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PAPER

New classes of carborane-appended 5-thio-D-glucopyranose derivatives†

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A series of carborane-appended 5-thio-D-glucopyranose (5-TDGP) derivatives containing one to two 5-TDGP moieties were synthesized *via* click cycloaddition reaction as well as following the traditional methods. Among the carboranyl-5-TDGP derivatives, the decapitated *nido*-carboranyl derivative **18** was found to be highly water-soluble and therefore its preliminary biodistribution study was conducted. A comparative biological evaluation of **18** *versus* its carboranyl-D-glucopyranose analog **19** with human hepatocellular carcinoma cells (SK-Hep1) indicated 5-TDGP to be a better boron carrier than normal D-glucopyranose. The carboranyl-5-TDGP **18** showed a nearly two fold increase in cellular boron accumulation than carboranyl-D-glucopyranose analog **19** over a period of 2 h. The accumulation of both **18** and **19** was found to occur in a temperature dependent manner. The higher accumulation of **18** suggested excellent promise for it to be a candidate for further evaluation as a future BNCT agent.

1. Introduction

5-Thio-D-glucopyranose (5-TDGP) is the nearest analog of D-glucopyranose (DGP), but differs from DGP by having a sulphur in place of the oxygen in the pyranose ring (Fig. 1).[1] Unlike DGP, 5-TDGP is a non-metabolite and essentially non-toxic with an LD_{50} value of 14 g kg^{-1}.[1b] When administered orally, a pronounced physiological effect of 5-TDGP on rats was observed with a sudden rise in blood D-glucose level and it was later observed that 5-TDGP was excreted unaltered in the urine.[1c] Furthermore, the use of 5-TDG for radiotherapy of leukemia has also been evaluated.[2] However, the difficulty of the multi-step synthetic procedure for 5-TDGP halted the chemistry of this thiosugar and its derivatives. Nonetheless, owing to the biological significance of this unique non-metabolic carbohydrate analog, extended investigation of the synthetic chemistry of 5-TDGP is warranted.

While carboranes are a class of boron-rich cluster compounds that could easily be functionalized and are resistant to biodegradation,[3,4] their deboronated derivatives are water-soluble thus making them important species in medicinal chemistry, including the boron neutron capture therapy (BNCT), as a source of boron atoms and as pharmacophores.[3–5] Thus, carborane–sugar conjugates have been synthesized and subsequently evaluated as boron delivery platforms for BNCT applications.[6] Due to the presence of carbohydrate-specific receptor proteins on the surface of tumor cells,[7] the combination of carbohydrate and carboranes enhances their uptake into tumor tissues. Secondly, the use of carbohydrates containing multiple hydroxyl groups compensates for the hydrophobicity of the carboranes, making them water-soluble and thereby enhancing their uptake into tumor tissues.[6d,8] Due to the structural similarity between DGP and 5-TDGP, the carboranyl-5-TDGP derivative is expected to be a non-metabolic conjugate that could deliver the required dosage of 10^9 B-10 atoms per tumor cell.[4] However, carboranyl-5-TDGP and its derivatives have never been synthesized and explored for medicinal applications, including BNCT. Therefore, we hereby report the synthesis and preliminary biological evaluation of carborane-5-TDGP derivatives as new boron bioconjugates.

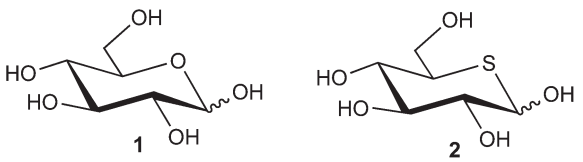

Fig. 1 D-Glucose and 5-thio-D-glucose.

2. Results and discussion

2.1. Synthesis

Unlike the widely available D-glucopyranose (**1**), the synthesis of 5-thio-D-glucopyranose (5-TDGP) (**2**) (Fig. 1) involves difficult multi-step procedures.[9a] However, a convenient literature

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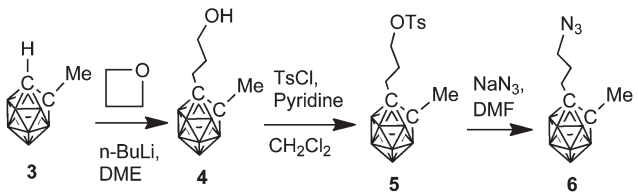
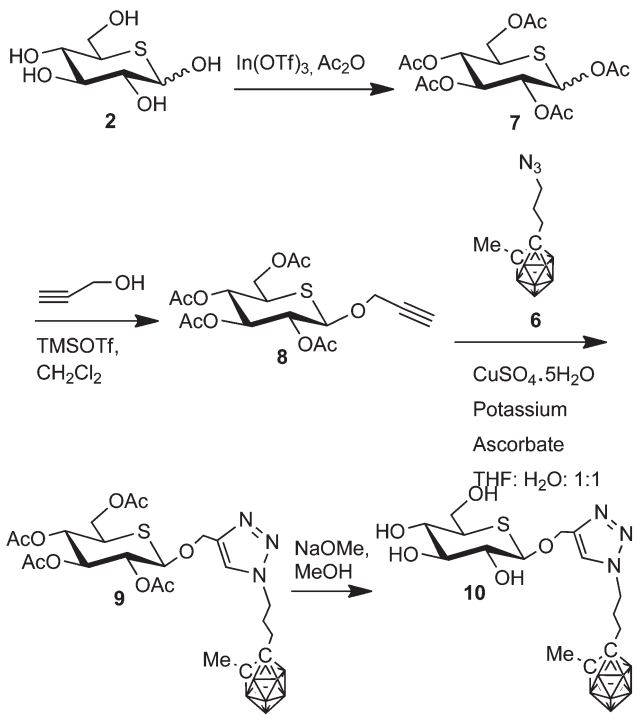
procedure that did not involve time-consuming purification and protection steps allowed us to synthesize 5-TDGP (**2**) on the multi-gram scale.[9b,c] The Cu^{I} catalyzed Huisgen-type 1,3-dipolar cycloaddition reaction between azides and alkynes (CuAAC), known as "Click" chemistry, typically leads to the formation 1,2,3-triazoles.[10] Therefore, this methodology was employed in our present work to synthesize new carborane-appended 5-TDGP derivatives. Thus, the carboranylazide **6** was prepared first from 1-methyl-*o*-carborane in three steps as shown in Scheme 1. 1-Methyl-*o*-carborane was treated with oxacyclobutane in the presence of *n*-BuLi to produce compound **4**. The primary alcohol group in **4** was treated with *para*-toluenesulfonyl chloride in the presence of pyridine and then the resulting compound **5** was converted to carboranylazide derivative **6** by reacting with sodium azide in DMF (Scheme 1).

