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Synthesis of Boron-Containing Derivatives of Pyropheophorbide *a* and Investigation of Their Photophysical and Biological Properties

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Abstract—Proceeding from pyropheophorbide *a* and 9-hydroxymethyl-*m*-carborane, 1-hydroxymethyl-*o*-carborane, and 3-amino-*o*-carborane new carboranylchlorins were prepared, and their photophysical and biological properties were investigated.

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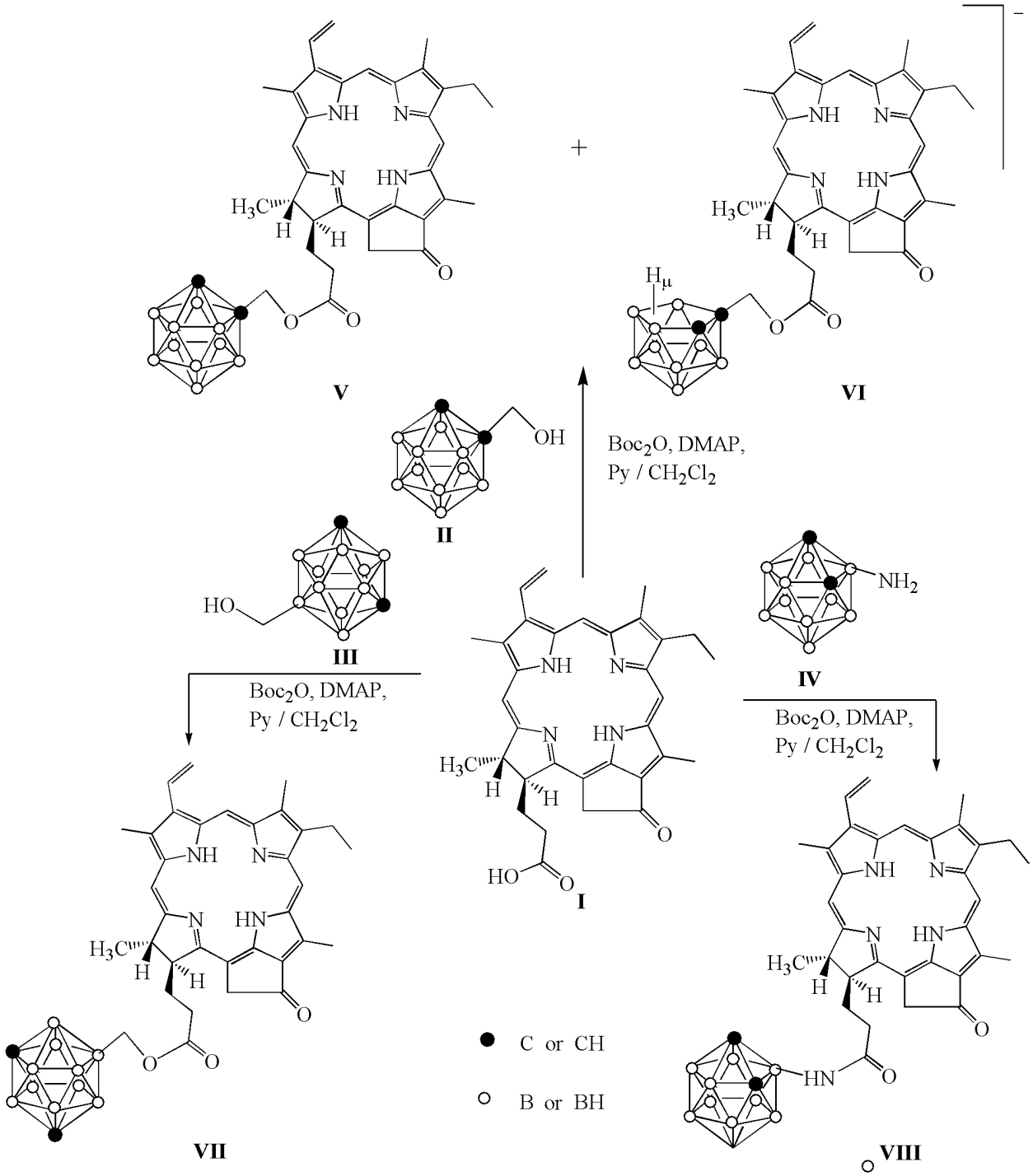
The modern development of cancer treatment using photodynamic (PDT) [1] and boron neutron capture therapy (BNCT) [2] depends to a considerable extent on preparation of new compounds with improved spectral, photophysical, and biological characteristics. Both these methods possess certain advantages and drawbacks. In order to combine PDT and BNCT compounds were synthesized and investigated based on porphyrins [3, 4] endowed with a double action: boron neutron capture and photosensitizing.

Recently a high interest is attracted by natural hydrogenated porphyrin analogs, chlorines. The choice of these compounds is due to their wide spread in the nature and relation to endogenous porphyrins thus suggesting that they would be of low toxicity and would be fast removed from the body. From this viewpoint pyropheophorbide *a* may be regarded as a convenient substance: It possesses a strong absorption in the region 667 nm required of the photosensitizers for PDT [1]. A modification of these natural pigments by introducing boron in a carborane structure provides an opportunity to apply the new compounds both to PDT and BNCT.

