11896 *Inorg. Chem.* **2009**, *48*, 11896–11902
DOI: 10.1021/ic902033c

Inorganic Chemistry
Article

# New Strategy for Synthesis of Mercaptoundecahydrododecaborate Derivatives via Click Chemistry: Possible Boron Carriers and Visualization in Cells for Neutron Capture Therapy

**Mohamed E. El-Zaria[†] and Hiroyuki Nakamura***

*Department of Chemistry, Faculty of Science, Gakushuin University, 1-5-1 Mejiro, Toshima-ku, Tokyo 171-8588, Japan. [†]Permanent address: Department of Chemistry, Faculty of Science, University of Tanta, 31527-Tanta, Egypt.*

Received October 14, 2009

A new method that utilizes the click cycloaddition reaction to functionalize $B_{12}H_{11}SH^{2-}$ (BSH) with organic molecules was investigated. *S,S*-Dipropargyl-$SB_{12}H_{11}^{-}$ (**1**) and *S*-propargyl-$SB_{12}H_{11}^{2-}$ (**4**) were prepared from $[(CH_3)_4N]_2B_{12}H_{11}SH$ and $[(CH_3)_4N]_2B_{12}H_{11}S(CH_2)_2CN$ (**2**) with propargyl bromide, respectively. Compound **1** or **4** reacted with various azides with mediation by Cu(II) ascorbate to give the corresponding bis-triazolo BSH derivatives (1-) or monotriazole BSH derivatives (2-), respectively, in excellent yields. The click cycloaddition reaction is very useful not only for the synthesis of various BSH-containing organic compounds for boron neutron capture therapy (BNCT) but also for the visualization of boron clusters in cells. We succeeded in the click cycloaddition reaction of compound **1** with Alexa Fluor 488 azide dye and found that **1** accumulated not in the cytoplasm but in the nuclei of HeLa cells.

## Introduction

Boron neutron capture therapy (BNCT) is a special type of radiotherapy for cancer treatment by using $^{10}B$ compounds. A nuclear reaction occurs when $^{10}B$ is irradiated with thermal neutrons. This reaction yields an unstable intermediate, $^{11}B$, which immediately undergoes fission to generate $^{7}Li$ and $^{4}He$ bearing approximately 2.4 MeV.[1,2] The particles dissipate their kinetic energies before traveling a distance equivalent to one cell diameter (~10 $\mu$m), enabling them to precisely kill tumor cells. Successful BNCT highly depends on the sufficient and selective boron delivery to the tumor cells. Therefore, the development of boron compounds that accumulate in the tumor cells in the appropriate concentrations is essential for BNCT. Various strategies have been studied to this end, including synthetic chemical approaches as well as biochemical and biophysical approaches.[3,4]

The disodium salt of mercaptoundecahydro-*closo*-dodecaborate ($Na_2B_{12}H_{11}SH$) has high boron content, an ionic nature, and significantly low toxicity based on its boron content. It was prepared for the first time more than 50 years ago.[5] The first clinical use of $Na_2B_{12}H_{11}SH$ was accomplished by Hatanaka in the 1960s for BNCT of patients with high-grade gliomas.[6,7] The potentially reactive sulfhydryl group in the $B_{12}H_{11}SH^{2-}$ (BSH) cluster is very important not only for transport into the brain tumor but also for functionalization with organic molecules.[8] Thus, numerous BSH-containing derivatives of biomolecules (e.g., porphyrins,[9−11] nitroimidazoles,[12] sugars,[13] and lipids[14−17]) have been synthesized

*To whom correspondence should be addressed. E-mail: hiroyuki.nakamura@gakushuin.ac.jp. Fax: +81-3-5992-1029.

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Published on Web 11/20/2009

for the development of tumor-targeting compounds. However, few strategies are available for the functionalization with organic molecules.

"Click chemistry" is a general term for a class of chemical transformations having a number of attractive features, including excellent functional group tolerance, high yield, and good selectivity under experimental conditions.[18,19] This is coupled with the use of readily available reagents and the avoidance of conventional chromatographic purification of the products. The Cu-catalyzed azide−alkyne cycloaddition reaction is one of the most reliable click reactions.[20,21] This reaction has enabled the practical and efficient preparation of 1,4-disubstituted-1,2,3-triazoles from an unprecedented range of substrates with excellent selectivity, which cannot be obtained with conventional Huisgen thermal approaches.[22] This has led to its application in a number of processes, including the synthesis of therapeutics,[23,24] protein-based biohybrids,[25,26] sugar arrays,[27] dendrimers,[28] and functional polymers.[29] The proposed mechanism for the Cu(I)-catalyzed reaction involves the addition of a Cu(I) acetylide to an azide in a stepwise sequence, giving a five-membered vinyl cuprate that yields the triazole products.[20,30] Recently, the facile synthesis of boronated chlorins,[31] hyaluronans,[32] and nucleotides[33,34] with click cycloaddition reactions was reported. In this paper, we report a simple and convenient procedure for the synthesis of BSH-containing organic molecules, based on the click cycloaddition reaction of organic azides with mono- and dipropargylic BSH derivatives. The current protocol enables us to synthesize a variety of BSH-containing biologically active compounds for BNCT.

