Synthesis of Carboranes Containing an Azulene Framework and *in Vitro* Evaluation as Boron Carriers

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3-(*o*-Carboranylhydroxymethyl)-7-isopropylazulene sodium carboxylate (**1**) and 3-(*o*-carboranylmethyl)-7-isopropylazulene sodium sulfonate (**2**) were synthesized from the palladium-catalyzed addition reaction of 1-carboranyltributylstannnane (**4**) to azulene aldehydes (**3** and **9**). Although the water solubility of **1** was of the order of 10^{-6} M, that of **2** was of the order of 10^{-3} M and was enough for clinical use. The cytotoxicity of **1** (IC_{50}) toward B-16 melanoma cells was of the order of 10^{-5} M, whereas that of **2** was of the order of 10^{-4} M. This value was close to that of BPA ($\sim 9 \times 10^{-3}$ M) which is utilized for clinical use. The boron uptake by B-16 cells was 0.17 μg of B/10^6 cells for **1** and 0.25 μg of B/10^6 cells for **2**. It is clear that compound **2** accumulates in B-16 melanoma cells with a significantly high level although it is highly water soluble and its cytotoxicity is significantly low.

Introduction

Recently, much attention has been paid to ^{10}B neutron capture therapy (BNCT), an alternative cancer therapy.[1] The interaction of boron-10 isotope and thermal neutron produces an α particle and recoils a lithium-7 ion bearing approximately 2.4 MeV (eq 1). The α particle

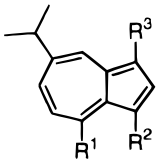
$$^{10}B + {}^{1}n \rightarrow {}^{7}Li + {}^{4}He + 2.4\ MeV \qquad (1)$$

and lithium ion dissipate their kinetic energy before traveling one cell diameter (9 and 5 μm), affording the potential for precise cell killing. The destructive effect is, therefore, highly localized to boron-loaded tissue, before the tumor area is irradiated by thermal neutron. A key requirement for BNCT is to develop a new boron-10 carrier that delivers an adequate concentration of ^{10}B atoms to tumors.[2] *o*-Carborane which has unique electronic and steric properties has been bonded to biologically active organic moieties, such as amino acids,[3] nucleosides,[4] porphyrins,[5] sugars,[6] and lipids,[7] to allow significant amounts of boron importation.

It has been reported that azulene derivatives, in which guaiazulene[8] is an active component of the essential oil of the plant *Guaiacum officinalis*,[9] have biologically active properties such as antiallergic, anti-inflammatory, and antiulcer activities. Especially, guaiazulene sodium sulfonate (GAS),[10] which was synthesized as a hydrophilic derivative of guaiazulene (GA),[9] has been utilized clinically as an antiinflammatory and antiulcer agent (Chart 1). Very recently, sodium 3-ethyl-7-isopropyl-1-azulenesulfonate (KT1-32; Chart 1) was synthesized by Yasunami and co-workers[11] and found to be a promising agent for the therapy of peptic ulcer.

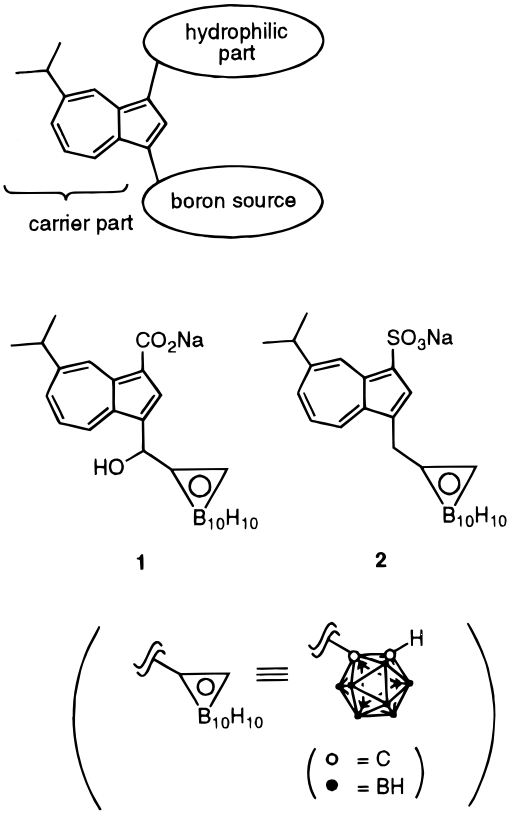
We thought that the azulene framework might be a good candidate for a ^{10}B carrier to deliver boron to tumor tissues because of their highly biologically active properties. The synthetic design of carboranes containing an azulene framework is shown in Chart 2. It was thought that *o*-carborane would be suitable as the boron source and sodium carboxylate and/or sulfonate would be utilized as the hydrophilic part (compounds **1** and **2** in Chart 2). Low cytotoxicity and high water solubility are required for a boron carrier for boron neutron capture therapy, and furthermore high uptake by cancer cells is essential for the carrier. In general, the former properties correspond to high hydrophilicity of the