In this connection the goal of this study was the synthesis of pyropheophorbide *a* (**I**) carborane derivatives. Initial pyropheophorbide *a* (**I**) was obtained from the lyophilized biomass of the microalga *Spirulina platensis* by a structural modification of its chlorophyll *a* [5, 6]. As boron-containing reagents we used *closo*-1-hydroxymethyl-*O*-carborane (**II**) [7], *closo*-9-hydroxymethyl-*m*-carborane (**III**) [8] where the functional hydroxymethyl groups were attached either to carbon atom (**II**) or to boron (**III**), and *closo*-3-amino-*O*-carborane (**IV**) [9]. Their addition to the carboxy group of the pyropheophorbide *a* was performed by the formation either of an ester group (**II** and **III**) or amide bond (**IV**) by the mixed anhydride method using di-*tert*-butyl pyrocarbonate in pyridine as the condensation agent.

The condensation of pyropheophorbide (**I**) and carborane **II** gave rise to equal amounts of two substances containing carboranes in *closo*- (**V**) and *nido*-forms (**VI**). From carborane **III** a single carboranyl derivative of pyropheophorbide *a* (**VII**) was obtained in a high yield containing the carborane in the *closo*-form. This outcome was apparently caused by the nonuniform electron density distribution on the carbon and boron atoms of the carborane polyhedron [10, 11]. The reaction of carborane **IV** yielded compound **VIII**.

All the carborane-containing derivatives of pyropheophorbide *a* were isolated by column chromatography. They are well soluble in chloroform, dichloromethane, and pyridine The structure of new compounds was confirmed by electronic, IR, ^{1}H and ^{11}B NMR, and mass spectra.



The electron absorption spectra of all compounds **V**–**VIII** were of chlorin type and has identical position of the bands in the visible region. In the spectra of all compounds the extinction coefficients were smaller than in the spectrum of initial pyropheophorbide *a* (**I**).

The IR spectra of freshly prepared samples of compounds **V**, **VII**, and **VIII** contained the absorption bands of B–H bond vibrations at 2591, 2599, and 2586 cm^{-1} respectively characteristic of *closo*-carboranes. In the IR spectrum of compound **VI** possessing the carborane in the *nido*-form the absorption band of B–H bond vibrations was observed at 2524 cm^{-1}. The IR spectrum of compound **VIII** contained the band characteristic of the stretching vibrations of the amide group carbonyl at 1680

cm^{-1} (amide I) and the band of the bending vibrations of the N–H bond at 1511 cm^{-1} (amide II). The stretching vibrations of C=O from the exocycle coincided with the absorption of the amide group carbonyl (amide **I**) at 1680 cm^{-1}.

^{1}H NMR spectra were registered for all compounds obtained. The spectra contained proton signals corresponding to chlorin ring confirming the structure of pyropheophorbide *a*, and also the proton signals from C–C$\underline{H}_2$ and B–C$\underline{H}_2$ groups as singlets at δ 3.85 and 3.99 ppm respectively. In the ^{1}H NMR spectrum of compound **VI** the signal of the bridging proton of the *nido*-dicarbaundecaborate anion appeared as a broadened singlet at δ –1.74 ppm.

^{11}B NMR spectrum revealed the presence of nine boron atoms in compound **VI** and of ten boron atoms in compounds **V, VII**, and **VIII**. In the ^{11}B NMR spectrum of compound **VI** a doublet of doublets was observed at –32.44 ppm belonging to B^{10} and confirming the presence of the *nido*-carborane polyhedron.

Spectral kinetic investigations of compounds obtained **I, V–VIII** were carried out. The absorption spectra of the compounds in chloroform solution of concentration C 5 × 10^{-6} mol l^{-1} measured in the range 350–700 nm are presented in Fig. 1.The positions of the

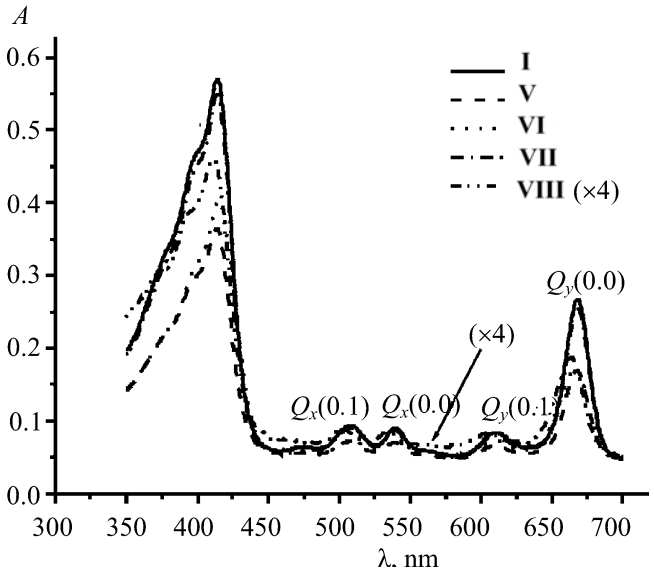


Fig. 1. Absorption spectra of pyropheophorbide *a* (**I**) and its carborane derivatives **V–VIII** in chloroform. The spectrum of compound **VIII** is four times enlarged. Concentration of pigment and its carborane derivatives 5×10^{-6} mol l^{-1}.

