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Synthesis of Boron Cluster Lipids: *closo*-Dodecaborate as an Alternative Hydrophilic Function of Boronated Liposomes for Neutron Capture Therapy

Jong-Dae Lee, Manabu Ueno, Yusuke Miyajima, and Hiroyuki Nakamura*

Department of Chemistry, Faculty of Science, Gakushuin University, Mejiro, Toshima-ku, Tokyo 171-8588, Japan

hiroyuki.nakamura@gakushuin.ac.jp

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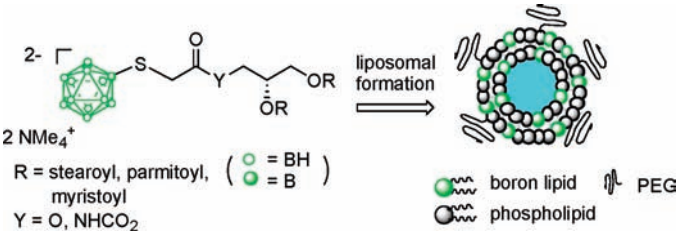
ABSTRACT



We succeeded in the synthesis of the double-tailed boron cluster lipids 4a–c and 5a–c, which have a $B_{12}H_{11}S$ moiety as a hydrophilic function, by S-alkylation of $B_{12}H_{11}SH$ (BSH) with bromoacetyl and chloroacetocarbamate derivatives of diacylglycerols for a liposomal boron delivery system on neutron capture therapy. Calcein encapsulation experiments revealed that the liposomes, prepared from the boron cluster lipid 4b, DMPC, PEG-DSPE, and cholesterol, are stable at 37 °C in FBS solution for 24 h.

Boron neutron capture therapy (BNCT) is a binary cancer treatment based on the nuclear reaction of two essentially nontoxic species, ^{10}B and thermal neutrons.[1] The neutron capture reaction by ^{10}B produces an α-particle and a lithium-7 ion bearing approximately 2.4 MeV, and these high linear energy transfer particles afford precise cell killing.[2−4] Therefore, high accumulation and selective delivery of boron into the tumor tissue are the most important requirements to achieve efficient neutron capture therapy of cancers. There are three most important parameters for development of boron compounds: (1) achieving tumor concentrations in the range of 20−35 μg ^{10}B/g; (2) a tumor/normal tissue differential greater than 3−5; (3) sufficiently low toxicity.[2,5] Recent promising approaches that meet these requirements entail the use of small boron molecules[2,6] and boron-conjugated biological complexes, such as monoclonal antibodies,[7] the epidermal growth factor,[8] and carborane oligo-

(1) Locher, G. L. *Am. J. Roentgenol.* **1936**, *36*, 1.
(2) Soloway, A. H.; Tjarks, W.; Barnum, B. A.; Rong, F-G.; Barth, R. F.; Codogni, I. M.; Wilson, J. G. *Chem. Rev.* **1998**, *98*, 1515.
(3) Barth, R. F.; Soloway, A. H.; Fairchild, R. G. *Cancer Res.* **1990**, *50*, 1061.
(4) Hawthorne, M. F. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 950.
(5) Barth, R. F.; Soloway, A. H.; Fairchild, R. G.; Brugger, R. M. *Cancer* **1992**, *70*, 2995.
(6) Our recent papers the for development of small boron molecules: (a) Nakamura, H.; Fukuda, H.; Girald, F.; Kobayashi, T.; Hiratsuka, J.; Akaizawa, T.; Nemoto, H.; Cai, J.; Yoshida, K.; Yamamoto, Y. *Chem. Pharm. Bull.* **2000**, *48*, 1034. (b) Takahashi, K.; Nakamura, H.; Furumoto, S.; Yamamoto, K.; Fukuda, H.; Matsumura, A.; Yamamoto, Y. *Bioorg. Med. Chem.* **2005**, *13*, 735. (c) Tietze, L. F.; Bothe, U.; Griesbach, U.; Nakaichi, M.; Hasegawa, T.; Nakamura, H.; Yamamoto, Y. *Chem. Bio. Chem.* **2001**, *2*, 326. (d) Lee, C.-H.; Oh, J. M.; Lee, J.-D.; Nakamura, H.; Ko, J.; Kang, S.-O. *Synlett* **2006**, 275. (e) Nakamura, H.; Kuroda, H.; Saito, H.; Suzuki, R.; Yamoti, T.; Maruyama, K.; Haga, T. *Chem. Med. Chem.* **2006**, *1*, 729.
(7) (a) Goldenberg, D. M.; Sharkey, R. M.; Primus, F. J.; Mizusawa, E. A.; Hawthorne, M. F. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 560. (b) Alam, F.; Soloway, A. H.; McGuire, J. E.; Barth, R. F.; Carey, W. E.; Adams, D. *J. Med. Chem.* **1985**, *28*, 522. (c) Pak, R. H.; Primus, F. J.; Rickard-Dickson, K. J.; Ng, L. L.; Kane, R. R.; Hawthorne, M. F. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 6986.
(8) (a) Gedda, L.; Olsson, P.; Carlsson, J. *Bioconjugate Chem.* **1996**, *7*, 584. (b) Yang, W.; Barth, R. F.; Adams, D. M.; Soloway, A. H. *Cancer Res.* **1997**, *57*, 4333.

