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CHEMISTRY

Synthesis and Antitumor Properties of Carborane Conjugates of 5-(4-Aminophenyl)-10,15,20-triphenylporphyrin

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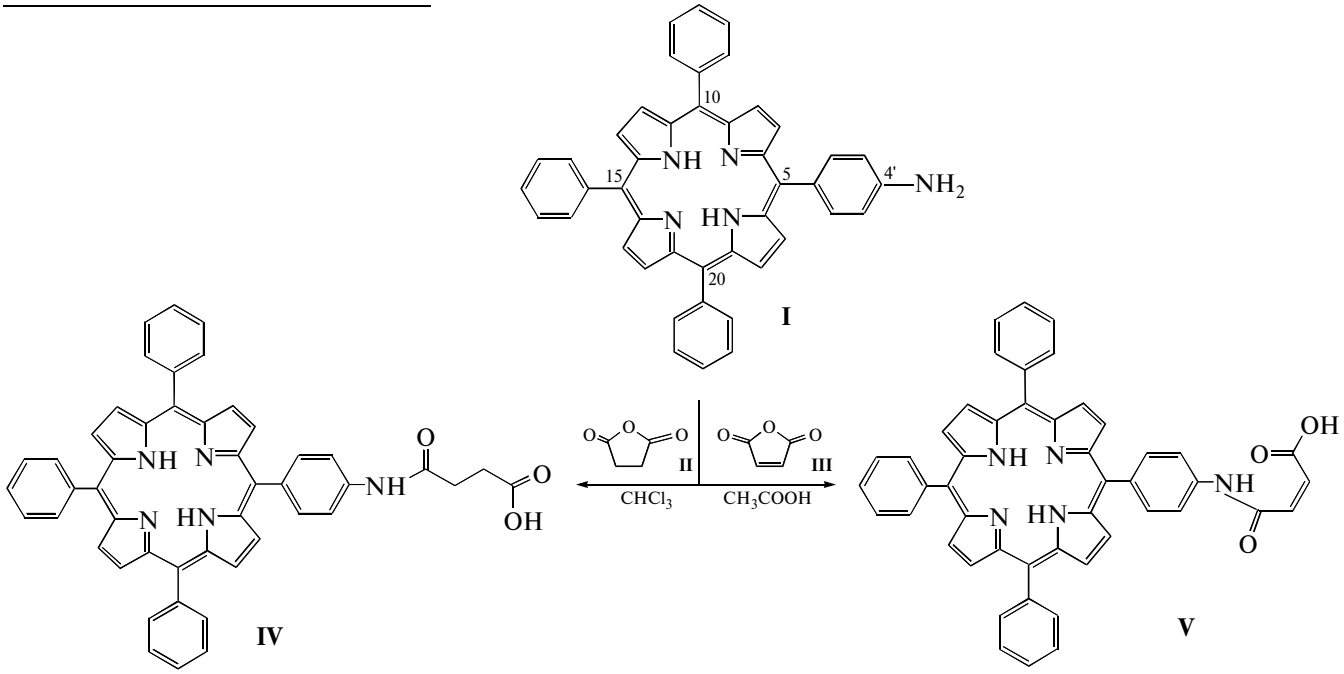
Porphyrins and their derivatives represent an important class of macroheterocycles with unique properties. The practical use of these compounds embraces important fields such as photosynthesis, non-linear optics, catalysis, and nanomaterials [1–7]. Porphyrins and other tetrapyrrole macrocycles are intensively used for the photodynamic therapy (PDT) of cancer and other diseases. This efficient therapy is based on the ability of porphyrins to be selectively accumulated in the tumor cells and generate active oxygen species ($^1O_2$, HO^-, $HO_2^{\cdot}$, and $O_2^{\cdot-}$) upon excitation with monochromatic light, which results in the tumor decay.

The optimization of the antitumor properties of photosensitizers implies the chemical modification of porphyrins. Previously we demonstrated that modification of porphyrin macrocycles by boron clusters considerably improves the antitumor properties of the photosensitizers [8, 9]. It is important that conjugation of carborane clusters to the periphery of macrocycles provides dual-action photo(radio)sensitizers that are suitable for both PDT and the boron neutron capture therapy (BNCT).

