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# Novel Derivatives of Chlorin $e_6$ with Hydrophilic Galactose Fragments and Carborane Polyhedra: Synthesis and Evaluation of Photodynamic Activity *in vitro*

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*In this study, we synthesized a series of carborane derivatives of chlorin $e_6$ modified with solubilizing galactose functional groups to produce amphiphilic photosensitizers with enhanced targeting properties based on the Warburg effect. Carborane polyhedra were introduced into the chlorin macrocycle via the formation of an amide bond between the carborane carboxylic acids and the 17-amino group of the chlorin macrocycle. A study of the dark and photoinduced cytotoxicity of the compounds synthesized demonstrated that all of them exhibit low dark cytotoxicity and exhibit significant photoinduced cytotoxicity against HeLa, A549, and HT-29 cells.*

**Ключевые слова:** Chlorin $e_6$, galactose, carborane, photodynamic therapy, boron neutron capture therapy, binary antitumor strategies.

# Новые производные хлорина $e_6$ с гидрофильными фрагментами галактозы и карборановыми полиэдрами: синтез и оценка фотодинамической активности *in vitro*

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*В настоящей работе получен ряд карборановых производных хлорина $e_6$, модифицированных солюбилизирующими галактозными функциональными группами, для получения амфифильных фотосенсибилизаторов с улучшенной таргетирующей способностью, основанной на эффекте Варбурга. Карборановые полиэдры были введены в макроцикл хлорина посредством образования амидной связи между карборановыми карбоновыми кислотами и 17-аминогруппой макроцикла хлорина. В результате исследования темновой и фотоиндуцированной цитотоксичности синтезированных соединений показано, что все они обладают низкой темновой цитотоксичностью и оказывают значительный фотоиндуцированный цитотоксический эффект в отношении клеток HeLa, A549 и HT-29.*

**Keywords:** Хлорин $e_6$, галактоза, карборан, фотодинамическая терапия, бор-нейтронозахватная терапия, бинарные противоопухолевые стратегии.

## Introduction

Cancer is a serious problem to human well-being and survival. Tetrapyrrole-containing compounds, especially those derived from natural sources, have attracted the particular attention in the preparation compounds with antitumor efficacy for photodynamic therapy (PDT). Among these, chlorin $e_6$ is a promising photosensitizer (PS) of second generation with high efficacy and minimal toxicity[1] possessing efficient properties for PDT clinical applications such as high phototoxic potential, and strong absorption in the red region of the visible spectrum which facilitate its deeper penetration into the cancer cells[2,3] as well as its high reactive oxygen species (ROS) generation ability. Numerous advantages of chlorin $e_6$ such as rapid and selective accumulation in tumor tissue, high therapeutic and diagnostic efficiency, almost complete elimination from the blood within 2-3 days, short period of increased skin photoactivity make these compounds the most utilized photosensitizers for PDT. Moreover, the structure of chlorin $e_6$ is well suited for design organic molecules of various functional diversity due to possibility of modifications its periphery serving as basic approach of novel PDT pharmaceuticals. PDT with chlorin $e_6$ photosensitizers demonstrated high efficiency in the treatment of malignant neoplasms of various localizations, including pancreatic,[4] melanoma,[5] bladder[6] and breast cancers.[7] Several chlorin $e_6$ photosensitizer drugs are available today to treat certain skin diseases, including acne, psoriasis, nonmelanoma skin cancer and precancerous skin changes, known as actinic keratosis.[8,9] It should be noted that recent research in PDT has focused on the development of approaches leading to the enhancing of specificity and uptake which is related to the synthesis of new photosensitizers. There are a number of reports on an increase in the selectivity of glycosylated PSs towards cancer cells.[10] Generally, cancer cells uptake higher level of glucose than normal cells in a phenomenon known as Warburg effect[11] and this strategy is widely used to increase the selectivity and bioavailability of PS for PDT. The conjugation of PSs with saccharides such as glucose,[12,13] galactose,[14-19] maltose[20,21] resulted in improvement of water solubility and rapid excretion from the body. Moreover, chlorin $e_6$ systems have been explored in various types of binary therapies like chemo-photodynamic therapy, photoimmunotherapy, and combined photodynamic-photothermal therapy. Among them the development of binary targeted anticancer therapy including PDT and boron neutron capture therapy (BNCT) are now the active field of the biomedical research. The combination of PDT with BNCT allows to overcome the disadvantages of traditional methods of cancer treatment such as surgery and chemotherapy as well as greatly enhance the therapeutic effect[22-25] since a binary therapy is more curative than a single therapy due to the synergistic effects of individual therapies. Cancer treatments with a single therapeutic agent often result in limited clinical effect due to tumour heterogeneity and drug resistance,[26] especially for more aggressive and metastasising cancer cells.[27] In this regard, boronated tetrapyrrole derivatives are promising dual sensitizers for BNCT and PDT owing to their low dark cytotoxicity, high boron content, and good tumor affinity. In earlier work,[28] it was demonstrated that the conjugation of the carborane cluster to the chlorin $e_6$ macrocycle yielded the derivative with a higher PS potency in the models of transplanted tumors compared to the boron-free chlorin $e_6$. Mechanistically, this effect was attributed to preferential localization of the carboranylchlorin in membrane organelles and its traverse across the membrane in an ionic form. So, the useful strategy to enhance the solubility and the targeting ability of carboranes is their conjugation with substrates that are known to possess anticancer or targeting properties. The use of conjugates of natural or synthetic tetrapyrrole compounds with boron polyhedra allows a number of advantages such as high boron concentration and tumor selectivity which ensure effective interaction with thermal neutrons as well as an accurate screening of drug delivery and tumor localization due to its fluorescence properties.[29,30] At the same time the hydrophobicity of both the chlorin macrocycle and the carborane polyhedron significantly reduces the bioavailability of the conjugates and complicates their applications as antitumor agents.[22-24,31-33] One way to overcome this disadvantage is to introduce carbohydrate units into the periphery of the chlorin macrocycle.[34] It was previously shown that functionalization of chlorin $e_6$ with galactose not only increases bioavailability, but also promotes conjugate active transport across the cell membrane.[14,15] It was previously shown that the presence of two galactose fragments on the periphery of the macrocycle promotes the solubility of the chlorin $e_6$ derivative in water not only if the molecule contains the chlorin macrocycle,[16,17] but also if the macrocycle contains bulky and hydrophobic terpene fragments, such as isosteviol and betulonic acid residues.[18] A similar approach can be used to obtain bioavailable chlorins with a carborane substituents on the periphery of the macrocycle. In the present work, new derivatives of chlorin $e_6$ with hydrophilic galactose fragments and boron polyhedra on the periphery of the macrocycle were synthesized and some their biological properties were studied.

## Experimental

### *Materials and methods*

$^1$H and $^{11}$B{$^1$H} NMR spectra of the synthesized compounds were recorded on Bruker AVANCE-II-300 (working frequency 300.17 MHz and 96.31 MHz for NMR $^1$H and $^{11}$B{$^1$H}, respectively) using standard impulse Bruker software for one and two-dimensional experiments. Infrared spectra were measured in KBr tablets on the "IR Prestige 21" device (Shimadzu). Mass spectra were recorded on the "Thermo-Finnigan LCQ Fleet" device. UV-Vis spectra were recorded on a spectrometer UV-1700 (Shimadzu) with the wavelength range of 200–1100 nm. The samples were analyzed in quartz cuvettes (10 mm thick). The reaction was controlled using TLC method on Sorbfil slides. Isolation of the reaction products was done using column chromatography on silica gel Alfa Aesar 70–230 mesh. Chlorin $e_6$ derivatives **1**,[19] carborane carboxylic acids **2**,[34] **3**,[35] **4**,[36] **5**,[34] **6**[37] were obtained according to literature methods.

## *Synthesis*

*General procedure for the preparation of compounds* ***7-11***. To the solution of compound **1** (600 mg, 0.51 mmol) in 30 mL $CH_2Cl_2$, corresponding carborane acid **2-6** (0.51 mmol), 2-chloro-1-methylpyridinium iodide (CMPI, 130 mg, 0.51 mmol) and 4-dimethylaminopyridine (DMAP, 63 mg, 0.51 mmol) were added. The mixture was reflux for 2 h (TLC: $CCl_4$–acetone, 2:1). The mixture was cooled, diluted with dichloromethane (70 mL), washed with 3% aqueous HCl (150 mL) and then washed with water until neutral p*H*, the organic layer was dried over anhydrous $Na_2SO_4$, and solvent was evaporated *in vacuo*. A residue after the evaporation was purified by column chromatography on a silica gel with $CCl_4$–acetone mixture (2:1 in vol. for compounds **7**, **8**), (1:1 in vol. for compound **9**) and (3:1 in vol. for compound **10**, **11**) as an eluent to afford expected chlorins as as a dark green crystalline powder.

