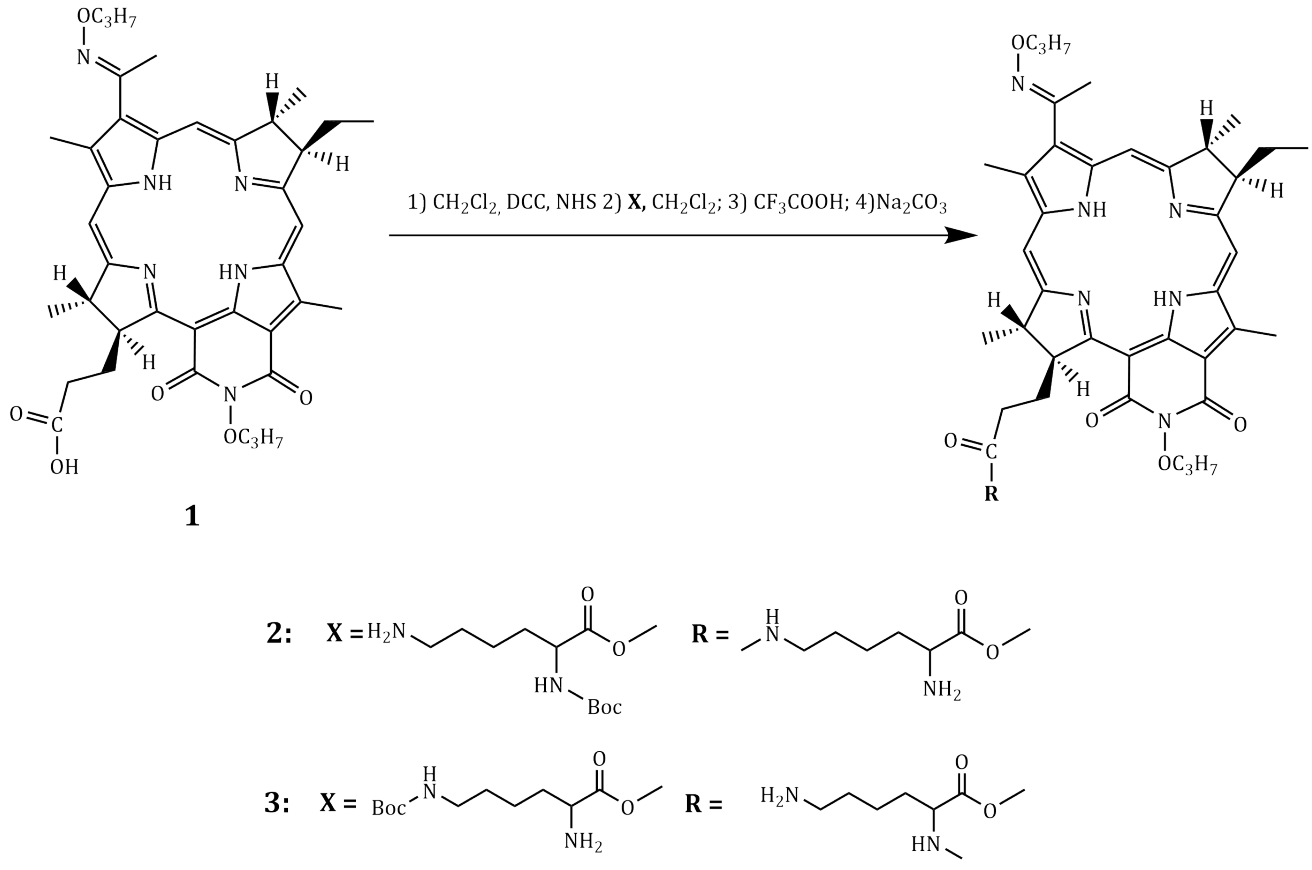
RESULTS AND DISCUSSION

The purpose of the first stage of the work was to obtain a lysine-containing derivative of bacteriopurpurinimide and the structural isomers of this conjugate, in which the amino acid is attached at both the α- and ε-amino groups.

Lysine is an essential amino acid and is involved in several metabolic processes within the cell. This is largely due to the presence of an amino group in the side chain, which is capable of protonation under physiological conditions. The resulting positive charge ensures the binding of the amino acid to the tumor and bacterial cell membranes. The electron-donating nitrogen atom of the ε-amino group of lysine, as well as the presence of α-NH_2– and α-COOH– groups in the molecule, allows the amino acid to create stable complexes with various metals, which increases the therapeutic and diagnostic potential of lysine-containing PSs. *O*-propyloxime-*N*-propoxybacteriopurpurinimide **1**, which is a derivative of natural bacteriochlorophyll *a*, whose synthesis was described by us earlier, was chosen as the lead compound in this work [34]. The pigment modification was carried out at the propionic acid residue in the 17th position of the macrocycle. In this work, based on the limited solubility of amino acid derivatives in organic solvents and the low solubility of bacteriochlorins in water, a synthesis strategy was chosen that included the initial preparation of an activated *O*-propyloxime-*N*-propoxybacteriopurpurinimide ester and the subsequent addition of amino acid derivatives.

For the activation of *O*-propyloxime-*N*-propoxybacteriopurpurinimide, we obtained a succinimide ester, which is stable both in aqueous media and in organic solvents. Dicyclohexylcarbodiimide was used as a condensing reagent (Scheme 1).

For the addition of lysine at the ε-amino group, the methyl ester of α-*N*-*tert*-butyloxycarbonyllysine was used, followed by the deprotection of the α-amino group of the attached amino acid by the action of trifluoroacetic acid (Scheme 1).



Scheme 1. Preparation of *O*-propyloxime-*N*-propoxybacteriopurpurinimide conjugates with lysine methyl ester.

The structure of compound **2** after isolation and chromatographic purification was confirmed by mass spectrometry (Fig. 4) and NMR spectroscopy (Fig. 5).

The mass spectrum contained the signal of the molecular ion ($[M]^+$ 837.58), as well as fragmentation typical of this bacteriopurpurinimide with the elimination of one ($M^+ - OC_3H_7$) or two oxypropyl groups ($M^+ - 2 - OC_3H_7$) (Fig. 4).

In turn, the NMR spectrum contained signals of the protons of both bacteriochlorin and amino acid residue, including the signals of the protons of the hydrocarbon side chain of lysine in the form of a multiplet in the region of 1.8–2.3 ppm and the amide proton signal at 8.25 ppm (Fig. 5).

The preparation of structural isomer **3**, in which the amide was formed due to the α-amino group of lysine, was carried out similarly to that of compound **2**; however, ε-*N*-*tert*-butyloxycarbonyl lysine methyl ester was used as the amino acid component (Scheme 1). The structure of lysine-bacteriopurpurinimide **3** was confirmed by mass spectrometry and NMR spectroscopy.

Since tin complexes are currently considered as an alternative to platinum-containing preparations, it was interesting to study the complexation of lysine with tin to then reproduce the metallation reaction on conjugate **2** [36–39]. For this purpose, the interaction of Boc-protected at the ε-amino group of lysine with trimethyltin chloride Me_3SnCl was realized. The reaction was carried out in the presence of triethylamine Et_3N (Scheme 2).

