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Synthesis, Characterization, and Biological Evaluation of o-Carboranyl Heterocyclic Compounds

조 선 대 학 교 대 학 원

화 학 과

정 유 진

오쏘-카보닐 헤테로고리 화합물의 합성, 특성 및 생물학적 평가

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이 논문을 이학석사학위신청 논문으로 제출함.

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정유진
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Mono- , bis-triazinyl 에 치환된 카보라닐계 화합물의 합성을 위한 방법으로 1,2-$C_2B_{10}H_{12}$ 를 기본골격으로 사용하였고 bis[di(methoxyethyl)amino] 와 bis(dimorpholino)triazine 을 치환기로 사용하였다. 6-Chloro-2,4-bis(morpholino)- (**1a**) 또는 bis[di(methoxyethyl)amino]-1,3,5-triazine (**1b**) 와 함께 propargyl alcohol 과 decaborane 을 반응시켜 mono- 또는 bis-substituted triazinyl-o-carboranes (**3-5**) 이다. Morpholino 와 methoxyethyl 작용기는 triazine 의 amine 단위에서 NMR spectroscopy 의 ^{1}H 와 ^{13}C 에 의해 확인할 수 있다. 그리고 triazinyl-o-carboranes 의 구조는 **3b**, **3c** 의 X-ray 결정구조를 통해 확인할 수 있다. 합성된 화합물들 중에서 한 가지는 물에 대한 용해도가 증가한 것을 볼 수 있다. Bis-triazinyl 단위체의 효과적인 위장으로부터 발생한 것으로 주변부의 극성 작용기에 의한 것이다. 또한 **5a** 는 HeLa cell 에서 높은 보론 흡수도를 보이고 B-16 melanoma cell 에서 낮은 독성을 가진다. 이것은 BNCT용 선구물질로서의 가능성이 크다는 것을 보여준다.

Abstract

Synthesis, Characterization, and Biological Evaluation of o-Carboranyl Heterocyclic Compounds

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A method for preparing mono- and bis-triazinyl-substituted carboranyl systems using 1,2-$C_2B_{10}H_{12}$ as cores and bis[di(methoxyethyl)amino] and bis(dimorpholino)triazine as substituents is described. Reaction of 6-chloro-2,4-bis(morpholino)- (**1a**) or bis[di(methoxyethyl)amino]-1,3,5-triazine (**1b**) with propargylalcohol and decaborane produced the corresponding mono- or bis-substituted triazinyl-o-carboranes (**3-5**). Morpholino and methoxyethyl functional groups on the amine units of triazine were identified by ^{1}H and ^{13}C NMR spectroscopy. Furthermore, the structures of the triazinyl-o-carboranes were established by X-ray diffraction studies of **3b**, and **3c**. Within the series of synthesized compounds, one showed increased water solubility arising from the effective camouflaging of the bis-triazinyl unit by the polar functional groups at the periphery. Furthermore, **5a** exhibited high boron uptake in HeLa and B-16 melanoma cells with low toxicity, showing promise as a BNCT agent.

Introduction

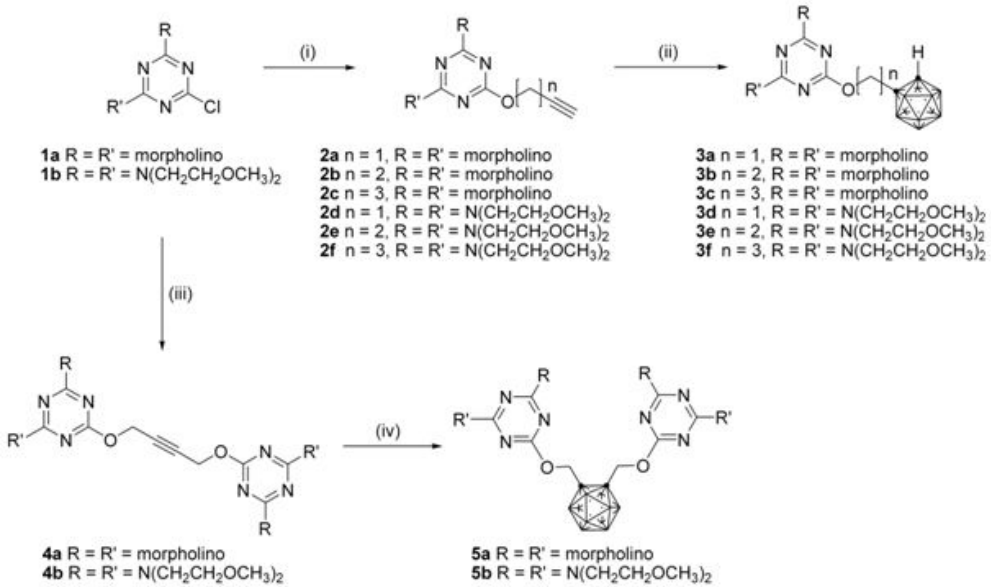
The class of *s*-triazine derivatives contains compounds possessing various types of biological activity. It has been suggested that one of these compounds, hexamethylmelamine, be used for the treatment of lung carcinoma.[1] Another 1,3,5-triazine derivative (5-azacytidine) is used for the treatment of acute lymphoblastic leukemia.[2,3] The antitumor activity of some 1,3,5-triazine derivatives is believed to be related to the fact that these compounds represent antimetabolites of pyrimidine bases and are capable of accumulating in tumor cells.[4]

