1-Carboranyl-3-(2-methylaziridino)-2-propanol. Synthesis, Selective Uptake by B-16 Melanoma, and Selective Cytotoxicity toward Cancer Cells

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1-Carboranyl-3-(2-methylaziridino)-2-propanol (MACB) was prepared from the reaction of 1-carboranyl-2,3-epoxypropane with metalated methylaziridines having copper $[Cu(CN)Li_2]_{1/2}$ or lead ($PbBu_3$) as the metal. MACB exhibited relatively high growth inhibition toward some cancer cells, and selective uptake of MACB by B-16 melanoma was accomplished.

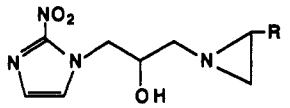
Introduction

The theoretical attractiveness of neutron capture therapy (NCT) versus other radio- and chemotherapic approaches for the treatment of cancer is as appealing now as when first proposed by Locher.[1a] The interaction of boron-10 and thermal neutron produces intense, ionizing radiation that is confined to single or adjacent cancer cells.

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

Much attention has been paid to the design and synthesis of boron-10 carriers that deliver adequate concentration of ^{10}B atoms to tumors.[1b-g] Thus biologically active and tumor-seeking organic moieties such as nucleosides,[1b,c] porphyrins,[1d] sugars,[1e] and lipids,[1g] have been bonded to carboranes.

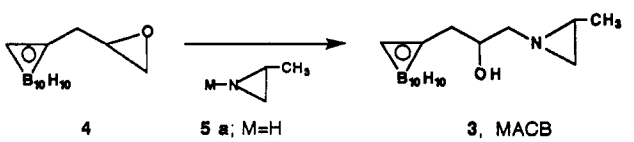
Cellular and molecular studies have indicated that under aerobic conditions α-(1-aziridinylmethyl)-2-nitro-1*H*-imidazole-1-ethanol (RSU-1069, 1) and α-[(2-methylaziridinyl)methyl]-2-nitro-1*H*-imidazole-1-ethanol (RSU1131, 2) alkylate DNA at the phosphate and purine bases via the aziridine group, a process that leads to DNA strand breakage.[2] Accordingly, it occurred to us that a carborane-containing aziridine group may become a potentially useful boron carrier for boron neutron capture therapy. We wish to report that the first synthesis of carboranylaziridine **3**, its selective uptake by B-16 melanoma, and its selective cytotoxicity toward cancer cells; the last observation has not been made with the previously known ^{10}B carriers.



1: R=H
2: R=CH_3

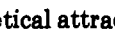
Synthetic Chemistry

1-Carboranyl-3-(methylaziridino)-2-propanol (**3**, MACB) contains both a carborane framework unstable under basic conditions and an aziridine group labile under acidic conditions. In fact, the thermal reaction[3] of **4** with the methylaziridine (**5a**) resulted in the decomposition of the carborane framework. We then examined the low-temperature reaction of **4** with several metalated aziridines (M = Li,[4] $AlEt_2$,[5] MgBr,[6] $PbBu_3$,[7] and CuLn[8]). The results are summarized in Table I. Among these reagents, the lead and copper aziridines gave a promising result (entries 4–7). The reaction with **5b** ($RPbBu_3$) proceeded smoothly, but the isolation of **3** from the reaction mixture and its purification required some cumbersome processes. The use of higher ordered amide cuprate $R_2Cu(CN)Li_2$ gave **3** in 50% isolated yield. On the other hand, the use of the corresponding lower ordered reagent resulted in 39% yield. Accordingly, we employed the higher order copper method for preparing **3** on a large scale.



