require high-tumor selectivity and should be essentially nontoxic. Therefore, the approach to use biological vehicles has become one of the recent trends to accumulate a large amount of ^{10}B in tumor tissues.

Liposomes, whose size typically ranges in mean diameters from 50 to 200 nm, display some unique pharmacokinetic characteristics. Liposomes exhibit preferential extravasation and accumulation at the site of solid tumors due to increased endothelial permeability and reduced lymphatic drainage in these tissues. which has been defined as enhanced permeability and retention effect (Matsumura et al., 1986; Maeda et al., 2000). Therefore, liposomes are efficient drug-delivery vehicles, because encapsulated drugs can be delivered to tumor selectively. Liposomal boron delivery system (BDS), in this context, is also considered to be promising for BNCT due to the possibility of carrying a large amount of ^{10}B compound. Two approaches have been investigated for the use of liposomes as boron-delivery vehicles: (1) encapsulation of boron compounds into liposomes, and (2) incorporation of boron-conjugated lipids into the liposomal bilayer. In this chapter, new technologies for liposomal BDS using both boron-encapsulation and boron-lipid liposome approaches are described.

8.2 BORON COMPOUND-ENCAPSULATED LIPOSOME APPROACH

8.2.1 Carcinoembryonic Antigen-Targeted Liposomes

Yanagie and coworkers first investigated a BSH-encapsulated liposome which was conjugated with a monoclonal antibody specific for carcinoembryonic antigen (CEA) (Yanagie et al., 1989, 1991). They prepared a new murine monoclonal antibody (2C-8) by injecting mice *i.p.* with CEA producing human pancreatic cancer cell line, AsPC-1. This anti-CEA monoclonal antibody was conjugated with large multilamellar liposomes incorporated $Cs_2{}^{10}BSH$. The liposome was prepared from egg yolk phosphatidylcholine, cholesterol, and dipalmitoylphosphatidylethanolamine (1/1/0.05), and $Cs_2{}^{10}BSH$ was encapsulated. The liposomes were treated with dithiothreitol and suspended in the *N*-hydroxysuccinimidyl-3-(2-pyridyldithio)propionate-treated antibody solution for conjugation. This immunoliposome was shown to bind selectively to human pancreatic carcinoma cells (AsPC-1) bearing CEA on their surface. The therapeutic effects of locally injected BSH-encapsulated immunoliposome on AsPC-1 xenografts in nude mice were evaluated. After intratumoral injection of the immunoliposomes, boron concentrations in tumor tissue and blood were 49.6 ± 6.6 and 0.30 ± 0.08 ppm, respectively. Thermal neutron irradiation (2×10^{12} n/cm^2) suppressed tumor growth in mice with intratumoral injection of BSH-encapsulated immunoliposomes and hyalinization and necrosis were found in the immunoliposome-treated tumors (Yanagie et al., 1997).

8.2.2 Various Boron Compounds-Encapsulated PEG Liposomes

Hawthorne and coworkers reported the preparation of boron-encapsulated liposomes from distearoylphosphatidylcholine (DSPC) and cholesterol. They encapsulated the hydrolytically stable borane anions $B_{10}H_{10}^{2-}$, $B_{12}H_{11}SH^{2-}$, $B_{20}H_{17}OH^{4-}$, $B_{20}H_{19}^{3-}$, and the normal form and photoisomer of $B_{20}H_{18}^{2-}$ in liposomes as their water-soluble sodium salts. Selective boron accumulation in tumor was observed in the use of these liposomes, although the boron compounds used do not normally exhibit affinity for tumors and are normally rapidly cleared from the body. The highest tumor concentrations achieved the therapeutic range (>15 μg of boron per gram of tumor), but more favorable results were obtained with the two isomers of $B_{20}H_{18}^{2-}$. These boron compounds have the capability to react with intracellular components after they have been deposited within tumor cells by the liposome, thereby preventing the borane ion from being released into blood (Shelly et al., 1992). The PEG-conjugated liposome was prepared with 5% PEG-200-distearoyl phosphatidylethanolamine and an apical-equatorial (*ae*) isomer of the $B_{20}H_{17}NH_3^{3-}$ ion, $[1\text{-}(2'\text{-}B_{10}H_9)\text{-}2\text{-}NH_3B_{10}H_8]^{3-}$, was encapsulated into the liposome. This liposome exhibited a long circulation lifetime due to escape from the

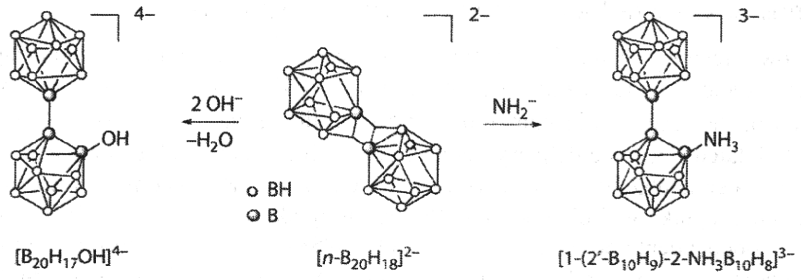


FIGURE 8.1 Hydrolysis and amination of n-$B_{20}H_{18}^{2-}$.

reticuloendothelial system (RES), resulting in the continued accumulation of boron in the tumor over the entire 48 h experiment and reaching a maximum of 47 μg of boron per gram of tumor (Feakes et al., 1994). Preparation of $B_{20}H_{17}OH^{4-}$ and $[1\text{-}(2'\text{-}B_{10}H_9)\text{-}2\text{-}NH_3B_{10}H_8]^{3-}$ from n-$B_{20}H_{18}^{2-}$ is shown in Figure 8.1.

8.2.3 Folate Receptor-Targeted Liposomes

Expression of folate receptor (FR) frequently is amplified among human tumors. Lee and coworkers developed boron-containing folate receptor-targeted liposomes (Pan, X. Q. et al., 2002). Two negatively charged boron compounds, $Na_2[B_{12}H_{11}SH]$ and $Na_3(B_{20}H_{17}NH_3)$, as well as five weakly basic boronated polyamines, SPD-5, SPM-5, ASPD-5, ASPM-5, and SPM-5,10, as shown in Figure 8.2, were incorporated into liposomes by a pH-gradient-driven remote-loading method with varying loading efficiencies. Greater loading efficiencies were obtained with lower molecular weight boron derivatives, using ammonium sulfate as the trapping agent, compared to those obtained with sodium citrate. The *in vitro* boron uptake of folate-conjugated liposomes was investigated using human KB squamous epithelial cancer cells. Higher cellular boron uptake (up to 1584 μg/10^9 cells) was observed with FR-targeted liposomes than with nontargeted control liposomes (up to 154 μg/10^9 cells).

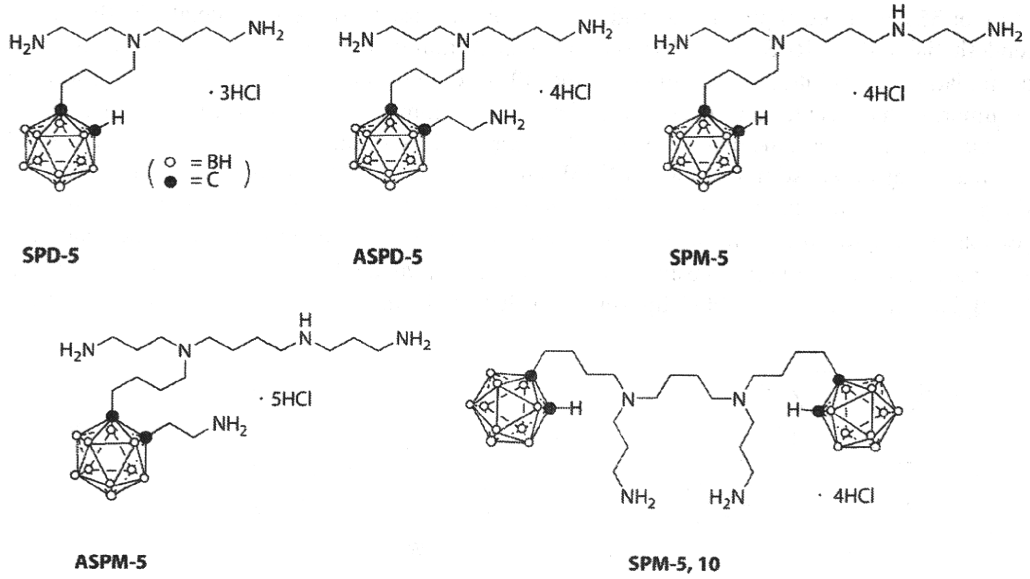


FIGURE 8.2 Structures of boronated polyamine derivatives.

8.2.4 Epidermal Growth Factor Receptor-Targeted Liposomes

Epidermal growth factor receptor (EGFR) tyrosine kinase plays a fundamental role in signal transduction pathways and the uncontrolled activation of this EGFR-mediated signaling may be due to overexpression of the receptors in numerous tumors. Kullberg and coworkers investigated EGF-conjugated PEGylated liposome delivery vehicle, containing water soluble boronated phenanthridine, WSP1, or water soluble boronated acridine, WSA1, for EGFR targeting. In the case of WSA1 a ligand dependent uptake was obtained and the boron uptake was as good as if free WSA1 was given. No ligand-dependent boron uptake was seen for WSP1-containing liposomes. *In vitro* boron uptake by glioma cells (6.29 ± 1.07 μg/g cells) was observed with WSA1-encapsulated EGF-
Q3 conjugated PEGylated liposomes (Kullberg et al., 2003)(Figure 8.3).

