

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

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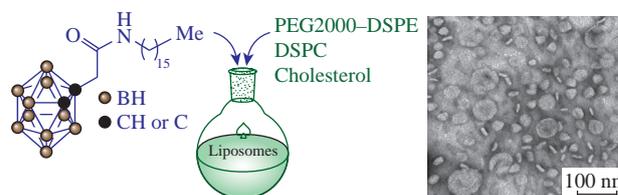
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Liposomes encapsulated with lipophilic derivative of 1,2-dicarba-*closo*-dodecaborane have been obtained and tested for toxicity to glioblastoma U87 cells and biodistribution on a U87MG xenograft mouse model. The liposomes are able to penetrate the tumor and provide boron concentration up to 1.5 $\mu\text{g g}^{-1}$ with tumor-to-muscle ratio up to 2.4.



Keywords: amide, *closo*-carborane, liposomes, U87 tumor model, toxicity, biodistribution, BNCT.

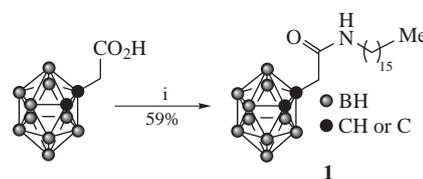
Boron neutron capture therapy (BNCT) represents a high-tech approach to cancer treatment based on two consecutive steps, namely (i) introduction of ^{10}B -containing compounds into tumor cells and (ii) irradiation with epithermal neutrons. The α -particles and ^7Li nuclei emitted as a result of the neutron capture are capable of local damaging the malignant cells.¹ Currently BNCT is used to treat melanomas, head and neck cancer as well as brain tumors. A limiting factor in its use is the lack of selective and low toxic boron delivery agents.^{2–4}

Employment of boron compounds in a liposomal form represents a way to reduce their toxicity and increase the selectivity. Liposomes are able to circulate in the bloodstream for a long time and capable of passive targeting.^{5–7} Thus, the possibility to introduce potential BNCT agents into the liposomes,^{8–15} micelles and other nanoscale aggregates^{16–23} is under active investigation. Boron-containing derivatives of cholesterol,^{24–26} glycerol ethers^{27,28} and lactose²⁹ have been synthesized. To improve the selectivity of delivery for the boron-containing liposomes, a functionalization with polyethylene glycol^{30,31} and tumor-targeting molecules^{32,33} is used. The reported drawbacks include accumulation of the boron-containing liposomes in liver³⁴ as well as their toxic effect³⁵ and induction of hemorrhage.³⁶ Stable liposomes containing sodium mercaptoundecahydro-*closo*-dodecaborate (sodium borocaptate,

BSH) within an aqueous core have been described in our article.³⁷

In this work, we obtained liposomes having a *closo*-carborane-derived amide within a lipid shell and assessed their applicability for the BNCT. Carborane-containing amide **1** was synthesized by coupling (1,2-dicarba-*closo*-dodecaboran-1-yl)acetic acid with cetylamine (Scheme 1). The molecule of amide **1** contains a lipophilic residue of the *closo*-carborane and a linear aliphatic moiety. So, it has high lipophilicity and good miscibility with the shell components of a liposome.

PEGylated liposomes **peg-lip-1** containing 10 wt% of amide **1** were prepared by the reverse-phase evaporation technique. The liposomes demonstrated high stability in an aqueous medium for at least 10 days (Figure S1, see Online Supplementary Materials).



Scheme 1 Reagents and conditions: i, $\text{Me}(\text{CH}_2)_{15}\text{NH}_2$, EtOCOCl , *N*-methylmorpholine, PhNEt_2 , THF, -15°C to room temperature.

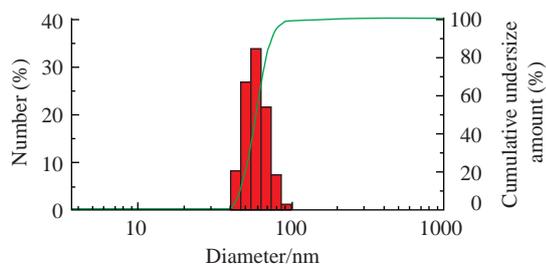


Figure 1 DLS data for carborane-containing liposomes **peg-lip-1**.

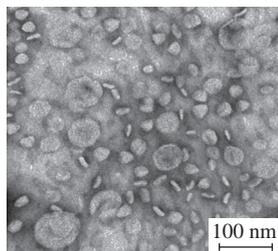


Figure 2 TEM image of carborane-containing liposomes **peg-lip-1** with 0.5% uranyl acetate as a contrast.