Currently, the delivery of boron-containing molecular fragments to tumor tissues and the accumulation of these agents within the framework of boron neutron capture therapy (BNCT) is the subject of a great deal of attention.[5] BNCT was first proposed as a potential cancer therapy in 1936,[6] but successful application of BNCT to the treatment of cancer still presents a challenge in medical research.[7] Additionally, most compounds that have developed for BNCT to date are not suitable due to their low water solubility, stability, and selectivity toward cancer cells.[8]

As part of our continuing search for new agents suitable for use in BNCT,[9] we have utilized an *o*-carborane framework not only as a boron carrier,[10] but also as a scaffold to construct bi-functional biologically active species.[11] However, since carboranes consist only of CH and BH units in the cage, they have lipophilic character.[12] Due to the lipophilic character of the carboranyl unit, introduction of a second functional group into the *o*-carboranyl triazine that endows the molecule with water solubility is highly desirable. To meet the requirements for BNCT agents, many attempts have been made in our laboratory to increase the water solubility of candidate molecules while maintaining high boron uptake and low toxicity.[9c,d] Among many candidates, the 1,3,5-triazine derivatives of the *o*-carboranyl system[9b,13] seem promising in that they show high boron uptake in cancer cells. Moreover, the

water-solubility of these molecules was found to be enhanced by introducing a second functionalgroup such as an functionalized amine moiety.[9b] We previously reported that tetrahydroisoquinolines (THIQ),[9a] 1,3,5-triazines,[9b,14] ethylamines,[15] and piperidines[9c] containing the *o*-carborane moiety were potential BNCT agents. Here, we report the synthesis and characterization of 1,3,5-triazinyl morpholine and di(methoxyethyl)amine derivatives and their related *o*-carborane moieties with good yields as potential BNCT agents as an extension of our ongoing investigations into the biological behavior of bio-molecules based on *o*-carboranes.

New *o*-carborane-based 1,3,5-triazine derivatives (75−78% for **2** and 36−38% for **4**) were synthesized as outlined in Scheme 1. As shown in Scheme 1, the starting materials **1** and alkynyloxy-1,3,5-triazine **2** and **4** can be easily prepared as previously described.[14,16]



Scheme 1. *Reagent and condition*: (i) alkynyl alcohol, *t*-BuOK, DMF, 70−75 °C, 5 h; (ii) decaborane ($B_{10}H_{14}$), *N*,*N*-dimethylaniline, toluene, reflux, 24 h; (iii) alkynyl alcohol, *t*-BuOK, DMF, 70−75 °C 24 h; (iv) decaborane, *N*,*N*-dimethylaniline, toluene, reflux, 36 h.

As shown in Table 1, compounds **2** and **4** exhibit characteristic absorption bands in the infrared spectra at 3025-3090 cm^{-1} reflecting the C-H bond of the alkynyl group. Key spectral data (infrared, ^{1}H, ^{13}C NMR spectroscopy) are summarized in Table 1. The ^{1}H NMR spectra of compounds **2** and **4** contain a broadened singlet due to CH groups of the propargyl fragment at 1.97-2.42 ppm, singlets reflecting OCH_2 groups at 4.35−4.89 (**2**) and 4.86 and 4.91 ppm (**4**), and multiplets due to protons of the morpholine substituent in the region of 1.94–2.43 (**2**) and 4.86 and 2.43 ppm (**4**). Treatment of **2** or **4** with decaborane ($B_{10}H_{14}$) and dimethylaniline in toluene produced the target compounds, **3** and **5**, respectively, in moderate yields (35–50%). Compounds **3** and **5** show characteristic absorption bands in the infrared spectra at 2586-2596 cm^{-1} for the B-H group (see Table 2). As shown in Table 2, in the ^{1}H NMR spectra of compound **3**, the proton chemical shift for the OCH_2 group (δ = 4.21−4.86 ppm) almost coincides with the value observed for the initial compound (**2**). Additionally, the signal produced by protons of the CH group of this carborane was observed in a weaker field (δ = 3.58−4.26 ppm) than the value for the corresponding fragment in the initial compound (δ = 3.55 ppm). In addition to the signals of protons of the morpholine substituent [δ = 3.65–3.77 (**3**), 3.68 and 3.74 ppm (**5**)], the spectra of compounds **3** and **5** contained a broad signal caused by B−H peaks of the *o*-carborane moieties from 0.5 to 3.4 ppm.