*Compound 7*. Yield: 327 mg (47%) as a dark green crystalline powder. UV-Vis ($CHCl_3$) $\lambda_{max}$ nm ($I_{relative}$, %): 662 (29), 606 (3), 525 (3), 500 (10), 402 (100). IR (KBr) ν $cm^{-1}$: 3059 (C–H, carborane), 2595 (B–H, carborane), 1736 (C=O, ester), 1653 (“amide-I”), 1601 (“chlorin band”), 1538 (“amide-II”). MS (ESI) for $MH^+$ ($C_{67}H_{99}B_{10}N_8O_{15}$), *m/z* (relative intensity of the peak in the isotopic cluster, %): for $C_{67}H_{99}{}^{10}B_6{}^{11}B_4N_8O_{15}$ found 1359.8 (~1%), calculated 1359.8 (1.0%); for $C_{67}H_{99}{}^{10}B_5{}^{11}B_5N_8O_{15}$ found 1360.7 (7 %) calculated 1360.8 (5.2%); for $C_{67}H_{99}{}^{10}B_4{}^{11}B_6N_8O_{15}$ found 1361.9 (26%), calculated 1361.8 (18.8%); for $C_{67}H_{99}{}^{10}B_3{}^{11}B_7N_8O_{15}$ found 1362.9 (60%), calculated 1362.8 (47.8%); for $C_{67}H_{99}{}^{10}B_2{}^{11}B_8N_8O_{15}$ found 1363.8 (100%), calculated 1363.8 (84.4%); for $C_{67}H_{99}{}^{10}B_1{}^{11}B_9N_8O_{15}$ found 1364.8 (95%), calculated 1364.8 (100.0 %); for $C_{67}H_{99}{}^{11}B_{10}N_8O_{15}$ found 1365.8 (72%), calculated 1365.8 (75.6%); for $^{12}C_{66}{}^{13}CH_{99}{}^{11}B_{10}N_8O_{15}$ found 1366.8 (28%), calculated 1366.8 (35.6 %); for $^{12}C_{65}{}^{13}C_2H_{99}{}^{11}B_{10}N_8O_{15}$ found 1367.8 (9%), calculated 1367.8 (11.9%); for $^{12}C_{64}{}^{13}C_3H_{99}{}^{11}B_{10}N_8O_{15}$ found 1368.7 (~3.0%), calculated 1368.8 (3.0%). $^1$H NMR ($CDCl_3$) $\delta_H$ ppm (*J*, Hz): –1.78 (1H, s, I-NH); –1.62 (1H, s, III-NH); 0.73 (6H, s), 1.04 (6H, s), 1.27 (6H, s) and 1.43 (6H, s) ($8^a$-$CH_3$, $9^a$-$CH_3$, $11^a$-$CH_3$, $12^a$-$CH_3$, $8^b$-$CH_3$, $9^b$-$CH_3$, $11^b$-$CH_3$, $12^b$-$CH_3$); 1.22–3.20 (10H, m, carborane BH); 1.74 (3H, t, 8(2)-$CH_3$, *J* = 6.2); 1.78 (3H, d, 18(1)-$CH_3$, *J* = 7.5); 2.19 – 2.35 (2H, m) and 2.47 – 2.75 (6H, m): (17(1)-$CH_2$, 17(2)-$CH_2$, 17(4)-$CH_2$, 17(5)-$CH_2$); 2.64 – 2.75 (2H, m), 3.05 – 3.25 3.18 (2H, m): 13(2)-$CH_2$, 13(3)-$CH_2$), 2.99 (4H, d, $CH_2$-$6^a$, $CH_2$-$6^b$, *J* = 5.0); 3.37 (3H, s, 7(1)-$CH_3$); 3.50 (3H, s, 2(1)-$CH_3$); 3.63 (3H, s, 12(1)-$CH_3$); 3.80 (3H, s, 15(3)-$CH_3$); 3.83-3.92 (3H, m, 8(1)-$CH_2$, $13^c$-$CH_2$); 4.05-4.20 (5H, m, $2^a$-H, $2^b$-H, $5^a$-H, $5^b$-H, $13^c$-$CH_2$), 4.24 (2H, d, $4^a$-H, $4^b$-H *J* = 7.8); 4.30 (1H, br.s, carborane CH); 4.40 (1H, m, 17-H); 4.43 (2H, d, $3^a$-H, $3^b$-H *J* = 7.7); 4.50 (1H, *br.s*, 18-H); 5.32 (1H, d, *J* = 18.0) and 5.44 (1H, d, *J* = 18.0) (15(1)-$CH_2$); 5.34 (2H, d, $1^a$-H, $1^b$-H, *J* = 4.8); 5.35 (1H, br.s, 17(3)-NH); 6.18 (1H, d, 3(2)-H (*cis*), *J* = 11.4); 6.38 (1H, d, 3(2)-H (*trans*), *J* = 17.7); 6.70 (1H, br.s, 17(6)-NH); 7.90 (1H, m, 13(1)-NH); 8.10 (1H, dd, 3(1)-H, *J* = 11.6, *J* = 17.9); 8.83 (1H, s, 20-H); 9.68 (1H, s, 5-H); 9.75 (1H, s, 10-H).$^{11}$B{$^1$H} NMR ($CDCl_3$) $\delta_B$ ppm (*J,* Hz): –2.3 (1B, br.s); –5.4 (1B, br.s); –9.9 (8B, br.s).

*Compound 8*. Yield: 306 mg (44%) as a dark green crystalline powder. UV-Vis ($CHCl_3$) $\lambda_{max}$ nm ($I_{relative}$, %): 662 (30), 606 (3), 529 (3), 501 (10), 403 (100). IR (KBr) ν $cm^{-1}$: 3059 (C–H, carborane), 2594 (B–H, carborane), 1736 (C=O, ester), 1653 (“amide-I”), 1601 (“chlorin band”), 1537 (“amide-II”). MS (ESI) for $MH^+$ ($C_{67}H_{99}B_{10}N_8O_{15}$) *m/z* (relative intensity of the peak in the isotopic cluster, %): for $C_{67}H_{99}{}^{10}B_5{}^{11}B_5N_8O_{15}$ found 1360.7 (5%), calculated 1360.8 (5.2%); for $C_{67}H_{99}{}^{10}B_4{}^{11}B_6N_8O_{15}$ found 1361.8 (25%), calculated 1361.8 (18.8%); for $C_{67}H_{99}{}^{10}B_3{}^{11}B_7N_8O_{15}$ found 1362.8 (57%), 1362.8 (47.%); for $C_{67}H_{99}{}^{10}B_2{}^{11}B_8N_8O_{15}$ found 1363.7 (95%), 1363.8 (84.4%); for $C_{67}H_{99}{}^{10}B^{11}B_9N_8O_{15}$ found 1364.7 (100%), calculated 1364.8 (100.0%); for $C_{67}H_{99}{}^{11}B_9N_8O_{15}$ found 1365.7 (70%), calculated 1365.8 (75.6%); for $^{12}C_{66}{}^{13}CH_{99}{}^{11}B_9N_8O_{15}$ found 1366.7 (37%), 1366.8 (35.6%); for $^{12}C_{65}{}^{13}C_2H_{99}{}^{11}B_9N_8O_{15}$ found 1367.7 (12%), calculated 1367.8 (11.9%). $^1$H NMR ($CDCl_3$) $\delta_H$ ppm (*J*, Hz): –1.91 (1H, s, I-NH); –1.75 (1H, s, III-NH); 0.71 (6H, s), 1.07 (6H, s), 1.26 (6H, s) and 1.45 (6H, s) ($8^a$-$CH_3$, $9^a$-$CH_3$, $11^a$-$CH_3$, $12^a$-$CH_3$, $8^b$-$CH_3$, $9^b$-$CH_3$, $11^b$-$CH_3$, $12^b$-$CH_3$); 1.30–3.18 (9H, m, carborane BH); 1.67–1.83 (6H, m, 8(2)-$CH_3$, 18(1)-$CH_3$); 1.90–2.15 (4H, m) and 2.20–2.47 (2H, m): (17(1)-$CH_2$, 17(2)-$CH_2$, 17(4)-$CH_2$, 17(5)-$CH_2$); 2.80–3.20 (6H, m): 13(2)-$CH_2$, 13(3)-$CH_2$, $13^c$-$CH_2$); 2.96 (4H, *br.s*, $CH_2$-$6^a$, $CH_2$-$6^b$); 3.35 (3H, s, 7(1)-$CH_3$); 3.53 (3H, s, 2(1)-$CH_3$); 3.61 (3H, s, 12(1)-$CH_3$); 3.83 (3H, s, 15(3)-$CH_3$); 3.78–3.91 (2H, m, 8(1)-$CH_2$); 4.05–4.20 (4H, m, $2^a$-H, $2^b$-H, $5^a$-H, $5^b$-H); 4.25 (1H, m, 17-H); 4.24 (2H, d, $4^a$-H, $4^b$-H *J* = 7.4); 4.42 (1H, m, 18-H); 4.43 (2H, d, $3^a$-H, $3^b$-H *J* = 7.7); 4.51 (1H, *br.s*, carborane CH); 4.53 (1H, br.s, carborane CH); 5.23 (1H, d, *J* = 18.3) and 5.53 (1H, d, *J* = 18.3) (15(1)-$CH_2$); 5.28 (1H, br.s, 17(3)-NH), 5.36 (2H, d, $1^a$-H, $1^b$-H, *J* = 4.1); 5.92 (1H, br.s, 17(6)-NH); 6.17 (1H, m, 17(6)-NH); 6.19 (1H, d, 3(2)-H (*cis*), *J* = 11.4); 6.38 (1H, d, 3(2)-H (*trans*), *J* = 18.0); 7.76 (1H, m, 13(1)-NH); 8.11 (1H, dd, 3(1)-H, *J* = 11.4, *J* = 17.7); 8.87 (1H, s, 20-H); 9.65 (1H, s, 5-H); 9.71 (1H, s, 10-H).$^{11}$B{$^1$H} NMR ($CDCl_3$) $\delta_B$ ppm (*J,* Hz): 4.2 (1B, br.s, $B^9$); –2.5 (1B, br.s); –10.0 (2B, br.s); –14.9 (6B, br.s).