Chart 1



GA: R^1=Me, R^2=H, R^3=Me
GAS: R^1=Me, R^2=SO_3Na, R^3=Me
KT1-32 : R^1=H, R^2=Et, R^3=SO_3Na

Chart 2



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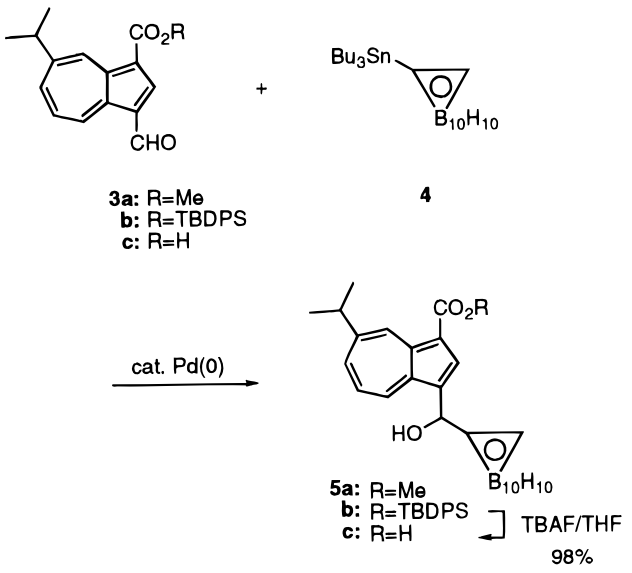
Scheme 1



compound, and the latter corresponds to high lipophilicity. Accordingly, a balance between lipo- and hydrophilicity, which is amphiphilic character, is needed for molecular design of the carrier. Here we wish to report the first synthesis of carboranylazulenes **1** and **2** using a new palladium-catalyzed reaction and their promising biological properties; cellular uptake of **1** and **2** by B-16 melanoma was significantly high in spite of their low cytotoxicity toward B-16 cells and high water solubility.

Synthetic Chemistry

It was thought at the beginning that the addition of *o*-carborane anion to azulene aldehyde would be a suitable approach to introduce carborane to the azulene framework by C−C bond formation. It is well known that the addition of lithiocarborane, which is readily prepared by the treatment of *o*-carborane with BuLi, to electrophiles, such as aldehydes, ketones, and RX, proceeds smoothly to give the corresponding C−C bond-forming product in good yield.[12] However, the reaction of azulene aldehyde **3a** (as shown in Scheme 1), which was prepared by methyl 7-isopropylazulene-1-carboxylate (**6**) with $POCl_3$ in DMF at 0 °C (Scheme 2),[13] with lithiocarborane resulted in the decomposition of the azulene framework. This result indicated that neutral conditions would be essential to introduce *o*-carborane to the azulene framework.

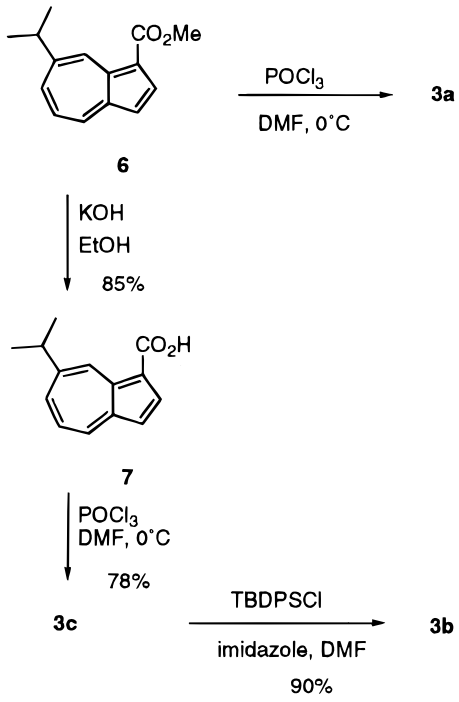
We recently reported that the reaction of *o*-carboranyltributylstannane (**4**) with aldehydes in the presence of catalytic amounts of $Pd_2dba_3{\cdot}CHCl_3$/2 dppe (dba = dibenzylideneacetone, dppe = 1,2-bis(diphenylphosphino)ethane) proceeds very smoothly under neutral conditions to give the corresponding C−C bond-forming products.[14] Thus, we examined the addition of **4** to azulene aldehyde **3a** using several palladium catalysts (Scheme 1). The results are summarized in Table 1. The use of dppe or dppp (1,3-bis(diphenylphosphino)-propane) as a ligand for the palladium catalyst gave **5a** in lower yields (entries 1 and 2). The use of dppb (1,2-bis(diphenylphosphino)butane) gave promising results (entries 3−6). The best result was obtained when 20 mol % of $Pd_2dba_3{\cdot}CHCl_3$/2 dppb was used as a catalyst (entry 5). Under these conditions, **5a** was obtained in 52% yield along with 34% yield of the recovered **3a**.