In the mass spectrum of metal complex **5**, isotopic splitting of the molecular ion signal was observed, confirming the presence of a tin atom in the molecule (Fig. 6).

The reaction of α-*N*-Boc-Lys with diphenyltin dichloride Ph_2SnCl_2 was carried out similarly under basic conditions. To confirm the structure, the matrix-assisted laser desorption/ionization (MALDI) mass spectrum of compound **6** was obtained, in which there was a molecular ion corresponding to the required mass, *m/z*: 568 $[M]^+$, with characteristic signals of tin isotopes (Fig. 7).

After the conditions of complexation for lysine derivatives were selected, we obtained an organotin complex based on the conjugate of dipropoxybacteriopurpurin-imide with lysine attached to the ε-amino group of the latter (ε-*N*-Lys-DPBP) **2** (Scheme 3).

For compound **7**, a mass spectrum was obtained (Fig. 8), where the expected signal of a molecular ion with an *m/z* value of 1001 $[M]^+$ and characteristic signals of tin isotopes was present.

As noted earlier, arginine, like lysine, is a positively charged amino acid under physiological conditions, since the guanidine group of the side chain is capable of protonation. Some works describe the

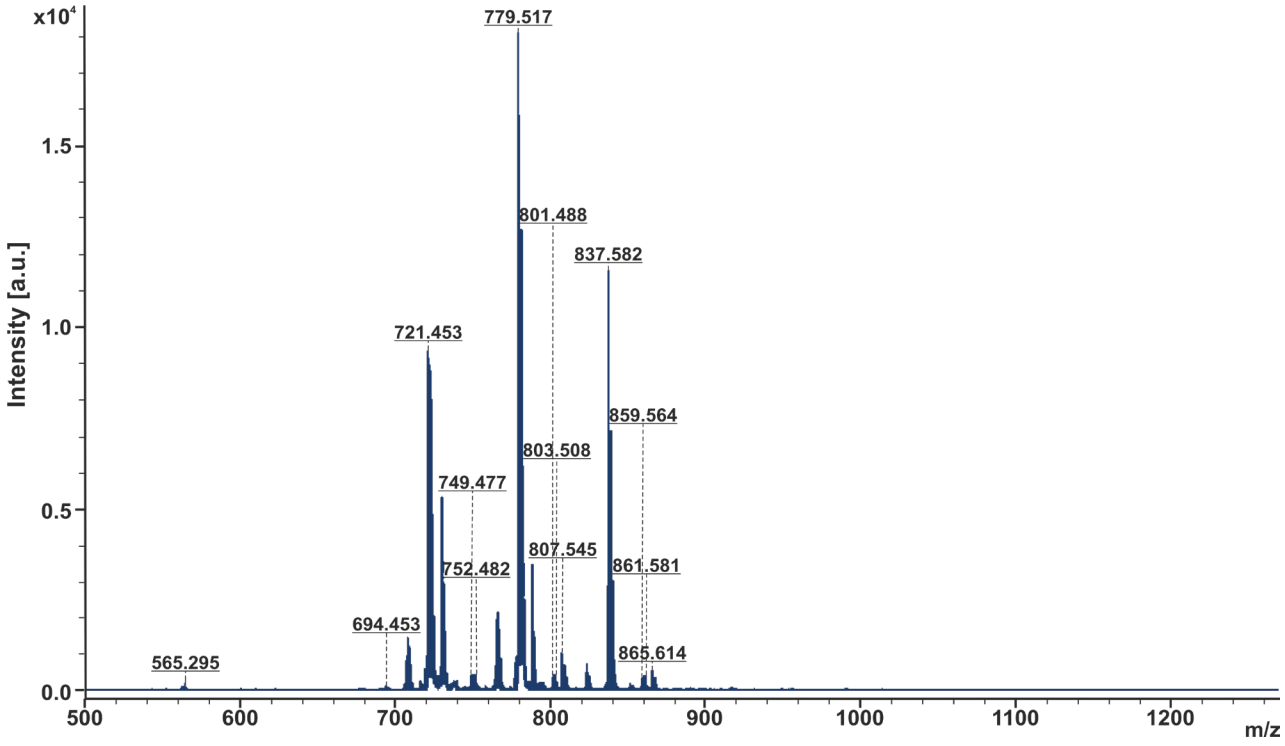


Fig. 4. MALDI TOF mass spectrum of compound **2**.

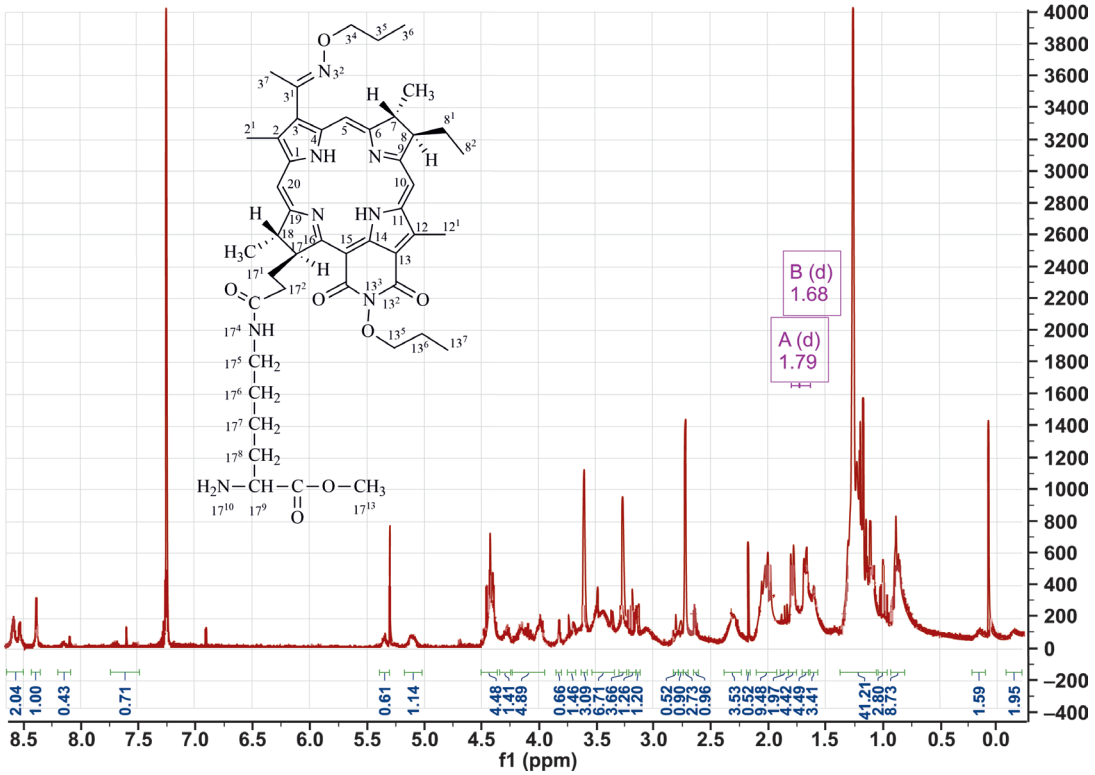
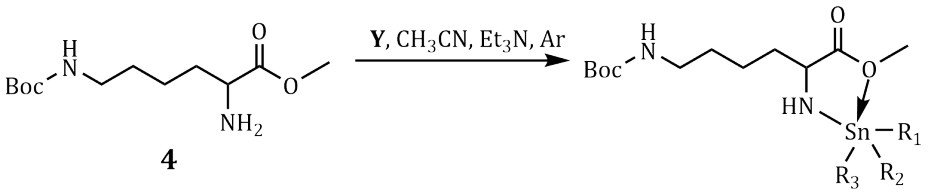


Fig. 5. ^{1}H NMR spectrum of compound **2**.