*Compound 9*. Yield: 261 mg (36%) as a dark green crystalline powder. UV-Vis ($CHCl_3$) $\lambda_{max}$ nm ($I_{relative}$, %): 662 (29), 607 (3), 528 (3), 500 (10), 403 (100). IR (KBr) ν $cm^{-1}$: 3075 (C–H, carborane), 2594 (B–H, carborane), 1736 (C=O, ester), 1655 (“amide-I”), 1605 (“chlorin band”), 1537 (“amide-II”). MS (ESI) for $MH^+$ ($C_{69}H_{100}B_{10}N_9O_{16}$) *m/z* (relative intensity of the peak in the isotopic cluster, %): for $C_{69}H_{100}{}^{10}B_5{}^{11}B_5N_9O_{16}$ found 1415.2 (4%), calculated 1415.8 (5.1%); for $C_{69}H_{100}{}^{10}B_4{}^{11}B_6N_9O_{16}$ found 1416.2 (17%), calculated 1416.8 (18.3%); for $C_{69}H_{100}{}^{10}B_3{}^{11}B_7N_9O_{16}$ 1417.8 (53 %), calculated 1417.8 (47.2%), for $C_{69}H_{100}{}^{10}B_2{}^{11}B_8N_9O_{16}$ found 1418.8 (75%), calculated 1418.8 (83.5%), for $C_{69}H_{100}{}^{10}B_1{}^{11}B_9N_9O_{16}$ found 1419.8 (100%), calculated 1419.8 (100.0%); for $C_{69}H_{100}{}^{11}B_{10}N_9O_{16}$ found 1420.7 (68%), calculated 1420.8 (76.7%); for $^{12}C_{68}{}^{13}C_1H_{100}{}^{11}B_{10}N_9O_{16}$ found 1421.8 (35%), calculated 1421.8 (36.9%); for $^{12}C_{67}{}^{13}C_2H_{100}{}^{11}B_{10}N_9O_{16}$ found 1422.8 (12 %), calculated 1422.8 (12.8%). $^1$H NMR ($CDCl_3$) $\delta_H$ ppm (*J*, Hz): –1.79 (1H, s, I-NH); –1.61 (1H, s, III-NH); 0.72 (6H, s), 1.05 (6H, s), 1.27 (6H, s) and 1.44 (6H, s) ($8^a$-$CH_3$, $9^a$-$CH_3$, $11^a$-$CH_3$, $12^a$-$CH_3$, $8^b$-$CH_3$, $9^b$-$CH_3$, $11^b$-$CH_3$, $12^b$-$CH_3$); 1.12–3.30 (9H, m, carborane BH); 1.73 (3H, d, 18(1)-$CH_3$, *J* = 6.6); 1.77 (3H, t, 8(2)-$CH_3$, *J* = 7.5); 2.11–2.31 (2H, m), 2.69 – 2.90 (4H, m) and 2.80–3.26 (2H, m) : (17(1)-$CH_2$, 17(2)-$CH_2$, 17(4)-$CH_2$, 17(5)-$CH_2$); 2.80–3.26 (4H, m): 13(2)-$CH_2$, 13(3)-$CH_2$,); 2.98 (4H, br.s, $CH_2$-$6^a$, $CH_2$-$6^b$); 3.37 (3H, s, 7(1)-$CH_3$); 3.51 (3H, s, 2(1)-$CH_3$); 3.63 (3H, s, 12(1)-$CH_3$); 3.82 (3H, s, 15(3)-$CH_3$); 3.82–3.93 (2H, m, 8(1)-$CH_2$); 4.05–4.33 (6H, m, $2^a$-H, $2^b$-H, $5^a$-H, $5^b$-H, carborane CH); 4.24 (2H, d, $4^a$-H, $4^b$-H *J* = 8.1); 4.37–4.57 (2H, m) (17-H, 18-H); 4.44 (2H, d, $3^a$-H, $3^b$-H *J* = 7.9); 5.34 (2H, d, $1^a$-H, $1^b$-H, *J* = 4.3); 5.30 (1H, d, *J* = 19.1) and 5.47 (1H, d, *J* = 19.1): (15(1)-$CH_2$); 5.55 (1H, d, C*H*=CH carborane, *J* = 12.9); 5.57 (1H, br.s, 17(3)-NH); 5.73 (1H, d, CH=C*H* carborane, *J* = 12.9), 6.19 (1H, d, 3(2)-H (*cis*), *J* = 11.7); 6.39 (1H, d, 3(2)-H (*trans*), *J* = 17.7); 7.42 (1H, m, 17(6)-NH); 7.90 (1H, m, 13(1)-NH); 8.11 (1H, dd, 3(1)-H, *J* = 11.9, *J* = 17.6); 8.83 (1H, s, 20-H); 9.69 (1H, s, 5-H); 9.74 (1H, s, 10-H); 9.93 (1H, m, $5^c$-NH-carborane). $^{11}$B{$^1$H} NMR ($CDCl_3$) $\delta_B$ ppm (*J,* Hz): –4.9 (3B, br.s); –11.2 (2B, br.s); –13.6 (5B, br.s).

*Compound* ***10***. Yield: 359 mg (50%) as a dark green crystalline powder. UV-Vis ($CHCl_3$) $\lambda_{max}$ nm ($I_{relative}$, %): 661 (30), 606 (3), 527 (3), 500 (10), 402 (100). IR (KBr) ν $cm^{-1}$: 3065 (C–H, carborane), 2575 (B–H, carborane), 1736 (C=O, ester), 1661 (“amide-I”), 1601 (“chlorin band”), 1539 (“amide-II”). MS (ESI) for $MH^+$ ($C_{70}H_{105}B_{10}N_8O_{15}$) *m/z* (relative intensity of the peak in the isotopic cluster, %): for $C_{70}H_{105}{}^{10}B_5{}^{11}B_5N_8O_{15}$ found 1402.7 (5%), calculated 1402.9 (5.1%), for $C_{70}H_{105}{}^{10}B_4{}^{11}B_6N_8O_{15}$ found 1403.7 (20%), calculated 1403.9 (18.5%), for $C_{70}H_{105}{}^{10}B_3{}^{11}B_7N_8O_{15}$ found 1404.8 (49%), calculated 1404.9 (47.1%); for $C_{70}H_{105}{}^{10}B_2{}^{11}B_8N_8O_{15}$: found 1405.8 (80%), calculated 1405.9 (83.6 %); for $C_{70}H_{105}{}^{10}B^{11}B_9N_8O_{15}$ found 1406.7 (100%), calcu-

lated 1406.9 (100.0%); for $C_{70}H_{105}{}^{11}B_{10}N_8O_{15}$ found 1407.8 (80%), calculated 1407.9 (76.8%); for $^{12}C_{69}{}^{13}CH_{105}{}^{11}B_{10}N_8O_{15}$ found 1408.8 (36%), calculated 1408.9 (37.1%); for $^{12}C_{68}{}^{13}C_2H_{105}{}^{11}B_{10}N_8O_{15}$ found 1409.9 (10%), calculated 1409.9 (12.8%). $^1$H NMR ($CDCl_3$) $\delta_H$ ppm (*J*, Hz): –1.77 (1H, s, I-NH); –1.59 (1H, s, III-NH); 0.72 (6H, s), 1.04 (6H, s), 1.26 (6H, s) and 1.44 (6H, s) ($8^a$-$CH_3$, $9^a$-$CH_3$, $11^a$-$CH_3$, $12^a$-$CH_3$, $8^b$-$CH_3$, $9^b$-$CH_3$, $11^b$-$CH_3$, $12^b$-$CH_3$); 1.00 (3H, d, *J* = 4. ) and 1.15 (3H, d, *J* = 6.9) ($16^c$-$CH_3$, $17^c$-$CH_3$); 1.08 – 3.30 (10H, m, carborane BH); 1.67–1.91 (6H, m, 18(1)-$CH_3$, 8(2)-$CH_3$); 2.11–2.20 (2H, m), 2.22–2.47 (4H, m) and 2.62–2.69 (2H, m): (17(1)-$CH_2$, 17(2)-$CH_2$, 17(4)-$CH_2$, 17(5)-$CH_2$); 2.59 (1H, d, *J* = 14.7) and 2.71 (1H, d, *J* = 14.7) ($13^c$-$CH_2$); 2.78–3.23 (4H, m): 13(2)-$CH_2$, 13(3)-$CH_2$,); 2.96 (4H, br.s, $CH_2$-$6^a$, $CH_2$-$6^b$); 3.36 (3H, s, 7(1)-$CH_3$); 3.50 (3H, s, 2(1)-$CH_3$); 3.63 (3H, s, 12(1)-$CH_3$); 3.82 (3H, s, 15(3)-$CH_3$); 3.76–3.96 (2H, m, 8(1)-$CH_2$); 4.06 (2H, t, $5^a$-H, $5^b$-H, *J* = 6.2) , 4.11 (2H, dd, $2^a$-H, $2^b$-H, *J* = 5.1, 2.2); 4.23 (2H, dd, $4^a$-H, $4^b$-H, *J* = 8.1, 1.1); 4.42 (2H, dd, $3^a$-H, $3^b$-H, *J* = 8.1, 2.2); 4.48 (1H, m) and 4.55 (1H, m) (17-H, 18-H); 5.34 (2H, d, $1^a$-H, $1^b$-H, *J* = 4.0); 5.41(1H, m) and 5.48 (1H, m,) (15(1)-$CH_2$);6.16 (1H, d, 3(2)-H (*cis*), *J* = 11.1); 6.37 (1H, d, 3(2)-H (*trans*), *J* = 18.0); 6.68 (1H, m, 17(3)-NH); 7.81 (1H, m, 13(1)-NH); 8.09 (1H, dd, 3(1)-H, *J* = 11.7, *J* = 18.0); 8.83 (1H, s, 20-H); 9.67 (1H, s, 5-H); 9.74 (1H, s, 10-H). $^{11}$B{$^1$H} NMR ($CDCl_3$) $\delta_B$ ppm (*J,* Hz): –4.4 (2B, br.s); –10.2 (8B, br.s).