Table 1. Reaction of **3a** with **4** in the Presence of Palladium Catalysts

entry	Pd catalyst (mol %)	phosphine ligand (mol %)	yield[a] of **5a** (%)	recovery of **3a** (%)
1	$Pd_2dba_3CHCl_3$ (5)	dppe (10)	4	53
2	$Pd_2dba_3CHCl_3$ (5)	dppp (10)	trace	>95
3	$Pd_2dba_3CHCl_3$ (5)	dppb (10)	26	70
4	$Pd_2dba_3CHCl_3$ (10)	dppb (20)	26	72
5	$Pd_2dba_3CHCl_3$ (20)	dppb (40)	52	34
6	$Pd_2dba_3CHCl_3$ (50)	dppb (100)	20	48

[a] Isolated yield based on **3a**.

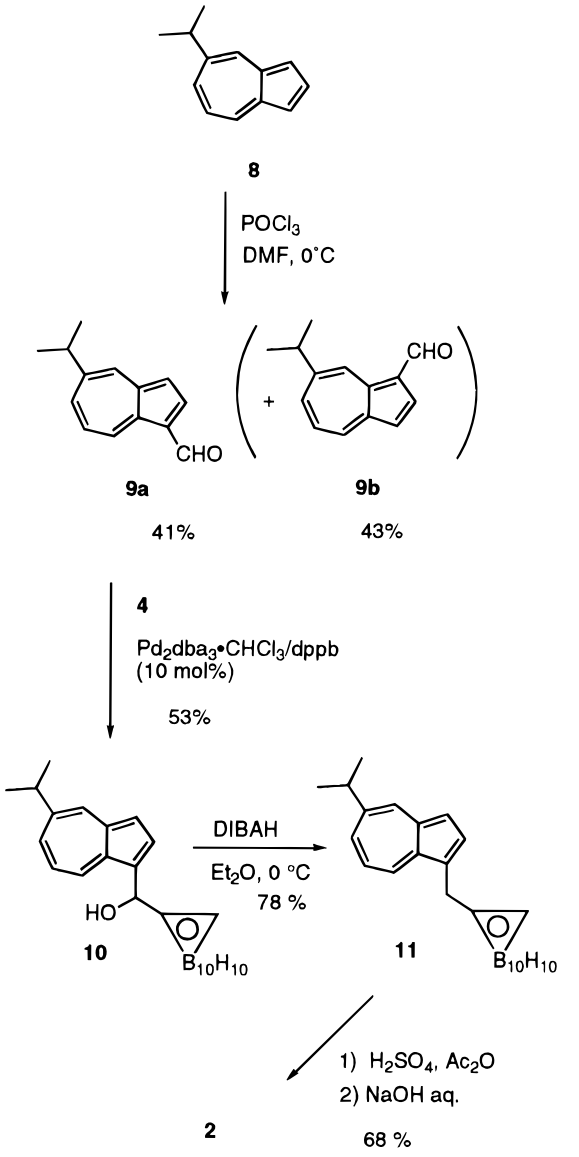
Scheme 2



Accordingly, we employed the $Pd_2dba_3{\cdot}CHCl_3$/2 dppb catalyst for synthesizing carboranes containing the azulene framework.

It was expected that the hydrolysis of the methyl ester protective group of **5a** would give the desired carboxylate **1**. However, treatment of **5a** with bases, such as KOH, NaOMe, and KOtBu, did not lead to the desired carboxylate but caused the retro-addition reaction of the carbinol group, giving the aldehyde **3a** and *o*-carborane.[15] Accordingly, we chose TBDPS as a protective group of the carboxylic acid, instead of a methyl group. Azulene ester **6** was deprotected by treatment with KOH in EtOH to afford carboxylic acid **7** in 85% yield. Formylation of **7** proceeded smoothly without protection of an acid functional group, giving **3c** in 78% yield. The carboxylic acid of **3c** was protected by the TBDPS group in the presence of imidazole in DMF, giving the corresponding silyl ester **3b** in 90% yield (Scheme 2). The reaction of **3b** with **4** using the $Pd_2dba_3{\cdot}CHCl_3$/2 dppb (20 mol %) catalyst in THF under reflux conditions gave carborane adducts **5b** (42%) and **5c** (6%) along with the recovered **3b** (32%) and desilylated product **3c** (19%). **5b** was converted readily to **5c** in 98% yield by treating with TBAF. Carboxylic acid **5c** was treated with ion exchange resin to afford sodium salt **1**. The water solubility of **5c** was 2.92×10^{-7} M, whereas that of **1** was 2.39×10^{-6} M, which were determined by ICP-AES. These solubility values are too low in comparison with

Scheme 3



those acceptable for administration by injection *in vivo*. Accordingly, we next synthesized carboranylazulene **2**, which has a sodium sulfonate group as a water soluble moiety and was expected to be more water soluble than the carboxylic acid derivatives.

