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Original article

New functionalized mercaptoundecahydrododecaborate derivatives for potential application in boron neutron capture therapy: Synthesis, characterization and dynamic visualization in cells

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ABSTRACT

A series of mercaptoundecahydrododecaborate ($B_{12}H_{11}SH^{2-}$, BSH) bearing mono- and dicarboxyalkyl derivatives was prepared, characterized, and their reactivity towards amidation and esterification in DMF was evaluated. Symmetrical alkylation of BSH was achieved by treatment with primary haloalkyl carboxylic acids in aqueous acetonitrile to produce *S,S*-bis(carboxyalkyl)sulfonium-undecahydro-*closo*-dodecaborate tetramethylammonium salts. Unsymmetrically substituted sulfonium salts were obtained through a similar treatment of cyanoethylthioether-undecahydro-*closo*-dodecaborate tetramethylammonium salt with haloalkyl carboxylic acid. Selective removal of the remaining cyanoethyl group upon treatment with tetramethylammonium hydroxide yielded *S*-carboxyalkyl-thioether-undecahydro-*closo*-dodecaborate ditetramethylammonium salts. *N,N′*-dicyclohexylcarbodiimide (DCC) activated amidation of *S,S*-bis(carboxyalkyl)sulfonium-undecahydro-*closo*-dodecaborate or *S*-carboxyalkyl-thioether-undecahydro-*closo*-dodecaborate tetramethylammonium salts with propargylamine provided the opportunity to install terminal acetylene groups for further conjugation. These compounds acted as powerful building blocks for the synthesis of a broad range of 1,4-disubstituted 1,2,3-triazole products in high yields, utilizing the Cu(I)-mediated click cycloaddition reaction. The synthesis of BSH-lipid with a two-tailed moiety was also achieved, by esterification of *S,S*-bis(carboxyethyl)sulfoniumundecahydro-*closo*-dodecaborate(1-) tetramethylammonium salt with 1,2-*O*-distearoyl-*sn*-3-glycerol, which may prove useful in the liposomal boron delivery system. The bio-compatibility of the azide-alkyne click reaction was then utilized by performing this reaction in cell culture. The distribution of BSH in HeLa cells could be visualized by treating the cells first with a BSH-alkyne compound and then with Alexa Fluor 488® azide dye. The BSH-dye conjugate, which did not wash out, revealed the distribution of boron in the HeLa cells. Cytotoxicity assays of these BSH derivatives revealed that the synthesized BSH-conjugated triazoles possessed low cytotoxicity in HeLa cancer cells. Of these compounds, BSH conjugated triazole **15** induced a significant increase in the level of boron accumulation in HeLa cells.

1. Introduction

"Click chemistry" is an attractive tool for medicinal chemistry applications because it is compatible with the aqueous biological environment and can be used for rapid synthesis of novel biologically active compounds [1]. Click reactions, requiring only benign reaction conditions and simple workup and purification procedures, can rapidly create molecular diversity through the use of reactive modular building blocks. By focusing research for new compounds only on those available through these reliable and efficient reactions, click chemistry may accelerate the process of discovery and optimization. The Cu(I)-catalyzed azide-alkyne cycloaddition reaction is one of the most reliable click reactions [2]. This reaction has enabled practical and efficient preparation of 1,4-disubstituted-1,2,3-triazoles from an unprecedented range of substrates with excellent

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selectivity, which could not otherwise be obtained using the traditional Huisgen thermal approaches [3]. Since its discovery the Cu(I)-catalyzed azide-alkyne cycloaddition reaction has been applied in many processes including the synthesis of therapeutics [4], protein-based biohybrids [5,6], sugar arrays [7], dendrimers [8] and functional polymers [9]. The proposed mechanism for this 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 which then yields the triazole products [2,10].

Boron neutron capture therapy (BNCT) is an anti-cancer treatment that involves the irradiation of ^{10}B-rich tumors with low energy neutrons [11]. Subsequent productions of high linear energy transfer particles, $^{4}He_2$ (α-particle) and $^{7}Li_3$, cause severe damage to tumor cells through ionization process. The advantage of this binary approach is in the differential dose that can be established between the tumor and its surrounding normal tissue, provided the compound which carries the target atom, is avidly taken up in tumor, yielding a high tumor to normal tissue ratio. Dodecaborates are undergoing a renaissance in the literature due to their unique structural and electronic properties and potential use in creating new diagnostics, therapeutics and electronically tunable materials [12–14]. In regards to compounds for use in BNCT, the mercaptoundecahydro-*closo*-dodecaborate disodium salt ($Na_2B_{12}H_{11}SH$, "BSH") exhibits a strong biological advantage over the related dicarba-*closo*-docedaborane ($C_2B_{10}H_{12}$, "carborane") compounds as it possesses a higher percentage of boron, is ionic in nature, and is significantly lower in toxicity based on its boron content. The presence of a potentially reactive sulfhydryl group in the BSH cluster is very important for the preparation of other tumor-seeking compounds [15]. For this purpose, numerous BSH-derivatives of biomolecules (e.g. pophyrins, nitroimidazoles, sugars, chlorins, and lipids) have been synthesized to date [16–22]. The goal of these efforts is the development of new boron delivery agents that are able to reach the stage for evaluation in a phase I clinical biodistribution study.

Although the main requirement for any new BNCT agent is to produce a sufficient amount of boron content at the target site, the agent must also possess low toxicity, water solubility, chemical stability, and if possible, a flexible high-yielding synthetic methodology. In order to meet these requirements, we explore in this paper procedures to prepare novel BSH-containing building blocks bearing free carboxylic acid and alkyne groups. Using these building blocks we explore procedures to covalently incorporate BSH into new compounds for treating cancer by BNCT. These compounds have been prepared with a view to their usefulness in wider bioconjugation and bioimaging applications.

An important step in the evaluation of new agents for BNCT is to investigate their uptake in cells *in vitro* or in larger organisms *in vivo*. Although carborane-containing compounds may be imaged directly using Raman microspectroscopy on a small scale (e.g. cobaltabisdicarbollide derivatives (COSAN) [23,24]), a molecular imaging approach using optical or radionuclear probes would be ideal for imaging in small animals and humans [18,25]. In either case, there is a clear need for carborane modalities that may be quickly and easily appended on to other useful groups using click chemistry. It will be shown that the simple BSH modalities described in this paper may also be appended onto a dye allowing BSH uptake to be detected in the cell, and that this reaction is able to take place inside the cell.

2. Results and discussion

2.1. Chemistry

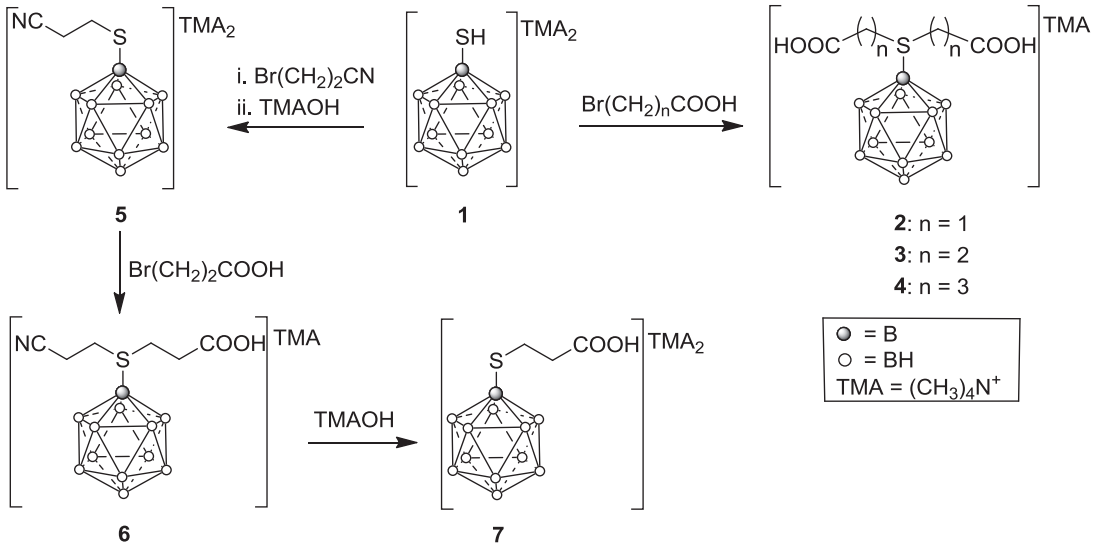
2.1.1. Functionalized BSH compounds as building blocks for organic reactions

Compounds bearing the BSH cluster are particularly attractive as boron-delivery drugs for BNCT. There is currently a great need for an improved synthetic pathway toward BSH-compounds that contain functional groups for conjugation to biomolecules [26]. Boron clusters bearing carboxylic groups can be used to prepare boronated antibodies as vehicles for the selective delivery of therapeutic quantities of ^{10}B to tumor for the purpose of BNCT [27]. The necessary BNCT boron concentration can also achieved by conjugating our BSH-derivatives to amino- or hydroxyl-dendrimers. To fulfill our aim, two synthetic strategies have been employed in the preparation of functionalized BSH according to Scheme 1. One is a direct synthetic route to prepare BSH bearing two symmetrical carboxylic acid groups, in which 1 equiv. of the BSH was allowed to react with 5 equiv. of bromoalkyl carboxylic acid to yield **2**–**4** in 80–87% yield. This simple one-step reaction gave significantly higher yields than that reported for BSH [28], which also employed a symmetrical carboxylic synthesis.