*Compound* ***11***. Yield: 330 mg (44%) as a dark green crystalline powder. UV-Vis ($CHCl_3$) $\lambda_{max}$ nm ($I_{relative}$, %): 664 (30), 607 (3), 526 (3), 500 (10), 402 (100). IR (KBr) ν $cm^{-1}$: 3063 (C–H, carborane), 2581 (B–H, carborane), 1736 (C=O, ester), 1661 ("amide-I"), 1601 ("chlorin band"), 1539 ("amide-II"). MS (ESI) $MH^+$ ($C_{75}H_{107}B_{10}N_8O_{15}$) *m/z* (relative intensity of the peak in the isotopic cluster, %): for $C_{75}H_{107}{}^{10}B_5{}^{11}B_5N_8O_{15}$ found 1464.8 (5%), calculated 1464.9 (4.9%); for $C_{75}H_{107}{}^{10}B_4{}^{11}B_6N_8O_{15}$ found 1466.0 (22%), calculated 1465.9 (17.9%); for $C_{75}H_{107}{}^{10}B_3{}^{11}B_7N_8O_{15}$ found 1466.9 (25%), calculated 1466.9 (46.0%); for $C_{75}H_{107}{}^{10}B_2{}^{11}B_8N_8O_{15}$ found 1468.0 (95%), calculated 1467.9 (82.%); for $C_{75}H_{107}{}^{10}B^{11}B_9N_8O_{15}$ found 1468.8 (100%), calculated 1468.9 (100.0%); for $C_{75}H_{107}{}^{11}B_{10}N_8O_{15}$ found 1496.9 (55%), calculated 1469.9 (78.7%); for $^{12}C_{74}{}^{13}CH_{107}{}^{11}B_{10}N_8O_{15}$ found 1471.0 (70%), calculated 1470.9 (39.6%); for $^{12}C_{73}{}^{13}C_2H_{107}{}^{11}B_{10}N_8O_{15}$ found 1471.8 (30%), calculated 1471.9 (14.2%); for $^{12}C_{72}{}^{13}C_3H_{107}{}^{11}B_{10}N_8O_{15}$ found 1473.0 (5%), calculated 1472.9 (3.9%). $^1$H NMR ($CDCl_3$) $\delta_H$ ppm (*J*, Hz): –1.66 (2H, s, I-NH/(I-NH)′); –1.49 (1H, s) and –1.46 (1H, s) (III-NH/(III-NH)′); 0.71 (6H, s), 0.85 (6H, s), 0.97 (6H, s), 1.08 (6H, s), 1.28 (6H, s), 1.31 (6H, s), 1.44 (6H, s) and 1.49 (6H, s) ($8^a$-$CH_3$/($8^a$-$CH_3$)′, $9^a$-$CH_3$/($9^a$-$CH_3$)′, $11^a$-$CH_3$/($11^a$-$CH_3$)′, $12^a$-$CH_3$/($12^a$-$CH_3$)′, $8^b$-$CH_3$/($8^b$-$CH_3$)′, $9^b$-$CH_3$/($9^b$-$CH_3$)′, $11^b$-$CH_3$/($11^b$-$CH_3$)′, $12^b$-$CH_3$/($12^b$-$CH_3$)′); 1.18–3.24 (20H, m, carborane BH); 1.69–1.85 (12H, m, 18(1)-$CH_3$/(18(1)-$CH_3$)′, 8(2)-$CH_3$/(8(2)-$CH_3$)′); 1.88 (3H, s) and 1.94 (3H, s) ($22^c$-$CH_3$/($22^c$-$CH_3$)′); 2.26–2.44 (8H, m) and 2.50-2.81 (8H, m) (17(1)-$CH_2$/(17(1)-$CH_2$)′, 17(2)-$CH_2$/(17(2)-$CH_2$)′, 17(4)-$CH_2$/(17(4)-$CH_2$)′, 17(5)-$CH_2$/(17(5)-$CH_2$)′); 2.91-3.10 (12H, m) and 3.12-3.30 (8H, m) (13(2)-$CH_2$/(13(2)-$CH_2$)′, 13(3)-$CH_2$/(13(3)-$CH_2$)′, $6^a$-$CH_2$/($6^a$-$CH_2$)′, $6^b$-$CH_2$/($6^b$-$CH_2$)′, $20^c$-$CH_2$/($20^c$-$CH_2$)′); 3.37 (3H, s) and 3.40 (3H, s) (7(1)-$CH_3$/(7(1)-$CH_3$)′); 3.49 (3H, s) and 3.58 (3H, s) (2(1)-$CH_3$/(2(1)-$CH_3$)′); 3.63 (3H, s) and 3.68 (3H, s) (12(1)-$CH_3$/(12(1)-$CH_3$)′); 3.82 (3H, s) and 3.85 (3H, s) (15(3)-$CH_3$/(15(3)-$CH_3$)′); 3.77–3.94 (6H, m, 8(1)-$CH_2$/(8(1)-$CH_2$)′, $13^c$-CH/($13^c$-CH)′); 4.03–4.21 (8H, m), 4.24–4.34 (4H, m), 4.42–4.55 (5H, m) and 4.59–4.71 (3H, m) (17-H/(17-H)′, 18-H/(18-H)′, $2^a$-H/($2^a$-H)′, $2^b$-H/($2^b$-H)′, $3^a$-H/($3^a$-H)′, $3^b$-H/($3^b$-H)′, $4^a$-H/($4^a$-H)′, $4^b$-H/($4^b$-H)′, $5^a$-H/($5^a$-H)′, $5^b$-H/($5^b$-H)′); 5.35 (2H, d, *J* = 3.9) and 5.41 (2H, d, *J* = 4.5) ($1^a$-H/($1^a$-H)′, $1^b$-H/($1^b$-H)′); 5.44–5.70 (6H, m, 15(1)-$CH_2$/(15(1)-$CH_2$)′, 17(3)-NH/(17(3)-NH)′); 5.27–5.76 (8H, m) and 6.40 (2H, t, *J* = 8.7) ($15^c$-CH/($15^c$-CH)′, $16^c$-CH/($16^c$-CH)′, $17^c$-CH/($17^c$-CH)′, $18^c$-CH/($18^c$-CH)′, $19^c$-CH/($19^c$-CH)′); 6.16 (2H, d, 3(2)-H (*cis*)/(3(2)-H (*cis*))′, *J* = 11.7); 6.40 (1H, d, *J* = 17.1) and 6.43 (1H, d, *J* = 17.4) (3(2)-H (*trans*)/(3(2)-H (*trans*))′); 7.85 (1H, m) and 7.93 (1H, m) (13(1)-NH/(13(1)-NH)′); 8.11 (1H, dd, *J* = 6.6, *J* = 11.1) and 8.17 (1H, dd, *J* = 7.2, *J* = 10.8) (3(1)-H/(3(1)-H)′); 8.80 (1H, s) and 8.90 (1H, s) (20-H/(20-H)′); 9.74 (1H, s) and 9.75 (1H, s) (5-H/(5-H)′); 9.76 (1H, s) and 9.83 (1H, s) (10-H/(10-H)′). $^{11}$B{$^1$H} NMR ($CDCl_3$) $\delta_B$ ppm (*J,* Hz): –3.5 (1B, br.s); –6.1 (1B, br.s); –10.3 (8B, br.s).

*General procedure for the preparation of deprotected compounds* ***12-16***. To the protected chlorin **7-11** (0.180 mmol) 5-10 mL 90% aqueous trifluoroacetic acid (TFA) was added. The resulting mixture was stirred at room temperature (~23 ºC) for 4 h. Next, the excess of TFA was evaporated at low pressure. A residue after the evaporation was chromatographed on silica gel (eluent: $CHCl_3$–$CH_3OH$, 1:1; TLC: $CHCl_3$–$CH_3OH$, 3:1).