Cetuximab, a recombinant chimeric monoclonal antibody, binds to the extracellular domain of the EGFR, thereby preventing the activation and subsequent dimerization of the receptor. Lee and coworkers developed cetuximab-immunoliposomes as an alternative immunoliposome for targeting of EGFR(+) glioma cells through a cholesterol-based membrane anchor, maleimido-PEG-cholesterol (Mal-PEG-Chol), to conjugated cetuximab to liposomes. BSH-encapsulated cetuximab-immunoliposomes were evaluated for targeted delivery to human EGFR gene transfected $F98_{EGFR}$ glioma cells. Much greater (~8-fold) cellular uptake of boron was obtained using cetuximab-immunoliposomes in EGFR(+) $F98_{EGFR}$ compared with nontargeted human IgG-immunoliposomes (Pan, et al., 2007).

8.2.5 Transferrin Receptor-Targeted Liposomes

Transferrin (TF) receptor-mediated endocytosis is a normal physiological process by which TF delivers iron to the cells and higher concentration of TF receptor has been observed on most tumor cells in comparison with normal cells. Maruyama and coworkers developed TF-conjugated PEG liposome. This liposome showed a prolonged residence time in the circulation and low RES uptake in tumor-bearing mice, resulting in enhanced extravasation of the liposomes into the solid tumor tissue, where the liposomes were internalized into tumor cells by receptor-mediated endocytosis (Ishida et al., 2001). The TF-conjugated PEG liposomes and PEG liposomes encapsulating ^{10}BSH were prepared and their tissue distributions in Colon 26 tumor-bearing mice after *i.v.* injection were compared with those of bare liposomes and free ^{10}BSH. When TF-PEG liposomes were injected at a dose of 35 mg ^{10}B/kg, a prolonged residence time in the circulation and low uptake by the reticuloendothelial system (RES) were observed in Colon 26 tumor-bearing mice, resulting in enhanced accumulation of ^{10}B into the solid tumor tissue. TF-PEG liposomes maintained a high ^{10}B level in the tumor, with concentrations over 30 μg of boron per gram of tumor for at least 72 h after injection. On the other hand, the plasma level of ^{10}B decreased, resulting in a tumor/plasma ratio of 6.0 at 72 h after injection. Administration of ^{10}BSH encapsulated in TF-PEG liposomes at a dose of 5 or 20 mg ^{10}B/kg and irradiation with 2×10^{12} neutrons/cm^2 for 37 min produced tumor growth suppression and improved long-term survival compared with boron-loaded PEG liposomes and bare liposomes, and free ^{10}BSH. Masunaga and coworkers evaluated biodistribution of ^{10}BSH and $Na_2{}^{10}B_{10}H_{10}$-encapsulated TF-PEG liposomes in SCC VII tumor-bearing mice (Masunaga et al.,

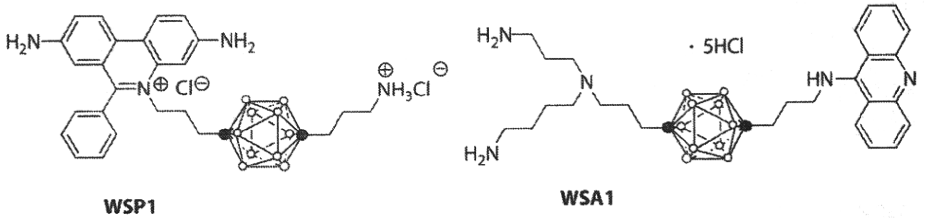


FIGURE 8.3 Structures of WSP1 and WSA1.

2006). The kinetics in the ^{10}B concentration in tumors loaded with both liposomes were similar except that ^{10}B concentrations were greater 24 h after the administration of $Na_2{}^{10}B_{10}H_{10}$ than ^{10}BSH in TF-PEG liposomes and ^{10}B concentration in tumors was 35.6 μg of boron per gram of tumor with injection of $Na_2{}^{10}B_{10}H_{10}$-encapsulated TF-PEG liposomes via the tail vein at a dose of 35 mg ^{10}B/kg. Q4

8.3 BORON LIPID–LIPOSOME APPROACH

Since a demonstration of liposomes as models for the biomembrane mimics were reported by Bangham (Bangham et al., 1965) and a first totally synthetic bilayer vesicle of didodecacyldimethylammonium bromide by Kunitake (Kunitake 1992; Kunitake et al., 1977), various self-organization bilayer membranes have been synthesized (Menger et al., 1995). Generally, amphiphiles of liposomal membranes consist of a long hydrocarbon chain, which is called a tail, and a hydrophilic part (Allen, 1998; Maruyama, 2000); (Torchilin et al., 2002); (Betageri et al., 1993). In the meanwhile, development of lipophilic boron compounds embedded within the liposome bilayer, provides an attractive method to increase the overall efficiency of incorporation of boron-containing species, as well as raise the gross boron content of the liposomes in the formulation. Various boron lipids have been developed recently and those are classified into two groups: *nido*-carborane conjugates (**1–3**) and *closo*-dodecaborane conjugates (**4–11**) as shown in Figures 8.4 and 8.5, respectively. Less toxicity has been observed in the boron lipids belonging to the latter group. Q5 Q6 Q7

8.3.1 *NIDO*-CARBORANE AMPHIPHILE

Hawthorne and coworkers first introduced *nido*-carborane as a hydrophilic moiety into the amphiphile and this single-tailed *nido*-carborane amphiphile was utilized for liposomal boron delivery using tumor-bearing mice (Feakes et al., 1995; Watson-Clark et al., 1998). They synthesized the *nido*-carborane amphiphile **1** (Figure 8.4) from the reaction of decaborane and 1-octadecyne followed by degradation of the resulting carborane cage under basic conditions. Boronated liposomes were prepared from DSPC, cholesterol, and **1**. After the injection of liposomal suspensions in BALB/c mice bearing EMT6 mammary adenocarcinomas, the time-course biodistribution of boron was examined. At the low injected doses normally used (5–10 mg ^{10}B/kg), peak tumor boron concentrations of 35 μg of boron per gram of tumor and tumor/blood boron ratios of approximately

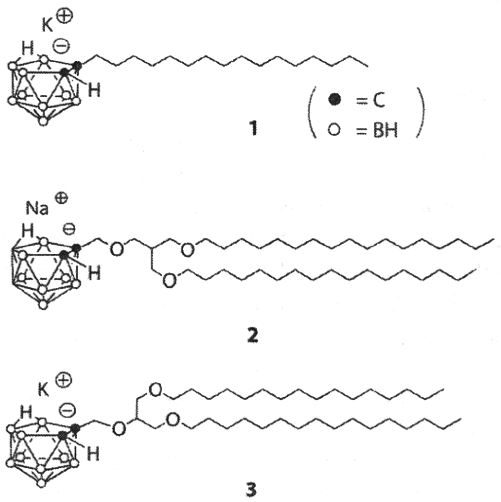


FIGURE 8.4 Structures of *nido*-carborane amphiphile and lipids.