The further reactivity of **2** for the synthesis of carborane-substituted 5-TDGP is illustrated in Scheme 2. Specifically, 5-TDGP **2** was converted to the corresponding pentacetylated derivative **7** in the presence of acetic anhydride and indium triflate.[11] The pentacetyl derivative **7** was then selectively functionalized at the anomeric position using 2-propyn-1-ol and TMSOTf in dichloromethane (DCM) to obtain the alkyne species **8** as shown in



Scheme 1 The synthesis of carboranylazide derivative **6**.

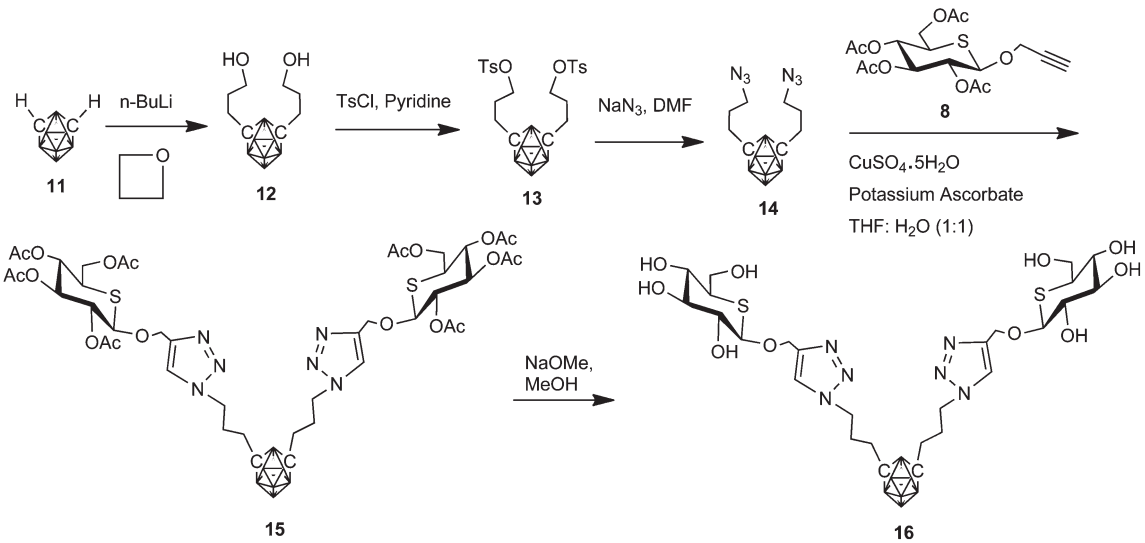


Scheme 2 The synthesis of new carboranyl-5-TDGP conjugate **10**.

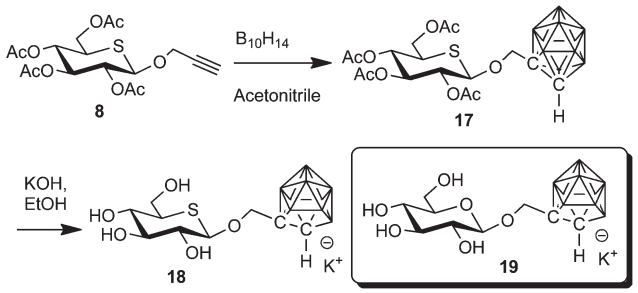
Scheme 2.[12] Subsequently, the new derivative of carboranyl-5-TDGP (**9**) was synthesized *via* reaction between **8** and **6** in the presence of potassium ascorbate and $CuSO_4{\cdot}5H_2O$ in THF and water. The reaction was very fast and was complete within an hour, producing **9** in good yield. The acetyl groups in **9** were deprotected by reacting it with sodium methoxide in methanol to isolate the new carboranyl-5-TDGP conjugate **10** in good yield (Scheme 2). While it is important to note that the controlled use of NaOMe in methanol did not deboronate the carborane cage in **9**, vigorous reaction conditions, such as reflux over a long period of time, led to complete degradation of the carborane cage along with the detachment of the 5-TDGP.

