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Synthesis of dodecaborate-conjugated cholesterols for efficient boron delivery in neutron capture therapy

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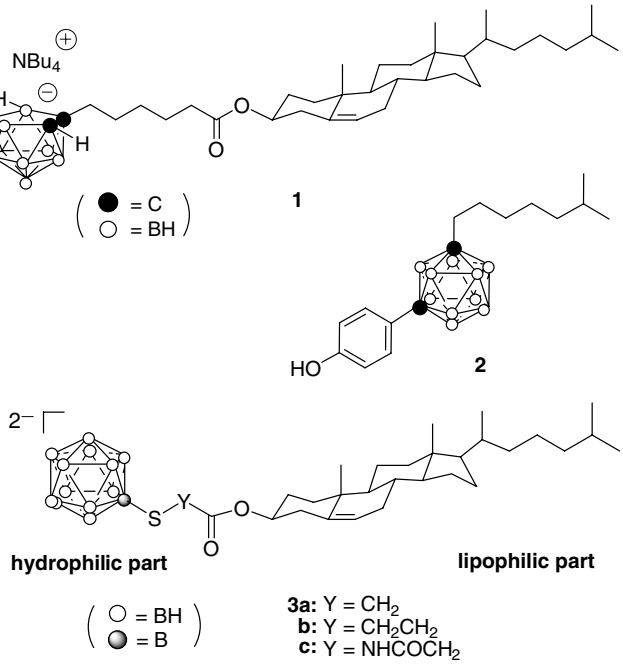
Abstract—Dodecaborate-conjugated cholesterols **3a–c** were synthesized for liposomal boron delivery systems in neutron capture therapy. The current synthesis is based on the S-alkylation protocol of the cyanoethyl-protected BSH with alkyl halides. The dodecaborate-conjugated cholesterol **3a** liposome, which was prepared from dimyristoylphosphatidylcholine (DMPC), cholesterol, dodecaborate-conjugated cholesterol **3a**, and polyethyleneglycol-conjugated distearoylphosphatidylethanolamine (PEG-DSPE) (1:0.5:0.5:0.1), exhibited higher cytotoxicity than BSH at the same boron concentration and IC_{50} values of the **3a** liposome and BSH toward colon 26 cells were estimated as 25 and 78 ppm of boron concentration, respectively.

Boron neutron capture therapy (BNCT) is a binary cancer treatment based on the nuclear reaction of two essentially nontoxic species, ^{10}B and thermal neutrons.[1] The high neutron capture cross section of ^{10}B allows us to generate an α-particle and a lithium-7 ion bearing approximately 2.4 MeV by neutron capture reaction, and these high linear energy transfer particles afford precise cell killing.[2–5] We have focused on boronated liposomes for boron delivery system.[6,7] 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, it is possible to functionalize liposomes by combination of lipid contents. Therefore, boron and drugs may be simultaneously delivered to tumor tissues for BNCT and chemotherapy of cancers. We recently reported boron ion cluster lipids which have a double-tailed moiety conjugated with boron ion clusters such as *nido*-carborane[8,9] and *closo*-dodecaborate[10] as a hydrophilic function, and investigated the stability, boron biodistribution, and BNCT effect of their liposomes.[9,11] Carborane-conjugated cholesterols (**1**)[12] and carborane-containing cholesterol mimic (**2**)[13,14] have been developed as an alternative content of liposomal membranes as shown in Scheme 1.



Scheme 1. Structures of boron cluster-conjugated cholesterols and the mimic.

We focused on the structure of mercaptoundecahydrododecaborate (BSH), which has been utilized for clinical

