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Liposomes loaded with lipophilic derivative of *closo*-carborane as a potential boron delivery system for boron neutron capture therapy of tumors

Alphiya R. Tsygankova, Dmitry A. Gruzdev, Vladimir V. Kanygin, Tatiana Ya. Guselnikova, Angelina A. Telegina, Anna I. Kasatova, Aleksandr I. Kichigin, Galina L. Levit, Ludmila V. Mechetina, Rinat A. Mukhamadiyarov, Ivan A. Razumov, Olga I. Solovieva, Olga Yu. Volkova, Andrey A. Ponomarev, Victor P. Krasnov and Evgenii L. Zavjalov

Experimental

General

(1,2-Dicarba-*closo*-dodecaboran-1-yl)acetic acid was obtained according to known procedure.[S1] Other reagents are commercially available.

The solvents were purified according to traditional methods and used freshly distilled. Melting point were obtained on a Stuart SMP3 apparatus (Barloworld Scientific, UK). The ^{1}H, ^{11}B, and ^{13}C NMR spectra were recorded on a Bruker Avance 500 (500, 160, and 126 MHz, respectively) spectrometer (Bruker Corp., USA) in $CDCl_3$ at 25°C with TMS and hexafluorobenzene as internal references and $BF_3 \cdot Et_2O$ as an external reference. Elemental analysis was performed using a PerkinElmer 2400 IIautomatic CHNS-O analyzer (PerkinElmer Inc., USA). Flash-column chromatography was performed using Silica gel 40 (230–400 mesh) (Alfa Aesar, UK).

Liposomes **peg-lip-1** were prepared using an ultrasonic disintegrator type UD-20 (TechPAN, Poland), a LABOROTA 4000 rotary evaporator (Heidolph Instruments, Germany), a Lipex extruder (LipexBiomembranes, Canada) equipped by Costar polycarbonate filters with 100-nm pores (Corning Inc., USA). The sizes and zeta-potential of liposomes were determined using a Zetasizer Nano ZS instrument (Malvern Panalytical, UK). Statistical analysis and graphical data presentation were performed in GraphPad Prism 7 (GraphPad Software) using the Mann-Whitney and Kruskal-Wallis tests.

The content of boron in liposomes, cells, organs and animal tissues was determined using an iCap-6500 ICP-OES CID spectrometer (Thermo Scientific, UK).

Cell culture

Human glioblastoma/astrocytoma cells of the U-87MG line were obtained at the Institute of Cytology and Genetics of the Russian Academy of Sciences (Siberian Branch) (ICG SB RAS) and frozen in cryotubes in 1 mL of freezing medium consisting of Dulbecco's modified Eagle medium / nutrient mixture F-12 (Thermo Fisher Scientific Inc., USA) (50%), fetal bovine serum (40%) and dimethyl

[S1] L.I. Zakharkin, V.I. Stanko, V.A. Brattsev, Yu.A. Chapovskii, A.I. Klimova, O.Yu. Okhlobystin, A.A. Ponomarenko, *Dokl. Akad. Nauk SSSR* 1964, **155**, 1119.

sulfoxide (10%). Cell suspension was frozen in cryotube (1 mL) at −60 °C for 48 h and then kept at −140 °C until further thawing. All cell experiments were performed under sterile conditions.

Cell counting was performed using a LUNA automated cell counter (Logos Biosystems, South Korea). The volume of probes of cells suspension was 20 μL.

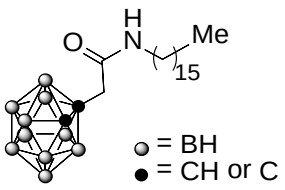
Biodistribution

The study was carried out in the Center for Shared Use "SPF-Vivarium" of the ICG SB RAS.