## Experimental Section

**General Remarks.** $^{1}$H NMR and $^{13}$C 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 and $^{13}$C NMR were expressed in parts per million (ppm, $\delta$ units), and coupling constant ($J$) values were expressed in units of hertz (Hz). $^{11}$B NMR spectra were recorded on a JEOL JNM-AL 300 spectrometer (96.3 MHz), and the chemical shifts were reported in $\delta$ units relative to external $BF_3 \cdot Et_2O$ in $CDCl_3$. IR ($cm^{-1}$) spectra were determined as KBr disk on a Shimadzu FTIR-8600PC spectrometer. Electron spray ionization (ESI) mass spectra were recorded on a Shimadzu LCMS-2010 eV spectrometer or Bruker Daltonics micro TOF-15 focus. Elemental analyses were performed by a CE instrument EA1110 CHNS-O automatic elemental analyzer. All compounds gave elemental analysis within ±0.4% of the theoretical values. Analytical thin layer chromatography (TLC) was performed on a glass plates of silica gel 60 $GF_{254}$ (Merck). Visualization was accompanied by UV light (254 nm), $I_2$, $KMnO_4$, or $PdCl_2$. Preparative TLC was carried out using 0.75 mm layers of silica gel 60 $GF_{254}$ (Merck) made from water slurries on glass plates of dimensions 20 × 20 $cm^2$, followed by drying in air at 100 °C. Column chromatography was conducted on silica gel (Merck Kieselgel 70−230 mesh). Most chemicals were of analytical grade and used without further purification. Azides ($C_6H_5$-$CH_2N_3$, *p*-Br-$C_6H_4CH_2N_3$, *p*-Me-$C_6H_4CH_2N_3$, *m*-CN-$C_6H_4$-$CH_2N_3$, *m*-MeO-$C_6H_4CH_2N_3$, $C_6H_5CH_2CH_2N_3$, and 3-azidopropyl-*o*-carborane), $[(CH_3)_4N]_2B_{12}H_{11}S(CH_2)_2CN$ **2**, and 1,2-*O*-distearoyl-*sn*-3-glycerol were prepared as described in literatures.[8,14,35,36]

***S,S*-Bis-(prop-2-ynyl)sulfonioundecahydro-*closo*-dodecaborate (1-) tetramethylammonium salt (1).** Bis-tetramethylammonium salt of BSH (1 g, 3.1 mmol) was dissolved in a mixture of acetonitrile/water (250 mL, 4:1) in a one-neck flask equipped with a dropping funnel. A solution of 3-bromo-1-propyne (1.3 mL, 17 mmol) in acetonitrile/water (40 mL, 4:1) was added dropwise at room temperature over a period of 10 min to the reaction mixture. After 24 h, the solvent was evaporated under vacuum, and the obtained solid was washed with ether and dissolved in acetonitrile. The inorganic salts were removed by filtration, and ether was added to the filtrate to precipitate the product **1** (840 mg, 84%) as the pale yellow solid. Crystallization from water is also possible for further purification, if necessary. mp over 300 °C. $^{1}$H NMR (300 MHz, $CD_3CN$): $\delta$ 3.89 (m, 4H, S-$CH_2$), 3.07 (s, 12H, $N(CH_3)_4$), 2.81 (m, 2H, C≡CH), 1.8−0.55 (m, 11H, $B_{12}H_{11}$). $^{13}$C NMR (75 MHz, $CD_3CN$): $\delta$ 77.87 (2C, C≡CH), 74.54 (2C, C≡CH), 56.23 (4C, $N(CH_3)_4$), 30.43 (2C, S-$CH_2$). $^{11}$B NMR (96.3 MHz; $CD_3CN$): $\delta$ −15.59 (bs, 1B, B1), −18.98 (d, $J_{BH}$ = 163.9 Hz, 11B, B2−12). IR (KBr, $cm^{-1}$): $\nu$(CH) 3263 (m), 3030 (m), 2960 (S), $\nu$(BH) 2495 (S), $\nu$(C≡C) 2123 (w), $\nu$(CH) 1485 (S), 1404 (W), 1286 (W), $\nu$(B−B) 1045 (S), $\nu$(CH) 995 (m), 948 (S), 821 (m), 719 (m), 673 (W). MS (ESI, negative): $m/z$ 251.2 ($M^-$). Elemental analysis calcd for $C_{10}H_{29}B_{12}NS$: C, 36.94; H, 8.99; N, 4.31%. Found: C, 36.65; H, 8.89; N, 4.25%.

***S*-(Prop-2-ynyl)thioundecahydro-*closo*-dodecaborate (2-) bis-tetramethylammonium salt (4).** To a solution of **3** (340 mg, 1.0 mmol) in acetone (20 mL) was added 1 equiv of a 25% solution of $(CH_3)_4NOH$ in methanol dropwise. The white precipitate of the product formed immediately. The precipitate was filtered off and dried to give **4** (350 mg, 97%) as a white solid. mp 250−251 °C. $^{1}$H NMR (300 MHz; DMSO): $\delta$ 3.58 (m, 2H, S-$CH_2$), 3.08 (s, 12H, $N(CH_3)_4$), 2.80 (m, 1H, C≡CH), 1.85−0.55 (m, 11H, $B_{12}H_{11}$). $^{13}$C NMR (75 MHz; DMSO): $\delta$ 77.87 (1C, C≡CH), 72.54 (1C, C≡CH), 56.34 (8C, $N(CH_3)_4$), 36.87 (1C, S-$CH_2$). $^{11}$B NMR (96.3 MHz; $CD_3CN$): $\delta$ −9.87 (bs, 1B, B1), −19.98 (d, $J_{BH}$ = 161.7 Hz, 11B, B2−11), −21.59 (bs, 1B, B12). IR

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(KBr, $cm^{-1}$): $\nu$(CH) 3265 (m), 3020 (m), 2965 (S), $\nu$(BH) 2495 (S), $\nu$(C≡C) 2125 (w), $\nu$(CH) 1485 (S), 1405 (W), 1286 (W), $\nu$(B−B) 1045 (S), $\nu$(CH) 995 (m), 948 (S), 821 (m), 719 (m), 673 (W). ESI-MS: $m/z$ 106.1 (106.1, $M^{2-}$). Elemental Analysis calcd for $C_{11}H_{38}B_{12}N_2S$: C, 36.68; H, 10.63; N, 7.78%. Found: C, 36.62; H, 10.49; N, 7.69%.