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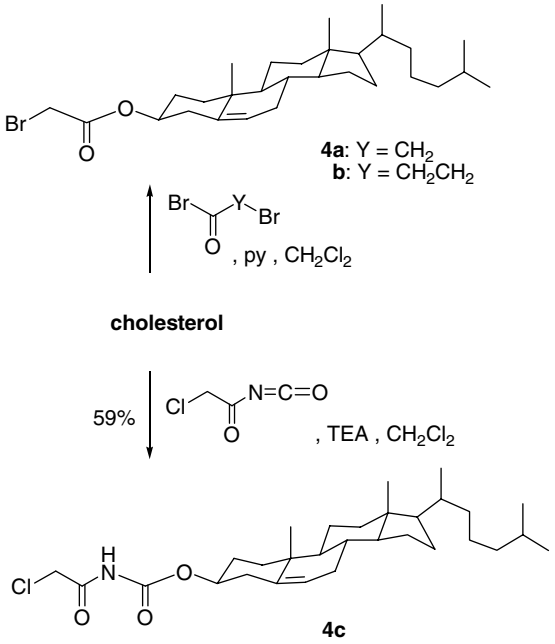
treatment on BNCT, as a water-soluble boron cluster of low toxicity. In this Letter, we report the synthesis of dodecaborate-conjugated cholesterols **3a**–**c** (Scheme 1) for liposomal boron delivery systems. The current synthetic strategy is based on the S-alkylation protocol of the cyanoethyl-protected BSH with alkyl halides.[15]

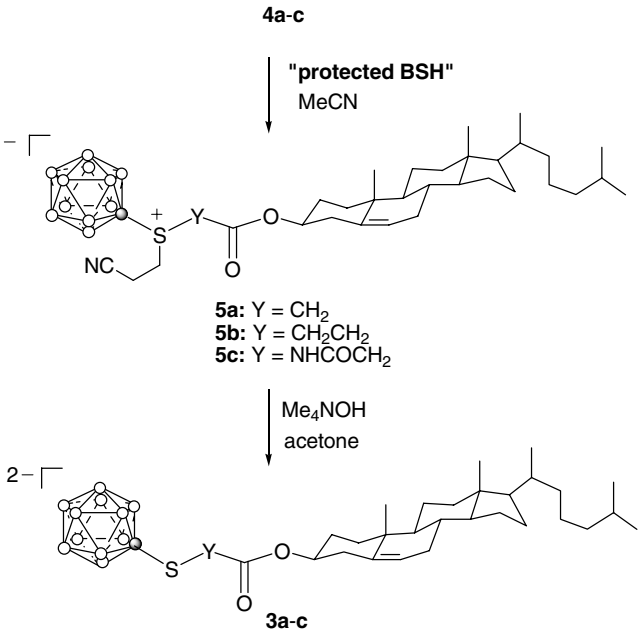
We first introduced the various linkers at the hydroxy group of cholesterol as shown in Scheme 2. Cholesterol was treated with bromoacetyl bromide in the presence of pyridine as a base to give the corresponding ester **4a** and with bromopropionyl chloride in the presence of triethylamine to **4b** in 83% and 81% yields, respectively. In a similar manner, chloroacetylcarbamate **4c** was also synthesized by treating with chloroacetyl isocyanate in the presence of trimethylamine.

We next examined the introduction of BSH into the cholesterol derivatives **4** as shown in Scheme 3. The S-alkylation of the protected BSH with **4a**–**c** proceeded in acetonitrile and the resulting sulfoniums **5a**–**c** were treated with an equivalent of tetramethylammonium hydroxide in acetone to give the corresponding thioesters **3a**–**c** in 73%, 54%, and 47% yields, respectively, in two steps.

A typical procedure for the synthesis of **3a** from **4a** is as follows: To a mixture of the protected BSH (200 mg, 0.51 mmol) in acetonitrile (10 mL) was added **4a** (275 mg, 0.55 mmol) in THF (10 mL) at room temperature, and the reaction mixture was heated at 60 °C and stirred for 14 h. The solvent was removed and the residue was purified by column chromatography on silica gel with ethyl acetate/acetone (5:1) to give **5a**, as a white solid, quantitatively. Sulfonium salt **5a** obtained was dissolved in acetone (5 mL) and a methanol solution of tetramethylammonium hydroxide (25% w/w, 186 mg) was then added with stirring. The white precipitate was filtered, washed with acetone, and dried in vacuo to give **3a** in 73% yield as a white solid.[16]



Scheme 2. Synthesis of cholesterol-linker conjugates **4a**–**c**.



Scheme 3. Synthesis of dodecaborate-conjugated cholesterols **3a**–**c**.