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The study was performed using immunodeficient SCID SPF mice (male, 8-10-week-old). Human glioblastoma cells (U87 line) were injected subcutaneously (100 thousand cells in 1 μL) followed by growing for 18-21 days. Liposomes **peg-lip-1** were injected intravenously (into the retroorbital sinus) at a concentration of 4 μg/g (relative to amide **1**). To assess the biodistribution, the animals were euthanized at equal time intervals (2, 4, 6 and 8 h after injection), the organs of interest (tumor, muscle, blood, brain, kidney, liver, and spleen) were taken off, then frozen and stored at –20°C prior to boron determination (2-4 animals per point were used).

Intact athymic male SCID mice at the age of 6 weeks were used in the experiment (20 individuals). Liposomes **peg-lip-1** at a concentration of 11.6 mg/mL (relative to amide **1**) were injected intravenously in a volume of 0.004 mL/g (into the retroorbital sinus). Analysis of the body weight of animals was performed 2 months after injection. Animals were euthanized 2.5 months after injection and histological changes of tissues were assessed.

***N*-(1,2-Dicarba-*closo*-dodecaboran-1-yl)acetyl-(1-hexadecyl)amine (1).** Ethyl chloroformate



(0.45 mL, 4.67 mmol) was added to a cold (−15 °C) solution of (1,2-dicarba-*closo*-dodecaboran-1-yl)acetic acid (0.95 g, 4.67 mmol) and *N*-methylmorpholine (0.51 mL, 4.67 mmol) in THF (29 mL). The mixture was stirred at −15 °C for 20 min then cetylamine (1.13 g, 4.67 mmol) and a solution of *N*,*N*-diethylaniline (1.39 g, 9.34 mmol) in THF (15 mL) were added. The reaction mixture was stirred at 20 °C for 20 h followed by evaporation *in vacuo*. The residue was taken up with EtOAc (50 mL) and the solution was successively washed with 1 N HCl (3×20 mL), saturated aqueous NaCl solution (3×30 mL), 5% aqueous $NaHCO_3$ solution (3×20 mL) and H_2O (2×30 mL). The organic layer was dried over Na_2SO_4 then evaporated. The residue was purified by flash column chromatography (eluent $CHCl_3$–MeOH 9 : 1). Yield 1.17 g (59%). Yellowish powder, mp 73.4-74.0 °C (hexane). ^{1}H NMR,δ: 0.88 (t, J = 6.9 Hz, 3 H, Me), 1.22-1.34 (m, 26 H, 13×CH_2), 1.46-1.53 (m, 2H, CH_2), 3.03 (s, 2H, CH_2CO), 3.22 (dt, J = 7.0, 6.6 Hz, 2H, CH_2), 4.60 (s, 1H, CH-carborane), 5.59 (br. s, 1H, NH) ppm. ^{11}B NMR,δ: −12.72 (2B), −11.84, −10.75 (2B), −9.59 (3B), −5.22, −2.20 ppm. ^{13}C NMR,δ: 14.11, 29.16, 29.24, 29.35, 29.49, 29.53, 29.61, 29.65 (2C), 29.68 (5C), 31.91, 39.98, 43.54, 58.56, 69.03, 166.02 ppm. Found (%): C 56.33; H 11.32; N 3.44. Calc. for $C_{20}H_{47}B_{10}NO$ (%): C 56.43; H 11.13; N 3.29.

Liposomes preparation

1,2-Distearoyl-*sn*-glycero-3-phosphocholine (DSPC) (1.00 g), 1,2-distearoyl-*sn*-3-phosphoethanolamine-*N*-[methoxy (polyethyleneglycol)-2000] (PEG2000-DSPE) (0.40 g), cholesterol

(0.25 g), and amide **1** (0.20 g) was dissolved in Et_2O–$CHCl_3$ 1 : 1 (v/v) mixture (21 mL). The solution was sonicated for 1 min, then organic solvents were removed by rotary evaporator (30 °C, 1 h). The resulting gel was diluted to a volume of 12 mL with 0.9% aqueous NaCl solution followed by sonification in an ice bath (5 min). Then 10-fold extrusion through polycarbonate filters with 100-nm pores at 60°C was carried out.