Expansion of absorption spectra of pyropheophorbide (**I**) and its carborane derivatives **V–VIII** into Gaussian components[a]

Component	*S*, %		Λ_{max}, nm		$w_{1/2}$, nm		*A*×10^2	
Expansion of absorption spectra of pyropheophorbide *a* (**I**) and its derivative **VII**								
	I	**VII**	**I**	**VII**	**I**	**VII**	**I**	**VII**
Q_x(0.1)	16.5	17.2	507	506.7	22.4	23.5	4.25	4.1
Q_x(0.0)	6.7	6.8	539.3	539.1	13.52	13.54	3.0	2.8
Q_y(0.1)	17.0	16.3	610.3	610.0	25.6	25.3	3.8	3.6
Q_y(0.0)	59.8	59.7	668.2	668.0	18.2	18.3	19.0	18.2
Expansion of absorption spectra of pyropheophorbide *a* derivatives **V** and **VI**								
	V	**VI**	**V**	**VI**	**V**	**VI**	**V**	**VI**
Q_x(0.1)	16.7	16.9	506.4	506.7	22.6	22.7	2.4	2.5
Q_x(0.0)	6.6	6.7	538.8	539.1	13.49	13.52	1.6	1.7
Q_y(0.1)	15.5	15.4	609.0	610.0	24.6	24.6	2.0	2.1
Q_y(0.0)	61.2	61.0	667.4	668.0	18.4	18.4	10.6	11.0
Expansion of absorption spectra of pyropheophorbide *a* derivative **VIII**								
Q_x(0.1)	13.7		503.2		22.5		0.61	
Q_x(0.0)	5.9		533.4		14.2		0.41	
Q_y(0.1)	22.7		603.8		31.6		0.72	
Q_y(0.0)	57.7		662.8		20.8		2.73	

[a] Q_x(0.1), Q_x(0.0), Q_y(0.1), Q_y(0.0) are typical notations of transitions in porphyrin molecules; *S* is the relative area under the Gaussian curves; λ_{max} is the position of the maximun of the absorption band; $w_{1/2}$ is the half-width of the absorption band; *A* is the amplitude of the absorption band.

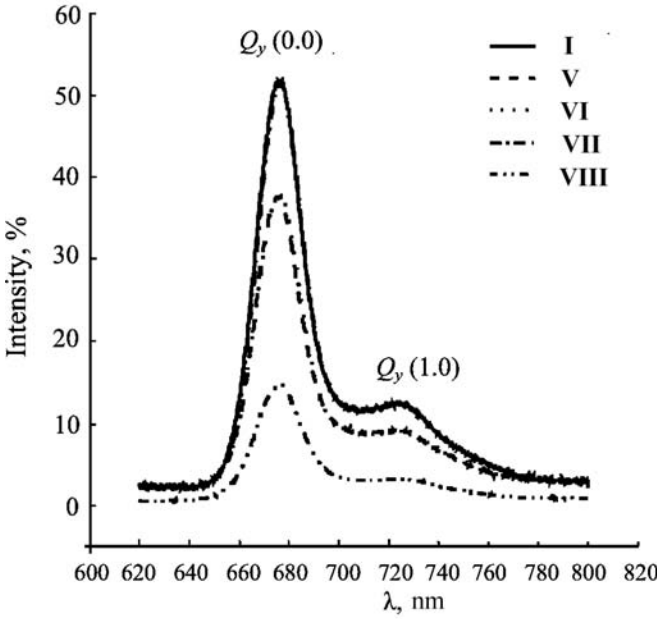


Fig. 2. Fluorescence spectra of pyropheophorbide *a* (**I**) and its carborane derivatives **V–VIII**. The fluorescence excitation was performed on a wavelength λ_{ex} 415 nm. Concentration of pyropheophorbide *a* and its derivatives in chloroform 5×10^{-6} mol l^{-1}. Relative quantum yield: 1 (**I**), 0.763 (**V**), 0.766 (**VI**), 0.987 (**VII**), 0.8 (**VIII**).

maxima of spectral bands in the spectra shown on Fig. 1 are designated according to the common nomenclature [12, 13]. To give more complete picture on the features of the absorption spectra in the region Q_x, Q_y the absorption was expanded into Gaussian components. The expansion parameters are given in the table. As seen from Fig. 1 the spectra of compounds **I, VII** and **V, VI** are nearly identical in pairs, whereas in the spectrum of compound **VIII** the optical density of the main bands is 4–5 times less, and the positions of the maxima are shifted to the blue region by 4–6 nm. Therefore for the sake of comparison of spectral characteristics the expansion of the spectra in the table is divided in three separate parts.

In the spectral range 500–700 nm the absorption spectra contain four bands with positions typical of $Q_x(0.1)$, $Q_x(0.0)$, $Q_y(0.1)$, and $Q_y(0.0)$ transitions in the metal-free porphyrins (D_{2h}-symmetry [12]). The positions of maxima, half-width, amplitude, and area under the Gausian bands curves simulating Q_x and Q_y transitions are identical in the spectra of compounds **I** and **VII** within the expansion accuracy (~5%). The spectra of carborane derivatives **V** and **VI** also coincide to a great extent. The main difference in the parameters of the Gaussian spectra of compounds **V, VI** and **I, VII** lies in the values of amplitudes Q_x and Q_y (Fig. 1). The reduced amplitudes of the Q_x, Q_y bands in the spectra of compounds **V, VI** may originate from the increased dipole moment of the transition from the ground state into the excited one. Finally, it follows from Fig. 1 and the table that the strongest alterations occurred in the absorption spectrum of carborane derivative **VIII**. These changes consist in the blue shift of the absorption bands maxima by 4–6 nm, a significant reduction in the amplitudes of the Gaussian bands, and the increase in intensity of the $Q_y(0.1)$ band at the expense of the partial decrease in the intensity of the $Q_x(0.1)$ band. This considerable loss in the amplitudes of Q_x and Q_y bands we believe to originate also from the large decrease in the dipole moment of transitions.