***S,S*-Bis[1-benzyl-(1,2,3-triazol-4-yl)methyl]sulfonioundecahydro-*closo*-dodecaborate (1-) tetramethylammonium salt (5).** To a solution of **1** (325 mg, 1 mmol) in acetonitrile (20 mL) were added $Cu(OAc)_2$ (50 mg, 0.27 mmol) and sodium ascorbate (100 mg, 0.5 mmol) at room temperature, and benzyl azide (333 mg, 2.5 mmol) was added dropwise with stirring. The reaction mixture was stirred for 2 h until complete consumption of **1** monitored by TLC ($MeOH/CH_2Cl_2$ 3:7). The mixture was filtered through a pad of Celite, and diethyl ether (10 mL) was added until a precipitate (inorganic salts) formed. This precipitate was removed by filtration, and another 200 mL of diethyl ether was added to the filtrate, whereupon a crystalline solid was precipitated. The finely crystalline product was filtered to give **5** (582 mg, 98%) as a white solid. For analysis a sample recrystallized again from acetonitrile/ether was obtained as colorless needles. mp 154−155 °C. $^1$H NMR (300 MHz, $CD_3CN$): $\delta$ 7.79 (s, 2H, CH-triazole), 7.34 (m, 10H, CH-phenyl), 5.45 (s, 4H, $CH_2$-benzyl), 4.39 (m, 4H, S-$CH_2$), 3.07 (s, 12H, $N(CH_3)_4$), 1.87−0.65 (m, 11H, $B_{12}H_{11}$). $^{13}$C NMR (75 MHz, $CD_3CN$): $\delta$ 136.36 (2C, C-triazole), 129.8, 129.27, 128.87 (12C, CH and C-phenyl), 125.97 (2C, CH-triazole), 56.2 (4C, (4C, $N(CH_3)_4$), 54.4 (2C, $CH_2$-benzyl), 37.35 (2C, S-$CH_2$). $^{11}$B NMR (96.3 MHz; $CD_3CN$): $\delta$ −14.88 (bs, 1B, B1), −20.17 (d, $J_{BH}$ = 151 Hz, 11B, B2−12). IR (KBr, $cm^{-1}$): $\nu$(CH) 3406 (m), 3020 (W), 2918 (W), $\nu$(BH) 2497 (S), $\nu$(C=C) 1627 (m), $\nu$(N=N) 1560 (W), $\nu$(CH) 1485 (S), 1415 (W), 1286 (W), $\nu$(B−B) 1045 (S), $\nu$(CH) 995 (m), 941 (S), 835 (m), 742 (m), 685 (W). ESI-MS: $m/z$ 517.5 (517.5, $M^-$). Elemental Analysis calcd for $C_{24}H_{43}B_{12}N_7S$: C, 48.74; H, 7.33; N, 16.58%. Found: C, 48.72; H, 7.28; N, 16.52%.

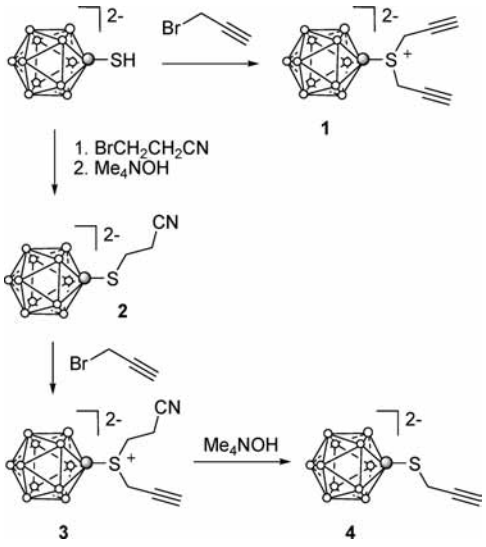
***S*-[1-Benzyl-1,2,3-triazol-4-yl)methyl]thioundecahydro-*closo*-dodecaborate (2-) bis-tetramethylammonium salt (12).** This compound was also prepared from **11** (473 mg, 1.0 mmol) using the procedure described for **4** to give a white solid of **11** (469 mg, 95%). Alternative procedure for synthesis of **12** is described as follows: To a solution of **4** (360 mg, 1 mmol) in acetonitrile-water (4:1, 20 mL) were added $Cu(OAc)_2$ (50 mg, 0.27 mmol) and sodium ascorbate (100 mg, 0.5 mmol) at room temperature, and benzyl azide (160 mg, 1.2 mmol) was added dropwise with stirring. The reaction mixture was stirred for 2 h until complete consumption of **4** monitored by TLC ($MeOH/CH_2Cl_2$ 3:7). The mixture was filtered off, and the solvent was removed under vacuum. The residue was purified by preparative TLC with $MeOH/CH_2Cl_2$ (3:7) as eluent to give **12** (369 mg, 77%) as a white solid. mp 235−236 °C. $^1$H NMR (300 MHz; $CD_3CN$): $\delta$ 7.68 (s, 1H, CH-triazole), 7.34 (m, 5H, phenyl), 5.45 (s, 2H, $CH_2$-benzyl), 3.59 (m, 2H, S-$CH_2$), 3.16 (s, 24H, $N(CH_3)_4$), 0.39−1.71 (m, 11H, $B_{12}H_{11}$). $^{13}$C NMR (75 MHz; $CD_3CN$): $\delta$ 137.52 (1C, CH-triazole), 129.65, 129.51, 129.15 (6C, CH and C-phenyl), 125.29 (1C, C-triazole), 54.47 (8C, $N(CH_3)_4$), 52.26 (1C, $CH_2$-benzyl), 37.52 (2C, S-$CH_2$). $^{11}$B NMR (96.3 MHz; $CD_3CN$): $\delta$ −10.75 (bs, 1B, B1), −20.17 (d, $J_{BH}$ = 86.7 Hz, 10B, B2−11), −22.58 (bs, 1B, B12) ppm. IR (KBr, $cm^{-1}$): $\nu$(CH) 3388 (m), 3020 (m), 2935 (W), $\nu$(BH) 2470 (S), $\nu$(C=C) 1622 (m), $\nu$(N=N) 1572 (W), $\nu$(CH) 1487 (S), 1417 (W), 1288 (W), $\nu$(B−B) 1049 (S), $\nu$(CH) 992 (m), 948 (S), 837 (m), 745 (m), 689 (W). MS (ESI, negative): $m/z$ 172.6 (172.6, $M^-/2$). Elemental analysis calcd for $C_{17}H_{37}B_{12}N_5S$: C, 43.82; H, 9.19; N, 14.19%. Found: C, 43.72; H, 9.17; N, 14.11%.