The final structural proofs of **3a-c** were obtained by an X-ray analysis, which were performed on the crystals of **3b** and **3c** (Figs. 1 and 2). Selected bond lengths, bond angles, and torsion angles are collected in Table 1. The crystal structures correspond well with the conformation and configuration derived from the NMR data. All three regions, that is, amine substituents, triazine ring, and tethered o-carborane group of **3a-c**, can be clearly assigned.

The biological activities, including the cytotoxicity and intracellular accumulation of the *o*-carboranyl aminoalcohol derivatives, were next investigated. As shown in Table 3, compounds **3a-f** and **5a** and **b** exhibited low cytotoxicity, with IC_{50} values (the half maximal inhibitory concentration) in the range of 9.02 to 44.57 μM for B-16 and 10.76 to 29.08 μM for HeLa cancer cells. Furthermore, the accumulated boron concentration of all compounds (**3a-f** and **5a** and **b**) were higher than that of *p*-boronophenylalanine (BPA). Among the *o*-carboranyl triazine derivatives tested, compound **5a** appeared to be a good candidate agent based on the three essential requirements for BNCT, good water solubility, low cytotoxicity, and high boron uptake.

In conclusion, the sequential replacement of three chlorine atoms on cyanuric chloride with cyclic or secondary amine nucleophiles provides the synthesis of a variety of alkynyl-oxo-substituted 1,3,5-*s*-triazine molecules. Thus, we have developed a general and versatile method for the preparation of triazines flanked with an *o*-carborane. In light of its operational simplicity and efficiency, this reliable method is expected to have a broad utility due to the scope of applications of the *s*-triazines. In particular, compounds **3a**, **3d** and **5a** showed lower toxicity over a wide range of boron concentrations up to 350 mg boron mL^{-1}.

Experimental Section

General consideration All manipulations were carried out using standard Schlenk techniques. Starting materials, **1a**, **2a**, and **3a**, were prepared as previously described.[9b,14] NMR spectra were collected using a JEOL (500 MHz) FT-NMR spectrometer and referenced based on the residual protons of the solvent ($CDCl_3$, 7.26 ppm). Infrared (IR) spectra were obtained on a JASCO FT/IR-5300 spectrophotometer. Low-resolution mass spectra were acquired with a Quattro AC spectrometer.

Determination of IC_{50}

The boron compound (20 mg) was dissolved in DMSO (1.0 mL), and the resulting solution was diluted with MEM (Modified Eagle Medium) (10% FCS), or BPA was directly dissolved in the same medium. In a Falcon 3072, 96-well culture plate, the cells (B-16 melanoma cancer cells from Tohoku University, Japan) (1×10^3 cells/well) were cultured in five wells with the medium containing boron compounds at various concentrations (1－100 ppm), and incubated for 3 d at 37 °C in a CO_2 incubator. DMSO is non-toxic at concentrations less than 0.5% and control experiments confirmed that DMSO was non-toxic at the concentrations used in the present experiments. After the 3 day incubation, the medium was removed, the cells were washed (3 times) with PBS (–) (phosphate-buffered saline), and the CellTiter 96 AQueous Non-Radioactive Cell Proliferation Assay [MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium] was used for counting cells on a Microplate reader. The results are presented in Table 2 as the concentration of agent that resulted in a cell culture with 50% of the cell number of the corresponding untreated culture (IC_{50}).

In vitro Boron Incorporation into B-16 Melanoma Cells

B-16 melanoma cells were cultured in Falcon 3025 dishes (150 mmϕ). When the cell population had increased to fill the dish (8.8×10^6 cells/dish), the boron compounds (1.0×10^{-4} M, 10.8 ppm boron) and BPA (1.0×10^{-3} M, 10.8 ppm boron) were added to the dishes. The cells were incubated for 3h at 37°C in medium (MEM, 10% FBS; 20 mL). The cells were washed (3 times) with Ca/Mg-free phosphate buffered saline [PBS (–)], collected by rubber policeman, digested with a mixture of 60% $HClO_4$–30% H_2O_2 (1:2) solution (2 mL), and then decomposed for 1h at 75 °C. After filtration through a membrane filter (Millipore, 0.22 mm), the boron concentration was determined using ICP-AES (Shimadzu, ICPS–1000–III). Each experiment was performed in triplicate. The average boron concentration of each fraction is indicated in Table 2.

Crystal Structure Determination

Crystals of **3b** and **3c** were obtained from toluene at -15 °C, sealed in glass capillaries under argon, and mounted on the diffractometer. Preliminary examination and data collection were performed using a Brucker SMART CCD detector system single-crystal X-ray diffractometer equipped with a sealed-tube X-ray source (40 kV × 50 mA) using graphite-monochromated Mo Kα radiation (λ = 0.7107 Å). Preliminary unit cell constants were determined with a set of 45 narrow-frame (0.3º in ω) scans. The double-pass method of scanning was used to exclude any noise. The collected frames were integrated using an orientation matrix determined from the narrow-frame scans. The SMART software package was used for data collection, and SAINT was used for frame integration.[17a] Final cell constants were determined by a global refinement of xyz centroids of reflections harvested from the entire data set.