As a continuation of our works on the modification of the porphyrin macrocycle with carboranes [11, 12] and to prepare new compounds with optimized activity, we developed the synthesis of boronated porphyrins with amide bonds. These bonds are stable against hydrolysis and are present in proteins and in numerous natural products and drugs. We acylated the amino group of 5-(4'-aminophenyl)-10,15,20-triphenylporphyrin (**I**) [13] with succinic (**II**) and maleic (**III**) anhydrides. This gave porphyrin conjugates containing substituted succinic (**IV**) and maleic (**V**) acid monoamides as pharmacophore groups (Scheme 1).



Scheme 1.

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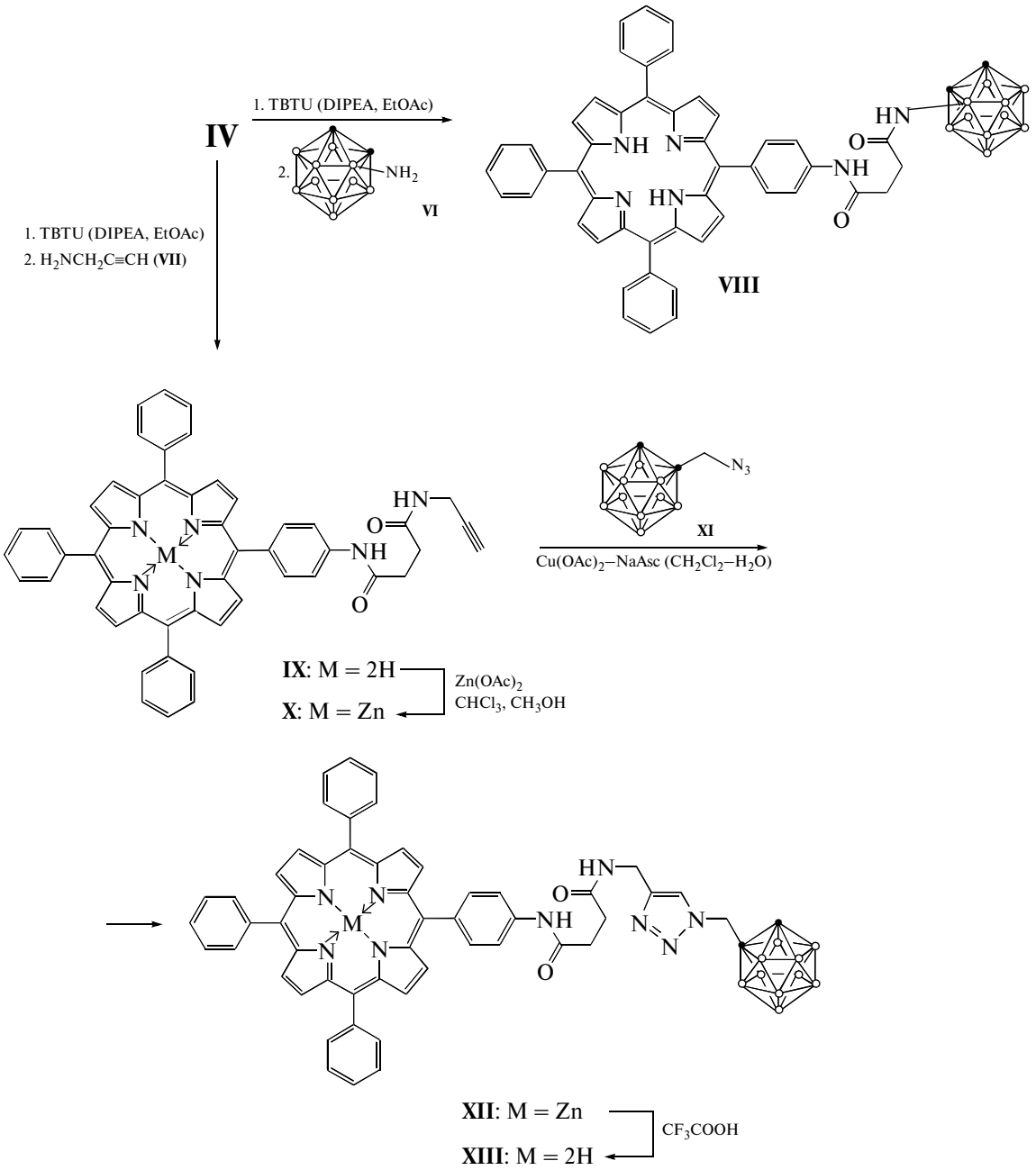
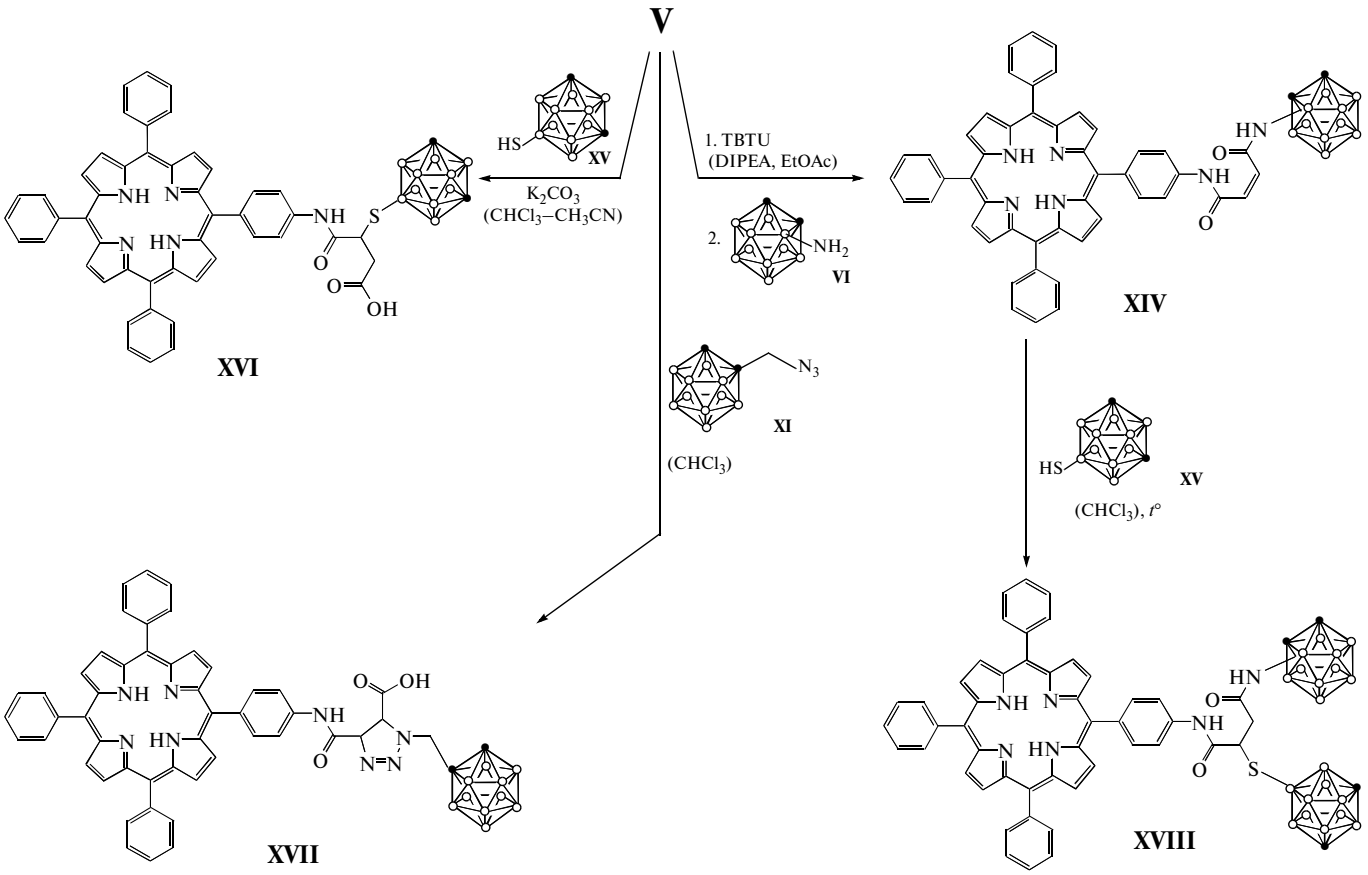


Scheme 2.

Porphyrins **IV** and **V** were used as precursors for the conjugation of carborane polyhedra at the periphery of the porphyrin macrocycle. The boron polyhedra were introduced into **IV** using functionalization of its carboxylic group activated by TBTU[1] with amines, in particular, 3-amino-*o*-carborane (**VI**) or propargylamine (**VII**). The reactions were carried out in ethyl acetate in the presence of diisopropylethylamine (DIPEA). This gave asymmetric porphyrin diamides (**VIII** and **IX**) (Scheme 2).