***S*-[1-(Benzyl-1,2,3-triazol-4-yl)methyl]-*S*-[1-(*m*-methoxybenzyl-1,2,3-triazol-4-yl)methyl]sulfonioundecahydro-*closo*-dodecaborate (1-) tetramethylammonium salt (14).** This compound was prepared from **13** (488 mg, 1 mmol) and benzyl azide (200 mg, 1.5 mmol), using the procedure described for **5** to give **14** (602 mg, 97%) as a white solid. mp 149−150 °C. $^1$H NMR (300 MHz; $CD_3CN$): $\delta$ 8.09, 7.89 (s, 2H, CH-triazole), 7.27, 6.85 (m, 9H, CH-phenyl), 5.47 (m, 4H, $CH_2$-benzyl), 4.35 (m, 4H, S-$CH_2$), 3.7 (s, 3H, O−$CH_3$), 3.11 (s, 12H, $N(CH_3)_4$), 1.87−0.56 (m, 11H, $B_{12}H_{11}$). $^{13}$C NMR (75 MHz; DMSO): $\delta$ 158.89 (2C, O-Cphenyl), 137.18, 136.84 (2C, C-triazole), 130.12, 129.97, 129.76, 125.35, 120.57, 119.89, 113.77, 113.45 (9C, CH and C-phenyl), 125.44 (2C, CH-triazole), 55.12 (1C, O−$CH_3$), 54.45 (4C, (4C, $N(CH_3)_4$), 52.72 (2C, $CH_2$-phenyl), 37.09, 36.67 (2C, S-$CH_2$). $^{11}$B NMR (96.3 MHz; $CD_3CN$): $\delta$ −12.45 (bs, 1B, B1), −20.65 (d, $J_{BH}$ = 151 Hz, 11B, B2−12). IR (KBr, $cm^{-1}$): $\nu$(CH) 3445 (m), 3030 (W), 2928 (W), $\nu$(BH) 2499 (S), $\nu$(C=C) 1605 (m), $\nu$(N=N) 1587 (W), $\nu$(CH) 1485 (S), 1413 (W), 1286 (W), $\nu$(B−B) 1047 (S), $\nu$(CH) 995 (m), 948 (S), 821 (m), 758 (m), 721 (W). MS (ESI, negative): $m/z$ 547.3 ($M^-$). Elemental analysis calcd for $C_{25}H_{45}B_{12}N_7OS$: C, 48.32; H, 7.3; N, 15.78%. Found: C, 48.09; H, 7.22; N, 15.56%.

***S,S*-Bis[1-(3-*o*-carboranylpropyl)-(1,2,3-triazol-4-yl)methyl]-sulfonioundecahydro-*closo*-dodecaborate (1-) tetramethylammonium salt (15).** This compound was prepared from **1** (325 mg, 1 mmol) and 3-azidopropyl-*o*-carborane (533 mg, 2.5 mmol), using the procedure described for **5** to give **15** (763 mg, 98%) as a white solid. mp 179−180 °C. $^1$H NMR (300 MHz; $CD_3CN$): $\delta$ 7.9 (s, 2H, CH-triazole), 4.3 (m, 4H, N−$CH_2$-), 4.3 (m, 4H, S-$CH_2$), 4.3 (m, 2H, CH-carborane), 3.08 (s, 12H, $N(CH_3)_4$), 2.25 (m, 4H, −$CH_2$−), 2.03 (m, 4H, $CH_2$-carborane), 1.85−0.58 (m, 22H, $B_{22}H_{22}$). $^{13}$C NMR (75 MHz; $CD_3CN$): $\delta$ 146.21 (2C, CH-triazole), 126.21 (2C, C-triazole), 76.16 (2C, C-carborane), 63.87 (2CH, CH-carborane) 56.15 (4C, (4C, $N(CH_3)_4$), 49.68 (2C, $CH_2$−N), 36.97 (2C, S-$CH_2$), 34.74 (2C, −$CH_2$−), 30.39 (2C, $CH_2$-carborane). $^{11}$B NMR (96.3 MHz; $CD_3CN$): $\delta$ −7.89, −10.9 (bs, 2B, B1), −14.52, −16.5, −19.41, −20.04, −20.49 (bs, 20B, B2−12). IR (KBr, $cm^{-1}$): $\nu$(CH) 3440 (m), 3043 (W), 2956 (W), $\nu$(BH) 2584, 2492 (S), $\nu$(C=C) 1620 (m), $\nu$(N=N) 1575 (W), $\nu$(CH) 1483 (S), 1413 (W), 1286 (W), $\nu$(B−B) 1045 (S), $\nu$(CH) 995 (m), 948 (S), 820 (m), 723 (m). MS (ESI, negative): $m/z$ 705.7 ($M^-$). Elemental analysis calcd for $C_{20}H_{63}B_{32}N_7S$: C, 30.81; H, 8.14; N, 12.57%. Found: C, 30.74; H, 8.03; N, 12.52%.

***S,S*-Bis[1-(1,2-*O*-distearoyl-*sn*-3-glycerol)-(1′,2′,3′-triazole-4yl)-methyl]sulfonioundecahydro-*closo*-dodecaborate (1-) tetramethylammonium salt (18).** A mixture of **1** (163 mg, 0.5 mmol), $Cu(OAc)_2$ (50 mg, 0.27 mmol), and sodium ascorbate (100 mg, 0.5 mmol) in acetonitrile (20 mL) was stirred at 50 °C for 5 min. While stirring, **17** (815 mg, 1.25 mmol) was added dropwise. The reaction mixture was stirred at 50 °C for 2 h until complete consumption of **1** monitored by TLC ($MeOH/CH_2Cl_2$ 3:7). The mixture was filtered off and diethyl ether (10 mL) was added until a precipitate (inorganic salts) formed. This precipitate was removed by filtration, and another 100 mL of diethyl ether was added to the filtrate, whereupon a crystalline solid was precipitated. The finely crystalline product was collected by filtration to give a white solid of **18** (700 mg, 86%). For analysis a sample recrystallized again from acetonitrile/ether was obtained as almost colorless needles. mp 62−63 °C. $^1$H NMR (300 MHz; $CDCl_3$): $\delta$ 7.66 (s, 2H, CH-triazole), 5.15 (m, 2H, CH−), 4.61 (m, 4H, N−$CH_2$−), 4.35 (m, 4H, S-$CH_2$), 4.33 (m, 4H, −$CHCH_2C$=O), 4.13 (m, 4H, $CHCH_2C$=O), 3.09 (s, 12H, $N(CH_3)_4$), 2.38 (m, 8H, −$CH_2CH_2C$=O), 1.85−0.55 (m, 11H, $B_{12}H_{11}$) 1.58 (m, 8H, $CH_2$), 1.22 (s, 80H, $CH_2$), 0.85 (t, 12H, $J_{CH}$ = 12.61 Hz, $CH_3$). $^{13}$C NMR (75 MHz; $CDCl_3$): $\delta$ 172.96, 172.83 (4C, CO), 136.85 (2C, CH-triazole), 127.0 (2C, C-triazole), 50.8 (4C, (4C, $N(CH_3)_4$), 38.69 (2C, S-$CH_2$), 69.83, 62.25, 34.15, 34.01, 31.89, 29.66, 29.59, 29.44, 29.33, 29.22, 29.07, 29.04, 28.88, 24.83, 24.78, 22.65, 14.07 (lipid-carbons). $^{11}$B NMR (96.3 MHz; $CDCl_3$): $\delta$ −12.56 (bs, 1B, B1), −20.32 (bs, 11B, B2−12). IR (KBr, $cm^{-1}$): $\nu$(CH) 2957 (m), 2920 (S), 2856 (S), $\nu$(BH) 2499 (m), $\nu$(C=O) 1741 (S), $\nu$(C=C) 1625 (m), $\nu$(N=N) 1572 (W), $\nu$(CH) 1487 (m), 1467 (m), 1275 (W), $\nu$(B−B) 1045 (S), $\nu$(CH) 995 (m), 948 (S), 721 (m). ESI-MS: $m/z$ 1551.5 (1551.5, $M^-$). Elemental analysis calcd for