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mers.[9] We have focused on boronated liposomes for the boron delivery system. A system involving the accumulation of boron in the liposomal bilayer is highly potent because drugs can be encapsulated into the vacant inner cell of a liposome. Furthermore, functionalization of liposomes is possible by combination of lipid contents. Therefore, boron and drugs may be simultaneously delivered to tumor tissues for BNCT and chemotherapy of cancers. Hawthorne and co-workers first introduced *nido*-carborane as a hydrophilic moiety into the amphiphile **1** (Figure 1) and examined

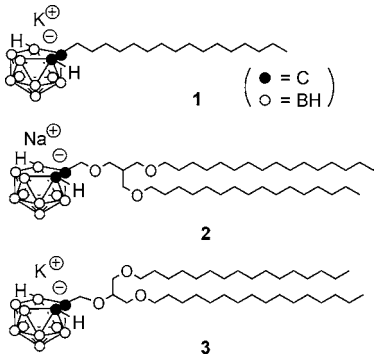
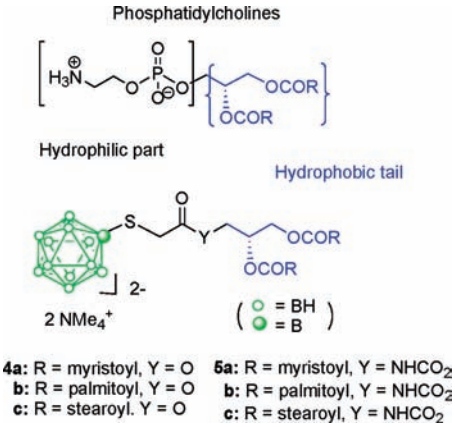


Figure 1. Structures of *nido*-carborane lipids.

liposomal boron delivery in mice using **1** and distearoylphosphatidylcholine (DSPC).[10] We recently developed the *nido*-carborane lipid **2**, which has a double-tailed moiety conjugated with *nido*-carborane as a hydrophilic function.[11] We investigated active targeting of the boronated liposomes to solid tumors by functionalization of transferrin on the surface of their liposomes and achieved a boron concentration of 22 μg of ^{10}B/g of tumor by the injection of the liposomes at 7.2 mg of ^{10}B/kg of body weight with longer survival rates of tumor-bearing mice after BNCT.[12] However, injection at higher boron concentrations resulted in mortality. Hawthorne and co-workers also recently reported synthesis of the *nido*-carborane lipid **3** and its unilamellar liposomes. They pointed out the high toxicity of the lipid **3** liposomes.[13]

To overcome this problem, we focused on mercaptoundecahydrododecaborate ($B_{12}H_{11}SH^{2-}$, BSH) as an alternative hydrophilic function of boron lipids. BSH is a water-soluble divalent anion cluster with significantly lowered toxicity and thus has been utilized for clinical treatment of BNCT. However, few synthetic examples of BSH derivatives as boron carriers have been reported so far due to the difficulty of their functionalizations.[14,15] Gabel and co-workers reported a novel "BSH activation" method, which enabled us to conduct S-alkylation of BSH under mild conditions.[16] In this paper, we report synthesis of *closo*-dodecaborate containing boron lipids **4a**−**c** and **5a**−**c** and their liposomal property (Scheme 1). Our design of the boron

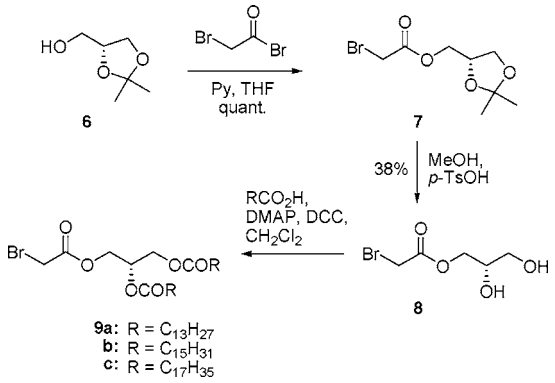
Scheme 1



lipids is based on biomimetic composition of phosphatidylcholines to meet a sufficiently low toxic requirement.