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5: Y = $(CH_3)_3SnCl$ R_1=R_2=R_3=CH_3

6: Y = $(Ph)_2SnCl_2$ R_1=R_2=Ph R_3=Cl

Scheme 2. Preparation of tin complexes with Boc-derivative of lysine (**5** and **6**).

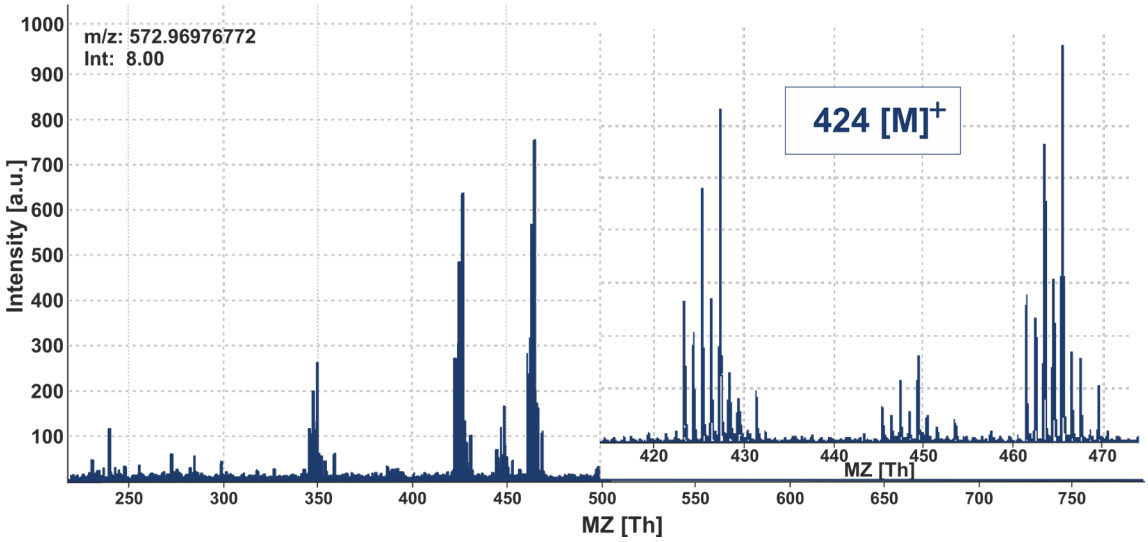


Fig. 6. MALDI TOF mass spectrum of metal complex **5**.

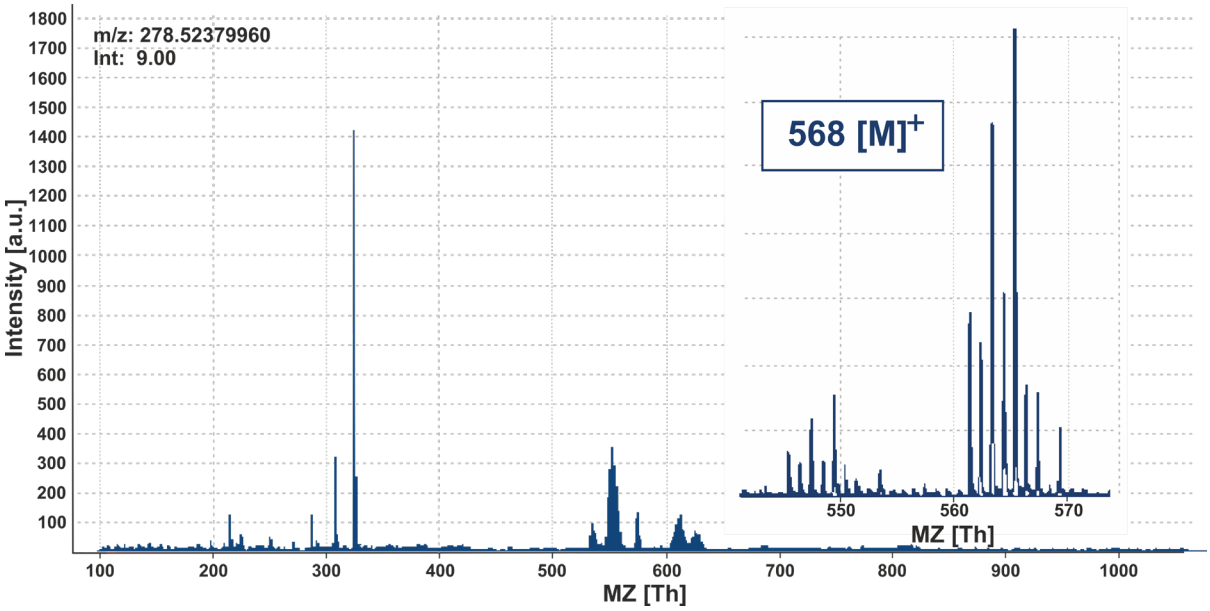
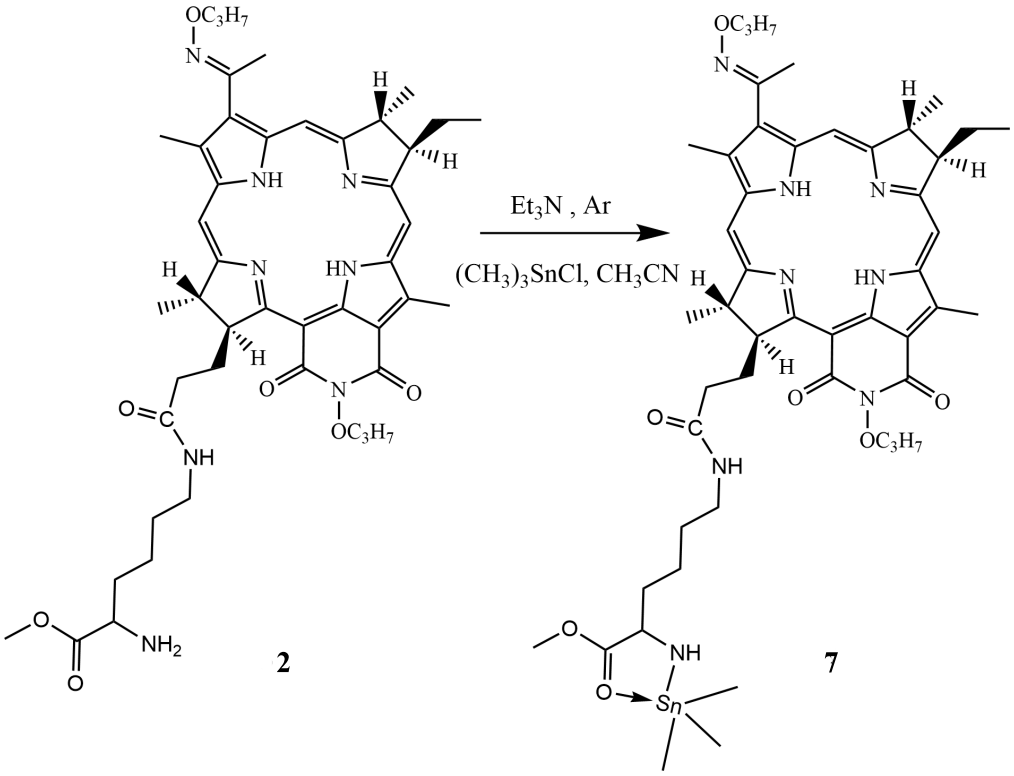


