Conjugates of natural chlorins and isobornylphenols with a different length of the spacer between the chlorin and terpenephenolic fragments: synthesis and antioxidant activity

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Conjugates containing chlorine and isobornylphenolic fragments, which are connected by spacers of different lengths, were synthesized from methyl pheophorbide *a*. The toxicity and antioxidant activity of the obtained compounds were studied. Derivatives of pyropheophorbide *a* containing the most distant from the macrocycle terpenephenolic fragment combine a low toxicity with the high antioxidant activity, and are the most promising for further designing of new medicinal agents for the treatment of diseases associated with a disorder in oxidation-reduction processes in the body.

Key words: methyl pheophorbide *a*, pyropheophorbide *a*, chlorin e_6, isobornylphenols, antioxidant activity (AOA), lipid peroxidation (LPO), human embryonic kidney cells 293 (HEK 293), SH-SY5Y neuroblastoma cells, viability.

Synthesis and study of compounds with an antioxidant activity is of interest in the context of search for new medicinal agents for the treatment of diseases associated with a disorder in oxidation-reduction processes in the body (radiation sickness, neurodegenerative and oncological diseases, *etc.*).[1—3] Porphyrins with substituents, which bring additional antioxidant properties, may be promising in this aspect.[4—7] One of synthetic pathways to obtain such porphyrins is a conjugation of the porphyrin macrocycle with molecules, which have their own antioxidant activity. Phenols with terpene substituents possess such properties. Synthesis of such conjugates from chlorophyll *a* derivatives has a number of significant advantages, the most important of which are a relatively low toxicity of these compounds[8,9] and the presence of several reaction centers that are convenient for use with preparative purposes. The biological activity of such conjugates may depend not only on the structure of a terpenephenolic fragment, but also on the manner of its conjugation with the porphyrin macrocycle, in particular on the position of the phenolic fragment in the macrocycle and the length of a spacer connecting the porphyrin and terpenephenol fragments. Taking all these facts into account, in the present work we report on the synthesis of some conjugates, containing chlorine and isobornylphenol fragments connected by spacers of various lengths, from methyl pheophorbide *a* **1** and the evaluation of toxicity and an antioxidant activity (AOA) of the obtained compounds.

Results and Discussion

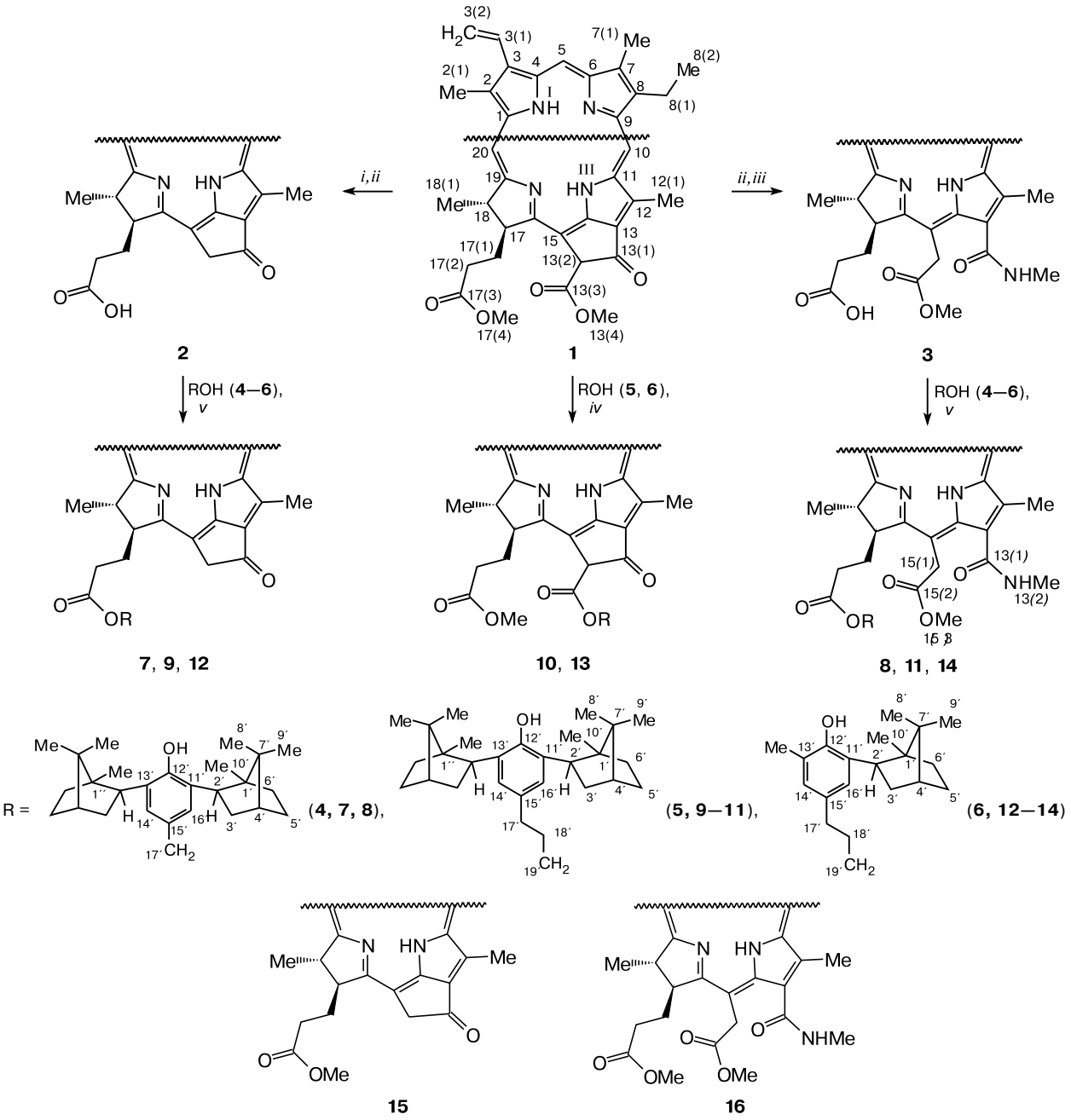
To conjugate chlorine fragments with a terpenephenol, an ester bond formation was carried out by using the Mukaiyama reagent for activation of the carboxyl groups in chlorins **2** and **3**, and also by a *one-pot* hydrolysis of the ester group at the exocycle of methyl pheophorbide *a* **1** with subsequent esterification (the carboxyl group, formed as a result of hydrolysis, was activated by the same re-

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 11, pp. 2157—2164, November, 2017.

agent) (Scheme 1). Terpenephenol derivatives **4**—**6**, containing hydroxyl groups separated from the phenol fragment by spacers of different lengths were used as esterifying alcohols. Conjugates **7** and **8** with relatively short spacers between the terpenephenol and macrocyclic parts were synthesized from chlorins **2** and **3** by treatment with terpenephenol alcohol **4**. The reaction of alcohol **5** with chlorines **1**—**3** allowed obtaining conjugates **9**—**11** with longer spacers. Using alcohol **6** for the esterification, derivatives **12**—**14** were prepared, which are analogs of conjugates **9**—**11**, but containing only the one terpene substituent at the terpenephenolic part of the molecule. Therefore, the combination of terpenephenolic alcohols with chlorins, having different positions of the esterified groups and a structure of the peripheral substituents, allows one to vary the length of a spacer between the macrocyclic and phenolic fragments.