Synthesis of the hydrophobic tail functions of **4** is shown in Scheme 2. Reaction of the chiral alcohol **6** with 1.2 equiv

Scheme 2



of bromoacetyl bromide gave the ester **7**, quantitatively, and the deprotection of **7** was carried out using catalytic amounts of *p*-TsOH in MeOH to give the corresponding diol **8** in

(9) (a) Cai, J.; Soloway, A. H.; Barth, R. F.; Adams, D. M.; Hariharan, J. R.; Wyzlic, I. M.; Radcliffe, K. *J. Med. Chem.* **1997**, *40*, 3887. (b) Fulcrand-El Kattan, G.; Lesnikowski, Z. J.; Yao, S.; Tanious, F.; Wilson, W. D.; Schinazi, R. F. *J. Am. Chem. Soc.* **1994**, *116*, 7494. (c) Nakanishi, A.; Guan, L.; Kane, R. R.; Kasamatsu, H.; Hawthorne, M. F. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 238.

(10) (a) Feakes, D. A.; Shelly, K.; Hawthorne, M. F. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 1367. (b) Watson-Clark, R. A.; Banquerigo, M. L.; Shelly, K.; Hawthorn, M. F.; Brahn, E. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 2531.

(11) Nakamura, H.; Miyajima, Y.; Takei, T.; Kasaoka, T.; Maruyama, K. *Chem. Commun.* **2004**, 1910.

(12) Miyajima, Y.; Nakamura, H.; Kuwata, Y.; Lee, J.-D.; Masunaga, S.; Ono, K.; Maruyama, K. *Bioconjugate Chem.* **2006**, *17*, 1314.

(13) Li, T.; Hamdi, J.; Hawthorne, M. F. *Bioconjugate Chem.* **2006**, *17*, 15.

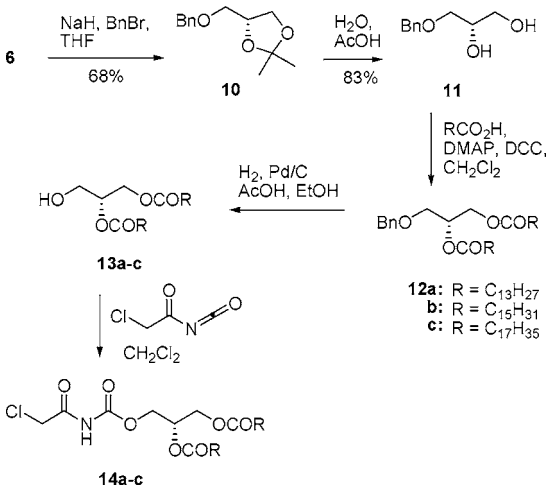
(14) (a) Holmberg, A.; Meurling, L. *Bioconjugate Chem.* **1993**, *4*, 570. (b) Swenson, D. H.; Laster, B. H.; Metzger, R. L. *J. Med. Chem.* **1996**, *39*, 1540. (c) Sano, T. *Bioconjugate Chem.* **1999**, *10*, 905. (d) Hoffmann, S.; Justus, E.; Ratajski, M.; Lork, E.; Gabel, D. *J. Organomet. Chem.* **2005**, *690*, 2757.

(15) Kultyshev, R. G.; Liu, J.; Liu, S.; Tjarks, W.; Soloway, A. H.; Shore, S. G. *J. Am. Chem. Soc.* **2002**, *124*, 2614.

38% yield. The ester formation of the diol **8** with various carboxylic acids was promoted by dicyclohexylcarbodiimide in the presence of catalytic amounts of *N*,*N*-dimethylaminopyridine in CH_2Cl_2 to afford the precursors **9a**−**c** in 61−75% yields.