**Scheme 1**




$C_{88}H_{179}B_{12}N_7O_8S$: C, 65.03; H, 11.1; N, 6.03%. Found: C, 64.91; H, 11.03; N, 5.89%.

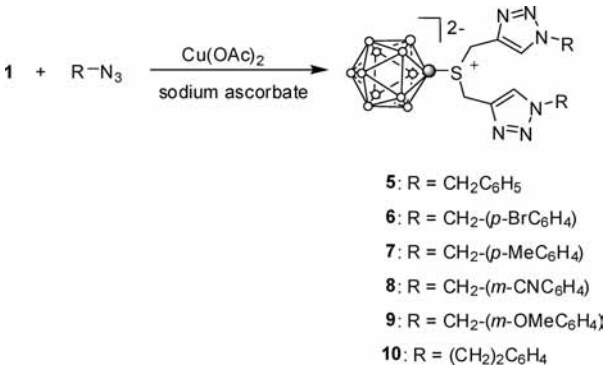
**Click Reaction in Cells.** The human cervical carcinoma cell line HeLa cells were plated on p35 dishes ($1 \times 10^4$ cells) containing 1 cm diameter glass coverslips and incubated at 37 °C for 24 h. After compound **1** treatment for 3 h, the cells were washed with PBS and fixed in 4% paraformaldehyde in PBS for 10 min. After washing with PBS, the cells were permeabilized with 0.1% Triton X-100 in PBS for 10 min, and blocked with 1% bovine serum albumin in PBS for 10 min. The click reactions with compound **1** and Alexa Fluor 488 azide (Alexa Fluor 488 5-carboxamide-(6-azidohexanyl), bis(triethylammonium salt), Invitrogen) were established with Click-iT Cell Reaction Buffer Kit (Invitrogen) according to the manufacturer's instructions. The cell nuclei were stained for 2 min with 100 nM DAPI. The cells were washed three times with PBS, mounted with Vectashield mounting medium (Vector), and analyzed under an Olympus IX71 fluorescence microscope.

## Results and Discussion

**Mono- and Dipropargylic BSH Derivatives.** The synthesis of mono- and dipropargylic BSH derivatives is shown in Scheme 1. The bis-tetramethylammonium salt of BSH was treated with 5.5 equiv of 3-bromo-1-propyne in acetonitrile/water (4:1 v/v) to give *S,S*-dipropargyl-$SB_{12}H_{11}^-$ **1** in 87% yield. For the synthesis of monopropargylic BSH derivative **4**, we utilized Gabel's BSH protection protocol.[8] BSH was first converted into *S,S*-dicyanoethyl-$SB_{12}H_{11}^-$, which was treated with tetramethylammonium hydroxide for cleavage of one of two cyanoethyl groups to give protected BSH **2**. *S*-alkylation of **2** with 3-bromo-1-propyne proceeded in acetonitrile/water (4:1 v/v) to give *S*-cyanoethyl-*S*-propargyl-$SB_{12}H_{11}^-$ **3** in 65% yield. The deprotection of **3** by treatment with $(CH_3)_4NOH$ affored *S*-propargyl-$SB_{12}H_{11}^-$ **4** in 95% yield.

**Click Chemistry of Mono- and Dipropargylic BSH Derivatives.** Click chemistry is an increasingly popular method for the rapid synthesis of novel biologically active compounds. By focusing research of new BNCT drugs on those that are available through these reliable and efficient reactions, click chemistry may accelerate the process of discovery and optimization. Three distinct features of click chemistry have called to our attention the possibility of applying this approach to the synthesis of BNCT agents: (1) the product is obtained in high yield without the need for further purification and without generating offensive byproducts; (2) the synthetic operation can be accomplished in a benign solvent, usually water; and (3) triazole units are heterocyclic structural motifs with considerable medicinal and agrochemical potential.[37]

**Scheme 2**




We first examined the click reaction of *S,S*-dipropargyl-$SB_{12}H_{11}^-$ **1** with 2.5 equiv of benzylazide at room temperature. By optimizing the reaction conditions, we found that $Cu(OAc)_2$ is a suitable source for the generation of Cu(I) species in situ in Huisgen's 1,3-dipolar cycloaddition reaction, as shown in Scheme 2. Sodium ascorbate proved to be an excellent reductant for this reaction.[38] Acetonitrile was found to be a suitable solvent because of the high solubility of both BSH-functionalized propynes and azides to give corresponding bis-triazoles **5** in 98% yield. As the reaction conditions were optimized, we employed them for further click reactions with various azides. The reactions proceeded smoothly in the presence of a semicatalytic amount of $Cu(OAc)_2$ and 0.5 equiv of sodium ascorbate at room temperature for 2 h, giving symmetric bis-triazolo BSH derivative (**5**−**10**) in 97−99% yields from **1**. Only the 1,4-disubstituted triazolo BSH derivatives were obtained (Scheme 2), in good agreement with the proposed reaction mechanism that the Cu(I) acetylide intermediate formed may undergo stepwise 1,3-dipolar cycloaddition with the azide, resulting in the regioselective product.[20,29]