The synthesis of carboranylazulene **2** is shown in Scheme 3. 5-Isopropylazulene (**8**)[16] was formylated by treatment with $POCl_3$ in DMF at 0 °C, and a mixture of **9a**,**b** was obtained in 41% and 43% yields, respectively. The reaction of **9a** with **4** in the presence of catalytic amounts of Pd_2dba_3·$CHCl_3$/2 dppb (10 mol %) in THF under reflux gave the addition product **10** in 53% yield. The use of smaller or larger amounts of the catalyst gave **10** in lower yields. Reduction of **10** with DIBAH in ether at 0 °C proceeded smoothly, giving **11** in 78% yield. The treatment of **11** with sulfonic acid in acetic anhydride solution followed by neutralization with sodium hydroxide gave **2** in 84% yield. The water solubility was dramatically increased; it was $>2.3 \times 10^{-3}$ M.

Biological Evaluation *in Vitro*

The growth inhibition of B-16 melanoma cells with **1** and **2** is shown in Figure 1. The cells were suspended in Eagle-MEM medium supplemented with 10% fetal calf serum (FCS) and cultured with different doses of each compound in Falcon dishes (60 mm o.d.) in a 5% CO_2 incubator at 37 °C for 3 days. The cells were trypsinated and counted by microscopic analysis. The IC_{50} value is defined as the dose that failed to inhibit cell growth by more than 50%. Compound **1** exhibited relatively high growth inhibition toward B-16 melanoma cells with an IC_{50} value of 2.6×10^{-5} M. However, compound **2** exhibited lower growth inhibition toward them with an IC_{50} value of 1.6×10^{-4} M (Table 2). In our previous study, the IC_{50} value of BPA,[3a] which is utilized clinically as a boron carrier for cancer therapy on the malignant melanoma[17] and brain tumors,[18] was 8.6×10^{-3}.[19] Therefore, it is clear that the cytotoxicity of compound **2** is low enough to be utilized for clinical use.

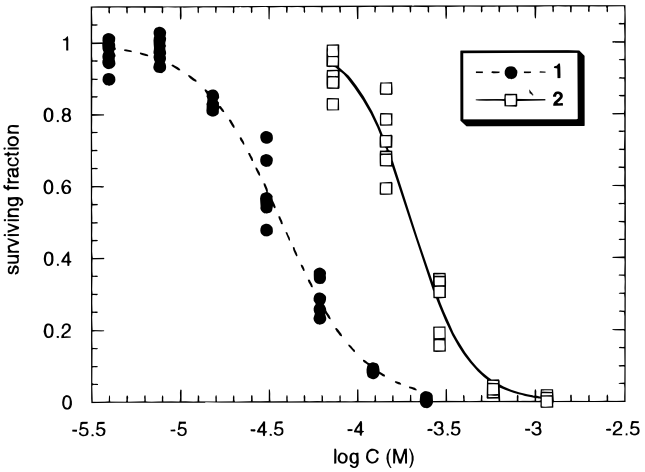


Figure 1. Growth inhibition of B-16 melanoma cells with **1** and **2** after incubation for 3 days.

Table 2. Relationship between Water Solubility, Cytotoxicity toward B-16 Melanoma Cells, and Boron Incorporation

compd	water solubility (M)	cytotoxicity IC_{50} (M)	administration (μg of B/mL)[a]	boron incorporation (μg of B/10^6 cells)[b]
1	2.39×10^{-6}	2.6×10^{-5}	2.81	0.17 ± 0.014
2	$>2.30 \times 10^{-3}$	1.6×10^{-4}	17.2	0.25 ± 0.013
BPA	nd[c]	8.6×10^{-3}	10.8[d]	0.31 ± 0.031

[a] Concentrations were based on their IC_{50} values. [b] Boron incorporated into the cells after 24 h incubation. Values are mean ± standard error of an average of three experiments. [c] Not determined. [d] 1.0×10^{-3} M BPA was administered.

Boron incorporation into B-16 cells was determined by using the ICP-AES method. The cells were cultured in Falcon dishes (150 mm o.d.) until they had filled the dishes ($4.0-5.0 \times 10^6$ cells/dish). The cells were incubated for 3−24 h with Eagle-MEM medium containing **1** (2.81 μg of B/mL) or **2** (17.2 μg of B/mL). As a comparative experiment, the cells were also incubated for 3−24 h with Eagle-MEM medium containing BPA (10.8 μg of B/mL). The concentration of the carriers was adjusted to that of their IC_{50} values. At 3, 12, and 24 h, the cells were washed three times with PBS(−) and processed for the determination of boron concentration by ICP-AES. The results are shown in Figure 2. Boron incorporations of **1** into B-16 cells at 3 and 12 h were similar to those of **2** at 3 and 12 h. However, the boron incorporation of compound **2** was higher than that of compound **1** after 24 h incubation. The tendency of compound **2** to incorporate into B-16 cells was very