Structure solution and refinement were carried out using the SHELXTL-PLUS software package.[17b]

Preparation of compounds 2 and 4

General Procedure: A DMF (50 mL) solution of 2,4-dimorpholino- or bis[di(methoxyethylamino)]-1,3,5-triazine **1** (10 mmol) and alkynyl alcohol (15 mmol for **2**) or 2-butyn-1,4-diol (8 mmol for **4**) was added to potassium *tert*-butoxide (1.2 mmol for **2** or 8.0 mmol for **4**) at room temperature. The reaction mixture was then stirred at room temperature for 1 h, followed by stirring at 70 °C for an additional 6 h. Next, the reaction mixture was cooled to room temperature and quenched with distilled H_2O (50 mL × 3). The reaction mixture was subsequently extracted with ethyl acetate (30 mL × 2). The organic layer was washed with H_2O (30 mL × 3), dried with anhydrous Na_2SO_4, and concentrated *in vacuo*. The residue was purified by flash column chromatography (ethyl acetate: *n*-hexane = 1:1) to give **2** and **4** in yields of 75-78% and 36-38%, respectively.

2a: Yield: 2.37 g (78%); LRMS: 305 (30%), $[M]^{+\bullet}$; IR (KBr pellet, cm^{-1}) ν(C−H) 3090; ^{1}H NMR ($CDCl_3$, ppm) δ 2.42 (t, *J* = 5.0 Hz, 1H), 3.70 (t, *J* = 10.0 Hz, 8H), 3.78 (t, *J* = 10.0 Hz, 8H), 4.89 (d, *J* = 5.0 Hz, 2H); ^{13}C NMR ($CDCl_3$, ppm) δ 43.9, 54.1, 66.8, 74.5, 78.6, 166.03, 170.0.

2b: Yield: 2.45 g (77%); LRMS: 319 (36%), $[M]^{+\bullet}$; IR (KBr pellet, cm^{-1}) ν(C−H) 3076; ^{1}H NMR ($CDCl_3$, ppm) δ 2.00 (t, *J* = 5.0 Hz, 1H), 2.67 (td, *J* = 15.0 and 6 Hz, 2H), 3.69 (t, *J* = 10.0 Hz, 8H), 3.78 (t, *J* = 10.0 Hz, 8H), 4.38 (t, *J* = 15.0 Hz, 2H); ^{13}C NMR ($CDCl_3$, ppm) δ 19.2, 43.9, 64.3, 66.8, 70.0, 79.2, 166.1, 170.5.

2c: Yield: 2.54 g (75%); LRMS: 333 (28%), $[M]^{+\bullet}$; IR (KBr pellet, cm^{-1}) ν(C—H) 3085; ^{1}H NMR ($CDCl_3$, ppm) δ 1.94 (m, 2H), 1.94 (t, *J* = 5.0 Hz, 1H), 1.97 (q, *J* = 15.0 Hz, 2H), 2.35 (td, *J* = 15.0 Hz and 5.0 Hz, 2H), 3.70 (t, *J* = 10.0 Hz, 8H), 3.78 (t, *J* = 10.0 Hz, 8H), 4.35 (t, *J* = 15.0 Hz, 2H); ^{13}C NMR ($CDCl_3$, ppm) δ 15.4, 28.0, 43.8, 65.2, 66.9, 68.8, 83.7, 166.1, 170.9.

2d: Yield: 3.05 g (77%); LRMS: 397 (26%), $[M]^{+\bullet}$; IR (KBr pellet, cm^{-1}) ν(C—H) 3083; ^{1}H NMR ($CDCl_3$, ppm) δ 2.43 (t, *J* = 3.0 Hz, 1H), 3.31 (s, 12H), 3.55 (t, *J* = 11.0 Hz, 8H), 3.74 (t, *J* = 11.0 Hz, 8H), 4.85 (d, *J* = 3.0 Hz, 2H); ^{13}C NMR ($CDCl_3$, ppm) δ 47.6, 47.9, 53.8, 58.9, 70.6, 71.2, 74.2, 79.1, 166.0, 169.5.

2e: Yield: 3.12 g (76%); LRMS: 411 (31%), $[M]^{+\bullet}$; IR (KBr pellet, cm^{-1}) ν(C—H) 3025; ^{1}H NMR ($CDCl_3$, ppm) δ 1.98 (t, *J* = 5.0 Hz, 1H), 2.65 (td, *J* = 15.0 Hz and 5.0 Hz, 2H), 3.32 (s, 12H), 3.55 (t, *J* = 10.0 Hz, 8H), 3.76 (t, *J* = 10.0 Hz, 8H), 4.36 (t, *J* = 15.0 Hz, 2H); ^{13}C ($CDCl_3$, ppm) δ 19.2, 47.7, 47.8, 58.8, 64.1, 69.9, 70.7, 71.3, 80.4, 166.1, 170.0.