*Compound* ***12***. Yield: 180 mg (83%) as a dark green crystalline powder. UV-Vis ($CHCl_3$-$C_2H_5OH$ 95% aqueous, 1:1) $\lambda_{max}$ nm ($I_{relative}$, %): 662 (30), 606 (3), 528 (3), 500 (10), 402 (100). IR (KBr) ν $cm^{-1}$: 3071 (C–H, carborane), 2588 (B–H, carborane), 1719 (C=O, ester), 1670 ("amide-I"), 1603 ("chlorin band"), 1545 ("amide-II"). MS (ESI) for $MH^+$ ($C_{55}H_{83}B_{10}N_8O_{15}$) *m/z* (relative intensity of the peak in the isotopic cluster, %): for $C_{55}H_{83}{}^{10}B_6{}^{11}B_4N_8O_{15}$ found 1199.8 (3%), calculated 1199.7 (1.1%); for $C_{55}H_{83}{}^{10}B_5{}^{11}B_5N_8O_{15}$ found 1200.9 (10%), calculated 1200.7 (5.7%); for $C_{55}H_{83}{}^{10}B_4{}^{11}B_6N_8O_{15}$ found 1201.8 (26%), calculated 1201.7 (20.3%); for $C_{55}H_{83}{}^{10}B_3{}^{11}B_7N_8O_{15}$ found 1202.8 (50%) calculated 1202.7 (50.8%); for $C_{55}H_{83}{}^{10}B_2{}^{11}B_8N_8O_{15}$ found 1203.8 (87%), calculated 1203.7 (87.7%); for $C_{55}H_{83}{}^{10}B^{11}B_9N_8O_{15}$ found 1204.7 (10%) calculated 1204.7 (100.0%); for $C_{55}H_{83}{}^{11}B_{10}N_8O_{15}$ found 1205.7 (71%), calculated 1205.7 (70.8%); for $^{12}C_{54}{}^{13}CH_{83}{}^{11}B_{10}N_8O_{15}$ found 1206.7 (30%), calculated 1206.7 (29.7%); for $^{12}C_{53}{}^{13}C_2H_{83}{}^{11}B_{10}N_8O_{15}$ found 1207.7 (10%), calculated 1207.7 (8.8%); for $^{12}C_{52}{}^{13}C_3H_{83}{}^{11}B_{10}N_8O_{15}$ found 1208.6 (5 %), calculated 1208.7 (2.0%). $^1$H NMR (DMSO-$d_6$) $\delta_H$ ppm (*J,* Hz): –2.01 (1H, s, I-NH); –1.76 (1H, s, III-NH); 1.14-3.18 (10H, m, carborane BH); 1.62-1.78 (6H, m, 8(2)-$CH_3$, 18(1)-$CH_3$); 1.91–2.36 (8H, m, 17(1)-$CH_2$, 17(2)-$CH_2$, 17(4)-$CH_2$, 17(5)-$CH_2$); 3.02–3.18 (10H, m, 13(2)-$CH_2$, 13(3)-$CH_2$, $6^a$-$CH_2$, $6^b$-$CH_2$, $13^c$-$CH_2$); 3.34 (3H, s, 7(1)-$CH_3$); 3.55 (6H, s, 2(1)-$CH_3$, 12(1)-$CH_3$); 3.72 (3H, s, 15(3)-$CH_3$); 3.51–3.80 (8H, m, α,β-$1^a$-OH, α,β-$1^b$-OH, α,β-$2^a$-OH, α,β-$2^b$-OH, α,β-$3^a$-OH, α,β-$3^b$-OH, α,β-$4^a$-OH, α,β-$4^b$-OH); 3.92-4.22 (6H, m), 4.34–4.54 (4H, m) and 4,56–4.68 (2H, m) (8(1)-$CH_2$, 17-H, 18-H, α,β-$2^a$-H, α,β-$2^b$-H, α,β-$3^a$-H, α,β-$3^b$-H, α,β-$4^a$-H, α,β-$4^b$-H, α,β-$5^a$-H, α,β-$5^b$-H); 5.01–5.20 (3H, m, α,β-$1^a$-H, α,β-$1^b$-H, $1^c$-CH); 5.31 (1H, d, *J* = 15.6) and 5.48 (1H, d, *J* = 18.0) (15(1)-$CH_2$); 6.21 (1H, d, 3(2)-H (*cis*), *J* = 11.7); 6.48 (1H, d, 3(2)-H (*trans*), *J* = 17.7); 7.99 (1H, m, 17(3)-NH); 8.25-8.43 (2H, m, 3(1)-H, 13(1)-NH); 9.13 (1H, s, 20-H); 9.77 (1H, s, 5-H); 9.81 (1H, s, 10-H). $^{11}$B{$^1$H} NMR (DMSO-$d_6$) $\delta_B$ ppm (*J,* Hz): –3.4 (1B, br.s); –6.2 (1B, br.s); –10.4 (8B, br.s).

*Compound* ***13***. Yield: 178 mg (82%) as a dark green crystalline powder. UV-Vis ($CHCl_3$-$C_2H_5OH$ 95 % aqueous, 1:1) $\lambda_{max}$ nm ($I_{relative}$, %): 662 (30), 605 (3), 528 (3), 500 (10), 402 (100). IR (KBr) ν $cm^{-1}$: 3065 (C–H, carborane), 2598 (B–H, carborane), 1719 (C=O, ester), 1678 ("amide-I"), 1605 ("chlorin band"), 1539 ("amide-II"). MS (ESI) for $(M+2H)^+$ ($C_{55}H_{84}B_{10}N_8O_{15}$) *m/z* (relative intensity of the peak in the isotopic cluster, %): for $C_{55}H_{84}{}^{10}B_6{}^{11}B_4N_8O_{15}$ found 1200.6 (2%), calculated 1200.7 (1.1%); for $C_{55}H_{84}{}^{10}B_5{}^{11}B_5N_8O_{15}$ found 1201.8 (9%), calculated 1201.7 (5.7%); for $C_{55}H_{84}{}^{10}B_4{}^{11}B_6N_8O_{15}$ found 1202.9 (23%), calculated 1202.7 (20.3%); for $C_{55}H_{84}{}^{10}B_3{}^{11}B_7N_8O_{15}$ found 1203.9 (44%), calculated 1203.7 (50.8%); for $C_{55}H_{84}{}^{10}B_2{}^{11}B_8N_8O_{15}$ found 1204.8 (82%), calculated 1204.7 (87.7%); for $C_{55}H_{84}{}^{10}B^{11}B_9N_8O_{15}$ found 1205.7 (100%), calculated 1205.7 (100.%); for $C_{55}H_{84}{}^{11}B_{10}N_8O_{15}$ found 1206.7 (66%), calculated 1206.7 (70.8%); for $^{12}C_{54}{}^{13}CH_{84}{}^{11}B_{10}N_8O_{15}$ found 1207.7 (35%), calculated 1207.7 (29.7%); for $^{12}C_{53}{}^{13}C_2H_{84}{}^{11}B_{10}N_8O_{15}$ found 1208.7 (13%), calculated 1208.7 (8.8%); for $^{12}C_{52}{}^{13}C_3H_{84}{}^{11}B_{10}N_8O_{15}$ found 1209.6 (5%), calculated 1209.7 (2.0%). $^1$H NMR (DMSO-$d_6$) $\delta_H$ ppm (*J,* Hz): –2.01 (1H, s, I-NH); –1.76 (1H, s,

III-NH); 1.47-3.15 (9H, m, carborane BH); 1.68 (3H, d, 18(1)-$CH_3$, $J$ = 7.2); 1.70 (3H, t, 8(2)-$CH_3$, $J$ = 7.4); 1.77–2.34 (8H, m, 17(1)-$CH_2$, 17(2)-$CH_2$, 17(4)-$CH_2$, 17(5)-$CH_2$); 2.94–3.17 (10H, m, 13(2)-$CH_2$, 13(3)-$CH_2$, α,β-6$^a$-$CH_2$, α,β-6$^b$-$CH_2$, 13$^c$-$CH_2$); 3.24-3.78 (8H, m, α,β-1$^a$-OH, α,β-1$^b$-OH, α,β-2$^a$-OH, α,β-2$^b$-OH, α,β-3$^a$-OH, α,β-3$^b$-OH, α,β-4$^a$-OH, α,β-4$^b$-OH); 3.34 (3H, s, 7(1)-$CH_3$); 3.55 (6H, s, 2(1)-$CH_3$, 12(1)-$CH_3$); 3.73 (3H, s, 15(3)-$CH_3$); 3.79-3.91 (2H, m, 8(1)-$CH_2$); 3.92-4.19 (4H, m), 4.41 (2H, m) and 4.66-4.92 (2H, m) (α,β-2$^a$-H, α,β-2$^b$-H, α,β-3$^a$-H, α,β-3$^b$-H, α,β-4$^a$-H, α,β-4$^b$-H, α,β-5$^a$-H, α,β-5$^b$-H); 4.37 (1H, d, $J$ = 9.3, 17-H); 4.61 (1H, q, $J$ = 7.7, 18-H); 5.00–5.38 (2H, m, α,β-1$^a$-H, α,β-1$^b$-H); 5.32 (1H, d, $J$ = 19.2) and 5.47 (1H, d, $J$ = 17.7) (15(1)-$CH_2$); 4.32–5.23 (2H, m, 2$^c$-CH, 1$^c$-CH); 6.21 (1H, d, 3(2)-H (*cis*), $J$ = 11.1); 6.48 (1H, d, 3(2)-H (*trans*), $J$ = 18.3); 7.40 (1H, m, 17(3)-NH); 7.93 (1H, m, 13(1)-NH); 8.35 (1H, dd, 3(1)-H, $J$ = 11.6, $J$ = 17.0); 9.14 (1H, s, 20-H); 9.77 (1H, s, 5-H); 9.81 (1H, s, 10-H). $^{11}B\{^1H\}$ NMR (DMSO-$d_6$) $\delta_B$ ppm (*J,* Hz): 4.4 (1B, br.s, B$^9$); –3.0 (1B, br.s); –9.6 (2B, br.s); –14.6 (6B, br.s).