The size and morphology of liposomes **peg-lip-1** were determined using dynamic light scattering (DLS) and transmission electron microscopy (TEM) (Figures 1 and 2). The TEM image reveals clusters of numerous polymorphic particles of different sizes, including lipid particles with a clear border and rarely occurred separately lying particles, multilamellar vesicles being absent. The majority of the stable lipid particles have a round or concave shape and demonstrate a narrow size distribution. The concave particles can account for the conglutination of inner walls of the liposomes in the presence of 0.5% uranyl acetate. An average size and ζ -potential of the liposomes were 73 ± 3 nm and -39.6 ± 0.9 mV, respectively, the concentration of boron was 2.3 ± 0.2 mg ml⁻¹ as determined using the ICP-OES analysis according to our procedure,³⁸ while the concentration of amide **1** was 11.6 ± 0.8 mg ml⁻¹.

It was found that liposomes **peg-lip-1** were non-toxic to human glioblastoma U87MG cells at concentrations of amide **1** up to 160 μ g ml⁻¹. Incubation of the U87MG cells with the liposomes at the amide **1** concentration equal to 100 μ g ml⁻¹ for 4 h resulted in significant boron accumulation, namely 0.08 μ g of boron per 10⁶ cells. Biodistribution of the liposomes was investigated on a model of severe combined immunodeficiency (SCID) mouse bearing a heterotopic U87MG xenograft after single-dose intravenous injection. Toxic effect of liposomes **peg-lip-1** was determined at a dose of 0.004 ml g⁻¹ related to body weight with 11.6 mg amide **1** per 1 ml. The liposomes accumulated significantly in liver, spleen and kidneys (Figure 3). The boron concentration ratios for tumor-to-muscle and tumor-to-brain distributions were 2.4 and 0.3, respectively, using 2–4 animals per point.

Histological changes of tissues at the same dose of 0.004 ml g⁻¹ related to body weight were investigated using intact athymic male six-week SCID mice ($n = 20$). The measurement of body weight was carried out within two months after injection and revealed no significant weight loss. Euthanasia was performed 2.5 months after the start of the experiment. The administration of liposomes **peg-lip-1** caused degeneration of liver cells and necrosis [Figure 4(a)] as well as blood circulation disorder and changes in the structure of spleen [Figure 4(b)]. Minor cerebral edema with isolated sites of minimal lymphocytic infiltration was also noted [Figure 4(c)] along with edema and hemorrhage in kidneys [Figure 4(d)].

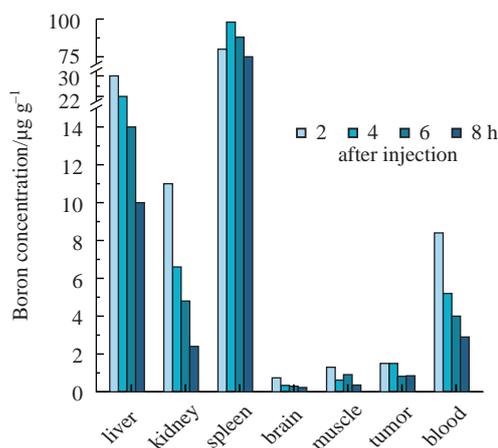


Figure 3 Biodistribution of carborane-containing liposomes **peg-lip-1** in mice ($n = 2-4$).

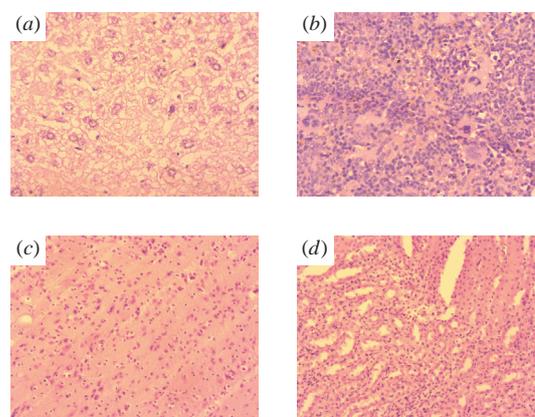


Figure 4 Histological sections for (a) liver, (b) spleen, (c) brain and (d) kidney of SCID mice two months after injection of carborane-containing liposomes **peg-lip-1** at a dose of 0.004 ml g⁻¹ related to body weight.

In general, high selectivity of boron accumulation and enhanced efficiency of BNCT has been achieved using long-term infusion of a drug.³⁹ Intraperitoneal or intratumoral injection of boron-containing compounds provides a high tumor-to-healthy tissue gradient.^{40–42} Therefore, an alternative route of administration for the liposomes, *e.g.*, intratumoral or intraarterial, could lead to an increase in the selectivity of boron accumulation.

In summary, a method has been developed for preparation of liposomes containing structurally simple *closo*-carborane derivative within a lipid shell. Initial biological assessment revealed that the new liposomes provided insufficient accumulation of boron in the tumor. The selectivity of biodistribution can be further increased using an alternative route of administration or an introduction of tumor-specific molecules into the structure of liposomes.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2021.09.022.

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