The chemical structures of the obtained compounds were confirmed by NMR, IR and UV-Vis spectroscopy methods and mass spectrometry. The peaks correspond-

Scheme 1



Reagents and conditions: *i*. collidine, reflux, 40 min; *ii*. 30% HCl, acetone, ~20 °C, 12 h; *iii*. $MeNH_2$, H_2O, THF, ~20 °C, 20 min; *iv* and *v*. 2-chloro-*N*-methylpyridinium iodide, 4-dimethylaminopyridine, reflux, 2 h, toluene (*iv*) or 0.5 h, CH_2Cl_2 (*v*).

ing to protonated molecular ions were observed in the mass spectra (ESI) for all obtained derivatives **7**—**14**. UV-Vis spectra contain absorption bands of chlorine chromophors. ^{1}H and ^{13}C NMR spectra of all compounds contain signals of the protons of the chlorine and isobornylphenolic fragments, while the intensity ratio of those signals in ^{1}H NMR spectra corresponds to conjugates with one terpenephenolic and one porphyrin fragment. Compound **6** is a mixture of enantiomers with the 1 : 1 ratio, so conjugates **12**—**14** prepared from **6** are also mixtures of diastereomers in the same ratio. ^{1}H and ^{13}C NMR spectra of compounds **12**—**14** contain a double set of signals, coinciding in a number of peaks and, in the case of ^{1}H NMR spectra, multiplicity and differing from each other by chemical shifts.

The antioxidant activity of porphyrin derivatives was evaluated *in vitro* as an ability to inhibit lipid peroxidation (LPO) in a brain of laboratory mices.[10—14] In this noncellular model system, compounds **7**—**10** and **12**—**16** demonstrated the high antioxidant activity at concentrations of 500 μmol L^{-1}, which inhibited LPO processes to a spontaneous level and lower (Table 1). When the concentration of compounds was decreased by an order of magnitude (up to 50 μmol L^{-1}), the high antioxidant activity in this model system remained only for compounds **9** and **12**, which are the derivatives of pyropheophorbide *a* with the most remote terpenephenolic and macrocyclic fragments. Their antioxidant activity was close to that of the reference drug, 2,6-diisobornyl-4-methylphenol (Dibornol), whose high antioxidant and membrane-protective activities were studied by us earlier.[7] The analysis of these data indicates that in some cases the introduction of a terpenephenolic fragment into the molecule results in a statistically significant increase of AOA over the starting porphyrin. For example, the conjugate **14** is more active than the non-terpenic chlorine **16**, while compounds **9**, **7**, and **12** are more active than corresponding pyro derivative **15**. The obtained data allow one to conclude that both porphyrin and terpenephenolic fragments contribute to AOA of the prepared conjugates, but a contribution of the latter is much higher. The highest AOA was observed for compounds with phenolic fragment most remote from the porphyrin macrocycle, which was achieved by esterification of the carboxyl group of the propionate substituent at position 17 with alcohols **5** and **6** with hydroxyl group separated from the terpenephenolic moiety by a spacer containing three methylene groups.

The effect of the derivatives of chlorophyll *a* **15**, **16** and their conjugates with terpenephenols **7**—**14** on the viability of the HEK293 normal cells and the tumor SH-SY5Y cells was evaluated by the ability of the cells to absorb trypan blue (IC_{50} value was used as a quantitative measure of the toxicity of the compounds; Table 2). It was found that chlorins **15** and **16**, having a similar structure of the macrocycle and lacking a terpenephenolic fragment, show a significant ability to inhibit the survival of HEK293 cells, while compound **16** also significantly inhibits the survival of SH-SY5Y cells. The conjugation of chlorins macrocycle with terpenephenol reduces the toxicity in most cases (relatively high toxicity remained only for methylamide derivatives **8** and **14**).

The analysis of results from the AOA and toxicity studies for the obtained derivatives indicates that the introduction of the terpenephenolic fragment on the

Table 1. Effects of compounds **7**—**16** in doses of 50 (I) and 500 μmol L^{-1} (II) on the content of secondary LPO products (TBA-reactive substances, TBA-RS) in the brain of laboratory mices*

Compound	TBA-RS/nmol L^{-1}	
	I	II
7	58.34±0.36	4.33±0.24
8	62.86±0.05	8.99±0.49
9	7.19±0.22	3.27±0.19
10	66.10±0.59	3.99±0.28
11	61.32±0.40	50.14±0.37
12	5.14±0.28	3.29±0.16
13	51.37±0.26	3.30±0.12
14	59.71±0.23	6.71±0.11
15	68.99±0.97	7.36±0.39
16	66.10±1.03	7.69±0.26
Dibornol	1.80±0.15	—

* The content of TBA-reactive substances in the control and intact samples is 63.95±0.29 and 20.22±1.19 nmol L^{-1}, respectively. For the control sample, LPO was initiated in the absence of the test compounds; for the intact sample, LPO was not initiated.

Table 2. The effect of chlorophyll *a* derivatives and their conjugates with terpenephenols **7**—**16** on the viability of HEK293 and SH-SY5Y cells

Compound	IC_{50}/μmol L^{-1}	
	HEK293	SH-SY5Y
7	44.0±4.8	38.9±2.4
8	1.9±0.7	4.2±0.3
9	>100	>100
10	>100	>100
11	>100	>100
12	>100	±6.0
13	>100	>100
14	13.1±.6	7.6±1.9
15	4.6±1.1	16.1±6.6
16	0.5±0.01	3.0±0.8

Note. To validate the procedure, 10% DMSO (inhibition of the viability of HEK293 and SH-SY5Y cells by 98±0.6 and 99±0.3%, respectively) was used as a reference.

periphery of chlorin macrocycle not only reduces a general toxicity of the compound, but also increases its AOA. The highest AOA combined with the lowest toxicity was demonstrated by pyropheophorbide *a* derivatives **9** and **12**, which contain the terpenephenolic fragment most remote from the macrocycle.

In summary, this work reports the synthesis of a series of based on methyl pheophorbide *a* **1** conjugates, that contain the chlorins and isobornylphenolic fragments linked by spacers of different lengths, and the evaluation of toxicity and the AOA of the obtained compounds. Derivatives of pyropheophorbide *a* **9** and **12** with the terpenephenolic fragment most remote from the macrocycle combine a low toxicity with the high AOA and are the most promising for further search for new medicinal agents for the treatment of diseases associated with a disorder in oxidation-reduction processes in the body.

Experimental

The chemical reaction progress was monitored by TLC on Sorbfil plates. Colorless reaction products **4**—**6** were detected by treatment of TLC plates with $KMnO_4$ solution (15 g of $KMnO_4$, 300 mL of H_2O and 0.5 mL of conc. H_2SO_4). ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance II 300 spectrometer (300.17 and 75.5 MHz) in $CDCl_3$ at room temperature. Diffuse reflectance IR spectra were recorded on a Shimadzu IR Prestige 21 IR Fourier spectrophotometer in the KBr pellets for solids and neat for liquids. Electrospray ionization (ESI) mass spectra were obtained on a Thermo Finnigan LCQ Fleet instrument using positive ions detection mode, the sample was injected as a chloroform—methanol solution. UV-Vis spectra were obtained for $CHCl_3$ solutions in quartz cells (10 mm) on a Shimadzu UV-1600 instrument. Elemental analysis was performed using an automatic elemental EA 1110 CHNS-O analyzer.