The boronation of amide **IX** containing a terminal triple bond was performed using 1,3-dipolar [2+3]-cycloaddition of azides to alkynes. This reaction occurs regioselectively when catalyzed by metal salts under mild conditions and is a convenient one-step method for converting a triple bond to heterocyclic 4-substituted 1,2,3-triazole pharmacophore. The loss of the catalyst via the introduction of copper ion into the coordination sphere of porphyrin **IX** was prevented by

[1] TBTU is *O*-(benzotriazol-1-yl)-N,N,N',N'-tetrabutyluronium tetrafluoroborate.



Scheme 3.

transforming **IX** to zinc complex **X**, which was made to react with 1-azidomethyl-*o*-carborane **XI** [14] to give carborane porphyrin **XII** with a triazole spacer. The reaction was carried out in the CH_2Cl_2–H_2O system at room temperature using $Cu(OAc)_2 \cdot 2H_2O$–sodium ascorbate (Asc) as the catalyst. Compound **X** was completely converted to triazole-containing porphyrin **XII**, which was isolated on a silica gel column (with chloroform as the eluent) in a 80% yield. Treatment of porphyrin **XII** with trifluoroacetic acid in CH_2Cl_2 afforded metal-free boronated porphyrin **XIII** (Scheme 2).

Compound **V** obtained by acylation of the amino group of porphyrin **I** with maleic anhydride contains two reactive sites, namely, the carboxylic group and the activated double bond, to which the carborane polyhedron can add. The reaction of the carboxylic group (TBTU activation) of **V** with aminocarborane **VI** taking place in ethyl acetate at 20°C in the presence of DIPEA resulted, as for porphyrin **IV**, in the formation of asymmetric carborane diamide **XIV** in 90% yield (Scheme 3).

In order to obtain stable compounds for biological assays, series of addition reactions to the activated double bond of porphyrin **V** with functionally substituted carboranes were carried out. In relation to 9-mercapto-*m*-carborane (**XV**), we demonstrated that **V** reacts rather easily with S-nucleophiles in the presence of potassium carbonate in a chloroform–acetonitrile mixture to give the corresponding thio derivative **XVI** in a 75% yield. Note that the reaction occurs as regioselective nucleophilic addition to the double bond, the initial attack of the carborane S-nucleophile being directed on the β-carbon atom relative to the carboxylic group of **V** (Scheme 3).

The reaction of **V** with 1-azidomethyl-*o*-carborane (**XI**) on refluxing in chloroform under argon for 15 h gave triazoline derivative **XVII** (yield 85%) having a free carboxylic group in position 4 of the triazoline ring (Scheme 3). This ring does not undergo intramolecular transformations with nitrogen evolution and aziridine formation under the reaction conditions.

We also studied the activity of boronated porphyrin **XIV** with respect to S-nucleophiles. The reaction of **XIV** with carborane **XV** performed on refluxing in chloroform yielded compound **XVIII** with two carborane polyhedra (Scheme 3), which may be useful for the BNCT of cancer.

Table. Physicochemical characteristics of compounds **IV**, **V**, **VII**, **IX**, **X**, **XII**–**XIV**, **XVI**–**XVIII**