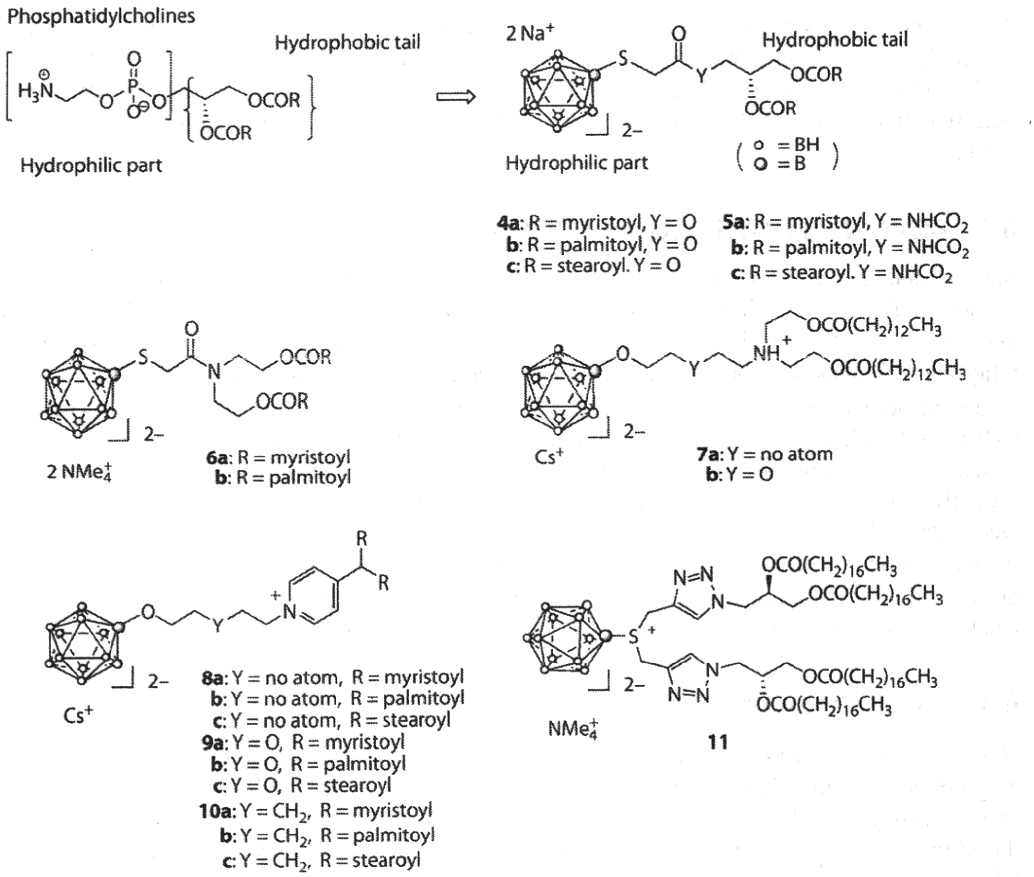


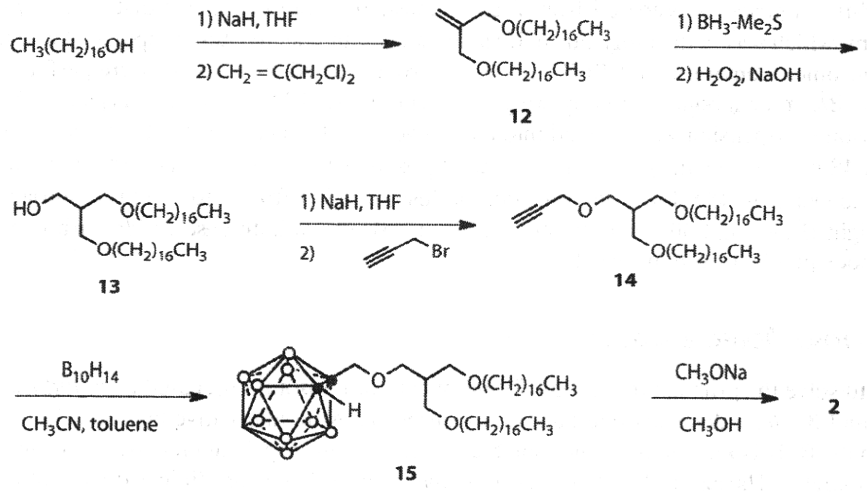
FIGURE 8.5 Structures of phosphatidylcholine and *closo*-dodecaborane lipids.

8 were achieved. These values are sufficiently high for the successful application of BNCT. The incorporation of both **1** and the hydrophilic species, $Na_3[1\text{-}(2'\text{-}B_{10}H_9)\text{-}2\text{-}NH_3B_{10}H_8]$, within the same liposome demonstrated significantly enhanced biodistribution characteristics, exemplified by maximum tumor boron concentration of 50 μg of boron per gram of tumor and tumor/blood boron ratio of 6.

8.3.2 *NIDO*-CARBORANE LIPIDS

Nakamura and coworkers developed *nido*-carborane lipid **2**, which consists of the *nido*-carborane moiety as the hydrophilic functionality conjugated with two long alkyl chains as the lipophilic functionality. Chemical synthesis of *nido*-carborane lipid **2** is shown in Scheme 8.1. Reaction of two equivalents of heptadecanol with 3-chloro-2-chloromethyl-1-propene using NaH as base gave the diether **12** in 93% yield and the hydroboration of **12** gave the corresponding alcohol **13** in 71% yield. The alcohol **13** was converted into the propargyl ether **14** in 48% yield by the treatment with propargyl bromide and the decaborane coupling of **6** was carried out in the presence of acetonitrile in toluene under reflux condition to give the corresponding *ortho*-carborane **15** in 80% yield. The degradation of the carborane cage by the treatment with sodium methoxide in methanol afforded the *nido*-carborane lipid **2** in 57% yield.

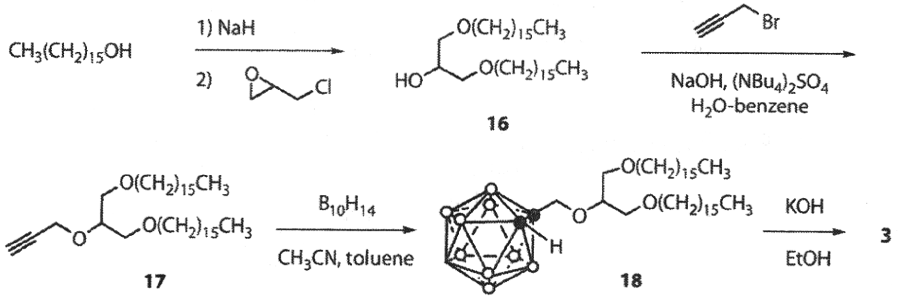
Analysis under a transmission electron microscope by negative staining with uranyl acetate showed stable vesicle formation of *nido*-carborane lipid **2**. The *nido*-carborane lipid **2** (CL) was incorporated into DSPC liposomes in a concentration-dependent manner (Nakamura et al., 2004).



SCHEME 8.1 Synthesis of *nido*-carborane lipid **2** (CL).

Furthermore, TF could be introduced to the surface of *nido*-carborane lipid liposomes (Tf(+)-PEG-CL liposomes) by coupling TF to the PEG-CO_2H moieties of Tf(−)-PEG-CL liposomes. The biodistribution of Tf(+)-PEG-CL liposomes injected intravenously into colon 26 tumor bearing BALB/c mice revealed that Tf(+)-PEG-CL liposomes accumulated in tumor tissues and stayed there for a sufficiently long time to increase tumor/blood boron ratio, although Tf(-)-PEG-CL liposomes were gradually released from tumor tissues with time. A boron concentration of 22 μg of boron per gram of tumor was achieved by injecting Tf(+)-PEG-CL liposomes (7.2 mg ^{10}B/kg) into tumor-bearing mice. As noted earlier, BSH-encapsulated Tf(+)-PEG liposomes accumulated in tumors at 35.5 μg of boron per gram of tissue 72 h after administration of 35 mg ^{10}B/kg. Therefore, ^{10}B delivery to tumor tissues by Tf(+)-PEG-CL liposomes would be more efficient than that by BSH-encapsulated Tf(+)-PEG liposomes based on dose-dependent drug delivery efficacy. However, significant acute toxicity was observed in 50% of the mice when Tf(+)-PEG-CL liposomes were injected at a dose of 14 mg ^{10}B/kg. Injection of Tf(+)-PEG-CL liposomes at a dose of 7.2 mg ^{10}B/kg and irradiation with 2×10^{12} neutrons/cm^2 for 37 min at the KUR atomic reactor suppressed tumor growth and the average survival rate of mice not treated with Tf(+)-PEG-CL liposomes was 21 days, whereas that of treated mice was 31 days (Miyajima et al., 2006).

Hawthorne and coworkers also synthesized *nido*-carborane lipid **3** as shown in Scheme 8.2. The reaction of two equivalents of 1-hexadecanol with epichlorohydrin using sodium hydride gave the corresponding alcohol **16**. Propargylation of **16** followed by the decaborane coupling afforded the carborane **18**. Finally, degradation of the carborane cage of **18** proceeded in the presence of KOH in ethanol to give the *nido*-carborane lipid **3**.



SCHEME 8.2 Synthesis of *nido*-carborane lipid **3**.

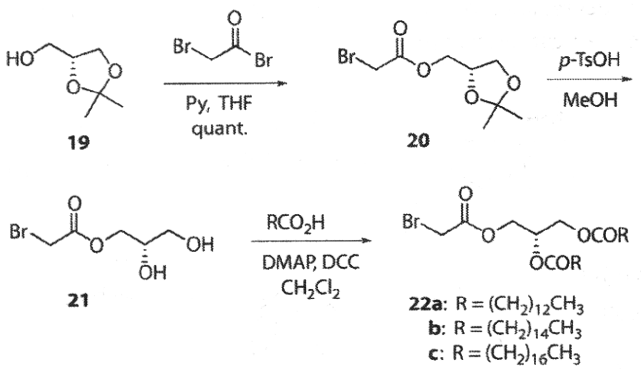
DSPC-free liposomes prepared from **3** and cholesterol exhibited a size distribution pattern of 40–60 nm, which was in the range normally associated with selective tumor uptake. Animal studies of the liposomes, containing **3**, DSPC, and cholesterol in varied proportions, were performed using male BALB/c mice (about 10 g body weight) bearing small EMT-6 tumors. Typically 200 μL of each liposome suspension was injected into the tail vein, and the behavior of the mice was followed for up to 48 h. Unfortunately, in each case, the liposomes were found to be very toxic: no mouse survived longer than 48 h following injection of doses ranging from 6 to 30 mg of boron per kg of body weight (Li et al., 2006).Due to the significant toxicity in both cases, *nido*-carborane framework is not suitable for use in BNCT.

8.3.3 *closo*-Dodecaborate Lipids

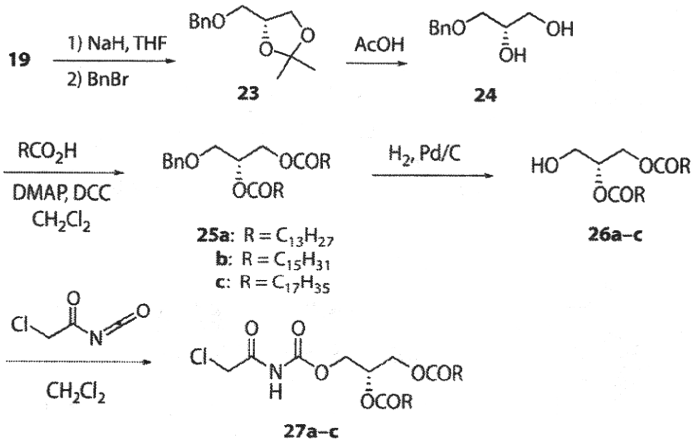
In order to solve the problem of the significant toxicity of liposomes prepared from *nido*-carborane lipids **2** and **3**, *closo*-dodecaborate has been focused on as an alternative hydrophilic function of boron lipids. BSH is known as a water-soluble divalent "*closo*-type" anion cluster and significantly lowered toxicity (Haritz et al., 1994), and thus has been utilized for clinical treatment of BNCT. Nakamura and coworkers succeeded in the synthesis of double-tailed *closo*-dodecaborate lipids **4a–c** and **5a–c**, which have a $B_{12}H_{11}S$-moiety as a hydrophilic function with chirality similar to natural phospholipids, such as distearoylphosphatidylcholine (DSPC), in their lipophilic tails (Lee et al., 2007; Nakamura et al., 2007a).