4 **5 a**; M=H
b; M=$PbBu_3$
c; $[Cu(CN)Li_2]_{1/2}$
3, MACB

Table I. Reactions of 4 with Metalated Aziridines

entry	5 (R= )	(equiv)	reaction temp and time	isolated yield of 3, % (recovery)[a]
1	RLi	(1.3)	−10 °C → rt,[c] 45 min	<5
2	$RAlEt_2$	(1.2)	rt, overnight	(100)
3	RMgBr	(1.2)	rt, 42 h	43
4	$RPbBu_3$	(3)	rt, 3 h	20[b] (80)
5	$RPbBu_3$	(3)	rt, 15 h	53[b] (28)
6	RCu(CN)Li	(3)	−78 °C → rt, 3 h	39 (38)
7	$R_2Cu(CN)Li_2$	(2)	−78 °C → rt, 3 h	50

[a] Recovery of the starting carborane, %. [b] Yield was determined by NMR. [c] Room temperature.

Table II. Growth Inhibition of Human Tumor and Normal Cell Lines

compound	cell line	IC_{50} ppm, 10^{-6} g dm^{-3}	IC_{50} M, mol dm^{-3}
3	B-16	1.40	5.45×10^{-6}
	HepG 2	2.13	8.28×10^{-6}
	TIG-1-20	4.70	1.83×10^{-5}
	IMR 32	8.20	3.19×10^{-5}
	1-87	15.5	6.02×10^{-5}
BPA	B-16	2100	8.55×10^{-3}
	TIG-1-20	2050	8.35×10^{-3}

Biological Evaluation in Vitro

The growth inhibition of several human tumor cell lines and normal cell (TIG-1-20) with **3** (MACB) and with *p*-(dihydroxyboryl)phenylalanine (BPA) is summarized in Table II. 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 60-mm-diameter dishes in a CO_2 incubator at 37 °C for 3 days. The cells were trypsinized and counted,

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Table III. The Comparison of Boron Incorporation into Tumor and Normal Cells Preincubated with 3 and BPA[a]

	B-16 (tumor cell)			TIG-1-20 (normal cell)			
compounds	2 h	24 h	increment[b]	2 h	24 h	increment[b]	accumulation ratio[c]
3	0.25 ± 0.03	0.26 ± 0.03	1.04	0.14 ± 0.02	0.11 ± 0.02	0.79	1.32
BPA	0.22 ± 0.03	0.28 ± 0.02	1.27	0.17 ± 0.01	0.26 ± 0.01	1.53	0.83

[a] Boron incorporated into the cells is shown in $\mu g/10^6$ cells. [b] The boron incorporation at 24 h is divided by that at 2 h. [c] The increment in B-16 is divided by that in TIG-1-20.

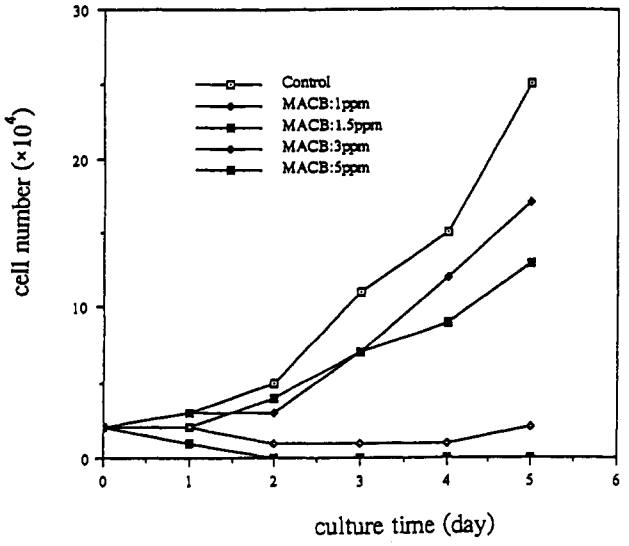

Figure 1. Kinetics of the growth inhibition of B-16 melanoma cells by 3 (MACB) in a 5-day Culture.

and results are presented as the concentration of agent that resulted in 50% of the cell number of untreated cultures. The kinetics of the growth inhibition of B-16 melanoma cells by 3 in a 5-day culture are shown in Figure 1. As evident, the cell growth was completely inhibited by adding 3 ppm (1.17×10^{-5} M) of 3.