Fluorescence spectra (Fig. 2) contain two bands $Q_y(0.0)$ and $Q_y(1.0)$ that are in a mirror symmetry to the absorption bands $Q_y(0.0)$ and $Q_y(0.1)$ (Fig. 1). Like the absorption spectra the fluorescence spectra of compound pairs **I, VII** and **V, VI** coincide within the pairs, but the fluorescence spectrum of compound **VIII** is strongly different in the intensities. The relative quantum efficiency of fluorescence of the carborane derivatives was calculated by formula (1) [14].

$$\varphi = \varphi_0(S/S_0)(\beta/\beta_0)(n^2/n_0^2). \quad (1)$$

Here φ_0 is the quantum efficiency of fluorescence of pyropheophorbide *a* (**I**) (taken equal to 1), S_0, S are areas under the fluorescence curve of pyropheophorbide *a* (**I**) and carborane derivatives **V–VIII**, β/β_0 is the relative absorption of pyropheophorbide *a* (**I**) (β_0) and derivatives **V–VIII** (β), n and n_0 are refractive indices.

It was established that the fluorescence quantum efficiency of compounds **I** and **VII** were identical (1.0 and 0.99), and those of compounds **V, VI**, and **VIII** were 0.76, 0.76, and 0.8 respectively. The carborane molecule addition to pyropheophorbide *a* reduced the fluorescence quantum efficiency by 20–24%. The decrease in the fluorescence efficiency can be ascribed either to the growing probability of a nonradiative deactivation of the excited state of the pyropheophorbide *a*, or by intramolecular electron transfer from the excited state of the pigment to the carborane. The existence of these processes can be observed by studying fluorescence spectra and the kinetics of fluorescence decay.

The fluorescence excitation spectra (λ_{fl} 680 nm) are shown in Fig. 3. As seen, in the region 300–750 nm the spectra are similar in the form and maxima positions to the absorption spectra (Fig. 1). The most significant

distinction is the change in the intensity ratio of the bands in the fluorescence excitation spectra compared to the absorption spectra.

Kinetics of fluorescence decay of compounds under study is presented on Fig. 4. The fluorescence decay fits to a single exponent, and therewith the fluorescence duration of compounds **I** and **VII** is the same within the accuracy of measurements (3%): 1660 and 1620 ps. The fluorescence duration (τ_{fl}) of carborane derivatives **V, VI**, and **VIII** in the monoexponential approximation is fairly close to that of compounds **I** and **VII** and amounts to 1550 ± 50 ps indicating the shortening of the fluorescence duration in carborane derivatives **V, VI**, and **VIII** by 7% compared to the τ_{fl} of initial pyropheophorbide *a* (**I**). The decrease in the relative fluorescence quantum efficiency for derivatives **V** and **VI** was 24%, for compound **VIII**, 20%, hence the proportionality between τ and φ was not observed.

The spectra and kinetic characteristics of initial pyropheophorbide *a* (**I**) and compound **VII** are virtually identical. This fact means that the addition of carborane at the boron atom does not considerably disturb the a conjugated π-system of chlorin molecule. The addition to **I** of carborane **IV** through an amide bond resulted in significant reduction of the bands intensity in the absorption (Fig. 1) and fluorescence (Fig. 2) spectra. The reason of the significant decrease in the bands intensity may be the diminishing of the dipole moments value corresponding to transitions from the ground to the excited state.

The lack of proportionality in the changes of τ and φ of compounds **V, VI**, and **VIII** may be due to the alteration of the rate constants of fluorescence (k_{fl}), intrinsic conversion (k_{intrin}), and intercombination conversion (k_{inter}). Actually, the parameters τ and φ are interrelated through expressions (2, 3).

$$\tau = 1/(k_{fl} + k_{intrin} + k_{inter}) = 1/\Sigma k_i \quad (2)$$

$$\varphi = k_{fl}/(k_{fl} + k_{intrin} + k_{inter}) = k_{fl}/\Sigma k_i \quad (3)$$

The decrease in the fluorescence duration of carborane derivatives **V, VI**, and **VIII** by 7% compared with the τ of initial pyropheophorbide *a* (**I**) means that Σk_i for compounds **V, VI**, and **VIII** increased by ~7% compared to Σk_i for **I**. The simultaneous considerably greater reduction of the φ value, e.g., by 20%, may result from the decrease of k_{fl} by ~14%. Taking the value of fluorescence quantum efficiency φ of the free pyropheophorbide *a* analogous to that of chlorophyll [15] equal 0.3, then the simultaneous decrease of τ and φ by ~7 and ~20% respectively after addition to molecule **I** of *closo*-1-hydroxymethyl-*O*-carborane (**II**) should require the reduction of k_{fl} by 14%, and increase in the sum (k_{intrin} + k_{inter}) by ~11%.

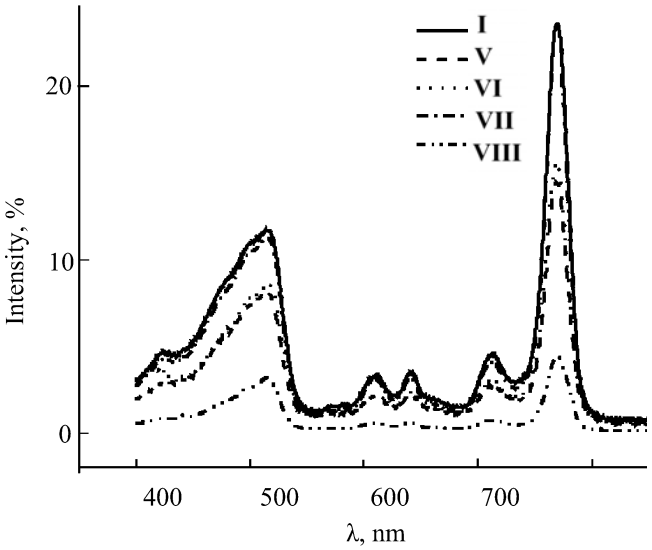


Fig. 3. Fluorescence excitation spectra of pyropheophorbide *a* (**I**) and its carborane derivatives **V–VIII**. Registration on a wavelength λ_{em} 680 nm. Concentration of all samples 5×10^{-6} mol l^{-1}.