2f: Yield: 3.18 g (75%); LRMS: 425 (28%), $[M]^{+\bullet}$; IR (KBr pellet, cm^{-1}) ν(C—H) 3080; ^{1}H NMR ($CDCl_3$, ppm) δ 1.96 (q, *J* = 15.0 Hz, 2H), 2.16 (t, *J* = 5.0 Hz, 1H), 2.33 (td, *J* = 15.0 Hz and 5 Hz, 2H), 3.33 (s, 12H), 3.55 (t, *J* = 10.0 Hz, 8H), 3.76 (t, *J* = 10.0 Hz, 8H), 4.35 (t, *J* = 15.0 Hz, 2H); ^{13}C NMR ($CDCl_3$, ppm) δ 15.4, 28.1, 65.0, 68.7, 70.8, 71.3, 75.2, 83.8, 166.1, 170.5.

4a: Yield: 2.22 g (38%); LRMS: 584 (37%), $[M]^{+\bullet}$; IR (KBr pellet, cm^{-1}) ν(C≡C) 2132; ^{1}H NMR ($CDCl_3$, ppm) δ 3.70 (t, *J* = 15.0 Hz, 16H), 3.78 (t, *J* = 15.0 Hz, 16H), 4.91 (s, 4H); ^{13}C NMR ($CDCl_3$, ppm) δ 43.9, 66.9, 77.6, 80.5, 166.0, 170.2.

4b: Yield: 2.26g (36%); LRMS: 768 (27%), $[M]^{+\bullet}$; IR (KBr pellet, cm^{-1}) ν(C≡C) 2110; ^{1}H NMR ($CDCl_3$, ppm) δ3.30 (s, 24H), 3.52 (t, *J* = 15.0 Hz, 16H), 3.71 (t, *J* = 15.0 Hz, 16H), 4.86 (s, 4H); ^{13}C NMR ($CDCl_3$, ppm) δ 54.1, 58.9, 58.9, 71.3, 81.4, 166.0, 170.0.

Preparation of compounds 3 and 5

General Procedure: Decaborane (0.88 g, 11 mmol) and *N*,*N*-dimethylaniline (1.75 g, 14 mmol) were added to a dried toluene (20 mL) solution containing alkynyloxy-1,3,5-triazines **2** or **4** (2.0 g, 10 mmol). The resulting solution was then heated at reflux for 24 h and filtered. Next, the filtrate was diluted with water (1:1), after which the precipitate was separated by filtration and recrystallized to give **3** or **5**.

3a: Yield: 2.11 g (50%). LRMS: 424† (100%), $[M]^{+\bullet}$; IR (KBr pellet, cm^{-1}) ν(B−) 2588; ^{1}H NMR ($CDCl_3$, ppm) δ 3.65 (t, *J* = 10.0 Hz, 8H), 3.70 (t, *J* = 10.0 Hz, 8H), 3.97 (br s, 1H), 4.86 (s, 2H) ^{13}C NMR ($CDCl_3$, ppm) δ 43.9, 58.1, 66.2, 66.7, 72.3, 165.8, 170.5.

3b: Yield: 2.14 g, (49%). LRMS: 438†(100%), $[M]^{+\bullet}$; IR (KBr pellet, cm^{-1}) ν(B−) 2596; ^{1}H NMR ($CDCl_3$, ppm) δ 2.67 (t, *J* = 12.0 Hz, 2H), 3.70 (t, *J* = 10.0 Hz, 8H), 3.77 (t, *J* = 10.0Hz, 8H), 3.84 (br s, 1H), 4.34 (t, *J* = 12.0 Hz, 2H); ^{13}C NMR ($CDCl_3$, ppm) δ 36.6, 43.9, 60.7, 66.8, 67.0, 72.4, 165.9, 170.1.

3c: Yield: 2.20 g, (49%). LRMS: 451† (100%), $[M]^{+\bullet}$; IR (KBr pellet, cm^{-1}) ν(B−H) 2591; ^{1}H NMR ($CDCl_3$, ppm) δ 1.93 (q, *J* = 12.0 Hz, 2H), 2.39 (t, *J* = 12.0 Hz, 2H), 3.58 (br s, 1H), 3.70 (t, *J* = 12.0 Hz, 8H), 3.76 (t, *J* = 12.0 Hz, 8H), 4.24 (t, *J* = 12.0 Hz, 2H); ^{13}C NMR ($CDCl_3$, ppm) δ 28.7, 305.2, 44.9, 61.6,

65.2, 66.8, 74.7, 166.0, 170.1.

3d: Yield: 2.42g (47%). LRMS: 515† (100%), $[M]^{+\bullet}$; IR (KBr pellet, cm^{-1}) ν(B−H) 2592; ^{1}H NMR ($CDCl_3$, ppm) δ 3.33 (s, 12H), 3.55 (t, *J* = 12.0 Hz, 8H), 4.26 (br s, 1H), 3.75 (t, *J* = 12.0 Hz, 8H), 4.82(s, 2H); ^{13}C NMR ($CDCl_3$, ppm) δ 47.9, 58.6, 66.1, 70.6, 71.0, 73.2, 166.0, 169.5.