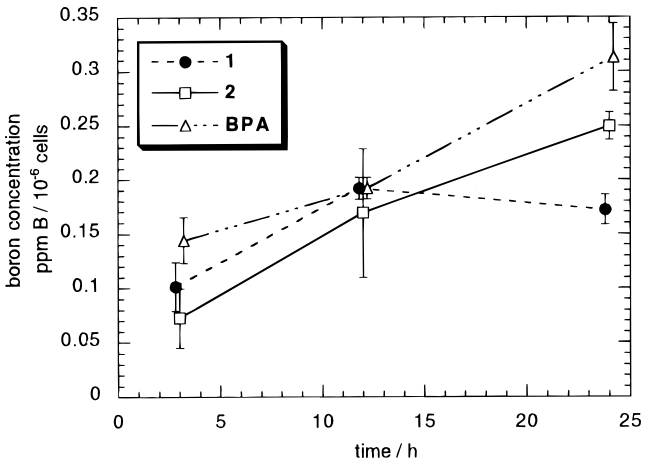


Figure 2. Boron incorporation into B-16 melanoma cells. Each point represents the mean ± SE of triplicate experiments.

similar to that of BPA. These results indicate that compound **2** accumulates into the B-16 cells with a significantly high level. The relationship between water solubility, cytotoxicity toward B-16 melanoma cells, and boron incorporation is summarized in Table 2.[20] Higher boron uptake with higher water solubility is presumably due to the amphiphilic character of the carborane-containing azulene derivative **2**.

Conclusion

We have succeeded in the synthesis of carboranylazulene derivatives using palladium-catalyzed addition reactions. This palladium-catalyzed reaction proceeds under essentially neutral conditions, and under these conditions the introduction of *o*-carborane to the azulene framework is achieved without decomposition of the azulene framework. Furthermore, it is proved by *in vitro* study that compound **2** accumulates highly into B-16 melanoma cells although it is highly water soluble and has low cytotoxicity. Judging from these points, it is considered that compound **2** is promising as a boron carrier for neutron capture therapy. The evaluation of compound **2** *in vivo* is now in progress.

Experimental Section

Materials. Melting points were determined on a MRK No. 8026 instrument and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Jeol GSX-270 spectrometer. The chemical shifts are reported in δ units relative to internal tetramethylsilane. IR spectra were recorded on a Shimadzu FTIR-8200A spectrometer. High-resolution mass spectra were recorded on a Jeol JMS-HX110 spectrometry. Most commercially supplied chemicals were distilled and stored over molecular sieves. The compound **6** was prepared from 2*H*-cyclohepta[*b*]furan-2-one, which was donated by Professor Y. Yasunami, Nihon University, according to the reported procedure.[21]

Source of Cells. B-16 melanoma cells were obtained from the Cancer Cell Repository, Institute of Development, Aging and Cancer, Tohoku University.

Methyl 3-Formyl-7-isopropylazulene-1-carboxylate (3a). Azulene aldehyde **3a** was obtained in 81% yield from methyl 7-isopropylazulene-1-carboxylate (**6**) according to the literature procedure:[21] red solid; mp 87−89 °C; IR (KBr) 2955, 1690, 1655, 1506, 1448, 1394, 1227, 1213, 1059, 885 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 10.27 (s, 1H), 9.72 (dd, J = 10.0, 2.0 Hz, 1H), 8.71 (s, 1H), 8.00 (d, J = 10.0 Hz, 1H), 7.82 (dd, J = 10.0 Hz, 1H), 3.98 (s, 3H), 3.32 (m, 1H), 1.46 (d, J = 7.0 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 186.97, 165.35, 154.35, 146.43, 145.51, 143.03, 141.02, 139.60, 138.14, 132.41, 123.94, 116.31, 51.29, 39.36, 24.56; HRMS calcd for $C_{16}H_{16}O_3$ m/z 256.1100, found m/z 256.1104.

7-Isopropylazulene-1-carboxylic Acid (7). To a solution of potassium hydroxide (10 g, 178.2 mmol) in water (60 mL) was added **6** (2.1 g, 9.3 mmol) in EtOH (50 mL) at room temperature, and the mixture was stirred under reflux for 2 h. The mixture was cooled to room temperature, and a large portion of the solvent was removed *in vacuo*. The resulting residue was diluted with water and acidified with HCl (6 N, 50 mL). The precipitate was collected by suction filtration, washed with water, and dried in a vacuum desiccator to give **7** (1.7 g, 85% yield) as a violet solid: mp 134−135 °C; IR (KBr) 2963, 2924, 2868, 1645, 1526, 1495, 1402, 1317, 1294, 1242, 1161, 1132, 1038, 918 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 9.79 (d, J = 2.0 Hz, 1H), 8.47 (d, J = 4.2 Hz, 1H), 8.38 (d, J = 10.0 Hz, 1H), 7.80 (dd, J = 10.0, 2.0 Hz, 1H), 7.46 (dd, J = 10 Hz, 1H), 7.23 (d, J = 4.2 Hz, 1H), 3.28 (m, 1H), 1.45 (d, J = 7.0 Hz, 6H).