Alcohols **5** and **6** were prepared according to the known procedure.[15] The spectral characteristics of **6** coincided with previously reported data.[15] Alcohol **4** was synthesized according to the published method.[16] Compounds **2**, **3**, **15**, and **16** were obtained according to the known procedure.[17]

4-(3-Hydroxypropyl)-2,6-di(1,7,7-trimethylbicyclo[2.2.1]-hept-*exo*-2-yl)phenol (5). White powder, m.p. 157—159 °C. Yield 73%. Found (%): C, 81.55; H, 10.97. $C_{29}H_{44}O_2$. Calculated (%): C, 82.02; H, 10.44. IR spectrum (neat), ν/cm^{-1}: 3604 (ArO<u>H</u>); 3145 (CH_2O<u>H</u>); 2949, 2875, 1456, 1384 (CH_2, Me); 1606 (C=C); 1182 (arom. C—O); 1049 (CH_2C—O); 790 (arom. C—H). ^1H NMR, δ: 0.84 (s, 6 H, C(10)H_3, C(10′)H_3); 0.88 (s, 6 H, C(9)H_3, C(9′)H_3); 0.94 (s, 6 H, C(8)H_3, C(8′)H_3); 1.39—1.48 (m, 5 H, H(5), H(5′), H(6), H(6′), CH_2O<u>H</u>); 1.58—1.69 (m, 4 H, H(3), H(3′), H(6), H(6′)); 1.85—1.95 (m, 5 H, H(4), H(4′), H(5), H(5′), H(18)); 2.26—2.32 (m, 2 H, 1 H(3), 1 H(3′)); 2.67 (t, 2 H, H(17), J = 8.1 Hz); 3.03 (t, 2 H, H(2), H(2′), J = 8.7 Hz); 3.69 (t, 2 H, H(19), J = 6.3 Hz); 4.74 (br.s, 1 H, ArO<u>H</u>); 7.01 (s, 2 H, H(14), H(16)). ^{13}C NMR, δ: 12.56 (C(10), C(10′)); 20.20 (C(8), C(8′)); 21.49 (C(9), C(9′)); 27.60 (C(5), C(5′)); 32.19 (C(17)); 34.03 (C(3), C(3′)); 34.76 (C(18)); 40.08 (C(6), C(6′)); 45.52 (C(4), C(4′)); 46.16 (C(2), C(2′)); 48.18 (C(7), C(7′)); 49.96 (C(1), C(1′)); 62.47 (C(19)); 125.38 (C(14), C(16)); 128.42, 131.72 (C(11), C(13), C(15)); 152.21 (C(12)).

Synthesis of compounds 10 and 13 (general procedure). A solution of methyl pheophorbide *a* **1** (0.08—0.66 mmol), 4-dimethylaminopyridine (DMAP) (the double molar excess with respect to compound **1**), 2-chloro-*N*-methylpyridinium iodide (5—10% molar excess relative to compound **1**), and terpenephenol **5** or **6** (50% of the number of moles of compound **1**) in toluene (25 mL) was refluxed for 2 h (control by TLC, eluent was CCl_4—acetone, 4 : 1). Then the reaction mixture was diluted with chloroform (100 mL), transferred into a separatory funnel, and washed with 5% aqueous HCl solution and distilled water until a neutral reaction of aqueous layer to remove the excess of DMAP and the products of conversion of 2-chloro-*N*-methylpyridinium iodide. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure at 40—50 °C. The residue was purified by chromatography on silica gel (eluent was CCl_4—acetone, ratio 70 : 1→1 : 1). The eluate containing the major reaction product was concentrated under reduced pressure and the residue was reprecipitated from its solution in chloroform with hexane.

Methyl (3*S*,4*S*)-21-{*O*-3-[(4-hydroxy-3-{(1*S*,2*R*,4*R*)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-yl}-5-{(1*R*,2*S*,4*S*)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-yl}phenyl)propyl]}carbamoyl-4,8,13,18-tetramethyl-20-oxo-9-ethenyl-14-ethylphorbin-3-propanoate (10) was prepared as a dark blue powder from methyl pheophorbide *a* **1** (47.9 mg, 0.08 mmol), DMAP (22.9 mg, 0.19 mmol), 2-chloro-*N*-methylpyridinium iodide (24.0 mg, 0.10 mmol), and terpenephenol **5** (19.1 mg, 0.04 mmol) in toluene (25 mL) in the yield of 25.6 mg (64%). MS (ESI), *m/z*: 999.6 $[MH]^+$. UV-Vis, λ/nm (I_{rel} (%)): 668.5 (41), 611.0 (8), 538.5 (10), 508.0 (11), 472.0 (6), 416.0 (100). ^1H NMR, δ: −1.65 (br.s, 1 H, N(III)H); 0.62 and 0.50 (both s, 3 H each, C(10′)H_3, C(10′′)H_3); 0.69 and 0.67 (both s, 3 H each, C(9′)H_3, C(9′′)H_3); 0.75 and 0.72 (both s, 3 H each, C(8′)H_3, C(8′′)H_3); 1.72 (t, 3 H, C(8(2))H_3, J = 8.2 Hz); 1.83 (d, 3 H, C(18(1))H_3, J = 7.3 Hz); 2.00—1.16 (m, 10 H, H(4′), H(4′′), C(5′)H_2, C(5′′)H_2, C(6′)H_2, C(6′′)H_2); 2.35—2.15 (m, 2 H, H(3′), H(3′′)); 2.79—2.45 (m, 4 H, C(17(1)H_2, C(17(2)H_2); 2.96—2.80 (m, 2 H, H(2′), H(2′′)), 3.22 (s, 3 H, C(7(1))H_3); 3.43 (s, 3 H, C(2(1)H_3); 3.58 (s, 3 H, C(12(1)H_3); 3.73 (s, 3 H, C(17(4)H_3); 3.77—3.65 (m, 2 H, C(8(1)H_2)); 4.40—4.26 (m, 3 H, H(17), C(17′)H_2)); 4.60 (s, 1 H, ArO<u>H</u>); 4.51 (q, 1 H, H(18), J = 7.3 Hz); 6.20 (d, 1 H, H(3(2))$_{trans}$, J = 12.0 Hz); 6.29 (d, 1 H, H(3(2))$_{trans}$, J = 18.0 Hz); 6.31 (s, 1 H, H(13(2))); 6.75 (s, 2 H, H(14′), H(16′)); 7.99 (dd, 1 H, H(3(1)), J = 18.0 Hz, J = 12.0 Hz); 8.60 (s, 1 H, H(20)); 9.36 (s, 1 H, H(5)); 9.52 (s, 1 H, H(10)). ^{13}C NMR, δ: 11.21 (C(7(1))), 12.09 (C(2(1))), 12.14 (C(12(1))), 12.41 and 12.46 (C(10′), C(10′′)), 17.44 (C(8(2))), 19.45 (C(8(1))), 19.75 and 19.93 (C(9′), C(9′′)), 21.19 and 21.32 (C(8′), C(8′′)), 23.20 (C(18)), 27.46 (C(5′), C(5′′)), 29.83 (C(17(1))), 30.89 (C(17(2))), 31.88 (C(17′)), 33.67 (C(5′), C(5′′)), 33.73 (C(18′)), 39.91 and 39.96 (C(6′), C(6′′)), 45.20 and 45.27 (C(2′), C(2′′)), 45.99 and 46.17 (C(4′), C(4′′)), 47.88 and 47.98 (C(7′), C(7′′)), 49.77 (C(1′), C(1′′)), 49.81 (C(18)), 50.15 (C(17)), 51.65 (C(17(4))), 65.02 (C(18′)), 93.12 (C(20)), 97.53 (C(5)), 104.37 (C(10)), 105.45 (C(15)), 122.72 (C(3(2))), 125.27 and 125.30 (C(14′), C(16′)), 128.21 (C(13)), 128.98 (C(11′), C(13′)), 129.17 (C(3(1))), 130.94 (C(12)), 130.97 (C(2)), 131.78 (C(15′)), 136.14 (C(7)), 136.22 (C(4)),

136.45 (C(3)), 137.96 141.97 (C(1)), 145.15 (C(8)), 149.69 (C(14)), 150.94 (C(9)), 152.14 (C(12′)), 155.55 (C(6)), 161.11 (C(16)), 169.12 (C(19)), 172.08 (C(17(3))), 173.34 (C(13(3))), 189.75 (C(13(1))).