In order to explore a new synthetic strategy for achieving greater water solubility, we chose to link two sugar moieties to a single carborane cage in which the steric crowding can be minimized by placing the cage carbons apart from each other. Thus, the *meta*-carborane **11** was functionalized using oxacyclobutane in the presence of *n*-BuLi in a procedure similar to that employed for the preparation of **4**. Subsequently, the two OH moieties in compound **12** were converted to tosyl groups using *para*-toluenesulfonyl chloride in the presence of pyridine to obtain **13**. Further reaction of **13** with sodium azide in DMF produced the diazide derivatives of *meta*-carborane **14**. As illustrated in Scheme 3, the acetylated *meta*-carboranylbis(5-TDGP) derivative **15** was prepared *via* reaction between the alkynyl-5-TDGP precursor **8** and the diazide derivative **14** in the presence of potassium ascorbate and $CuSO_4{\cdot}5H_2O$ in THF and water. Subsequent deprotection of the acetyl groups in **15** using NaOMe in methanol yielded a new *meta*-carboranylbis-(5-TDGP) derivative **16** (Scheme 3). Despite the presence of two 5-TDGP moieties, compound **16** was found to be sparingly soluble in water.

The highly water-soluble form of carborane-appended 5-TDGP, compound **18** was prepared from compound **8** (Scheme 4). Reaction between **8** and decaborane ($B_{10}H_{14}$) in acetonitrile, resulted in the formation of the acetylated carborane-appended 5-TDGP derivative **17**. Compound **17** was subjected to deboronation of the appended carborane cage in the presence of ethanolic potassium hydroxide that led to the formation of the cage-opened *nido*-carborane-appended 5-TDGP derivative **18** which was found to be highly water soluble. It is important to note that during the deboronation step KOH also deprotected the OH moieties of the 5-TDGP thus eliminating the additional deprotection step. Removal of one B vertex from the *closo*-1,2-$C_2B_{10}H_{11}$-cage of **17** made the hydrophilic [*nido*-7,8-$C_2B_9H_{11}]^-$-cage fragment of **18** (Scheme 4).[5,12] All compounds reported in this paper were characterized by ^1H, ^{13}C and ^{11}B NMR spectra and IR spectra, as well as the melting point measurements of the solid products. The IR spectra of all *closo*-carborane containing compounds showed strong bands between 2588–2601 cm^{-1} where as the *nido*-carborane containing compound **18** showed the characteristic band at 2518 cm^{-1} corresponding to ν(B–H). The mass spectral data of all the new compounds confirmed their formation (see ESI†). For comparative biological evaluation, the normal D-glucopyranose analogue **19** (Scheme 4) was also prepared according to the procedure described in the literature.[12,13] The biological evaluation of both **18** and **19** was carried out systematically and the results are presented below.



Scheme 3 The synthesis of *meta*-carborane-substituted bis(5-TDGP) conjugate **16**.



Scheme 4 The synthesis of water-soluble carboranyl-5-thio-D-glucopyranose **18**.

2.2. Biological evaluation of 18 and 19

The carboranyl-5-TDGP (**18**) and carboranyl-D-glucopyranose (**19**) were dissolved in growth medium to a final concentration of 2.5 mM, and applied to SK-Hep1 human hepatocellular carcinoma cells. The medium was aspirated from the cells and replaced with medium containing the compounds, and cells were incubated at 37 °C or maintained on ice for 1 or 2 h. The pre- and post-harvest phase-contrast images of SK-Hep1 cells after treatment with the 5-thio-D-glucose derivative **18** and D-glucose derivative **19** are shown in Fig. 2. Cells appeared healthy and intact after the two-hour treatment, and were harvested as intact single-cell suspensions, suggesting that the sugar–boron derivatives were non-toxic to the cells at the 2.5 mM concentration administered. Both compounds **18** and **19** (Scheme 4) were found to accumulate in a time- and temperature-dependent manner in the human hepatocellular carcinoma cells, but the carboranyl-5-TDGP (**18**) accumulated faster than the D-glucose analogue (**19**). While carboranyl-5-TDGP **18** yielded 81 ± 11 ng of cell-associated boron after 1 h and 123 ± 12 ng after 2 h, the D-glucose analogue (**19**) yielded 44 ± 9 and 66 ± 7 ng of boron per 5×10^5 cells, after 1 and 2 h, respectively (Fig. 3). The corresponding values for **18** and **19** at 4 °C were much lower at 1 and 2 h, with 19 ± 6, 12 ± 1, 3 ± 0 and 6 ± 1 ng boron per 5×10^5 cells, respectively.

Further calculations indicate ~1×10^{11} ^{10}B atoms per cell have been accumulated *via* the carboranyl-5-TDGP (**18**) at 37 °C whereas ~8×10^{10} ^{10}B atoms per cell have been accumulated *via* the D-glucose analogue (**19**) at 37 °C. Although both these derivatives exceeded the desired BNCT requirement of 10^9 ^{10}B atoms per cell, the accumulation of carboranyl-5-TDGP (**18**) is one order higher than the D-glucose analogue (**19**) which is significant for successful treatment of cancer *via* BNCT. Furthermore, the temperature-dependence of accumulation suggests that the compounds are transported into the cells rather than simply binding to the surface. The precise mechanism(s) by which the compounds enter the cells remains to be determined.

Detailed descriptions of the growth of the cell culture and preparation of the biodistribution assay and measurement are given in the experimental section.

3. Conclusions

Carboranyl-5-TDGP derivatives were synthesized *via* both traditional approaches as well as by employing [3 + 2] click cycloaddition reaction. Despite the presence of two 5-TDGP moieties, the *meta*-carboranyl derivative **16** was found to be sparingly soluble in water. However, the decapitated *nido*-carboranyl-5-TDGP derivative **18** was found to be highly water soluble. A comparative biological analysis of **18** *versus* its D-glucopyranose analog **19** indicated a nearly twofold increase in boron accumulation in the SK-Hep1 cells due to the presence of 5-TDGP moieties in **18**. The transportation of both sugar and thiosugar derivatives into the cancer cell happens in a temperature dependent manner and substantial accumulation of carboranyl-5-TDGP derivative **18** indicates that 5-TDGP could be a good candidate for further evaluation as a BNCT agent.