3e: Yield: 2.48 g (47%). LRMS: 530† (100%), $[M]^{+\bullet}$; IR (KBr pellet, cm^{-1}) ν(B−H) 2588; ^{1}H NMR ($CDCl_3$, ppm) δ 2.70 (t, *J* = 12.0 Hz, 2H), 3.32 (s, 12H), 3.55 (t, *J* = 10.0 Hz, 8H), 3.76 (t, *J* =10.0 Hz, 8H), 3.79 (br s, 1H), 4.35 (t, *J* = 12.0 Hz, 2 H); ^{13}C NMR ($CDCl_3$, ppm) δ 36.4, 47.8, 60.8, 63.8, 70.7, 71.1, 72.5, 166.0, 169.6.

3f: Yield: 2.60g (48%). LRMS: 544† (100%), $[M]^{+\bullet}$; IR (KBr pellet, cm^{-1}) ν(B−H) 2594; ^{1}H NMR ($CDCl_3$, ppm) δ 1.92 (q, *J* = 12.0 Hz, 2H), 2.36 (t, *J* = 12.0 Hz, 2H), 3.35 (s, 12H), 3.53 (t, *J* = 10.0 Hz, 8H), 3.73 (br s, 1H), 3.75 (t, *J* = 10.0Hz 8H), 4.21 (t, *J* = 12.0 Hz, 2H); ^{13}C NMR ($CDCl_3$, ppm) δ 28.8, 35.2, 58.9, 61.6 , 64.9, 70.7, 71.2, 74.9, 166.0, 170.2.

5a: Yield: 2.44 g (35%). LRMS: 702† (100%), $[M]^{+\bullet}$; IR (KBr pellet, cm^{-1}) ν(B−H) 2586; ^{1}H NMR ($CDCl_3$, ppm) δ 3.68 (t, *J* = 15.0 Hz, 16H), 3.74 (t, *J* = 15 Hz, 8H) 5.00 (s, 4H); ^{13}C NMR ($CDCl_3$, ppm) δ43.9, 64.7, 66.8, 72.6, 165.9, 169.7.

5b: Yield: 3.62 g (41%). LRMS: 888† (100%), $[M]^{+\bullet}$; IR (KBr pellet, cm^{-1}) ν(B−H) 2593; ^{1}H NMR ($CDCl_3$, ppm) δ 3.31 (s, 24H), 3.55 (t, *J* = 12.0 Hz, 16H), 3.75 (t, *J* = 12.0 Hz, 16H), 5.05 (s, 4H); ^{13}C NMR ($CDCl_3$, ppm) δ 47.2, 59.0, 64.2, 70.6, 71.2, 75.6, 166.0, 169.3.

†These masses correspond to the maximum intensity peak of a fragment showing the expected isotope distribution pattern for 10 boron atoms with natural abundance of boron-10 and boron-11.

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Table 1. Yields and Key Spectral Data for **2** and **4**

Entry	No.	Yield (%)	IR(cm^{-1})	^{1}H-NMR					^{13}C-NMR				
			C≡C*H*	C≡C*H*	-OC*H*$_3$	-NC*H*$_2$-	-C*H*$_2$-C≡C	-OC*H*$_2$-	-*C*≡CH	-OCH$_3$	-NCH$_2$-	-OCH$_2$-	-CH$_2$-C≡C
1	**2a**	78	3090	2.42(t)		3.70(t)	4.89(d)	4.89(d), 3.78(t)	78.6, 74.5		54.1	66.8, 54.1	66.8
2	**2b**	77	3076	2.00(t)		3.70(t)	2.67(td)	4.38(t), 3.78(t)	79.2, 70.0		43.9	66.8, 64.3	19.2
3	**2c**	75	3085	1.97(t)		3.70(t)	1.97(td)	4.35(t), 3.78(t)	83.7, 68.8		43.8	66.9, 65.2	28.1
4	**2d**	77	3083	2.43(t)	3.31(s)	3.55(t)	4.85(d)	4.85(d), 3.75(t)	79.1, 74.2	53.8	58.9	71.2, 70.6	71.2
5	**2e**	76	3025	1.98(t)	3.32(s)	3.55(t)	2.75(td)	4.36(t), 3.76(t)	80.4, 71.3	58.8	64.1	69.9, 64.1	19.2
6	**2f**	75	3080	21.6(t)	3.33(s)	3.55	1.96(td)	4.35(t), 3.76(t)	83.8, 75.2	58.9	65.0	71.3, 70.8	28.0
7	**4a**	38	2132			3.70(t)	4.91(s)	4.91(d), 3.77(t)	80.5		43.9	77.6, 66.9	77.6
8	**4b**	36	2110		3.30(s)	3.53(t)	4.86(s)	4.86(d, 3.72(t)	81.4	58.9	54.1	71.3, 71.0	71.0