Fig. 7. MALDI TOF mass spectrum of compound **6.**



Scheme 3. Preparation of tin complex ε-*N*-Lys-DPBP **7**.

accumulation of PSs containing a guanidine group in mitochondria, which increases the photoinduced cytotoxicity of such pigments. In our work, arginine was the second amino acid introduced into the PS molecule according to the method described above for lysyl-DPBP (Scheme 4).

The ^{1}H NMR spectrum of the obtained conjugate **8** contains a broadened signal of the protons of the amino and imino groups of the guanidine fragment of arginine at 8.26 ppm (Fig. 9).

It is known from the literature that the target molecules for interacting with the components of

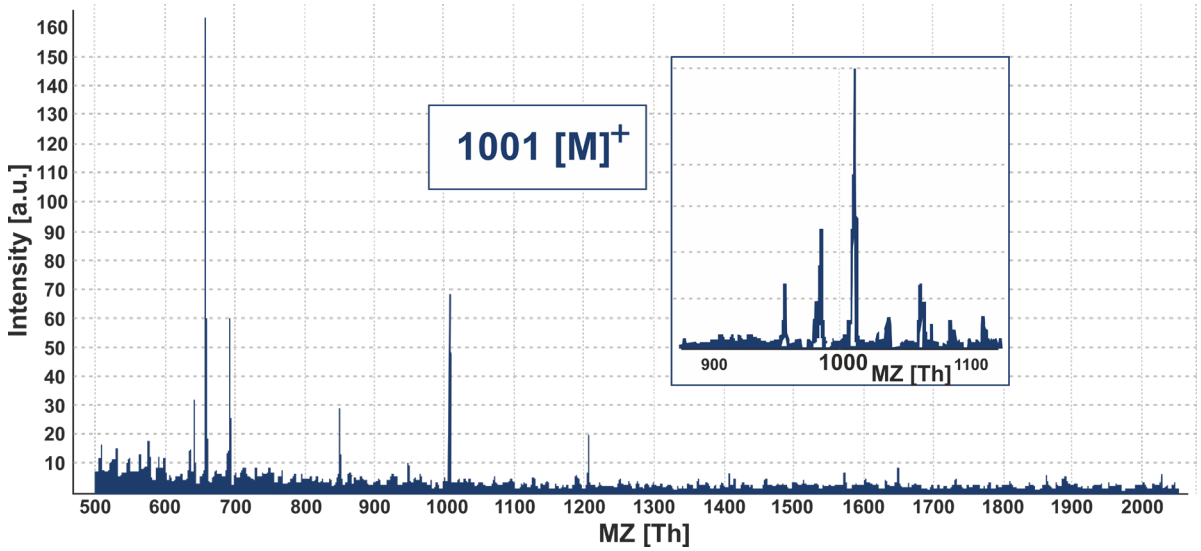
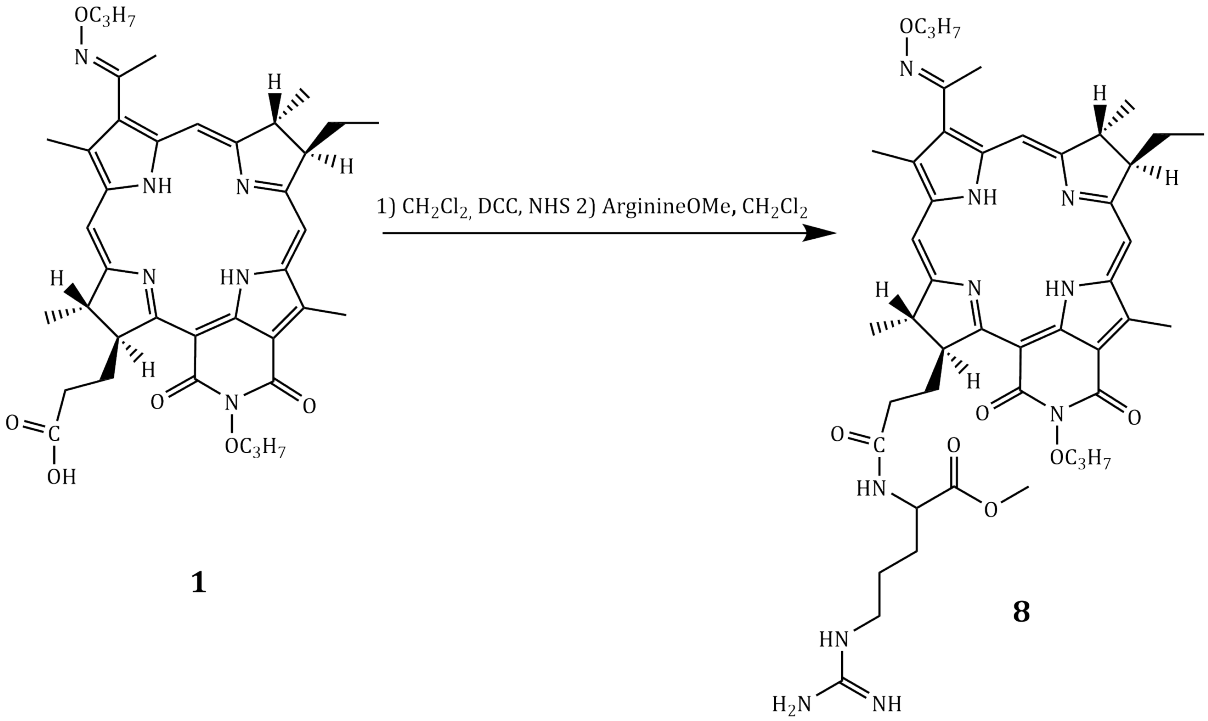


Fig. 8. MALDI TOF mass spectrum of compound **7.**



Scheme 4. Synthesis of *O*-propyloxime-*N*-propoxybacteriopurpurinimide with arginine methyl ester.

the glutathione antioxidant system are methionine derivatives: MSO and BSO, the addition of which into the leader *O*-propyloxime-*N*-propoxybacteriopurpurinimide was implemented in this work (Scheme 5). The amidation of the propionic acid residue at position 17[3] of the macrocycle was carried out according to the traditional procedure in the presence of the activating agent 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (Scheme 5).

