COMMUNICATION

ChemComm
www.rsc.org/chemcomm

Synthesis and vesicle formation of a nido-carborane cluster lipid for boron neutron capture therapy†

Hiroyuki Nakamura,*[a] Yusuke Miyajima,[a] Toshiaki Takei,[b] Satoshi Kasaoka[c] and Kazuo Maruyama[c]

[a] *Department of Chemistry, Faculty of Science, Gakushuin University, Tokyo 171-8588, Japan. E-mail: hiroyuki.nakamura@gakushuin.ac.jp; Fax: 81 3 5992 1029; Tel: 81 3 3986 0221*

[b] *Institute for Molecular Biology, Gakushuin University, Tokyo 171-8588, Japan*

[c] *Department of Biopharmaceutics, School of Pharmaceutical Sciences, Teikyo University, Kanagawa 199-0195, Japan*

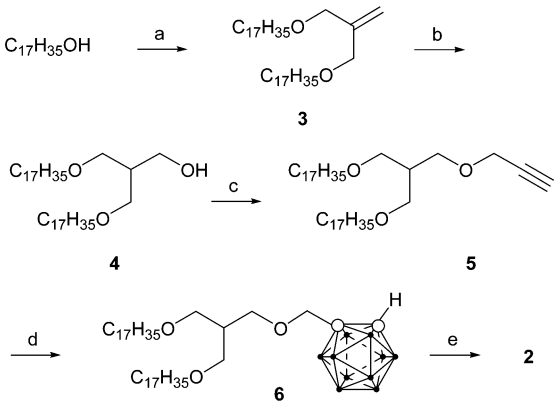
Received (in Cambridge, UK) 23rd April 2004, Accepted 21st June 2004
First published as an Advance Article on the web 29th July 2004

The nido-carborane lipid 2, which has a double-tailed moiety, was synthesized from heptadecanol in 5 steps. Analysis in a transmission electron microscope by negative staining with uranyl acetate showed that the lipid 2 formed a stable vesicle in which calcein was encapsulated. The lipid 2 was incorporated into distearoylphosphatidylcholine (DSPC) liposomes at a very high concentration.

High accumulation and selective delivery of boron into tumor tissues are the most important requirements to achieve efficient neutron capture therapy of cancers. Various boron carriers have been synthesized for these purposes.[1–4] A drug delivery system[5–9] has also been studied for the selective boron delivery.[10–12] In this system, boron compounds are encapsulated into the liposome and delivered into tumor tissues. As an alternative boron delivery system, the accumulation of boron into the liposome bilayer is highly potent because the density of molecules accumulated into the bilayer is much higher than that of those encapsulated in the inner cell of a liposome. Hawthorne and coworkers first introduced a nido-carborane into the amphiphile **1** as a hydrophilic part (Fig. 1), and examined liposomal boron delivery in mice using the amphiphile **1** and distearoylphosphatidylcholine (DSPC).[13,14] We designed the nido-carborane lipid **2**, which has a double-tailed moiety, for the purposes of high boron accumulation into liposomes and stable formation of boron-clusters containing liposomes. Here we report the synthesis and the vesicle formation of the nido-carborane lipid **2**. Furthermore, we found that **2** was also accumulated into the DSPC liposome and the DSPC–PEG liposome at a very high concentration.[15–19]

The synthetic route of the nido-carborane lipid **2** is shown in Scheme 1. The reaction of two equivalents of heptadecanol with 3-chloro-2-chloromethyl-1-propene using NaH as a base gave the diether **3** in 93% yield and the hydroboration of **3** gave the corresponding alcohol **4** in 71% yield. The alcohol **4** was converted into the propargyl ether **5** in 58% yield by treatment with propargyl bromide and the decaborane coupling of **5** was carried out in the presence of acetonitrile in toluene under reflux conditions to give the corresponding ortho-carborane **6** in 80% yield. Degradation of the carborane cage by treatment with sodium methoxide in methanol afforded the nido-carborane lipid **2** in 57% yield.

We next examined the vesicle formation of the nido-carborane lipid **2**. The nido-carborane lipid **2** (1.0 mg) was suspended in a calcein solution in water (0.5 mg/5 mL) and the suspension was heated until the solution became clear. After the solution was cooled to room temperature, the solution, again suspended, was passed through a Sephadex G-75 column chromatograph (25 mL) eluted with water. Each 2 mL of five fractions was separated and analyzed in a transmission electron microscope by negative staining with uranyl acetate. Fig. 2a and 2b show the transmission electron micrographs of the vesicle formation of **2** in the fractions 1 and 2, respectively. The size of the boron cluster vesicles in the fraction 1 was estimated at between 400 and 600 nm and that in the fraction 2 was estimated at between 150 and 200 nm.



Scheme 1 The synthesis of the boron lipid **2**. *Reagents*: a) 1. NaH, THF, 2. CH_2=C(CH_2Cl)$_2$, 93%; b) 1. BH_3·Me_2S, 2. H_2O_2, NaOH, 71%; c) 1. NaH, THF, 2. propargyl bromide, 58%; d) $B_{10}H_{14}$, CH_3CN, toluene, 80%; e) NaOMe, MeOH, 57%.