Compound **2**, isolated in 80% yield, was clearly identified by ESI-MS (*m/z* 291.2, $[M]^-$). The spectrum displayed the molecular ion in a ^{10}B/^{11}B isotopic distribution pattern characteristic of polyhedral boranes. The ^{1}H NMR spectrum of **2** clearly indicated the presence of the acidic protons (7.77 ppm), methylene group (3.89 ppm), tetramethylammonium cation (3.07 ppm) and the B–H cluster protons (1.93–0.56 ppm, overlapping broad singlets) in the expected integral ratios. The ^{13}C NMR spectrum also confirmed the product, featuring resonances consistent with C=O, $N(CH_3)_4$ and methylene groups (116.9, 56.0 and 44.3 ppm, respectively). The ^{11}B NMR spectrum contained only two broad signals (−15.1 and −19.0 ppm) in a 1:11 pattern, which is consistent with a disubstituted rather than monosubstituted derivative. The IR spectrum of **2** contained the characteristic strong absorption bands located at 2495 and 1753 cm^{-1}, which were assigned to the B–H and C=O groups, respectively.

To probe the effect of alkyl chain length on the alkylation reaction, additional BSH-derivatives **3** and **4** were prepared using the same method as for **2**, and were isolated in 85% and 87% yield, respectively. The ^{1}H NMR spectra of **3** and **4** contained resonances for the BS-CH_2 groups between 3.36 and 3.31 ppm. However, the resonances assigned to the CH_2–COOH protons at 2.35 and 1.92 ppm, respectively, were upfield shifted with respect to the corresponding protons in **2**. The negative-ion ESI mass spectra of **3** and **4** gave the expected molecular ion peaks (*m/z* 319.2 and 347.3, respectively) in typical isotopic distribution patterns.

A second synthetic route was explored where only one carboxylic group was incorporated into the final BSH product. This route proceeded by a stepwise reaction via *S*-cyanoethylmercaptoundecahydrododecaborate (**5**) [15], which was then treated with bromopropanoic acid to give **6** in 79% yield. Subsequent alkaline removal of the cyanoethyl protecting group gave **7** in greater than 95% yield (Scheme 1). Alkylation of **1** with all of the primary carboxylic halides investigated invariably led to simultaneous appearance of mono- and bisalkylated products, often in the continued presence of unreacted thiol. With an excess of halide, bisalkylated sulfonium salts are obtained as a rule. It was found that the cyanoethyl group could be used as a convenient protective group for the sulfur of $B_{12}H_{11}SH^{2-}$ [15]. In the ^{11}B NMR spectrum of **6**, two resonances (−14.9 and −19.0 ppm, 1:11 pattern) were observed, consistent with the disubstituted structure, whereas the spectrum of **7** exhibited four resonances (−11.4, −19.5, −20.3 and −22.4 ppm, 1:5:5:1 pattern) after deprotection, which was expected for the monosubstituted final product. The NMR signals for the ethylene group were upfield shifted in both the ^{1}H and ^{13}C NMR spectra of **7** upon removal of the cyanoethyl group in **6** by TMAOH. The ESI mass spectrum of **6** showed a disubstituted isotopic pattern with molecular ion peaks at *m/z* 300.2. The ESI mass spectrum of **7** showed a monosubstituted isotopic pattern with a



Scheme 1. General pathway for synthesis of compounds **2**–**7**.

molecular ion peak at *m/z* 122.8 consistent with the doubly charged molecular anion $[M]^{2-}$. This was further confirmed by microanalysis.

2.1.2. Amidation and esterification of S,S-alkylated BSH

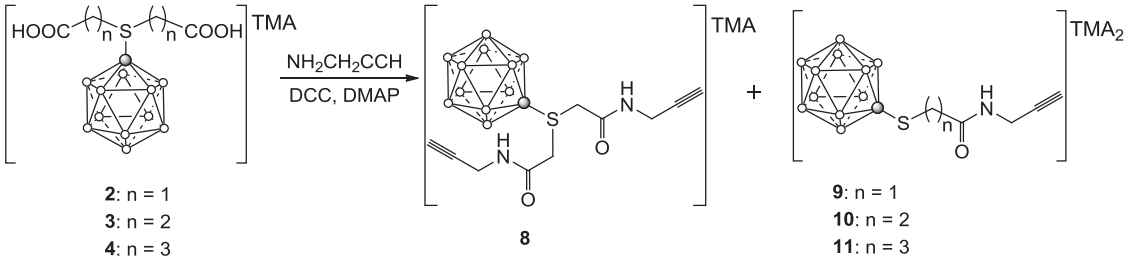
With the BSH-carboxylic acid derivatives in hand, BSH derivatives bearing terminal alkynes were then accessed by coupling the acids to propargylamine, under well-established amide-coupling conditions (*N,N′*-dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)pyridine (DMAP), Schemes 2 and 3). These compounds could then be exploited as versatile BSH-building blocks using the Cu(I)-mediated click reaction.

The reactions of **2**–**4** with propargylamine proceeded according to Scheme 2. The conditions are considered relatively mild since the reactions were performed at room temperature. Each amidation reaction was performed in *N,N′*-dimethylformamide (DMF) solution as this solvent was capable of dissolving both the BSH acids and propargylamine. The resulting products (**8**–**11**) were purified by column chromatography using methanol/dichloromethane (1:4) eluent. The formation of both mono and disubstituted BSH-amide products from pure disubstituted BSH-acid starting materials indicated the effect of basicity on the disubstituted BSH derivatives. This cleavage of S–C bonds is common when using basic media, or when strongly electron-withdrawing substituents are attached to the BSH cage [15,12]. The reaction of dodecaborates containing carboxymethyl groups (**2**) leads to compound **8** as major product while compounds **3** and **4** produce **10** and **11**, respectively. This can be attributed to the absence of β-hydrogen on compound **2** [12].