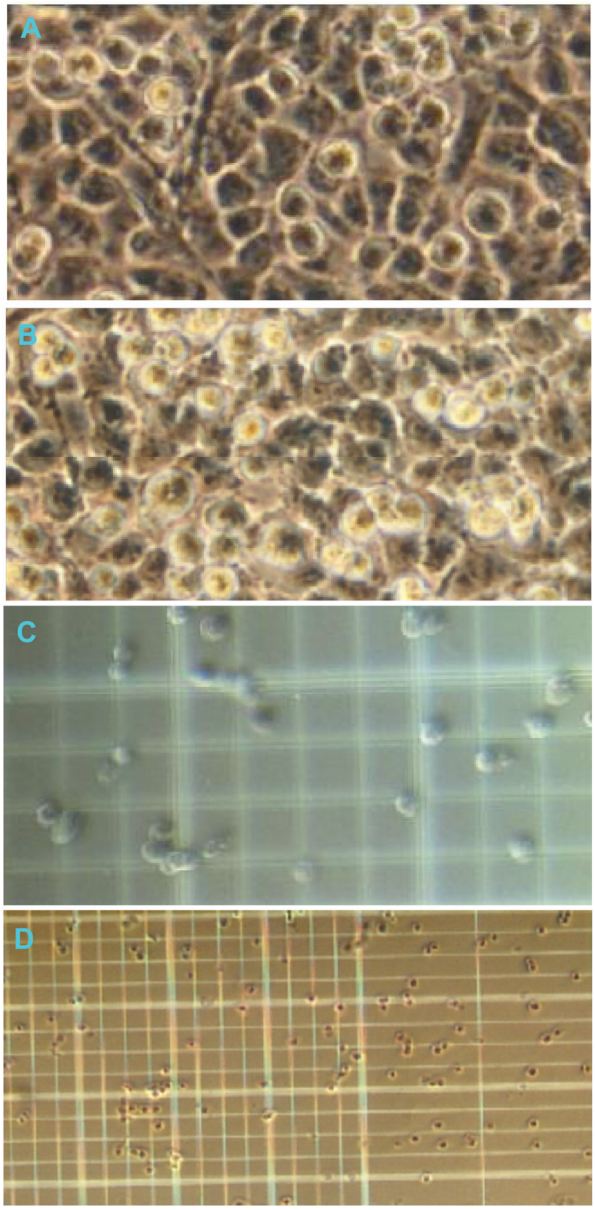


Fig. 2 Phase-contrast images of SK-Hep1 cells: (A) after 2 h treatment with 5-thio-D-glucose derivative **18**; (B) after 2 h treatment with D-glucose derivative **19**; (C) post harvest appearance of cells after 2 h treatment with 5-thio-D-glucose derivative **18**; (D) post harvest appearance of original SK-Hep1 cells (A, B and C 200X; D, 100X).

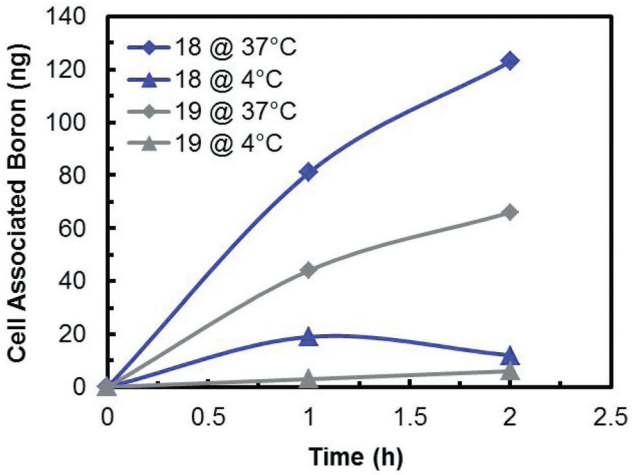


Fig. 3 The temperature-dependent accumulation of carborane-appended thiosugar and sugar derivatives (**18** and **19**) to SK-Hep1 human hepatocellular carcinoma cells. Cell pellets were assayed for boron content by ICP-MS as described in the experimental section. The values indicated on the *y*-axis were the average of triplicate determinations for each time point and condition, and are reported as nanogram boron per 5×10^5 cells. Values (average and SD) for each are reported in the results section.

4. Experimental section

4.1. General methods

The starting material, 5-thio-D-glucopyranose (**1**) was synthesized by the improved method as described in the literature.[9b,c] All reactions were generally performed under argon in oven-dried flasks using Schlenk lines. Solvents and reagents were added by syringes. Solvents were dried using standard procedures. Reagents were used as purchased without further purification. Products were all purified by column chromatography on silica gel (70–230 mesh), and yields refer to analytically pure samples. While the ^{1}H NMR spectra were recorded on a Fourier-Transform multinuclear NMR spectrometer at 500 and 300 MHz, the ^{13}C NMR spectra were recorded at 125 and 75 MHz. The chemical shifts are reported relative to tetramethylsilane (TMS) (^{1}H: δ = 0.00 ppm) and $CDCl_3$ (^{13}C: δ = 77.0 ppm). The integrals are in accordance with the assignments, the coupling constants (*J*s) are all given in Hz, and all of the ^{13}C NMR spectra were proton–decoupled. The ^{11}B NMR spectra were recorded at 64.2, 96.3 and 160.5 MHz and the chemical shifts are relative to $BF_3{\cdot}Et_2O$ standard. The Infrared (IR) spectra of all compounds were recorded on an FT-IR spectrophotometer. Melting points of the solids were measured using standard apparatus and the data were uncorrected. Mass spectral analyses were carried out using ES and EI spectrometers.