Compound	UV/Vis, λ_{max}, nm ($\varepsilon \times 10^{-3}$)	IR (KBr, ν, cm^{-1})	^1H NMR (δ, ppm, $CDCl_3$)	^{11}B NMR (δ, ppm, $CDCl_3$)	MS, *m/z*
IV	646.5, 590.0, 546.0, 514.5, 419.5	3437 (NH), 1713 (CO in OH), 1659 (amide I), 1597 (amide II)	8.82 (s, 8H, β-pyrrole), 8.10 (m, 8H, Ph), 7.87 (m, 2H, Ph), 7.74 (m, 9H, Ph), 2.67 (t, 2H, *J* 5.60, CH_2–CH_2), 2.64 (t, 2H, *J* 5.60, CH_2–CH_2), –2.77 (s, 2H, NH)	–	730 $[M+1]^+$
V	646.20, 590.40, 550.20, 514.80, 418.20	3322 (NH), 1710 (CO– in COOH), 1630 (amide I), 1539 (amide II)	8.90 (s, 2H, β-pyrrole), 8.85 (s, 6H, β-pyrrole), 8.22 (m, 10H, Ph), 7.88 (m, 9H, Ph), 6.67 (d, 1H, *J* 12.8 Hz, CH=CH), 6.42 (d, 1H, *J* 12.8 Hz, CH=CH), 6.11 (s, 1H, NH), –2.66 (s, 2H, porphyrin NH)	–	728 $[M+1]^+$
VII	645.0 (5.22), 589.5 (6.00), 548.6 (10.06), 514.0 (20.57), 418.0 (401.10)	3317 (NH), 3060 (carborane CH), 2594 (BH), 1665 (amide I), 1597 (amide II)	–	–	870 $[M+1]^+$
IX	647.0 (3.1), 591.5 (3.0), 522.6 (5.4), 515.0 (11.6), 419.0 (231.0)	–	8.88 (s, 2H, β-pyrrole), 8.18 (s, 6H, β-pyrrole), 8.18 (m, 8H, Ph), 7.93 (m, 2H, Ph), 7.75 (m, 9H, Ph), 4.13 (d.d, 2H, *J* 2.6, 7.9 Hz, NH**CH$_2$**C≡CH), 3.37 (t, 1H, *J* 2.6 Hz, C≡CH), 2.89 (m, 2H, CH_2), 2.79 (s, 2H, NH), 2.76 (m, 2H, CH_2), –2.80 (2H, porphyrin NH)	–	767 $[M+1]^+$
X	590.0, 549.0, 420.0	–	8.80 (s, 2H, β-pyrrole), 8.83 (s, 6H, β-pyrrole), 8.05 (m, 8H, Ph), 7.90 (m, 2H, Ph), 7.27 (m, 9H, Ph), 4.10 (d.d, 2H, *J* 2.6, 7.9 Hz, NH**CH$_2$**C≡CH), 3.39 (t, 1H, *J* 2.6 Hz , ≡CH), 2.89 (m, 2H, CH_2), 2.79 (s, 2H, NH), 2.76 (m, 2H, CH_2)	–	830 $[M+1]^+$
XII	590.0 (0.32), 549.0 (1.24), 420.0 (250.0)	3304 (NH), 3055 (carborane CH), 2583 (BH), 1661 (amide I), 1597 (amide II)	8.95 (s, 9H, β-pyrrole and =CH–), 8.22 (m, 8H, Ph), 8.03 (m, 2H, Ph), 7.75 (m, 11H, Ph and 2 × NH), 5.15 (s, 2H, C–CH_2–N), 3.66 (m, 4H, CH_2–CH_2), 2.94 (s, 1H, carborane CH)	2.29 (1B, *J* 150 Hz), –4.95 (1B, *J* 150 Hz), –9.10 (2B, *J* 154 Hz), –11.49 (2B, *J* 159 Hz), –13.03 (4B, *J* 180 Hz)	1031 $[M+1]^+$
XIII	646.0 (2.41), 590.0 (3.24), 550.2 (5.61), 514.0 (11.23), 419.0 (177.69)	3320 (NH), 3054 (carborane CH), 2583 (BH), 1665 (amide I), 1597 (amide II)	–	–	969 $[M+1]^+$
XIV	646.2 (5.12), 590.4 (5.90), 550.2 (10.0), 514.8 (20.3), 418.2 (401.1)	3318 (NH), 3057 (carborane CH), 2596 (BH), 1627 (amide I), 1598 (amide II)	8.85 (s, 8H, β-pyrrole), 8.20 (m, 8H, Ph), 7.95 (m, 2H, Ph), 7.76 (m, 9H, Ph), 6.66 (d, 1H, *J* 12.0 Hz, –CH=CH–), 6.35 (d, 1H, *J* 12.0 Hz, –CH=CH–), 4.51 (s, 2H, NH), 4.12 (s, 1H, carborane CH), 3.48 (s, 1H, carborane CH), 2.9–0.88 (m, 10 H, BH), –2.77 (s. 2H, porphyrin NH)	–4.07 (d, 3B, *J* 149 Hz), –7.03 (s, 1B), –10.54 (d, 1B, *J* 159 Hz), –12.67 (d, 2B, *J* 163 Hz), –14.25 (d, 2B, *J* 165 Hz), –14.60 (d, 1B, *J* 155 Hz)	869 $[M+1]^+$
XVI	647.0 (6.75), 591.0 (7.20), 550.0 (10.5), 515.0 (18.8), 419.0 (382.7)	3356 (NH), 3060 (carborane CH), 2601 (BH), 1728 (CO– in COOH), 1626 (amide I), 1592 (amide II)	8.79 (s, 9H, β-pyrrole, NH), 8.21 (m, 8H, Ph), 7.76 (m, 11H, Ph), 5.57 (t, 1H, *J* 8.2 Hz CH_2–**CH**–S), 3.94 (d, 2H, *J* 8.2 Hz, CH_2), 2.94 (s, 1H, carborane CH), 2.8–0.9 (m, 10H, BH), –2.76 (s, 2H, porphyrin NH)	–0.88 (s, 1B, B9), –6.02 (d, 2B, *J* 167 Hz), –9.57 (d, 1B, *J* 144 Hz), –13.04 (d, 3B, *J* 168 Hz), –13.92 (d, 2B, *J* 167 Hz), –17.54 (d, 1B, *J* 180 Hz)	903 $[M+1]^+$