***tert*-Butyldiphenylsilyl 3-Formyl-7-isopropylazulene-1-carboxylate (3b).** To a mixture of **7** (1.8 g, 8.4 mmol) in anhydrous DMF (15 mL) was added phosphorous oxychloride (2.4 mL, 25.4 mmol) in anhydrous DMF solution (15 mL) at 0 °C, and the mixture was allowed to warm to room temperature over 12 h. The mixture was poured into water (200 mL), and the pH of the solution was adjusted to ca. pH 10 by adding KOH solution (12 N, 150 mL). The aqueous solution was washed three times with ether (50 mL) and then acidified with HCl (pH 3). The precipitate was collected by suction filtration, washed with water, and dried in a vacuum desiccator to give crude product **3c** (1.6 g, 6.60 mmol, 78% yield) as a violet solid. The product was used for further manipulation without any further purification. To a solution of crude carboxylic acid **3c** (0.5 g, 2.06 mmol) and imidazole (211 mg, 3.10 mmol) in DMF (5 mL) was added TBDPSCl (0.81 g, 3.10 mmol) at room temperature, and the mixture was stirred for 2 h. The reaction was quenched with water (30 mL); the mixture was extracted with ether, dried over anhydrous $MgSO_4$, and concentrated. Purification by column chromatography on silica gel (hexane/ethyl acetate = 8:1) gave **3b** (0.90 g, 1.87 mmol, 90% yield) as a red solid: mp 109 °C; IR (KBr) 2960, 2856, 1682, 1510, 1400, 1209, 1173, 1115, 1059, 953 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 10.34 (s, 1H), 9.87 (d, J = 2.0 Hz, 1H), 9.74 (dd, J = 10.0, 2.0 Hz, 1H), 8.55 (s, 1H), 7.99 (d, J = 10.0 Hz, 1H), 7.84 (dd, J = 10.0 Hz, 1H), 7.82 (m, 4H), 7.44 (m, 6H), 3.26 (m, 1H), 1.29 (d, J = 7.0 Hz, 6H), 1.24 (s, 9H). Anal. ($C_{31}H_{32}O_3Si$) C, H.

Reaction of 1-Carboranyltributylstannane (4) with 3a. A mixture of **3a** (40 mg, 0.16 mmol), 1-carboranyltributylstannane (**4**) (101 mg, 0.23 mmol), $Pd_2(dba)_3$·$CHCl_3$ (32 mg, 0.031 mmol), and diphenylphosphinobutane (26 mg, 0.062 mmol) was dissolved in THF (1.5 mL) under Ar and stirred for 14 h under reflux. The solvent was removed *in vacuo*, and the residue was purified by column chromatography on silica gel (hexane/ethyl acetate = 5:1) to give **5a** (32 mg, 0.081 mmol, 52% yield) as a violet needle: mp 203 °C; IR (KBr) 3610, 2579, 2350, 2330, 1670, 1654, 1649, 1453, 1229, 777 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 9.78 (d, J = 2.0 Hz, 1H), 8.38 (dd, J = 10.0, 2.0 Hz, 1H), 8.31 (s, 1H), 7.84 (dd, J = 10.0, 2.0 Hz, 1H), 7.50 (dd, J = 10.0 Hz, 1H), 5.92 (d, J = 3.0 Hz, 1H), 4.00 (bs, 1H), 3.94 (s, 3H), 3.25 (m, 1H), 2.80 (d, J = 3.0 Hz, 1H), 1.43 (d, J = 7.0 Hz, 6H). Anal. ($C_{18}H_{28}O_3B_{10}$) C, H.

Reaction of 1-Carboranyltributylstannane (4) with 3b. A mixture of **3b** (500 mg, 1.04 mmol), 1-carboranyltributylstannane (**4**) (901 mg, 2.08 mmol), $Pd_2(dba)_3$·$CHCl_3$ (215 mg, 0.208 mmol), and diphenylphosphinobutane (335 mg, 0.832 mmol) was dissolved in THF (10 mL) under Ar and stirred for 40 h under reflux. The solvent was removed *in vacuo*, and the residue was purified by column chromatography on silica gel (hexane/ethyl acetate = 5:1) to give **5b** (273 mg, 0.437 mmol, 42% yield) first and **5c** (23 mg, 0.061 mmol, 6%), along with the recovered **3b** (162 mg, 0.336 mmol, 32%) and desilylated product **3c** (48 mg, 0.20 mmol, 19%), respectively.

***tert*-Butyldiphenylsilyl 3-(*o*-carboranylhydroxymethyl)-7-isopropylazulene-1-carboxylate (5b):** violet needle; mp 214 °C; IR (KBr) 3368, 3082, 2961, 2932, 2860, 2608, 2573, 2361, 1645, 1429, 1223, 700 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 9.74 (d,

J = 2.0 Hz, 1H), 8.50 (s, 1H), 8.42 (d, J = 10.0 Hz, 1H), 7.80−7.83 (m, 6H), 7.53 (dd, J = 10.0, 2.0 Hz, 1H), 7.41−7.46 (m, 6H), 5.97 (d, J = 3.0 Hz, 1H), 4.01 (bs, 1H), 3.19 (m, 1H), 2.66 (d, J = 3.0 Hz, 1H), 1.34 (d, J = 7.0 Hz, 6H), 1.23 (s, 9H). Anal. ($C_{33}H_{44}O_3B_{10}Si\cdot H_2O$) C, H.