Synthesis of the hydrophobic tail functions of **22** is shown in Scheme 8.3. Reaction of the chiral alcohol **19** with 1.2 equivalents of bromoacetyl bromide gave the ester **20**, quantitatively, and the deprotection of **20** was carried out using catalytic amounts of *p*-TsOH in MeOH to give the corresponding diol **21**. Ester formation from diol **21** using 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 **22a–c** in 61–75% yields. Synthesis of the hydrophobic tail functions of **27** is shown in Scheme 8.4. The chiral alcohol **19** was first protected with benzyl bromide using NaH and the resulting dioxolane **23** was converted into the diol **24** using aqueous AcOH in 83% yield. Ester formation of **24** using various carboxylic acids was carried out in a similar manner to give **25a–c**, quantitatively. Deprotection of the benzyl group of **25a–c** by hydrogenation gave the corresponding alcohols **26a–c** (89– > 99% yields), which then reacted with chloroacetyl isocyanate in CH_2Cl_2 to give **27a–c** in 74–98% yields.

Introduction of BSH into the hydrophobic tail functions **22** and **27** was examined using the "protected BSH (**28**)," which was prepared according to the Gabel's protocol (Gabel et al., 1993), as shown in Scheme 8.5. S-Alkylation of **28** with **22a–c** proceeded in acetonitrile at 70°C for 12–24 h, giving the corresponding *S*-dialkylated products **29**, which were immediately treated



SCHEME 8.3 Synthesis of hydrophobic tail functions **22a–c**.



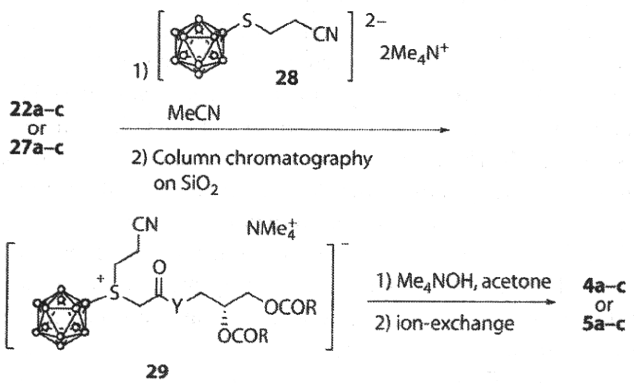
SCHEME 8.4 Synthesis of hydrophobic tail functions **27a–c**.

with tetramethylammonium hydroxide (1 equiv.) in acetone to give **4a–c** in 76–91% yields, as tetramethylammonium salts. In a similar manner, the **5a–c** were obtained from **27a–c** in 54–83% yields.

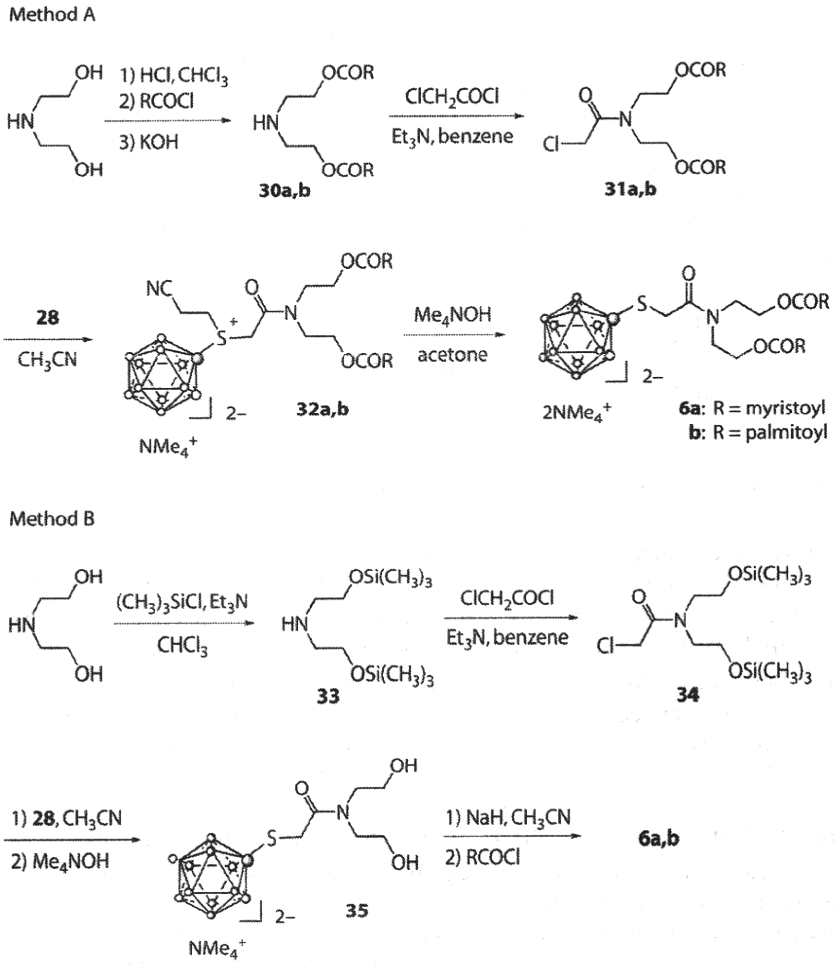
Calcein-encapsulation experiments revealed that the liposomes, prepared from boron cluster lipids **4**, DMPC, PEG-DSPE, and cholesterol, are stable at 37°C in FBS solution for 24 h.

The time-dependent biodistribution experiment of boronated liposomes prepared from closo-dodecaborate lipid **4c** and injected intravenously into colon 26 tumor bearing BALB/c mice (20 mg ^{10}B/kg) showed high ^{10}B accumulation in the tumor tissue (23 μg of boron per gram of tumor) 24 h after injection (Nakamura et al., 2009). In addition to determining ^{10}B concentration in various organs, neutron irradiation of the mice was carried out 24 h after administration of the boron liposomes in the JAEA atomic reactor (JRR-4). Tumor growth rate in mice administered with boron liposomes was significantly suppressed, although the administration of saline did not reduce tumor growth after neutron irradiation (Ueno et al., 2010).

Gabel and coworkers synthesized *closo*-dodecaborate lipids **6a** and **6b** as shown in Scheme 8.6. The introduction of the boron cluster was achieved by alkylation with "protected BSH (**28**)" and subsequent alkaline removal of the cyanoethyl protecting group (Gabel et al., 1993). In method A, 2 equivalents of the chloroanhydride of the fatty acids was allowed to react with diethanolamine. The resulting products *N,N*-(2-dimyristoyloxyethyl)- and *N,N*-(2-dipalmitoyloxyethyl)-amine (**30a** and **30b**, respectively) were reacted with chloroacetylchloride in the presence of triethylamine to obtain the chloroacetamides **31a** and **31b**. The reaction of **31a,b** and the tetramethylammonium salt of **28**



SCHEME 8.5 Synthesis of *closo*-dodecaborate lipids **4** and **5**.

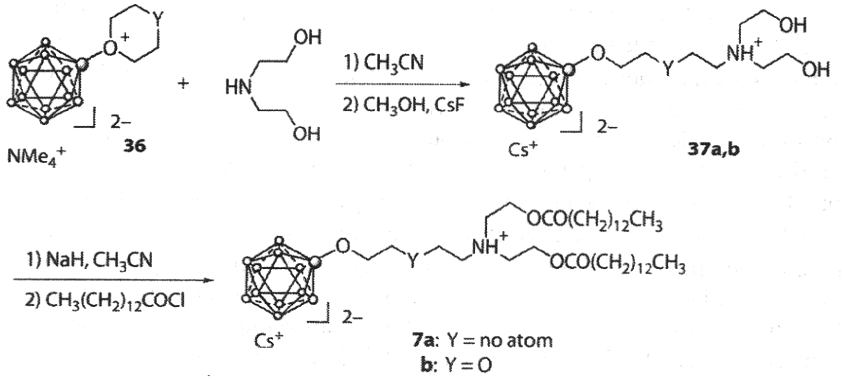


SCHEME 8.6 Synthesis of *closo*-dodecaborate lipids **6**.

produced sulfonium salts **32a,b**. The products **6a** and **6b** were obtained from the reaction of sulfonium salts **32a,b** with tetramethylammonium hydroxide in acetone. The yields of lipids **6a** and **6b** are 48–55% (overall yield from diethanolamine 25–28%).

The lipids were also able to be obtained through method B (Scheme 8.6). Two equivalents of chlorotrimethylsilane were reacted with diethanolamine in the presence of triethylamine. The resulting trimethylsilyloxy derivative **33** was reacted with chloroacetylchloride in the presence of triethylamine to give **34** in 84% yield. The alkylation of **34** with **28,** followed by deprotection with tetramethylammonium hydroxide in acetone, and subsequent esterification with the alkanoylchloride gave the products **6a** and **6b**. The overall yield of **6a** from diethanolamine was 46%.

Differential scanning calorimetry showed that **6a** and **6b** alone exhibit a main phase transition at 18.8 and 37.9°C, respectively. These temperatures were quite comparable to the transition temperatures of DMPC and DPPC, (24.3 and 41°C, respectively). Liposomes prepared from boron lipids, DSPC, and cholesterol (1:1:1 mole ratio) were successfully prepared by thin film hydration and extrusion. The mean diameters of the liposomes containing **6a** and **6b** in combination with DSPC and cholesterol were found to be 135 and 123 nm, respectively. The liposomes had ξ-potentials of −67 and −63 mV, respectively, reflecting the double negative charge of the head group. Liposomes prepared from **6a** were slightly less toxic to V79 Chinese hamster cells (IC_{50} = 5.6 mM) than

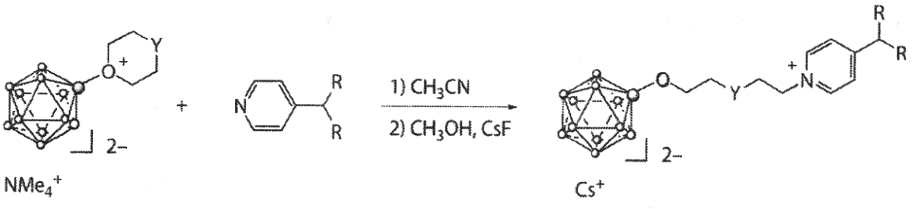


SCHEME 8.7 Synthesis of *closo*-dodecaborate lipids **7**.

unformulated BSH (IC_{50} = 3.9 mM), while liposomes prepared from **6b** were not toxic even at 30 mM (Justus et al., 2007).

Schaffran and coworkers synthesized new boron-containing lipids, which consist of a diethanolamine frame with two myristoyl chains bonded as esters (Schaffran et al., 2009b). Butylene or ethyleneoxyethylene units provide a link between the doubly negatively charged dodecaborate cluster and the amino function of the frame, obtained by nucleophilic attack of diethanolamine on the tetrahydrofuran and dioxane derivatives, respectively, of *closo*-dodecaborate (Scheme 8.7). The thermotropic behavior was found to be different for the two lipids, with the butylene lipid **7a** showing sharp melting transitions at surprisingly high temperatures. Toxicity *in vitro* and *in vivo* varied greatly, with the butylene derivative **7a** being more toxic than the ethyleneoxyethylene derivative **7b**.

Furthermore, Schaffran and coworkers developed pyridinium lipids with the dodecaborate cluster. The lipids consist of a pyridinium core with C12, C14, and C16 chains as lipid backbone, connected through the nitrogen atom through a butylene, pentylene, or ethyleneoxyethylene linker to the oxygen atom on the dodecaborate cluster as headgroup (Schaffran et al., 2009a). Synthesis of pyridinium lipids with the dodecaborate cluster is shown in Scheme 8.8. The lipids were obtained by nucleophilic attack of 4-(bisalkylmethyl)pyridine on the tetrahydrofurane **36**, the dioxane **37**, and a newly prepared tetrahydropyrane derivative **38**, respectively, of *closo*-dodecaborate. All of these boron lipids form closed vesicles in addition to some bilayers in the pure state and in the presence of helper lipids. The thermotropic behavior was found to be increasingly complex and polymorphic with increasing alkyl chain length. Except for two lipids (**9a** and **9b**), all lipids showed low *in vitro* toxicity, and longer alkyl chains led to a significant decrease in toxicity. The choice of the



SCHEME 8.8 Synthesis of tetrahydropyran derivatives **8–10** of the *closo*-dodecaborate cluster.

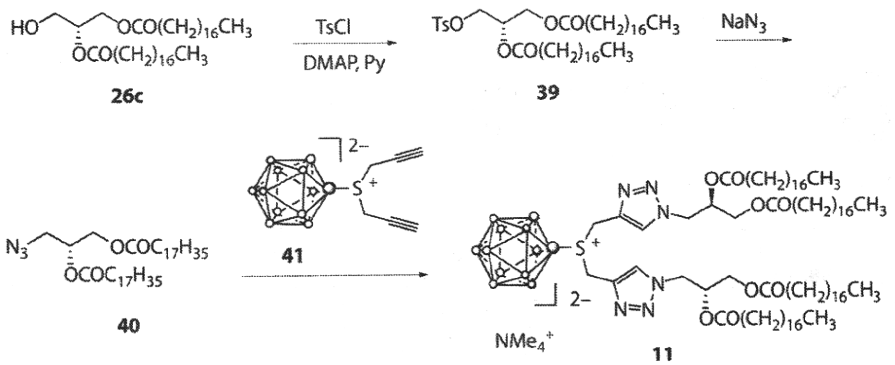
linker played no major role with respect to their ability to form liposomes and their thermotropic properties, but the toxicity was influenced by the linkers in the case of short alkyl chains.

El-Zaria and Nakamura developed a new method that utilizes the click cycloaddition reaction to functionalize BSH with organic molecules (El-Zaria et al., 2009; El-Zaria et al., 2010). *S,S*-bis(propynyl)sulfonioundecahydro-*closo*-dodecaborate (1-) tetramethylammonium salt (*S,S*-dipropargyl-$SB_{12}H_{11}^-$: **41**) was prepared from BSH with propargyl bromide. Compound **41** acts as a powerful building block for the synthesis of a broad spectrum of 1,4-disubstituted 1,2,3-triazole products in high yields based on the click cycloaddition reaction mediated by Cu(II) ascorbate. The reactions require only benign reaction conditions and simple workup and purification procedures; an unsymmetric bis-triazole BSH derivative could also be synthesized by the stepwise click reaction. Synthesis of the *closo*-dodecaborate lipid with four-tailed moieties was achieved by the click cycloaddition reaction of **41** with 3-*O*-azidoacetyl-1,2-*O*-distearoyl-*sn*-3-glycerol **40**, which was readily prepared from the corresponding alcohol **26c** in two steps as shown in Scheme 8.9.

8.4 BORON CHOLESTEROL–LIPOSOME APPROACH

Cholesterol is indispensable for the formation of stable liposomes, especially in blood circulation. Therefore, the development of boronated cholesterol derivatives is considered to be an alternative approach to boron embedment in the liposome bilayer. The first boronated cholesterol derivatives were reported by Feakes et al. (1999) They introduced a *nido*-carborane into the cholesterol framework through ether or ester bonds. Although they synthesized *nido*-carborane conjugated cholesterols **42a–b** (Figure 8.6), the evaluation of their liposomes has not been reported yet.

Tjarks and coworkers developed ortho-carboranyl phenol **43** as a cholesterol mimic, which was utilized as a lipid bilayer component for the construction of nontargeted and receptor-targeted boronated liposomes. The major structural feature of the boronated cholesterol mimic is the physicochemical similarity between cholesterol and carborane frameworks (Endo et al., 1999). Cholesterol analog **43** was stably incorporated into non-, FR-, and vascular endothelial growth factor receptor-2 (VEGFR-2)-targeted liposomes. No major differences in appearance, size distribution, and lamellarity were found among conventional DPPC/cholesterol liposomes, nontargeted, and FR-targeted liposomal formulations of this carboranyl cholesterol derivative. FR-targeted boronated liposomes were taken up extensively by FR-overexpressing KB cells *in vitro*, and the uptake was effectively blocked in the presence of free folate. There was no apparent *in vitro* cytotoxicity in FR-overexpressing KB cells and VEGFR-2-overexpressing 293/KDR cells when these were incubated with boronated FR- and (VEGFR-2)-targeted liposomes, respectively, although the former accumulated extensively in KB cells and the latter effectively interacted with VEGFR-2 by causing autophosphorylation and protecting 293/KDR cells from SLT (Shiga-like toxin)-VEGF cytotoxicity (Thirumamagal et al., 2006).



SCHEME 8.9 Synthesis of the *closo*-dodecaborate lipid **11** by the click cycloaddition reaction.

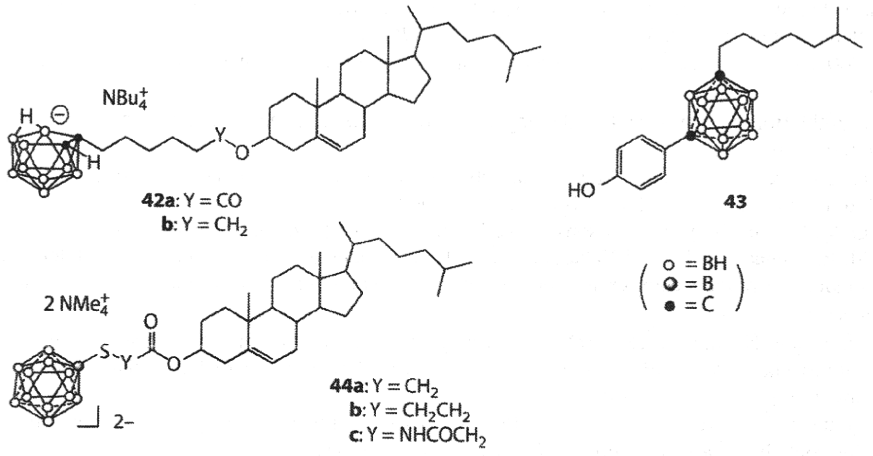


FIGURE 8.6 Structures of cholesterol-boron cluster conjugates (**42** and **44**) and ortho-carboranyl phenol **43**.

Nakamura, Gabel, and coworkers developed *closo*-dodecaborate conjugated cholesterols **44a–c**. The closo-dodecaborate conjugated cholesterol **44a** liposome, which was prepared from dimyristoylphosphatidylcholine, cholesterol, **44a**, and PEG-conjugated distearoylphosphatidylethanolamine (1:0.5:0.5:0.1), exhibited higher cytotoxicity than BSH at the same boron concentration and the IC_{50} values of **44a** liposome and BSH toward colon 26 cells were estimated to be 25 and 78 ppm of boron concentration, respectively (Nakamura et al., 2007b).

8.5 SUMMARY

Recent development of liposomal BDS are summarized in this chapter. Two approaches to encapsulation of boron compounds into liposomes and incorporation of boron-conjugated lipids into the liposomal bilayer have been investigated. Since the leakage upon storage has been observed in boron-encapsulated liposomes, the combination of both approaches would be more potent to carry a large amount of ^{10}B compounds into tumor. In general drug delivery systems, liposomes are used for selective delivery of drugs to tumors in an effort to avoid the drugs from undesirably accumulating in other organs. However, a large amount of liposomes that are administrated accumulate in the liver and may cause severe side effects for patients. BDS, in this context, is a safer system because boron compounds delivered with liposomes are nontoxic unless neutron capture reaction of boron takes place. Therefore, the boron compounds accumulated in other organs will not cause side effects for the patient under condition that the boron compounds are nontoxic. Liposomal drug delivery system is variable depending on tumoral blood vessel formations and diffusion processes for the liposomes to reach deeper portion in tumors, thus it is important to choose the target cancers that are considered to be suitable for treatment with BDS. In this regards, liposomal BDS targeted to brain tumor is not a suitable strategy although brain tumors still are the major target for BNCT. Since successful BNCT is highly dependent on the selective and significant accumulation of boron-10 in tumor cells, liposomal BDS would be one of the efficient approaches for the treatment of a variety of cancers with BNCT.

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第69回日本血液学会
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合同シンポジウム2
疾患治療を目的としたDDS
最近の進歩

がん中性子捕捉療法と次世代ホウ素デリバリーシステム

中 村 浩 之

Key words : Boron neutron capture therapy (BNCT), DDS, Cancer, Liposome

1. はじめに

高齢化の進む我が国の死亡原因の第一位はがんであり，その年間死亡者数はおよそ30万人である。がん検診の普及，早期診断・早期治療，さらには初期治療としての手術・放射線・化学療法の進歩によって，ある程度治癒率の改善がみられるものの，化学療法では全身的な副作用との戦い，放射線治療では照射野内の正常組織損傷の問題が常に存在する。このような中で，化学療法と放射線療法の両方の原理を上手く利用したホウ素中性子捕捉療法（BNCT: boron neutron capture therapy）が注目されている[1,2]。

低エネルギーの熱中性子はエネルギーの高い高速中性子とは異なり，人体には無害である。しかしながら熱中性子とホウ素10との反応は，リチウムとヘリウム（α線）を生じ，これらのエネルギーは2.79 MeVとおよそ細胞1つを殺傷するのに十分であり，その飛程は細胞1つの直径（5〜9 μm）である（式1）。したがって，予めホウ素分子をがん細胞にのみ選択的に取り込ませそこへ中性子照射を行えば，がん細胞のみを選択的に破壊することができる（Fig. 1）。これを利用するのがBNCTである。

$$^{10}B + {}^{1}n \rightarrow {}^{7}Li + {}^{4}He + 2.79\ MeV \qquad (1)$$

では，なぜホウ素分子なのか？中性子を原子核に照射した際に，中性子を捕捉する大きさ“中性子捕捉断面積”を主な元素について比較した（Table 1）[3]。中性子捕捉断面積はバーン（1 barn $=10^{-24}$ cm^2）という単位で表される。^{135}Xe, ^{149}Sm, ^{151}Eu, ^{157}Gdなどがきわめて大きい値を示している。^{10}Bの中性子捕捉断面積は3,837バーンとそれほど大きな値は示していないのに，中性子捕捉療法に有望であるのは主に次に挙げる4つの理由からである。(i) ^{10}Bは非放射性で天然のホウ素に約20%含まれるため入手容易である。(ii) 上で述べたように核反応の際のα線の飛程が1個の細胞内に限られる。(iii) ホウ素の広範な化学反応性と安定性により種々の生物活性分子や生体関連物質への導入が可能である。(iv) 重金属のような高い毒性を示さない。一方，生体中の元素も中性子を捕捉して放射線を生じるが，その中性子捕捉断面積は^{10}Bよりも数桁小さな値なので（Table 1）通常は無視できる。しかしながら水素と窒素は生体中に高濃度に存在するため，中性子の照射線量に大きく影響する。したがってこれらの影響を最小限にするためにも，腫瘍組織内の^{10}B濃度が20〜35 μg/g，もしくは^{10}B原子が10^9個/細胞であれば，放射線量のおよそ85%が^{10}Bの中性子捕捉反応から生じると計算されている[4]。最終的には照射できる中性子線量の上限は，水素と窒素が中性子を捕捉して出す放射線に周囲の正常組織がどれほど耐えられるかに依存する。このためにも^{10}Bががん細胞に選択

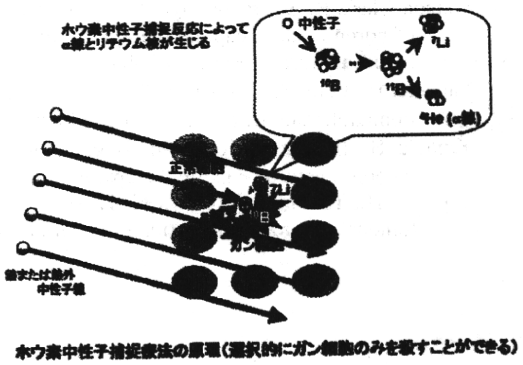

Fig. 1 Concept of BNCT (authorized by FFAG−DDS Research Organization)

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Table 1 Capture Cross Section Values of Various Nuclides for Thermal Neutrons

nuclide	cross section capture value[a]	nuclide	cross section capture value[a]
^{6}Li	942	H	0.332
^{10}B	3838	C	0.0037
^{113}Cd	20,000	N	1.75
^{135}Xe	2,720,000	O	<0.0002
^{149}Sm	41,500	P	0.19
^{151}Eu	59,002	S	0.52
^{157}Gd	240,000	Na	0.536
174Hf	400	K	2.07

[a] Cross section capture values in barns.

的に集積することが必要であり，実際に臨床上の立場から腫瘍組織内 ^{10}B 濃度が 30 μg/g 以上，^{10}B 濃度の腫瘍組織/血液および腫瘍組織/正常組織の比がいずれも 5 以上が望ましいとされている。

$$^{1}H+^{1}n\rightarrow^{2}H+\gamma 2.23\ MeV \quad (2)$$

$$^{14}N+^{1}n\rightarrow^{14}C+^{1}p 0.63\ MeV \quad (3)$$

2. BNCTの特徴

一般的な放射線療法であるX線やガンマ線を用いた治療法では，腫瘍部位に狙いを定めても，直径数 mm くらいの大きさの中は均一に照射される。したがって，体外から照射する放射線を用いると，近接する腫瘍細胞と正常細胞はほぼ同じ物理的なダメージを受けることになる。脳腫瘍の治療では広い範囲の正常脳組織内に点在する腫瘍細胞だけを選択的に治療する必要があるが，腫瘍部だけに絞り込んで照射しようとしても，その周辺にある正常脳組織の障害は避けられない。一方，BNCTでは体内であらかじめ送り込んでおいた ^{10}B の中性子捕捉反応で発生する α 線と ^{7}Li 粒子は，発生してから止まるまでの距離（飛程）が，ほぼ細胞1個分の長さと短いため，腫瘍細胞で発生した α 線と ^{7}Li 粒子も周囲の正常脳組織にほとんど影響を与えない。さらに，BNCTで発生する α 線と ^{7}Li 粒子はX線やガンマ線に比べて生物学的な効果が2~3倍程度高く，治療効果が高いことが期待される。したがって正常脳組織にあまりダメージを与えないで腫瘍組織を細胞選択的に破壊することができる。また，通常1回（2時間以内）の照射で治療が終了するのもBNCTの特徴である。

3. 脳腫瘍 BNCT の世界初の成功例

BNCTの概念は，1936年に Locher によって最初に提唱された[5]。その後，1951年から米国ブルックヘブン国立研究所（BNL）において悪性神経膠腫を対象とした最初の試験治療研究が Farr および Sweet らによって開始され，10年間で45例の BNCT が行われた。また，1953年からマサチューセッツ工科大学（MIT）でも治療が開始され，18例の BNCT が行われたが，ホウ素化合物の腫瘍選択性と中性子遮蔽の不十分さ故に治療成績が悪く，1961年に治療が中断された[6]。

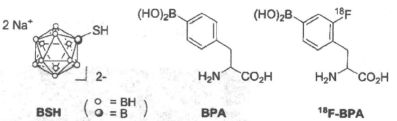

Fig.2 Structures of BSH, BPA, and ^{18}F-BPA.

一方，日本では1959年から BNCT に関する基礎研究が始められ，1968年に帝京大学の（故）畠中らは，Fig. 2 に示すように非常に低毒性であるホウ素イオンクラスター（BSH: mercaptoundecahydrododecaborate）を用いて世界で初めて脳腫瘍の BNCT に成功した[7]。BSH は分子内に12個のホウ素原子を含む20面体の特異な化学構造を有しており，それ自身はがん細胞に対する選択性は低いが，高水溶性・低毒性である（Fig. 2）。健全な脳には血液脳関門現象（blood-brain barrier）があり，血液中の水溶性物質は正常な脳組織には取り込まれにくいが，脳腫瘍はこの血液脳関門が壊れているため BSH のような水溶性の化合物が脳組織内に取り込まれると考えられている。畠中らの成功以来，日本はこの分野をリードしてきており，現在まで脳腫瘍の治療実績は250症例を越えている。悪性度の高い膠芽細胞腫（glioblastoma）では2年生存率がおよそ40%，5年生存率ではおよそ20%であるが，比較的悪性度の低い星状細胞腫（astrocytoma）では，5年生存率はおよそ60%と，標準的な治療の~15%に比べ BNCT 治療効果は優れていると云える。

4. 悪性黒色腫への BNCT

1987年，神戸大学の三島らはアミノ酸誘導体である BPA（*p*-boronophenylalanine）を用いて悪性黒色腫（メラノーマ）の BNCT に成功した[8]。BPA は必須アミノ酸であるフェニルアラニンの類縁体として増殖の盛んながん細胞に選択的に取り込まれると考えられている。BPA は，中性領域下での溶解性が低いため，臨床では D-フルクトースとの複合体として用いられている。現在まで悪性黒色腫の治療実績はおよそ30症例で5年生存率は60%を越えており，非常に治療効果が高い。また外科的手術と異なり機能温存できることから QOL の高さも BNCT の利点である。

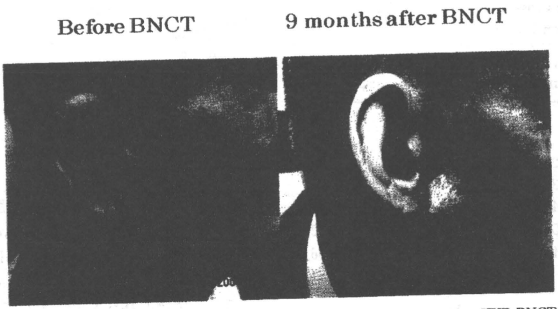

Fig. 3 BNCT for parotid cancer patient (authorized by Prof. K. Ono at KUR BNCT research group).

5. PET診断用 ^{18}F-BPA開発およびBPAとBSHの併用療法と適応拡大

1994年，京都府医大の今堀・上出らにより ^{18}F-BPAを用いたPET（positron emission tomography）診断法が開発され，あらかじめ腫瘍部位のホウ素蓄積量を見積もることができるようになると同時に多くの悪性腫瘍に集積することも分かった[9]。さらに，京都大の小野らと今堀らの共同研究によってBPAを用いた悪性神経膠腫の世界最初のBNCTを実施した。BPAは増殖しているがん細胞には選択的に取り込まれるものの，休止期腫瘍細胞への取り込みは低いことが弱点であったが，BSHとの併用により克服できるようになった。

2001年，大阪大の加藤・由良らは小野らと共同して世界に先駆けて頭頸部悪性腫瘍のBNCTに成功した。頭頸部悪性腫瘍は現在でも手術が中心であり，審美障害，嚥下・咀嚼障害などの機能障害が後遺することがある。彼らは，再発耳下腺癌患者に対し，BSHとBPAの併用BNCTを行ったところ，9ヶ月後にはがんが完全に消失し，皮膚への放射線障害もほとんど見られなかった（Fig. 3；京大炉BNCT研究グループ・小野教授より提供）。この成功をきっかけにBNCTの適応拡大が進められており，口腔多発癌患者や外側咽頭後リンパ節転移患者へBNCTが施され経過も良好である。さらに，川崎医科大の平塚らは，術前照射を行いBNCTと外科手術の組み合わせで高い治療効果を報告しており，京都大の鈴木・小野らは肝臓癌，肺癌，胸壁腫瘍へBNCTを適応拡大している（Fig. 4；京大炉BNCT研究グループ・小野教授より提供）[10~12]。

一方，ホウ素薬剤である ^{10}B濃縮したBSHとL体BPAは，海外からの輸入に頼っていたため，臨床に必要なホウ素薬剤の確保がしばしば困難であった。大阪府立大の切畑らはステラケミファ（株）と共同で，^{10}B濃縮したBSHとBPAの国産化に成功し，現在ではこの2つのホウ素薬剤のGMPレベルでの供給体制が整った。

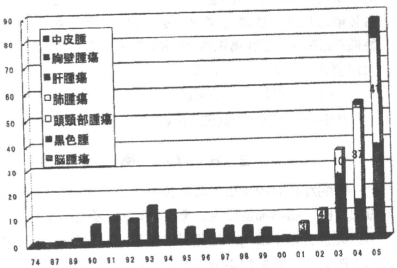

Fig. 4 Cases of BNCT at Kyoto University reactor institute (authorized by Prof. K. Ono at KUR BNCT research group).

6. 次世代ホウ素デリバリーシステム

さて，BNCTにおいて ^{10}Bを含む分子を如何にしてがん細胞にのみ選択的に高濃度で送り込むかが治療効果の決め手となることは云うまでもない。実際の臨床では，BSHとL体BPAを併用して，腫瘍内ホウ素濃度が25～100 ppm，腫瘍/血液のホウ素濃度比ならびに腫瘍/正常組織のホウ素濃度比が2～3で行われている。はじめに述べたように，BNCTの望まれる条件（腫瘍内ホウ素濃度が30 ppm以上でなお且つ，腫瘍/血液ならびに腫瘍/正常組織のホウ素濃度比が5以上）を達成するために，さまざまなホウ素キャリアーの開発研究が行われてきた。

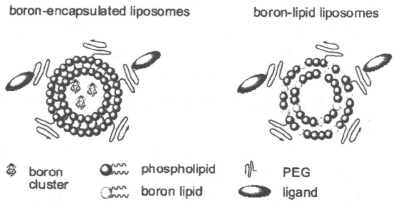

Fig. 5 Boron-encapsulated liposomes and boron-lipid liposomes

ホウ素キャリアーに望まれることは，(i) 500 mg/kg 程度の濃度で投与が可能なくらい毒性が低いこと，(ii) 十分に水溶性であること，(iii) 腫瘍細胞への蓄積が選択的であること，が挙げられる。これらの条件を満たすためには，従来の抗がん剤開発とは全く異なるアプローチが必要になってくる。我々は，リポソーム DDS（drug delivery system）を用いたホウ素デリバリーがこれらの条件を満たす有力なアプローチであると考え研究を進めてきた。

リポソーム DDS を用いたホウ素デリバリーの方法として Fig. 5 に示すように，大きく 2 つの戦略に分けられる。一つは，ホウ素薬剤をリポソーム内に封入する方法である。この方法は，一般的なリポソームを用いた DDS を応用するものであり，BSH などのホウ素化合物を封入する。もう一つの方法として，我々はホウ素をリポソーム膜に埋め込む方法を考えた。この方法では，リポソーム内にさらに抗がん剤などの薬剤を封入することができるため，化学療法との複合治療が期待できる。いずれの場合も，リポソーム膜を PEG 化することで EPR（enhanced permeability and retension）効果を高めたり[13,14]，さまざまな分子をリポソーム膜に結合させることにより，能動的にターゲティングできるような機能を持たせることが可能となってきた。

6.1. ホウ素薬剤内封型リポソーム

リポソームを用いたホウ素デリバリーは，1991 年に東大の柳衛らによって最初に報告された[15]。彼らは，エッグ PC（phosphatidylcholine），コレステロール，DTP-DPPE（3-(2-pyridyldithio)propionyl-dipalmitoylphosphatidylethanolamine）(1:1:0.05) からリポソームを調製し，BSH を封入した後，そのリポソームと anti-human CEA（carcinoembryonic antigen）モノクロナール抗体を結合させ，BSH 内封イムノリポソームを合成した。AsPC-1（ヒト膵臓がん）細胞を移植したヌードマウスに対し，BSH 封入イムノリポソームをホウ素濃度およそ 7.8 mg/kg（マウスの体重 20 g と仮定して計算）投与し 1 時間後に中性子照射したマウスでは，腫瘍増殖が 50% 以下に抑えられた[16]。

1992 年，Hawthorne らは DSPC（distearoyl phosphatidylcholine）とコレステロールを用いて，BSH だけでなくさまざまなホウ素イオンクラスターを封入したリポソームを報告した。それらの中でも $Na_3[1\text{-}(2'\text{-}B_{10}H_9)\text{-}2\text{-}NH_3B_{10}H_8]$ を内封した PEG 化リポソームを，EMT6 細胞を移植した BALB/c マウスに対し，ホウ素濃度 22 mg/kg で投与したところ，血液中ホウ素濃度は 6 時間後で 87.2 ppm，48 時間後でも 19.3 ppm であった。一方，腫瘍内ホウ素濃度は，6 時間後で 27.4 ppm であったのに対し，48 時間後では 46.7 ppm と時間の経過とともに EPR 効果によりホウ素が蓄積することがわかった。また 48 時間後のホウ素濃度の腫瘍組織/血液（T/B）比は 2.4 であった[17,18]。

Lee らはさらに腫瘍組織部位に集積したホウ素封入リポソームを細胞選択的にかつ能動的に取り込ませるために，表面に葉酸修飾したホウ素封入リポソームを開発した[19]。葉酸結合タンパクである folate receptor（FR）は正常組織では非常に限られた部位でのみ発現している糖タンパクである一方，多くのがんにおいて過剰発現が認められている[20]。葉酸はこの FR に対し，非常に高い結合力（$K_a = \sim 10^{10}\ M^{-1}$）を有するため，表面に葉酸が結合したリポソームは FR を過剰発現している卵巣がんなどに選択的に取り込まれることが報告されている。葉酸を結合させたリポソーム内に BSH を封入し，培養細胞である KB 細胞に 2 時間接触させたところ，細胞内ホウ素濃度は培地中のホウ素濃度依存的に高くなり，同条件下で葉酸を加えた場合，細胞内ホウ素濃度が低下したことから，葉酸結合型ホウ素リポソームの細胞内取り込みが，葉酸受容体である FR を介していることが示唆されている。

Kullberg らは，ホウ素化合物をリポソームに封入した EGF 結合型ホウ素リポソームを開発した[21]。細胞増殖因子の 1 つである EGF（上皮細胞増殖因子）は，細胞表面に発現しているその受容体と結合して，細胞増殖シグナルを伝達する。この EGF 受容体は多くの腫瘍細胞表面で高発現している。そこで，EGF をリポソーム表面に結合させることにより，この EGF－EGF 受容体の相互作用を利用して，腫瘍細胞への能動的なターゲティングを狙うものである[22]。いずれの場合も，動物実験に関してはまだ報告されていない。

丸山らは，Transferrin（TF）－TF 受容体の相互作用を利用して，TF をリポソーム表面に結合させた TF－PEG リポソームを開発し，腫瘍細胞への能動的なターゲティングに成功した[23]。TF は，血液中に 2.5 mg/ml 含まれ