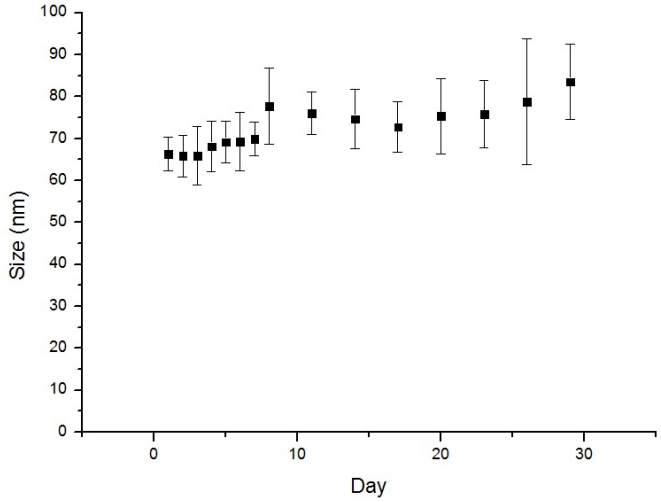


Figure S1 Stability of liposomes **peg-lip-1** over time

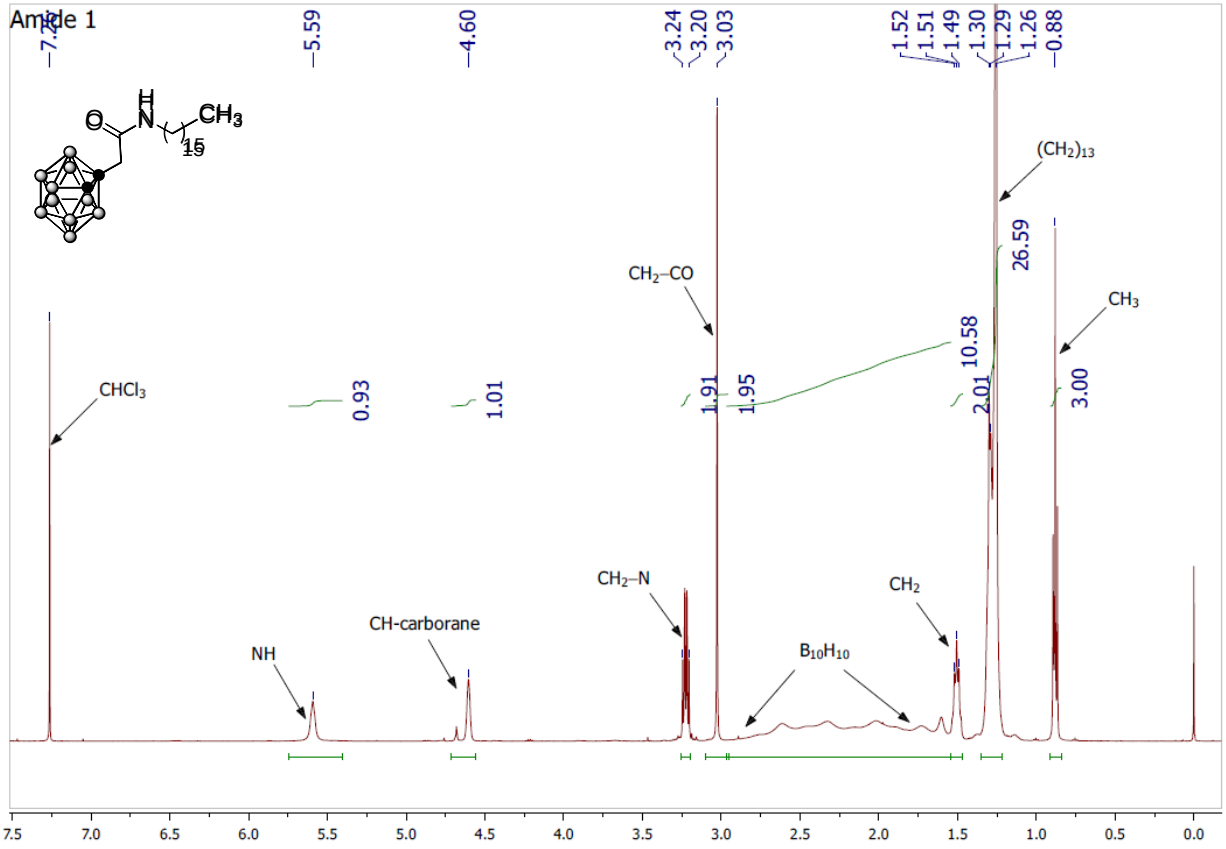