4.2. Cell culture

Human hepatocellular carcinoma cells (SK-Hep1) were chosen because of their well-characterized aggressive growth and accelerated nutrient uptake rates, reflective of highly malignant cancer,[14] and the emerging interest in pursuing BNCT for hepatocellular carcinoma (HCC).[15] SK-Hep1 were grown in Dulbecco's Modified Essential Medium (DMEM) supplemented with 2 mM L-glutamine and 10% (v/v) fetal bovine serum (FBS), and were maintained in a humidified atmosphere of 5% CO_2/95% air at 37 °C, as previously described.[10f,14] Cells were seeded into 12-well culture plates at a concentration of 4×10^4 cells per well and allowed to attach and grow to 90% confluence prior to initiation of experiments.

4.3. Biodistribution assay and measurement

The carboranyl-5-TDGP **18** and carboranyl-D-glucopyranose **19** were dissolved in growth medium to a final concentration of

2.5 mM. The medium was aspirated from the cells and replaced with medium containing **18** and **19**, and cells were incubated at 37 °C or maintained on ice for 1 or 2 h. All assays were performed in triplicate for each time point and temperature. After each time period, the medium was aspirated and the cell monolayers were rinsed twice with 1 mL of phosphate-buffered saline (PBS). The cells were scraped into 500 μL of PBS with a plastic spatula and triturated 10 to 15 times with a pipette to yield a single cell suspension. Cell pellets were obtained by centrifugation of the cell suspension at 500 × *g* for 2 min, followed by aspiration of the supernatant. Cell pellets were stored at −80 °C until assayed for boron content by ICP-MS. The cell pellets were then digested with one mL of high purity nitric acid and quantitative measurements were performed on a Perkin Elmer Sciex Elan 6000 Inductively Coupled Plasma Mass Spectrometer. Results are reported as ng boron per 5×10^5 cells.

4.4. Synthesis and analytical data of compounds

4: Dimethoxyethane (DME) (20 mL) was added to the carborane precursor **3** (500 mg, 3.164 mmol), to which *n*-BuLi (2.0 mL, 3.32 mmol) was slowly added at 0 °C with constant stirring over a period of 30 min. The resulting mixture was stirred at room temperature for an additional 30 min and then cooled to 0 °C in order to add dropwise the oxacyclobutane (0.31 mL, 4.74 mmol) in 5 mL of DME. The mixture was stirred at room temperature for 24 h and then quenched with 10 mL of 1 M HCl and extracted with diethyl ether. The organic layer was washed with water, dried over anhydrous $MgSO_4$, filtered, and concentrated using a rotary evaporator. The residue was purified by silica gel column chromatography using 100% ethyl acetate (EtOAc) to isolate 350 mg of the pure compound **4**. Yield: 51%. Colorless solid. Melting Point: 61–64 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.71 (t, 2H, J = 5.85 Hz), 2.36–2.33 (m, 2H), 2.0 (s, 3H), 1.85–1.82 (m, 2H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 77.8, 74.8, 61.5, 32.3, 32.0, 23.1. ^{11}B NMR (Proton decoupled, 64.2 MHz): −4.37, −5.57, −10.53. IR (KBr): 3279, 2946, 2875, 2570, 1952, 1449, 1389, 1345, 1181, 1061, 1028, 950, 730 cm^{-1}. ES-MS (*m*/*z*): Cald: 216.33 Found: 215.3 (M^+ − 1, 100%).

5: Compound **4** (2 g, 9.25 mmol) was dissolved in dichloromethane (80 mL), to which pyridine (10 mL) and *p*-toluenesulfonyl chloride (3.62 g, 18.5 mmol) were added and the resulting mixture was stirred at room temperature for 24 h. This mixture was then washed with 2 N NaOH solution and extracted with dichloromethane. The organic layer was washed with water, dried over anhydrous $MgSO_4$, filtered, and concentrated using a rotary evaporator. The residue was purified by silica gel column chromatography using 20% EtOAc in hexane to obtain 2.56 g of the pure compound **5**. Yield: 75%. Colorless solid. Melting Point: 92–94 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.8 (d, 2H, J = 8.17 Hz), 7.38 (d, 2H, J = 8.10 Hz), 4.0 (t, 2H, J = 5.70 Hz), 2.47 (s, 3H), 2.27–2.23 (m, 4H), 1.99 (s, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 145.3, 132.6, 130.0, 127.8, 76.6, 74.9, 68.9, 31.4, 28.8, 23.1, 21.6. ^{11}B NMR (Proton decoupled, 64.2 MHz): −5.55, −10.33. IR (KBr): 2946, 2587, 1931, 1595, 1453, 1414, 1180, 1096, 1020, 931, 837, 740 cm^{-1}. ES-MS (*m*/*z*): Cald: 370.52. Found: 393.3 (M + Na, 100%).

6: Sodium azide (1.26 g, 19.43 mmol) was added to the anhydrous DMF (15 mL) solution of compound **5** (2.4 g, 6.477 mmol) and the resulting mixture was stirred at room temperature for 24 h. The solvent was then removed and the residue purified by silica gel column chromatography using 25% EtOAc in hexane to produce 1.70 g of the pure carboranylazide **6** in almost quantitative yield. Colorless liquid. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 4.0 (t, 2H, J = 6.25 Hz), 2.30–2.27 (m, 2H), 2.0 (s, 3H), 1.88–1.82 (m, 2H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 77.2, 74.8, 50.4, 32.4, 28.9, 23.1. ^{11}B NMR (Proton decoupled, 160.5 MHz): −3.91, −5.33, −8.84, −9.44, −10.18, −10.64. IR (neat): 2943, 2589, 2100, 1452, 1351, 1241, 1183, 1021, 950, 739 cm^{-1}. ES-MS (*m*/*z*): Cald: 241.35. Found: 241.3 (M^+, 100%).