Methyl (3*S*,4*S*)-21-{*O*-3-[(4-hydroxy-3-{(1*S*,2*R*,4*R*)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-yl}-5-methyl]}carbamoyl-4,8,13,18-tetramethyl-20-oxo-9-ethenyl-14-ethylphorbin-3-propanoate (13) was prepared as a dark blue powder from methyl pheophorbide *a* **1** (398.3 mg, 0.66 mmol), DMAP (101.2 mg, 0.83 mmol), 2-chloro-*N*-methylpyridinium iodide (101.0 mg, 0.40 mmol), and terpenephenol **6** (102.4 mg, 0.33 mmol) in toluene (25 mL) in the yield of 104.1 mg (36%). MS (ESI), *m/z*: 877.6 $[MH]^+$. UV-Vis, λ/nm (I_{rel} (%)): 668.5 (50), 611.0 (9), 562.0 (3), 538.5 (10), 508.0 (10), 476.5 (2), 414.0 (100). ^{1}H NMR, δ*: −1.63 (br.s, 1 H, N(III)H); 0.62/0.53 (s, 3 H, C(10′)H_3)); 0.65/0.63 (s, 3 H, C(9′)H_3)); 0.75/0.68 (s, 3 H, C(8′)H_3)); 1.72 (t, 3 H, C(8(2))H_3), J = 8.3 Hz), 1.83 (d, 3 H, C(18(1))H_3, J = 9.0 Hz); 2.06/2.05 (s, 3 H, C(13′(1))H_3); 2.78—2.14 and 2.00—1.16 (both m, 6 H and 5 H, C(17(1))H_2, C(17(2))H_2, C(3′)H_2, H(4′), C(5′)H_2, C(6′)H_2); 2.92 (t, 1 H, H(2′), J = 8.3 Hz); 3.24 (s, 3 H, C(7(1))H_3); 3.43 (s, 3 H, C(2(1))H_3); 3.58 (s, 3 H, C(12(1))H_3); 3.73 (s, 3 H, C(17(4))H_3); 3.77—3.65 (m, 2 H, C(8(1))H_2); 4.40—4.22 (m, 3 H, H(17), C(17′)H_2); 4.46 (s, 1 H, ArOH), 4.50 (q, 1 H, H(18), J = 8.3 Hz); 6.20 (d, 1 H, H(3(2))$_{cis}$, J = 12.0 Hz); 6.30 (s, 1 H, H(13(2))); 6.31 (d, 1 H, H(3(2))$_{trans}$, J = 18.0 Hz); 6.56 (s, 1 H, H(14′)); 6.76 (s, 1 H, H(16′)), 8.00 (dd, 1 H, H(3(1)), J = 18.0 Hz, J = 12.0 Hz); 8.60 (s, 1 H, H(20)); 9.38 (s, 1 H, H(5)); 9.54 (s, 1 H, H(10)). ^{13}C NMR, δ: 11.22 (C(7(1))), 12.10 (C(2(1))), 12.13 (C(12(1))), 12.29/12.32 (C(10′)), 15.99 (C(13(1′))), 17.43 (C(8(2))), 19.45 (C(8(1))), 19.86/20.01 (C(9′)), 21.17/21.30 (C(8′)), 23.16 (C(18(1))), 27.42/27.45 (C(5′)), 29.82 (C(17(2))), 31.01/30.69 (C(17(1))), 31.36/31.41 (C(18′)), 33.90/33.94 (C(3′), C(17′)), 39.98/40.02 (C(6′)), 45.28/45.35 (C(2′)), 45.59 (C(4′)), 47.80/47.90 (C(7′)), 49.51/49.53 (C(1′)), 50.13 (C(18)), 51.12 (C(17)), 51.64 (C(17(4))), 64.97/65.03 (C(19′)), 65.06 (C(13(2))), 93.11 (C(20)), 97.53 (C(5)), 104.40 (C(10)), 104.44 (C(15)), 122.36/122.76 (C(3(2))), 125.78, 215.85 and 127.89 (C(14′), C(15′), C(16′)), 128.56 (C(13)), 129.00 (C(11′)), 129.10 (C(13′)), 129.10 (C(3(1))), 129.18, 128.56 (C(12)), 131.61 (C(2)), 131.81 (C(15′)), 136.16 (C(7)), 136.27 (C(4)), 136.47 (C(3)), 137.95 (C(11)), 141.99 (C(11)), 145.20 (C(8)), 149.70 (C(14)), 151.00 (C(9)), 151.06 (C(12′)), 155.60 (C(6)), 161.10 (C(16)), 169.14 (C(13(3))), 172.10 (C(19)), 173.34 (C(17(3))), 189.78 (C(13(1))).

Synthesis of compounds 7—9, 11, 12 and 14 (general procedure). A solution of carboxychlorin **2** or **3** (0.25—0.30 mmol), 2-chloro-*N*-methylpyridinium iodide (equimolar amount to carboxychlorin), terpene alcohol **4**, **5** or **6** (0.15—0.20 mmol), and DMAP (0.50—0.60 mmol) in dichloromethane (25 mL) was refluxed for 30 min. Then the reaction mixture was cooled down and diluted with dichloromethane (100 mL) and washed with 5% aqueous HCl solution and distilled water until a neutral reaction of aqueous layer to remove the excess of DMAP and the products of conversion of 2-chloro-*N*-methylpyridinium iodide. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure at 40—50 °C. Then the residue was purified by chromatography on silica gel (eluent was CCl_4—acetone, ratio 60 : 1→1 : 1). The eluate containing the desired product was concentrated under reduced pressure and the residue was reprecipitated from its solution in chloroform with hexane.

(3*S*,4*S*)-{*O*-[(4-Hydroxy-3-{(1*S*,2*R*,4*R*)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-yl}-5-{(1*R*,2*S*,4*S*)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-yl}phenyl)methyl]}-4,8,13,18-tetramethyl-20-oxo-9-ethenyl-14-ethylphorbin-3-propanoate (7) was obtained as a dark blue powder from compound **2** (137.4 mg, 0.26 mmol) and compound **4** (66.4 mg, 0.17 mmol) in 110.8 mg (71%) yield. MS (ESI), *m/z*: 913.8 $[MH]^+$. UV-Vis, λ/nm (I_{rel} (%)): 668.0 (45), 610.5 (9), 539.5 (10), 509.0 (12), 473.0 (6), 414.0 (100). ^{1}H NMR, δ: −1.69 (br.s, 1 H, N(I)H); 0.46 (br.s, 1 H, N(III)H); 0.94, 0.89, 0.86, 0.79, 0.77 (all s, 3 H, 3 H, 3 H, 6 H, C(8′)H_3, C(8′′)H_3, C(9′)H_3, C(9′′)H_3, C(10′)H_3, C(10′′)H_3), 2.79—2.56, 2.45—2.06, 2.02—1.20 (all m, 2 H, 6 H, 16 H, C(3′)H_2, C(3′′)H_2, H(4′), H(4′′), C(5′)H_2, C(5′′)H_2, C(6′)H_2, C(6′′)H_2, C(17(1))H_2, C(17(2))H_2, C(8(2))H_3, C(18(1))H_3); 3.04 and 2.94 (both t, 1 H each, H(2′), H(2′′), J = 8.3 Hz); 3.26 (s, 3 H, C(2(1))H_3); 3.44 (s, 3 H, C(7(1))H_3); 3.71 (s, 3 H, C(12(1))H_3); 3.74 (q, 2 H, C(8(1))H_2, J = 8.3 Hz); 4.30 (br.d, 1 H, H(17), J = 7.3 Hz), 4.49 (q, 1 H, H(18), J = 6.4 Hz); 4.90 (s, 1 H, ArOH); 5.06 and 5.01 (both d, 1 H each, C(17′)H_2, J = 12.0 Hz); 5.25 and 5.08 (both d, 1 H each, C(13(2))H_2, J = 19.2 Hz); 6.20 (d, 1 H, H(3(2))$_{cis}$, J = 11.0 Hz); 6.31 (d, 1 H, H(3(2))$_{trans}$, J = 17.4 Hz); 7.13 and 7.11 (both s, 1 H each, H(14′), H(16′)); 8.03 (dd, 1 H, H(3(1)), J = 18.3 Hz, J = 11.0 Hz); 8.68 (s, 1 H, H(20)); 9.39 (s, 1 H, H(5)); 9.50 (s, 1 H, H(10)). ^{13}C NMR, δ: 11.26 (C(7(1))), 12.07 (C(2(1))), 12.11 (C(12(1))), 12.52 (C(10′), C(10′′)), 17.43 (C(8(2))), 19.50 (C(8(1))), 20.11 (C(9′), C(9′′)), 21.30 (C(8′), C(8′′)), 23.13 (C(18(1))), 29.71 (C(17(1))), 31.35 (C(17(2))), 33.98 (C(3′), C(3′′)), 40.00 (C(6′), C(6′′)), 46.07 (C(2′), C(2′′)), 45.38 (C(4′), C(4′′)), 48.12 (C(7′), C(7′′)), 48.12 (C(13(2))), 49.97 (C(18)), 49.97 (C(1′), C(1′′)), 51.68 (C(17)), 67.34 (C(17′)), 93.07 (C(20)), 97.20 (C(5)), 104.11 (C(10)), 106.09 (C(15)), 122.54 (C(3(2))), 126.29 (C(14′), C(16′), C(15′)), 128.31 (C(13)), 128.85 (C(11′), C(13′)), 128.91 (C(3(1))), 129.28 (C(12)), 130.60 (C(2)), 131.55 (C(15′)), 135.83 (C(7)), 136.11 (C(4)), 136.22 (C(3)), 137.92 (C(11)), 141.50 (C(1)), 144.07 (C(8)), 149.06 (C(14)), 150.78 (C(9)), 152.03 (C(12′)), 154.31 (C(6)), 160.44 (C(16)), 171.43 (C(19)), 172.99 (C(17(3)), 196.15 (C(13(1)).