3-(*o*-Carboranylhydroxymethyl)-7-isopropylazulene-1-carboxylic Acid (5c). To a solution of **5b** (200 mg, 0.32 mmol) in THF (3 mL) was added tetrabutylammonium fluoride in THF (1.0 M, 0.5 mL, 0.5 mmol) at room temperature, and the mixture was stirred for 6 h. The reaction was quenched with water; the mixture was extracted with ethyl acetate, dried over $MgSO_4$, and concentrated. Purification by silica gel column chromatography (hexane/ethyl acetate = 5:1) gave carboxylic acid **5c** (119 mg, 0.31 mmol, 98%) as a violet solid: mp 110 °C; IR (KBr) 2963, 2579, 1651, 1531, 1456, 1435, 1238, 1080, 1003, 914, 800 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.83 (s, 1H), 8.49 (s, 1H), 8.45 (d, J = 10.0 Hz, 1H), 7.90 (d, J = 10.0 Hz, 1H), 7.58 (t, J = 10.0 Hz, 1H), 5.96 (s, 1H), 4.04 (bs, 1H), 3.30 (m, 1H), 1.46 (d, J = 7.0 Hz, 6H). Anal. ($C_{17}H_{26}O_3B_{10}$) C, H.

Formylation of 5-Isopropylazulene (8). To a mixture of 7-isopropylazulene (**8**) (0.89 g, 5.2 mmol) in anhydrous DMF (10 mL) was added phosphorous oxychloride (0.48 mL, 5.2 mmol) in anhydrous DMF solution (10 mL) at 0 °C, and the mixture was stirred for 2 h. The reaction mixture was poured into water (200 mL), and the pH of the solution was adjusted to ca. pH 10 by adding aqueous KOH solution (12 N, 100 mL). The mixture was extracted with ether, dried over anhydrous $MgSO_4$, and then concentrated. The resulting residue, which contained two regioisomers, was chromatographed on a silica gel column using hexane/ethyl acetate (5:1) as an eluent to give 1-formyl-7-isopropylazulene (**9b**) (0.45 g, 2.27 mmol, 43% yield) first and then 1-formyl-5-isopropylazulene (**9a**) (0.42 g, 2.12 mmol, 41% yield), respectively.

1-Formyl-5-isopropylazulene (9a): violet solid; mp 68 °C; IR (KBr) 1645, 1499, 1466, 1414, 1398, 1391, 1261, 1040, 791 cm^{-1}; ^1H NMR ($CDCl_3$) δ 10.32 (s, 1H), 9.47 (d, J = 10.0 Hz, 1H), 8.46 (s, 1H), 8.23 (d, J = 4.0 Hz, 1H), 7.81 (d, J = 10.0 Hz, 1H), 7.59 (t, J = 10.0 Hz, 1H), 7.25 (d, J = 4.0 Hz, 1H), 3.18 (m, 1H), 1.39 (d, J = 7.0 Hz, 6H). Anal. ($C_{14}H_{14}O$) C, H.

Reaction of 1-Carboranyltributylstannane (4) with 9a. A mixture of **9a** (43.0 mg, 0.217 mmol), 1-carboranyltributylstannane (**4**) (188 mg, 0.434 mmol), $Pd_2(dba)_3\cdot CHCl_3$ (44.8 mg, 0.044 mmol), and diphenylphosphinobutane (37.0 mg, 0.087 mmol) was dissolved in CH_3CN (2.2 mL) at room temperature under Ar and stirred for 14 h. The solvent was removed *in vacuo*, and the residue was purified by column chromatography on silica gel (hexane/ethyl acetate = 10:1) to give **10** (39.4 mg, 0.115 mmol, 53% yield) as a violet solid: mp 123 °C; IR (KBr) 3078, 2964, 2573, 1647, 1508, 1458, 1420, 1225, 1180, 1070, 1049, 1018 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.35 (d, J = 2.0 Hz, 1H), 8.29 (d, J = 10.0 Hz, 1H), 7.90 (d, J = 4.0 Hz, 1H), 7.65 (dd, J = 10.0, 2.0 Hz, 1H), 7.29 (d, J = 4.0 Hz, 1H), 7.24 (t, J = 10.0 Hz, 1H), 6.04 (d, J = 3.0 Hz, 1H), 4.00 (bs, 1H), 3.15 (m, 1H), 2.54 (d, J = 3.0 Hz, 1H), 1.40 (d, J = 7.0 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 144.5, 144.3, 141.5, 137.9, 136.5, 134.9, 133.0, 124.6, 124.2, 116.4, 70.1, 59.9, 39.0, 28.4, 24.8, 24.5; HRMS calcd for $C_{16}H_{26}OB_{10}$ m/z 344.2915, found m/z 344.2920.