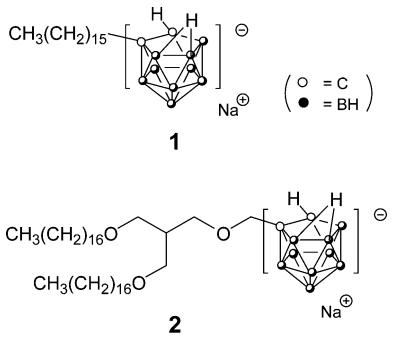


Fig. 1 Structures of the boron cluster lipids **1** and **2**.

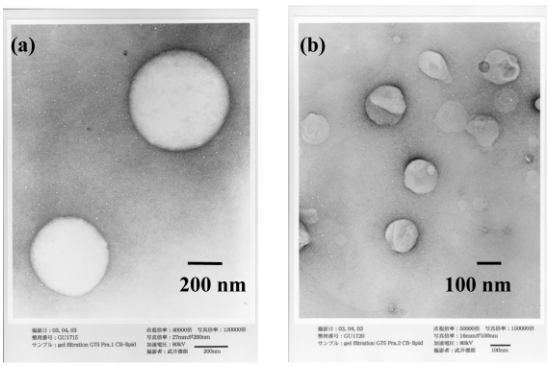


Fig. 2 Transmission electron micrographs of the vesicle formation from the nido-carborane lipid **2** in the fractions 1 (a) and 2 (b) after Sephadex G-75 column chromatography.

† Electronic supplementary information (ESI) available: experimental procedures and spectral data for the compounds **2–6**. See http://www.rsc.org/suppdata/cc/b4/b406141a/

DOI: 10.1039/b406141a

The stability of the boron cluster vesicles in a foetal bovine serum (FBS), which can be considered as a model of blood, was examined using the vesicle solution of the fraction 2. To an FBS was added a boron cluster vesicle fraction (the volume ratio FBS : vesicle solution = 9 : 1) and the mixture was incubated at 37 °C with stirring. The fluorescence of the FBS solution was measured at 0–18 h. The results are shown in Fig. 3. The fluorescence intensity is plotted on the ordinate and the incubation time is plotted on the horizontal axis. The black squares show the fluorescence intensity of the FBS solution containing the vesicle fraction and the black circles show that of the solution after destruction of the vesicles by addition of Triton X-100. No increase of the fluorescence intensity of the FBS solutions was observed during 18 h. Therefore, the calcein encapsulated into the boron cluster vesicle was not released. This result indicates that the boron cluster vesicle prepared from the nido-carborane lipid **2** is quite stable in the FBS solution at 37 °C.

We examined the effect of the accumulation ratio of DSPC and the lipid **2** on the liposome formation under various concentrations. Bare liposomes were prepared from DSPC, cholesterol (CH) and **2** (1 : 1 : *x*, *x* = 0–1). PEG liposomes were prepared from DSPC, CH, **2** and PEG-distearoylphosphatidylethanolamine (DSPE) (1 : 1 : *x*: 0.11, *x* = 0–1). The lipid concentration was estimated by phosphorus assay.[20] Boron content was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES). The results are shown in Fig. 4. The concentration ratio of **2** to DSPC in preparation of the liposomes is plotted on the horizontal axis and the ratio of DSPC and **2** in the liposomes obtained is plotted on the ordinate. Very interestingly, the ratio of DSPC and the nido-carborane lipid **2** of the liposomes increased in proportion to the increase of the mixing ratio of **2** to DSPC in the solution. Furthermore, it was observed that the nido-carborane lipid **2** was incorporated into the liposome membranes with five times higher concentration than DSPC.

In conclusion, we succeeded in the synthesis of the nido-carborane lipid **2**, which is a new class of bilayer membrane lipid, and investigated the stable vesicle formation of the lipid **2** in FBS solution and the high incorporation into distearoylphosphatidylcholine (DSPC) liposomes. We believe that the current nido-carborane lipid possesses a high potency not only for boron delivery systems

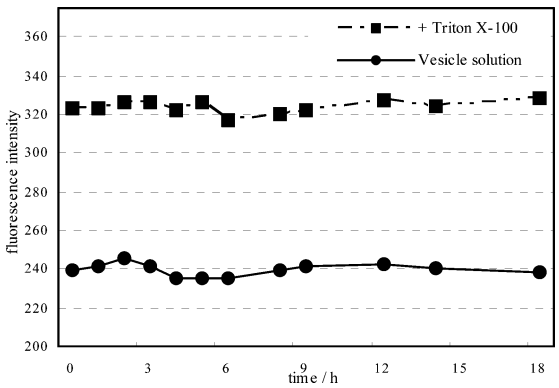


Fig. 3 The fluorescence of the vesicle of the fraction 2, in which calcein was encapsulated by the nido-carborane lipid **2**, in FBS. The square plot shows the fluorescence intensity of the FBS solution containing the vesicle fraction 2 and the circle plot shows that of the solution after destruction of vesicles by the addition of Triton X-100.