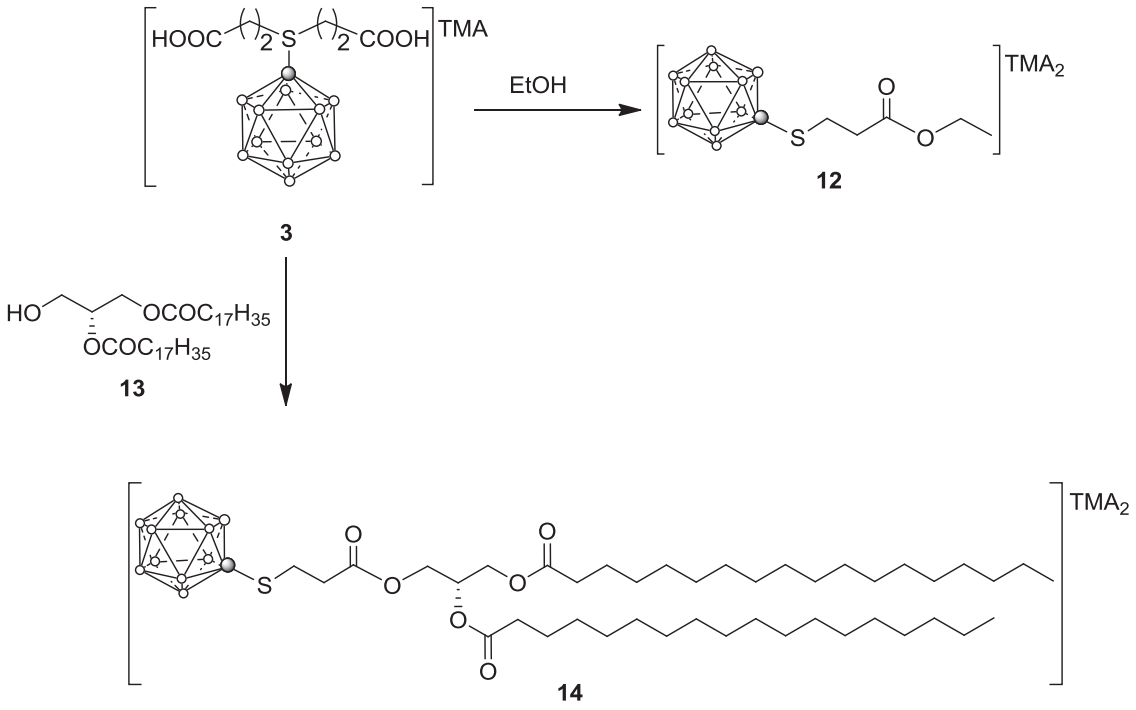
The ^{1}H NMR spectrum of **8** exhibited resonances distinctly different to those observed in the ^{1}H NMR spectrum of the precursor **2**. In particular, the presence of propargylamide was identified by resonances at 6.78, 3.86 and 3.39 ppm, corresponding to the NH, CH_2 and C≡CH groups, respectively, whereas the resonance corresponding to COOH was not observed. The ^{13}C NMR spectrum also indicated the presence of proparylamide with additional resonances at 81.1, 72.4, and 56.2 ppm corresponding to the *C*≡CH, *C*≡CH, and *CH*$_2$*C*≡*C*H groups, respectively. The negative-ion ESI mass spectrum contained the expected molecular ion peak (*m/z* 369.2), and the IR spectrum revealed bands characteristic of C≡C (2123 cm^{-1}) and amide NH (1645 cm^{-1}) groups. The ^{11}B NMR spectrum of **8** contained two broad singlets at *ca.* −19 and −15 ppm in the 1:11 pattern typical of disubstituted BSH derivatives. In contrast, the ^{11}B NMR spectrum of **9** contained signals in the 1:5:5:1 pattern, typical of monosubstituted BSH derivatives. The identity of **9** was further confirmed by ESI-MS (*m/z* 134.5, $[M]^{2-}$) and microanalysis. Similarly, compounds **10** and **11** were identified and characterized by NMR, IR, ESI-MS, and elemental microanalysis. Despite multiple attempts, single crystals of **8**–**11** could not be grown.

In the course of selecting ideal reaction solvents for the amide coupling reactions, it was discovered that in the presence of DMAP (3–10 mol%) the DCC-activated esterification of BSH-carboxylic acids with alcohol was accelerated. The ester product could be formed in good yield at room temperature with minimal side-product formation (Scheme 3). For example, under these conditions the targeted synthesis of ethyl ester **12** from **3** could be achieved in high yield (83%) by treatment with ethanol.

To demonstrate the wider utility of this esterification reaction,



Scheme 2. Synthesis of compounds **8**–**11**.



Scheme 3. Synthesis of compounds **12** and **14**.

the esterification of **3** to produce a compound for liposomal born delivery was attempted. The liposomal boron delivery system has attracted much attention because of its capability of delivering high therapeutic amounts of boron to tumor tissue [29–33]. Lipid carriers extravasate through the highly permeable microvessels of tumors and remain locked in the interstitial fluid compartment due to the lack of functional lymphatic drainage [34]. Liposomes may therefore function as 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 have been developed in our laboratory and others [22–30,35–38]. Following the successful preparation of **12**, the versatile BSH-carboxylic acid **3** was treated with 1,2-*O*-distearoyl-*sn*-3-glycerol (**13**) in the presence of DMAP and DCC at 40 °C. This yielded the target BSH-lipid ester **14** in 89% yield. In this case, a higher temperature (40 °C) was necessary to dissolve the lipid **13** in DMF.

The ester products **12** and **14** were identified in their ^{1}H NMR spectra by resonances corresponding to the ester methylene group ($COOCH_2$) appearing at 4.35 ppm, with associated absences of acidic and alcoholic OH resonances. Evidence in support of structures **12** and **14** was also obtained by ESI mass spectrometry. The spectra of **12** and **14** showed the expected doubly charged negative molecular ions *m/z* 274.2 and *m/z* 433.2, respectively, in the typical ^{10}B/^{11}B isotopic pattern.

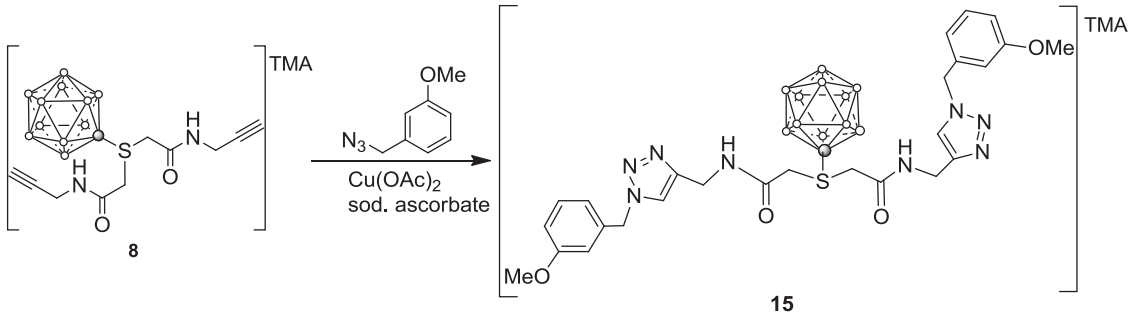
2.1.3. Click chemistry of BSH derivatives

Though a variety of click reactions have been reported in the literature, the Huisgen 1,3-dipolar cycloaddition of azides and alkynes plays a particularly important role in organic synthesis, and has become a common coupling procedure in all chemical disciplines in recent times. Four distinct observations have drawn our attention to the possibility of applying click chemistry to the synthesis of BNCT agents: (1) This reaction is high yielding without generating offensive byproducts and requires only minimal purification; (2) The reaction proceeds in benign solvent, usually water; (3) Triazole units are heterocyclic structural motifs with considerable medicinal and agrochemical potential [39]; (4) It is possible to generate a plethora of new compounds quickly and reliably, which thereby accelerates the process of drug discovery.

In the course of reaction optimization, it was found that $Cu(OAc)_2$/sodium ascorbate mixture was an excellent system for the *in situ* production of Cu(I) catalyst [40] and allowed the preparation of a broad spectrum of 1,4-disubstituted 1,2,3-triazole products in high yield. Acetonitrile-water (1:1) was the solvent of choice, which permitted greater solubility of both the BSH-functionalized alkynes and azides in the reaction mixture, and conforms to many click reaction conditions reported in the literature. Most of the reactions in this work gave excellent product yields ranging from 91% to 98% (room temperature, 6 h). In particular, the *S,S*-bis(1,4-disubstituted-1,2,3-triazole)thioundecahydro-*closo*-dodecaborate product (**15**) was obtained in 98% yield after only 2 h, by treatment of **8** with *m*-methoxy-benzylazide [41,42] (Scheme 4). Only the 1,4-disubstituted triazole isomer was obtained, which is in agreement with the proposed reaction mechanism wherein the Cu(I)-acetylide intermediate undergoes a stepwise addition with the azide, resulting in a regioselective product [2,10].

Preliminary attempts to synthesize *S*-[1,4-disubstituted-1,2,3-triazole]thioundecahydro-*closo*-dodecaborate(2-) ditetramethylammonium salts (**16** and **17**) by direct coupling of **10** with azides in aqueous solution were unsuccessful, due to the poor aqueous solubility of the azide. However, the direct coupling of equimolar amounts of **10** and either benzylazide or *p*-bromo-benzylazide proceeded reliably in a mixture of acetonitrile-water (4:1) over 2 h, furnishing compounds **16** and **17** in 91% and 96% yield, respectively (Scheme 5). The products were isolated by preparative TLC using dichloromethane/methanol eluent.

