Chemical transformations of bacteriochlorophyll *a* and its medical applications*

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The review is devoted to methods of targeted chemical modifications of bacteriochlorophyll *a*. These methods provide an approach to the synthesis of derivatives with improved photophysical properties and increased hydrophilicity and are suited to the design of conjugates with other biologically active molecules utilized for fluorescence diagnosis and binary cancer therapy.

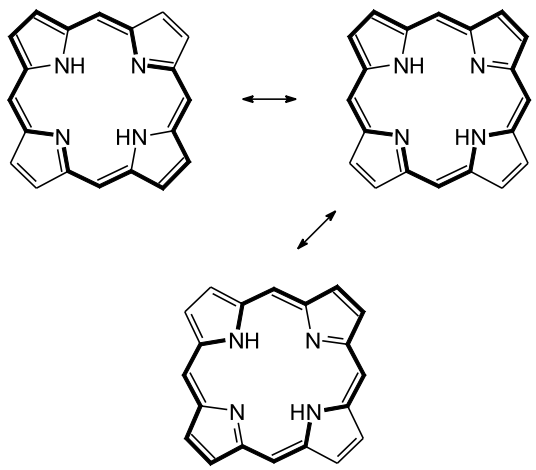
Key words: bacteriochlorophyll *a*, fluorescence diagnosis, photosensitizer, photodynamic therapy, bacteriochlorins, bacteriopurpurins, bacteriopurpurinimides.

Hydrogenated porphyrin analogs — chlorophylls and bacteriochlorophylls — play an important role in nature, in particular, they are involved in photosynthesis and related processes. These compounds have attracted great interest due to a wide range of useful properties of their modified derivatives. The unique ability of bacteriochlorins to selectively accumulate in tumor tissues and induce fluorescence or photodynamic effects under laser irradiation is successfully employed in fluorescence diagnosis (FD) and photodynamic therapy (PDT) of malignant tumors.[1—5]

Porphyrin systems follow Hückel's rules for aromaticity. Of 22 π electrons of the macrocycle, only 18 ($4n + 2$) electrons are responsible for aromaticity and are delocalized in accordance with the resonance structures presented in Scheme 1.

Therefore, the presence of two peripheral double bonds is not a necessary condition for the retention of aromaticity. Upon the reduction of these two bonds, the aromaticity of the bacteriochlorins that formed is retained and a change in the symmetry leads to a considerable bathochromic shift of the long-wavelength Q_2 band at 770 nm. As can be seen in Fig. 1, the chemical modification of the bacteriochlorin macrocycle, including the introduction of the additional

Scheme 1



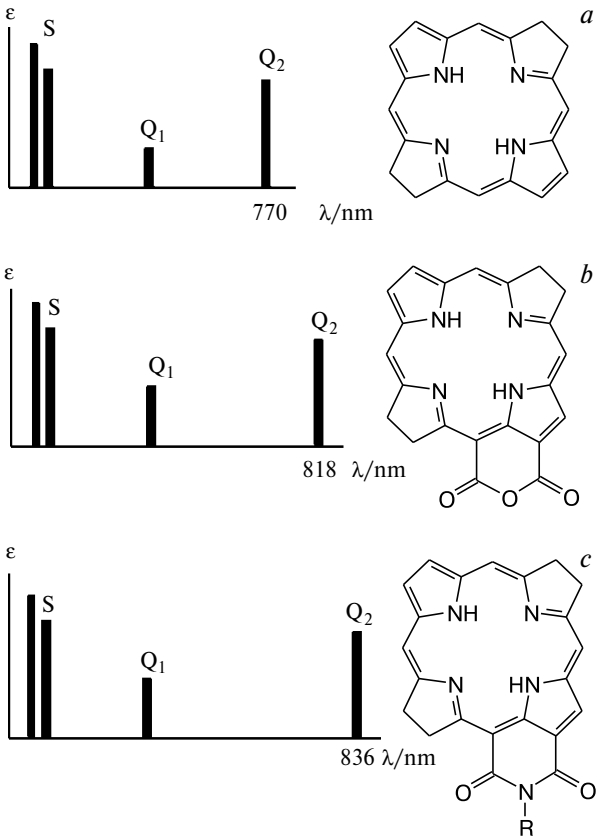


Fig. 1. Absorption spectra of bacteriochlorophyll *a* (bacteriochlorin type) (*a*) and its derivatives of the bacteriopurpurin (*b*) and bacteriopurpurinimide (*c*) types (S is the Soret band, Q_1 and Q_2 are absorption bands).

* Dedicated to Academician of the Russian Academy of Sciences N. S. Zefirov on the occasion of his 80th birthday.

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annulated ring *E* (anhydride or imide rings), causes further changes in the spectroscopic characteristics.

Bacteriochlorophylls represent an independent group of naturally occurring chlorophylls and are widespread in nature, primarily in numerous photosensitizing bacteria.[6,7] Pigments differ in the degree of hydrogenation of the macrocycle and the nature of substituents. Of particular interest is bacteriochlorophyll *a*, the absorption maxima of which not only are shifted to the red end of the spectrum but also have the highest extinction coefficients.

The source of bacteriochlorophyll *a* is the biomass of purple bacteria, such as *Rhodobacter sphaeroides*, *Rh. roseapersiana*, and *Rh. xapsulata*, which produce only one type of bacteriochlorophylls, thus making the isolation and purification of the pigment much easier.[8–11]

The design of highly efficient photosensitizers (PS) based on bacteriochlorophyll *a* for application in medicine, in particular, in the cancer treatment, is a complex problem that includes the development of methods for the synthesis of stable derivatives with improved spectroscopic and photophysical properties, the construction of new amphiphilic molecules with an optimal ratio of hydrophobic to hydrophilic substituents, and the enhancement of the efficiency and selectivity of the photodynamic action of sensitizers.

The molecular structure of the pigment offers wide possibilities for its targeted functionalization (Fig. 2).

The annulation of the six-membered anhydride ring to the main macrocycle leads to an increase in the overall stability of the system.[12]

The synthesis of bacteriopurpurin is based on the allomerization of bacteriochlorophyll *a* (Scheme 2). In our

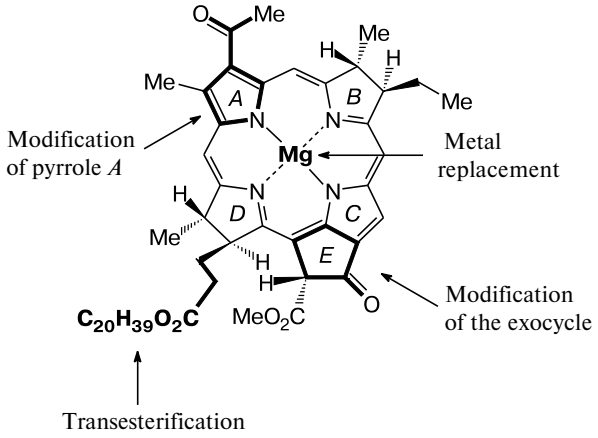
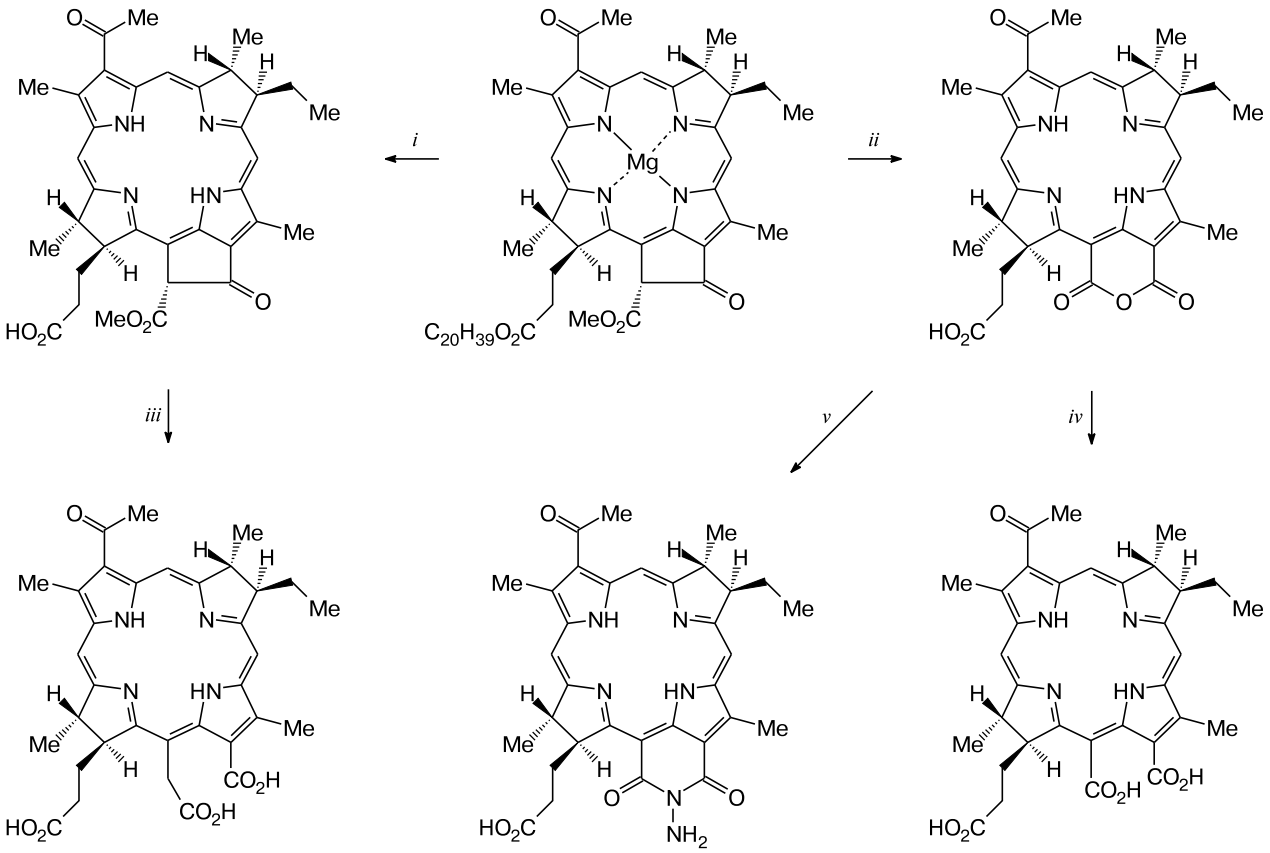


Fig. 2. Possible chemical modifications of bacteriochlorophyll *a*.