To assess the photoinduced activity of DPBP derivatives with the methyl esters of MSO (DPBP-MSO) **9** and BSO (DPBP-BSO) **10**, the HeLa cellline was chosen [40]. *O*-propyloxime-*N*-propoxybacteriopurpurinimide was taken as a reference drug, and the survival of tumor cells was assessed visually using an inverted microscope and the MTT test; the results of which are shown in the table[1].

[1] Biological tests were performed at the P.A. Herzen Moscow Oncological Research Institute, Branch of the Federal State Budgetary Institution of the National Medical Research Center of Radiology of the Ministry of Health of Russia under the leadership of the Head of the Department, Cand. Sci. A.A. Pankratov.

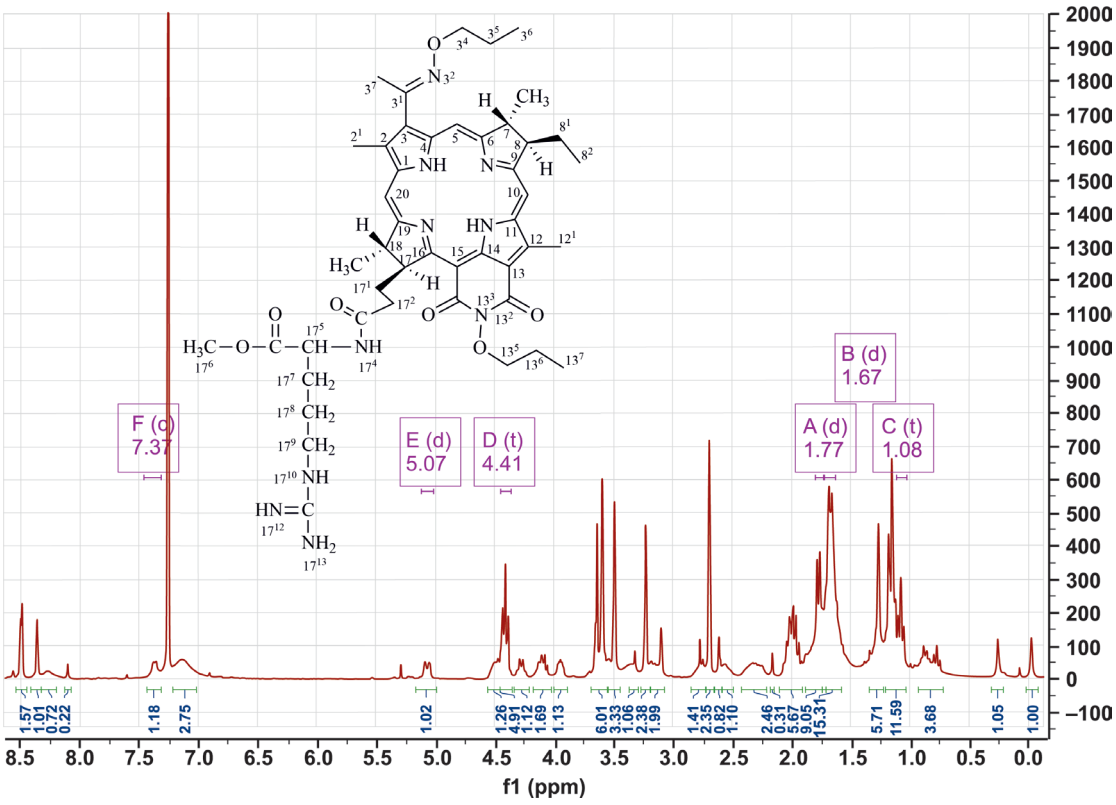
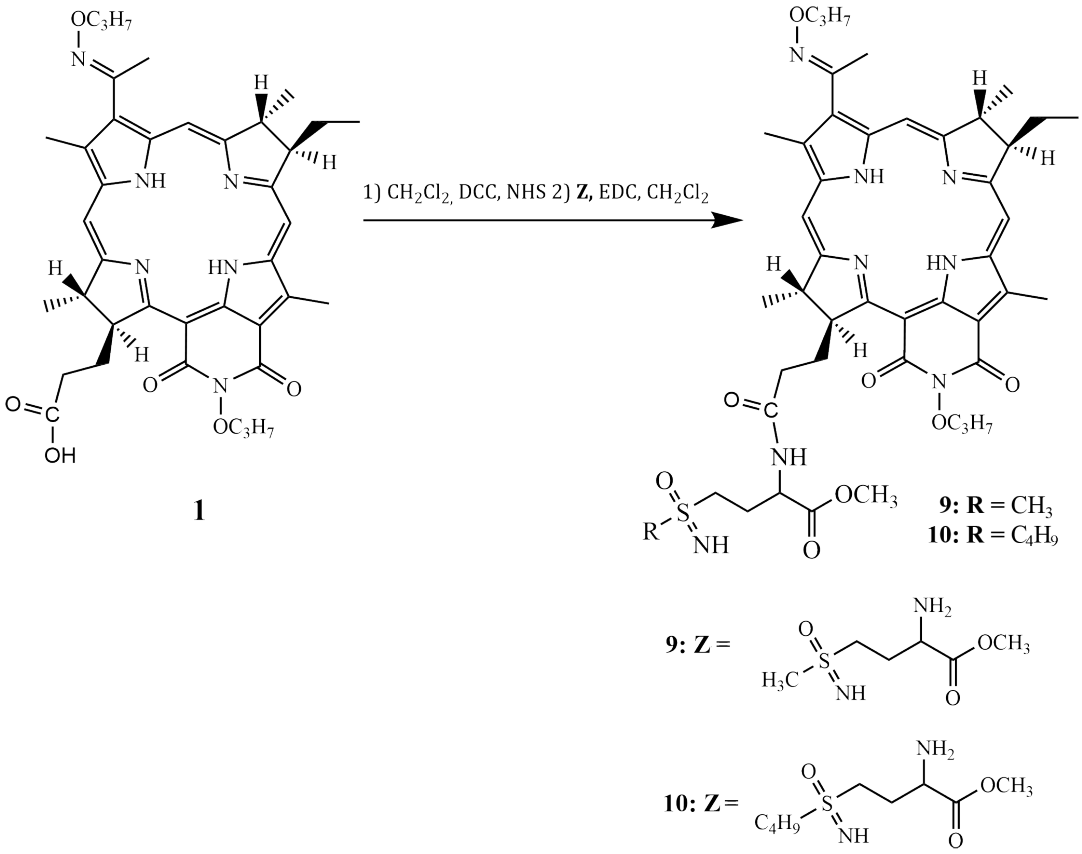


Fig. 9. ^{1}H NMR spectrum of compound **8**.