The 2D $^{13}C/^{1}H$ NMR spectrum of **3b** and the assignment are shown in Figure 1 and Table 1, respectively. Whereas monodimensional ^{1}H spectra did not resolve the resonances of the extra protons of the linker, this could be achieved with the two-dimensional spectrum.

We next examined the cytotoxicity of liposomes containing **3a**. The liposome was prepared from dimyristoylphosphatidylcholine (DMPC), cholesterol, dodecaborate-conjugated cholesterol **3a**, and polyethyleneglycol-conjugated distearoylphosphatidylethanolamine (PEG-DSPE) (1:0.5:0.5:0.1), by the reverse-phase evaporation (REV) method.[17] The liposomes obtained were subjected to extrusion 10 times through a polycarbonate membrane of 100 nm pore size, using an extruder device thermostated at 60 °C. Purification was accomplished by ultracentrifuging at 200,000*g* for 60 min at 4 °C, and the pellets obtained were resuspended in cell culture medium. Boron concentration was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES). Figure 2 shows dose-dependent cell survival curves using colon 26 mouse colonectal cancer cells. The dodecaborate-conjugated cholesterol **3a** liposome exhibited higher cytotoxicity than BSH at the same boron concentration and IC_{50} values of the **3a** liposome and BSH were estimated as 25 and 78 ppm of boron concentration, respectively. The higher cytotoxicity of cholesterol **3a** liposome may be due to the higher lipophilicity and higher molecular weight of **3a** (and its liposome) in comparison with BSH.[18] It should be noted that total dose volume of cholesterol **3a** liposome for administration was much larger than that of BSH although boron concentrations were similar.

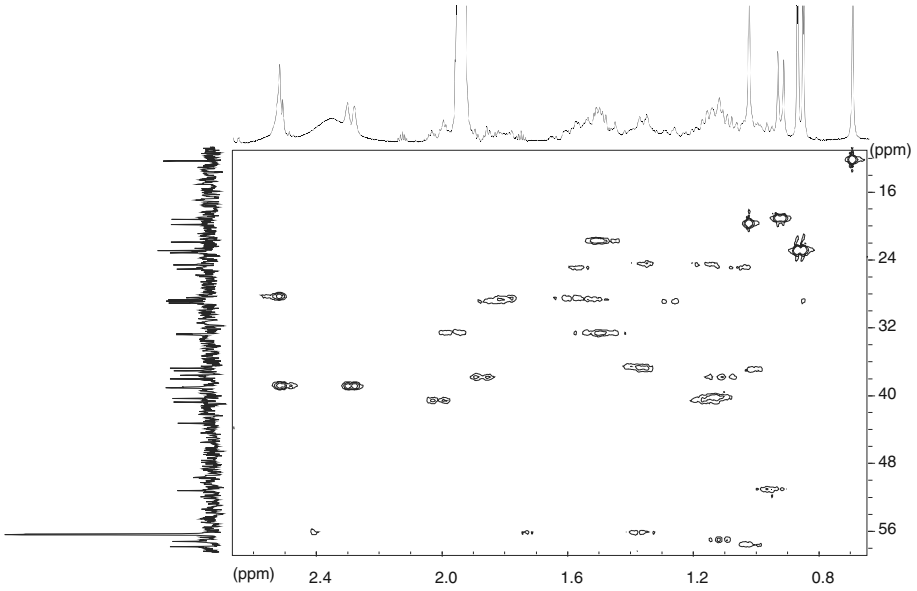


Figure 1. HSQC $^{13}C/^{1}H$ spectrum of **3b**.

Table 1. HSQC $^{13}C/^{1}H$ spectrum of **3b** as tetramethylammonium salt

Assignment	^{13}C δ (ppm)	^{1}H δ (ppm)
1 CH_2	37.8	1.87/1.11
2 CH_2	28.5	1.80/1.59
3 CH–C–CO	74.1	4.47
4 C_2	38.85	2.29
5 $=C_q$	141.1	—
6 =CH	123.0	5.38
7 CH_2	32.5	1.97/1.53
8 CH	32.6	1.48
9 CH	51.0	0.96
10 C_q	37.4	—
10′ CH_3	19.7	1.03
11 CH_2	21.7	1.50
12 CH_2	40.5	2.02/1.17
13 C_q	43.0	—
13′ CH_3	12.1	0.70
14 CH	57.6	1.04
15 CH_2	24.4	1.36/1.16
16 CH_2	28.9	1.84/1.28
17 CH	56.9	1.11
18 CH	36.5	1.39
18′ CH_3	19.0	0.93
19 CH_2	36.9	1.37/1.01
20 CH_2	24.9	1.58/1.06
21 CH_2	40.1	1.13
22 CH	28.6	1.52
23 CH_3	23.0/22.7	0.864/0.860
S–CH_2	28.3	2.52
CH_2–CO	38.8	2.51
$N(CH_3)_4$	56.2/56.1/56.0	3.11