Scheme 2



Reagents and conditions: *i*. HCl, CF_3CO_2H/Me_2CO, argon. *ii*. 1) NaOH, O_2; 2) HCl. *iii*. 1) NaOH, argon; 2) H^+. *iv*. 1) NaOH; 2) H^+. *v*. $NH_2NH_2 \cdot H_2O$.

studies, we used the purple non-sulfur bacterium *Rh. capsulatus* strain B10, which was grown under chemoheterotrophic conditions,[13] as a producer of bacteriochlorophyll *a*, the content of which was 10—14 mg of the pigment per gram of the dry biomass.

The introduction of the exocycle leads to a bathochromic shift of the Q_2 band from 760 to 818 nm. However, the anhydride ring is stable only in neutral and acidic media, but it is rapidly cleaved in the presence of bases to form bacteriochlorin *p* exhibiting absorption at 770 nm. Therefore, it was necessary to transform the anhydride into a more stable derivative.

At the time when we started our research, methods have been developed for the replacement of an oxygen atom in the exocycle of bacteriopurpurin with the nitrogen atom using the reaction with alkylamines.[14,15]

However, cycloimides prepared by this method cannot be used for the synthesis of other *N*-substituted cyclic imides.

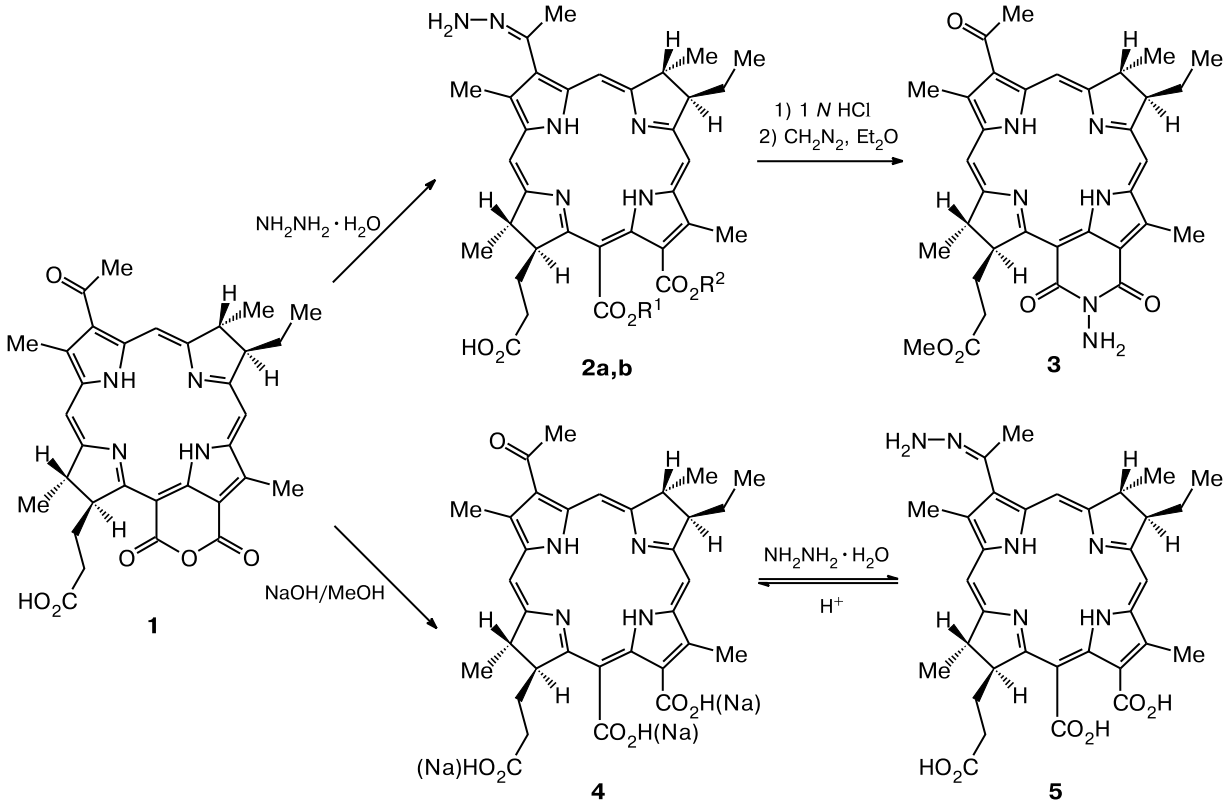
Radically new opportunities were found when performing this reaction with bifunctional reagents, such as hydrazine hydrate and hydroxylamine (see Scheme 2), instead of alkylamines. First, the use of strong nucleophilic reagents made the synthesis of cycloimides much easier. Second, due to the presence of an amino or hydroxy group at the nitrogen atom in the cycloimides thus synthesized, allowed us to prepare series of modified bacteriopurpurinimides based on one compound, which greatly simplified the search for new most efficient PS.[16,17]

An investigation of the reaction of bacteriopurpurin (**1**) with hydrazine hydrate (Scheme 3) showed that the first step results in the very rapid opening of the anhydride ring to form monohydrazides **2a,b**, which is accompanied by the transformation of the acetyl group into the hydrazone moiety. The suggestion that the reaction generates hydrazone followed by its hydrolysis in an acidic medium was confirmed by the reaction of bacteriochlorin *p* (**4**) with hydrazine. Product **5** was isolated without the addition of acid. The mass spectrum of the latter confirmed the formation of hydrazone, which is easily hydrolyzed to the starting bacteriochlorin *p* (**4**) in the presence of hydrochloric acid.

During the further course of the reaction, the absorption band at 750 nm is shifted to 836 nm, which attests to the spontaneous cyclization of monohydrazides. This reaction proceeds much faster in the presence of hydrochloric acid in the reaction mixture.

N-Aminobacteriopurpurinimide (**3**) was a key compound in the synthesis of numerous derivatives of bacteriochlorophyll *a*, which have been synthesized in our laboratory.

Scheme 3



2: $R^1 = NHNH_2$, $R^2 = OH$ (**a**); $R^1 = OH$, $R^2 = NHNH_2$ (**b**)

Tetrapyrroles bearing one or several positively charged groups are known to have antimicrobial activity when used in PDT.[18,19] This is associated with inactivation of viruses, bacteria, yeast fungi, and simplest reactive oxygen species, which are generated by photosensitizers under illumination.[20—22]

Attempts to transform the exocyclic amino group of bacteriopurpurinimide **3** into the cationic trimethylammonium group using the reactions of this compound with both iodomethane and dimethyl sulfate failed. A more successful approach is based on the acylation of the *N*-amino derivative of **3** with isonicotinic acid chloride followed by the quaternization of the nitrogen atom in the pyridine ring of compound **6** with iodomethane giving salt **7** (Scheme 4).

Cationic PS **7** is much more hydrophilic compared to the starting bacteriopurpurinimides **3** and **6**, with the result that it is more soluble in aqueous alcoholic solutions. Therefore, compound **7** is convenient for biological testing and is promising for subsequent medical applications.

The antimicrobial activity of cycloimide **7** and its synthetic precursor **6** was tested by photoinduced suppression of bioluminescence of a genetically engineered strain of Gram-negative luminescent bacteria.

As opposed to cycloimide **6** lacking charged groups, PS **7** triggers photoinduced suppression of bioluminescence of the bacterial strain, the degree of which increases with an increase in the concentration of the photosensitizer and the radiation dose.

On the one hand, white light had no effect on the intensity of bacterial bioluminescence. On the other hand, in the absence of irradiation, PS **7** did not induce a significant suppression of bioluminescence intensity.

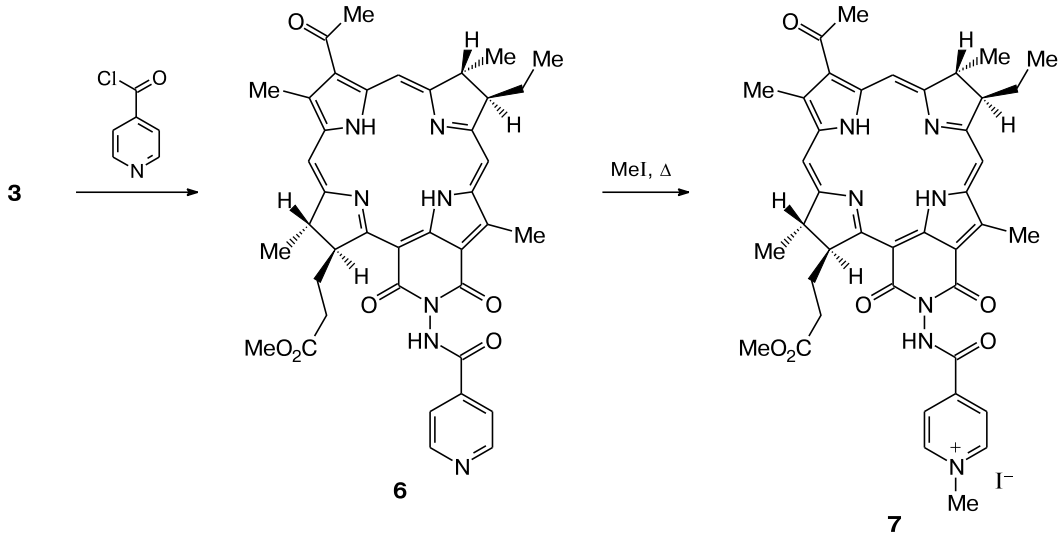
A study of antifungal activity showed that cationic photosensitizer **7** not only has antibacterial activity but also exhibits high efficiency in the inactivation of the *Candida guilliermondii* yeasts. Taking into account the fact that the long-wavelength absorption maximum of cycloimide **7** is observed at 830 nm, such PS combined with the appropriate laser source of radiation can be used for photosensitization of dense microorganism cultures.