Scheme 5. Synthesis of the conjugate of *O*-propyloxime-*N*-propoxybacteriopurpurinimide with methyl ester of L-MSO and L-(S,R)-BSO.

The presented results show a 5- and 6-fold increase in the photoinduced cytotoxicity of the DPBP-MSO and DPBP-BSO derivatives compared to the parent compound DPBP. The half-maximal inhibitory concentration, IC_{50}, changed insignificantly upon irradiation in the presence of PS in the incubation medium and after washing the cells from PS, which indicates a high internalization of the pigment by tumor cells. Additionally, dark cytotoxicity increased fourfold in DPBP-MSO and DPBP-BSO compared to the initial compound DPBP.

EXPERIMENTAL

Conjugate of *O*-propyloxime-*N*-propoxybacteriopurpurinimide with lysine methyl ester (2). Compound **1** (1 equiv, 28.7 μmol, 20 mg) was dissolved in 2 mL of methylene chloride, 1,3-dicyclohexylcarbodiimide (1.5 equiv, 43.1 μmol, 9 mg) was added and stirred at 0°C for 30 min. Afterward, *N*-hydroxysuccinimide (1.5 equiv, 43.1 μmol, 8 mg) was added to the reaction mixture and stirred for 24 h, after which the solvent was removed in a vacuum. The residue was dissolved in ethyl acetate, the resulting precipitate was collected by filtration on a Schott filter, and the solvent was removed in vacuo. The obtained activated ester of *O*-propyloxime-*N*-propoxybacteriopurpurinimide (1 equiv, 25.2 μmol, 20 mg) was dissolved in 2 mL of methylene chloride, and the methyl ester of α-*N*-*tert*-butyloxycarbonyl lysine (2 equiv, 50.4 μmol, 13.8 mg) was added, after which the reaction mixture was stirred for 24 h. Subsequently, trifluoroacetic acid (2 equiv, 8.5 μL) was added, and the mixture was stirred for another 24 h at room temperature. The progress of the reaction was monitored chromatographically (R_f = 0.55, CH_2Cl_2/CH_3OH, 40/1, v/v). The solvent was removed in vacuo and compound **2** was purified by preparative TLC (CH_2Cl_2/CH_3OH, 40/1, v/v). The product yield was 11 mg (55%).

Mass spectrum, *m/z*: calculated for $C_{46}H_{62}N_8O_7$ $[M]^+$: 838.47, found: 837.48.

^{1}H NMR spectrum (δ, ppm): 8.6 (H, s, 10-H), 8.54 (H, s, 5-H), 8.4 (H, s, 20-H), 8.25 (H, m, 17^4-NH), 5.10 (H, m, 17-H), 4.47 (2H, m, 17^9-CH_2), 4.45 (4H, t, *J* = 6.7 Hz, 3^4-CH_2, 13^5-CH_2), 4.28 (H, m, 18-H), 4.15 (2H, m, 7-H), 3.99 (H, m, 8-H), 3.63 (3H, s, 17^{13}-CH_3), 3.61 (3H, s, 12-CH_3), 3.43 (2H, m, 17^5-CH_2), 3.27 (3H, s, 2-CH_3), 2.72 (3H, s, 3^1-CH_3), 2.30 (4H, m, 17^2-CH_2^a, 17^1-CH_2^a, 17^2-CH_2^b, 17^1-CH_2^b), 2.05 (2H, m, 17^6-CH_2), 2.01 (6H, m, 3^5-CH_2, 13^6-CH_2, 8^{1a}, 8^{1b}-H), 1.86 (2H, m, 17^8-CH_2), 1.79 (3H, d, *J* = 7.2 Hz, 7-CH_3), 1.68 (3H, d, *J* = 6.2 Hz, 18-CH_3), 1.6 (2H, m, 17^7-CH_2), 1.17 (6H, m, 13^7-CH_3, 3^6-CH_3), 1.11 (3H, m, 8^2-CH_3), and 0.16 (s, NH), −0.13 (s, NH).

Conjugate of *O*-propyloxime-*N*-propoxybacteriopurpurinimide with lysine methyl ester (3). The activated *O*-propyloxime-*N*-propoxybacteriopurpurinimide ester (1 equiv, 25.2 μmol, 20 mg) was dissolved in 2 mL of methylene chloride, and ε-*N*-*tert*-butyloxycarbonyl lysine (2 equiv, 50.4 μmol, 13.8 mg) was added. The reaction mixture was stirred for 24 h, after which a twofold excess of trifluoroacetic acid was added, and the reaction was left for another 24 h. The progress of the reaction was monitored chromatographically (R_f = 0.6, CH_2Cl_2/CH_3OH, 35/1, v/v). The solvent was removed in vacuo and compound **3** was purified by preparative TLC (CH_2Cl_2/CH_3OH, 35/1, v/v). The product yield was 10 mg (50%).

Mass spectrum, *m/z*: calculated for $C_{46}H_{62}N_8O_7$ $[M]^+$: 838.47, found: 837.58.

^{1}H NMR spectrum (δ, ppm): 8.62 (H, s, 10-H), 8.52 (H, s, 5-H), 8.42 (H, s, 20-H), 8.17 (H, m, 17^4-NH), 5.13 (H, m, 17-H), 4.44 (4H, t, *J* = 6.5 Hz, 3^4-CH_2, 13^5-CH_2), 4.30 (H, m, 18-H), 4.17 (2H, m, 7-H), 4.00 (H, m, 8-H), 3.75 (2H, m, 17^5-CH_2), 3.66 (3H, s, 17^{13}-CH_3), 3.62 (3H, s, 12-CH_3), 3.15 (2H, m, 17^9-CH_2), 3.28 (3H, s, 2-CH_3), 2.73 (3H, s, 3^1-CH_3), 2.28 (4H, m, 17^2-CH_2^a, 17^1-CH_2^a, 17^2-CH_2^b, 17^1-CH_2^b), 2.06 (2H, m, 17^8-CH_2), 2.00 (6H,

Photoinduced cytotoxicity of compounds **1**, **9** and **10** in HeLa cells *in vitro*

No.	PS	Experiment options		
		Exposure in the presence of PS in the incubation medium	Exposure without PS in the incubation medium	Dark cytotoxicity
		IC_{50} value, nM		
1	**DPBP**	111 ± 8	129 ± 24	2141 ± 763
2	**DPBP-BSO**	20 ± 0.7	21 ± 2	485 ± 63
3	**DPBP-MSO**	16 ± 1	20 ± 5	563 ± 258

m, 3^5-CH_2, 13^6-CH_2, 8^{1a}, 8^{1b}-H), 1.86 (2H, m, 17^6-CH_2), 1.8 (3H, d, J = 6.9 Hz, 7-CH_3), 1.68 (3H, d, J = 5.9 Hz, 18-CH_3), 1.61 (2H, m, 17^7-CH_2), 1.18 (6H, m, 13^7-CH_3, 3^6-CH_3), 1.11 (3H, m, 8^2-CH_3), 0.08 (s, NH), and −0.22 (s, NH).