8: To a solution of compound **7** (600 mg, 1.47 mmol) in 5 mL of dry CH_2Cl_2 were added oven-dried 4 Å molecular sieves, 2-propyne-1-ol (0.27 mL, 4.43 mmol), and trimethylsilyl triflate (0.55 mL, 2.95 mmol) at 0 °C and then the reaction mixture was stirred at room temperature for one hour. After completion of the reaction, the mixture was quenched with saturated $NaHCO_3$ solution and then the reaction mixture was extracted with dichloromethane. The organic layer was washed with water, dried over anhydrous $MgSO_4$, filtered, and concentrated using a rotary evaporator. The crude product was purified by silica gel column chromatography using 20–25% EtOAc in hexane to get 250 mg of the pure compound **8** as colorless solid. Yield: 42%. Melting Point: 73–75 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.50 (t, 1H, J = 9.85 Hz), 5.32 (t, 1H, J = 10.0 Hz), 5.19 (dd, 1H, J_1 = 3.05 Hz, J_2 = 10.22 Hz), 5.12 (d, 1H, J = 3.03 Hz), 4.41–4.37 (m, 2H), 4.06 (dd, 2H, J_1 = 3.15 Hz, J_2 = 12.04 Hz), 3.46–3.42 (m, 1H), 2.45 (t, 1H, J = 2.28 Hz), 2.08 (s, 3H), 2.06 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.5, 170.0, 169.6, 169.5, 77.9, 77.3, 75.6, 74.3, 72.0, 70.6, 61.1, 55.4, 38.7, 20.7, 20.6, 20.5. IR (KBr): 3278, 2978, 2922, 2119, 1748, 1436, 1380, 1213, 1032, 969, 896 cm^{-1}. ES-MS (*m*/*z*): cald: 402.42, found: 425.1 (M^+ + Na, 100%).

9: Compound **8** (30 mg, 0.074 mmol) was added to carboranylazide **6** (36 mg, 0.149 mmol) in the reaction flask containing $CuSO_4 \cdot 5H_2O$ (74.4 mg, 0.298 mmol) and potassium ascorbate (128 mg, 0.596 mmol) in THF (3 mL) and H_2O (3 mL). After stirring this mixture at room temperature for 3 h, aqueous ammonium chloride solution was added to the mixture and was then extracted with EtOAc. The organic layer was washed with water, dried with $MgSO_4$, and the solvent was removed using a rotary evaporator. Purification of the compound was carried out by silica gel column chromatography using 10% MeOH in ethyl acetate to isolate 40 mg of pure compound **9**. Yield: 83%. Colorless liquid. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.6 (s, 1H), 5.52 (t, 1H, J = 10.0 Hz), 5.33 (dd, 1H, J_1 = 9.50 Hz, J_2 = 11.0 Hz), 5.17 (dd, 1H, J_1 = 3.0 Hz, J_2 = 10.50 Hz), 4.95 (d, 2H, J = 3.0 Hz), 4.45–4.40 (m, 4H), 4.11 (dd, 1H, J_1 = 3.5 Hz, J_2 = 12.0 Hz), 3.55–3.52 (m, 1H), 2.29–2.26 (m, 4H), 2.11 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 2.0 (s, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.6, 169.9, 169.8, 169.5, 143.9, 122.9, 78.7, 76.4, 75.0, 74.6, 72.3, 70.7, 61.8, 61.3, 49.3, 38.5, 32.1, 29.9, 23.1, 20.8, 20.7, 20.5. IR (neat): 2914, 2850, 2588, 2100, 1741, 1444, 1351, 1260, 1028, 1179, 1028, 733 cm^{-1}. ^{11}B NMR (Proton decoupled, 160.5 MHz): −3.99, −5.57, −9.60, −10.33,

−10.89. ES-MS (*m*/*z*): Cald: 645.78. Found: 644.4 (M^+ − 1, 100%).

10: Sodium methoxide (1.6 mg, 0.0309 mmol) in methanol (15 mL) was added to **9** (40 mg, 0.0619 mmol) and the resulting mixture was stirred at room temperature for 1 h. This mixture was then quenched with DOWEX resin and the solvent was removed using a rotary evaporator. Purification of the product was carried out by silica gel column chromatography using 10% MeOH in ethyl acetate to obtain 25 mg of the pure compound **10** as a colorless liquid. Yield: 86%. ^{1}H NMR (d_4-MeOH, 500 MHz): δ 8.0 (s, 1H), 4.99 (d, 1H, J = 12.5 Hz), 4.70 (d, 1H, J = 3.0 Hz), 4.48 (d, 1H, J = 12.0 Hz), 4.48 (t, 1H, J = 6.5 Hz), 3.92 (d, 1H, J = 3.50 Hz), 3.90 (d, 1H, J = 3.50 Hz), 3.86–3.82 (m, 1H), 3.74–3.71 (m, 1H), 3.63–3.55 (m, 1H), 3.09–3.05 (m, 4H), 2.40–2.34 (m, 1H), 2.23–2.17 (m, 1H), 2.0 (s, 3H). ^{13}C NMR (d_4-MeOH, 125 MHz): δ 144.2, 124.1, 81.7, 77.5, 75.7, 75.6, 74.5, 60.8, 60.7, 48.8, 43.6, 31.5, 22.0. IR (neat): 3486, 2920, 2849, 2361, 1635, 1470, 1400, 1104, 806 cm^{-1}. ^{11}B NMR (Proton decoupled, 160.5 MHz): −4.49, −5.92, −9.06, −9.74, −10.72. ES-MS (*m*/*z*): Cald: 477.63. Found: 477.3 (M^+, 100%).