In recent years, gold nanoparticles have been successfully used for the delivery of anticancer agents in chemotherapy[23,24] and the delivery of photosensitizers for PDT of cancer.[25—27] An important advantage of gold nanoparticles is their ability to accumulate in tumor tissues *via* the passive targeting by extravasation of nanosized materials from defective tumor vessels.[28—30]

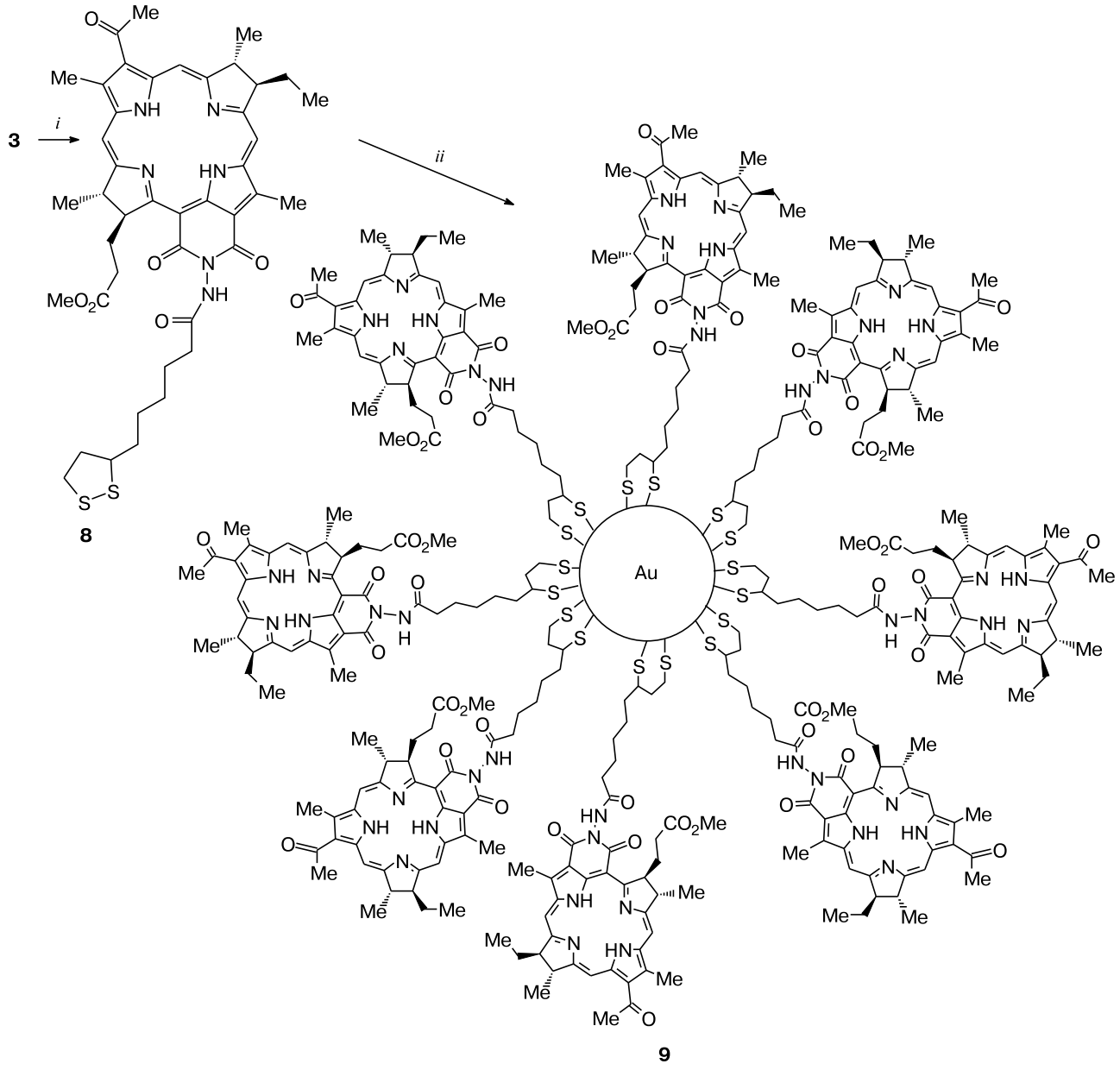
We used bacteriopurpurinimide **3** to synthesize a new sulfur-containing derivative of bacteriochlorophyll *a*, in which the exocyclic amino group is acylated with a lipoic acid residue. The latter is a biogenic compound, which acts in the body as a cofactor of pyruvate dehydrogenase and alpha-ketoglutarate dehydrogenase complexes. A disulfide group in the lipoic acid molecule imparts aurophilic properties to the pigment, and the latter was immobilized on the surface of gold nanoparticles through the S—Au bond formation (Scheme 5).

The shape and the size of the resulting particles coated with the immobilized photosensitizer (PS-Au) were determined by dynamic light scattering and transmission electron microscopy. Nanostructured PS, which was obtained as spheres with a hydrodynamic radius of 100—110 nm, absorbed light at 824 nm and exhibited intense fluorescence at 830 nm. This made it possible to study the kinetics of the distribution of this compound in organs and in normal and tumor tissues of tumor-bearing animals. A comparison of the biological properties of the free (PS) and immobilized (PS-Au) pigments in experiments in rats with sarcoma M-1 demonstrated that the immobilization of bac-

Scheme 4



Scheme 5



Reagents and conditions: *i*. Lipoic acid, CH_2Cl_2, EEDQ (ethyl 2-ethoxy-1(2*H*)-quinolinecarboxylate), 48 h.
ii. Gold nanoparticles, 24 h.

teriopurpurinimide on gold nanoparticles leads to an increase in the circulation time of nanostructured PS in the bloodstream and enhances its accumulation in the tumor stroma.

Using *N*-aminobacteriopurpurinimide **3** and the naphthalimide dye, we synthesized bichromophoric systems. The latter serve as models for theranostics — drugs, which, apart from the ability to selectively accumulate in cancer cells, exhibit intense fluorescence, thus making it possible to track the accumulation sites of the drug.[31]

The FD method is based on the ability of a photosensitizer to exhibit fluorescence upon activation with light of a specific wavelength and, consequently, to act as a cancer cell marker. However, the intrinsic fluorescence of bacteriochlorins is weak, which limits their application as diagnostic agents.[32] An enhancement of the fluorescence properties of PS based on natural pigments is achieved by the synthesis of bacteriochlorin conjugates of fluorophores. The latter are compounds of different nature exhibiting strong fluorescence, for example, such as carbocyanine

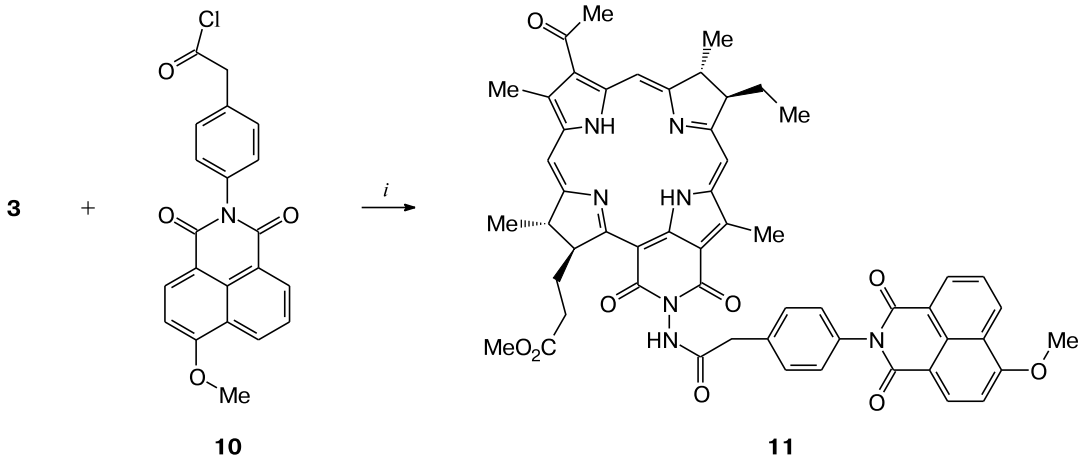
and naphthalimide dyes. The latter dyes are characterized by a narrow absorption band and exhibit fluorescence with a large Stokes shift, which should provide efficient fluorescence diagnosis of tumors.

On the one hand, the use of natural bacteriochlorophyll *a* derivatives in theranostics holds promise for enhancing the efficiency of the photodynamic action on tumor cells due to an increase in the depth of the photoinduced damaging effect. On the other hand, due to a considerable difference in the light absorption by the dye and the photosensitizer, the fluorescence diagnosis and the photodynamic action can be utilized separately by employing wavelength-tunable lasers.

The acylation of *N*-aminobacteriopurpurin **3** with naphthalimidecarboxylic acid chloride **10** gave bichromophoric system **11**, in which the natural pigment (ABPI) and the fluorophore (NI1) are linked by a rather short and rigid spacer (Scheme 6).