Figure S2 ^{1}H NMR spectrum of compound **1**

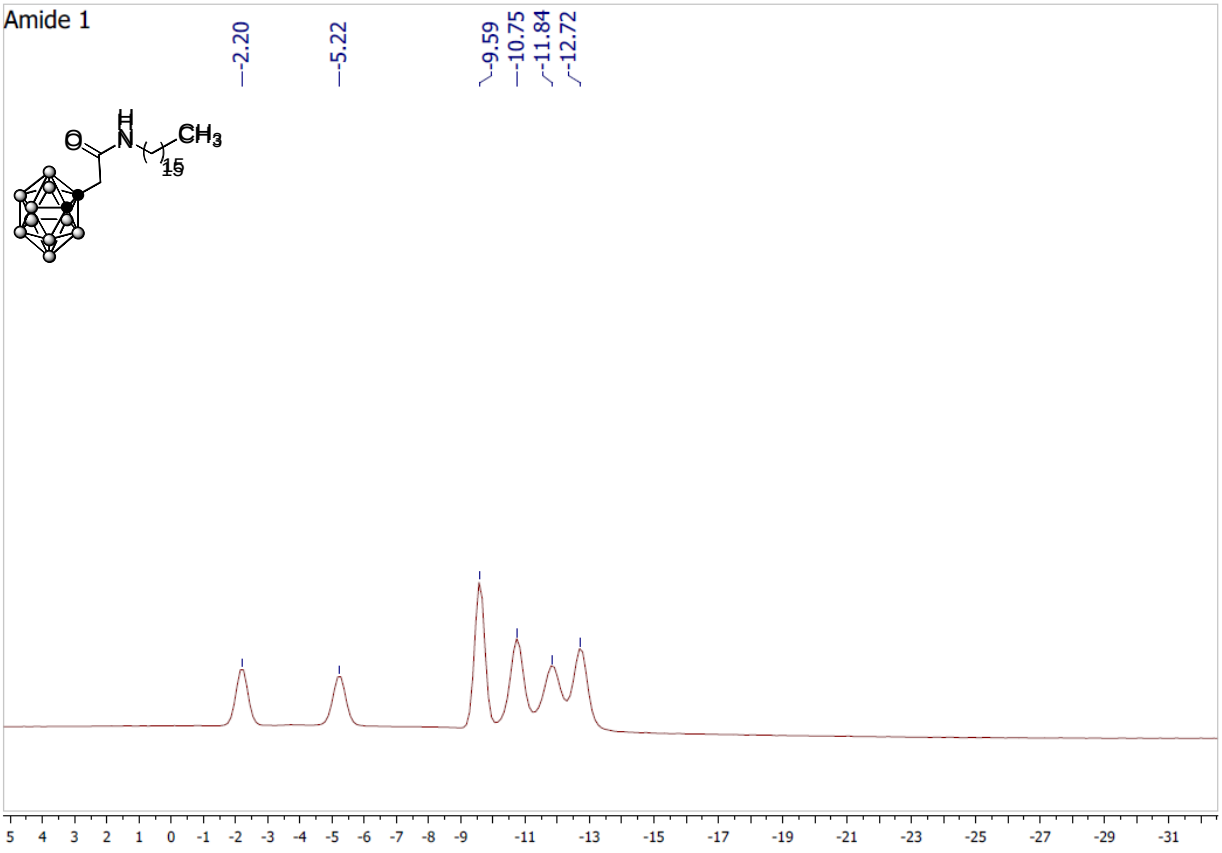