1-(*o*-Carboranylmethyl)-5-isopropylazulene (11). To a mixture of **10** (380 mg, 1.98 mmol) in dried ether (20 mL) was added diisobutylaluminum hydride in hexane (1 M, 20 mL, 20 mmol) dropwise at −78 °C, and the mixture was allowed to warm to room temperature and stirred for 1 day. The reaction mixture was cooled to 0 °C, ethanol (10 mL) was added, and then the mixture was poured into aqueous H_2SO_4 solution (1 N, 60 mL). The mixture was extracted with hexane, dried over $MgSO_4$, and concentrated. Purification by silica gel column chromatography (hexane/ethyl acetate = 10:1) gave **11** (508.8 mg, 1.55 mmol, 78%) as a violet oil: IR (CCl_4) 3063, 2963, 2930, 2970, 2590, 1578, 1464, 1443, 1412, 1396, 1101, 1065, 1018 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.25 (d, J = 10.0 Hz, 1H), 8.20 (s, 1H), 7.68 (d, J = 4.0 Hz, 1H), 7.62 (d, J = 10.0 Hz, 1H), 7.25 (d, J = 4.0 Hz, 1H), 7.20 (t, J = 10.0 Hz, 1H), 4.06 (s, 2H), 3.14 (m, 1H), 3.12 (bs, 1H), 1.40 (d, J = 7.0 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 143.5, 140.8, 138.3, 137.7, 136.5, 136.0, 133.0, 123.6, 120.7, 115.9, 76.1, 59.2, 38.9, 35.4, 24.7; HRMS calcd for $C_{16}H_{26}B_{10}$ m/z 328.2966, found m/z 328.2976. Anal. ($C_{16}H_{26}B_{10}$) C, H.

3-(*o*-Carboranylmethyl)-7-isopropylazulene Sodium Sulfonate (2). To a mixture of **11** (722 mg, 2.2 mmol) in acetic anhydride (20 mL) was added concentrated H_2SO_4 (0.5 mL) dropwise at 0 °C, and the mixture was immediately poured into cold water (100 mL). The mixture was filtered, and aqueous NaOH (25%, 10 mL) was added to the filtrate. The violet precipitate was filtered off, dissolved in ethyl acetate, washed with aqueous NaOH (4%), and dried over Na_2SO_4. The solvent was removed *in vacuo*, and sodium sulfonate **2** was obtained (646 mg, 1.50 mmol, 68% yield) as a violet solid: mp 189−191 °C; IR (KBr) 3061, 2976, 2581, 1418, 1177, 1051, 1024 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.32 (d, J = 2.0 Hz, 1H), 8.37 (d, J = 10.0 Hz, 1H), 8.04 (s, 1H), 7.85 (dd, J = 10.0, 2.0 Hz, 1H), 7.45 (t, J = 10.0 Hz, 1H), 4.31 (bs, 1H), 4.08 (s, 2H), 3.25 (m, 1H), 1.40 (d, J = 7.0 Hz, 6H). Anal. ($C_{16}H_{25}O_3B_{10}SNa\cdot H_2O$) C, H, S.

Determination of IC_{50}. The boron compound **1** (10 mg) was dissolved in 1 mL of DMSO, and the resulting solution was diluted with Eagle's MEM (10% FCS), or the boron compound **2** was directly dissolved in the same medium. In five Falcon 3002 culture dishes (60 mm o.d.), the cells (1×10^5 cells/dish) were cultured with the medium containing boron compounds at each concentration (**1** 1.6, 3.1, 6.3, 12.5, 25, 50, and 100 ppm; **2** 32, 63, 125, 250, and 500 ppm) and incubated for 3 days at 37 °C in a CO_2 incubator. It is known that DMSO is nontoxic at a concentration lower than 0.5%. We also confirmed by the control experiment that DMSO was nontoxic at the concentrations shown above. The medium was removed, and the cells were washed three times with PBS(−) (phosphate-buffered saline) and then trypsinized for counting cells on a hemacytometer. The surviving fraction of the cells at each concentration was calculated by dividing the survival cell number by the average of those of the control experiment. The concentration of the boron compounds was plotted as the abscissa and the surviving fraction as the ordinate (Figure 1). The IC_{50} values were obtained from these curves.

Boron Incorporation into B-16 Cells. B-16 melanoma cells were cultured in Falcon 3025 dishes (150 mm o.d.). When the cells had filled up the dishes, the cell number was counted ($4.0-5.0 \times 10^6$ cells/dish). Since all glassware and living organisms contain very trace amounts of boron, one dish was for the control experiment in which any synthesized compounds were not added. The boron compound **1** (2.6×10^{-5} M, 2.81 μg/mL), **2** (1.6×10^{-4} M, 17.2 μg/mL), and BPA (1.0×10^{-3} M, 10.8 μg/mL) were added to the dishes. The cells were incubated for 3−24 h at 37 °C in 20 mL of medium (Eagle-MEM, 10% FCS). The cells were washed three times with Ca Mg free phosphate-buffered saline (PBS(−)), collected by rubber policeman, digested with 7 mL of 60% $HClO_4$−30% H_2O_2 solution, and then decomposed for 1 h at 75 °C. After filtration with membrane filter (Millipore, 0.22 μm), the boron concentration was determined by using ICP-AES (Shimadzu, ICPS-1000-III). The boron concentration of the control experiment was subtracted from the boron concentrations of the cells of each dish. Three replications of each experiment were carried out. The average boron concentration of each fraction is indicated in Figure 2.

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