The ^{1}H and ^{13}C NMR spectra of the BSH-conjugates **15**–**17** revealed resonances similar to their corresponding BSH and azide precursors, with additional resonances corresponding to newly formed triazole groups. The ^{1}H NMR spectra of **15**–**17** contained signals for the aromatic triazole CH in the range 8.09–6.84 ppm.



Scheme 4. Synthesis of compound **15**.

The ^{1}H NMR spectrum of **15** also contained a singlet characteristic of the methoxy protons at 2.22 ppm. The presence of aromatic groups was confirmed by the ^{13}C NMR spectra of **15**–**17**, which contained aromatic carbons in the range 169.2–114.6 ppm. The ^{11}B NMR spectra of **15**–**17** were virtually unchanged from that of their corresponding precursors **8** and **10**, indicating that the S–C bonds were stable to cleavage under these reaction conditions, as expected. This was further confirmed by the ESI mass spectra, which did not contain ions characteristic of such degradation. Distinct changes in the IR spectra also confirm triazole formation. The IR spectra of precursors **8** and **10** contained a weak absorption band at 2125 cm^{-1}, attributed to the vibrational mode of the C≡C group. The IR spectra of the free azides exhibited a strong absorption band at 2160–2120 cm^{-1} due to the asymmetric stretching of the azide group, which occurred as a doublet. As expected, such bands in the IR spectra of the products **15**–**17** were absent, and the presence of medium and weak absorption bands at 1651–1625 and 1551 cm^{-1}, characteristic of C=C and N=N groups, respectively, further confirm triazole formation. The BSH vibrational modes ν(B–H) and ν(B–B) did not change significantly after conjugation. For compounds **15**–**17**, ν(B–H) lay in the 2482–2503 cm^{-1} region, and ν(B–B) lay in the region 1047–1050 cm^{-1}. Only minor differences are observed in the IR spectrum of the parent $B_{12}H_{12}^{2-}$ compound (ν(B–H) 2486–2462 cm^{-1}; ν(B–B) 1073–1057 cm^{-1}) [43], indicating that intracluster bonding was not perturbed by functionalization of the icosahedron.

A fluorescent triazolo-carborane could be prepared by reaction of Alexa Fluor 488® azide with compound **9** (Scheme 6). Successful formation of the boronated Alexa Flour conjugate (**18**) was confirmed by ESI mass spectrometry, elemental analysis and ^{1}H NMR spectroscopy. The aromatic protons of the dye moiety (δ 7–8 ppm) were easily identified, while the remaining dye proton environments overlapped with the boron cluster region (δ 1–4 ppm).

2.2. Biology

2.2.1. Cell toxicities of BSH conjugated triazoles (*15*–*18*)

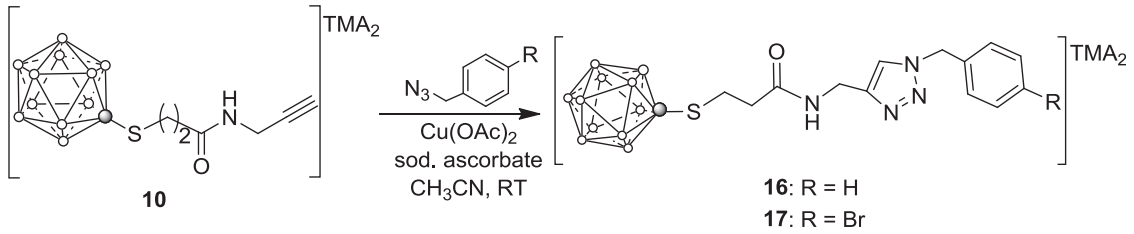
The *in vitro* toxicity of the synthesized BSH conjugated triazoles (**15**–**18**) toward HeLa cancer cells was examined by means of a cell viability assay using 3′-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). Of the boronated triazoles, the BSH-conjugated Alexa fluor **18** showed slightly lower cytotoxicity in relation to compounds **15**–**17** (Table 1), However, all the BSH-conjugated mono-triazoles (**15**–**18**) were significantly less cytotoxic than the parent BSH (IC_{50} 27–72 μM *vs.* 2.5 μM). The more hydrophilic BSH conjugated mono-triazoles **16** and **17** (IC_{50} = 49 and 51 mM, respectively) were found to be slightly more cytotoxic toward HeLa cells than BSH conjugated di-triazole **15** (IC_{50} = 27 μM). These results indicate that conjugation of only one triazole group to the polyhedral borane anion resulted in reduced cytoxicity.

2.2.2. Intracellular boron concentrations of BSH conjugated triazoles (*15*–*18*)

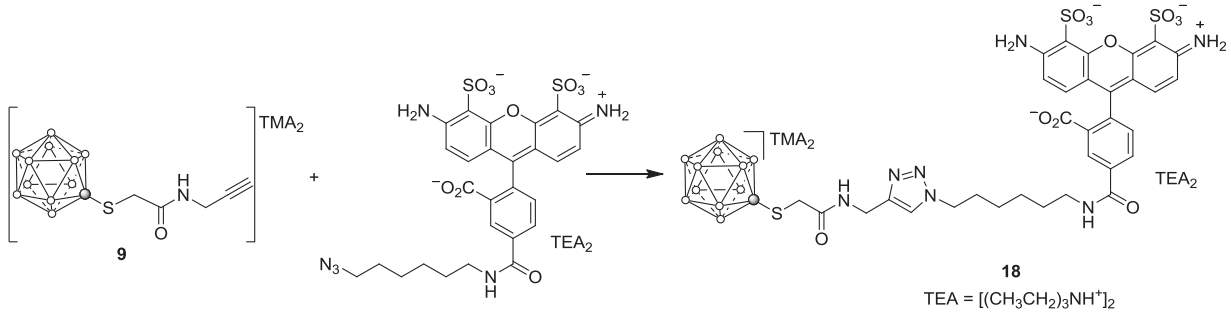
The levels of intracellular accumulation of the BSH conjugated triazoles were examined by determination of boron concentrations in HeLa cells by ICP-AES (inductively coupled plasma atomic emission spectroscopy). As shown in Table 1, the resulting intracellular accumulation of boron after treatment of the cells with boronated triazoles **16**–**18** (612–1542 ppm) was at least two-fold higher than after treatment with the parent compound BSH (310 ppm). Interestingly, compound **15**, which incorporates two triazole groups, showed the highest intracellular boron accumulation (1452 ppm), roughly twice that of the other compounds **16**–**18** (612–743 ppm). This result suggests the additional triazole group promoted uptake, perhaps due to increased lipophilicity.

2.2.3. Visualization of compound *9* by click cycloaddition reaction with Alexa Fluor 488® azide in HeLa cells

The imaging of biomolecules within living systems requires the means to distinguish the target from the surrounding components, usually by use of a spectroscopic probe. Click chemistry has prevailed in applications where toxicity is irrelevant or when visualizing biomolecules in fixed cells [44,45]. The click cycloaddition reaction of compound **9** with Alexa Fluor 488® azide, a dye which emits maximum fluorescence at 520 nm with excitation at 495 nm,



Scheme 5. Synthesis of compounds **16** and **17**.



Scheme 6. Synthesis of compound **18**.

Table 1
The *in vitro* toxicity and intracellular uptake of BSH and BSH conjugated triazoles (**15**–**18**).

Compound	IC_{50} (μM)	Boron concentration (ppm)
BSH	2.5 ± 1.6	310 ± 2.4
15	27 ± 2.1	1452 ± 1.4
16	49 ± 0.5	743 ± 1.7
17	51 ± 0.6	675 ± 0.8
18	72 ± 1.3	612 ± 0.9

HeLa cancer cells were incubated for 72 h in the presence of each BSH-conjugated triazole, and the ratios of viable cells were determined by MTT assay. The drug concentration required to inhibit cell growth by 50% (IC_{50}) was determined from semi logarithmic dose–response plots, and results represent the means ± SDs of triplicate samples.

was examined inside HeLa cells. The cells were plated on dishes containing 1 cm-diameter glass coverslips and incubated at 37 °C for 24 h. They were then treated with a solution of compound **9** (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 then stained for 2 min with 100 nM 4′,6-diamino-2-phenylindole (DAPI). Fluorescence microscopy images are shown in Fig. 1.