Figure S3 ^{11}B NMR spectrum of compound **1**

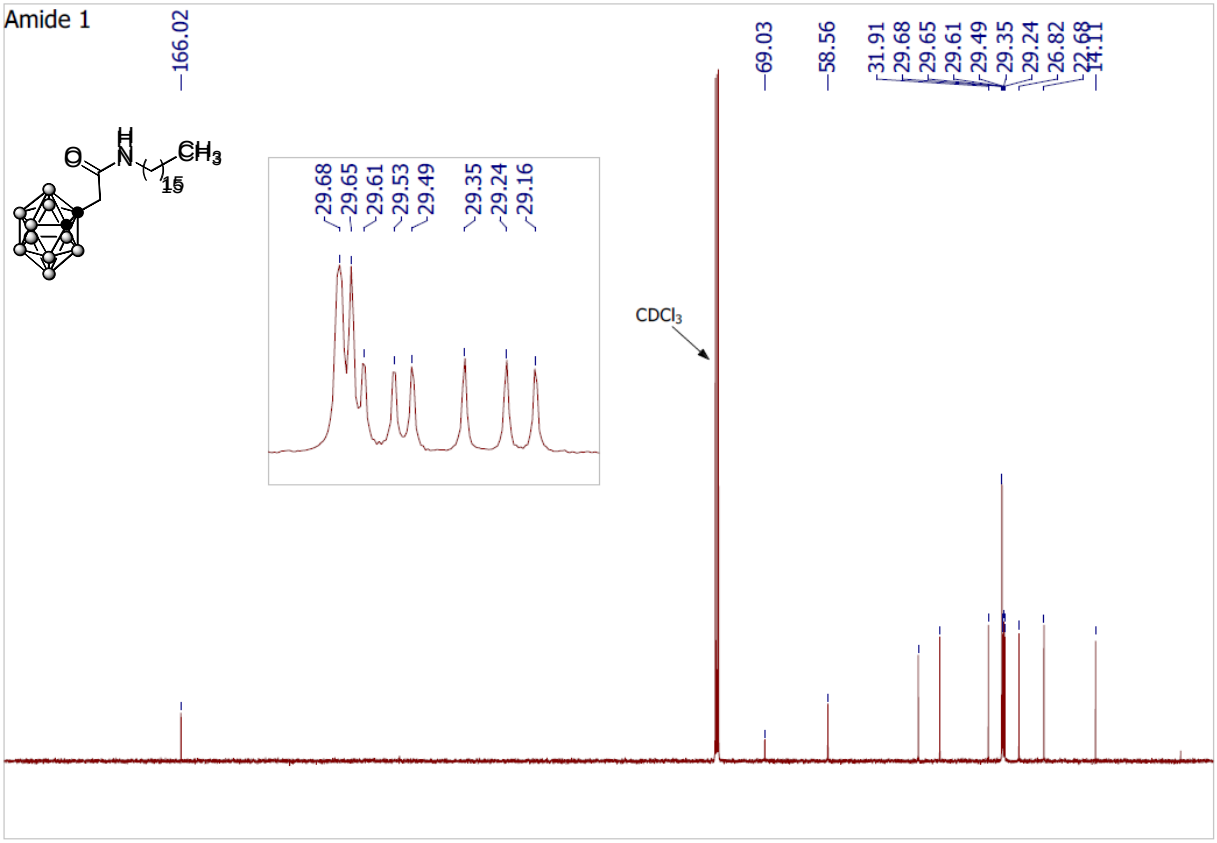


Figure S4 ^{13}C NMR spectrum of compound **1**