The complex of trimethyltin (IV) with ε-*N*-Boc-lysine (5). Methyl ester of ε-*N*-Boc-lysine **4** (1 equiv, 1 mmol, 260 mg) was dissolved in 4 mL of acetonitrile, and 100 μL of triethylamine was added. After 30 min, trimethyltin chloride (1 equiv, 1 mmol, 200 mg) was added and stirred for a day. The progress of the reaction was monitored chromatographically. Afterward, the reaction mixture was extracted in the H_2O/CH_2Cl_2 system to remove excess triethylamine, and the resulting organic extract was chromatographed using preparative TLC in the dichloromethane–methanol system (20/1, v/v). The yield was 130 mg (50%).

Mass spectrum, *m/z*: calculated for $C_{15}H_{32}N_2O_4{}^{118}Sn$ $[M]^+$: 424.14 (100.0%), 422.14 (74.8%), 420.14 (42.2%), 423.14 (36.8%), 421.14 (29.3%), 428.14 (16.9%), 425.14 (16.7%), 426.14 (14.3%), 429.14 (2.8%), 416.14 (2.8%), 427.14 (2.3%), 418.14 (1.9%), 419.14 (1.3%); found: 424.14 $[M]^+$ (100%), 422.14 (74.1%), 420.14 (40.2%), 423.14 (32.1%), 421.14 (28.3%), 428.14 (15.9%), 425.14 (14.0%), 426.14 (14.3%), 429.14 (2.8%), 416.14 (2.8%), 427.14 (2.6%), 418.14 (1.9%), and 432.14 (1.3%).

Diphenyltin (IV) complex with ε-*N*-Boc-lysine (6). ε-*N*-Boc-lysine methyl ester (1 equiv, 1 mmol, 260 mg) was dissolved in 4 mL of acetonitrile, and 100 μL of triethylamine was added; after 30 min, diphenyltin dichloride (1 eq, 1 mmol, 343 mg) was added and stirred for a day. The progress of the reaction was monitored chromatographically.

Next, the reaction mixture was extracted in the H_2O–CH_2Cl_2 system to remove excess triethylamine, and the resulting organic extract was chromatographed using preparative TLC in the dichloromethane–methanol system (30/1, v/v). The yield was 57 mg (22%).

Mass spectrum, *m/z*: calculated for $C_{24}H_{33}ClN_2O_4{}^{118}Sn$ $[M]^+$: 568.12 (100.0%), 566.11 (78.7%), 567.12 (45.3%), 564.11 (40.2%), 565.12 (31.6%), 570.11 (28.4%), 569.12 (25.3%), 568.11 (21.4%), 570.12 (19.5%), 572.12 (17.5%), 569.11 (13.8%), 571.12 (11.5%), 566.12 (7.4%); found: 566.32 (100%), 568.11 (70.7%), 567.12 (42.3%), 564.11 (36.2%), 565.12 (31.6%), 570.11 (28.4%), 569.12 (25.3%), 568.11 (21.4%), 570.12 (19.5%), 572.12 (17.5%), 569.11 (13.8%), and 571.12 (1.5%).

Synthesis of the tin (IV)-complex of ε-Lys-DPBP conjugate (7). Compound **2** (1 equiv, 0.01 mmol, 10 mg) was dissolved in 4 mL of acetonitrile, and 50 μL of triethylamine was added; after 30 min, trimethyltin chloride (2.5 equiv, 0.025 mmol, 5 mg) was added and stirred for a day. The progress of the reaction was monitored chromatographically. Afterward, the reaction mixture was extracted in the H_2O–CH_2Cl_2 system to remove excess triethylamine, and the resulting extract was chromatographed using preparative TLC in the dichloromethane–methanol system (30/1, v/v). The yield was 2 mg (20%).

Mass spectrum, *m/z*: calculated for $C_{49}H_{70}N_8O_7{}^{118}Sn$ 1002.44 (100.0%), 1000.44 (75.9%), 1001.44 (59.5%), 1003.44 (49.0%), 999.44 (41.9%), 998.44 (38.6%), 1006.44 (15.6%), 1004.44 (14.8%), 1004.45 (13.3%), 1002.45 (10.2%), 1007.45 (8.6%), 1005.44 (7.2%), 1003.45 (6.1%); found: 1001.44 (100%), 1000.44 (70.1%), 1002.44 (58.5%), 1003.44 (48.0%), 999.44 (41.9%), 998.44 (38.6%), 1006.44 (13.1%), 1004.44 (14.8%), 1004.45 (7.3%), and 1002.45 (5.2%).

Conjugate of *O*-propyloxime-*N*-propoxybacteriopurpurinimide with arginine (8). Conjugate **8** was prepared from 20 mg of compound **1** by a procedure similar to the preparation of dipropoxybacteriopurpurinimide conjugates with lysine **2** and **3** to obtain activated DPBP succinimide ester. The yield of the target compound **8** was 14.6 mg (73%).

Electronic spectrum, λ_{max}, nm (relative peak intensity): 368, 420, 544, 798 (1 : 0.50 : 0.37 : 0.44).

Mass spectrum, *m/z*: calculated for $C_{46}H_{62}N_{10}O_7$ $[M]^+$: 867.01, found: 867.6.

^{1}H NMR spectrum (δ, ppm): 8.50 (H, s, 10-H), 8.49 (H, s, 5-H), 8.36 (H, s, 20-H), 8.26 (3H, br.s, 17^{12}-NH, 17^{13}-NH_2), 7.37 (H, d, J = 8.5 Hz, 17^4-NH), 7.14 (br.s, 17^{10}-NH), 5.07 (H, d, J = 9.5 Hz, 17-H), 4.49 (H, m, 17^5-H), 4.41 (4H, t, J = 6.6 Hz, 3^4-CH_2, 13^5-CH_2), 4.28 (H, m, 18-H), 4.14 (2H, m, 7-H), 3.99 (H, m, 8-H), 3.64 (3H, s, 17^6-CH_3), 3.60 (3H, s, 12-CH_3), 3.35 (2H, m, 17^7-H) 3.23 (3H, s, 2-CH_3), 3.13 (2H, m, 17^8-H), 2.69 (3H, s, 3^1-CH_3), 2.57 (2H, m, 17^9-H), 2.31 (4H, m, 17^2-$CH_2{}^a$, 17^1-$CH_2{}^a$, 17_2-$CH_2{}^b$, 17^1-$CH_2{}^b$), 1.99 (6H, m, 3^5-CH_2, 13^6-CH_2, 8^{1a}, 8^{1b}-H), 1.77 (3H, d, J = 7.1 Hz, 7-CH_3), 1.67 (3H, d, J = 6.8 Hz, 1^8-CH_3) 1.16 (6H, m, 13^7-CH_3, 3^6-CH_3), 1.08 (3H, t, J = 7.28 Hz, 8^2-CH_3), and 0.26 (s, NH), −0.02 (s, NH).