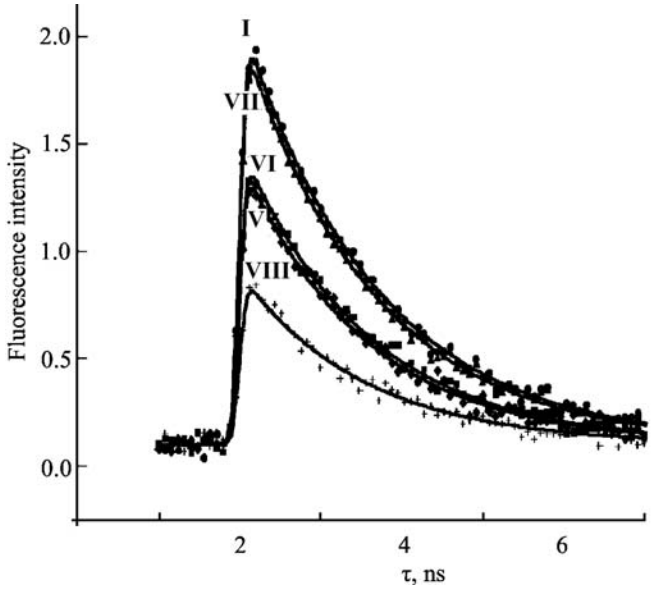


Fig. 4. Kinetics of fluorescence decay of compounds **I, V–VIII**. Pulse fluorimeter, λ_{excit} 532 nm, $\Delta t_{pulse} \approx 20$ ps, $\lambda_{reg} > 640$ nm. Experimental data are depicted by marks, full lines are monoexponential approximation of the experimental data. The fluorescence intensity is normalized with respect to the fraction of absorbed energy at l 532 nm, the signal of derivative **VIII** is double enlarged for the sake of clearness.

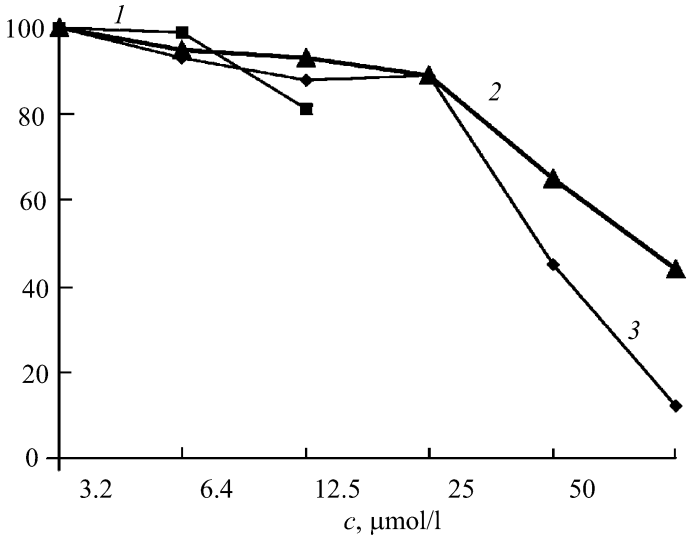


Fig. 5. The results of investigation of compounds **I, V**, and **VI** toxicity with respect to cells of the large intestine epithelium cancer (line HCT 116). Incubation time 72 h. (*1*) **I**; (*2*) **V**; (*3*) **VI**.

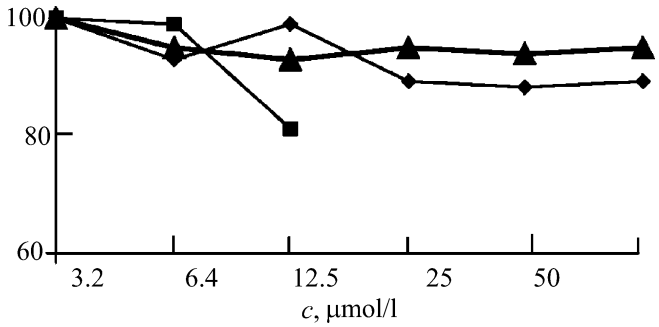


Fig. 6. The results of investigation of compounds **I, V**, and **VI** toxicity with respect to nonneoplastic cells (human skin fibroblasts). Incubation time 72 h. (*1*) **I**; (*2*) **V**; (*3*) **VI**.

Another probable reason of the decrease in the fluorescence quantum efficiency φ of carborane derivatives **V, VI**, and **VIII** as compared with the φ value of the initial pyropheophorbide *a* may be an intramolecular electron transfer from the excited state of the chlorin fragment of the molecule to its carborane part. Actually it was formerly shown [16] that in a tetraphenylporphyrin carborane derivative prepared by acylating four amino groups of 5,10,15,20-tetra(*p*-aminophenyl)porphyrin with 9-*O*-carboranylacetyl chloride the intramolecular electron transfer occurred with a probability ~0.8. Therewith the kinetics of fluorescence decay were two-component: τ_1 300 ps and τ_2 2.5 ns.