We also examined the synthesis of monotriazole BSH derivative (**12**). Initial attempts to synthesize **12** by direct coupling of **4** with benzylazide in aqueous solution were unsuccessful because of the poor solubility of the azide. We found that the mixture of acetonitrile and water effectively promoted the reaction: the direct coupling of equimolar amounts of **4** and benzylazide proceeded in the presence of $Cu(OAc)_2$ and sodium ascorbate at room temperature in a mixture of acetonitrile and water (4:1 v/v) for 2 h. Compound **12** was isolated in 77% yield after purification by preparative TLC using $CH_2Cl_2$/MeOH (3:7 v/v) as eluent (Scheme 3). Another indirect route for the synthesis of **12** is the coupling reaction of **3** with benzylazide in acetonitrile under the same reaction conditions to

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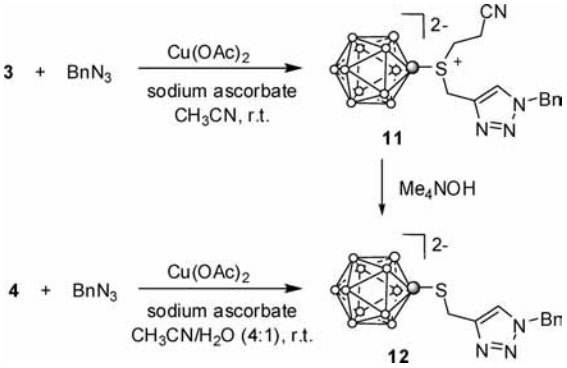
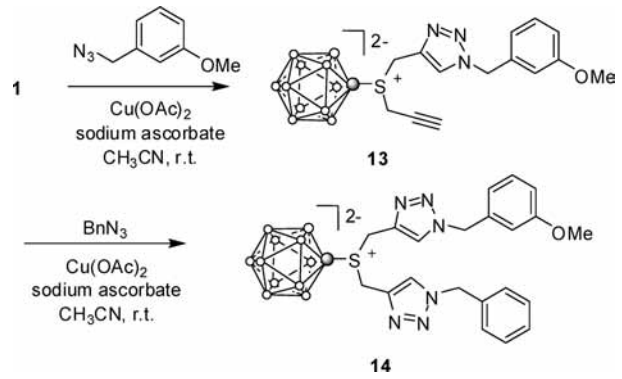
**Scheme 3**




**Scheme 4**

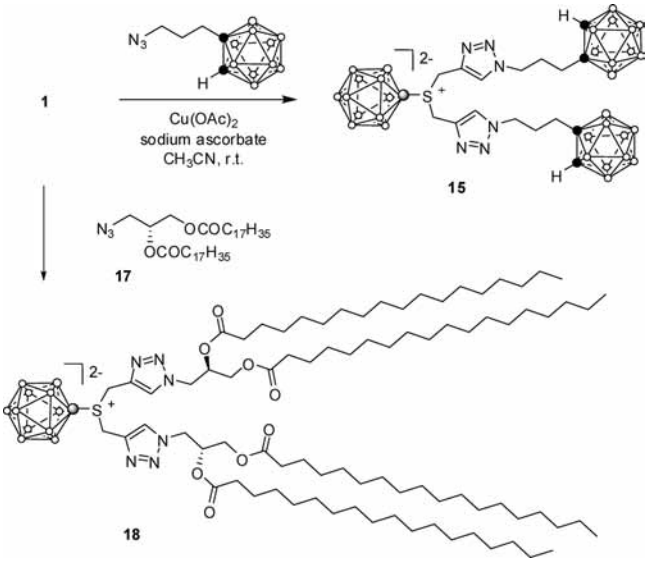



give **11** in 98% yield. Treatment of **11** with an equimolar amount of $(CH_3)_4NOH$ in acetone gave **12** in 95% yield (Scheme 3). Although the indirect method proceeds in two steps, compound **12** was isolated by reprecipitation from diethyl ether without the need for chromatographic workup.

The synthesis of an unsymmetric bis-triazolo BSH derivative (**13**) was also achieved by the stepwise click reactions of **1** with two different azides, as shown in Scheme 4. Treatment of **1** with an equimolar amount of *m*-methoxybenzylazide gave **13** in 88% yield. This provides an opportunity to intentionally preserve the unreacted propyne group for additional click reactions. Such compounds are important because the free propyne group can be used in click chemistry based fluorescence labeling methods for the visualization of boron compounds in cells incubated with azide-containing dyes. Similarly, the reaction of equimolar amounts of **13** with benzylazide gave BSH bearing two different triazole units (**14**) in 97% yield (Scheme 4). The reaction appears to be very safe and does not require any special precautions.

**Candidates for Boron Carriers for BNCT.** An efficient BNCT agent should be able to deliver a therapeutic amount of $^{10}B$ to tumors (> 20 $\mu$g/g) with high selectivity and low systemic toxicity.[1,4] The advantage of boron cluster containing compounds is that they can deliver high concentrations of boron to tumor cells with tolerable toxicity per molecule of BNCT agent administered. Polyhedral borane anions, such as *closo*-$B_{10}H_{10}^{2-}$ and *closo*-$B_{12}H_{12}^{2-}$, as well as carboranes, such as *closo*-$C_2B_{10}H_{12}$ and their corresponding *nido*-$C_2B_9H_{12}^{-}$, have been utilized for this purpose because of their known chemistry, hydrophilic/lipophilic property, high boron content, and chemical stability. Efforts to improve the efficacy of