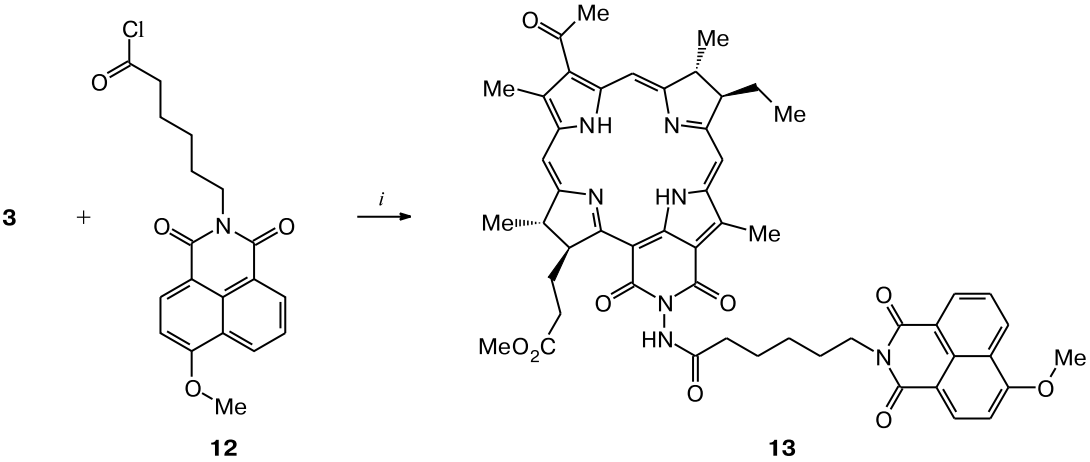
In order to evaluate the effect of the length and the nature of the spacer on the intramolecular interaction between the subunits and on the photonics of the bichromophoric system, conjugate **13** was synthesized in a similar way by the reaction with acid chloride **12** (Scheme 7). It should be noted that an increase in the spacer length and the minimization of the effect of the steric factor resulted in a decrease in the reaction time and an increase in

Scheme 6



i. C_5H_5N, argon.

Scheme 7



i. C_5H_5N, argon.

the yield of the target compound (the yields of compounds **11** and **13** were 45 and 61%, respectively).

The structures of target conjugates **11** and **13** were confirmed by mass spectrometry and ^{1}H NMR spectroscopy. The intramolecular energy transfer was investigated by the analysis of electronic absorption and fluorescence spectra.

The integration of two photoactive subunits having individual spectroscopic characteristics to one bichromophoric system can lead to a considerable change in the photonics of the conjugate as a whole compared to the starting chromophores due to the donor to acceptor energy transfer that takes place in the system. To minimize this process, the fluorescence of the donor should not overlap with the absorption of the acceptor. As can be seen in Fig. 3, this overlap occurs in conjugates **11** and **13** in the range of 400—550 nm.

Meanwhile, it is known that 1,8-naphthalimides containing alkoxy substituents at the C(4) atom have a fluorescence maximum in the blue region at 430—470 nm.

Therefore, it was expected that conjugates **11** and **13** whould be promising theranostics, which are suited to both the fluorescence diagnosis due to high fluorescence of the naphthalimide and the photodynamic action on the tumor through the absorption of bacteriopurpurinimide in the near-IR region.

However, contrary to expectations, the fluorescence of the naphthalimide dye involved in the conjugate appeared to be two orders of magnitude lower compared to that of the free dye (the fluorescence quantum yield of the free dye is 0.58, whereas the corresponding value of the dye in conjugate **11** is 0.0068) (Fig. 4). This is due to the fact that the energy of light with a wavelength of 430 nm, which was used to irradiate the system, was absorbed by both chromophores and, as a consequence, only a part of the total excitation energy was converted to the emission energy of the naphthalimide subunit. Besides, the fluorescence quantum yield of conjugate **11** was 25 times lower than that of a solution containing an equimolar mixture of the starting chromophores (Table 1). This is indicative of strong fluorescence quenching of naphthalimide in the conjugate due to the radiationless energy transfer (the efficiency of the energy transfer for compound **11** is 96%).

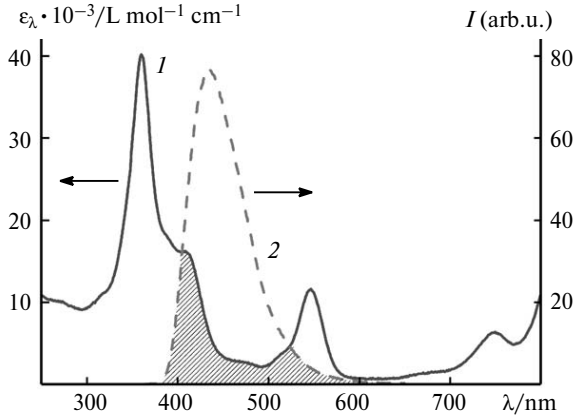


Fig. 3. Electronic absorption spectrum of aminobacteriopurpurinimide ABPI (*1*) and the fluorescence spectrum of the naphthalimide dye NI1 (*2*) in acetonitrile.

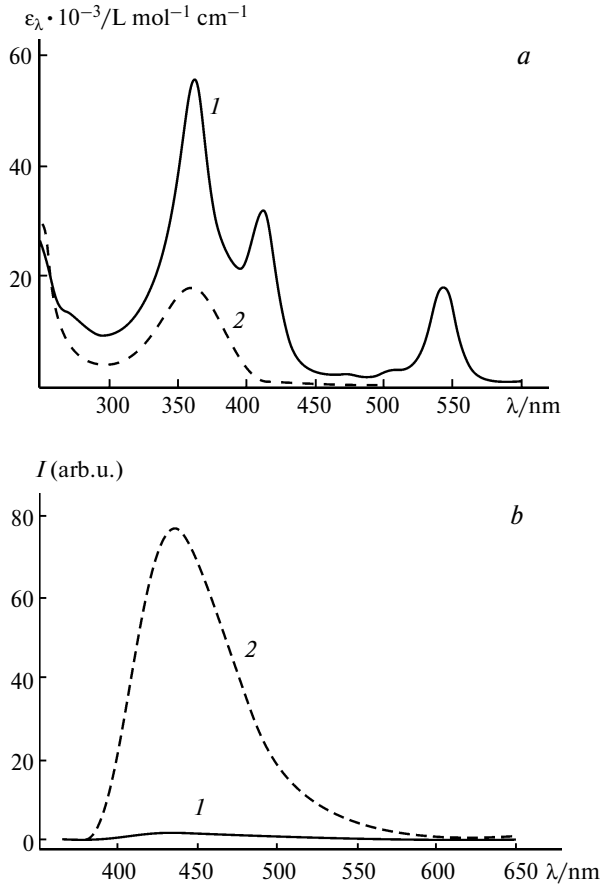


Fig. 4. Electronic absorption spectra (*a*) and fluorescence spectra (*b*) of conjugate **11** (*1*) and naphthalimide **10** (*2*) in acetonitrile ($C = 5.8 \cdot 10^{-6}$ mol L^{-1}).

Table 1. Spectroscopic and photophysical characteristics of bacteriopurpurin—imidenaphthalimide conjugates and their naphthalimide precursors

Compound	φ	λ/nm	R_0/Å	E(%)
10	0.58	434	—	—
3 + **10**	0.17	436	—	—
11	0.0068	436	13.0	96
12	0.56	446	—	—
3 + **12**	0.11	448	—	—
13	0.055	448	13.2	50

Note: φ is the quantum yield, λ is the wavelength, R_0 is the distance between the rings, and E is the efficiency of the energy transfer.

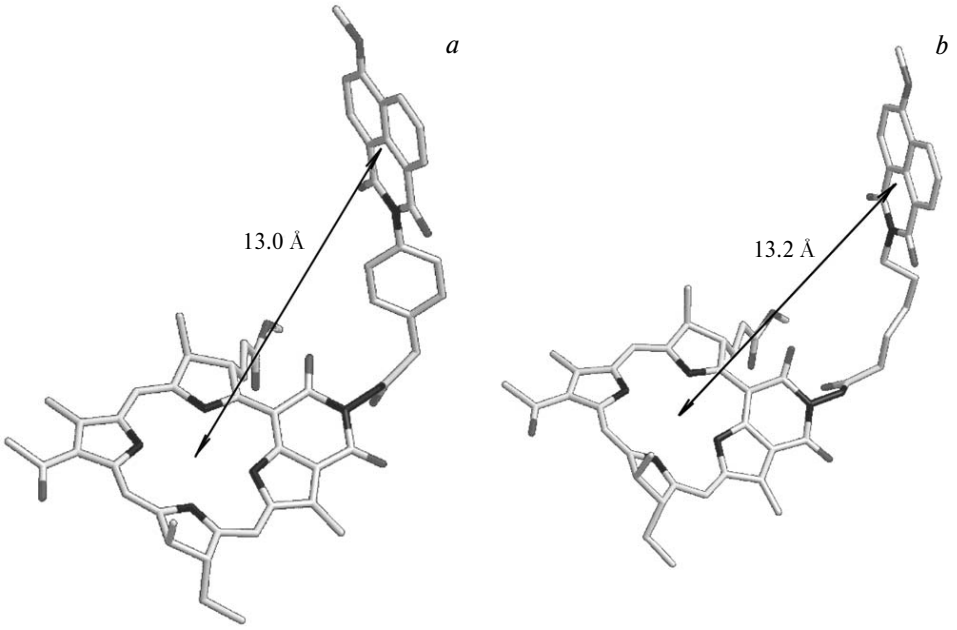


Fig. 5. Three-dimensional structures of conjugates **11** (*a*) and **13** (*b*).

The calculation of the bond lengths and the selection of the optimal conformations for the conjugate molecules by computer modeling provided an estimate for the distances between the rings in conjugates **11** (R_0 = 13 Å) and **13** (R_0 = 13.2 Å) (Fig. 5, *a* and *b*, respectively), which should correspond to 95% energy transfer.

However, as shown earlier, the flexibility of the spacer may influence the energy transfer in purpurinimide conjugates of carbocyanine dyes. In the case under consideration, the replacement of the quite rigid aromatic spacer in compound **11** with the flexible pentamethylene moiety in conjugate **13**, on the one hand, can facilitate the mutual orthogonal arrangement of the chlorin and naphthalimide rings, at which the efficiency of the energy transfer is close to zero, and, on the other hand, leads to the absence of a π—π interaction between the aromatic rings in chromophores because of the insufficient spacer length, resulting in fluorescence quenching.

The fluorescence quantum yields of naphthalimide and an equimolar mixture of chromophores are given in Table 1. It can be seen that the fluorescence quantum yield of conjugate **13** is two times lower than that of the equimolar mixture of the chromophores, which corresponds to 50% efficiency of the energy transfer (compared to 96% efficiency of the energy transfer for conjugate **11**).

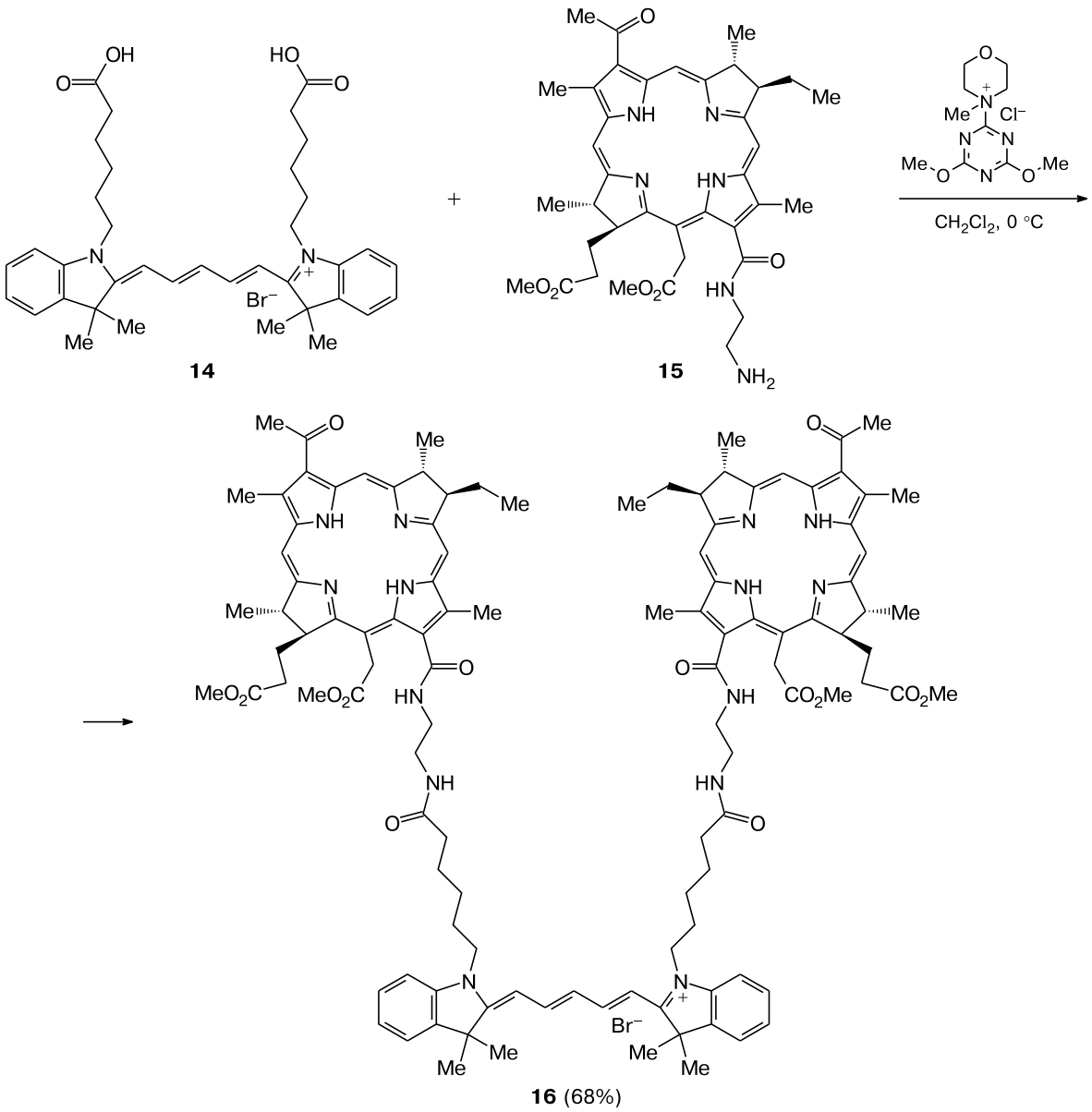
Since the distances between the chromophores in conjugates **11** and **13** and the fluorescence quantum yields of naphthalimides **10** and **12** are very similar (see Table 1), a considerable decrease in the efficiency of the energy transfer in conjugate **13** can be attributed solely to conformational changes in the molecule such that the mutual arrangement of the chromophores is unfavorable for the intramolecular energy transfer.

Despite the promising properties of naphthalimide dyes, including chemical stability, the rigid planar molecular structure, high molar extinction coefficients, and a large Stokes shift, there are limitations to their application for cancer therapy. Functional derivatives of naphthalic acid imide cannot be used for fluorescence imaging of deep-lying and pigmented tumors, including melanoma, because of the short-wavelength absorption of these compounds. Therefore, in the next step of our studies, we used a carbocyanine dye as a fluorescent label for the design of models for theranostics.

The reaction of the dicarboindocyanine dye Cy 5.5 (**14**) with bacterioaminoamide **15** in a molar ratio of 1 : 2 gave conjugate **16** (Scheme 8). *N*-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-*N*-methylmorpholinium chloride (DMTMM) was used as an efficient condensing agent for the formation of the amide bond between the subunits. The course of the reaction was monitored by chromatography because R_f of the target product substantially differs from this parameter for the starting compounds.

In order to determine the primary structure of conjugate **16**, we assigned the signals of all protons and carbon atoms (except for quaternary carbons) in the 1D ^{1}H and ^{13}C NMR spectra and measured COSY, TOCSY, ^{1}H,^{13}C-HSQC, ^{1}H,^{13}C-HMBC, and ROESY spectra. The secondary structure of the conjugate in chloroform was suggested based on 2D ROESY spectra, which show ^{1}H—^{1}H couplings that can occur in a nonplanar structure (Fig. 6).

Scheme 8



The optimal conformation of the conjugate was determined using the computer-aided analysis with the Sybyl 8 package (Tripos Inc., St. Louis, USA) for calculations of intermolecular interactions. Initially, we chose three conformations having the minimum internal energy, which are shown in Fig. 7. The full energy minimization of the molecule using the ICM-Pro software (Version 3.7-2b) in terms of the BPMC variance optimization method (variational Monte-Carlo method) demonstrated that the conformation **A** has the minimum energy (–338.21 kcal mol^{-1}).

The molecular energy of the conformation **B** is somewhat higher (–333.64 kcal mol^{-1}). Therefore, taking into account the NMR spectroscopic data, which correlate with the structure **A** (see Fig. 7), we suggested that the structures **B** and **A** exist in equilibrium in a solution of conjugate **16**, with the latter structure slightly predominating.

The electronic spectrum of triad **16** is a superposition of the absorption spectra of its individual components (Fig. 8). The absorption spectrum of conjugate **16** is identical to the spectrum of the corresponding mixture of free bacteriochlorin and the dye. This is indicative of the absence of a pigment-pigment interaction between the main states of the subunits in the conjugate.

The fluorescence spectrum of conjugate **16** obtained with excitation at the absorption band maximum of the bacteriochlorin component of the conjugate (515 nm)

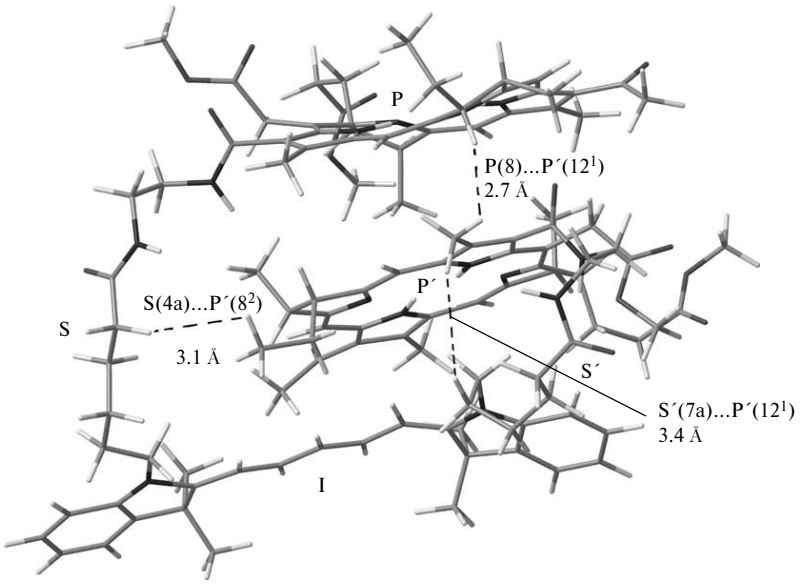


Fig. 6. Three-dimensional structure of triad **16** (two parallel planes of the tetrahydroporphyrin macrocycles (P and P´) linked to the conjugated system of the indodicarbocyanine dye (I) through spacer groups (S and S´)). The distances between selected hydrogen atoms and specific proton-proton interactions (dashed lines) are indicated.

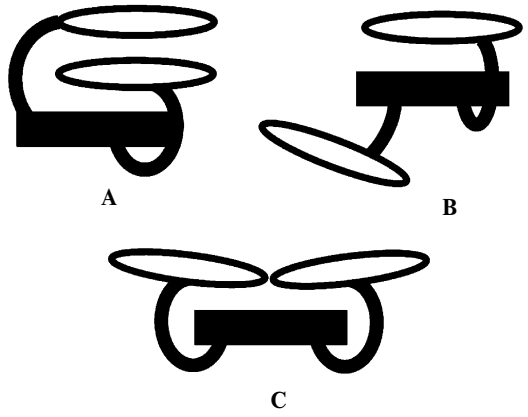


Fig. 7. Schematic representation of three possible conformations **A**—**C** of triad **16**.

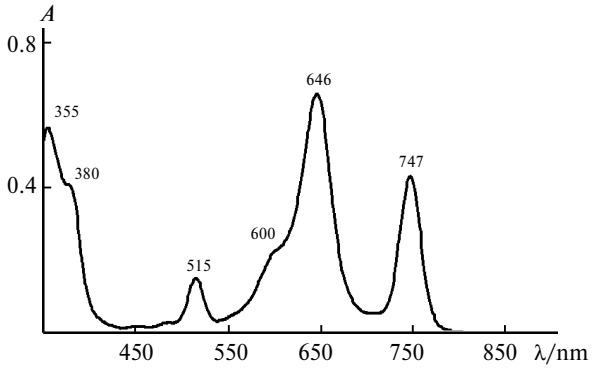


Fig. 8. Absorption spectrum of triad **16** in acetone. The absorption maxima at λ = 515 and 747 nm belong to bacterioaminoamide **15**; at 646 nm, to dye **14**.

shows the only fluorescence peak characteristic of free bacteriochlorin (Fig. 9, *a*), whereas the spectrum obtained with excitation at the absorption band maximum of the carbocyanine component (646 nm) shows two bands corresponding to the fluorescence of bacteriochlorin (756 nm) and the dye (672 nm), the intensity of the latter in the conjugate being lower than the fluorescence of the individual compound (Fig. 9, *b*).

An analysis of the fluorescence spectra provides evidence for the intramolecular energy transfer from the carbocyanine dye to bacteriochlorin, the efficiency of this transfer being ~96%. As can be seen in Table 2, the fluorescence quantum yield (Φ_F) of the carbocyanine dye in triad **16** is 2.5% of this parameter for free dye **14**.

It should be noted that the fluorescence quenching (3.5-fold) for bacteriochlorin subunits occurs also in the triad due apparently to the spatial proximity of these subunits and the interaction between the π-electron systems of the macrocycles.

The photosensitizing activity of bacteriochlorins in conjugate **16**, which was estimated from the quantum yields of singlet oxygen generation (Φ_Δ), was also 2.5—3 times lower compared to the free pigment (see Table 2).

However, the quantum yields of singlet oxygen generation (20—30%) and the fluorescence quantum yields (0.035) are, on the whole, rather high. Therefore, this substance is promising for both the photodynamic action on tumors and its fluorescence imaging.

We used *N*-aminobacteriopurpurinimide **3** as a precursor in the synthesis of boron-containing conjugates of the bacteriochlorophyll *a* series. The attachment of boron polyhedra to natural chlorophyll derivatives enables the

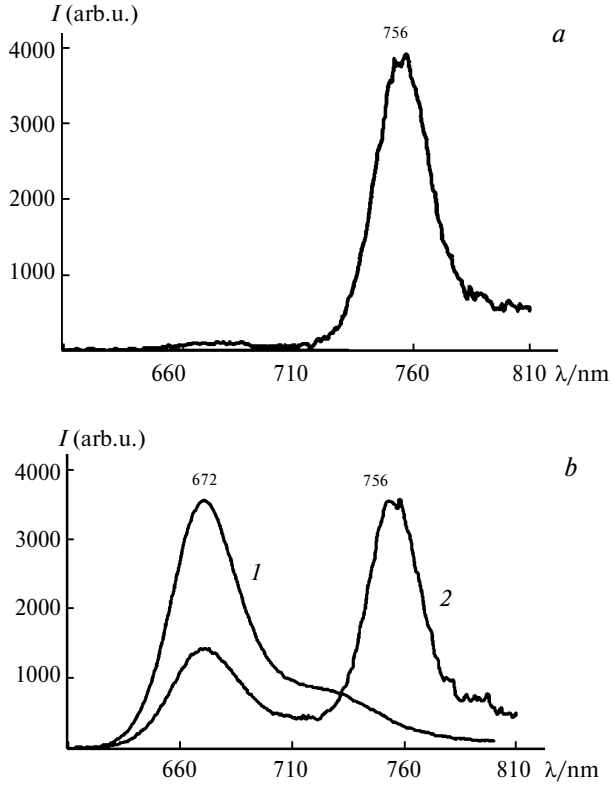


Fig. 9. Fluorescence spectra in acetone of conjugate **16** (excitation wavelength 515 nm) (*a*), the free dye (*1*) and the conjugate (*2*) under irradiation at 646 nm (*b*).

targeted delivery of boron atoms to tumors, which is used for the design of agents for boron neutron capture therapy (BNCT) of cancer.[33,34]

The presence of the exocyclic amino group allowed us to develop two procedures for the synthesis. One of these procedures involves the formation of the amide bond between the macrocycle and boron clusters; another procedure is based on the preparation of conjugates by the reaction of purpurinimide **3** with oxonium derivatives of metallocarborane (Scheme 9).[35–37]

The former approach was implemented in the reaction of cycloimide **3** with carboranylcarboxylic acids using various methods of activation of the carboxyl group. Conjugate **19** was synthesized by the reaction of the starting aminobacteriopurpurinimide **3** with dodecaborate-containing carboxylic acid chloride **17** in the presence of triethylamine; conjugate **20**, by the reaction of the same cycloimide **3** with acid **18** activated by dicyclohexylcarbodiimide.

However, the yields of boron-containing conjugates synthesized by this method were not higher than 20%. The approach involving the nucleophilic ring opening in the oxonium derivative of bis(dicarbollide)cobalt **21** in the presence of diisopropylethylamine (DIPEA) proved to be more efficient. Conjugate **22** containing two boron polyhedra was synthesized in 64% yield. This compound shows absorption at 830 nm and can be considered as a promising PS for combined PDT—BNCT of cancer.

One of the possible ways to increase the selectivity of the photodynamic action of PS molecules is to attach ligands, which have specific receptors on the tumor cell surface. This leads to an increase in the selectivity of binding to the cell membrane, the enhancement of the efficiency of the photodynamic action, and, as a consequence, a decrease in the drug concentration.

It is known that cancer cells are characterized by high expression of galectins (such as galectin-1 and galectin-3). These proteins contain a carbohydrate-recognition domain having high affinity for β-galactosides. Therefore, carbohydrate substituents in bacteriochlorins can provide the vector delivery of PS into cancer cells *via* receptor-mediated endocytosis.

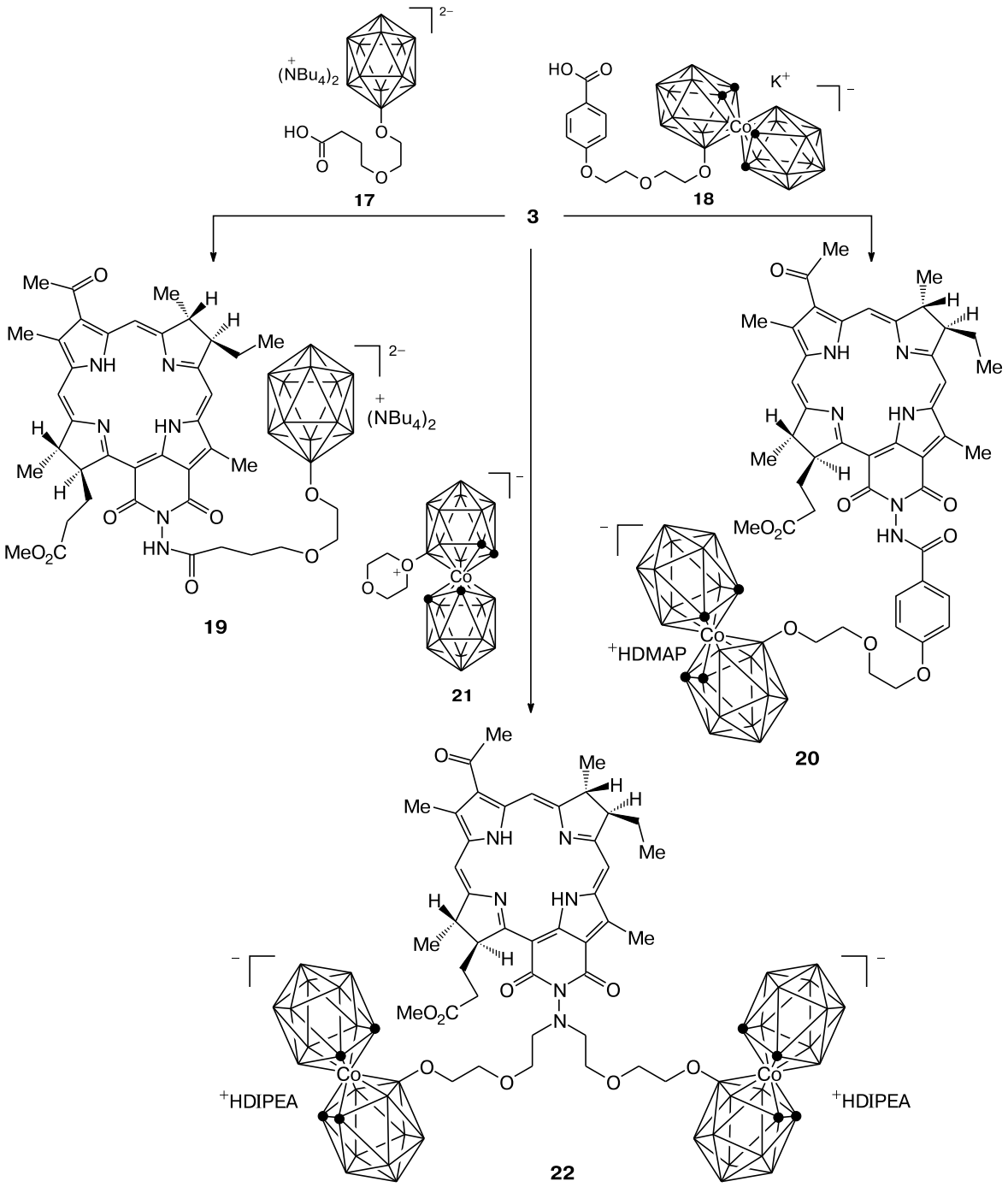
We developed a scheme for the synthesis of bacteriopurpurinimide conjugates of carbohydrate residues, which involved the click reaction between acetylene derivatives of bacteriochlorins, on the one hand, and a lactose deriva-

Table 2. Fluorescence quantum yields (Φ_F) and the quantum yields of singlet oxygen generation (Φ_Δ) by triad **16** and its components in acetone

Sample	Fluorescence		Singlet oxygen generation	
	λ_{ex}[a]/nm	Φ_F	λ_{ex}[b]/nm	Φ_Δ
Free dye	600	0.80±0.04	647	<0.005
Free bacteriochlorin	515	0.13±0.01	747	0.80±0.05
Dye in the triad	600	0.020±0.05[c]	—	—
Triad	600	0.035±0.005[d]	647	0.20±0.03
Triad	515	0.036±0.005[d]	747	0.30±0.04

[a] The fluorescence excitation wavelength corresponding to the absorption maximum of the triad components: 600 and 515 nm for the dye and bacteriochlorin, respectively.
[b] The excitation at the absorption maximum of the triad components: 647 and 747 nm for the dye and bacteriochlorin, respectively.
[c] For the carbocyanine dye in the triad (λ = 672 nm).
[d] For bacteriochlorin in the triad (λ = 756 nm).

Scheme 9



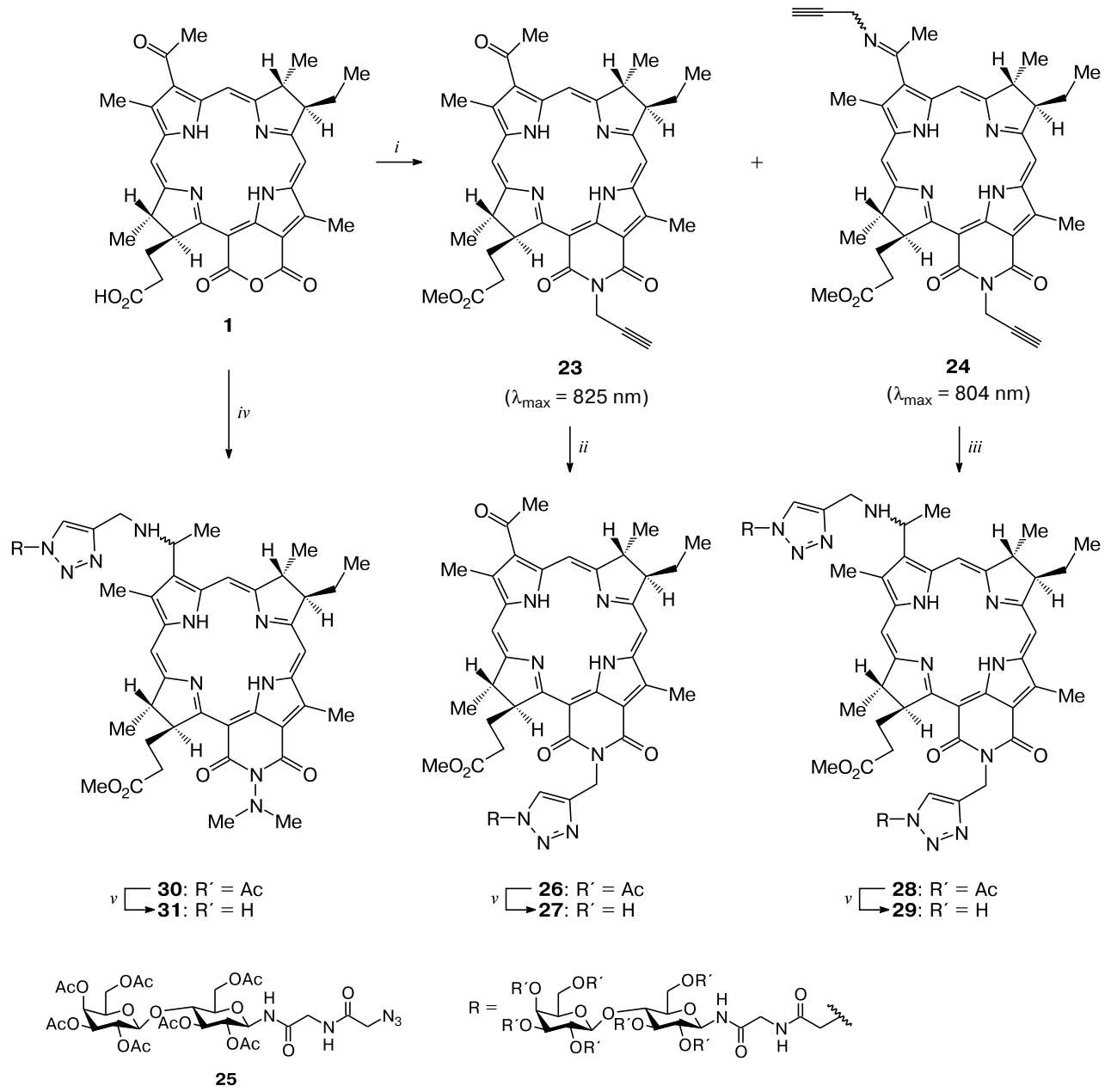
DIPEA is diisopropylethylamine, DMAP is 4-dimethylaminopyridine

tive containing the azide group, on the other hand, as the key step.[38,39]

In this scheme, cycloimides containing a propargyl group as a substituent at different positions of the mactrocycle were generated from bacteriopurpurin **1** serving as a key compound. In fact, the presence of two highly electrophilic centers (the acetyl group and the anhydride exocycle) in the bacteriopurpurin molecule results in the ambiguous course of the reaction with propargylamine giving, apart from cycloimide **23**, dialkynyl product **24** (Scheme 10).

It was shown that the ratio of products **23** and **24** can be varied by changing the amount of propargylamine and

Scheme 10



Reagents and conditions: *i*. 1) CH_2N_2; 2) $H_2NCH_2C{\equiv}CH$, $CHCl_3$, Δ. *ii*. **25**, CuI (10 mol.%), DIPEA, CH_2Cl_2. *iii*. 1) $NaBH_4$, MeOH; 2) **25**, CuI (10 mol.%), DIPEA, CH_2Cl_2. *iv*. 1) $NH_2NH_2 \cdot H_2O$; 2) HCl; 3) CH_2N_2; 4) MeI/DIPEA; 5) $H_2NCH_2C{\equiv}CH$; 6) $NaBH_4$/MeOH; 7) **25**, CuI. *v*. 1) MeONa/MeOH; 2) H^+.

the reaction time. For instance, the reaction of bacteriopurpurin methyl ester with a 8-fold excess of propargylamine under reflux in chloroform for 16 h gave cycloimide **23** in 60% yield and Schiff base **24** in 20% yield. An increase in the excess of propargylamine (40 equiv.) and the reaction time to 40 h resulted in the formation of dipropargyl derivative **24** as the major product. The subsequent chromatographic separation of the reaction mixture afforded cycloimides **23** and **24**. These compounds were introduced into the reaction with azide derivative of lactose **25**, which was prepared by the successive acylation of aminolactoside peracetate with glycine and azidoacetic acid.

The cycloaddition proceeded in a similar way as that with chlorin.[40] However, the formation of a Cu complex of bacteriopurpurinimide was not observed in the course

of the reaction. This is consistent with the fact known in the chemistry of porphyrins, according to which the ease of the formation of metal complexes decreases in the series porphyrins—chlorins—bacteriochlorins. Glycoconjugate **26** was synthesized in 80% yield.

Bacteriopurpurinimide containing two lactose residues was synthesized based on cycloimide **24**. For this purpose, the latter was reduced with sodium borohydride to form a mixture of diastereomers. The latter were introduced into the coupling reaction with lactoside **25** to prepare conjugate **28** in 70% yield.

N,*N*-Dimethylaminobacteriopurpurinimide was used as the starting compound for the targeted introduction of a carbohydrate moiety into the pyrrole ring *A*. The reaction of this compound with propargylamine under reflux in chloroform gave the corresponding Schiff base, which was reduced with sodium borohydride. The resulting mixture of diastereomers was introduced into the coupling with the azide derivative of lactose peracetate **25** to form conjugate **30** in 83% yield.

Highly hydrophilic carbohydrate-containing conjugates **27**, **29**, and **31** in the series of natural bacteriochlorins were synthesized for the first time by removing the acetyl protecting groups in compounds **26**, **28**, and **30**.

The NMR spectra of lactosylbacteriopurpurinimides **26**, **28**, and **30** show signals assigned to the macrocycle, triazole, and a carbohydrate moiety. This allowed us not only to unambiguously establish the structures of the compounds but also to determine the tautomeric forms and conformations.

The photoinduced activity of the resulting carbohydrate-containing bacteriochlorins was evaluated in the transformed cell line Hep2 (larynx epidermoid carcinoma). It was shown that bacteriopurpurinimide derivatives are, as a whole, weakly active, the position of the carbohydrate residue in the mactrocycle having a certain effect on the photocytotoxicity of the compounds (Table 3). Bacteriopurpurinimide **31** containing a disaccharide residue in the pyrrole ring *A* exhibits moderate activity (IC_{50} = 2.24 μmol L^{-1}), whereas pigments containing a carbohydrate moiety in the exocycle (compound **27**) or two disaccharide residues (compound **29**) are inactive.

The low activity of glycoconjugates of natural bacteriochlorins (lower than that of nonglycosylated chlorin e_6 (IC_{50} = 0.16 μmol L^{-1})) may be attributed to high hydrophilicity of the resulting pigments and the presence of the triazole ring.

The use of hydroxylamine provided radically new opportunities for the synthesis of cycloimides of the bacteriochlorophyll *a* series and their subsequent chemical transformations.

The presence of two reaction centers (the anhydride ring and the acetyl group) in the bacteriopurpurin molecule resulted in the stepwise reaction with hydroxylamine (Scheme 11). The first step affords oxime **32**, and only then (in the presence of an excess of the reagent) the second hydroxylamine molecule reacts with the anhydride ring to form *N*-hydroxybacteriopurpurinimide oxime **37**.

The presence of a labile hydrogen atom in the latter compound makes it possible to substantially extend the range of cycloimides using the acylation and alkylation reactions. Of most interest is *O*-methyl derivative **42** that is generated in high yield by the reaction of diazomethane with cycloimide **37**.

The investigation revealed two interesting facts. The acidity of the hydrogen atom in the oxime moiety is so low that the latter is not involved in the reaction with diazomethane. In addition, the detailed analysis (TLC, mass spectrometry) of *N*-methoxy derivative **42** demonstrated that this compound is a mixture of two isomers with very similar R_f values. The isomers were isolated and characterized by ^{1}H NMR spectroscopy, including 1D NOE spectroscopy, and it was concluded that compound **42** exists in two stereoisomeric forms (*syn* and *anti*).

Compound **42** has good photophysical properties. Thus, the singlet oxygen quantum yield (in a 1% Cremophor emulsion in water) is rather high (φ_Δ = 0.57), due to which the pigment showed significant photoinduced cytotoxicity in the cell line A549 (IC_{50} = 0.97 μmol L^{-1}).

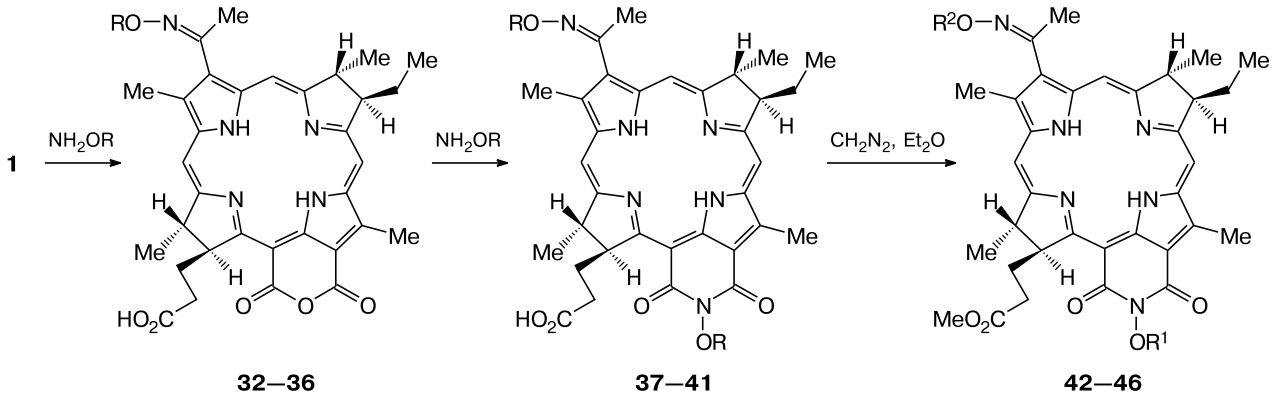
However, the study of the pharmacokinetics of this compound showed that it has some properties, which limit its further application. Thus, this compound has a low selectivity index (1.2—1.6), and the dynamics of changes in the PS concentration in the tumor, including short-term accumulation (20—80 min) and rapid excretion, interferes with efficient PDT.[41]

Taking into account that alkoxyamines are often not commercially available, we developed a versatile proce-

Table 3. Results of biological *in vitro* assays of glycosylated bacteriopurpurinimides **27**, **29**, and **31** in relation to chlorin e_6 trimethyl ester

Compound	Macrocycle type	Carbohydrate residue type	IC_{50} /μmol L^{-1}
27	Bacteriopurpurinimide	13^2-*N*-Lac	—
29	Bacteriopurpurinimide	di-Lac	—
31	Bacteriopurpurinimide	3-Lac	2.24
Chlorin e_6 trimethyl ester	Chlorin e_6	—	0.16

Scheme 11



32—36 **37—41** **42—46**

R = H (**32**, **37**), Me (**33**, **38**), Et (**34**, **39**), Bu (**35**, **40**), C_6H_{13} (**36**, **41**)
R^1 = Me, R^2 = H (**42**); $R^1 = R^2$ = Me (**43**); $R^1 = R^2$ = Et (**44**); $R^1 = R^2$ = Bu (**45**); $R^1 = R^2 = C_6H_{13}$ (**46**)

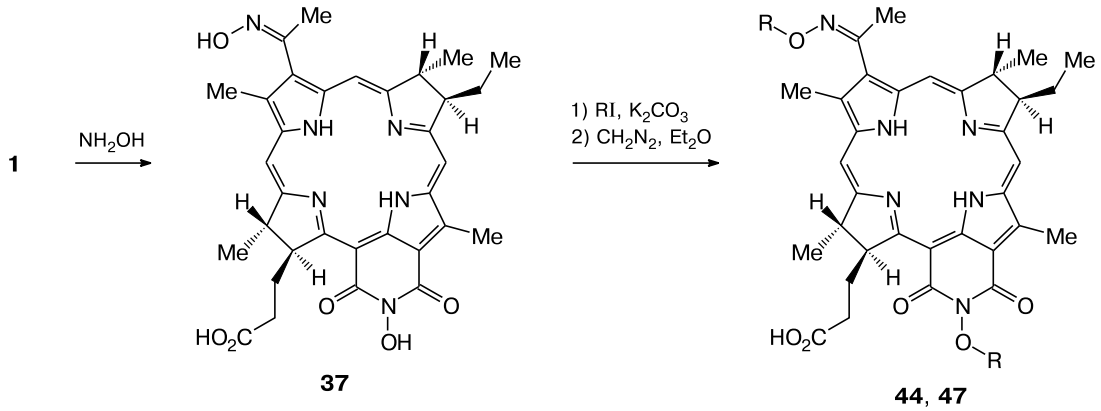
dure for the synthesis of *N*-alkoxybacteriopurpurinimide alkyloximes, involving the synthesis of *N*-hydroxybacteriopurpurinimide oxime and the reaction of the latter with appropriate alkyl iodides in the presence of potassium carbonate (Scheme 12).

This approach allowed us to prepare a homologous series of bacteriopurpurinimides containing alkyl substituents of different length in order to perform biological screening assays and search for the most efficient PS.[42]

To enhance the overall hydrophobicity of the molecule, we synthesized cycloimides **43**—**47** (see Schemes 11 and 12) containing alkoxy groups both in the exocycle and the pyrrole ring *A*. Despite the fact that hydrophilic compounds are more suitable for the preparation of solutions for intravenous administration, the ability of these compounds to cross cell membranes and invade vitally important target cells is rather limited. It is known[43,44] that hydrophobic PS generally penetrate inside the tumor and cancer cells, whereas hydrophilic PS are accumulated in blood vessels and stroma at the tumor periphery. The amphiphilicity of pigments can be varied by changing the length of alkyl side chains, which, in turn, has an effect on the photoinduced activity of PS. It was shown[45] that PS containing side chains composed of three and six methylene units exhibit the maximum activity both *in vitro* and *in vivo*.

Initially, we synthesized *N*-ethoxybacteriopurpurinimide *O*-ethyloxime methyl ester[46] and studied its biological properties. The presence of two ethyl groups in this compound imparts certain hydrophobicity to the molecule. However, this hydrophobicity is insufficient for the efficient transmembrane transfer in cancer cells, resulting in low photoinduced antitumor activity. In addition, the pigment is characterized by low selectivity of accumulation

Scheme 12



37 **44**, **47**

R = Et (**44**), Pr (**47**)

in the tumor (the selectivity index is not higher than 2) and the absence of complete cure of animals.[47]

The above-mentioned drawbacks stimulated the search for new highly efficient PS with increased hydrophobicity due to an increase in the carbon chain of side substituents.

We suggested that a nanostructured aqueous dispersion based on *N*-propoxybacteriopurpurinimide *O*-propyloxime methyl ester using Cremophor ELP as the solubilizing agent, which disaggregates PS and is not inherently toxic, is PS of choice.

The photodynamic efficiency of this PS was evaluated in *in vivo* experiments, involving the systemic administration of the agent in tumor-bearing animals at doses from 1.0 to 10.0 mg kg^{-1} and the optical irradiation of the pathological area 0.25—8 h after the administration of PS in the spectral range of 789—831 nm at an energy density from 45 to 360 J cm^{-2}. Photodynamic therapy using this agent showed high photoinduced anticancer activity, which was manifested in 100% tumor growth retardation and 90% cure of animals due to the selective accumulation in the tumor and rapid excretion from the body.[48]

Therefore, in our studies we implemented one of the main principles of the drug design by performing a chemical modification of a lead compound, *viz.*, *N*-hydroxybacteriopurpurinimide oxime, through the introduction of substituents at the periphery of the macrocycle with the retention of the pharmacophore structure.[49]

* * *

The current situation with research in the area considered in the present review is such that, despite the interesting spectroscopic, photophysical, and biological properties of bacteriochlorophyll *a* derivatives and considerable prospects of their use in biology and medicine, chemical modifications of this pigment and the use of this compound for the construction of new materials have received little attention in the literature. It cannot be said with certainty whether this is due to difficulties associated with the growing of the biomass and insufficient quantities of the biomass produced with a high bacteriochlorophyll *a* content, chemical instability and photolability of the pigment, or the complexity of the synthesis of its derivatives. However, long-standing experience gained in the chemistry of porphyrins and related compounds at the Moscow Technological University, Institute of Fine Chemical Technologies, and the participation in fundamental research on the development of chemical and biotechnological methods of modification of biologically active compounds contributed significantly to the chemistry of tetrahydroporphyrins.

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