Carboranylaziridine 3 exhibited relatively high growth inhibition toward B-16 melanoma and Hep G 2 (liver cancer cell), with IC_{50} values of the order of 10^{-6} M. Very interestingly, these values were significantly lower than those of TIG-1-20 (human fetal lung normal cell) (ca. the order of 10^{-5} M), indicating that MACB possesses selective cytotoxicity toward certain cancer cells. Such selective cytotoxicity was not observed with the boron carriers reported previously. For example, *p*-(dihydroxyboryl)-phenylalanine (BPA) which has been clinically used to treat a patient for skin cancer[10] exhibited essentially same cytotoxicity toward B-16 and TIG-1-20 (Table II). Accordingly, there is a possibility that 3 may serve as an anticancer agent. The cytotoxicity of 3 toward 1-87 (lung cancer) and IMR 32 (brain tumor) were lower than that toward TIG-1-20. The stability of 3 in B-16 melanoma cells was examined. After incubation of 3, organic materials were extracted with ether. HPLC analysis revealed the absence of 3 in the organic phase.

Boron accumulation in B-16 and TIG-1-20 cells was measured by using the ICP (induced coupled plasma) method. The cells [$(4.5–5.0) \times 10^6$] were incubated for 1–24 h with Eagle-MEM medium containing 3 (1.5 ppm) or BPA·HCl (200 ppm). The concentrations of the carriers were adjusted to those of their IC_{50} values. At 1, 2, 3, 6, and 24 h, the cells were washed three times with PBS-(–) (Ca–Mg-free phosphate-buffered saline, 5 mL), and processed for boron measurement by ICP (Figure 2 and Table III). Regardless of 3 or BPA, the boron incorporation into B-16 cells at 24 h increased slightly in comparison with

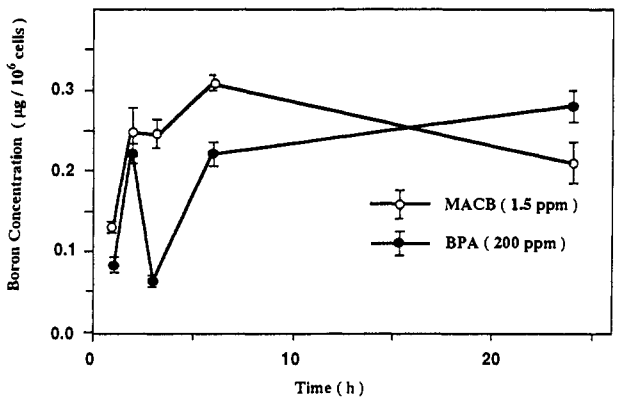

Figure 2. Boron incorporation into B-16 melanoma cell preincubated with 3 (MACB) and BPA.

that at 2 h. Very interestingly, the incorporation into TIG-1-20 after 24 h decreased by using 3, although it increased with BPA. As shown in the last column of Table III, the accumulation ratio of the tumor to normal cells was 1.32 with 3 and 0.83 with BPA. It is clear that the selective uptake of boron by the tumor cells has been realized with 3.

Experimental Section

Materials. Melting points were determined on a Yamato MP-21 capillary melting point apparatus and uncorrected. ^1H and ^{13}C NMR spectra were recorded on a JEOL GSX-270 spectrometer. The chemical shifts are expressed in parts per million downfield from the tetramethylsilane internal standard. IR spectra were recorded on a Hitachi M-52 spectrophotometer. High-resolution mass spectra were recorded on a JEOL JMS-HX110. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl under nitrogen immediately prior to use. Butyllithium in hexane solution was purchased and titrated prior to use. Most commercially supplied chemicals were distilled and stored over molecular sieves.

The Source of Cells. B-16 melanoma cells were obtained from Professor Mishima's laboratory, