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

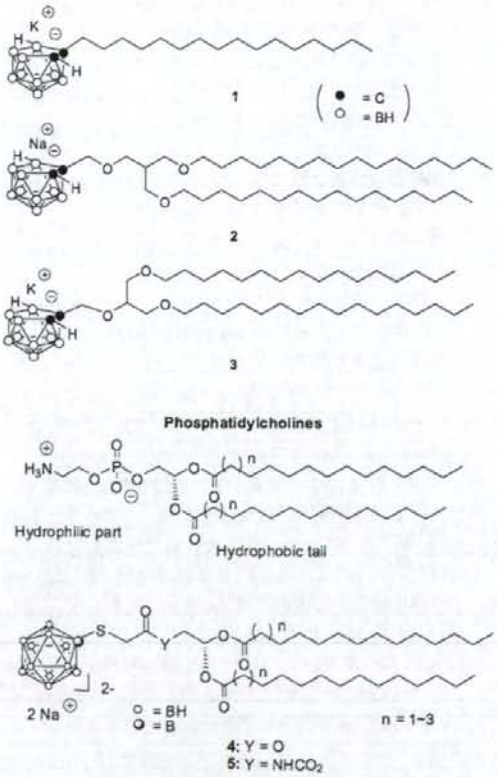

Fig. 6 Structures of *nido*-carborane lipids 1-3 and closo-dodecaborate lipids 4 and 5.

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。

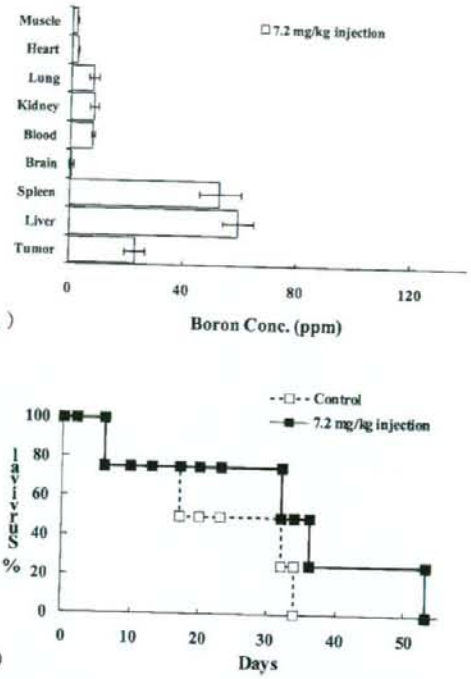

Fig. 7 (a) Boron concentration in various tissues at 72h after administration. (b) Survival curve of tumor-bearing mice after neutron irradiation. The mice were injected with 7.2 mg ^{10}B/kg of the Tf(+)-PEG-CL-liposome and incubated for 72 h before irradiation. Control indicates survival rates of tumor-bearing 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

おり，リンカー部位にエステル基（脂質4）またはカルバメート基（脂質5）を有し，BSHとSを介して結合している。これらのホウ素脂質から調整したリポソームは，正常マウスに対しホウ素濃度で20 mg/kgでは急性毒性は見られなかった。現在，NEDO「次世代DDS型悪性腫瘍治療システム」開発事業（平成17～19年度，プロジェクトリーダー：松村明教授・筑波大学）にて実用化に向けて研究を進めている。

6.3. 今後の展望

BNCTのためのホウ素キャリアーの開発には，いわゆるナノモルレベルで薬理効果が要求される抗がん剤のようなドラッグデザインではなく，ミリモルレベルで投与できるのに十分な低毒性であり，なおかつ腫瘍細胞に集積することが必要とされる。そのために，ここ十数年で低毒性小分子ホウ素化合物の開発だけでなく，本稿でも紹介したようにリポソームを用いたホウ素デリバリーシステムの開発が盛んに研究されてきた。BNCTにおいて1950年代に開発されたBSH，BPAという2剤以外には，まだ臨床応用されたホウ素薬剤は残念ながら登場してない。現在，核燃料の問題からBNCTに適応できる小型加速器の開発が精力的に行われている。熱中性子源が原子炉から加速器に移行できれば都市部病院併設型加速器によるBNCTが可能となることから，将来放射線療法の一般的治療法の一つになるであろう。そのためにも治療効果の高いホウ素デリバリーシステムの開発が期待される。

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AUTHOR'S PROOF

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Synthesis of *closo*-Dodecaboryl Lipids and their Liposomal Formation for Boron Neutron Capture Therapy

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Abstract High accumulation and selective delivery of boron into tumor tissues are the most important requirements to achieve efficient neutron capture therapy of cancers. We focused on liposomal boron delivery system to achieve a large amount of boron delivery to tumor. We succeeded in the synthesis of the double-tailed boron cluster lipids **4a–c** and **5a–c**, which has a $B_{12}H_{11}S$-moiety as a hydrophilic function, by *S*-alkylation of $B_{12}H_{11}SH$ with bromoacetyl and chloroacetocarbamate derivatives of diacylglycerols. Size distribution of liposomes prepared from the boron cluster lipid **4b**, dimyristoylphosphatidylcholine, polyethyleneglycol-conjugated distearoylphosphatidylethanolamine, and cholesterol was determined as 100 nm in diameter by an electrophoretic light scattering spectrophotometer. Calcein-encapsulation experiments revealed that these boronated liposomes are stable at 37 °C in fetal bovine serum solution for 24 h.

Keywords *closo*-dodecaborate · boron ion cluster lipid · boron neutron capture therapy · liposome · mercaptoundecahydrododecaborate · BSH

Introduction

Boron neutron capture therapy (BNCT) was first proposed as a binary approach to cancer treatment in 1936 [1]. This therapy is based on the capture reaction of thermal neutrons using no radioactive ^{10}B, which produces an α-particle and a lithium-7 nuclei ion with approximately 2.4 MeV. These high-linear energy transfer particles dissipate their kinetic energy before traveling one cell diameter (5–9 μm) in biological tissues ensuring their potential for precise cell killing [2–4]. Their destructive effect is highly observed in boron-loaded tissues. Therefore, the successful treatment of cancer by BNCT demands the selective and marked accumulation of ^{10}B in malignant tumor tissues. The amount of ^{10}B required to obtain fatal tumor cell damage has been calculated to be more than 30 μg/g of tumor tissue, owing to the low contact probability between thermal neutrons and ^{10}B [5]. Therefore, the marked accumulation and selective delivery of boron into tumor tissues are the most important requirement to achieve an effective BNCT of cancers. Although mercaptoundecahydrododecaborate (BSH) [6, 7] and L-4-dihydroxyboronylphenylalanine [8, 9] have been utilized for BNCT, new boron-10 carriers that deliver an adequate concentration of ^{10}B atoms to tumors should be developed to achieve a potent and extensive cancer therapy [10]. Recent promising approaches that meet these requirements entail the use of small boron molecules [11–16], such as porphyrins [17–22], nucleosides [23–28], and amino acids [29–39], and boron-conjugated biological complexes, such as monoclonal antibodies [40–46], epidermal growth factors [47–50], and carborane oligomers [51–55].

Recently, Maruyama and co-workers have developed transferrin-loaded (Tf) Polyethyleneglycol (PEG) liposomes as a new type of target-sensitive liposome [56–60]. PEG units have a heightened effect of prolonged residence time in the circulation and escaping ability from reticulo-endothelial-system (RES) uptake, resulting in an enhanced extravasation of liposomes into solid tumor tissues. Furthermore, this liposome is internalized into tumor cells by receptor-mediated endocytosis and taken up into endosome-like intracellular vesicles. They applied this

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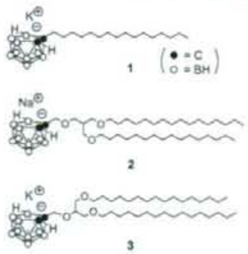


Fig. 1 The *nido*-carborane as a hydrophilic moiety into the amphiphile **1**

"active targeting" system to the selective boron delivery for BNCT. In this system, boron compounds are encapsulated in liposomes and delivered to tumor tissues [61].

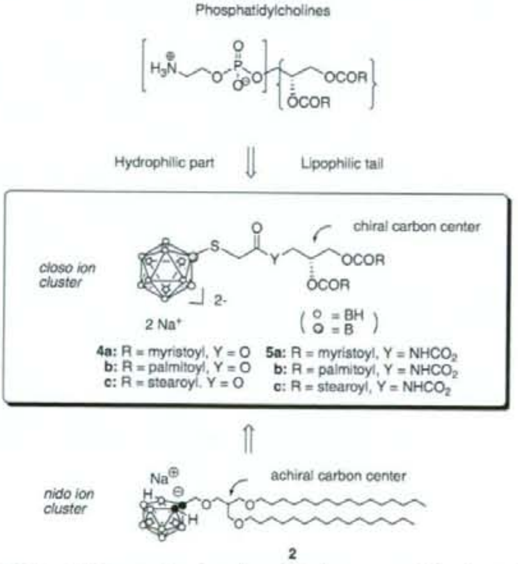
As an alternative boron delivery system, a system involving the accumulation of boron in the liposomal bilayer is highly potent because drugs can be encapsulated into the vacant inner cell of a liposome. Furthermore, functionalization of liposomes is possible by combination of lipid contents. Therefore, 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** (Fig. 1) and examined liposomal boron delivery in mice using **1** and distearoylphosphatidylcholine (DSPC) [62, 63]. We recently developed the *nido*-carborane lipid **2**, which has a double-tailed moiety conjugated with *nido*-carborane as a hydrophilic function [64]. This lipid has a symmetric carbon in the lipophilic alkyl chain. 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 ^{10}B/g tumor by the injection of the liposomes at 7.2 mg ^{10}B/kg body weight with longer survival rates of tumor-bearing mice after BNCT [65]. However, the injection of higher boron concentrations resulted in mortality of mice. We thought that this high toxicity may be due to a structure of "*nido*-type" carborane cage, although the cytotoxic mechanism of *nido*-carborane has not been studied in detail. 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 [66].

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 "*closo*-type" anion cluster and 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 difficulty of their functionalizations [67–71]. Gabel and co-workers [72] reported a novel "BSH-activation" method, which enabled us to conduct *S*-alkylation of BSH under mild conditions. Our design of the boron lipids is based on biomimetic composition of phosphatidylcholines combined with the *closo*-type boron anion cluster to meet a sufficiently low toxic requirement [73, 74]. In this paper, we describe full accounts of the synthesis of *closo*-dodecaborate containing boron lipids **4a–c** and **5a–c** and their liposomal property (Scheme 1).

Results and Discussion

Synthesis of closo-Dodecaborate Containing Boron Lipids **4a–c** *and* **5a–c** Synthesis of the hydrophobic tail functions of **4** is shown in Scheme 2. Reaction of the chiral alcohol **6** with 1.2 equiv 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 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 chloroacetyl isocyanate followed by deprotection of the acetal group; however, the chloroacetylcarbamate moiety decomposed under the acidic condition of *p*-TsOH in



Scheme 1 Scheme showing *closo*-dodecaborate containing boron lipids **4a–c** and **5a–c** and their liposomal property Q2

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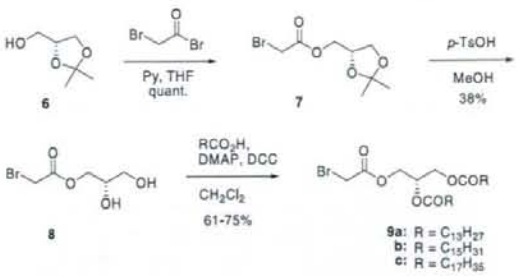


Q2 **Scheme 2** Synthesis of the hydrophobic tail functions of **4**

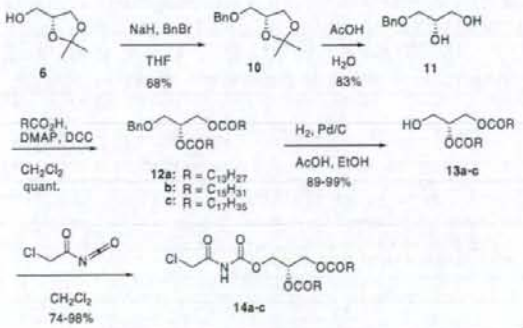
MeOH. Therefore, **6** was protected with benzylbromide using NaH, and the resulting dioxolane **10** was converted into the diol **11** using aqueous AcOH in 83% yield. The ester formation of **11** with various carboxylic acids was carried out in a similar manner to give **12a–c**, quantitatively. 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 (**17**)," which was developed by Gabel and co-workers [72]. As shown in Scheme 4, BSH was treated with 2-iodopropionitrile (2 equiv.) to give the monocation **16**, which underwent the dealkylation in the presence of base such as tetramethylammonium hydroxide to afford **17** in 80% yield.

Finally, *S*-alkylation of **17** 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, the **5a–c** were obtained from **12a–c** in 54–83% yields. Ion-exchange reactions Q4 proceeded readily to afford their sodium salts (Scheme 5).

Size Distributions of the Boronated Liposomes We examined formation 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 **4b** (1:1–*X*:0.1:*X*=0–1, molar ratio) by the reverse-phase evaporation (REV) method [75]. The liposomes obtained were subjected to extrusion 10 times through a polycarbonate membrane of a 100 nm pore size, using an extruder devise 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 phosphate-buffered saline (PBS) buffer. Liposome size was measured with an electrophoretic light scattering spectrophotometer. The size distribution of the liposomes composed of **4b** before and after extrusion is shown in Fig. 2. Interestingly, increase of the contents of **4b** in liposome resulted in the wider size distribution, and two major maximum distributions were observed in the liposome with 100% boron lipid **4b** (Fig. 2d). The sizes of maximum 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 of polydispersity index values, respectively.

We next investigated the time-dependent stability of the boronated lipsomes of **4b** in a 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, the release of aqueous contents of liposomes can be monitored by an increase of fluorescent intensity [76]. 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, 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 Fig. 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 DSPC, cholesterol, PEG-DSPE, and the *nido*-carborane lipid **2** (*X*=0.5, Fig. 3d). These results indicate that the current boron lipids are more suitable for stable liposomal formation than the previous *nido*-carborane lipid **2** presumably due to the similar structure of lipophilic double tail moiety to the natural phospholipids. As the boron lipid **4b** has a similar chirality to phosphatidylcholines in the lipophilic double tail moiety, both lipids would tend to pack together densely and thus form stable liposomes.



Scheme 3 Synthesis of the hydrophobic tail functions of **5** Q2

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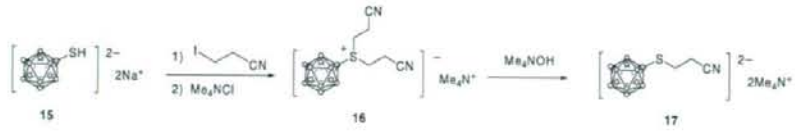
Q2 **Scheme 4** BSH treatment with 2-iodopropionitrile giving monocation **16** and dealkylation giving **17**



15 16 17

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. A key for the current synthesis is *S*-alkylation of the activated BSH with bromoacetyl and chloroacetocarbamate derivatives of diacylglycerols. The liposomes prepared from the boron cluster lipid **4b** were stable in FBS, although the liposome prepared from the *nido*-carborane lipid **2** was not stable at 50% concentration (X=0.5), presumably due to the similar structure of lipophilic double tail moiety to the natural phospholipids. *In vivo* biodistribution and BNCT studies of the boronated liposomes of **4** and **5** are in progress in our laboratory.

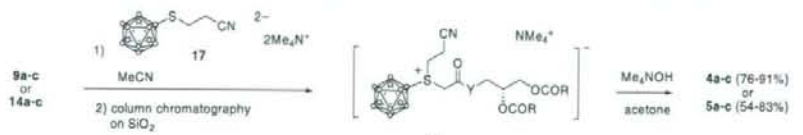
Experimental

General ^{1}H NMR, ^{13}C NMR, and ^{11}B NMR spectra were measured on a JEOL JNM-AL 300 (300 MHz) and Varian Unity-Inova 400 (400 MHz) spectrometers. Chemical shifts of ^{1}H NMR were expressed in parts per million downfield from $CHCl_3$ as an internal standard (δ=7.26) in $CDCl_3$. Chemical shifts of ^{13}C NMR were expressed in parts per million downfield from $CDCl_3$ as an internal standard (δ= 77.0) in $CDCl_3$. Analytical thin layer chromatography (TLC) was performed on glass plates (Merck Kieselgel 60 F_{254}, layer thickness 0.2 mm). Column chromatography was performed on silica gel (Merck Kieselgel 70–230 mesh). All reactions were carried out under argon atmosphere using standard Schlenk techniques. Most chemicals and solvents were of analytical grade and used without further purification. Infrared (IR) spectra were recorded on a Shimadzu FT-IR 8200A spectrometer. Mass spectrometry data were collected by Shimadzu LCMS-2010 EV spectrometer. $[B_{12}H_{11}SCH_2CH_2CN]^{2-}$·2($Me_4N^+$) was prepared from $Na_2B_{12}H_{12}$ according to the literature procedure [72].

Synthesis of ((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl 2-bromoacetate (7) To a stirred solution of (*R*)-(−)-2,2-dimethyl-1,3-dioxolane-4-methanol **6** (1.2 ml, 10.0 mmol) and pyridine (0.97 ml, 1.2 equiv) in 50 ml of THF, which was cooled to 0 °C, was added a bromoacetyl bromide (1.0 ml, 1.2 equiv) at 0 °C. The reaction temperature was maintained at 0 °C for 30 min, after which the reaction mixture was warmed slowly to room temperature. After being stirred for an additional 4 h, the solid was removed by filtration through celite, the filtrate was concentrated under reduced pressure, and the product was purified by flash column chromatography eluting EtOAc/hexane (1:1). Yield: 2.5 g (10.0 mmol, >99%) of colorless oil. $[\alpha]_D^{28}$= +2.7(*c*1.0, $CHCl_3$); IR (KBr pellet, cm^{-1}) ν(C=O) 1743, ν(C–H) 2,889, 2,939, 2,960, 2,988; ^{1}H NMR (ppm, $CDCl_3$) δ 1.38 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 3.78 (dd, 2H, $^2J_{C\text{-}H}$=8.4 Hz, $^3J_{C\text{-}C\text{-}H}$=6.0 Hz, OCH_2CH), 3.89 (s, 2H, CH_2Br), 4.10 (dd, 2H, $^2J_{C\text{-}H}$= 8.4 Hz, $^3J_{C\text{-}C\text{-}H}$=6.4 Hz, OCH_2CH), 4.19 (dd, 2H, $^2J_{C\text{-}H}$= 11.4 Hz, $^3J_{C\text{-}C\text{-}H}$=6.4 Hz, $CHCH_2OC(=O)CH_2Br$), 4.26 (dd, 2H, $^2J_{C\text{-}H}$=11.4 Hz, $^3J_{C\text{-}C\text{-}H}$=4.8 Hz, $CHCH_2OC(=O)CH_2Br$), 4.33–4.38 (m, 1H, CH); ^{13}C NMR (ppm, $CDCl_3$) δ 25.3, 26.6, 32.2, 66.0, 73.2, 99.9, 167.0; Anal. Calcd for $C_8H_{13}O_4Br_1$: C, 37.96; H, 5.18. Found: C, 38.58; H, 5.19.

Synthesis of (S)-2,3-Dihydroxypropyl 2-Bromoacetate (8) To a stirred solution of compound **7** (2.53 g, 10.0 mmol) in 20 ml of methanol was added a catalytic TsOH (0.17 g, 0.1 equiv). The reaction mixture was stirred for 30 min at room temperature. The resulting solution was concentrated to dryness, and the residue was dissolved in CH_2Cl_2. After drying (Na_2SO_4) and filtration through celite, the filtrate was concentrate under reduced pressure, and the product was purified by flash column chromatography eluting EtOAc/hexane (2:1). Yield: 0.81 g (3.8 mmol, 38%) of pale yellow oil. $[\alpha]_D^{28}$= +0.75 (*c* 1.0, $CHCl_3$); IR (KBr pellet, cm^{-1}) ν(C=O) 1734, ν(C-H) 2,887, 2,958, ν(O–H) 3,335; ^{1}H NMR (ppm, $CDCl_3$) δ 3.65 (dd, 2H, $^2J_{C\text{-}H}$=11.2 Hz, $^3J_{C\text{-}C\text{-}H}$=6.0 Hz, $HOCH_2CH$), 3.75 (dd, 2H, $^2J_{C\text{-}H}$= 12.2 Hz, $^3J_{C\text{-}C\text{-}H}$=4.0 Hz, $HOCH_2CH$), 3.88 (s, 2H, CH_2Br), 3.97–4.03 (m, 1H, CH), 4.25 (dd, 2H, $^2J_{C\text{-}H}$=11.4 Hz,

Q2 **Scheme 5** Synthesis of **9a–c** or **14a–c** with purification by column chromatography on SiO_2



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Q3 Q5

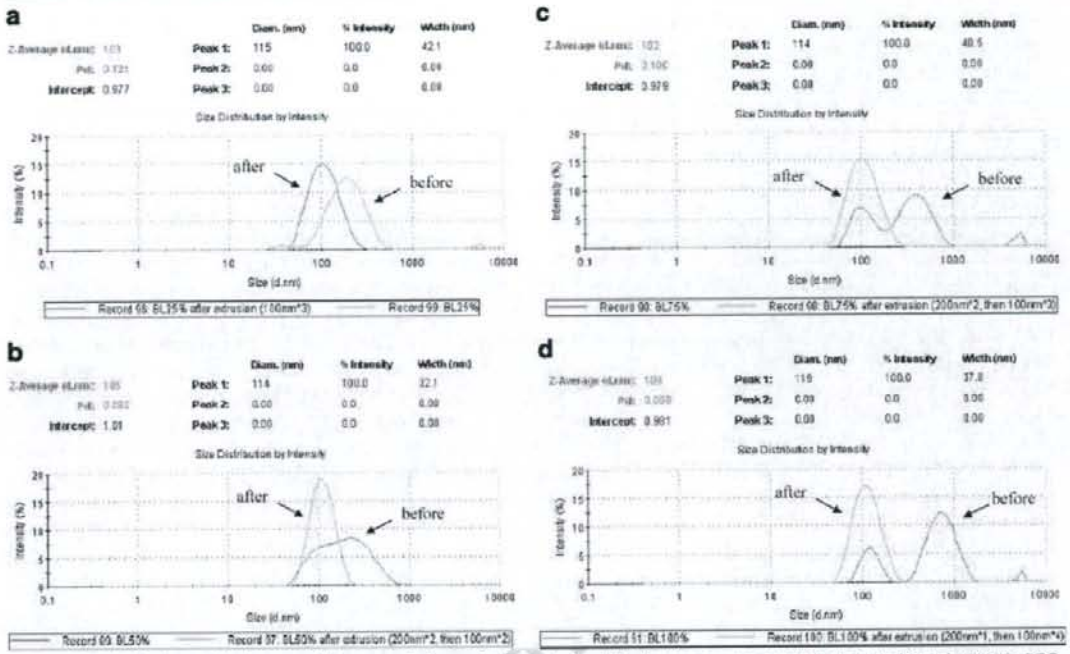

Fig. 2 Size distributions of the boronated liposomes before and after extrusion. The liposomes were prepared from cholesterol, DMPC, PEG-DSPE, and **4b**, (1:1–*X*:0.1:*X*, molar ratio). **a** *X*=0.25, **b** *X*=0.5, **c** *X*=0.75, **d** *X*=1.0

$^{3}J_{C\text{–}C\text{–}H}$=6.4 Hz, $CHCH_2OC(=O)CH_2Br$), 4.31 (dd, 2H, $^{2}J_{C\text{–}H}$=11.6 Hz, $^{3}J_{C\text{–}C\text{–}H}$=4.4 Hz, $CHCH_2OC(=O)CH_2Br$); ^{13}C NMR (ppm, $CDCl_3$) δ 25.8, 63.1, 66.6, 69.7, 167.7; Anal. Calcd for $C_5H_9O_4Br_1$: C, 28.19; H, 4.26. Found: C, 28.15; H, 4.24.

General Procedure for Synthesis of 1,2-O-diacyl-3-O-bromoacetyl-sn-glycerols **9** To a stirred solution of 3-*O*-bromoacetyl-*sn*-glycerol **8** (0.64 g, 3.0 mmol), DMAP (0.07 g, 0.2 equiv) in 50 ml of dry CH_2Cl_2 at 0 °C were added DCC (1.36 g, 2.2 equiv) and the carboxylic acid (2.2 equiv), and the resulting suspension was stirred for 12 h. The solid was removed by filtration through celite, the filtrate was concentrated under reduced pressure, and the product was purified by column chromatography using the solvent indicated as the eluent.

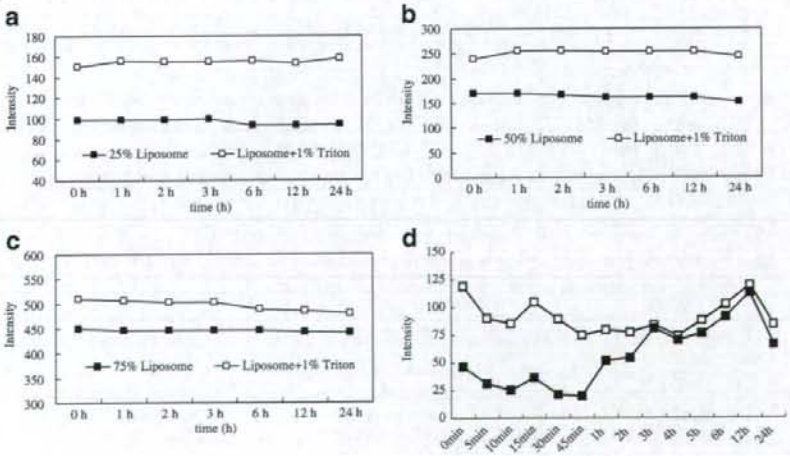

Fig. 3 Fluorescence intensities of calcein-encapsulated liposomes composed of the *closo*-dodecaborate lipid **4b** with various ratios (**a** *X*=0.25, **b**=0.5, **c**=0.75) and the *nido*-carborane lipid **2** (**d** *X*=0.5) in fetal bovine serum (*FBS*). The *white plots* show the fluorescence intensity of the FBS solution, and the *black plots* show that of the solution after destruction of liposomes by the addition of Triton X-100. The difference in fluorescence intensity between the *black and white plots* indicates calcein release from liposomes

3-*O*-bromoacetyl-1,2-*O*-dimyristoyl-*sn*-3-glycerol (**9a**) obtained as a white powder in 61% (1.15 g, 1.8 mmol) yield by column chromatography eluting with EtOAc/ hexane (1:20). Mp.: 30–31 °C; $[\alpha]_D^{28}=+0.65$ (*c* 1.0, $CHCl_3$); IR (KBr pellet, cm^{-1}) ν(C=O) 1746, ν(C–H) 2,855, 2,924, 2,957; ^1H NMR (ppm, $CDCl_3$) δ 0.88 (t, 6H, $^3J_{C\text{-}C\text{-}H}=$ 6.4 Hz, CH_3), 1.26 (s, 40H, CH_2), 1.59–1.63 [m, 4H, $CH_2CH_2C(=O)$], 2.32 [td, 4H, $^2J_{C\text{-}H}=8.0$ Hz, $^3J_{C\text{-}C\text{-}H}=$ 4.0 Hz, $CH_2C(=O)$], 3.84 (s, 2H, CH_2Br), 4.17 (dd, 2H, $^2J_{C\text{-}H}=11.8$ Hz, $^3J_{C\text{-}C\text{-}H}=6.0$ Hz, OCH_2CH), 4.26 (dd, 2H, $^2J_{C\text{-}H}=12.0$ Hz, $^3J_{C\text{-}C\text{-}H}=6.0$ Hz, $BrCH_2C(=O)OCH_2CH$), 4.31 (dd, 2H, $^2J_{C\text{-}H}=12.0$ Hz, $^3J_{C\text{-}C\text{-}H}=4.4$ Hz, OCH_2CH), 4.41 (dd, 2H, $^2J_{C\text{-}H}=11.8$ Hz, $^3J_{C\text{-}C\text{-}H}=4.4$ Hz, $BrCH_2C(=O)OCH_2CH$), 5.27–5.34 (m, 1H, *CH*); ^{13}C NMR (ppm, $CDCl_3$) δ 14.1, 22.7, 24.8, 25.2, 29.0, 29.1, 29.2, 29.4, 29.5, 29.6, 29.7, 31.9, 33.9, 34.0, 61.8, 63.9, 71.2, 166.8, 172.9, 173.2; Anal. Calcd for $C_{33}H_{61}O_6Br_1$: C, 62.54; H, 9.70. Found: C, 62.58; H, 9.70.

3-*O*-bromoacetyl-1,2-*O*-dipalmitoyl-*sn*-3-glycerol (**9b**) obtained as a white powder in 73% (1.51 g, 2.2 mmol) yield by column chromatography eluting with EtOAc/ hexane (1:20). Mp.: 39–40 °C; $[\alpha]_D^{28}=+0.45$ (*c* 1.0, $CHCl_3$); IR (KBr pellet, cm^{-1}) ν(C=O) 1,744, ν(C–H) 2,851, 2,918, 2,957; ^1H NMR (ppm, $CDCl_3$) δ 0.88 (t, 6H, $^3J_{C\text{-}C\text{-}H}=$ 6.8 Hz, CH_3), 1.26 (s, 48H, CH_2), 1.61–1.63 [m, 4H, $CH_2CH_2C(=O)$], 2.32 [td, 4H, $^2J_{C\text{-}H}=7.6$ Hz, $^3J_{C\text{-}C\text{-}H}=$ 4.0 Hz, $CH_2C(=O)$], 3.84 (s, 2H, CH_2Br), 4.17 (dd, 2H, $^2J_{C\text{-}H}=12.0$ Hz, $^3J_{C\text{-}C\text{-}H}=6.0$ Hz, OCH_2CH), 4.26 (dd, 2H, $^2J_{C\text{-}H}=12.0$ Hz, $^3J_{C\text{-}C\text{-}H}=6.0$ Hz, $BrCH_2C(=O)OCH_2CH$), 4.31 (dd, 2H, $^2J_{C\text{-}H}=12.0$ Hz, $^3J_{C\text{-}C\text{-}H}=4.4$ Hz, OCH_2CH), 4.41 (dd, 2H, $^2J_{C\text{-}H}=11.8$ Hz, $^3J_{C\text{-}C\text{-}H}=4.4$ Hz, $BrCH_2C(=O)OCH_2CH$), 5.27–5.32 (m, 1H, *CH*); ^{13}C NMR (ppm, $CDCl_3$) δ 14.1, 22.7, 24.8, 25.2, 29.0, 29.1, 29.2, 29.4, 29.5, 29.6, 29.7, 31.9, 33.9, 34.0, 34.1, 61.8, 63.9, 71.2, 166.8, 172.9, 173.2; Anal. Calcd for $C_{37}H_{69}O_6Br_1$: C, 64.42; H, 10.08. Found: C, 64.28; H, 10.06.

3-*O*-bromoacetyl-1,2-*O*-distearoyl-*sn*-3-glycerol (**9c**) obtained as a white powder in 75% (1.68 g, 2.3 mmol) yield by column chromatography eluting with EtOAc/ hexane (1:20). Mp.: 44–46 °C; $[\alpha]_D^{28}=+0.55$ (*c*1.0, $CHCl_3$); IR (KBr pellet, cm^{-1}) ν(C=O) 1,744, ν(C–H) 2,851, 2,918, 2,957; ^1H NMR (ppm, $CDCl_3$) δ 0.88 (t, 6H, $^3J_{C\text{-}C\text{-}H}=$ 6.8 Hz, CH_3), 1.26 (s, 56H, CH_2), 1.58–1.65 [m, 4H, $CH_2CH_2C(=O)$], 2.32 [td, 4H, $^2J_{C\text{-}H}=7.6$ Hz, $^3J_{C\text{-}C\text{-}H}=$ 4.0 Hz, $CH_2C(=O)$], 3.84 (s, 2H, CH_2Br), 4.17 (dd, 2H, $^2J_{C\text{-}H}=11.8$ Hz, $^3J_{C\text{-}C\text{-}H}=6.0$ Hz, OCH_2CH), 4.26 (dd, 2H, $^2J_{C\text{-}H}=12.0$ Hz, $^3J_{C\text{-}C\text{-}H}=6.0$ Hz, $BrCH_2C(=O)OCH_2CH$), 4.31 (dd, 2H, $^2J_{C\text{-}H}=12.0$ Hz, $^3J_{C\text{-}C\text{-}H}=4.4$ Hz, OCH_2CH), 4.41 (dd, 2H, $^2J_{C\text{-}H}=12.0$ Hz, $^3J_{C\text{-}C\text{-}H}=4.0$ Hz, $BrCH_2C(=O)OCH_2CH$), 5.27–5.32 (m, 1H, *CH*); ^{13}C NMR (ppm, $CDCl_3$) δ 14.1, 22.7, 24.8, 25.2, 29.0, 29.1, 29.2, 29.3, 29.4, 29.5, 29.6, 29.7, 31.9, 33.9, 34.0, 34.1, 61.8, 63.9, 68.5, 166.8, 172.8, 173.2; Anal. Calcd for $C_{41}H_{77}O_6Br_1$: C, 66.01; H, 10.40. Found: C, 66.22; H, 10.40.

*Synthesis of (R)-4-((Benzyloxy)Methyl)-2,2-Dimethyl-1,3-Dioxolane (***10***)* NaH (0.26 g, 11 mmol) was washed with hexane and dissolved in THF (20 ml). To this solution was added (*R*)-(−)-2,2-dimethyl-1,3-dioxolane-4-methanol **6** (1.2 ml, 10.0 mmol) at 0 °C and the reaction mixture was stirred for 30 min. Benzyl bromide (1.43 ml, 12 mmol) was then added at 0 °C, and the reaction mixture was warmed slowly to room temperature. After being stirred for an additional 2 h, the saturated NH_4Cl solution was added, then the product was extracted with Et_2O (100 ml×3). The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Purification by column chromatography on SiO_2 with EtOAc/hexane (1:10) gave **10** as colorless oil (2.12 g, 9.5 mmol, 95%). $[\alpha]_D^{28}=-19.8$ (*c* 1.0, $CHCl_3$); IR (KBr pellet, cm^{-1}) $\nu(C{=}C_{aryl})$ 698, 739, 1,053, 1,097, 1,371, 1,381, 1,454, ν(C–H) 2,866, 2,936, 2,988, 3,032; ^1H NMR (ppm, $CDCl_3$) δ 1.36 (s, 3H, CH_3), 1.42 (s, 3H, CH_3), 3.47 (dd, 2H, $^2J_{C\text{-}H}=9.8$ Hz, $^3J_{C\text{-}C\text{-}H}=$ 5.6 Hz, $CHCH_2O$), 3.55 (dd, 2H, $^2J_{C\text{-}H}=9.8$ Hz, $^3J_{C\text{-}C\text{-}H}=5.6$ Hz, $CHCH_2O$), 3.74 (dd, 2H, $^2J_{C\text{-}H}=9.8$ Hz, $^3J_{C\text{-}C\text{-}H}=6.4$ Hz, OCH_2CH), 4.05 (dd, 2H, $^2J_{C\text{-}H}=$ 8.0 Hz, $^3J_{C\text{-}C\text{-}H}=6.4$ Hz, OCH_2CH), 4.27–4.33 (m, 1H, *CH*), 4.57 (d, 2H, $^2J_{C\text{-}H}=6$ Hz, CH_2Ph), 7.25–7.34 (m, 5H, CH_2Ph); ^{13}C NMR (ppm, $CDCl_3$) δ 25.3, 26.7, 66.8, 71.0, 73.4, 74.7, 127.6, 127.7, 127.9, 128.3, 128.4, 137.9; Anal. Calcd for $C_{13}H_{18}O_3$: C, 70.24; H, 8.16. Found: C, 71.04; H, 8.18.

*Synthesis of (S)-3-(Benzyloxy)Propane-1,2-Diol (***11***)* ~~(Martin SF, et al. J Org Chem. 1994;59:4805–20)~~ To a Q6 solution of acetic acid (14 ml) and water (6 ml) was added **10** (2.0 ml, 9.0 mmol), and the reaction mixture was stirred at 65 °C for 1 h. The reaction mixture was neutralized with saturated aqueous $NaHCO_3$ solution (ca. 20 ml) and extracted with dichloromethane (20 ml×2). The organic layer was dried over anhydrous $MgSO_4$ and concentrated. Purification by column chromatography on SiO_2 with EtOAc/hexane (1:1) gave **11**, quantitatively (1.64 g, 9 mmol) as pale yellow oil. IR (KBr pellet, cm^{-1}) $\nu(C{=}C_{aryl})$ 700, 739, 1,036, 1,043, 1,074, 1,454, 1,497, ν(C–H) 2,870, 2,924, ν(O–H) 3,364; ^1H NMR (ppm, $CDCl_3$) δ 3.53 (dd, 2H, $^2J_{C\text{-}H}=9.8$ Hz, $^3J_{C\text{-}C\text{-}H}=6.0$ Hz, $CHCH_2O$), 3.57 (dd, 2H, $^2J_{C\text{-}H}=9.8$ Hz, $^3J_{C\text{-}C\text{-}H}=4.4$ Hz, $CHCH_2O$), 3.60–3.72 (m, 2H, $HOCH_2CH$), 3.86–3.92 (m, 1H, *CH*), 4.55 (s, 2H, CH_2Ph), 7.30–7.38 (m, 5H, CH_2Ph); ^{13}C NMR (ppm, $CDCl_3$) δ 63.9, 70.7, 71.6, 73.5, 127.7, 127.8, 128.4, 137.7; Anal. Calcd for $C_{10}H_{14}O_3$: C, 65.91; H, 7.74. Found: C, 65.25; H, 7.75.

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General Procedure for Synthesis of 1,2-O-Diacyl-3-O-Benzyl-sn-Glycerol (**12**) ~~*(Martin SF, et al. J Org Chem. 1994;59:4805–20)*~~ To a solution of **11** (1.82 g, 10 mmol) and DMAP (1.22 g, 10 mmol) in dichloromethane (50 ml) was added dicyclohexylcarbodiimide (6.19 g, 30 mmol), and the mixture was stirred for 30 min. A carboxylic acid was then added and the resulting suspension was stirred for 12 h. The solid was removed by filtration through celite, and the filtrate was concentrated under reduced pressure. Purification by column chromatography on SiO_2 with EtOAc/hexane (1:10) gave **12**.

1,2-*O*-Dimyristoyl-3-*O*-benzyl-*sn*-glycerol (**12a**) obtained as a white powder in quantitative yield. Mp.: 32–33 °C; IR (KBr pellet, cm^{-1}) ν(C=O) 1,736, ν(C–H) 2,853, 2,920, 2,955; ^{1}H NMR (ppm, $CDCl_3$) δ 0.88 (t, J=6.8 Hz, 6H, CH_3), 1.25 (s, 40H, CH_2), 1.56–1.63 (m, 4H, CH_2), 2.30 [td, 4H, $^2J_{C-H}$=16.8 Hz, $^3J_{C-C-H}$=7.6 Hz, CH_2C(=O)], 3.56–3.62 (m, 2H, $CHCH_2OBn$), 4.19 [dd, 2H, $^2J_{C-H}$=11.2 Hz, $^3J_{C-C-H}$=8.0 Hz, $CHCH_2C$(=O)], 4.34 [dd, 2H, $^2J_{C-H}$=12.0 Hz, $^3J_{C-C-H}$=4.0 Hz, $CHCH_2C$(=O)], 4.54 (d, 2H, $^{gem}J_{C-H}$=5.6 Hz, CH_2Ph), 5.22–5.27 (m, 1H, CH), 7.29–7.35 (m, 5H, C_6H_5); ^{13}C NMR (ppm, $CDCl_3$) δ 14.1, 14.2, 22.7, 24.9, 29.1, 29.3, 29.5, 29.7, 29.8, 32.0, 34.1, 34.4, 62.6, 68.3, 69.9, 73.3, 127.7, 128.5, 137.7, 173.2, 173.5; Anal. Calcd for $C_{38}H_{65}O_5$: C, 75.83; H, 10.88. Found: C, 75.70; H, 10.92.

1,2-*O*-Dipalmitoyl-3-*O*-benzyl-*sn*-glycerol (**12b**) obtained as a white powder in 91% yield. Mp.: 40–41 °C; IR (KBr pellet, cm^{-1}) ν(C=O) 1,736, ν(C–H) 2,851, 2,918, 2,955; ^{1}H NMR (ppm, $CDCl_3$) δ 0.88 (t, J=6.8 Hz, 6H, CH_3), 1.25 (s, 48H, CH_2), 1.56–1.63 (m, 4H, CH_2), 2.30 (td, 4H, $^2J_{C-H}$=16.8 Hz, $^3J_{C-C-H}$=7.6 Hz, CH_2C(=O)), 3.56–3.62 (m, 2H, $CHCH_2OBn$), 4.19 (dd, 2H, $^2J_{C-H}$=11.2 Hz, $^3J_{C-C-H}$=8.0 Hz, $CHCH_2C$(=O)), 4.34 (dd, 2H, $^2J_{C-H}$=12.0 Hz, $^3J_{C-C-H}$=4.0 Hz, $CHCH_2C$(=O)), 4.54 (d, 2H, $^{gem}J_{C-H}$=5.6 Hz, CH_2Ph), 5.22–5.27 (m, 1H, CH), 7.28–7.36 (m, 5H, C_6H_5); ^{13}C NMR (ppm, $CDCl_3$) δ 14.2, 22.7, 24.9, 25.0, 29.0, 29.1, 29.3, 29.4, 29.5, 29.6, 29.7, 31.9, 34.1, 34.3, 62.6, 68.2, 70.0, 73.3, 127.6, 127.7, 128.4, 137.7, 173.1, 173.4; Anal. Calcd for $C_{42}H_{73}O_5$: C, 76.66; H, 11.18. Found: C, 77.48; H, 11.13.

1,2-*O*-Distearoyl-3-*O*-benzyl-*sn*-glycerol (**12c**) obtained as a white powder in quantitative yield. Mp.: 49–50 °C; IR (KBr pellet, cm^{-1}) ν(C=O) 1734, ν(C–H) 2851, 2918, 2957; ^{1}H NMR (ppm, $CDCl_3$) δ 0.88 (t, J=6.8 Hz, 6H, CH_3), 1.25 (s, 56H, CH_2), 1.56–1.63 (m, 4H, CH_2), 2.30 [td, 4H, $^2J_{C-H}$=16.8 Hz, $^3J_{C-C-H}$=7.6 Hz, CH_2C(=O)], 3.56–3.62 (m, 2H, $CHCH_2OBn$), 4.19 [dd, 2H, $^2J_{C-H}$=11.2 Hz, $^3J_{C-C-H}$=8.0 Hz, $CHCH_2C$(=O)], 4.34 [dd, 2H, $^2J_{C-H}$=12.0 Hz, $^3J_{C-C-H}$=4.0 Hz, $CHCH_2C$(=O)], 4.54 (d, 2H, $^{gem}J_{C-H}$=5.6 Hz, CH_2Ph), 5.22–5.27 (m, 1H, CH), 7.29–7.35 (m, 5H, C_6H_5); ^{13}C NMR (ppm, $CDCl_3$) δ 14.1, 22.7, 24.9, 25.0, 29.0, 29.1, 29.3, 29.4, 29.5, 29.6, 29.7, 31.9, 34.1, 34.3, 62.3, 68.2, 70.0, 73.3, 127.6, 127.8, 128.4, 137.7, 173.1, 173.4; Anal. Calcd for $C_{46}H_{81}O_5$: C, 77.37; H, 11.43. Found: C, 77.96; H, 11.43.

Typical Procedure for Synthesis of 1,2-O-Dimyristoyl-sn-Glycerol (**13a**) ~~*(Martin SF, et al. J Org Chem. 1994;59:4805–20)*~~ A solution of 1,2-*O*-dimyristoyl-3-*O*-benzyl-*sn*-glycerol **12a** (6.0 g, 10.0 mmol), 5% Pd/C (600 mg), and glacial acetic acid (20 ml) in ethanol (100 ml) was stirred under hydrogen atmosphere at room temperature. The reaction progress was monitored by TLC. When the reaction was complete, the reaction mixture was diluted with CH_2Cl_2, and the catalysts were removed by celite filtration. The filtrate was evaporated under reduced pressure, and the crude product was purified by column chromatography on SiO_2 with the EtOAc/hexane (1:3) to give **13c**, quantitatively, as a white solid (5.1 g, 10.0 mmol). Mp.: 56–58 °C; IR (KBr pellet, cm^{-1}) ν(C=O) 1,707, 1,717, 1,732. ν(C–H) 2,851, 2,918, 2,957, ν(O–H) 3,649; ^{1}H NMR (ppm, $CDCl_3$) δ 0.88 (t, J=6.8 Hz, 6H, CH_3), 1.26 (s, 40H, CH_2), 1.56–1.63 (m, 4H, CH_2), 2.05 (t, 1H, J=6.4 Hz, CH_2OH), 2.32, [td, 2H, $^2J_{C-H}$=7.6 Hz, $^3J_{C-C-H}$= 7.6 Hz, CH_2CH_2C(=O)], 2.35 [td, 2H, $^2J_{C-H}$=7.2 Hz, $^3J_{C-C-H}$=7.6 Hz, CH_2CH_2C(=O)], 3.72–3.74 (m, 2H, $CHCH_2OH$), 4.24 [dd, 2H, $^2J_{C-H}$=12.0 Hz, $^3J_{C-C-H}$= 5.6 Hz, $CHCH_2C$(=O)], 4.32 [dd, 2H, $^2J_{C-H}$=12.0 Hz, $^3J_{C-C-H}$=4.4 Hz, $CHCH_2C$(=O)], 5.06–5.11 (m, 1H, CH); ^{13}C NMR (ppm, $CDCl_3$) δ 14.1, 22.7, 24.8, 24.9, 29.0, 29.1, 29.2, 29.3, 29.5, 29.6, 29.7, 31.9, 34.1, 34.3, 61.5, 62.0, 72.1, 173.4, 173.8; Anal. Calcd for $C_{31}H_{59}O_5$: C, 72.75; H, 11.62. Found: C, 72.42; H, 11.64.

1,2-*O*-Dipalmitoyl-*sn*-glycerol (**13b**) was synthesized from **12b** (6.6 g, 10.0 mmol) using the procedure described for **13a** to give **13b** (5.3 g, 93%) as a white solid. Mp.: 62–64 °C; IR (KBr pellet, cm^{-1}) ν(C=O) 1,697, 1,717, 1,734, ν(C–H) 2,851, 2,918, 2,957, ν(O–H) 3,649; ^{1}H NMR (ppm, $CDCl_3$) δ 0.88 (t, J=6.8 Hz, 6H, CH_3), 1.25 (s, 48H, CH_2), 1.58–1.66 (m, 4H, CH_2), 2.08 (t, 1H, J=6.4 Hz, CH_2OH), 2.32, [td, 2H, $^2J_{C-H}$=7.6 Hz, $^3J_{C-C-H}$=7.6 Hz, CH_2CH_2C (=O)], 2.35 [td, 2H, $^2J_{C-H}$=7.2 Hz, $^3J_{C-C-H}$=7.6 Hz, CH_2CH_2C(=O)], 3.71–3.74 (m, 2H, $CHCH_2OH$), 4.24 [dd, 2H, $^2J_{C-H}$=12.0 Hz, $^3J_{C-C-H}$=5.6 Hz, $CHCH_2C$ (=O)], 4.32 [dd, 2H, $^2J_{C-H}$=12.0 Hz, $^3J_{C-C-H}$=4.4 Hz, $CHCH_2C$(=O)], 5.06–5.11 (m, 1H, CH); ^{13}C NMR (ppm, $CDCl_3$) δ 14.1, 22.7, 24.8, 24.9, 29.0, 29.1, 29.2, 29.3, 29.4, 29.5, 29.6, 29.7, 31.9, 34.1, 34.3, 61.6, 61.9, 72.1, 173.4, 173.8; Anal. Calcd for $C_{35}H_{67}O_5$: C, 74.02; H, 11.89. Found: C, 74.17; H, 11.90.

1,2-*O*-Distearoyl-*sn*-glycerol (**13c**) was synthesized from **12c** (7.2 g, 10.0 mmol) using the procedure described for **13a** to give **13c** (5.6 g, 89%) as a white solid. Mp.: 71–72 °C; IR (KBr pellet, cm^{-1}) ν(C=O) 1,697, 1,717, 1,734, ν(C–H) 2,851, 2,918, 2,957, ν(O–H) 3,649; ^{1}H NMR (ppm,

$CDCl_3$) δ 0.88 (t, J=6.8 Hz, 6H, CH_3), 1.26 (s, 40H, CH_2), 1.56–1.63 (m, 4H, CH_2), 2.04 (t, 1H, J=6.4 Hz, CH_2OH), 2.33, [dd, 4H, $^2J_{C-H}$=16.6 Hz, $^3J_{C-C-H}$=8.0 Hz, CH_2CH_2C(=O)], 3.72–3.75 (m, 2H, $CHCH_2OH$), 4.24 [dd, 2H, $^2J_{C-H}$=12.0 Hz, $^3J_{C-C-H}$=5.6 Hz, $CHCH_2C$(=O)], 4.32 [dd, 2H, $^2J_{C-H}$=12.0 Hz, $^3J_{C-C-H}$=4.4 Hz, $CHCH_2C$(=O)], 5.06–5.11 (m, 1H, CH); ^{13}C NMR (ppm, $CDCl_3$) δ 14.1, 22.7, 24.8, 24.9, 29.0, 29.1, 29.2, 29.3, 29.4, 29.5, 29.6, 29.7, 31.9, 34.1, 34.3, 61.6, 61.9, 72.1, 173.4, 173.8; Anal. Calcd for $C_{39}H_{75}O_5$: C, 75.07; H, 12.11. Found: C, 75.01; H, 12.08.

*Typical Procedure for Synthesis of 3-O-chloroacetylcarbamoyl-1,2-O-dimyristoyl-sn-glycerols (*__14a__*)* To a solution of **13a** (5.1 g, 10.0 mmol) in CH_2Cl_2 (50 ml) was added
Q6 chloroacetyl isocyanate ~~(Speziale AJ, et al. J Org Chem. 1963, 28, 1805–11)~~ (1.4 g, 12.0 mmol) at 0 °C, and the mixture was stirred for 6 h at 40 °C. After the reaction mixture was concentrated under reduced pressure, the product was purified by column chromatography on SiO_2 with CH_2Cl_2/CH_3OH (50:1) to give **14a** (4.7 g, 74%) as a white solid: Mp.: 55–57 °C; $[\alpha]_D^{28}$= +2.1 (c1.0, $CHCl_3$); IR (KBr pellet, cm^{-1}) ν(C=O) 1,732, 1,786, ν(C–H) 2,851, 2,920, 2,955, ν(N–H) 3,280; ^1H NMR (ppm, $CDCl_3$) δ 0.88 (t, J=6.4 Hz, 6H, CH_3), 1.25 (s, 40H, CH_2), 1.57–1.61 (m, 4H, CH_2), 2.32 [td, 4H, $^2J_{C-H}$=11.2 Hz, $^3J_{C-C-H}$=7.6 Hz, 4H, CH_2C(=O)], 4.28 (dd, $^2J_{C-H}$=11.2 Hz, $^3J_{C-C-H}$=7.6 Hz, 2H, CH_2), 4.29 (dd, $^2J_{C-H}$=11.2 Hz, $^3J_{C-C-H}$=4.4 Hz, 2H, CH_2), 4.48 (s, 2H, CH_2Cl), 5.22–5.26 (m, 1H, CH), 7.92 (s, 1H, NH); ^{13}C NMR (ppm, $CDCl_3$) δ 14.1, 22.6, 24.8, 29.0, 29.1, 29.2, 29.3, 29.4, 29.6, 29.7, 31.9, 33.9, 34.1, 43.5, 61.6, 64.4, 68.0, 150.7, 166.5, 172.9, 173.2; Anal. Calcd for $C_{34}H_{62}N_1O_7Cl_1$: C, 64.58; H, 9.88; N, 2.22. Found: C, 64.26; H, 9.98; N, 2.27.

3-*O*-Chloroacetylcarbamoyl-1,2-*O*-dipalmitoyl-*sn*-glycerol (**14b**) was synthesized from **13b** (5.7 g, 10.0 mmol) using the procedure described for **14a** to give **14b** (5.9 g, 86%) as a white solid: Mp.: 64–65 °C; $[\alpha]_D^{28}$= +1.45 (c1.0, $CHCl_3$); IR (KBr pellet, cm^{-1}) ν(C=O) 1,736, 1,786, ν(C–H) 2,851, 2,918, 2,950, ν(N–H) 3,358; ^1H NMR (ppm, $CDCl_3$) δ 0.88 (t, J=6.4 Hz, 6H, CH_3), 1.28 (s, 48H, CH_2), 1.57–1.61 (m, 4H, CH_2), 2.33 [td, 4H, $^2J_{C-H}$=11.2 Hz, $^3J_{C-C-H}$=8.0 Hz, 4H, CH_2C(=O)], 4.30 (dd, $^2J_{C-H}$=12.0 Hz, $^3J_{C-C-H}$=4.8 Hz, 2H, CH_2), 4.32 (dd, $^2J_{C-H}$=12.0 Hz, $^3J_{C-C-H}$=4.4 Hz, 2H, CH_2), 4.49 (s, 2H, CH_2Cl), 5.25–5.29 (m, 1H, CH), 7.93 (s, 1H, NH); ^{13}C NMR (ppm, $CDCl_3$) δ 14.0, 22.6, 24.8, 29.0, 29.1, 29.2, 29.3, 29.4, 29.5, 29.6, 29.7, 31.9, 33.9, 34.1, 41.9, 43.6, 61.7, 64.4, 68.4, 150.9, 166.8, 169.1, 172.8, 173.2; Anal. Calcd for $C_{38}H_{69}N_1O_7Cl_1$: C, 66.40; H, 10.12; N, 2.04. Found: C, 66.44; H, 10.07; N, 2.04.

3-*O*-Chloroacetylcarbamoyl-1,2-*O*-distearoyl-*sn*-glycerol (**14c**) was synthesized from **13c** (6.3 g, 10.0 mmol) using the procedure described for **14a** to give **14c** (7.4 g, >99%) as a white solid: Mp.: 71–73 °C; $[\alpha]_D^{28}$= +1.35 (c1.0, $CHCl_3$); IR (KBr pellet, cm^{-1}) ν(C=O) 1,734, 1,767, 1,786, ν(C–H) 2,851, 2,918, 2,950, ν(N–H) 3364; ^1H NMR (ppm, $CDCl_3$) δ 0.88 (t, J=6.0 Hz, 6H, CH_3), 1.25 (s, 56H, CH_2), 1.57–1.61 (m, 4H, CH_2), 2.33 [td, 4H, $^2J_{C-H}$=11.6 Hz, $^3J_{C-C-H}$=7.2 Hz, 4H, CH_2C(=O)], 4.28 (dd, $^2J_{C-H}$=12.0 Hz, $^3J_{C-C-H}$=4.4 Hz, 2H, CH_2), 4.29 (dd, $^2J_{C-H}$=12.0 Hz, $^3J_{C-C-H}$=4.4 Hz, 2H, CH_2), 4.48 (s, 2H, CH_2Cl), 5.25–5.29 (m, 1H, CH), 7.95 (s, 1H, NH); ^{13}C NMR (ppm, $CDCl_3$) δ 14.1, 22.7, 24.8, 29.0, 29.1, 29.3, 29.4, 29.5, 29.6, 29.7, 29.9, 31.9, 34.0, 34.3, 41.9, 43.5, 61.7, 64.5, 68.4, 150.7, 166.5, 172.9, 173.2; Anal. Calcd for $C_{42}H_{78}N_1O_7Cl_1$: C, 67.75; H, 10.56; N, 1.88. Found: C, 68.86; H, 10.66; N, 1.86.

General Procedure for Synthesis of 4 and 5 To a solution of $B_{12}H_{11}SCH_2CH_2CN \cdot 2TMA$ **17** (300 mg, 0.8 mmol) in CH_3CN (70 ml) was added 3-*O*-bromoacetyl-1,2-*O*-diacyl-*sn*-glycerol **9** or 3-*O*-chloroacetylcarbamoyl-1,2-*O*-diacyl-*sn*-glycerol **14** (1.2 equiv) at room temperature, and the resulting suspension was stirred for 1 day at 70 °C. The reaction mixture was concentrated under reduced pressure and purified by column chromatography on SiO_2 with EtOAc/acetone (5:1) as the eluent to give the compounds **18**, which were immediately dissolved in a minimum volume of acetone at room temperature, and an equimolar amount of tetramethylammonium hydroxide (25% solution in methanol) was added. The precipitate was filtered, and the white solids obtained were washed with dry acetone several times and dried under vacuum to give pure products.

1,2-*O*-dimyristoyl-*sn*-glycero-3-*O*-acetyl-$SB_{12}H_{11} \cdot 2TMA$ (**4a**) was obtained from **9a** (0.63 g, 1.0 mmol) as a white powder (0.67 g, 76% yield): MS(ESI, negative) m/z=363.3 ([M-2TMA]/2); Mp. 228–230 °C; $[\alpha]_D^{28}$= +8.7 (c0.50, CH_3CN); IR (KBr pellet, cm^{-1}) ν(C=O) 1734, ν(B–H) 2496, ν(C–H) 2853, 2922, 2957; ^1H NMR (ppm, CD_3CN) δ 0.88 (t, 6H, $^3J_{C-C-H}$=6.8 Hz, CH_3), 1.27 (s, 40H, CH_2), 1.52–1.62 [m, 4H, CH_2CH_2C(=O)], 2.27 (dd, 2H, $^2J_{C-H}$=6.4 Hz, $^3J_{C-C-H}$=2.8 Hz, OCH_2CH), 2.31 (dd, 2H, $^2J_{C-H}$=7.6 Hz, $^3J_{C-C-H}$=2.8 Hz, OCH_2CH), 3.09 (s, 24H, NCH_3), 3.16 (s, 2H, CH_2S), 4.13 (dd, 2H, $^2J_{C-H}$=10.6 Hz, $^3J_{C-C-H}$=6.4 Hz, $SCH_2C(=O)OCH_2CH$), 4.29 (dd, 2H, $^2J_{C-H}$=12.0 Hz, $^3J_{C-C-H}$=3.6 Hz, $SCH_2C(=O)OCH_2CH$), 5.18–5.22 (m, 1H, CH); ^{13}C NMR (ppm, CD_3CN) δ 14.1, 23.1, 25.4, 29.5, 29.7, 29.8, 30.0, 30.1, 32.4, 34.4, 35.4, 55.9, 56.0, 56.1, 62.6, 62.8, 69.7, 173.4, 173.5, 173.7; Anal. Calcd for $C_{41}H_{96}B_{12}N_2O_6S_1$: C, 56.28; H, 11.06; N, 3.20; S, 3.66. Found: C, 56.21; H, 11.04; N, 3.26; S, 3.76.

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1,2-*O*-dipalmitoyl-*sn*-glycero-3-*O*-acetyl-$SB_{12}H_{11}$·2TMA (**4b**) was obtained from **9b** (0.69 g, 1.0 mmol) as a white powder (0.75 g, 81% yield): MS(ESI, negative) m/z=391.4 ([M-2TMA]/2); Mp. 219–221 °C; $[\alpha]_D^{28}$= +14.4 (*c* 0.50, CH_3CN); IR (KBr pellet, cm^{-1}) ν(C=O) 1,736, ν(B–H) 2,494, ν(C–H) 2,851, 2,920, 2,957; ^1H NMR (ppm, CD_3CN) δ 0.88 (t, 6H, $^3J_{C\text{-}C\text{-}H}$=6.8 Hz, CH_3), 1.27 (s, 48H, CH_2), 1.49–1.61 [m, 4H, $CH_2CH_2C(=O)$], 2.27 (dd, 4H, $^2J_{C\text{-}H}$= 7.4 Hz, $^3J_{C\text{-}C\text{-}H}$=2.8 Hz, OCH_2CH), 2.30 (dd, 2H, $^2J_{C\text{-}H}$= 7.4 Hz, $^3J_{C\text{-}C\text{-}H}$=2.8 Hz, OCH_2CH), 3.10 (s, 24H, NCH_3), 3.17 (s, 2H, CH_2S), 4.13 [dd, 2H, $^2J_{C\text{-}H}$=11.6 Hz, $^3J_{C\text{-}C\text{-}H}$= 6.8 Hz, $SCH_2C(=O)OCH_2CH$], 4.29 [dd, 2H, $^2J_{C\text{-}H}$= 12.2 Hz, $^3J_{C\text{-}C\text{-}H}$=3.6 Hz, $SCH_2C(=O)OCH_2CH$], 5.17–5.22 (m, 1H, *CH*); ^{13}C NMR (ppm, CD_3CN) δ 14.1, 23.1, 25.4, 29.4, 29.5, 29.7, 29.8, 30.0, 30.1, 32.4, 34.5, 35.4, 55.9, 56.0, 56.1, 62.6, 62.8, 69.7, 173.4, 173.5, 173.7; Anal. Calcd for $C_{45}H_{104}B_{12}N_2O_6S_1$: C, 58.05; H, 11.26; N, 3.01; S, 3.44. Found: C, 58.48; H, 11.28; N, 3.00; S, 3.44.

1,2-*O*-distearoyl-*sn*-glycero-3-*O*-acetyl-$SB_{12}H_{11}$·2TMA (**4c**) was obtained from **9c** (0.75 g, 1.0 mmol) as a white powder (0.90 g, 91% yield): MS(ESI, negative) m/z=419.4 ([M-2TMA]/2); Mp. 215–217 °C; $[\alpha]_D^{28}$= +34.4 (*c*0.50, CH_3CN); IR (KBr pellet, cm^{-1}) ν(C=O) 1736, ν(B–H) 2,496, ν(C–H) 2,851, 2,918, 2,957; ^1H NMR (ppm, CD_3CN) δ 0.88 (t, 6H, $^3J_{C\text{-}C\text{-}H}$=6.8 Hz, CH_3), 1.27 (s, 56H, CH_2), 1.52–1.61 [m, 4H, $CH_2CH_2C(=O)$], 2.27 (dd, 2H, $^2J_{C\text{-}H}$=5.2 Hz, $^3J_{C\text{-}C\text{-}H}$=2.4 Hz, OCH_2CH), 2.31 (dd, 2H, $^2J_{C\text{-}H}$=7.6 Hz, $^3J_{C\text{-}C\text{-}H}$=2.8 Hz, OCH_2CH), 3.09 (s, 24H, NCH_3), 3.16 (s, 2H, CH_2S), 4.13 [dd, 2H, $^2J_{C\text{-}H}$= 11.6 Hz, $^3J_{C\text{-}C\text{-}H}$=6.4 Hz, $SCH_2C(=O)OCH_2CH$], 4.29 [dd, 2H, $^2J_{C\text{-}H}$=12.2 Hz, $^3J_{C\text{-}C\text{-}H}$=3.6 Hz, $SCH_2C(=O)OCH_2CH$], 5.16–5.24 (m, 1H, *CH*); ^{13}C NMR (ppm, CD_3CN) δ 13.2, 22.2, 28.6, 28.7, 28.8, 28.9, 29.0, 31.5, 33.5, 33.6, 55.0, 55.1, 55.2, 61.6, 61.7, 61.9, 68.8, 172.5, 172.6, 172.9; Anal. Calcd for $C_{49}H_{112}B_{12}N_2O_6S_1$: C, 59.61; H, 11.44; N, 2.84; S, 3.25. Found: C, 59.58; H, 11.61; N, 2.84; S, 3.20.

1,2-*O*-dimyristoyl-*sn*-glycero-3-*O*-acetylcarbamoyl-$SB_{12}H_{11}$·2TMA (**5a**) was obtained from **14a** (0.63 g, 1.0 mmol) as a white powder (0.49 g, 54% yield): MS(ESI, negative): m/z=384.8 ([M-2TMA]/2); Mp. 229–231 °C; $[\alpha]_D^{28}$= +20.4 (*c*0.50, CH_3CN); IR (KBr pellet, cm^{-1}) ν(C=O) 1739, 1774, ν(B–H) 2484, ν(N–H) 3450, ν(C–H) 2851, 2922, 2957; ^1H NMR (ppm, CD_3CN) δ 0.87 (t, 6H, $^3J_{C\text{-}C\text{-}H}$=6.8 Hz, CH_3), 1.26 (s, 40H, CH_2), 1.53–1.58 [m, 4H, $CH_2CH_2C(=O)$], 2.28 (dd, 2H, $^2J_{C\text{-}H}$=6.4 Hz, $^3J_{C\text{-}C\text{-}H}$= 2.8 Hz, OCH_2CH), 2.31 (dd, 2H, $^2J_{C\text{-}H}$=7.6 Hz, $^3J_{C\text{-}C\text{-}H}$= 2.8 Hz, OCH_2CH), 3.09 (s, 24H, NCH_3), 3.15 (s, 2H, CH_2S), 4.14 [dd, 2H, $^2J_{C\text{-}H}$=10.6 Hz, $^3J_{C\text{-}C\text{-}H}$=6.4 Hz, $SCH_2C(=O)OCH_2CH$], 4.29 [dd, 2H, $^2J_{C\text{-}H}$=12.0 Hz, $^3J_{C\text{-}C\text{-}H}$=3.6 Hz, $SCH_2C(=O)OCH_2CH$], 5.18–5.22 (m, 1H, *CH*), 9.67 (s, 1H, *NH*); ^{13}C NMR (ppm, CD_3CN) δ 14.1, 23.1, 25.4, 29.5, 29.7, 29.8, 30.0, 30.1, 32.4, 34.4, 35.4, 55.9, 56.0, 56.1, 62.6, 62.8, 69.7, 173.4, 173.5, 173.7; Anal. Calcd for $C_{42}H_{96}B_{12}N_3O_7S_1$: C, 55.01; H, 10.55; N, 4.58; S, 3.50. Found: C, 54.85; H, 10.60; N, 4.58; S, 3.69.

1,2-*O*-dipalmitoyl-*sn*-glycero-3-*O*-acetylcarbamoyl-$SB_{12}H_{11}$·2TMA (**5b**) was obtained from **14b** (0.69 g, 1.0 mmol) as a white powder (0.59 g, 61% yield): MS (ESI, negative): m/z=412.9 ([M-2TMA]/2); Mp. 185–187 °C; $[\alpha]_D^{28}$= +12.7 (*c*0.50, CH_3CN); IR (KBr pellet, cm^{-1}) ν(C=O) 1739, 1772, ν(B–H) 2,488, ν(N–H) 3,435, ν(C–H) 2,851, 2,918, 2,957; ^1H NMR (ppm, CD_3CN) δ 0.87 (t, 6H, $^3J_{C\text{-}C\text{-}H}$=6.8 Hz, CH_3), 1.27 (s, 48H, CH_2), 1.49–1.61 [m, 4H, $CH_2CH_2C(=O)$], 2.27 (dd, 4H, $^2J_{C\text{-}H}$=7.4 Hz, $^3J_{C\text{-}C\text{-}H}$= 2.8 Hz, OCH_2CH), 2.30 (dd, 2H, $^2J_{C\text{-}H}$=7.4 Hz, $^3J_{C\text{-}C\text{-}H}$= 2.8 Hz, OCH_2CH), 3.08 (s, 24H, NCH_3), 3.15 (s, 2H, CH_2S), 4.13 [dd, 2H, $^2J_{C\text{-}H}$=11.6 Hz, $^3J_{C\text{-}C\text{-}H}$=6.8 Hz, $SCH_2C(=O)OCH_2CH$], 4.28 [dd, 2H, $^2J_{C\text{-}H}$=12.2 Hz, $^3J_{C\text{-}C\text{-}H}$=3.6 Hz, $SCH_2C(=O)OCH_2CH$], 5.17–5.22 (m, 1H, *CH*), 9.69 (s, 1H, *NH*); ^{13}C NMR (ppm, CD_3CN) δ 14.1, 23.1, 25.4, 29.4, 29.5, 29.7, 29.8, 30.0, 30.1, 32.4, 34.5, 35.4, 55.9, 56.0, 56.1, 62.6, 62.8, 69.7, 173.4, 173.5, 173.7; Anal. Calcd for $C_{46}H_{104}B_{12}N_3O_7S_1$: C, 56.77; H, 10.77; N, 4.32; S, 3.30. Found: C, 56.76; H, 11.04; N, 4.39; S, 3.28.