Methyl 2-{*O*-[(4-hydroxy-3-{(1*S*,2*R*,4*R*)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-yl}-5-{(1*R*,2*S*,4*S*)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-yl}phenyl)methyl]carbamoylethyl}-18-[(*N*-methyl)carbamoyl]chlorin-20-yl)acetate (8) was obtained as a dark blue powder from compound **3** (189.0 mg, 0.30 mmol) and compound **4** (87.9 mg, 0.22 mmol) in 134.1 mg (61%) yield. MS (ESI), *m/z*: 1002.7 $[MH]^+$. UV-Vis, λ/nm (I_{rel} (%)): 663.5 (21), 606.0 (3), 554.5 (2), 527.5 (4), 500.5 (10), 402.5 (100). ^{1}H NMR, δ: −1.78 (br.s, 1 H, N(I)H); −1.58 (br.s, 1 H, N(III)H); 0.79 and 0.78 (both s, 6 H, 12 H, C(8′)H_3, C(8′′)H_3, C(9′)H_3, C(9′′)H_3, C(10′)H_3, C(10′′)H_3); 2.68—2.53, 2.29—2.09, 1.92—1.22 (all m, 1 H, 4 H, 19 H, C(3′)H_2, C(3′′)H_2, H(4′), H(4′′), C(5′)H_2, C(5′′)H_2, C(6′)H_2, C(6′′)H_2, C(17(1))H_2, C(17(2))H_2, C(8(2))H_3, C(18(2))H_3); 2.95 (t, 2 H, H(2′), H(2′′), J = 8.2 Hz); 3.28 (d, 1 H, C(13(2))H_3, J = 4.6 Hz); 3.34 (s, 3 H, C(2(1))H_3); 3.52 (s, 3 H, C(7(1))H_3); 3.57 (s, 3 H, C(12(1))H_3); 3.84 (s, 3 H, C(15(3))H_3); 3.82 (q, 2 H, C(8(1))H_2, J = 6.5 Hz); 4.39 (br.d, 1 H, H(17), J = 10.1 Hz);

* Hereinafter the symbol "/" indicates the signals from diastereomers differing by the chemical shift values.

4.50 (q, 1 H, H(18), J = 7.3 Hz); 4.95 (s, 1 H, ArOH); 5.06 and 4.99 (both d, 1 H each, C(17′)H_2, J = 11.9 Hz); 5.50 and 5.24 (both d, 1 H each, C(15(1))H_2, J = 19.2 Hz); 6.17 (d, 1 H, H(3(2))$_{cis}$, J = 11.9 Hz); 6.36 (br.t, 1 H, C(13(1))NH, J = 4.6 Hz); 6.38 (d, 1 H, H(3(2))$_{trans}$, J = 17.4 Hz); 7.09 (s, 2 H, H(14′), H(16′)), 8.12 (dd, 1 H, H(3(1)), J = 17.1 Hz, J = 11.9 Hz); 8.84 (s, 1 H, H(20)); 9.66 (s, 1 H, H(5)); 9.71 (s, 1 H, H(10)). ^{13}C NMR, δ: 11.34 (C(7(1))), 11.95 (C(2(1))), 12.17 (C(12(1))), 12.53 (C(10′), C(10′′)), 17.70 (C(8(2))), 19.70 (C(8(1))), 20.12 (C(9′), C(9′′)), 21.32 (C(8′), C(8′′)), 23.04 (C(18(1))), 27.25 (C(13(2))), 27.49 (C(5′), C(5′′)), 29.74 (C(17(2))), 31.55 (C(17(1))), 33.96 (C(3′), C(3′′)), 40.00 (C(6′), C(6′′)), 46.05 (C(2′), C(2′′)), 45.36 (C(4′), C(4′′)), 48.13 (C(7′), C(7′′)), 49.35 (C(18)), 49.98 (C(1′), C(1′′)), 52.10 (C(15(3))), 53.18 (C(17)), 67.25 (C(17′)), 93.76 (C(20)), 98.80 (C(5)), 101.45 (C(10)), 102.10 (C(15)), 121.66 (C(3(2))), 126.17 (C(14′), C(16′), C(15′)), 128.09 (C(13)), 128.88 (C(11′), C(13′)), 129.51 (C(3(1))), 129.98 (C(12)), 130.21 (C(2)), 134.65 (C(7)), 134.85 (C(4)), 135.09 (C(3)), 136.00 (C(11)), 139.02 (C(1)), 144.67 (C(8)), 148.82 (C(14)), 148.80 (C(9)), 149.06 (C(12′)), 154.27 (C(6)), 166.86 (C(16)), 168.96 (C(19)), 170.03 (C(13(1))), 173.03 (C(17(3))), 174.23 (C(15(2))).