Conjugate of *O*-propyloxime-*N*-propoxybacteriopurpurinimide with L-MSO methyl ester (9). Compound **1** (1 equiv, 35.9 μmol, 20 mg) was dissolved in 1 mL of methylene chloride, and EDC HCl (1.5 equiv, 53.8 μmol, 11 mg) was added, after which the reaction mixture was stirred for 15 min at 0°C. Next, a solution of L-MSO methyl ester (4 equiv, 143.6 μmol, 33 mg) in 1 mL of methylene chloride was added, and the reaction mixture was stirred for another 48 h in an inert argon atmosphere without access to light. The product was extracted with a 4% aqueous hydrochloric acid–chloroform mixture (250/25, v/v), then the extract was purified

by preparative TLC in dichloromethane–methanol (45/1, v/v). The yield was 8 mg (40%).

Mass spectrum, *m/z*: calculated for $C_{45}H_{60}N_8O_8S$ $[M]^+$: 872.43, found: 872.38.

Electronic spectrum, λ_{max}, nm: (relative peak intensity): 368, 419, 543, 798 (1 : 0.50 : 0.39 : 0.42).

^{1}H NMR spectrum (δ, ppm): 8.51 (s, H, 5-H), 8.44 (s, H, 10-H), 8.41 (s, H, 20-H), 5.04 (d, *J* = 8.7 Hz, H, 17-H), 4.35 (m, 4H, -$OCH_2CH_2CH_3$), 4.07 (m, 2H, 18-H, 7-H), 3.92 (m, H, 8-H), 3.58 (s, 3H, OCH_3), 3.53 (s, 3H, 12-CH_3), 3.2 (s, 3H, 2-CH_3), 3.07 (s, H, CH_3–S–CH_2–CH_2–CH–), 2.72 (m, 2H, CH_3–S–CH_2–), 2.64 (s, 3H, CH_3–S), 1.97 (m, 2H, CH_3–S–CH_2–CH_2–), 1.71 (d, *J* = 7.1 Hz, 3H, 7-CH_3), 1.63 (d, *J* = 7.0 Hz, 9H, -$OCH_2CH_2CH_3$, 18-CH_3), 1.0 (t, *J* = 7.2 Hz, 3H, 8^2-CH_3), 0.1 (s, H, NH), and −0.2 (s, H, NH).

Conjugate of *O*-propyloxime-*N*-propoxy-bacteriopurpurinimide with L-(S,R)-BSO methyl ester (10).The preparation of conjugate 10 from 25 mg of compound **1** was carried out similarly to the preparation of compound **9**. The yield was **9** mg (36%).

Mass spectrum, *m/z*: calculated for $C_{48}H_{66}N_8O_8S$ $[M]^+$: 914.47, found: 914.69.

Electronic spectrum, λ_{max}, nm (relative peak intensity): 368, 420, 545, 799 (1 : 0.47 : 0.34 : 0.46).

^{1}H NMR spectrum (δ, ppm): 9.06 (s, H, S=NH), 8.60 (s, H, 5-H), 8.53 (s, H, 10-H), 8.45 (s, H, 20-H), 5.12 (d, *J* = 8.5 Hz, H, 17-H), 4.44 (m, 4H, O–CH_2–CH_2–CH_3), 4.33 (m, 3H, 13^1-CH_3), 4.17 (m, 2H, 18-H, 7-H), 4.00 (m, H, 8-H), 3.88 (m, 2H, O–CH_2–CH_2–CH_3), 3.66 (s, 3H, O–CH_3), 3.62 (s, 3H, 12-CH_3), 3.29 (s, 3H, 2-CH_3), 3.24 (t, *J* = 7.4 Hz, H, NH–CH), 2.73 (d, *J* = 7.6 Hz, 7H, CH_2–S–CH_2, 3^1-CH_3), 2.71 (m, 2H, 17^2-CH_2), 2.33 (m, 2H, 8^1-CH_2), 2.04 (m, 2H, NH–CH–CH_2), 1.79 (d, *J* = 7.3 Hz, 3H, 7-CH_3), 1.70 (d, *J* = 6.8 Hz, 9H, O–CH_2–CH_2–CH_3), 1.26 (s, 2H, S–CH_2–CH_2–CH_2–CH_3), 1.18 (m, 2H, S–CH_2–CH_2–CH_2–CH_3), 1.09 (t, *J* = 7.3 Hz, 3H, 8^2–CH_3), 0.89 (s, 3H, $(CH_2)_3$–CH_3), 0.15 (s, H, NH), and −0.14 (s, H, NH).

Investigation of the photoinduced activity of compounds (9) and (10) *in vitro*.

Cytotoxicity was assessed using the standard MTT test. The cells were cultured under standard conditions at 37°C in a humidified atmosphere with 5% CO_2 in DMEM medium supplemented with L-glutamine (2 mM) and fetal calf serum (10%, *PanEko*, Russia). Photoinduced efficacy was assessed as follows: HeLa cells were seeded in a 96-well flat-bottomed microtiter plate (*Costar*, USA). Test compounds were added 24 h after inoculation. The concentration varied in the range of 0.05–28.00 μM. Subsequently, the cells were irradiated with a halogen lamp through a KS-19 broadband filter that transmits light with a wavelength of more than 720 nm. The power density was 21.0 ± 1.0 mW/cm^2, and the calculated light dose was 10 J/cm^2. Irradiation was performed with and without the removal of PS from the medium. After irradiation, the cells were incubated under standard conditions for 24 h. To analyze the PS cytotoxicity, the cells were placed in darkened conditions for 24 h. The survival rate was assessed by visual inspection and colorimetry using the MTT test. Inhibition of cell growth by more than 50% was considered biologically significant. This value was calculated as the mean of three independent tests.

CONCLUSIONS

In the present work, several conjugates of *O*-propyloxim-*N*-propoxybacteriopurpurinimide with amino acids and their derivatives, such as lysine, arginine, MSO, and BSO were prepared. The structure of all the obtained compounds was reliably confirmed by a complex of physicochemical methods of analysis. The chelating ability of the DPBP conjugate with lysine was shown, and the Sn(IV) complex was obtained. Biological tests were carried out, and the high photoinduced cytotoxicity of DPBP derivatives with MSO and BSO was shown.

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Authors' contributions

A.F. Mironov– scientific consulting at all stages of the work;

P.V. Ostroverkhov – collecting and processing materials on the lysine derivatives of bacteriopurpurinimide;

N.S. Kirin – collecting and processing materials on the arginine derivatives of bacteriopurpurinimide;

V.A. Pogorilyy – collecting and processing materials on the synthesis and study of the properties of the conjugates of bacteriopurpurinimide with MSO and BSO;

S.I. Tikhonov – collecting and processing materials on the synthesis and study of the properties of metal complexes of bacteriopurpurinimides with external chelating groups;

A.A. Tsygankov– management of the biotechnological part of the work, including the cultivation of biomass and isolation of bacteriochlorophyll *a*;

O.O. Chudakova – collecting and processing materials to optimize the conditions for cultivating biomass and isolating bacteriochlorophyll *a*;

M.A. Grin – concept and design of the study, collecting and processing materials.

The authors declare no conflicts of interest.

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