HeLa cells treated with both the DMSO vehicle (without compound **9**) and Alexa Fluor 488® azide did not show any fluorescence other than DAPI (blue), indicating that the Alexa Fluor 488® azide did not accumulate in HeLa cells in its unconjugated form. However, cells treated sequentially with compound **9** first then Alexa Fluor 488® azide second showed both the characteristic Alexa Fluor (green) and DAPI (blue) fluorescences after the PBS wash. This promising result shows that the click reaction with Alexa Fluor 488® azide proceeded successfully within the cells. As the Alexa Fluor 488® dye became conjugated to the intracellular BSH derivative **9** and was trapped, the trapped conjugate could then be observed, directly revealing the distribution of **9** inside the cell. As shown in Fig. 1, the Alexa Fluor 488® fluorescence was not coincident with the DAPI fluorescence, which is nucleus-specific. Therefore, these data show the accumulation of the BSH-Alexa Fluor 488® conjugate (and therefore BSH compound **9**) to be confined to the cytoplasm. The same result was obtained when we tested the cellular uptake of compound **9**Alexa Fluor 488® conjugate (**18**). These key preliminary results will be important for further assessing the uptake of new agents for BNCT on the cellular level.

3. Conclusions

We have developed new methods to functionalize BSH with organic molecules using click chemistry. Our method focused on the synthesis of two classes of BSH building blocks, containing either terminal functionalized carboxyl groups or propyne groups, for covalent incorporation into structures for use in the treatment of cancer by BNCT. Compounds **2**–**4** and **7** acted as powerful precursors for the high-yield synthesis of a broad spectrum of bioconjugate-BSH compounds, utilizing amidation, esterification and Cu(I)-mediated click cycloaddition reactions. The click reactions studied here required only benign reaction conditions and simple workup and purification procedures. All BSH-conjugated triazoles were isolated directly as pure solids (*i.e.* without chromatographic separations), meeting the requirements for large-scale applications. Additionally, DCC-activated esterification appeared to be a highly versatile, controllable, and relatively mild technique for modification of *S*-carboxyalkyl-thioether-undecahydro-*closo*-dodecaborate ditetramethylammonium salts, and it will be extremely useful in the future design of novel BSH-based biomolecules. As the BSH cluster is relatively hydrophilic, its lipid-conjugates can efficiently form biomembranes and liposomes. The BSH-lipid **14** prepared in this work by DCC-mediated esterfication may serve as a useful model for future preparations of such macro-structures. We successfully demonstrated the click reaction of compound **9** with Alexa Fluor 488® azide inside HeLa cells,

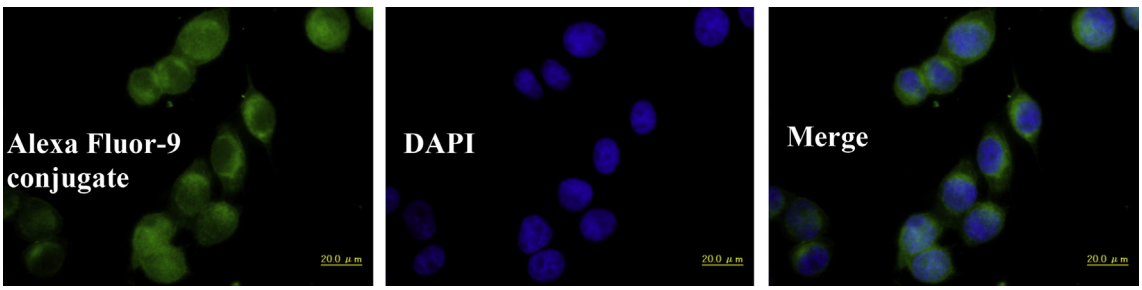


Fig. 1. Click chemistry in cells. HeLa cells were incubated for 3 h with compound **9** (1 mM). After fixation and permeabilization of the cells, a click reaction between **9** and Alexa Fluor 488 azide was performed yielding the Alexa Fluor-**9** conjugate within the cell. The cell nuclei were stained with DAPI and visualized under a fluorescence microscope. Fluorescence images for treated HeLa cells at 520 nm (*left*), 461 nm (*middle*) and merged image (*right*).

clearly proving that compound **9** accumulated in the cytoplasm of the cells. The click cycloaddition reaction is very useful not only for the synthesis of various BSH-containing organic compounds for BNCT but also for the visualization of boron clusters in cells.

4. Experimental section

4.1. Materials and methods

All chemicals and reagents were commercially available and were used as received. Solvents used were reagent grade or higher, and used without further purification. Bis(tetramethylammonium-cyanoethyl-thioetherundecahydro-*closo*-dodecaborate (**5**), azides ($C_6H_5CH_2N_3$, *p*-Br-$C_6H_4CH_2N_3$, *m*-OMe-$C_6H_4CH_2N_3$), and 1,2-*O*-distearoyl-*sn*-3-glycerol (**13**) were prepared as described in the literature [15,20,40]. Analytical thin layer chromatography (TLC) was performed on glass plates of silica gel 60 GF_{254} (Merck). Visualization was accompanied by UV light (254 nm) and $PdCl_2$ stain. Preparative TLC was carried out using 0.75 mm layers of silica gel 60 GF_{254} (Merck) on glass plates of dimensions 20 × 20 cm, prepared from water slurries followed by drying in air at 100 °C. Column chromatography was conducted on silica gel 60 (Merck 70–230 mesh). Elemental microanalyses were performed on a CE instrument EA1110 CHNS–O automatic elemental analyzer. All compounds gave elemental analysis within ±0.3% of the theoretical values. ^{1}H NMR and ^{13}C NMR spectra were measured on JEOL JNM-AL 300 (300 MHz) or Bruker AV 600 spectrometers. Chemical shifts are expressed in parts per million (ppm, δ units), and coupling constants are expressed in hertz (Hz). ^{11}B NMR spectra were recorded on a JEOL JNM-AL 300 spectrometer (96.3 MHz) and the chemical shifts are reported in δ units relative to external standard $BF_3 \cdot Et_2O$ in $CDCl_3$. Transmission IR spectra (cm^{-1}, KBr disc) were obtained on a Shimadzu FTIR-8600PC spectrometer. Electro-spray ionization (ESI) mass spectra were recorded on a Shimadzu LCMS-2010 eV spectrometer.

4.1.1. Synthesis of tetramethylammonium salt of compound **2**

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 bromoacetic acid (2.36 g, 17 mmol) in acetonitrile/water (40 mL, 4:1) was added dropwise to the reaction mixture over a period of 10 min at room temperature. After 24 h the solvent was evaporated under vacuum, the residue was washed with ether, and the obtained solid was redissolved in acetonitirile. The inorganic salts were removed by filtration, and the product was triturated with ether. The solid was filtered off to yield **1** as a white solid (907 mg, 80%). Crystallization from water was also possible. ^{1}H NMR (300 MHz, CD_3CN): δ 7.73 (bs, 2H, COOH), 3.89 (s, 4H, S–CH_2), 3.07 (s, 12H, $N(CH_3)_4$), 1.93–0.56 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (75 MHz, CD_3CN): δ 166.9 (2C, C=O), 56.0 (4C, $N(CH_3)_4$), 44.3 (2C, S–CH_2). ^{11}B NMR (96.3 MHz, CD_3CN): δ −15.1 (bs, 1B), −19.1 (d, J_{BH} = 75.6 Hz, 11B). IR (KBr, cm^{-1}) ν: 3263, 3030, 2960, 2495, 1753, 1485, 1040 (S), 995, 948, 821, 719, 673. ESI-MS: *m/z* 291.2 $[M]^-$. Elemental Analysis Calcd for $C_8H_{29}B_{12}O_4NS$: C, 26.32; H, 8.01; N, 3.84%. Found: C, 26.29; H, 7.98; N, 3.81%.