1,2-*O*-distearoyl-*sn*-glycero-3-*O*-acetylcarbamoyl-$SB_{12}H_{11}$·2TMA (**5c**) was obtained from **14c** (0.74 g, 1.0 mmol) as a white powder (0.86 g, 83% yield): MS(ESI, negative) m/z=440.9 ([M-2TMA]/2); Mp. 181–183 °C; $[\alpha]_D^{28}$= +125.4 (*c*0.50, CH_3CN); IR (KBr pellet, cm^{-1}) ν(C=O) 1,740, 1,774, ν(B–H) 2,490, ν(C–H) 2,851, 2,918, 2,957, ν(N–H) 3,425; ^1H NMR (ppm, CD_3CN) δ 0.88 (t, 6H, $^3J_{C\text{-}C\text{-}H}$=6.8 Hz, CH_3), 1.27 (s, 56H, CH_2), 1.52–1.61 [m, 4H, $CH_2CH_2C(=O)$], 2.27 (dd, 2H, $^2J_{C\text{-}H}$=5.2 Hz, $^3J_{C\text{-}C\text{-}H}$= 2.4 Hz, OCH_2CH), 2.31 (dd, 2H, $^2J_{C\text{-}H}$=7.6 Hz, $^3J_{C\text{-}C\text{-}H}$= 2.8 Hz, OCH_2CH), 3.06 (s, 24H, NCH_3), 3.11 (s, 2H, CH_2S), 4.13 [dd, 2H, $^2J_{C\text{-}H}$=11.6 Hz, $^3J_{C\text{-}C\text{-}H}$=6.4 Hz, $SCH_2C(=O)OCH_2CH$], 4.29 [dd, 2H, $^2J_{C\text{-}H}$=12.2 Hz, $^3J_{C\text{-}C\text{-}H}$=3.6 Hz, $SCH_2C(=O)OCH_2CH$], 5.20–5.25 (m, 1H, *CH*), 9.72 (brs, 1H, *NH*); ^{13}C NMR (ppm, CD_3CN) δ 14.1, 14.6, 23.1, 25.4, 29.5, 29.7, 29.8, 29.9, 30.0, 30.1, 32.3, 34.4, 34.5, 38.9, 55.8, 55.9, 56.0, 62.6, 63.8, 69.4, 160.5, 169.7, 172.2, 173.6; Anal. Calcd for $C_{50}H_{113}B_{12}N_3O_7S_1$: C, 58.29; H, 11.06; N, 4.08; S, 3.11. Found: C, 58.68; H, 11.14; N, 4.20; S, 3.19.

Preparation of Calcein-Encapsulated Boronated Liposomes Boronated liposomes were prepared from cholesterol, dimyristoylphosphatidylcholine (DMPC), and boron cluster lipids (**4a–c** and **5a–c**) (1:1–*X*:*X*, *X*=0–1, molar ratio) by the REV method. The representative procedure for the preparation of 25% of **4c** containing liposomes is shown as follows: A mixture of DMPC (17.0 mg), cholesterol (19.3 mg), and **4c** (24.4 mg) were dissolved in 10 ml of chloroform/diisopropylether mixture (1:1, *v*/*v*) in a round-

bottom flask. An aqueous solution of calcein (100 mM, 5 ml) was added to the lipid solution to form an emulsion. The volume ratio of the aqueous phase to the organic phase was maintained at 1:2. The emulsion was sonicated for 1 min, and then the organic solvent was removed under vacuum in a rotary evaporator at 37 °C with broken down repeatedly to obtain a suspension of liposomes. The liposomes obtained were subjected to extrusion ten times through a polycarbonate membrane of 100-nm pore size, using an extruder device (Lipex Biomembrane, Canada) thermostated at 60 °C. Purification was accomplished by ultracentrifuging at 200,000×*g* for 60 min at 4 °C (Hitachi himac 65β, P50AT2 rotor), and the pellets obtained were resuspended in PBS buffer. Liposome size was measured with an electrophoretic light scattering spectrophotometer (ELS-700, Otsuka Electronics, Tokyo).

Stability of Boronated Liposomes in Fetal Bovine Serum The calcein-encapsulated boronated liposomes were added to a fetal bovine serum (FBS) (volume ratio: FBS/liposome solution=9:1), and the mixture was incubated at 37 °C with stirring. The fluorescence intensity of the FBS solutions was measured at 0–24 h using an excitation wavelength of 490 nm with emission-wave length of 520 nm. The fluorescence intensity was also measured at each fraction after degradation of liposomes by the addition of a 1% aqueous solution of Triton X-100 to the FBS solution.

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中性子捕捉がん治療のための次世代ホウ素デリバリーシステム

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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。

熱中性子は人体には無害であるが、ホウ素 10 と反応することにより、リチウムとヘリウム（α線）を生じる。これらの粒子エネルギーは 2.79MeV とおよそ 1 つの細胞を破壊するのに十分なエネルギーである（式 1）。この核反応を利用しがんを殺傷するのが BNCT である。BNCT は、世界に先駆け日本で初めて臨床応用に成功を収めて以来、世界をリードしてきた分野であるが、原子炉からの熱・熱外中性子線を利用しているため、対応できる治療患者数および地域が限られている。現在、病院内設置可能な BNCT 用加速器の開発が日本をはじめ全世界で進められており、加速器から十分な熱中性子が得られるようになれば、都市型病院への併設が可能となることから BNCT は細胞選択的放射線療法として一般に普及することが期待される。

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

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）。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 3837 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 20～35μg/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 [1]。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。

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
^{174}Hf	400	K	2.07

[a] Cross section capture values in barns.

2．ホウ素デリバリーシステム

近年、ホウ素のがん組織への有効な送達法としてドラッグデリバリーシステムの利用が注目されている [2]。リポソーム DDS を用いたホウ素デリバリーの方法として、大きく２つの戦略に分けられる（Figure 1）。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 [3-5]。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、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。

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（Figure 2）を用いたものであった [6]。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 に低下したものの T/N 比は 8.4 であった。

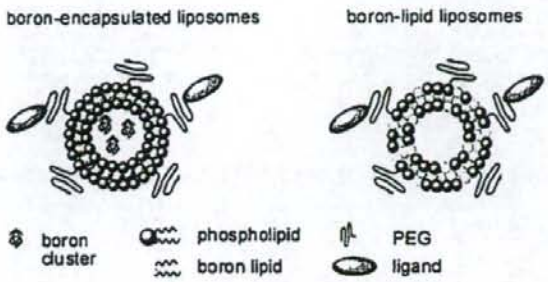

Figure 1. Boron-encapsulated liposomes and boron-lipid liposomes.

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[7]。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[8]。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ホウ素濃度で 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 mgB/kg 投与した場合では 22 ppm、14.4 mgB/kg 投与の場合では 40 ppm であった。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 を開発している[9]。

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 を設計した[10]。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おり、リンカー部位にエステル基(脂質 4)またはカルバメート基(脂質 5)を有し、BSH と S を介して結合している。これらのホウ素脂質から調整したリポソームは、正常マウスに対しホウ素濃度で 20 mg/kg では急性毒性は見られなかった[11]。

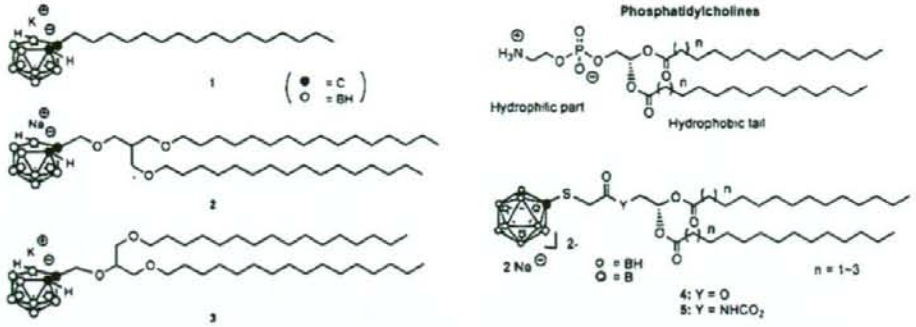

Figure. 2. Structures of *nido*-carborane lipids **1-3** and closo-dodecaborate lipids **4** and **5**

3．BNCT 効果

ホウ素脂質 4 (n = 16)、DSPC、DSPE-PEG2000、コレステロール（それぞれ 0.25:0.75:0.1:1.0）から調整したホウ素リポソームを用いて上記と同様に Colon 26 細胞を移植した BALB/c マウスに尾静脈より投与し (20 mgB/kg) ホウ素分布を調べたところ、投与 24 時間後に腫瘍内ホウ素濃度が 22 ppm であった (Figure 3)。そこで、投与 24 時間後に中性子照射を行い腫瘍の経時的変化を調べたところ、Figure 4 に示すようにホウ素リポソームを投与したマウスでは、中性子照射 1 週間後には腫瘍の委縮が見られ増殖抑制が見られた。

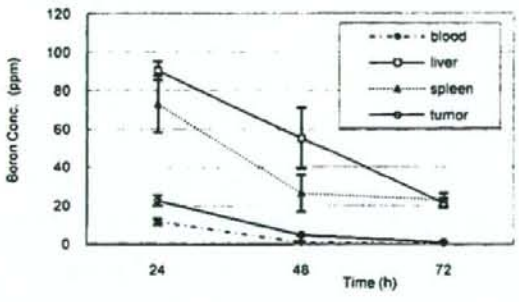

Figure 2. Time course of biodistribution of boron liposomes prepared from **4c** in tumor-bearing mice.

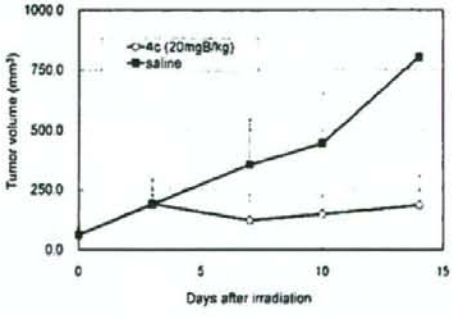

Figure 3. Tumor growth curve of mice bearing colon 26 tumors after injection of 20mg ^{10}B/kg of boronliposomes, thermal neutron irradiation with 0.9-1.4 x 10^{12} n/cm^2.

4．おわりに

BNCT のためのホウ素キャリアーの開発には、いわゆるナノモルレベルで薬理効果が要求される抗がん剤のようなドラッグデザインではなく、ミリモルレベルで投与できるのに十分な低毒性であり、なおかつ腫瘍細胞に集積することが必要とされる。そのために、低毒性小分子ホウ素化合物の

開発だけでなく、リポソームを用いたホウ素デリバリーシステムの開発が十数年前から盛んに研究されてきた。BNCT において 1950 年代に開発された BSH、BPA という 2 剤以外には、まだ臨床応用されたホウ素薬剤は残念ながら登場してない。現在、核燃料の問題から BNCT に適応できる小型加速器の開発が精力的に行われている。熱中性子源が原子炉から加速器に移行できれば都市部病院併設型加速器による BNCT が可能となることから、将来放射線療法の一般的治療法の一つになるであろう。そのためにも治療効果の高いホウ素デリバリーシステムの開発が期待される。

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