*Compound* ***14***. Yield: 181 mg (80%) as a dark green crystalline powder from 130 mg (0.09 mmol) of compound **9**. UV-Vis ($CHCl_3$-$C_2H_5OH$ 95 % aqueous, 1:1) $\lambda_{max}$ nm ($I_{relative}$, %): 662 (30), 606 (3), 529 (3), 500 (10), 401 (100). IR (KBr) ν cm$^{-1}$: 3076 (C–H, carborane), 2594 (B–H, carborane), 1719 (C=O, ester), 1674 ("amide-I"), 1609 ("chlorin band"), 1539 ("amide-II"). MS (ESI) for $(MH)^+$ ($C_{57}H_{83}B_{10}N_8O_{15}$) *m/z* (relative intensity of the peak in the isotopic cluster, %): for $C_{57}H_{84}{}^{10}B_6{}^{11}B_4N_9O_{16}$ found 1254.4 (1%), calculated 1254.7 (1.1%); for $C_{57}H_{84}{}^{10}B_5{}^{11}B_5N_9O_{16}$ found 1255.7 (4%), calculated 1255.7 (5.6%); for $C_{57}H_{84}{}^{10}B_4{}^{11}B_6N_9O_{16}$ found 1256.8 (9%), calculated 1256.7 (20.0%); for $C_{57}H_{84}{}^{10}B_3{}^{11}B_7N_9O_{16}$ found 1257.5 (20%), 1257.7 (50.2%); for $C_{57}H_{84}{}^{10}B_2{}^{11}B_8N_9O_{16}$ found 1258.9 (82%), calculated 1258.7 (87.0 %); for $C_{57}H_{84}{}^{10}B^{11}B_9N_9O_{16}$ found 1259.8 (100%), calculated 1259.7 (100.0 %); for $C_{57}H_{84}{}^{11}B_{10}N_9O_{16}$ found 1260.8 (100%), calculated 1260.7 (71.9%); for $^{12}C_{56}{}^{13}CH_{84}{}^{11}B_{10}N_9O_{16}$ found 1261.7 (56%), calculated 1261.7 (31.1%); for $^{12}C_{55}{}^{13}C_2H_{84}{}^{11}B_{10}N_9O_{16}$ found 1262.7 (23%), calculated 1262.7 (9.5%); for $^{12}C_{54}{}^{13}C_3H_{84}{}^{11}B_{10}N_9O_{16}$ found 1263.7 (7%), calculated 1263.7 (2.2%). $^1H$ NMR (DMSO-$d_6$) $\delta_H$ ppm (*J,* Hz): –2.00 (1H, s, I-NH); –1.74 (1H, s, III-NH); 1.49–3.12 (9H, m, carborane BH); 1.63-1.75 (6H, m, 18(1)-$CH_3$, 8(2)-$CH_3$); 2.04–2.25 (8H, m, 17(1)-$CH_2$, 17(2)-$CH_2$, 17(4)-$CH_2$, 17(5)-$CH_2$); 3.12–3.27 (8H, m, 13(2)-$CH_2$, 13(3)-$CH_2$, α,β-6$^a$-$CH_2$, α,β-6$^b$-$CH_2$); 3.36 (6H, s, 7(1)-$CH_3$, 2(1)-$CH_3$); 3.55 (3H, s, 12(1)-$CH_3$); 3.72 (3H, s, 15(3)-$CH_3$); 3.58–3.91 (8H, m, α,β-1$^a$-OH, α,β-1$^b$-OH, α,β-2$^a$-OH, α,β-2$^b$-OH, α,β-3$^a$-OH, α,β-3$^b$-OH, α,β-4$^a$-OH, α,β-4$^b$-OH); 3.93–4.20 (2H, m, 8(1)-$CH_2$); 3.93–4.20 (4H, m) and 4.26–4.55 (4H, m) (α,β-2$^a$-H, α,β-2$^b$-H, α,β-3$^a$-H, α,β-3$^b$-H, α,β-4$^a$-H, α,β-4$^b$-H, α,β-5$^a$-H, α,β-5$^b$-H); 4.39 (1H, d, 17-H, $J$ = 9.0); 4.60 (1H, q, 18-H, $J$ = 6.9); 4.76–5.20 (6H, m, α,β-1$^a$-H, α,β-1$^b$-H, 1$^c$-CH, 2$^c$-CH, 13$^c$-CH, 14$^c$-CH); 5.32 (1H, d, $J$ = 19.8) and 5.49 (1H, d, $J$ = 20.4) (15(1)-$CH_2$); 6.21 (1H, d, 3(2)-H (*cis*), $J$ = 10.8); 6.48 (1H, d, 3(2)-H (*trans*), $J$ = 17.7); 7.97 (1H, m, 17(3)-NH); 8.34 (1H, dd, 3(1)-H, $J$ = 11.9, $J$ = 17.3); 8.65 (1H, m, 13(1)-NH); 9.12 (1H, s, 20-H); 9.73 (1H, m, 5$^c$-NH-carborane); 9.77 (1H, s, 5-H); 9.80 (1H, s, 10-H). $^{11}B\{^1H\}$ NMR (DMSO-$d_6$) $\delta_B$ ppm (*J,* Hz): –5.4 (3B, br.s); –11.0 (2B, br.s); –13.6 (5B, br.s).

*Compound* ***15***. Yield: 188 mg (84%) as a dark green crystalline powder. UV-Vis ($CHCl_3$-$C_2H_5OH$ 95% aqueous, 1:1) $\lambda_{max}$ nm ($I_{relative}$, %): 661 (30), 605 (3), 528 (3), 499 (10), 401 (100). IR (KBr) ν cm$^{-1}$: 3099 (C–H, carborane), 2577 (B–H, carborane), 1719 (C=O, ester), 1676 ("amide-I"), 1603 ("chlorin band"), 1541 ("amide-II"). MS (ESI) for $(MH)^+$ ($C_{58}H_{89}B_{10}N_8O_{15}$) *m/z* (relative intensity of the peak in the isotopic cluster, %): for $C_{58}H_{89}{}^{10}B_5{}^{11}B_5N_8O_{15}$ found 1242.4 (3%), calculated 1242.8 (6.2%); for $C_{58}H_{89}{}^{10}B_4{}^{11}B_6N_8O_{15}$ found 1243.18 (6%), calculated 1243.8 (22.3%); for $^{12}C_{57}{}^{13}CH_{89}{}^{10}B_4{}^{11}B_6N_8O_{15}$ found 1244.1 (20%), calculated 1244.8 (13.2%); for $C_{58}H_{89}{}^{10}B_3{}^1B_7N_8O_{15}$ found 1244.9 (44%), calculated 1244.7 (42.7%); for $C_{58}H_{89}{}^{10}B_2{}^{11}B_8N_8O_{15}$ found 1245.91 (88%), calculated 1245.8 (97.1%); for $C_{58}H_{89}{}^{10}B^{11}B_9N_8O_{15}$ found 1246.8 (100%), calculated 1246.7 (100.0%); for $C_{58}H_{89}{}^{11}B_{10}N_8O_{15}$ found 1247.7 (85%), calculated 1247,8 (80.5%); for $^{12}C_{57}{}^{13}CH_{89}{}^{11}B_{10}N_8O_{15}$ found 1248.7 (46%), calculated 1248.7 (34.9%); for $^{12}C_{56}{}^{13}C_2H_{89}{}^{11}B_{10}N_8O_{15}$ found 1249.7 (18 %), calculated 1249.7 (10.7%); for $^{12}C_{55}{}^{13}C_3H_{89}{}^{11}B_{10}N_8O_{15}$ found 1250.7 (6%), calculated 1250.7 (1.7%). $^1H$ NMR (DMSO-$d_6$) $\delta_H$ ppm (*J,* Hz): –2.00 (1H, s, I-NH); –1.74 (1H, s, III-NH); 1.09 (6H, d, 16$^c$-$CH_3$, 17$^c$-$CH_3$, $J$ = 6.0); 1.40–3.20 (10H, m, carborane); 1.69 (3H, d, 18(1)-$CH_3$, $J$ = 6.9); 1.70 (3H, t, 8(2)-$CH_3$, $J$ = 7.2); 1.87–2.34 (8H, m, 17(1)-$CH_2$, 17(2)-$CH_2$, 17(4)-$CH_2$, 17(5)-$CH_2$); 2.55–2.75 (2H, m, 13$^c$-$CH_2$); 3.00–3.21 (9H, m, 13(2)-$CH_2$, 13(3)-$CH_2$, α,β-6$^a$-$CH_2$, α,β-6$^b$-$CH_2$, 15$^c$-CH); 3.34 (3H, s, 7(1)-$CH_3$); 3.55 (6H, s, 2(1)-$CH_3$, 12(1)-$CH_3$); 3.72 (3H, s, 15(3)-$CH_3$); 3.59–3.92 (10H, m, 8(1)-$CH_2$, α,β-1$^a$-OH, α,β-1$^b$-OH, α,β-2$^a$-OH, α,β-2$^b$-OH, α,β-3$^a$-OH, α,β-3$^b$-OH, α,β-4$^a$-OH, α,β-4$^b$-OH); 3.91–4.20 (4H, m) and 4.28–4.52 (4H, m) (α,β-2$^a$-H, α,β-2$^b$-H, α,β-3$^a$-H, α,β-3$^b$-H, α,β-4$^a$-H, α,β-4$^b$-H, α,β-5$^a$-H, α,β-5$^b$-H); 4.40 (1H, d, 17-H, $J$ = 9.3); 4.60 (1H, q, 18-H, $J$ = 7.5); 5.01–5.18 (2H, m, α,β-1$^a$-H, α,β-1$^b$-H); 5.31 (1H, d, $J$ = 17.1) and 5.48 (1H, d, $J$ = 18.9) (15(1)-$CH_2$); 6.21 (1H, d, 3(2)-H (*cis*), $J$ = 11.7); 6.48 (1H, d, 3(2)-H (*trans*), $J$ = 18.0); 7.93 (1H, m, 17(3)-NH); 8.26–8.42 (2H, m, 13(1)-NH, 3(1)-H); 9.13 (1H, s, 20-H); 9.77 (1H, s, 5-H); 9.80 (1H, s, 10-H). $^{11}B\{^1H\}$ NMR (DMSO-$d_6$) $\delta_B$ ppm (*J,* Hz): –4.8 (2B, br.s); –10.8 (8B, br.s).