**Scheme 5**




BNCT have focused on the development of novel boronated agents that have high boron content and exhibit selective uptake by tumor cells and favorable subcellular distribution.[39] Two drugs, $Na_2B_{12}H_{11}SH$ and *p*-boronophenylalanine (BPA), are used for the clinical treatment of glioblastoma and malignant melanoma patients, respectively, in BNCT.[40] Recently, the combination of BPA and BSH is utilized as BNCT's "cocktail" protocol for the treatment of glioblastoma and head and neck cancer patients.[41] Furthermore, the *tetrakis*-carborane carboxylate ester of 2,4-bis-($\alpha$,$\beta$-dihydroxyethyl)deuteroporphyrin-IX (BOPP)[42] was developed as an alternative candidate for boron carriers for BNCT. However, a phase I clinical study revealed its toxic side effects, most notably thrombocytopenia, which led to the limitation of its tolerable dose in humans.[43] The BNCT cocktail approach that utilizes a combination of BPA and BOPP has been demonstrated in mice bearing human undifferentiated thyroid cancer cells.[44]

Using the BNCT cocktail approach as reference, we synthesized high-boron-content compound **15** from **1** using the click cycloaddition reaction. Compound **1** was treated with 2.5 equiv of 3-azidopropyl-*o*-carborane[36] in the presence of $Cu(OAc)_2$ and sodium ascorbate in acetonitrile at room temperature to afford the bis-carborane-conjugated dodecaborate **15** in 98% yield (Scheme 5). The advantage of such compound bearing a high percentage of boron by weight (ca. 45%) is that it can potentially deliver high therapeutic amounts of boron to target

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tumor cells with tolerable toxicity. Moreover, it possesses the properties of two different boron cluster units (BSH and *o*-carborane). This new strategy in the synthesis of boronated compounds may be used to optimize BNCT with a cocktail of different boron clusters in one BNCT agent.

In addition to the BNCT cocktail approach, the liposomal boron delivery system has been attracting attention because it can deliver high therapeutic amounts of boron to tumor tissue.[45−49] Lipid carriers extravasate through the highly permeable microvessels of the tumors and remain locked in the interstitial fluid compartment because of the lack of functional lymphatic drainage.[50] Liposomes might therefore be useful vehicles for transporting boron to tumor tissue. Boron-containing lipids constitute very interesting building blocks for the construction of boron-containing liposomes and several approaches toward the synthesis of such lipids intended for incorporation into liposomes have been developed in our laboratory and others.[14−16,51−53]

In the present study, we demonstrated a new route for the preparation of a *closo*-dodecaborate lipid with a four-tailed moiety using click chemistry. For BSH coupling, it was necessary to prepare the requisite azide derivative of the lipid. The reaction of 1,2-*O*-distearoyl-*sn*-3-glycerol[14] with 1.2 equiv of *p*-tolylsulfonyl chloride gave **16** in 76% yield, which reacted with 5 equiv of sodium azide to obtain **17** in 90% yield after purification by column chromatography. The click cycloaddition reaction of **1** with azidolipid **17** was then performed in acetonitrile at 50 °C to give corresponding boronated lipid **18** in 86% yield (Scheme 5). In this case, a high temperature (50 °C) is necessary compared to the other cycloaddition reactions described in this paper because of the insolubility of azidolipid **17** in acetonitrile at room temperature.

**Spectroscopic Studies.** To gain information about the structures of the boronated compounds, NMR, IR, mass spectrometry, and elemental analysis were conducted. $^1$H NMR signals for aliphatic hydrogens (C≡CH and $CH_2$) of **1** and **3** appeared at about 3.89 and 2.81 ppm, respectively, whereas $^{13}$C NMR signals for CH, C, and $CH_2$ groups appeared at about 77.85, 74.5, and 30.5 ppm, respectively. These signals showed shifts to the high field region in both $^1$H and $^{13}$C NMR spectra of **4** upon removal of the cyanoethyl group in **3** by $(CH_3)_4NOH$. The $^1$H and $^{13}$C NMR spectra of the boronated compounds (**5**−**15** and **18**) revealed signals characteristic of both $B_{12}H_{11}S^{2-}$ derivatives and azide derivatives, with new signals corresponding to the triazole proton and the methylene group at N1 position of triazole. In the $^1$H NMR spectrum of **13**, the signal at 2.85 ppm indicated the presence of a free alkyne group that is available for further coupling, as expected from the click cycloaddition reaction of **1** with *m*-methoxybenzyl azide in a 1:1 ratio. The structure of compound **16** was also confirmed by $^1$H NMR spectroscopy, which showed new signals of the aromatic CH doublet at 7.92, 7.77, 7.4, and 7.34 ppm and a $CH_3$ singlet at 2.22 ppm. These signals disappeared in the $^1$H NMR spectrum of azide **17**, and a new signal of the methylene group ($-CH_2N_3$) appeared at 3.43 ppm. The $^{11}$B NMR spectra presented a characteristic shielding pattern over a quite remarkable range of about −19 to −15 ppm for **1**, **3**, **5**−**11**, **13**, **14**, and **18** and of about −9.5 to −22 ppm for **4** and **12**, showing only minor differences in the overall $^{11}$B cluster shielding patterns. The $^{11}$B NMR spectrum of **15** consisted of singlets at −7.8, −10.9, −14.5, and −16.5 ppm and multiplets in the range of −19.4 to −20.5 ppm with relative areas of 1:1:2:1:27. The high field singlets were assigned to three equatorial boron atoms, one apical boron atom of carboranes, and one apical boron atom of BSH cluster, respectively. The low field multiplets were assigned to 26 equatorial boron atoms of carboranes and the BSH cluster, as well as one apical boron atom of the BSH cluster.

Boronated compounds have characteristic stretching modes that are suitable for study by IR spectroscopy. The IR spectra of **1**, **3**, **4**, and **13** contained a weak absorption band located at about 2125 cm$^{-1}$, which could be attributed to the vibrational mode of the C≡C group. Free azide **17** exhibited a strong absorption band at 2160−2120 cm$^{-1}$ because of the asymmetric stretching of the azide group, which occurred as a doublet. These were replaced with new medium and weak absorption bands within regions 1641−1602 and 1590−1555 cm$^{-1}$, which are characteristic of C=C and N=N groups, respectively. The $\nu$(B−H) or the $\nu$(B−B) were not sensitive to the click reactions. For compounds **1**, **3**, **4**−**15**, and **18**, $\nu$(B−H) lay in the 2584−2499 cm$^{-1}$ region, whereas $\nu$(B−B) varied from 1049 to 1045 cm$^{-1}$. Among the frequencies of the $B_{12}H_{12}^{2-}$ moiety {$\nu$(B−H) 2486 to 2462 cm$^{-1}$; $\nu$(B−B) 1073 to 1057 cm$^{-1}$},[54] only slight differences were found among the compounds, indicating that intracluster bonding was not perturbed by the substitution of the icosahedron.