3-{(3*S*,4*S*)-{*O*-[(4-Hydroxy-3-{(1*S*,2*R*,4*R*)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-yl}-5-{(1*R*,2*S*,4*S*)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-yl}phenyl)}propyl]}-4,8,13,18-tetramethyl-20-oxo-9-ethenyl-14-ethylphorbin-3-propanoate (9) was obtained as a dark blue powder from compound **2** (141.8 mg, 0.27 mmol) and compound **5** (70.1 mg, 0.17 mmol) in 60.1 mg (38%) yield. MS (ESI), m/z: 941.6 $[MH]^+$. UV-Vis, λ/nm (I_{rel} (%)): 668.5 (42), 610.5 (8), 540.0 (10), 508.5 (11), 472.0 (6), 414.5 (100). ^{1}H NMR, δ: −1.65 (br.s, 1 H, N(I)H); 0.50 (br.s, 1 H, N(III)H); 0.93, 0.88, 0.84, 0.79, 0.76 (all s, 3 H, 3 H, 3 H, 3 H, 6 H, C(8′)H_3, C(8′′)H_3, C(9′)H_3, C(9′′)H_3, C(10′)H_3, C(10′′)H_3); 2.82—2.63, 2.43—2.09, 1.98—1.20 (all m, 2 H, 5 H, 17 H, C(3′)H_2, C(3′′)H_2, H(4′), H(4′′), C(5′)H_2, C(5′′)H_2, C(6′)H_2, C(6′′)H_2, C(17(1))H_2, C(17(2))H_2, C(17′)H_2, C(8(2))H_3, C(18(1))H_3); 2.57—2.50 (m, 2 H, C(18′)H_2); 2.93 (t, 2 H, C(17′)H_2, J = 8.2 Hz); 3.03 and 2.59 (both t, 1 H each, H(2′), H(2′′), J = 7.3 Hz); 3.28 (s, 3 H, C(2(1))H_3); 3.45 (s, 3 H, C(7(1))H_3); 3.71 (s, 3 H, C(12(1))H_3); 3.73 (q, 2 H, C(8(1))H_2, J = 7.3 Hz); 4.14—3.97 (m, 2 H, C(19′)H_2); 4.35 (br.d, 1 H, H(17), J = 8.3 Hz); 4.54 (q, 1 H, H(18), J = 6.4 Hz); 4.64 (s, 1 H, ArOH); 5.31 and 5.15 (both d, 1 H each, C(13(2))H_2, J = 20.2 Hz); 6.21 (d, 1 H, H(3(2))$_{cis}$, J = 11.9 Hz); 6.32 (d, 1 H, H(3(2))$_{trans}$, J = 17.4 Hz); 6.87 (s, 2 H, H(14′), H(16′)); 8.05 (dd, 1 H, H(3(1)), J = 17.4 Hz, J = 11.9 Hz); 8.60 (s, 1 H, H(20)); 9.42 (s, 1 H, H(5)); 9.54 (s, 1 H, H(10)). ^{13}C NMR, δ: 11.23 (C(7(1)), 12.05 (C(2(1)), 12.12 (C(12(1)), 12.50/12.59 (C(10′), C(10′′)), 17.44 (C(8(2))), 19.46 (C(8(1))), 20.12/20.21 (C(9′), C(9′′)), 21.39/21.50 (C(8′), C(8′′)), 23.18 (C(18(1))), 27.53 (C(5′), C(5′′)), 29.89 (C(17(2))), 30.52 (C(17(1))), 32.10 (C(17′)), 33.93 (C(3′), C(3′′)), 34.02 (C(18′)), 40.00 (C(6′), C(6′′)), 45.42 (C(2′), C(2′′)), 46.06 (C(4′), C(4′′)), 48.10 (C(7′), C(7′′), C(13(2)), 49.90 (C(1′), C(1′′)), 50.00 (C(18)), 51.80 (C(17)), 64.02 (C(19′)), 92.99 (C(20)), 97.18 (C(5)), 104.05 (C(10)), 106.04 (C(15)), 122.51 (C(3(2))), 125.27 (C(14′), C(16′)), 128.31 (C(13)), 128.38 (C(11′), C(13′)), 129.24 (C(3(1))), 130.50 (C(12)), 130.97 (C(2)), 131.51 (C(15′)), 135.84 (C(7)), 136.04 (C(4)), 136.17 (C(3)), 137.86 (C(11)), 141.52 (C(1)), 144.96 (C(8)), 149.01 (C(14)), 150.76 (C(9)), 152.24 (C(12′)), 155.17 (C(6)), 160.32 (C(16)), 171.38 (C(19)), 173.09 (C(17(3))), 196.13 (C(13(2))).

Methyl 2-(*O*-3-[(4-hydroxy-3-{(1*S*,2*R*,4*R*)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-yl}-5-{(1*R*,2*S*,4*S*)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-yl}phenyl)propyl]carbamoylethyl}-18-[(*N*-methyl)carbamoyl]chlorin-20-yl)acetate (11) was obtained as a dark blue-green powder from compound **3** (180.1 mg, 0.29 mmol) and compound **5** (94.5 mg, 0.22 mmol) in 128.8 mg (57%) yield. MS (ESI), m/z: 1030.7 $[MH]^+$. UV-Vis, λ/nm (I_{rel} (%)): 663.5 (29), 607.5 (4), 557.0 (2), 528.5 (4), 500.0 (10), 403.0 (100). ^{1}H NMR, δ: −1.76 (br.s, 1 H, N(I)H); −1.54 (br.s, 1 H, N(III)H); 0.84, 0.82, 0.79 (all s, 3 H, 12 H, 6 H, C(8′)H_3, C(8′′)H_3, C(9′)H_3, C(9′′)H_3, C(10′)H_3, C(10′′)H_3); 2.62—2.49, 2.34—2.08, 1.92—1.21 (all m, 3 H, 5 H, 20 H, C(3′)H_2, C(3′)H_2, H(4′), H(4′′), C(5′)H_2, C(5′′)H_2, C(6′)H_2, C(6′′)H_2, C(17(1))H_2, C(17(2))H_2, C(17′)H_2, C(18′)H_2, C(8(2))H_3, C(18(1))H_3); 3.03—2.91 (m, 2 H, H(2′), H(2′′)); 3.28 (d, 1 H, C(13(2))H_3, J = 4.6 Hz); 3.35 (s, 3 H, C(2(1))H_3); 3.53 (s, 3 H, C(7(1))H_3); 3.58 (s, 3 H, C(12(1))H_3); 3.83 (s, 3 H, C(15(3))H_3); 3.82 (q, 2 H, C(8(1))H_2, J = 7.3 Hz); 4.15—3.96 (m, 2 H, C(19′)H_2), 4.41 (br.d, 1 H, H(17), J = 9.2 Hz); 4.53 (q, 1 H, H(18), J = 7.3 Hz); 4.75 (s, 1 H, ArOH); 5.54 and 5.28 (both d, 1 H each, C(15(1))H_2, J = 19.2 Hz); 6.18 (d, 1 H, H(3(2))$_{cis}$, J = 11.9 Hz); 6.36 (br.t, 1 H, C(13(1))NH, J = 4.6 Hz); 6.39 (d, 1 H, H(3(2))$_{trans}$, J = 18.3 Hz); 6.90 (s, 2 H, H(14′), H(16′)), 8.12 (dd, 1 H, H(3(1)), J = 18.3 Hz, J = 11.9 Hz); 8.85 (s, 1 H, H(20)); 9.66 (s, 1 H, H(5)); 9.72 (s, 1 H, H(10)). ^{13}C NMR, δ: 11.35 (C(7(1))), 11.98 (C(2(1))), 12.20 (C(12(1))), 12.51 (C(10′), C(10′′)), 17.74 (C(8(2))), 19.72 (C(8(1))), 20.13 (C(9′), C(9′′)), 21.32 (C(13(2))), 21.40 (C(8′), C(8′′)), 27.30 (C(5′), C(5′′)), 29.71 (C(17(2))), 30.44 (C(17(1))), 31.29 (C(17′)), 33.93 (C(3′), C(3′′)), 33.94 (C(18′)), 40.00 (C(6′), C(6′′)), 45.41 (C(2′), C(2′′)), 45.41 (C(15(3))), 46.07 (C(4′), C(4′′)), 48.11 (C(7′), C(7′′)), 49.31 (C(18)), 49.92 (C(1′), C(1′′)), 52.12 (C(17)), 63.93 (C(19′)), 93.65 (C(20)), 98.85 (C(5)), 101.49 (C(10)), 102.02 (C(15)), 121.63 (C(13(2))), 125.26 (C(14′)H, C(16′)H), 128.40 (C(13)), 128.43 (C(11′), C(13′)), 129.54 (C(3(1))), 129.92 (C(12)), 130.19 (C(2)), 130.98 (C(15′)), 134.60 (C(7)), 134.98 (C(4)), 136.09 (C(3)), 138.93 (C(11)), 142.10 (C(1)), 144.79 (C(8)), 149.10 (C(14)), 150.14 (C(9)), 152.26 (C(12′)), 155.50 (C(6)), 161.96 (C(16)), 168.93 (C(19)), 170.15 (C(13(1))), 173.11 (C(15(2))), 174.28 (C(17(3))).