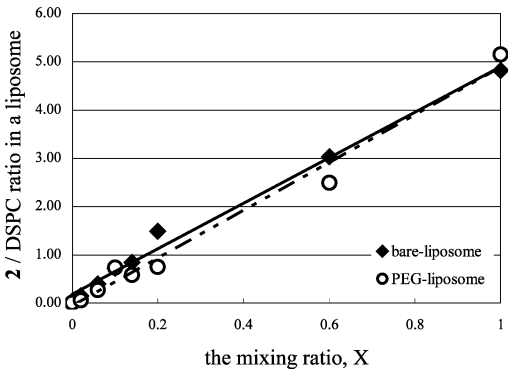


Fig. 4 Incorporation of the nido-carborane lipid **2** into liposomal membranes. The bare-liposome was prepared from DSPC, CH, and **2** (the mixing ratio of 1 : 1 : *x*; *x* = 0–1), and the PEG-liposome was prepared from DSPC, CH, **2**, and PEG-DSPE (the mixing ratio of 1 : 1 : *x*: 0.11, *x* = 0–1).

in boron neutron capture therapy but also for application to self-organization nanomaterials. Biodistribution studies of the nido-carborane lipid **2** containing DSPC liposomes in mice bearing tumors are in progress in our laboratory.

Notes and references

1 M. F. Hawthorne, *Angew. Chem., Int. Ed. Engl.*, 1993, **32**, 950.
2 A. H. Soloway, W. Tjarks, B. A. Barnum, F.-G. Rong, R. F. Barth, I. M. Codogni and J. G. Wilson, *Chem. Rev.*, 1998, **98**, 1515.
3 A. Nakanishi, L. Guan, R. R. Kane, H. Kasamatsu and M. F. Hawthorne, *Proc. Natl. Acad. Sci. USA*, 1999, **96**, 238.
4 H. Nakamura, M. Sekido and Y. Yamamoto, *J. Med. Chem.*, 1997, **40**, 2825; H. Nakamura, M. Fujiwara and Y. Yamamoto, *Bull. Chem. Soc. Jpn.*, 2000, **73**, 231; H. Nakamura, H. Fukuda, F. Girard, T. Kobayashi, H. Nemoto, J. Cai, K. Yoshida and Y. Yamamoto, *Chem. Pharm. Bull.*, 2000, **48**, 1034.
5 A. Chonn and P. R. Cullis, *Curr. Opin. Biotechnol.*, 1995, **6**, 698.
6 T. M. Allen, *Drugs*, 1998, **56**, 747.
7 K. Maruyama, *Biol. Pharm. Bull.*, 2000, **23**, 791.
8 V. P. Torchilin and V. Weissig, *Liposomes*, Oxford University Press, New York, 2000.
9 G. V. Betageri, S. A. Jenkins and D. L. Parsons, *Liposome Drug Delivery Systems*, TECHNOMIC, Basel, 1993.
10 K. Shelly, D. A. Feakes, M. F. Hawthorne, P. G. Schmidt, T. A. Krisch and W. F. Bauer, *Proc. Natl. Acad. Sci. USA*, 1992, **89**, 9039.
11 H. Yanagie, T. Tomita, H. Kobayashi, Y. Fujii and Y. Nonaka, *Br. J. Cancer*, 1997, **75**, 660.
12 M. Johnson, N. Bergstrand and K. Edwards, *J. Liposome Res.*, 1999, **9**, 53.
13 D. A. Feakes, K. Shelly and M. F. Hawthorne, *Proc. Natl. Acad. Sci. USA*, 1995, **92**, 1367.
14 R. A. Watson-Clark, M. L. Banquerigo, K. Shelly, M. F. Hawthorne and E. Brahn, *Proc. Natl. Acad. Sci. USA*, 1998, **95**, 2531.
15 K. Maruyama, T. Takizawa, N. Takahashi, T. Tagawa, K. Yagaike and M. Iwatsuru, *Adv. Drug Deliv. Rev.*, 1997, **24**, 235.
16 K. Maruyama, N. Takahashi, T. Tagawa, K. Nagaike and M. Iwatsuru, *FEBS Lett.*, 1997, **413**, 177.
17 O. Ishida, K. Maruyama, H. Tanahashi, M. Iwatsuru, K. Sasaki, M. Eriguchi and H. Yanagie, *Pharm. Res.*, 2001, **18**, 1042.
18 A. Klibanov, K. Maruyama, A. M. Beckerleg, V. P. Torchilin and L. Hung, *Biochim. Biophys. Acta*, 1991, **1062**, 142.
19 A. Klibanov and L. Hung, *Liposome Res.*, 1992, **2**, 321.
20 C. H. Fiske and Y. Subbarow, *J. Biol. Chem.*, 1925, **66**, 375.