12: A solution of *n*-BuLi (18.22 mL, 1.6 M in hexane) was slowly added to a solution of *meta*-carborane **11** (2 g, 13.88 mmol) in dry THF (80 mL) at 0 °C. The resulting mixture was stirred at room temperature for 30 min and to which a solution of oxacyclobutane (2.9 mL, 44.44 mmol) in 10 mL dry THF was added dropwise at 0 °C. The mixture was then warmed to room temperature and stirred for about 20 h. At this point, 50 mL of 1M HCl was added to the mixture and stirred at room temperature for 1 h. The mixture was then extracted with ethyl acetate three times. The organic layer was concentrated and the crude reaction mixture was purified by silica gel column chromatography using 20–30% ethyl acetate in hexane to isolate 2.82 g of pure compound **12** as a colorless solid. Yield: 78%. Melting point: 64–67 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.64 (t, 4H, J = 6.0 Hz), 2.13–2.10 (m, 4H), 1.72–1.66 (m, 4H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 75.5, 61.9, 33.5, 32.8. ^{11}B NMR (Proton decoupled, 160.5 MHz): −7.25, −11.10, −13.56. IR (KBr): 3299, 2940, 2878, 2588, 1962, 1715, 1451, 1330, 1066, 1027, 937 cm^{-1}. ES-MS (*m*/*z*): Cald: 260.38. Found: 261.3 (M^+ + 1, 100%).

13: To a solution of **12** (2.82 g, 10.83 mmol) in pyridine (20 mL) and DCM (80 mL) at 0 °C *para*-toluenesulfonylchloride (8.25 g, 43.32 mmol) was slowly added with constant stirring. The resulting mixture was slowly warmed to room temperature and stirred at this temperature for 24 h. After this period, the mixture was washed with 2 N NaOH solution and the organic layer was dried with $MgSO_4$, filtered and then concentrated using a rotary evaporator. The crude product was purified by silica gel column chromatography using 20% ethyl acetate in hexane to isolate 4 g of the pure compound **13** as a colorless solid. Yield: 68%. Melting point: 70–71 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.83 (d, 4H, J = 8.0 Hz), 7.43 (d, 4H, J = 8.0 Hz), 4.0 (t, 4H, J = 6.0 Hz), 2.53 (s, 6H, Me), 2.02–1.99 (m, 4H), 1.75–1.71 (m, 4H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 145.1, 132.8, 130.0, 127.9, 74.5, 69.1, 32.8, 29.1, 21.7. ^{11}B NMR (Proton decoupled, 160.5 MHz): −7.29, −11.03, −13.64. IR (KBr): 2962, 2897, 2601, 1919, 1805, 1596, 1455, 1359, 1174, 1094, 1013, 975, 925 cm^{-1}. ES-MS (*m*/*z*): Cald: 568.76. Found: 569.3 (M^+, 100%).

14: Sodium azide (1.46 g, 22.496 mmol) was added to the solution of **13** (2.02 g, 3.749 mmol) in dry DMF (20 mL). The resulting mixture was stirred at room temperature for 24 h. The solvent DMF was removed *in vacuo* and the product residue was extracted with DCM. The organic layer was dried with $MgSO_4$, filtered and then concentrated by using a rotary evaporator. The resulting crude product residue was purified by silica gel column chromatography using 80% ethyl acetate in hexane to obtain 600 mg of pure compound **14** as a colorless liquid. Yield: 51%. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.24 (t, 4H, J = 6.50 Hz), 2.03–2.0 (m, 4H), 1.67–1.55 (m, 4H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 75.0, 50.7, 34.1, 29.3. ^{11}B NMR (Proton decoupled, 160.5 MHz): −7.19, −11.12, −13.54. IR (KBr): 3439, 2941, 2597, 2099, 1741, 1573, 1385, 1239, 1031 cm^{-1}. ES-MS (*m*/*z*): Cald: 310.41. Found: 240.1 (M^+ − 5N, 100%).

15: THF (3 mL) and H_2O (3 mL) were added to **14** (50 mg, 0.16 mmol) and **3** (194 mg, 0.48 mmol). Then $CuSO_4{\cdot}5H_2O$ (160.79 mg, 0.64 mmol), and potassium ascorbate (276 mg, 1.29 mmol) were added to it and the resulting mixture was stirred at room temperature for 24 h. After adding NH_4Cl solution, the mixture was extracted with ethyl acetate. The organic fraction was washed with water, dried with $MgSO_4$, and then the solvent was removed using a rotary evaporator. Purification of the product was carried out by silica gel column chromatography using 10% MeOH in ethyl acetate to obtain 100 mg of pure compound **15** as a colorless solid. Yield: 56%. Melting Point: 147–151 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.59 (s, 2H), 5.55 (t, 2H, J = 9.75 Hz), 5.38 (dd, 2H, J_1 = 9.65 Hz, J_2 = 10.70 Hz), 5.22 (dd, 2H, J_1 = 6.0 Hz, J_2 = 10.0 Hz), 5.05–4.97 (m, 6H), 4.47 (dd, 2H, J_1 = 5.0 Hz, J_2 = 12.0 Hz), 3.66–3.56 (m, 8H), 2.16 (s, 8H), 2.0 (s, 24H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.6, 170.0, 169.8, 169.5, 143.7, 122.8, 78.7, 74.6, 72.3, 70.9, 61.8, 61.3, 49.4, 38.6, 33.7, 30.3, 20.8, 20.7, 20.6. IR (KBr): 3194, 2963, 2599, 1750, 1436, 1378, 1225, 1074, 1023 cm^{-1}. ^{11}B NMR (Proton decoupled, 160.5 MHz): −7.28, −11.0, −13.6. ES-MS (*m*/*z*): Cald: 1115.24. Found: 1116.5 (M^+ + 1, 100%).