In conclusion, we succeeded in the synthesis of dodecaborate-conjugated cholesterols **3a**–**c**. The BSH protection protocol was effective for conjugation of BSH into cholesterol. Liposomal formulations of **3a**–**c** and in vivo biodistribution of their liposomes are now under investigation.

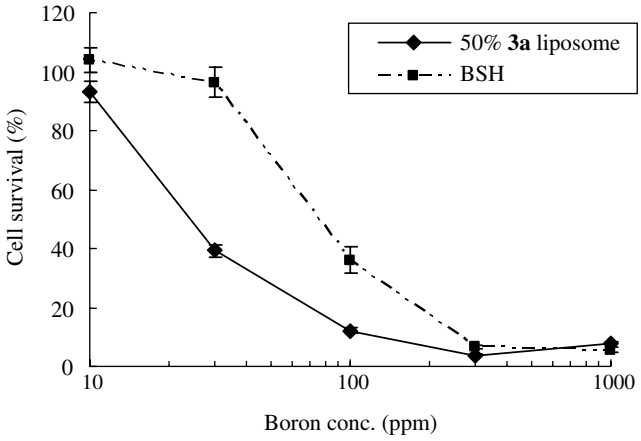


Figure 2. Cell survivals of colon 26 mouse colorectal cancer cells with the **3a** liposome and BSH after incubation for 3 days.

Acknowledgment

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16. Spectral data for compound **3a**: ^{1}H NMR (CD_3CN, 400 MHz) δ 5.37 (br d, $J = 4.8$ Hz, 1H), 4.42 (m, 1H), 3.11 (s, 2H), 3.07 (s, 12H), 2.57 (d, $J = 6.8$ Hz, 2H), 2.02–1.76 (m, 5H), 1.86–0.92 (m, 21H), 1.02 (s, 3H), 0.91 (d, $J = 6.4$ Hz, 3H), 0.86 (d, $J = 2.0$ Hz, 3H), 0.84 (d, $J = 2.0$ Hz, 3H), 0.69 (s, 3H); MS (ESI, negative) $[(M-2TMA)/2]^-$ m/z 300*. Compound **3b**: ^{11}B NMR (CD_3CN, 200 MHz): δ −6.13 (s, 1B), −14.46 (s, 5B), −15.37 (s, 5B), −17.33 (s, 1B) ppm. IR (KBr, ν, cm^{-1}): 3028, 2950, 2867 (C–H), 2484 (B–H) 1653 (C=C). MS (ESI): positive 74 $[cat]^+$, 837* $[3cat^+ + A^{2-}]^+$, 665* $[2cat^+ + H^+ + A^{2-}]^+$, negative 307.5* $[A]^{2-}$, 688* $[A^{2-} + cat^+]^-$; Compound **3c**: ^{1}H NMR (CD_3CN, 400 MHz) δ 9.88 (br s, 1H), 5.41 (br m, 1H), 4.48 (m, 1H), 3.12 (br s, 3H), 3.07 (s, 24H), 2.37 (d, $J = 6.4$ Hz, 2H), 2.01–1.80 (m, 4H), 1.68–0.92 (m, 21H), 1.03 (s, 3H), 0.92 (d, $J = 6.4$ Hz, 3H), 0.87 (d, $J = 1.6$ Hz, 3H), 0.85 (d, $J = 1.6$ Hz, 3H), 0.69 (s, 3H); MS (ESI, negative) $[(M-2TMA)/2]^-$ $m/z = 322$*. The masses marked with * represent the most intense masses of an ensemble of peaks corresponding to the boron and carbon isotope distribution.
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