4.1.2. Synthesis of tetramethylammonium salt of compound **3**

This compound was prepared as described for compound **2** using bromopropanoic acid (2.60 g, 17 mmol) and isolated as a white solid (1.04 g, 85%). ^{1}H NMR (300 MHz, CD_3CN): δ 7.77 (bs, 2H, COOH), 3.88 (m, 4H, S–CH_2), 3.07 (s, 12H, $N(CH_3)_4$), 2.81 (m, 4H, CH_2C=O) 1.60–0.55 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (75 MHz, CD_3CN): δ 172.1 (2C, C=O), 56.2 (4C, $N(CH_3)_4$), 36.9 (2C, S–CH_2), 31.3 (2C, CH_2). ^{11}B NMR (96.3 MHz, CD_3CN): δ −14.9 (bs, 1B), −19.8 (d, J_{BH} = 153.9 Hz, 11B). IR (KBr, cm^{-1}) ν: 3263, 3030, 2960, 2495, 1732, 1485, 1049, 948, 837, 721. ESI-MS: *m/z* 319.2 $[M]^-$. Elemental Analysis Calcd for $C_{10}H_{33}B_{12}O_4NS$: C, 30.55; H, 8.46; N, 3.56%. Found: C, 30.49; H, 8.42; N, 3.50%.

4.1.3. Synthesis of tetramethylammonium salt of compound **4**

This compound was prepared as described for compound **1** using bromobutanoic acid (2.84 g, 17 mmol) and isolated as a white solid (1.14 g, 87%). NMR (300 MHz, CD_3CN): δ 9.97 (bs, 2H, COOH), 3.31 (m, 4H, S–CH_2), 3.06 (s, 12H, $N(CH_3)_4$), 2.35 (m, 4H, CH_2C=O), 1.92 (4H, CH_2), 1.72–0.53 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (75 MHz, CD_3CN): δ 173.9 (2C, C=O), 56.2 (4C, $N(CH_3)_4$), 40.7 (2C, S–CH_2), 32.5 (2C, CH_2), 22.5 (2C, CH_2). ^{11}B NMR (96.3 MHz, CD_3CN): δ −15.6 (bs, 1B), −19.8 (d, J_{BH} = 163.9 Hz, 11B). IR (KBr, cm^{-1}) ν: 3359, 3028, 2954, 2445, 1732, 1481, 1049, 948 (S), 837, 721. ESI-MS: *m/z* 347.3 $[M]^-$. Elemental Analysis Calcd for $C_{12}H_{37}B_{12}O_4NS$: C, 34.22; H, 8.85; N, 3.33%. Found: C, 34.18; H, 8.81; N, 3.27%.

4.1.4. Synthesis of tetramethylammonium salt of compound **6**

This compound was prepared as described for compound **1** using compound **5** (0.50 g, 1.3 mmol) and bromopropanoic acid (3.44 g, 22.63 mmol) and isolated as a white solid (394 mg, 79%). NMR (300 MHz, CD_3CN): δ 7.93 (bs, 1H, COOH), 3.32 (m, 2H, CH_2), 3.1 (s, 24H, $N(CH_3)_4$), 3.0–2.98 (m, 4H, CH_2), 1.93–0.99 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (75 MHz, CD_3CN): δ 174.4 (1C, C=O), 118.0 (1C, CN), 56.1 (8C, $N(CH_3)_4$), 37.0 (1C, CH_2), 35.1 (1C, CH_2), 31.1 (1C, CH_2), 16.2 (1C, CH_2). ^{11}B NMR (96.3 MHz, CD_3CN): δ −14.9 (bs, 1B, B1), −20.2 (d, J_{BH} = 152.2 Hz, 11B). IR (KBr, cm^{-1}) ν: 3353, 3026, 2951, 2512, 1716. ESI-MS: *m/z* 300.2 $[M]^-$. Elemental Analysis Calcd for $C_{10}H_{32}B_{12}O_2N_2S$: C, 32.10; H, 8.62; N, 7.49%. Found: C, 31.99; H, 8.55; N, 7.43%.

4.1.5. Synthesis of bis-tetramethylammonium salt of compound **7**

To a solution of **6** (374 mg, 1.0 mmol) in acetone (20 mL), 1 equiv. of tetramethylammonium hydroxide solution (25% solution in methanol) was added dropwise. The white precipitate that immediately formed was filtered off and dried to give **7** as a white solid (382 mg, 97%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.90 (bs, 1H, COOH), 3.13 (s, 24H, $N(CH_3)_4$), 2.36 (t, J = 9 Hz, 2H, S–CH_2), 1.90 (t, J = 9 Hz, 2H, CH_2C=O), 1.58–0.26 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (75 MHz, DMSO-d_6): δ 175.4 (1C, C=O), 54.4 (8C, $N(CH_3)_4$), 42.8 (1C, S–CH_2), 32.2 (1C, CH_2). ^{11}B NMR (96.3 MHz, DMSO-d_6): δ −11.4 (bs, 1B, B1), −19.8 (d, J_{BH} = 75.2 Hz, 10B), −22.4 (bs, 1B). IR (KBr, cm^{-1}) ν: 3421, 3020, 2954, 2457, 1651, 1558, 1049, 949, 840, 721. ESI-MS: *m/z* 122.8 $[M]^{2-}$. Elemental Analysis Calcd for $C_{11}H_{40}B_{12}O_2N_2S$: C, 33.51; H, 10.23; N, 7.11%. Found: C, 33.49; H, 10.19; N, 7.08%.

4.1.6. Synthesis of tetramethylammonium salt of compound **8**

Propargylamine (39.0 μL, 0.60 mmol) was added to a solution of compound **2** (100 mg, 0.27 mmol), *N,N′*-dicyclohexylcarbodiimide (DCC) (30.0 mg, 1.90 mmol), and 4-dimethylaminopyridine (DMAP) (17.0 mg, 0.14 mmol) in dry DMF (5 mL) at room temperature. The mixture was stirred at room temperature for 6 h and the reaction progress monitored by TLC. The solvent was removed under reduced pressure and the crude product was purified by chromatography on silica gel using dichloromethane-methanol (4:1) as the mobile phase to yield **8** as a white solid (73 mg, 63%), R_f = 0.37. ^{1}H NMR (300 MHz, CD_3CN): δ 6.78 (bs, 2H, NH_2), 3.86 (s, 4H, CH_2N), 3.39 (m, 2H, C≡CH), 3.13 (s, 12H, $N(CH_3)_4$), 2.97 (m, 4H, S–CH_2), 1.73–0.60 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (75 MHz, CD_3CN): δ 164.9 (2C, C=O), 81.1, 72.4 (4C, C≡CH), 56.2 (4C, $N(CH_3)_4$), 46.6 (2C, S–CH_2), 40.2 (4C, N–CH_2). ^{11}B NMR (96.3 MHz, CD_3CN): δ −14.8 (bs, 1B), −20.0 (d, J_{BH} = 149.3 Hz, 11B). IR (KBr, cm^{-1}) ν: 3251, 3021, 2952, 2489, 2123, 1684, 1645, 1485, 1040, 995, 948, 821, 719. ESI-MS: *m/z* 369.2 $[M]^-$. Elemental Analysis Calcd for $C_{18}H_{49}B_{12}O_2N_4S$: C, 41.95; H, 9.58; N, 10.87%. Found: C, 41.91; H, 9.56; N, 10.84%.

*4.1.7. Bis-tetramethylammonium salt of compound **9***

This compound was isolated from the crude reaction mixture of compound **8** as a white solid (34 mg, 30%), R_f = 0.19, ^{1}H NMR (300 MHz, CD_3CN): δ 6.48 (bs, 1H, NH), 4.13 (m, 2H, CH_2N), 3.44 (m, 1H, S–CH_2), 3.01 (s, 24H, $N(CH_3)_4$), 2.48 (m, 2H, C≡CH), 1.93–1.1 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (75 MHz, CD_3CN): δ 169.3 (1C, CO), 66.1 (1C, C≡CH), 56.2 (8C, $N(CH_3)_4$), 51.0 (1C, CH), 31.5, 26.5 (2C, CH_2). ^{11}B NMR (96.3 MHz, CD_3CN): δ −10.8 (bs, 1B), −15.0 (d, J_{BH} = 193.6 Hz, 11B), −20.7 (bs, 1B). IR (KBr, cm^{-1}) ν: 3245, 3024, 2956, 2495, 2127, 1705, 1657. ESI-MS: *m/z* 134.5 $[M]^{2-}$. Elemental Analysis Calcd for $C_{13}H_{41}B_{12}N_3OS$: C, 37.42; H, 9.90; N, 10.07%. Found: C, 37.23; H, 10.04; N, 10.26%.