*Compound* ***16***. Yield: 193 mg (82%) as a dark green crystalline powder. UV-Vis ($CHCl_3$-$C_2H_5OH$ 95% aqueous, 1:1) $\lambda_{max}$ nm ($I_{relative}$, %): 662 (30), 607 (3), 528 (3), 500 (10), 402 (100). IR (KBr) ν cm$^{-1}$: 3099 (C–H, carborane), 2583 (B–H, carborane), 1719 (C=O, ester), 1678 ("amide-I"), 1601 ("chlorin band"), 1543 ("amide-II"). MS (ESI) for $(M+2H)^+$ ($C_{63}H_{92}B_{10}N_8O_{15}$) *m/z* (relative intensity of the peak in the isotopic cluster, %): for $C_{63}H_{92}{}^{10}B_6{}^{11}B_4N_8O_{15}$ found 1304.5 (2%), calculated 1304.8 (1.1%); for $C_{63}H_{92}{}^{10}B_5{}^{11}B_5N_8O_{15}$ found 1305.4 (6%), calculated 1305.8 (5.4%); for $C_{63}H_{92}{}^{10}B_4{}^{11}B_6N_8O_{15}$ found 1307.0 (31%), calculated 1306.8 (19.3%); for $C_{63}H_{92}{}^{10}B_3{}^{11}B_7N_8O_{15}$ found 1307.9 (65%), calculated 1307.8 (48.8%); for $C_{63}H_{92}{}^{10}B_2{}^{11}B_8N_8O_{15}$ 1308.8 (96%), calculated 1308.8 (85.5%), for $C_{63}H_{92}{}^{10}B^{11}B_9N_8O_{15}$ found 1309.7 (100%), calculated 1309.8 (100.0%); for $C_{63}H_{92}{}^{11}B_{10}N_8O_{15}$ found 1310.7 (75%), calculated 1310.8 (74.0%); for $^{12}C_{62}{}^{13}CH_{92}{}^{11}B_{10}N_8O_{15}$ found 1311.7 (41%), calculated 1311.8 (33.6%); for $^{12}C_{61}{}^{13}C_2H_{92}{}^{11}B_{10}N_8O_{15}$ found 1312.7 (15%), calculated 1312.8 (10.8%); for $^{12}C_{60}{}^{13}C_3H_{92}{}^{11}B_{10}N_8O_{15}$ found 1313.7 (5%), calculated 1313.8 (2.6%). $^1H$ NMR (DMSO-$d_6$) $\delta_H$ ppm (*J,* Hz): –2.02 (1H, s, I-NH); –1.75 (1H, s, III-NH); 1.15-3.30 (10H, m, carborane); 1.62–1.74 (6H, m, 18(1)-$CH_3$, 8(2)-$CH_3$); 2.18 (3H, s, 22$^c$-$CH_3$); 1.82–2.15 (8H, m, 17(1)-$CH_2$, 17(2)-$CH_2$, 17(4)-$CH_2$, 17(5)-$CH_2$); 2.64–2.96 (10H, m, 13(2)-CH2, 13(3)-$CH_2$, α,β-6$^a$-$CH_2$, α,β-6$^b$-$CH_2$, 20$^c$-$CH_2$); 3.33 (3H, s, 7(1)-$CH_3$); 3.55 (6H, s, 2(1)-$CH_3$, 12(1)-$CH_3$); 3.68 (3H, s,15(3)-$CH_3$); 3.36-3.73 (8H, m, α,β-1$^a$-OH, α,β-1$^b$-OH, α,β-2$^a$-OH, α,β-2$^b$-OH, α,β-3$^a$-OH, α,β-3$^b$-OH, α,β-4$^a$-OH, α,β-4$^b$-OH); 3.72–4.19 (9H, m) and 4.39–4.53 (2H, m) (8(1)-$CH_2$, 13$^c$-CH, α,β-2$^a$-H, α,β-2$^b$-H, α,β-3$^a$-H, α,β-3$^b$-H, α,β-4$^a$-H, α,β-4$^b$-H, α,β-5$^a$-H, α,β-5$^b$-H); 4.34 (1H, d, 17-H, $J$ = 10.2); 4.59 (1H, q, 18-H, $J$ = 7.1); 4.89–5.09 (2H, m, α,β-1$^a$-H, α,β-1$^b$-H); 5.28 (1H, d, $J$ = 18.6) and 5.46 (1H, d, $J$ = 19.2) (15(1)-$CH_2$); 6.21 (1H, d, 3(2)-H (*cis*), $J$ = 11.7); 6.47 (1H, d, 3(2)-H (*trans*), $J$ = 17.7); 7.16-7.41 (10H, m, 15$^c$-CH, 16$^c$-CH, 17$^c$-CH, 18$^c$-CH, 19$^c$-CH); 7.76 (1H, m, 17(3)-NH); 8.00 (1H, m, 13(1)-NH); 8.33 (1H, dd, 3(1)-H, $J$ = 17.9, $J$ = 11.3); 9.13 (1H, s, 20-H); 9.76 (1H, s, 5-H); 9.80 (1H, s, 10-H). $^{11}B\{^1H\}$ NMR (DMSO-$d_6$) $\delta_B$ ppm (*J,* Hz): –6.5 (2B, br.s); –10.5 (8B, br.s).

## *Cytotoxicity Study*

Determination of dark and photoinduced cytotoxicity of the synthesized compounds was carried out according to a previously described method[38] using human cell lines of cervical adenocarcinoma (HeLa) (BioloT, Russia), lung adenocarcinoma (A549) (BioloT, Russia) and colon adenocarcinoma (HT-29) (BioloT, Russia).

*Dark cytotoxicity assessment*. An appropriate amount of the compound was dissolved in DMSO (Amresco, USA) to obtain a stock solution with a concentration of 20 mmol/L. Working solutions were prepared by serial dilution of the stock solution in DMSO. Then, 1 μL of the solution at the appropriate concentration and 199 μL of nutrient medium containing 5,000 cells for HeLa or A549 lines, and 20,000 cells for HT-29 were added to each well of a sterile 96-well culture plate. The final concentrations of the compound were 0.1, 1, and 10 μmol/L, with a DMSO concentration of 0.5% (v/v). DMSO at 0.5% (v/v) was added to the control group. The cells were incubated with the compound for 72 h at 37 °C, 100% humidity, in an atmosphere of 5% $CO_2$. Cell viability was assessed using the fluorometric microculture cytotoxicity assay (FMCA)[39]. After removing the nutrient medium, the cells were washed with PBS (PanEco, Russia), and then 100 μL of fluorescein diacetate solution (Sigma, USA) were added. The cells were incubated at 37 °C and 5% $CO_2$ for 40 minutes. Fluorescence intensity was measured using a CLARIOstar microplate reader (BMG LABTECH, Germany) at wavelengths of 485 nm (excitation) and 520 nm (emission). Cell viability was calculated as the ratio of fluorescence intensity of treated cells to that of the corresponding control, expressed as a percentage. Experiments were performed in six biological replicates. Samples were assessed for artifacts using Grubbs' test. Statistical significance of differences was determined using Student's t-test in Microsoft Excel (2010). Results are presented as mean survival values ± standard deviation.

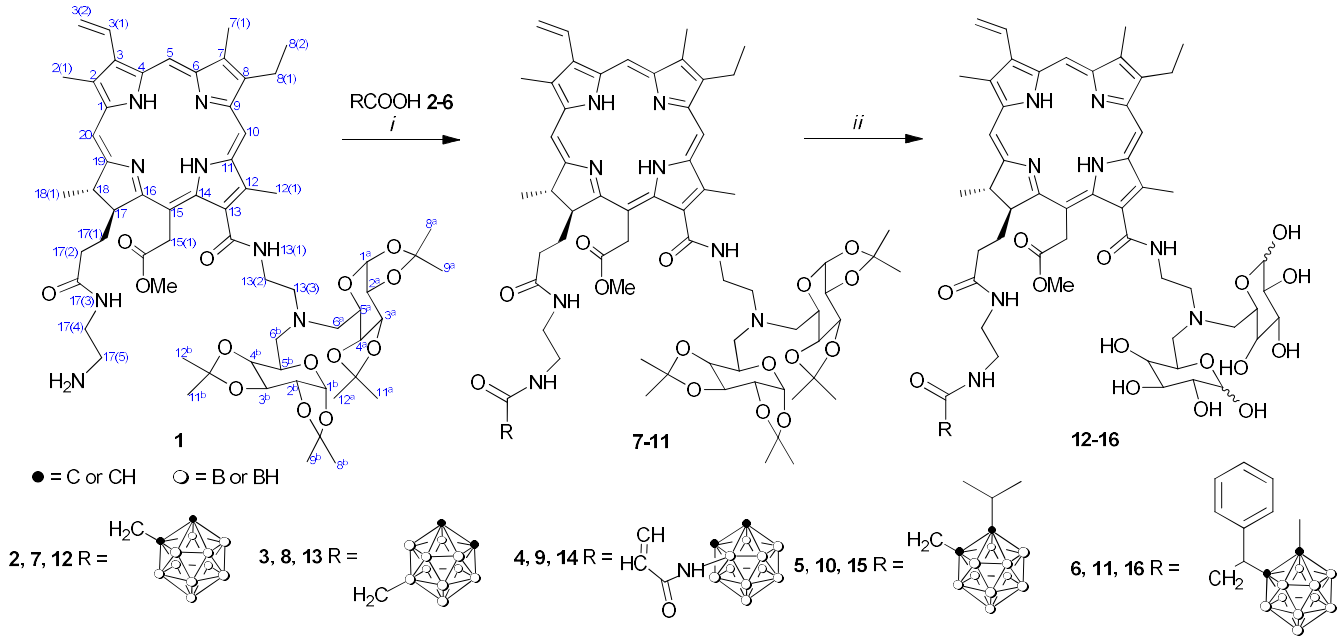
*Photoinduced cytotoxicity* was assessed using the fluorometric microculture cytotoxicity assay (FMCA), similar to the dark cytotoxicity assay. Two hours after compound addition, cells were irradiated with 660 nm light (LED source, light exposure 12 J/cm$^2$) for 20 min. The cells were then incubated for 70 hours in the dark at 37 °C, 100% humidity, in an atmosphere containing 5% $CO_2$.

## Results and Discussion

### *Synthesis*

The synthesis of carborane conjugates of chlorin $e_6$ macrocycle containing hydrophilic galactose substituents aiming to create boronated agents for use in PDT and BNCT was carried out using 13-diisopropylidene galactose-substituted aminochlorin **1**.[19] The conjugation of carborane polyhedra with chlorin $e_6$ macrocycle **1** was proceeded via the formation of an amide bond during the reactions of carbon and boron-substituted *o*-carborane carboxylic acids **2**–**6**[34-37] with amino group in position 17 of chlorin $e_6$ (Scheme 1). The reactions were carried out in the presence of Mukaiyama (2-chloro-1-methylpyridinium iodide, CMPI) reagent in $CH_2Cl_2$-DMAP system to afford *closo*-boronated conjugates **7**-**11** in 36–50% yield. The treatment of conjugates **7**-**11** with 90% TFA resulted in the formation of the deprotected conjugates **12**-**16** containing *o*-carborane and hydrophilic digalactose fragments in their structure (Scheme 1) in 80–84% yield.