3-{(3*S*,4*S*)-{*O*-[(4-Hydroxy-3-{(1*S*,2*R*,4*R*)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-yl}-5-methyl}propyl]}-4,8,13,18-tetramethyl-20-oxo-9-ethenyl-14-ethylphorbin-3-propanoate (12) was obtained as a dark blue powder from compound **2** (149.5 mg, 0.28 mmol) and compound **6** (72.1 mg, 0.23 mmol) in 79.4 mg (42%) yield. MS (ESI), m/z: 819.5 $[MH]^+$. UV-Vis, λ/nm (I_{rel} (%)): 664.0 (31), 608.0 (3), 554.5 (2), 529.0 (4), 501.5 (9), 403.0 (100). ^{1}H NMR, δ: −1.66 (br.s, 1 H, N(I)H); 0.48 (br.s, 1 H, N(III)H); 0.93/0.88, 0.81/0.79, 0.84, 0.74 (all s, 3 H each, C(8′)H_3, C(9′)H_3, C(10′)H_3), 2.40/2.27 (s, 3 H, C(13(1′))H_3); 2.82—2.68, 2.43—2.09, 1.98—1.20 (all m, 1 H, 7 H, 13 H, C(3′)H_2, H(4′), C(5′)H_2, C(6′)H_2, C(18′)H_2, C(17(1))H_2, C(17(2))H_2, C(8(2))H_3, C(18(1))H_3); 2.47 (t, 2 H, C(17′)H_2, J = 8.2 Hz); 3.10/3.00 (t, 1 H, H(2′), J = 8.3 Hz); 3.26 (s, 3 H, C(2(1))H_3); 3.44 (s, 3 H, C(7(1))H_3); 3.70 (s, 3 H, C(12(1))H_3); 3.76—3.66 (m, 2 H, C(8(1))H_2); 4.11—3.98 (m, 2 H, C(19′)H_2), 4.35 (br.d, 1 H, H(17), J = 8.3 Hz); 4.52 (q, 1 H, H(18), J = 6.4 Hz); 4.60 (s, 1 H, ArOH); 5.31 and 5.15 (both d, 1 H each, C(13(2))H_2, J = 20.2 Hz); 6.21 (d, 1 H, H(3(2))$_{cis}$,

J = 12.0 Hz); 6.32 (d, 1 H, H(3(2))$_{trans}$, J = 18.0 Hz); 6.88 (s, 2 H, H(14′), H(16′)), 8.03 (dd, 1 H, H(3(1)), J = 18.0 Hz, J = 12.0 Hz); 8.59 (s, 1 H, H(20)); 9.40 (s, 1 H, H(5)); 9.51 (s, 1 H, H(10)). ^{13}C NMR, δ: 11.23 (C(7(1))), 12.02 (C(2(1))), 12.11 (C(12(1))H_3), 12.41/12.48 (C(10′)), 16.14/16.24 (C(13′(1))), 17.43 (C(8(2))), 19.47 (C(8(1))), 20.21 (C(9′)), 21.36 (C(8′)), 23.19 (C(18(1))), 27.49 (C(5′)), 29.85 (C(17(2))), 31.65 (C(17(1))), 34.17 (C(3′)), 34.64 (C(18′)), 40.06 (C(6′)), 45.50 (C(2′)), 45.76 (C(4′)), 47.99 (C(7′)), 49.63 (C(1′)), 50.01 (C(18)), 51.74 (C(17)), 62.46 (C(17′)), 64.09 (C(18′)), 68.00 (C(19′)), 93.01 (C(20)), 97.20 (C(5)), 104.07 (C(10)), 106.04 (C(15)), 122.53 (C(3(2))), 125.81 and 125.95 (C(14′), C(16′)), 128.25 (C(13)), 128.78/128.87 (C(11′), C(13′)), 129.06 (C(3(1))), 129.23 (C(12)), 130.49 (C(2)), 131.53/131.71 (C(15′)), 135.86 (C(7)), 136.06 (C(4)), 136.19 (C(3)), 137.86 (C(11)), 141.55 (C(1)), 144.98 (C(8)), 149.01 (C(14)), 150.77 (C(9)), 151.18 (C(12′)), 155.21 (C(6)), 160.29 (C(16)), 171.41 (C(19)), 173.12 (C(17(3))), 196.18 (C(13(1))).

Methyl 2-(*O*-3-[(4-hydroxy-3-{(1*S*,2*R*,4*R*)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-yl}-5-methylphenyl)propyl]carbamoylethyl}-18-[(*N*-methyl)carbamoyl]chlorin-20-yl)acetate (14) was obtained as a blue-green powder from compound **3** (184.6 mg, 0.30 mmol) and compound **6** (72.5 mg, 0.24 mmol) in 88.7 mg (41%) yield. MS (ESI), m/z: 908.7 $[MH]^+$. UV-Vis, λ/nm (I_{rel} (%)): 663.5 (33), 608.0 (3), 557.5 (1), 529.5 (2), 501.5 (8), 403.0 (100). ^{1}H NMR, δ: −1.76 (br.s, 1 H, N(I)H); −1.54 (br.s, 1 H, N(III)H); 0.82, 0.83/0.80, 0.74 (all s, 3 H each, C(8′)H_3, C(9′)H_3, C(10′)H_3); 2.17/2.14 (s, 3 H, C(13′(1))H_3); 2.60—2.41, 2.34—2.08, 1.92—1.21 (all m, 3 H, 5 H, 20 H, C(3′)H_2, H(4′), C(5′)H_2, C(6′)H_2, C(18′)H_2, C(17′)H_2, C(17(1))H_2, C(17(2))H_2, C(8(2))H_3, C(18(1))H_3); 3.02/2.97 (t, 1 H, H(2′), J = 8.3 Hz); 3.28 (d, 3 H, C(13(2))H_3, J = 4.6 Hz); 3.35 (s, 3 H, C(2(1))H_3); 3.53 (s, 3 H, C(7(1))H_3); 3.58 (s, 3 H, C(12(1))H_3); 3.83 (s, 3 H, C(15(3))H_3); 3.88—3.76 (m, 2 H, C(8(1))H_2); 4.12—3.95 (m, 2 H, C(19′)H_2); 4.40 (br.d, 1 H, H(17), J = 9.2 Hz); 4.52 (q, 1 H, H(18), J = 7.3 Hz); 4.67/4.58 (s, 1 H, ArO<u>H</u>); 5.56/5.54 and 5.28/5.25 (both d, 1 H each, C(15(1))H_2, J = 19.2); 6.18 (d, 1 H, H(3(2))$_{cis}$, J = 12.0 Hz); 6.36 (br.t, 1 H, C(13(1))NH, J = 4.6 Hz); 6.40 (d, 1 H, H(3(2))$_{trans}$, J = 18.0 Hz); 6.88 (br.s, 1 H, H(16′)); 6.68 (br.s, 1 H, H(14′)); 8.13 (dd, 1 H, H(3(1)), J = 18.0 Hz, J = 12.0 Hz); 8.85 (s, 1 H, H(20)); 9.67 (s, 1 H, H(5)); 9.72 (s, 1 H, H(10)). ^{13}C NMR, δ: 11.37 (C(7(1))), 11.97 (C(2(1))), 12.20 (C(2(1))), 12.39 (C(10′)), 16.14/16.17 (C(13′(1))), 17.75 (C(8(2))), 19.72 (C(8(1))), 20.21 (C(9′)), 21.37 (C(9′)), 23.08 (C(18(1))), 27.27 (C(13(2))), 27.44/27.52 (C(5′)), 29.71 (C(17(2))), 30.29/30.38 (C(17(1))), 31.22/31.26 (C(17′)), 31.64/31.60 (C(3′)), 34.09/34.16 (C(18′)), 37.81 (C(15(1))), 40.06/40.02 (C(3′)), 45.69 (C(2′)), 45.50/45.45 (C(15(3))), 45.75 (C(4′)), 48.00 (C(7′)), 49.34 (C(18)), 49.63 (C(1′)), 52.11 (C(17)), 63.96/64.02 (C(19′)), 93.64 (C(20)), 98.85 (C(5)), 101.49 (C(10)), 102.05 (C(15)), 121.65 (C(3(2))), 125.81 (C(14′), C(16′)), 128.00/128.06 (C(13)), 128.78/128.87 (C(11′), C(13′)), 129.51 (C(3(1))), 129.92 (C(12)), 130.19 (C(2)), 131.67/131.71 (C(15′)), 134.58 (C(3)), 134.96/135.01 (C(4)), 136.11 (C(3)), 134.86 (C(11)), 138.95 (C(1)), 144.79 (C(8)), 149.13 (C(14)), 151.18 (C(9)), 151.21 (C(12′)), 154.29 (C(6)), 161.96 (C(16)), 166.68 (C(19)), 168.88 (C(13(1))), 170.15 (C(15(2))), 173.17/174.25 (C(17(3))).