The structural features of tetraphenylporphyrin carborane derivative [16] unlike those of compounds **V**–**VIII** reported here are as follows. In the presence of a hydrogenated pyrrole ring in compounds **V–VIII** under investigation the number of π-electrons in the conjugated system of chlorin is changed. But the retention of a number of spectral parameters indicates that the internal 16-membered ring remains a chromophore [12]. Besides the tetraphenylporphyrin carborane derivative [16] contains four carborane polyhedrons linked through amide bonds via benzene rings in the *meso*-positions of the porphyrin macrocycle. The substances **V–VIII** contain a single carborane polyhedron linked by ester (**V–VII**) or amide (**VIII**) bond that is a substituent in the β-position of the hydrogenated core of the pyropheophorbide *a*.

These significant structural differences in the carborane derivatives are revealed also in their photophysical characteristics. The fluorescence efficiency of the tetraphenylporphyrin carborane derivative is 5 times less than that of the free pigment, and the kinetics of fluorescence decay are two-component [16]. In our case the fluorescence efficiency of the pyropheophorbide *a* carborane derivatives is reduced only by 20–24%, and fluorescence decay kinetics fits to a single exponent. Therefore it may be concluded that the variation of τ and φ in compounds under study is due to the redistribution of the intramolecular constants of the deactivation of the pyropheophorbide *a* excited state caused by its bonding to the carbon atom of carborane behaving as an electron-acceptor substituent [10, 11, 17]. Bonding with *closo*-9-hydroxymethyl-*m*-carborane (**III**) through boron did not affect the photophysical characteristics of the pyropheophorbide *a*.

The carborane systems exhibit a characteristic dependence of inductive effect of the carboranyl groups on the substituent position in the polyhedron. It was shown by studies [10, 11, 17] that in the carborane series **II**–**IV** the largest electron-donor effect belonged to the 9-*m*-carboranyl group (σ_i –0.16), the 1-*O*-carboranyl group possessed a strong electron-acceptor inductive effect (σ_i +0.38), and the 3-*O*-carboranyl group was a weak electron-acceptor (σ_i +0.11). These inductive effects presumably also affect the spectral kinetic characteristics of pyropheophorbide *a* carborane derivatives.

As already mentioned, the characteristic feature of compound **VIII** where the *closo*-3-amino-*O*-carborane is linked to the pyropheophorbide *a* molecule by an amide bond was a strong reduction in intensity of the bands in the absorption and fluorescence spectra. This is caused by a strong perturbation effect of the carborane on the

matrix element of the complete dipole moment of the transition $S_0 > S_n$. We believe that the optimization of the amide bond length based on this compound should permit a synthesis of a model structure capable of an efficient intramolecular charge separation.

A study was performed on cytotoxic activity of compounds **I, V–VIII** with respect to nonneoplastic cells on the culture of human skin fibroblasts and to tumor cells of the large intestine epithelium cancer (line HCT 116) (Fig. 5, 6) [test on the cells ability to reduce the salt 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide after the cells incubation for 72 h (MTT–test)] [18]. No toxicity of compounds **I, V–VIII** with respect to nonneoplastic cells was found. Pyropheophorbide *a* (**I**) is sparingly soluble in water at concentration higher than 12 μmol l^{-1} therefore its effect on the cells was estimated only within this range. Its toxicity was not revealed. The most toxic for the tumor cells was the pyropheophorbide *a* derivative where the carborane polyhedron was present in the *nido*-form **VI**. Compound **V** was less toxic for the tumor cells. Compounds **V** and **VI** are soluble in water up to the concentration 50 μmol l^{-1} and are toxic at concentrations over 12.5 μmol l^{-1}. Compounds **VII** and **VIII** were not toxic for the tumor cells. The results obtained suggest that further research on compounds **V–VIII** is promising for their application to boron neutron capture therapy.

EXPERIMENTAL

All reactions were carried out in anhydrous solvents. The reaction progress and the homogeneity of individual compounds obtained was monitored by TLC on Silufol UV-254 plates (Kavalier, Czechia) in solvent systems chloroform (A), chloroform–methanol, 9:1, (B). The purification was performed by column chromatography on silica gel 60 Merck (0.040–0.063 mm). Electronic spectra were measured on a Jasco UV-7800 instrument in dichloromethane. IR spectra were recorded on an IR Fourier spectrophotometer Bruker Equinox-55 from films. ^{1}H NMR spectra of compounds **VII** and **VIII** solutions in $CDCl_3$ and ^{11}B NMR spectra (operating frequency 128.38 MHz) were registered on a spectrometer Bruker Avance-400. ^{1}H NMR spectra of compounds **V** and **VI** were taken on a spectrometer Bruker MSL-300. Mass spectra were obtained on a mass spectrometer with a time-of-flight base VISION 2000 (Termobioanalysis corp., Finnigan, USA) equipped with a pulse nitrogen laser of 3B class, radiation with a wave-length 337 nm, using MALDI method with 2,4,6-trihydroxyacetophenone matrix.

Spectral kinetic measurements on the absorption spectra were performed on a double-beam spectrometer Hitachi-557 (Japan). Fluorescence and fluorescence excitation spectra were measured on a spectrofluorimeter Hitachi-850 (Japan). The fluorescence spectra were measured at excitation on a wavelength 415 nm. The observation of fluorescence excitation spectra was carried out by registration of fluorescence signal on a wavelength 680 nm. The optical slit width was 5 nm. The measurement of the lifetime of the excited states of compounds under study was performed using the fluorimetry with the picosecond laser excitation [19]. The samples were excited with light pulse ~20 ps long on a wavelength 532 nm. The fluorescence signal was registered from the face of the cell applying electron-optical chamber Agat SF-3M (Russia) coupled with a multidetector CCD Hamamatsu C7041 (Japan). The time resolution of the system provided a possibility to measure the kinetics of fluorescence decay in the time range 5 ps–10 ns. The accumulation of experimental data till the desired signal/noise ratio was performed automatically. The mathematical treatment of the fluorescence decay kinetics was done by convolution of a model curve of the type $F(t) = \Sigma A_i \, exp(-t/\tau_i)$ with a spread function of the type $\varphi(t)$ of Gaussian form (A_i, τ_i are amplitudes and durations of the i component of kinetics, $\Sigma A_i = 1$).