Table. (Contd.)

Compound	UV/Vis, λ_{max}, nm ($\varepsilon \times 10^{-3}$)	IR (KBr, ν, cm^{-1})	^1H NMR (δ, ppm, $CDCl_3$)	^{11}B NMR (δ, ppm, $CDCl_3$)	MS, *m/z*
XVII	646.0 (4.75), 591.0 (5.61), 551.0 (8.20), 515.0 (14.6), 419.0 (354.16)	3318 (NH), 3060 (carborane CH), 2594 (BH), 1678–1599 (CO– in COOH, amide I), 1512 (amide II)	10.20 (s, 1H, NH), 8.84 (s, 8H, β-pyrrole), 8.22 (m, 8H, Ph), 7.95 (m, 2H, Ph), 7.75 (m, 9H, Ph), 6.34 (d, 1H, *J* 13.6 Hz, CH–CH–N), 6.21 (d, 1H, *J* 13.6 Hz, CH–CH–N), 4.51 (s, 2H, CH_2), 3.48 (s, 1H, carborane CH), 2.90–0.88 (m, 10H, BH)	–4.00 (d, 3B, *J* 151 Hz), –8.79 (d, 1B, *J* 154 Hz), –11.47 (d, 1B, *J* 183 Hz), –12.94 (d, 4B, *J* 179 Hz), –14.44 (d, 1B, *J* 180 Hz)	927 $[M+1]^+$
XVIII	619.0 (2.31), 592.0 (2.91), 551.0 (2.96), 514.0 (5.02), 419.0 (111.0)	3318 (NH), 3060 (carborane CH), 2594 (BH), 1728 (CO– in COOH), 1626 (amide I), 1592 (amide II)	8.89 (s, 2H, β-pyrrole), 8.88 (s, 2H, β-pyrrole), 8.85 (s, 4H, β-pyrrole), 8.32 (m, 2H, Ph), 8.21 (m, 6H, Ph), 7.76 (m, 11H, Ph), 4.68 (s, 1H, NH), 3.08 (s, 1H, NH), 3.59 (dd, 1H, *J* 18.6, 9.42 Hz CH_2–**CH**–S), 3,44 (s, 4H, carborane CH), 3.10 (d.d, 2H, **CH_2**–CH–S, *J* 18.6, 9.42 Hz), –2.80 (2H, porphyrin NH)	–0.37 (s, 1B), –0.77 (s, 1B), –4,23 (d, 4B, *J* 160 Hz), –9.54 (d, 2B, *J* 163 Hz), –13.92 (d, 2B, *J* 169 Hz), –14.63 (d, 4B, *J* 170 Hz), –15.32 (d, 4B, *J* 180 Hz), –18.40 (d, 2B, *J* 161 Hz)	1043 $[M+1]^+$