*4.1.8. Synthesis of bis-tetramethylammonium salt of compound **10***

This compound was prepared as described for compound **9** using compound **3** (100 mg, 0.25 mmol), propargylamine (39.0 μL, 0.60 mmol), DCC (40 mg, 1.90 mmol) and DMAP (19.0 mg, 0.15 mmol) and isolated as a white solid (74 mg, 82%), R_f = 0.23. ^{1}H NMR (300 MHz, CD_3CN): δ 6.82 (bs, 1H, NH), 4.37 (s, 2H, CH_2N), 3.86 (m, 2H, S–CH_2), 3.10 (s, 24H, $N(CH_3)_4$), 2.57 (m, 1H, C≡CH), 2.39 (m, 2H, S–CH_2), 1.93–0.56 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (75 MHz, CD_3CN): δ 162.3 (1C, C═O), 72.1 (1C, C≡CH), 56.2 (8C, $N(CH_3)_4$), 54.6 (1C, S–CH_2), 38.6 (1C, N–CH_2), 35.3 (1C, N–CH_2), 25.6 (1C, N–CH_2). ^{11}B NMR (96.3 MHz, CD_3CN): δ −11.3 (bs, 1B), −20.2 (d, J_{BH} = 91.1 Hz, 10B), −22.2 (bs, 1B). IR (KBr, cm^{-1}) ν: 3336, 3020, 2951, 2499, 2127, 1734, 1485, 1040, 995, 948, 821, 719. ESI-MS: *m/z* 141.0 $[M]^{2-}$. Elemental Analysis Calcd for $C_{14}H_{43}B_{12}N_3OS$: C, 38.99; H, 10.05; N, 9.74%. Found: C, 38.87; H, 10.01; N, 9.72%.

*4.1.9. Synthesis of bis-tetramethylammonium salt of compound **11***

This compound was prepared as described for compound **9** using compound **4** (100 mg, 0.24 mmol), propargyl amine (38.0 μL, 0.60 mmol), DCC (39 mg, 1.90 mmol) and DMAP (18.0 mg, 0.15 mmol) and isolated as a white solid (91 mg, 86%), R_f = 0.28. ^{1}H NMR (300 MHz, CD_3CN): δ 6.85 (bs, H, NH), 4.31 (s, 2H, CH_2), 3.82 (m, 2H, CH_2), 3.11 (s, 24H, $N(CH_3)_4$), 2.63 (m, 1H, C≡CH), 2.42, 2.38 (m, 4H, CH_2), 1.93–0.56 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (75 MHz, CD_3CN): δ 173.4 (1C, C═O), 68.4 (1C, C≡CH), 56.2 (8C, $N(CH_3)_4$), 54.1 (1C, CH_2), 37.5 (1C, CH_2), 35.3 (1C, N–CH_2), 33.2, 25.6 (2C, CH_2). ^{11}B NMR (96.3 MHz, CD_3CN): δ −11.4 (bs, 1B), −20.3 (d, J_{BH} = 75.2 Hz, 10B), −22.4 (bs, 1B). IR (KBr, cm^{-1}) ν: 3339, 3023, 2986, 2468, 2129, 1732, 1635, 1040 (S), 995, 948, 821, 719. ESI-MS: *m/z* 148.5 $[M]^{2-}$. Elemental Analysis Calcd for $C_{15}H_{45}B_{12}N_3OS$: C, 40.46; H, 10.19; N, 9.44%. Found: C, 40.39; H, 10.08; N, 9.32%.

*4.1.10. Synthesis of bis-tetramethylammonium salt of compound **12***

Ethanol (0.5 mL) was added to a solution of compound **3** (100 mg, 0.25 mmol), DCC (40.0 mg, 1.90 mmol), and DMAP (19.0 mg, 0.14 mmol) in dry DMF (5 mL) at room temperature. The mixture stirred at room temperature for 6 h and the reaction monitored by TLC. The solvent was removed under reduced pressure and the crude product was purified by chromatography on silica gel using dichloromethane-methanol (4:1) as the mobile phase to yield **12** as a colorless oil (89 mg, 83%), R_f = 0.24. ^{1}H NMR (300 MHz, CD_3CN): δ 4.35 (m, 4H, O–CH_2), 4.05 (m, 2H, S–CH_2), 3.87 (m, 2H, CH_2CO), 3.13 (s, 24H, $N(CH_3)_4$), 2.51 (m, 3H, CH_3), 1.93–0.44 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (75 MHz, CD_3CN): δ 173.8 (1C, CO), 64.9 (1C, OCH_2), 56.3 (4C, $N(CH_3)_4$), 37.5 (1C, S–CH_2), 34.7 (1C, CH_2), 23.6 (1C, CH_3). ^{11}B NMR (96.3 MHz, CD_3CN): δ −10.7 (bs, 1B), −20.7 (d, J_{BH} = 121.6 Hz, 10B), −22.7 (bs, 1B). IR (KBr, cm^{-1}) ν: 2967, 2502, 1725, 1045, 747. ESI-MS: *m/z* 274.2 $[M]^-$, 137 $[M]^{2-}$. Elemental Analysis Calcd for $C_{13}H_{44}B_{12}N_2O_2S$: C, 36.97; H, 10.50; N, 6.63%. Found: C, 36.85; H, 10.42; N, 6.51%.

*4.1.11. Synthesis of bis-tetramethylammonium salt of compound **14***

This compound was prepared as described for compound **12** using compound **13** (191 mg, 0.31 mmol), compound **3** (100 mg, 0.25 mmol), DCC (40.0 mg, 1.90 mmol), and DMAP (19.0 mg, 0.14 mmol) in dry DMF (5 mL) at 40 °C and isolated as a white solid (272 mg, 89%), R_f = 0.22. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.15 (m, 1H, CH), 4.39 (m, 2H, O–CH_2), 4.33 (m, 2H, $CHCH_2C$═O), 4.13 (m, 2H, $CHCH_2C$═O), 4.07 (m, 2H, S–CH_2), 3.85 (m, 2H, CH_2CO), 3.09 (s, 24H, $N(CH_3)_4$), 2.45 (m, 3H, CH_3), 2.38 (m, 4H, CH_2CH_2C═O), 1.85–0.55 (m, 11H, $B_{12}H_{11}$) 1.58 (m, 4H, CH_2), 1.22 (s, 40H, CH_2), 0.85 (t, 6H, J_{CH} = 12.61 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.0, 172.8, 172.2 (3C, CO), 50.8 (1C, O–CH_2), 56.5 (8C, $N(CH_3)_4$), 38.7 (2C, S–CH_2), 69.8, 62.3, 34.2, 34.0, 31.9, 29.7, 29.6, 29.4, 29.3, 29.2, 29.1, 29.0, 28.9, 24.8, 24.8, 22.7, 14.1. ^{11}B NMR (96.3 MHz, $CDCl_3$): δ −11.3 (bs, 1B), −20.2 (d, J_{BH} = 91.1 Hz, 10B), −22.2 (bs, 1B). IR (KBr, cm^{-1}) ν: 2957, 2920, 2856, 2499, 1741, 1625, 1572, 1487, 1467, 1275, 1045, 995, 948, 721. ESI-MS: *m/z* 433.2 $[M]^{2-}$. Elemental Analysis Calcd for $C_{51}H_{116}B_{12}N_2O_6S$: C, 60.33; H, 11.52; N, 2.76%. Found: C, 60.05; H, 11.30; N, 2.62%.

*4.1.12. Synthesis of tetramethylammonium salt of compound **15***

To a solution of **8** (515 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. With stirring, *m*-methoxybenzylazide (408 mg, 2.5 mmol) was then added dropwise. The reaction mixture was stirred for 6 h until complete consumption of **3** was indicated by TLC (methanol/dichloromethane 1:4). The mixture was filtered through a pad of celite and diethyl ether (10 mL) was added until a precipitate of inorganic salts formed. This precipitate was removed by filtration and additional diethyl ether (200 mL) was added to the filtrate, whereupon a crystalline solid precipitated. The fine crystalline product was filtered off to give **15** (765 mg, 98%) as a white solid. mp 201–202 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.23 (bs, 2H, NH), 8.09 (s, 2H, $CH_{Arom.}$), 7.25, 6.84 (m, 8H, $CH_{Arom.}$), 6.85 (bs, 2H, NH), 5.48 (s, 4H, CH_2-benzyl), 4.05 (bs, 4H, N–CH_2), 4.34 (m, 4H, S–CH_2), 3.70 (s, 6H, O–CH_3), 3.09 (s, 12H, $N(CH_3)_4$), 1.85–0.55 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (75 MHz, DMSO-d_6): δ 163.1 (2C, C═O), 160.8 (2C, O-$C_{Arom.}$), 138.0 (2C, C-triazole), 130.9, 121.0, 114.8, 114.6, 56.2 (2C, O–CH_3), 54.6 (4C, (4C, $N(CH_3)_4$), 46.5, 32.7, 31.4. ^{11}B NMR (96.3 MHz, DMSO-d_6): δ −14.8 (bs, 1B, B1), −20.6 (d, J_{BH} = 149.3 Hz, 11B, B2-12). IR (KBr, cm^{-1}) ν: 3444, 3359, 3020, 2503, 1689, 1650, 1500, 1487, 1050. ESI-MS: *m/z* 707.4 $[M]^-$. Elemental Analysis Calcd for $C_{31}H_{57}B_{12}N_9O_4S$: C, 47.63; H, 7.35; N, 16.13%. Found: C, 47.56; H, 7.29; N, 16.04%.