The antioxidant activity of porphyrin derivatives was evaluated *in vitro* by the ability to inhibit LPO in a substrate obtained from the brain of laboratory mices.[10,11] After the extraction, the brain was homogenized (10%) in saline solution (pH 7.4) and centrifuged for 10 min (centrifuge CM-6M, 1600 g, 3000 rpm). Then the supernatant (S1)[10,12] containing water, proteins, DNA, RNA, and lipids (cholesterol, galactolipids, individual phospholipids, and gangliosides) was taken. The studied compounds were added to the supernatant as solutions in acetone (the final concentration 50 and 500 μmol L^{-1}). After 30 min, LPO was initiated by addition of a freshly prepared solution of $FeCl_2$ and ascorbic acid,[13] and the test samples were incubated for 1 h at 37 °C under slow stirring. The content of the secondary products of LPO reacting with 2-thiobarbituric acid (TBA-reactive products) was determined[11,14,18] using a ThermoSpectromic Genesys 20 spectrophotometer (USA) at λ = 532 nm, the extinction coefficient of $1.56 \cdot 10^5$ L mol^{-1} cm^{-1} was used in calculations. A series of four samples was used in each experiment.

The effect of compounds on the viability of HEK293 and SH-SY5Y cells was evaluated by staining cells with trypan blue. The method is based on the ability of the dye to penetrate into the interior of the cell through damaged membranes, while living cells do not stain.[19] Human embryonic kidney cells 293 (HEK293) and neuroblastoma cells (SH-SY5Y) (Russian Collection of Cell Cultures, Institute of Cytology of the Russian Academy of Sciences, St. Petersburg) were seeded in 24-well plates ($1.5 \cdot 10^5$ cells mL^{-1}) in the DMEM (Biolot, Russia) medium, containing 10% fetal bovine serum (Invitrogen, USA), 2 mM of L-glutamine, 50 μg mL^{-1} of gentamycin sulfate (PanEko, Russia), and cultured under 5% CO_2 atmosphere at 95% humidity. After 24 h from the reseeding, the test compounds were added to the cells, and the cells were incubated for 48 h. Immediately before the experiments, the stock solutions of test compounds (100 mmol L^{-1} in 100% DMSO) were diluted with the complete culture medium to the final concentrations of 0.5, 5, and 50 μg mL^{-1}; the final concentration of DMSO was 0.1%. When the incubation was finished, the cells were trypsinized (0.25% trypsin solution, 0.02% Versene solution, 3 min, 37 °C), and differential counting of live and dead cells was performed using trypan blue (0.01%) in the Goryaev chamber. The results were expressed as the percentage of living cells relative to the total number of cells in the experimental and control groups. The data obtained from two independent experiments were presented as the mean values of three measurements for each concentration ± standard deviation with respect to the control values (0.1% DMSO), which were assumed as 100%. To calculate the IC_{50} values (the concentration of a compound, which inhibits cell viability by 50%), a non-linear regression of the logarithm of the compound concentrations and normalized values for the inhibition percentage of viability (GraphPad Prism v.5.0, GraphPad Software Inc., USA) was performed.

This work was performed using the equipment of the "Chemistry" Center for Collective Use at the Institute of Chemistry of Komi Scientific Centre, Ural Branch of the Russian Academy of Sciences and the "Molecular Biology" Center for Collective Use at the Institute of Biology of Komi Scientific Centre, Ural Branch of the Russian Academy of Sciences. The used animals were obtained from the scientific collection of experimental animals at the Institute of Biology of Komi Scientific Centre, Ural

Branch of the Russian Academy of Sciences (http://www.ckp-rf.ru/usu/471933/).

This work was financially supported by the Russian Science Foundation (Project No. 16-13-10367).

References

1. M. V. Kamyshentsev, P. D. Shabanov, V. E. Stefanov, *Obzory po klinicheskoi farmakologii i ledarstvennoy terapii* [*Reviews on Clinical Pharmacology and Drug Therapy*], 2002, **1**, No. 1, 29 (in Russian).
2. V. I. Kaledin, N. G. Kolosova, A. M. Gonchar, A. Yu. Grishapova, A. E. Prosenko, *Sibirskii ekologicheskii zhurnal* [*Siberian Ecological Journal*], 2004, No. 1, 19 (in Russian).
3. E. B. Burlakova, *Russ. J. Gen. Chem.*, 2007, **77**, 1983.
4. E. V. Buravlev, I. Yu. Chukicheva, O. G. Shevchenko, K. Yu. Suponitsky, A. V. Kutchin, *Russ. Chem. Bull.*, 2016, **65**, 1232.
5. E. V. Buravlev, I. Yu. Chukicheva,O. G. Shevchenko, A. V. Kutchin, *Russ. Chem. Bull.*, 2017, **66**, 297.
6. E. V. Buravlev, I. Yu. Chukicheva, O. G. Shevchenko, K. Yu. Suponitskii, A. V. Kutchin, *Russ. Chem. Bull.*, 2017, **66**, 91.
7. O. G. Shevchenko, S. N. Plyusnina, L. N. Shishkina, I. Y. Chukicheva, I. V. Fedorova, A. V. Kuchin, *Biochemistry (Moscow) Suppl. Ser. A: Membr. Cell Biol.*, 2013, **7**, 302.
8. M. O. Senge, *Photodiagn. Photodyn. Ther.,* 2012, **9**, 170.
9. E. S. Nyman, P. H. Hynninen, *J. Photochem. Photobiol., B*, 2004, **73**, 1.
10. C. I. Acker, R. Brandão, A. R. Rosário, C. W. Nogueira, *Environ. Toxicol. Pharmacol.*, 2009, **28**, 280.
11. S. T. Stefanello, A. S. Prestes, T. Ogunmoyole, S. M. Salman, R. S. Schwab, C.R. Brender, L. Dornelles, J. B. T. Rocha, F. A. A. Soares, *Toxicol. In Vitro*, 2013, **27**, 1433.
12. N. A. V. Belle, G. D. Dalmolin, G. Fonini, M. A. Rubim, J. B. T. Rocha, *Brain Res.*, 2004, **1008**, 245.
13. R. Chawla, R. Arora, R. Kumar, A. Sharma, J. Prasad, S. Singh, R. Sagar, P. Chaudhary, S. Shukla, G. Kaur, R. K. Sharma, S. C. Puri, K. L. Dhar, G. Handa, V. K. Gupta, G. N. Qazi, *Mol. Cell. Biochem.*, 2005, **273**, 193.
14. T. Asakawa, S. Matsushita, *Lipids*, 1980, **15**, 137.
15. I. Yu. Chukicheva, O. V. Sukrusheva, L. I. Mazaletskaya, A. V. Kutchin, *Russ. J. Org. Chem.*, 2016, **52**, 813.
16. E. V. Buravlev, I. Yu. Chukicheva, I. A. Dvornikova, A. V. Churakov, A. V. Kutchin, *Russ. J. Org. Chem.*, 2012, **48**, 938.
17. L. A. Tulaeva, D. V. Belykh, N. M. Yakovleva, I. A. Selkova, A. V. Rocheva, A. V. Kutchin, *Izv. Vyssh. Uchebn. Zaved. Khim. Khim. Tekhnol.*, 2006, **49**, 82 (in Russian).
18. J.-S. Kim, *Food Nutr. Sci.*, 2013, **4**, 177.
19. K. S. Louis, A. C. Siegel, *Methods Mol. Biol.*, 2011, **740**, 7.

Received April 28, 2017;
in revised form July 24, 2017