ている鉄輸送タンパクであり，細胞表面に発現しているTF受容体と結合することにより，細胞内に鉄を送り込むが，腫瘍細胞の多くでこのTF受容体が高発現している。Colon 26マウス大腸がん細胞を移植したマウスを用いた生体内分布実験では，血中ホウ素濃度はBSHをそれぞれ封入したTF－PEGリポソームおよびTF－PEGリポソームの両方とも経時的に低下した。一方，腫瘍内ホウ素濃度はPEGリポソームでは48時間後は35 ppmに到達し，その後時間の経過とともに低下していき，72時間後には20 ppmとなったのに対し，TF－PEGリポソームの場合72時間後においてもおよそ35 ppmと高い蓄積性を示した。また，ホウ素濃度のT/N比は，48時間でホウ素濃度のT/N比は2.5，72時間後では6.0と非常に高い値が得られた。さらに，ホウ素濃度20 mg/kg投与した担がんマウスに対し，72時間後に中性子照射を行ったところ，中性子照射100日後ではPEGリポソームを投与したマウスの生存率は20%であったのに対し，TF－PEGリポソームを投与したマウスの生存率は70%とTFを結合したことによる能動的ターゲティング効果が顕著に見出された。

また，増永・小野らはこのTF－PEGリポソーム技術を応用して，$Na_2B_{10}H_{10}$（GB）を封入したTF－PEGリポソームのSCC VIIマウス扁平上皮がん細胞に対するBNCT効果を検討した[24]。彼らは，増殖期にある細胞（P-cell）だけでなく静止状態の細胞（Q-cell）に対するBNCT効果を ***in vitro*** で検討したところ，TF－PEGリポソームの方がPEGリポソームよりも効果的であり，Q-cellにも有効性が見出された。さらに，SCC VII細胞を移植したマウスを用いてdecahydrodecaborate（GB）およびBSHを封入したTF－PEGリポソームの生体内ホウ素分布を調べたところ，腫瘍内ホウ素濃度は投与後（投与ホウ素濃度：35 mg/kg）24時間で蓄積量が最大となり，BSH封入TF－PEGリポソームでは21.1 ppmであったのに対し，GB封入TF－PEGリポソームでは35.6 ppmとGB封入リポソームの方が腫瘍集積性が高いことがわかった。しかしながら，T/B比はいずれの場合もおよそ0.5と，血中の方がホウ素濃度が高い結果となった。

6.2. ホウ素脂質型リポソーム

このように，多面体構造のホウ素クラスターイオンを封入したリポソームを用いて，高い治療効果が達成できる可能性が示されてきた。しかしながら，使用されているホウ素封封入リポソームは非常に高いイオン濃度であり高浸透圧的な溶液であることから，これ以上の高濃度化は困難であると同時に，このような条件下でのリポソーム膜安定性の問題が生じている。一方，リポソームの脂質二分子膜は，分子間相互作用により自己集合化しているため密度が高く，この二分子膜へホウ素分子を導入できれば，非常に高濃度でホウ素をデリバリーできると考えられる。さらに，リポソーム膜内にホウ素を導入させることで，リポソーム内に抗がん剤などさまざまな薬剤が封入できることから，BNCTと化学療法の複合治療が可能となる。

リポソーム膜内にホウ素を導入したホウ素リポソームの最初の報告は，Hawthorneらによって開発された一本鎖ホウ素イオンクラスター脂質1（Fig. 6）を用いたものであった[25]。この化合物は炭素鎖16の脂溶性部位と水溶性の ***nido*** 型カルボラン部位からなる両親媒性分子である。彼らは，DSPC，コレステロール，***nido*** 型カルボラン脂質1からリポソームを調製した。EMT6細胞を移植したマウスを用いて生体内ホウ素分布を調べたところ，投与ホウ素濃度6 mg/kgでは腫瘍内ホウ素濃度が投与後6時間で22 ppm，その後16～30時間はおよそ34 ppmで一定であった。48時間後には25 ppmに低下したもの

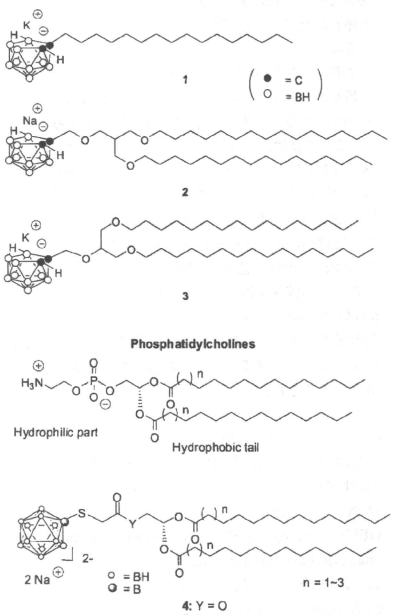

Fig. 6 Structures of ***nido***-carborane lipids 1-3 and closo-dodecaborate lipids 4 and 5.

のT/N比は8.4であった。

一方，我々はリポソーム膜へ効率良くかつ安定に生成するためには，二分子膜を形成しているリン脂質のように脂溶性部位が二本鎖であれば良いと考えた。そこで，二本鎖ホウ素イオンクラスター脂質2を設計した[26]。電子顕微鏡で確認したところ，合成したイオン性ホウ素クラスター脂質2から150～200 nmの大きさのベシクルが形成していることが分かった。これは世界で初めてのホウ素脂質ベシクルである。

このように安定なベシクルを形成することが分かったので，このホウ素イオンクラスター脂質2とDSPC，コレステロールを用いて，能動的ターゲティングを指向したTF結合型ホウ素クラスターリポソームを合成し，担癌マウスを用いた体内分布ならびに中性子捕捉治療を行った[27]。ホウ素クラスターリポソームおよびトランスフェリン修飾型ホウ素クラスターリポソームを用いてマウス内での各臓器における分布を経時的に測定したところ，血液中の濃度変化はトランスフェリン修飾型ホウ素クラスターリポソームおよび非修飾型ホウ素クラスターリポソームともに速やかに低下した。一方，肝臓・腎臓・脾臓ではトランスフェリン修飾型ホウ素クラスターリポソームの方がより高濃度で蓄積していることがわかった。肺では両者とも血中濃度の低下に伴って低下することがわかった。興味深いことに，腫瘍ではトランスフェリン非修飾型ホウ素クラスターリポソームが時間に伴って濃度が減少しているのに対し，トランスフェリン修飾型ホウ素クラスターリポソームでは時間経過と関係なく蓄積しており，72時間後でもトランスフェリン非修飾型ホウ素クラスターリポソームのおよそ3倍の濃度であることがわかった。

次に，担癌マウスを用いて中性子捕捉治療効果について検討した。左足にColon 26細胞を移植したBALB/cマウス（生後6週間，16～18 g）にトランスフェリン修飾型ホウ素クラスターリポソームを^{10}B濃度で7.2 mg/kg，14.4 mg/kgそれぞれ静脈投与し，72時間後各臓器を分画しホウ素濃度をプロンプトγ法により測定した。7.2 mg/kg投与したマウスでは，72時間後，筋肉・心臓・脳ではホウ素蓄積はほとんど見られなかった。肺・血液ではおよそ10 ppm，脾臓・肝臓では非常に高いホウ素蓄積が見られた。腫瘍内ホウ素蓄積量を見てみると7.2 mg/kg投与した場合では22 ppm，14.4 mg/kg投与の場合では40 ppmであった。

さらに，トランスフェリン修飾型ホウ素クラスターリポソームを^{10}B濃度で7.2 mg/kg投与した担癌マウスを72時間後，京都大学原子炉において中性子照射した。照射後の生存曲線をFig. 7(b)に示したが，ホウ素クラスターリポソームを投与していないマウスでは，中性子照射後の平均寿命が22日であったのに対し，ホウ素クラスターリポソームを^{10}B濃度で7.2 mg/kg投与したマウスでは，平均寿命32日とおよそ1.5倍延命効果が見られた。

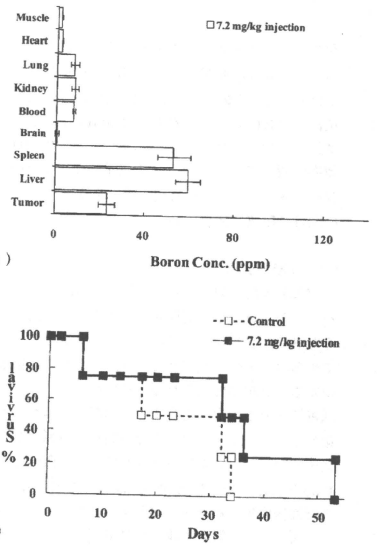

Fig. 7 (a) Boron concentration in various tissues at 72h after administration. (b) Survival curve of tumor-bearing mice after neutron irradiation. The mice were injected with 7.2 mg ^{10}B/kg of the Tf(+)-PEG-CL-liposome and incubated for 72 h before irradiation. Control indicates survival rates of tumor-bearing mice after neutron irradiation without administration of Tf(+)-PEG-CL liposomes.

これらの結果は，米国NCI（National Cancer Institute）のNanotech News for Cancer Therapyで紹介された[28]。最近，Hawthorneらも同様な二本鎖ホウ素イオンクラスター脂質3（Fig. 6）を開発している[29]。

このように，二本鎖ホウ素イオンクラスター脂質は安定なホウ素リポソームを形成し，腫瘍へも効率よく集積することが分かった。しかしながら，ホウ素濃度で14.4 mg/kg投与した場合に急性毒性が一部のマウスに見られたことから，我々はより低毒性なホウ素脂質の開発を目指し次世代ホウ素イオンクラスター脂質4および5（Fig. 6）を設計した[30]。この脂質は，脂溶性部位に生体リン脂質（Phosphatidylcholines）と同じ立体構造を有して