Pyropheophorbide *a* (*closo*-*O*-carboran-1-yl)-methyl ester (V) and (*nido*-7,8-dicarbaundecaboran-7-yl)methyl ester (VI). To a solution of 34.5 mg (0.06 mmol) of pyropheophorbide *a* (**I**) in a mixture of 2 ml of dichloromethane and 2 ml of pyridine in an argon flow at cooling to –5°C was added 20 mg (0.09 mmol) of di-*tert*-butyl pyrocarbonate, and the mixture was stirred for 30 min. Then 13 mg (0.075 mmol) of *closo*-1-hydroxymethyl-*O*-carborane (**II**) and 10 mg (0.078 mmol) of *N*,*N*-dimethylaminopyridine were added. The reaction mixture was stirred for 24 h at 20°C. The solvents were distilled off, the reaction product was subjected to column chromatography on silica gel. The first fraction was eluted with chloroform, R_f 0.8 (A), then the polarity of the eluent was raised, and the second fraction was eluted with a mixture chloroform–methanol, 9:1, R_f 0.6 (B). The solvent was removed, the residues were ground with petroleum ether, the precipitates were separated and dried.

Compound **V**. Yield 15.7 mg (35%), R_f 0.8 (A). Electronic spectrum, λ_{max}, nm ($\varepsilon \times 10^{-3}$): 667.50 (38.9),

608.00 (5.3), 537.00 (7.5), 509.50 (7.2), 418.00 (77). IR spectrum, ν, cm^{-1}: 3335 (NH), 3077 (CH of carborane), 2962, 2928, 2873 (CH), 2591 (BH), 1735 (CO of ester), 1672 (CO of exocycle). ^{1}H NMR spectrum, δ, ppm: 9.46 s (1H, H^{10}), 9.36 s (1H, H^5), 8.55 s (1H, H^{20}), 7.98 d.d (1H, 3-C$\underline{H}$=CH$_2$, *J* 17.55, 11.94 Hz), 6.25 d.d (1H, 3-CH=C$\underline{H}^A$H^B, *J* 17.97 Hz), 6.15 d.d (1H, 3-CH=CH$^A\underline{H}^B$, *J* 11.55 Hz), 5.25 d.d (2H, 13-H^2, *J* 20.16 Hz), 4.45 m (1H, H^{18}), 4.31 m (1H, H^{17}), 3.85 s (2H, CCH$_2$), 3.66 m (2H, 8-C$\underline{H}_2$CH$_3$), 3.60 s (2H, 17-H^5), 3.63, 3.40, 3.21 s (3H each, 12,2,7-CH$_3$), 2.56 m (2H, 17-H^1), 2.13 m (2H, 17-H^2), 1.83 d (3H, 18-CH$_3$, *J* 6.42 Hz), 1.69 t (3H, 8-CH$_2$C$\underline{H}_3$, *J* 7.29 Hz), –1.71 s (2H, NH). Mass spectrum: *m/z* 691.3 [*M* + 1]$^+$.

Compound **VI**. Yield 15 mg (34%), R_f 0.6 (B). Electronic spectrum, λ_{max}, nm (ε × 10^{-3}): 668.00 (27.3), 610.50 (6.57), 541.5 (6.80), 510.00 (6.60), 417.50 (79.7). IR spectrum, ν, cm^{-1}: 3293 (NH), 3096 (CH of carborane), 2961, 2925, 2867 (CH), 2524 (BH), 1720 (CO of ester), 1678 (CO of exocycle). ^{1}H NMR spectrum, δ, ppm: 9.10 s (1H, H^{10}), 8.52 s (1H, H^5), 8.50 s (1H, H^{20}), 7.83 d.d (1H, 3-C$\underline{H}$=CH$_2$, *J* 17.58, 11.434 Hz), 6.22 d (1H, 3-CH=C$\underline{H}^A$H^B, *J* 11.64 Hz), 6.12 d (1H, 3-CH=CH$^A\underline{H}^B$, *J* 11.88 Hz), 5.25 d.d (2H, 13-H^2, *J* 20.1 Hz), 4.48 m (1H, H^{18}), 4.29 m (1H, H^{17}), 4.0 m (2H, 8-C$\underline{H}_2$CH$_3$), 3.45 s (2H, 17-H^5), 3.33, 3.04, 2.72 s (3H each, 12,2,7-CH$_3$), 2.40 m (2H, 17-H^1), 2.13 m (2H, 17-H^2), 1.83 d (3H, 18-CH$_3$, *J* 6.8 Hz), 1.56 t (3H, 8-CH$_2$C$\underline{H}_3$, *J* 6.84 Hz), –1.75 br.s (1H, H$_\mu$ of *nido*-carborane), –2.61 s (2H, NH). ^{11}B NMR spectrum, δ, ppm (CD$_3$Cl$_3$): –9.44 d (1B, B$^{3(6)}$, *J* 130 Hz), –10.40 d (1B, B$^{6(3)}$, *J* 130 Hz), –14.53 d (1B, B$^{9(11)}$, *J* 178 Hz), –15.89 d (1B, B$^{11(9)}$, *J* 166 Hz), –17.21 d (1B, B$^{2(4)}$, *J* 141 Hz), –18.62 d (1B, B$^{4(2)}$, *J* 134 Hz), –22.02 d (1B, B^5, *J* 147 Hz), –32.44 d.d (1B, B^{10}, J_{BH} 132, J_{BH} 51 Hz), –36.72 d (1B, B^1, *J* 146 Hz). Mass spectrum: *m/z* 681.4 [*M* + 1]$^+$.