16: Sodium methoxide (0.05 mg) was added to a solution of an acetylated derivative of **15** (220 mg, 0.197 mmol) in 3 mL of dry methanol and the resulting mixture was then stirred at room temperature for 1 h. Subsequently, the mixture was quenched with DOWEX resin and the solvent removed on a rotary evaporator. Purification of the product was carried out by silica gel column chromatography using 10% MeOH in ethyl acetate to isolate 100 mg of pure compound **16** as a colorless liquid. Yield: 65%. ^{1}H NMR (d_4-MeOH, 300 MHz): δ 8.0 (s, 2H), 4.88 (s, 2H), 4.70–4.63 (m, 2H), 4.99–4.95 (m, 2H), 4.35 (s, 2H), 4.12–4.09 (m, 2H), 3.91–3.81 (m, 4H), 3.74–3.71 (m, 2H), 3.65–3.47 (m, 4H), 3.32 (s, 2H), 3.07–3.06 (m, 2H), 2.0 (s, 6H). ^{13}C NMR (d_4-MeOH, 75 MHz): δ 144.0, 124.1, 81.8, 75.7, 75.0, 74.4, 60.8, 49.0, 43.6, 33.3, 30.1 cm^{-1}. ^{11}B NMR (Proton decoupled, 96.3 MHz): −11.11. IR (neat): 3397, 2918, 2872, 2594, 1598, 1386, 1224, 1071, 736 cm^{-1}. ES-MS (*m*/*z*): Cald: 778.95. Found: 779.4 (M^+, 100%).

17: Decaborane (214 mg, 1.75 mmol) was dissolved in dry acetonitrile (12 mL) and refluxed at 90 °C for 3 h. To this, a solution of **8** (640 mg, 1.59 mmol) in 6 mL of toluene was added *via* an addition funnel and again refluxed at 100 °C for 24 h. The solvent was then removed under vacuum and the

product in the crude reaction mixture was purified by silica gel column chromatography using 20–30% ethyl acetate in hexane to isolate 550 mg of the pure compound **17** as a colorless solid. Yield: 66%. Melting Point: 60–62 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.40 (t, 1H, J = 9.87 Hz), 5.29 (t, 1H, J = 10.75 Hz), 5.16 (dd, 1H, J_1 = 3.09 Hz, J_2 = 10.19 Hz), 4.82 (d, 1H, J = 3.05 Hz), 4.38 (dd, 1H, J_1 = 4.96 Hz, J_2 = 12.09 Hz), 4.28 (d, 1H, J = 11.0 Hz), 4.09 (dd, 1H, J_1 = 3.21 Hz, J_2 = 12.0 Hz), 3.97 (br, s, 1H, cage-H), 3.81 (d, 1H, J = 10.97 Hz), 3.37–3.34 (m, 1H), 2.10 (s, 3H), 2.09 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.4, 169.8, 169.6, 169.4, 96.3, 80.6, 74.2, 71.7, 70.3, 60.8, 58.0, 38.9, 20.6, 20.5. IR (KBr): 3062, 2963, 2596, 1747, 1378, 1261, 1220, 1098, 1037, 801 cm^{-1}. ^{11}B NMR (Proton decoupled, 96.3 MHz): −2.74, −4.32, −8.88, −11.83, −13.14. ES-MS (m/z): Cald: 520.61. Found: 544.2(M^+ + Na, 100%).

18: Compound **17** (230 mg, 0.44 mmol), dissolved in 5 mL of THF, was added to a solution of potassium hydroxide (544 mg, 9.7 mmol) in 10 mL of ethanol and refluxed overnight. The resulting mixture was cooled to room temperature and solid CO_2 was added to it. The insoluble part was filtered through a Buchner funnel. The filtrate was concentrated and purified by silica gel column chromatography using dichloromethane, 10% acetone in dichloromethane to acetone as eluents to obtain 90 mg of the pure compound **18** as a colorless liquid. Yield: 54%. ^{1}H NMR (d_4-MeOH, 500 MHz): δ 4.72 (d, 1H, J = 1.95 Hz), 4.54 (d, 1H, J = 1.90 Hz), 3.92–3.88 (m, 2H), 3.84–3.80 (m, 2H), 3.70–3.67 (m, 4H), 3.62–3.55 (m, 4H), 3.13–3.09 (m, 2H), 3.06–3.02 (m, 2H), 2.18 (s, 2H). ^{13}C NMR (d_4-MeOH, 125 MHz): δ 81.0, 80.8, 76.1, 75.7, 75.4, 74.7, 74.5, 69.2, 60.8, 60.7, 54.6. IR (neat): 3519, 2971, 2921, 2518, 1697, 1621, 1394, 1127, 1066, 1026, 913 cm^{-1}. ^{11}B NMR (Proton decoupled, 160.5 MHz): −10.78, −14.49, −16.17, −18.60, −20.14, −21.19, −33.35, −37.75. ES-MS (m/z): Cald: 380.75. Found: 342.4 (M^+ − K, 100%).

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