Table 2. Yields and Key Spectral Data for **3** and **5**

Entry	No.	Yield (%)	IR (cm^{-1}) B-H	^{1}H-NMR					^{13}C-NMR				
				C-H (Carb)	OCH_3	NCH_2-	CH_2-Carb	OCH_2-	*C*/*CH* (Carb)	OCH_3	NCH_2-	OCH_2-	CH_2-Cab
1	**3a**	50	2588	3.97(br s)		3.65(t)	4.86(s)	4.86(s), 3.70(t)	72.3, 66.2		43.9	66.7, 66.2	66.7
2	**3b**	49	2596	3.84(br s)		3.77(t)	2.67(t)	4.34(t), 3.77(t)	72.4, 64.0		43.9	67.0, 66.8	36.6
3	**3c**	49	2591	3.58(br s)		3.70(t)	2.39(t)	4.24(t), 3.70(t)	74.7, 65.2		43.9	66.8, 61.6	35.2
4	**3d**	47	2592	4.26(br s)	3.33(s)	3.55(t)	4.82(s)	4.82(s), 3.75(t)	73.2, 66.1	58.6	47.9	71.0, 70.6	71.0
5	**3e**	47	2588	3.79(br s)	3.32(s)	3.76(t)	2.70(t)	4.35(t), 3.76(t)	72.5, 63.8	60.8	47.8	71.1, 70.7	36.4
6	**3f**	48	2594	3.73(br s)	3.35(s)	3.53(t)	2.36(t)	4.21(t), 3.75(t)	74.9, 61.6	58.9	47.7	71.2, 70.7	35.2
7	**5a**	35	2586			3.68(t)	5.00(s)	5.00(s), 3.74(t)	66.8		43.9	66.8	72.6
8	**5b**	41	2593		3.31(s)	3.65(t)	5.05(s)	5.05(s), 3.75(t)	75.6	59.0	47.2	70.6	71.2

Table 3. Cytotoxicity (IC_{50}) and Boron Uptake for B-16 Melanoma and HeLa Cancer Cells

Entry	Compd	IC_{50} (μM)		Boron Uptake (μg B/10^6 cells)	
		B-16	HeLa	B-16	HeLa
1	**3a**	26.89 (± 7.75)	23.71 (± 4.09)	0.37 ±0.65	0.25 ± 0.34
2	**3b**	15.95 (± 2.68)	29.08 (± 4.45)	0.35 ± 0.63	0.26 ± 0.37
3	**3c**	9.02 (± 1.42)	16.83 (± 3.94)	0.19 ± 0.027	0.17 ± 0.014
4	**3d**	20.31 (± 1.66)	19.42 (± 2.17)	0.37 ± 0.065	0.22 ± 0.15
5	**3e**	13.28 (± 1.32)	16.17 (± 0.38)	0.35 ± 0.14	0.28 ± 0.19
6	**3f**	13.14 (± 6.30)	19.84 (± 1.92)	0.23 ± 0.047	0.25 ± 0.41
7	**5a**	44.57 (± 6.69)	10.76 (± 0.21)	0.51 ± 0.18	0.16 ± 0.22
8	**5b**	15.06 (± 1.78)	18.02 (± 0.77)	0.16 ± 0.11	0.18 ± 0.11
9	**BPA**	44.95 (± 0.30)	35.48 (± 1.45)	0.07 ± 0.028	0.02 ± 0.013