Notes: **I**, 5-(*p*-Aminophenyl)-10,15,20-triphenylporphyrin; **IV**, 5-[4'-(3"-(carboxypropanoyl)- aminophenyl]-10,15,20-triphenylporphyrin; **V**, 5-[4'-(3"-carboxypropenoyl)aminophenyl]-10,15,20-triphenylporphyrin; **VIII**, 5-[4'-(*N*-carboran-3-yl)succinamidophenyl-10,15,20-triphenylporphyrin; **IX**, 5-[4'-*N*-(propargyl)succinamidophenyl-10,15,20-triphenylporphyrin; **X**, zinc 5-[4'-*N*-(propargyl) succinamidophenyl-10,15,20-triphenylporphyrin; **XII**, zinc 5-{4'-*N*-(*o*-carboran-1"-yl)methyl]triazolyl}succinamidophenyl-10,15,20-triphenylporphyrin; **XIII**, 5-{[4'-*N*-(*o*-carboran-1"-yl)methyl]triazolyl}succinamidophenyl-10,15,20-triphenylporphyrin; **XIV**, 5-{4'-[*N*-(*o*-carboran-3"-yl)maleinamido]phenyl}-10,15,20-triphenylporphyrin, **XVI**, 5-{4'-[3"-(*m*-thiocarboran-9"-yl)carboxypropanoyl]-aminophenyl}-10,15,20-triphenylporphyrin; **XVII**, 5-{4'-[5"-carboxy-1-(*o*-carboran-1"-yl)methyl](triazolin-4"-yl)carbonylamino]phenyl}-10,15,20-triphenylporphyrin; **XVIII**, 5-{4'-[2"-(*m*-carboran-9"-yl)thio)-N^4-(*o*-carboran-3"-yl)succinamido-N^1] phenyl}-10,15,20-triphenylporphyrin. For compounds **I**, **VII**, and **VIII**, see the text.

The structures of all of the synthesized compounds were confirmed by UV/Vis, IR, ^1H and ^{11}B NMR spectroscopy and mass spectrometry (table). The IR spectra of carborane-containing porphyrins (**VIII**, **XII**, **XIII**, **XIV**–**XVIII**) show an absorption band at 2580–2600 cm^{-1} corresponding to –BH stretching vibrations. In addition, amide I and amide II bands occur at 1627 and 1598 cm^{-1}, respectively.

The antitumor characteristics of porphyrins **V**, **XIV**, and **XVIII** were assessed by measuring the dark toxicity and the ability to cause light-induced death of cultured human tumor cells (HCT116 colon cancer cells). The dark cytotoxicity was determined in the MTT test [15] after cell incubation with compounds **V**, **XIV**, and **XVIII** for 72 h. The 50% inhibition of cell growth was observed at rather high concentrations, the IC_{50} values being >12.5 μM for **V**, >10 μM for **XIV**, and >25 μM for **XVIII**. The low dark cytotoxicity enables the use of these compounds in the PDT of cancer.

The experimental PDT was carried out after 30 to 60 min incubation of the cells with 10 μM of **V**, **XIV**, or **XVIII** and removal of the compounds from the culture medium [9]. The illumination of cells loaded with compound **V** caused necrosis of 100% of the cells within 5 to 7 min after the end of light exposure. The necrosis was detected based on typical morphology (cell shadows) and on the inclusion of propidium iodide and counting of the percentage of colored cells using a flow type cytofluorimeter [9]. After illumination of the cells incubated with compound **XIV**, the cell death was slower and the morphology of the damaged cells was indicative of apoptosis; the cells rounded and gradually detached from the substrate. Within 24 h after the end of light exposure, ~90% of the cells were detached from the solid substrate; however, the morphological pattern of the death differed from that of photoinduced necrosis. Finally, illumination of the cells incubated with compound **XVIII** induced a much slower damage: after 24–48 h many cells changed insignificantly and remained linked to the substrate.

Thus, compounds **V**, **XIV**, and **XVIII** showed no dark toxicity. The action of light on the cells loaded with non-boronated porphyrin **V** caused fast (during the first minutes) necrosis. In the presence of monocarboranylporphyrin **XIV**, the death developed more slowly and by a mechanism other than PDT-induced necrosis, while in the presence of dicarboranylporphyrin **XVIII**, the cell damage was even less efficient. The results show that the boron polyhedra change the photosensitizer properties of porphyrins. The addition of one boron polyhedron to the macrocycle can switch the death mechanism from necrosis to apoptosis, which is significant for the selection of the therapy protocol. The addition of the second boron polyhe-

dron can reduce the photoactivity, probably, due to retarded transport into the cells or for other reasons, which need to be elucidated for determining the further strategy of the design of boronated porphyrins as therapeutic agents.

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