The negative-ion ESI mass spectra of compounds **1**, **3**, **5**−**11**, **13**−**15**, and **18** showed only the signal of a singly charged ion whose mass and typical isotopic pattern of boron isotopes ($^{10}$B and $^{11}$B) suggest the molecular formula at $m/z = M^-$. The ESI mass spectra of **4** and **12** showed only the signal of the doubly charged molecular anion that was attributed to $m/z = M^-/2$.

**Visualization of Compound 1 by Click Cycloaddition Reaction with Alexa Fluor 488 Azide in HeLa Cells.** The development of technology for the chemical modification of compounds in, or on, living cells under physiological conditions has become an important issue for the dynamic imaging of drugs in medicinal chemistry. We examined the click cycloaddition reaction of compound **1** with Alexa Fluor 488 azide, which emits maximum

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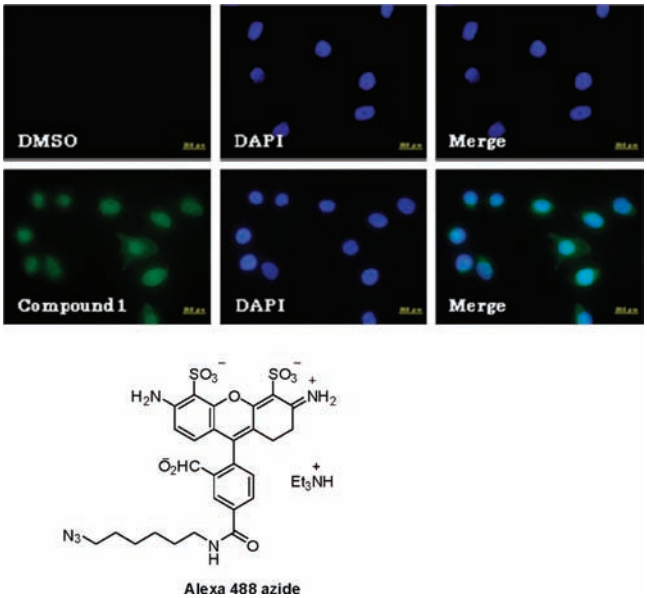


**Figure 1.** Click chemistry in cells. HeLa cells were incubated for 3 h with compound **1** (1 mM). After fixation and permeabilization of the cells, click reactions with compound **1** and Alexa Fluor 488 azide were performed, and the cell nuclei were stained with DAPI. The cells were analyzed under a fluorescence microscope.

fluorescence at 520 nm with excitation at 495 nm, in HeLa cells. The cells were plated on dishes containing 1 cm diameter glass coverslips and incubated at 37 °C for 24 h. Then, they were treated with compound **1** (1 mM) for 3 h. After fixing the cells with 4% paraformaldehyde in PBS for 10 min, the click cycloaddition reaction was performed with Alexa Fluor 488 azide. Cell nuclei were stained for 2 min with 100 nM 4′,6-diamino-2-phenylindole (DAPI). Fluorescence microscopy images are shown in Figure 1. HeLa cells treated with DMSO (without compound **1**) and Alexa Fluor 488 azide did not show any fluorescence except DAPI images (blue). In contrast, cells treated with compound **1** and Alexa Fluor 488 azide showed green fluorescence. The results indicate that compound **1** accumulating in the cells reacted with Alexa Fluor 488 azide in the cells and that the Alexa Fluor 488-conjugated *closo*-dodecaborates were illuminated by the fluorescence microscope. Interestingly, the fluorescence of the Alexa Fluor 488-conjugated *closo*-dodecaborates was observed mainly in the nuclei. Therefore, it was revealed that compound **1** accumulated not in the cytoplasm but in the nuclei of cells.

## Conclusions

We have developed a new method to functionalize BSH with organic molecules. Our method focused on the synthesis of two classes of BSH terminal functionalized propyne groups: (*S,S*-dipropargyl-$SB_{12}H_{11}^-$: **1**) and (*S*-propargyl-$SB_{12}H_{11}^{2-}$: **4**). Compounds **1** and **4** acted as powerful building blocks 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 current reactions require only benign reaction conditions and simple workup and purification procedures, and an unsymmetric bis-triazolo BSH derivative could also be synthesized by the stepwise click reaction. Compound **1** with 3-azidopropyl-*o*-carborane gave a high-boron-content compound having two different boron clusters (one BSH and two *o*-carboranes). The synthesis of BSH lipid with four-tailed moieties was also achieved by the click cycloaddition reaction of **1** with 3-*O*-azidoacetyl-1,2-*O*-distearoyl-*sn*-3-glycerol, which may be useful in the liposomal boron delivery system for neutron capture therapy. Finally, we demonstrated the click reaction of compound **1** with Alexa Fluor 488 azide in HeLa cells and found that compound **1** accumulated not in the cytoplasm but in the nuclei of the cells. We believe that this study not only provides synthetic applications but also clarifies the biological mechanism of BSH derivatives for neutron capture therapy.

**Acknowledgment.** We wish to acknowledge Dr. H.-S. Ban (Gakushuin Uinversity) for his contribution of biological experiments. This work was supported in part by Health and Labour Sciences Research Grants for Research on Nanotechnical Medical from the Ministry of Health, Labour and Welfare (20100201). M. E. El-Zaria thanks the Japan Society for the Promotion of Science (JSPS, ID No. P08363) for the financial support.

**Note Added after ASAP Publication.** This article was released ASAP on November 20, 2009. Reference 31 was updated and the correct version was posted on November 25, 2009.

**Supporting Information Available:** Detailed experimental procedures and characterization data of compounds **3**, **6**−**11**, **13**, **16**, and **17**. $^{11}B$, $^{1}H$, $^{13}C$, and $^{13}C$ dept 135° NMR spectra of compounds (**1**−**18**). This material is available free of charge via the Internet at http://pubs.acs.org.