All the newly synthesized compounds were isolated by column chromatography and their structures were confirmed by UV-vis, IR and $^1$H, $^{11}$B{$^1$H} NMR spectroscopy and ESI mass spectrometry. The IR spectra of carborane chlorin $e_6$ conjugates **7**–**16** exhibited intense bands at 2577–2598 cm$^{-1}$ and bands at 3059–3099 cm$^{-1}$ corresponding to the stretching vibrations of the carborane BH and CH groups in the *closo*-carborane polyhedra, respectively. The absorption band at 1736 cm$^{-1}$ (for compounds **7**–**11**) and 1719 cm$^{-1}$ (for compounds **12**–**16**) corresponded to the stretching vibrations of C=O ester bond. In the IR spectra of compounds **7**-**16** the characteristic intense absorption




**Scheme 1**. Synthesis of galactose-substituted chlorin $e_6$ conjugates with carborane polyhedra **7**-**16**. (i): RCOOH, CMPI, DMAP, $CH_2Cl_2$, reflux, 2 h; (ii): TFA 90 % aqueous, ~23°C, 4 h.

bands at 1603–1679 $cm^{-1}$ and 1537–1543 $cm^{-1}$ were observed assigned to the stretching vibration of amide I and amide II groups, respectively. In the $^1$H NMR spectra the protons of the chlorin macrocycle in compounds **7**–**11** exhibited the signals in the downfield region at δ 10.0 – 8.5 ppm (*meso*-protons) and in the high-field region at δ –1.46 – (–2.02) ppm (protons of inner NH-groups). Proton signals corresponding to the galactose fragments and isopropylidene protecting group of galactose fragments are also observed supporting the presence of galactose substituents in the molecules, as well as the stability of the isopropylidene protection groups under the reaction conditions. In addition, in the $^1$H NMR spectra of compounds **7**–**9**, carborane CH protons were detected in the range of δ 4.05–4.53 ppm. The formation of an amide bond during the synthesis of compounds **7**–**11** was confirmed by the presence of a broadened multiplet signal of the NH-amide proton at position 17(3) at δ=6.70 ppm for compound **7**, at 6.17 ppm for compound **8**, at 7.42 ppm for compound **9**, at 6.68 ppm for compound **10**, and at 5.57 ppm for compound **11**. The peaks corresponding to protonated $[MH]^+$ or hydrated $[M+2H]^+$ with typical for compounds containing boron atoms isotopic composition were observed in mass spectra of all the prepared derivatives **7**-**16**.

## *Cellular investigations*

It was previously shown[16-18] that derivatives of chlorin $e_6$ with galactose fragments on the periphery of the macrocycle have water solubility (0.1–10 mg/mL), which depends on the presence, structure and location of hydrophobic substituents on the periphery of the macrocycle. In aqueous solutions, galactose derivatives of chlorin $e_6$ were present as uniformly distributed single molecules or small luminescent aggregates, which was confirmed by coloration and the appearance of red luminescence when illuminated with violet light (λ = 405 nm), *i.e.,* a true solution was formed. *Closo*-carboranes are known to serve as rigid lipophilic moieties commonly used to create amphiphilic systems. Modification of carboranes with polar or nonpolar substituents makes them versatile building blocks for the production of a variety of effective biological systems. However, the presence of two hydrophilic galactose entities in the structure of the synthesized amphiphilic chlorins containing carborane clusters (compounds **12**-**16)** did not ensure their ability to form solutions in water.

The dark and photoinduced cytotoxic activity of novel derivatives of chlorin $e_6$ with hydrophilic galactose fragments and carborane polyhedra **12**-**16** was investigated. A fluorimetric microculture cytotoxic assay (FMCA) was used to estimate the viability of HeLa, A549 and HT-29 cells. FMCA was based on the measurement of the fluorescence intensity of fluorescein, which is a product of the hydrolysis of the non-fluorescent fluorescein diacetate by intracellular esterases [39]. The data on survival of HeLa, A549 and HT-29 cells after dark and photoinduced exposure of the studied compounds **12**-**16** at three concentrations of 0.1, 1.0 and 10 μmol/L are presented in Table 1.

**Table 1**. Cell survival (A549, HeLa and HT-29 cells) after dark and photoinduced exposure of compounds **12**-**16** in DMEM/F12 nutrient medium supplemented with 10% fetal bovine serum (light source – LEDs, λ=660 nm, light exposure value 12 J/$cm^2$).

| Compounds | 0.1 μmol/L dark/photo | 1.0 μmol/L dark/photo | 10 μmol/L dark/photo |
|---|---|---|---|
| | A549 | | |
| **12** | 105.64±0.94 / 91.5±1.1 | 104.98±0.61 / 87.4±1.2 | 97.9±1.2 / 1.60±0.17* |
| **13** | 97.9±1.2 / 93.4±1.7 | 96.5±2.9 / 91.2±1.8 | 96.78±0.86 / 1.40±0.10* |
| **14** | 105.7±1.8 / 99.93±0.85 | 104.1±1.6 / 85.1±2.6 | 96.57±0.82 / 0.99±0.07* |
| **15** | 100.46±0.38 / 95.42±0.42 | 99.57±0.81 / 89.39±0.77 | 93.35±0.69 / 1.17±0.03* |
| **16** | 101.79±0.98 / 99.6±1.2 | 101.24±0.85 / 95.9±1.0 | 91.59±0.52 / 1.18±0.06* |
| | HeLa | | |
| **12** | 106.9±1.3 / 108.62±0.82 | 110.5±4.4 / 20.5±2.3* | 81.1±2.9 / 2.06±0.12* |
| **13** | 89.7±2.4 / 100.7±2.0 | 91.8±4.4 / 26.1±1.4* | 95.4±3.2 / 1.15±0.06* |
| **14** | 103.9±1.3 / 88.53±0.74 | 98.5±4.7 / 21.2±3.1* | 70.6±4.3 / 2.10±0.24* |
| **15** | 102.0±4.3 / 92.5±2.3 | 103.2±2.1 / 27.6±1.9* | 86.0±2.6 / 0.97±0.05* |
| **16** | 112.2±2.0 / 97.9±2.1 | 104.5±3.3 / 29.9±2.2* | 79.9±4.8 / 3.00±0.31* |
| | HT-29 | | |
| **12** | 102.58±0.87 / 85.7±1.4 | 101.5±2.1 / 87.2±4.0 | 88.9±3.3 / 3.44±0.42* |
| **13** | 93.1±1.8 / 103.9±2.7 | 95.2±1.6 / 91.2±2.2 | 103.1±1.2 / 0.84±0.03* |
| **14** | 107.4±1.1 / 102.8±2.5 | 105.8±1.6 / 73.4±2.8 | 92.6±1.5 / 2.25±0.10* |
| **15** | 103.16±0.47 / 103.2±3.0 | 104.88±0.57 / 92.6±2.2 | 94.87±0.38 / 3.42±0.38* |
| **16** | 111.2±1.1 / 95.4±1.1 | 108.7±1.3 / 90.2±2.2 | 92.7±1.3 / 2.98±0.19* |

Note: * - differences with the control (dark cytotoxicity) are statistically significant at $p < 0.05$ (Student's t-test).

When cells were treated with the compounds **12**-**16** at concentrations of 0.1, 1.0 and 10 μmol/L for 72 h without exposure to light, more than 70% of the cells survived, indicating their low intrinsic cytotoxicity. A minor but statistically significant decrease in cell survival is observed only when the concentration of the compound increases from 1.0 to 10 μmol/L. It should be noted that no statistically significant decrease in cell survival was observed for increasing of concentration from 1.0 to 10 μM for any cell line in a case of compound **13**. The viability of HeLa, A549 and HT-29 cells with assurance decreases after photo-induced exposure to compounds **12**–**16** at a concentration of 10 μmol/L, and in the case of HeLa cells, viability with certainty decreases starting from a concentration of 1.0 μmol/L. All the obtained carborane conjugates **12**–**16** showed comparable values of dark and photoinduced toxicity regardless of the functional environment of the *o*-carborane polyhedra associated with the chlorin $e_6$ macrocycle. These results demonstrated that all the compounds obtained have a stronger photoinduced effect on HeLa cells than A549 and HT-29 cells.

## Conclusions

Chlorins $e_6$ due to their efficient photophysical properties are considered as attractive photosensitizers for photodynamic therapy of cancer and other therapeutic applications. In the present work a series of carborane derivatives of chlorin $e_6$ modified with solubilizing galactose functionalities was prepared to achieve amphiphilic photosensitizers with improved targeting ability based on the Warburg effect. Carborane polyhedra were introduced into the chlorin macrocyle via the amide bond formation between carborane carboxylic acids with 17-amino group of the chlorin macrocycle. The dark and photoinduced cytotoxicity studies of prepared compounds were conducted across three different cancer cell lines (HeLa, A549 and HT-29 cells). It was shown that all compounds synthesized have low dark cytotoxicity and have a significant photoinduced effect towards HeLa, A549 and HT-29 cells. The low dark cytotoxicity and significant photoinduced cytotoxic effect demonstrated with carborane galactose derivatives of chlorin $e_6$ make it possible to consider synthesized conjugates as promising candidates for further investigation in the binary antitumor strategies such as PDT and BNCT.

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**Author contribution**. Marina V. Mal'shakova: Conceptualization, Investigation, Writing - Original Draft, Writing - Review and Editing; Victoria M. Alpatova: Investigation; Yana I. Pylina: Investigation, Writing - Original Draft, Writing - Review and Editing, Formal analysis; Valentina A. Ol'shevskaya: Conceptualization, Methodology, Investigation, Writing - Original Draft, Writing - Review and Editing, Visualization; Alexander V. Kuchin: Conceptualization, Writing - Review and Editing; Dmitry V. Belykh: Conceptualization, Methodology, Supervision, Investigation, Writing - Original Draft, Writing - Review and Editing, Visualization.

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