Synthesis of the hydrophobic tail functions of **5** is shown in Scheme 3. We first examined the reaction of **6** with

Scheme 3

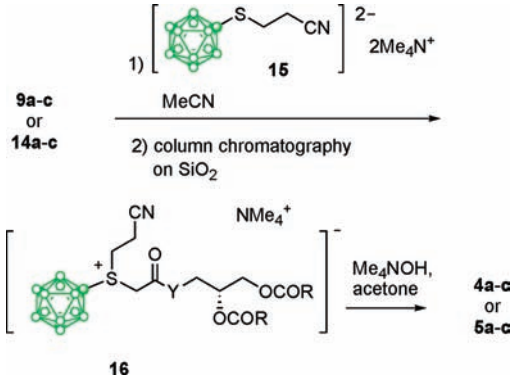


chloroacetyl isocyanate followed by deprotection of the acetal group; however, the chloroacetylcarbamate moiety decomposed under the acidic condition of *p*-TsOH in MeOH. Therefore, **6** was protected with benzylbromide using NaH and the resulting dioxolane **10** was converted into the diol **11** using aqueous AcOH in 83% yield. The ester formation of **11** with various carboxylic acids was carried out in a similar manner to give **12a**−**c**, quantitatively. Deprotection of the benzyl group of **12a**−**c** by hydrogenation gave the corresponding alcohols **13a**−**c** (89−>99% yields), which reacted with chloroacetyl isocyanate in CH_2Cl_2 to give **14a**−**c** in 74−98% yields.

Introduction of BSH into the hydrophobic tail functions **9** and **12** was examined using the "activated BSH (**15**)", which was prepared according to Gabel's protocol, as shown in Scheme 4. S-Alkylation of **15** with **9a**−**c** proceeded in acetonitrile at 70 °C for 12−24 h, giving the corresponding S-dialkylated products **16a**−**c**, which were immediately treated with tetramethylammonium hydroxide (1 equiv) in acetone to give **4a**−**c** in 76−91% yields, as tetramethylammonium salts. In a similar manner, **5a**−**c** were obtained from **12a**−**c** in 54−83% yields.

We examined formation and membrane stability of the liposomes using the boron cluster lipid **4b**. Liposomes were prepared from cholesterol, dimyristoylphosphatidylcholine (DMPC), polyethyleneglycol-conjugated distearoylphosphatidylethanolamine (PEG-DSPE), and the boron cluster lipid

Scheme 4



4b (1:1 − *X*:0.1:*X* = 0−1, molar ratio), by the reverse-phase evaporation (REV) method.[17] The liposomes obtained were subjected to extrusion 10 times through a polycarbonate membrane of 100 nm pore size, using an extruder device thermostated at 60 °C. Purification was accomplished by ultracentrifuging at 200 000*g* for 60 min at 4 °C, and the pellets obtained were resuspended in PBS buffer. Liposome size was measured with an electrophoretic light scattering spectrophotometer. The size distribution of the liposomes composed of **4b** is shown in Figure 2. The sizes of maximum

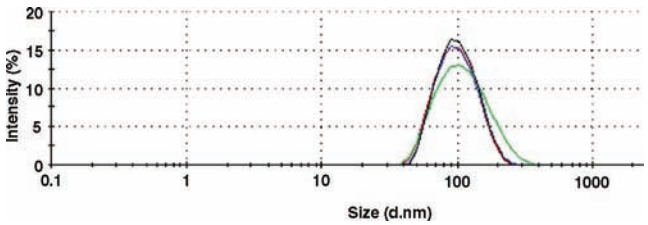


Figure 2. Size distributions of the boronated liposomes after extrusion. **4b** ratio: *X* = 0.25 (red), 0.5 (green), 0.75 (blue), and 1 (black).

distribution of the liposomes against **4b** contents were 103 (*X* = 0.25), 105 (*X* = 0.5), 102 (*X* = 0.75), and 108 nm (*X* = 1) with 0.121, 0.092, 0.106, and 0.089 as polydispersity index (PDI) values, respectively. The liposomes composed of **4a**, **4c**, and **5a**−**c** also gave similar size distributions (the data are not shown).

We next investigated the time-dependent stability of the boronated lipsomes of **4b** in fetal bovine serum (FBS). Fluorescent probes, such as calcein, are self-quenching at high concentrations, and the leakage of these fluorophores into the external medium results in the relief of self-quenching and an increase in the fluorescence. Therefore,

(16) (a) Gabel, D.; Moller, D.; Harfst, S.; Rosler, J.; Ketz, H. *Inorg. Chem.* **1993**, *32*, 2276. (b) Lechtenberg, B.; Gabel, D. *J. Organomet. Chem.* **2005**, *690*, 2780. (c) Azev, Y.; Lork, E.; Duelcks, T.; Gabel, D. *Tetrahedron Lett.* **2004**, *45*, 3249.

(17) Szoka, F.; Papahadjopoulos, D. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 4194.