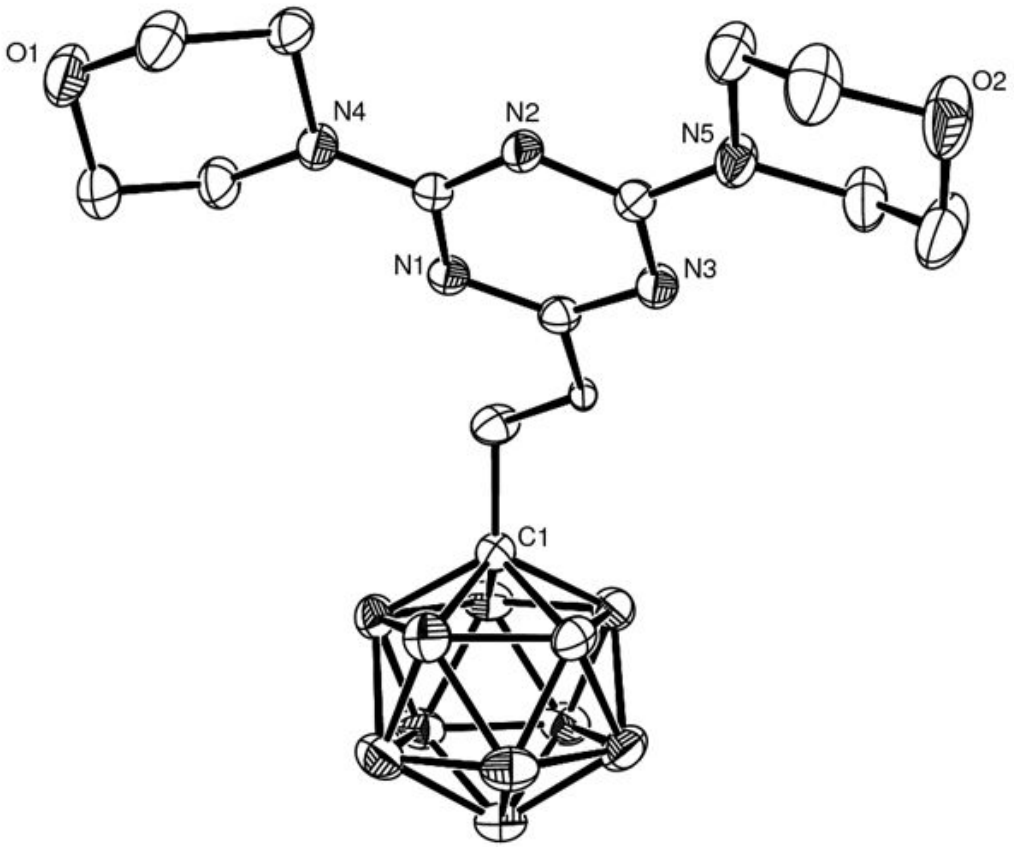

Figure 1. Molecular structure of **3b** with thermal ellipsoids drawn at the 30% level and H atoms are omitted for clarity.

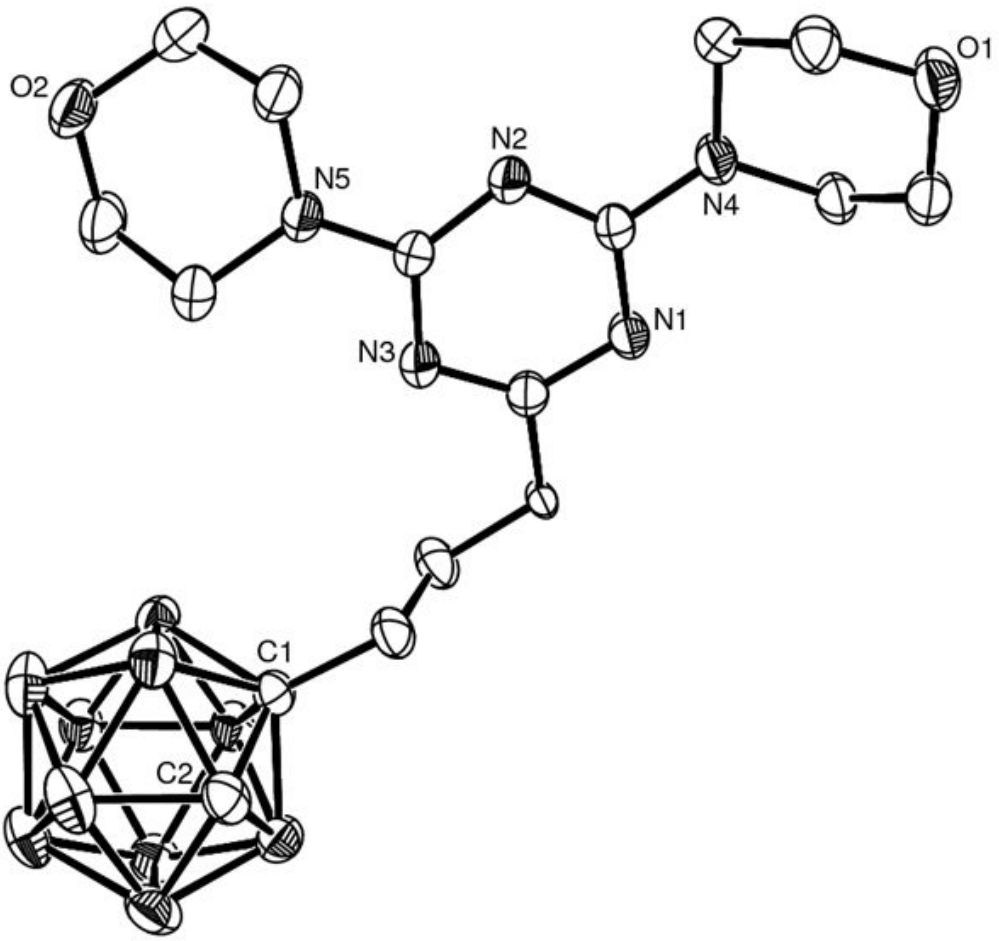

Figure 2. Molecular structure of **3c** with thermal ellipsoids drawn at the 30% level and H atoms are omitted for clarity.

감사의 글

랩 생활을 시작한지가 엊그제 같은데 벌써 2년이라는 시간이 흘렀습니다. 제게 2년이라는 시간은 정말 감사함으로 가득한 시간이었습니다.

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