*4.1.13. Synthesis of bis-tetramethylammonium salt of compound **16***

This compound was prepared as described for compound **15** using compound **10** (431 mg, 1 mmol) and benzylazide (332 mg, 2.5 mmol) and isolated as a white solid (513 mg, 91%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.85 (bs, 1H, $CH_{Arom.}$), 7.34 (m, 5H, $CH_{Arom.}$), 7.06 (bs, H, NH), 5.52 (s, 2H, CH_2-benzyl), 4.38 (bs, 2H, N–CH_2), 3.13 (s, 24H, $N(CH_3)_4$), 2.35 (m, 2H, S–CH_2), 2.26 (m, 2H, S–CH_2), 1.26–0.47 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (75 MHz, DMSO-d_6): δ 170.1, 162.9, 136.6 (1C, CH-triazole), 129.8, 129.8, 129.4, 129.3, 129.1 (6C, CH and C-phenyl), 125.7 (1C, C-triazole), 56.3, 56.2, 56.2, 54.6 (8C, $N(CH_3)_4$), 52.6 (1C, CH_2-benzyl), 38.6, 35.3 (C, S–CH_2). ^{11}B NMR (96.3 MHz, DMSO-d_6): δ −10.8 (bs, 1B), −17.0 (d, J_{BH} = 193.3 Hz, 10B), −20.6 (bs, 1B). IR (KBr, cm^{-1}) ν: 3568, 3386, 2488, 1725, 1651, 1551, 1049, 948. MS (ESI, negative): *m/z* 207.6 $[M]^{-2}$. Elemental Analysis Calcd for $C_{21}H_{50}B_{12}N_6OS$: C, 44.68; H, 8.93; N, 14.89%. Found: C, 44.61; H, 8.88; N, 14.81%.

*4.1.14. Synthesis of bis-tetramethylammonium salt of compound **17***

This compound was prepared from **10** (431 mg, 1 mmol) and *p*-bromobenzylazide (530 mg, 2.5 mmol) using the procedure

described for **15**, to give **17** as a white solid (617 mg, 96%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.96 (bs, 1H, $CH_{Arom.}$), 7.54 (m, 2H, $CH_{Arom.}$), 7.28 (m, 2H, $CH_{Arom.}$), 6.83 (bs, H, NH), 5.50 (s, 2H, CH_2-benzyl), 4.41 (m, 2H, N–CH_2), 3.17 (s, 2H, S–CH_2), 3.09 (s, 24H, $N(CH_3)_4$), 1.71–0.59 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (75 MHz, DMSO-d_6): δ 169.2, 135.8 (2C, C-triazole), 132.8, 130.9, 122.7 (12C, CH and C-phenyl), 126.2 (2C, CH-triazole), 56.3 (4C, 4C, $N(CH_3)_4$), 53.7 (2C, CH_2-benzyl), 37.4 (2C, S–CH_2). ^{11}B NMR (96.3 MHz, DMSO-d_6): δ −10.7 (bs, 1B), −20.7 (d, J_{BH} = 121.6 Hz, 11B), −22.7 (bs, 1B). IR (KBr, cm^{-1}) ν: 3442, 3032, 2928, 2482, 1705, 1625, 1555, 1487, 1407, 1289, 1047, 995, 949, 817, 717. MS (ESI, negative): *m/z* 247.6 $[M]^{2-}$. Elemental Analysis Calcd for $C_{21}H_{49}B_{12}BrN_6OS$: C, 39.20; H, 7.68; N, 13.06%. Found: C, 39.05; H, 7.49; N, 12.97%.

4.1.15. Synthesis of compound **9**- Alexa Fluor 488® conjugate (**18**)

To a solution of **9** (6.4 mg, 0.03 mmol) in DMSO (1 mL) were added $Cu(OAc)_2$ (2.5 mg, 0.01 mmol) and sodium ascorbate (5 mg, 0.03 mmol) at room temperature. A solution of Alexa Fluor 488® azide (3.0 mg, 3.48 × 10^{-3} mmol) in DMSO (0.5 mL) was added dropwise to the reaction mixture while stirring under argon atmosphere. The light-sensitive dye was protected from light during this and all subsequent reaction and purification steps. After 6 h the reaction mixture was diluted with water (10 mL) and passed through a Sep-Pak C18 Cartridge. The green coloured fraction was eluted off with methanol/H_2O (8:2, 2 mL), concentrated by rotary evaporation and lyophilised to provide **18** (4.2 mg, 94%) as a dark green solid. ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.94 (bs, 1H, NH), 8.86 (bs, 1H, CH), 8.45 (m, 2H, 2CH), 8.04 (bs, 1H, CH), 7.73 (m, 1H, CH), 7.21 (bs, 2H, NH_2) 7.16 (bs, 1H, CH), 6.57 (bs, 1H, NH), 4.55 (t, J = 8 Hz, 2H, CH_2N), 4.35 (m, 2H, CH_2N), 3.76 (m, 1H, S–CH_2), 3.38 (m, 2H, CH_2N), 3.07 (s, 24H, $N(CH_3)_4$), 2.99 (m, 6H, $3CH_2$), 1.93–1.1 (m, 19H, $B_{12}H_{11}$ + $4CH_2$), 0.96 (t, J = 12 Hz, 9H, $3CH_3$). ^{11}B NMR (192,3 MHz, DMSO-d6): δ −11.2 (bs, 1B), −15.4 (d, J_{BH} = 190.1 Hz, 11B), −21.3 (bs, 1B). MS (ESI, negative): *m/z* 231.28 $[M]^{4-}$/4. Elemental Analysis Calcd for $C_{52}H_{97}B_{12}N_{11}O_{11}S_3$: C, 48.86; H, 7.65; N, 12.05%. Found: C, 48.67; H, 7.53; N, 12.00%.

4.2. Biology

4.2.1. Cell viability (MTT) assay

Human cervical carcinoma HeLa cells were used for the cell viability assay. The cells (5 × 10^3 cells per well of a 96-well plate) were incubated at 37 °C for 72 h in RPMI-1640 medium (100 μL) containing various concentrations of the BSH and BSH conjugated triazoles. After the incubation, 3′-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) in PBS (5 mg/mL, 10 mL) was added to each well, and the cells were further incubated at 37 °C for 4 h. After the removal of the medium, DMSO (100 μL) was added and the absorbance at 570 nm was determined with a microplate reader. The drug concentration required to reduce cell viability by 50% (IC_{50}) was determined from semi logarithmic dose–response plots.

4.2.2. Boron accumulation study

HeLa cells (1 × 10^6 cells) were incubated at 37 °C for 3 h in medium containing BSH and BSH conjugated triazoles. After the incubation, the cells were washed three times with PBS to remove any surface bound boron before digesting with perchloric acid/hydrogen peroxide solution (2 mL) at 70 °C for 6 h. Boron content in the digested solutions were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES).

4.2.3. Click reaction in HeLa cells

Human cervical carcinoma HeLa cells were plated on p35 dishes (1 × 10^4 cells) containing 1 cm diameter glass coverslips and incubated at 37 °C for 24 h. After incubating the cells with compound **9** 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 reaction between compound **9** and Alexa Fluor 488® azide (Alexa Fluor 488® 5-carboxamide-(6-azidohexanyl), bis(-triethylammonium salt), Invitrogen) was 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.

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