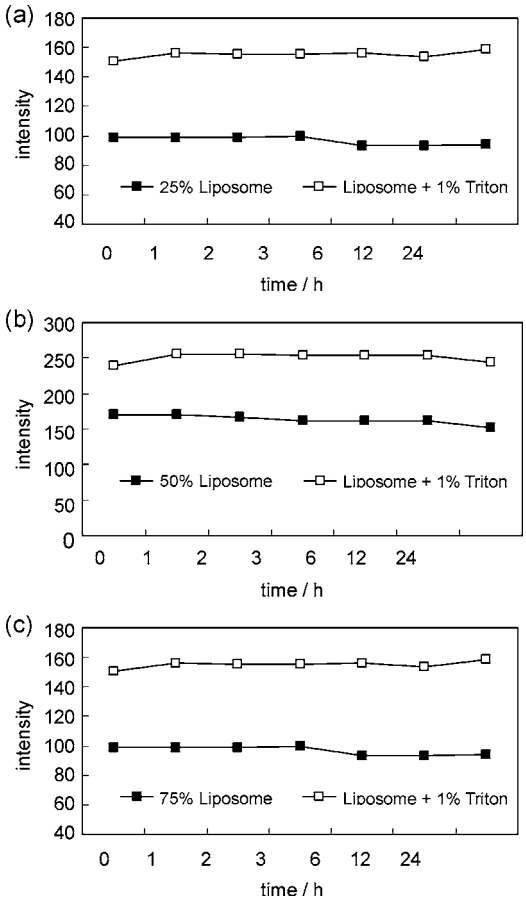


Figure 3. Time-dependent fluorescence intensities of calcein-encapsulated liposomes composed of **4b** with various ratios (X: (a) = 0.25, (b) = 0.5, (c) = 0.75) in FBS. Fluorescence intensity is plotted on the vertical axis, and incubation time is plotted on the horizontal axis. The black plots show the fluorescence intensity of the FBS solution containing liposomes, and the white plots show that of the solution after destruction of liposomes by the addition of Triton X-100.

the release of aqueous contents of liposomes can be monitored by an increase of fluorescent intensity.[18] We prepared the boronated liposomes at various concentrations of the boron cluster lipid **4b** using calcein (100 mM), and a liposome solution (the volume ratio of FBS/liposome solution = 9:1) was added to FBS and incubated at 37 °C with stirring. The fluorescence of the FBS solutions was measured at 0−24 h. The results of the liposomes of **4b** with various ratios (X = 0.25, 0.5, and 0.75) are shown in Figure 3. No increase in the fluorescence intensity of the FBS solutions was observed within 24 h; therefore, the boronated liposomes were stable in the FBS solution at 37 °C at least for 24 h. However, the increase of the fluorescence intensity was observed in the case of the liposome prepared from cholesterol, PEG-DSPE, and **4b** (X = 1).

Furthermore, to investigate the accumulation ratios of the boron cluster lipid **4b** and phospholipids including DMPC and PEG-DSPE on liposome formation, we determined boron and phosphorus concentrations of the liposome solutions by inductively coupled plasma atomic emission spectroscopy (ICP-AES). The concentration ratios of **4b** to the phospholipids on the liposomes prepared from cholesterol, DMPC, PEG-DSPE, and **4b** (1:1 − X:0.1:X, molar ratio) were 0.84 (X = 0.25) and 2.6 (X = 0.50). Therefore, it was observed that the boron lipid **4b** was incorporated into the liposome membranes at a higher concentration than phospholipids.

In conclusion, we succeeded in the synthesis of the double-tailed boron cluster lipids **4** and **5**, which have a $B_{12}H_{11}S$ moiety as a hydrophilic function, by S-alkylation of BSH with bromoacetyl and chloroacetocarbamate derivatives of diacylglycerols. The liposomes prepared from the boron cluster lipid **4b**, DMPC, PEG-DSPE, and cholesterol were stable in FBS. We also investigated toxicity of the boron liposomes and found that no mouse died after injection with the boron liposomes at a dose of 15 mg of boron per kilogram of weight for up to three weeks, although 50% of the mice injected with the boron liposomes prepared from the lipid **2** died at a dose of 14 mg of boron per kilogram of weight within 48 h (n = 4). In vivo biodistribution and BNCT studies of the boronated liposomes of **4** and **5** are in progress in our laboratory.

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Supporting Information Available: Detailed experimental procedures and characterization data for compounds **4**, **5**, and **7**−**14**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) Düzgüneş, N.; Bagatolli, L. A.; Meers, P.; Oh, Y.-K.; Straubinger, R. M. In *Liposomes*; Torchilin, V. P., Weissig, V., Eds.; Oxford: Oxford, 2002; pp 105−119.