Compounds **VII** and **VIII** were similarly prepared.

Pyropheophorbide *a* (*closo*-*m*-carboran-9-yl)methyl ester (VII) was obtained from 0.06 mmol of compound **I** and 0.075 mmol of compound **III**. The reaction product was subjected to column chromatography on silica gel, eluent chloroform–methanol, 9:1, first fraction, R_f 0.8 (B). The solvent was removed, the residue was ground with petroleum ether, the precipitate was separated and dried. Yield 37.3 mg (84%). Electronic spectrum, λ_{max}, nm (ε ×10^{-3}): 668.50 (35.7), 610.50 (6.67), 540.00 (7.74), 509.00 (8.34), 414.50 (77.1). IR spectrum, ν, cm^{-1}: 3395 (NH), 3059 (CH of carborane), 2961, 2926, 2868 (CH), 2599 (BH), 1732 (CO of ester), 1687 (CO of exocycle). ^{1}H NMR spectrum, δ, ppm: 9.46 s (1H, H^{10}), 9.35 s (1H, H^5), 8.54 s (1H, H^{20}), 7.98 d.d (1H, 3-C$\underline{H}$=CH$_2$, *J* 17.8, 11.48 Hz), 6.25 d.d (1H, 3-CH=CH$^A\underline{H}^B$, *J* 17.72 Hz), 6.15 d (1H, 3-CH=C$\underline{H}^A$H^B, *J* 11.56 Hz), 5.25 d.d (2H, 13-H^2, *J* 19.92 Hz), 4.49 m (1H, H^{18}), 4.28 m (1H, H^{17}), 3.99 s (2H, BCH$_2$), 3.66 m (2H, 8-C$\underline{H}_2$CH$_3$), 3.60 s (2H, 17-H^5), 3.65, 3.39, 3.21 s (3H each, 12,2,7-CH$_3$), 2.56 m (2H, 17-H^1), 2.31 m (2H, 17-H^2), 1.80 d (3H, 18-CH$_3$, *J* 7.12 Hz), 1.68 t (3H, 8-CH$_2$C$\underline{H}_3$, *J* 7.72 Hz), –1.72 s (2H, NH). ^{11}B NMR spectrum, δ, ppm: –0.59 C (1B, B^9), –6.70 d (2B, B^{5,12}, J_{BH} 162 Hz), –10.41 d (1B, B^{10}, J_{BH} 150 Hz), –13.62 d (4B, B^{4,8,6,11}, J_{BH} 166 Hz), –17.39 d (1B, B^3, J_{BH} 179 Hz), –19.47 d (1B, B^2, J_{BH} 179 Hz). Mass spectrum: *m/z* 691.7 [*M* + 1]$^+$.

N-(*closo*-*O*-Carboran-3-yl)pyropheophorbide *a* (VIII) was obtained from 0.06 mmol of compound **I** and 0.075 mmol of compound **IV**. The reaction product was subjected to column chromatography on silica gel, eluent chloroform (first fraction). Yield 24 mg (56%), R_f 0.8 (A). Electronic spectrum, λ_{max}, nm (ε × 10^{-3}): 663.8 (12.49), 606.0 (3.05), 535.4 (3.44), 504.4 (3.27), 412.6 (40.37). IR spectrum, ν, cm^{-1}: 3349 (NH), 3078 (CH of carborane), 2586 (BH), 1680 (amide **I** and CO of exocycle), 1511 (amide II). ^{1}H NMR spectrum, δ, ppm: 9.44 s (1H, H^{10}), 9.36 s (1H, H^5), 8.52 s (1H, H^{20}), 7.99 d.d (1H, 3-C$\underline{H}$=CH$_2$, *J* 17.6, 11.6 Hz), 6.25 d (1H, 3-CH=CH$^A\underline{H}^B$, *J* 18 Hz), 6.19 d (1H, 3-CH=C$\underline{H}^A$H^B, *J* 11.46 Hz), 5.02 d (1H, 13-H^2, *J* 17 Hz), 5.14 d (1H, 13-H^2, *J* 17 Hz), 4.45–4.32 m (1H, H^{18}), 4.20–4.18 m (1H, H^{17}), 3.65 m (2H, 8-C$\underline{H}_2$CH$_3$), 3.6 s (2H, 17-H^5), 3.52, 3.39, 3.22 s (ïO 3H, 12,2,7-CH$_3$), 2.5–2.3 m (2H, 17-H^1, 2H, 17-H^2), 1.8 d (3H, 18-CH$_3$, *J* 7.12 Hz), 1.67 t (3H, 8-CH$_2$-C$\underline{H}_3$, *J* 7.29 Hz), 0.062 s (1H NH), –1.56 s (1H, NH). ^{11}B NMR spectrum, δ, ppm: –4.32 d (2B, B^{9,12}, *J* 149 Hz), –6.14 s (1B, B^3), –10.96 d (2B, B^{8,10}, *J* 142 Hz), –14.61 d (4B, B^{4,7,5,11}, *J* 160 Hz), –18.35 d (1B, B^6, *J* 155 Hz). Mass spectrum: *m/z* 676.4 [*M* + 1]$^+$.

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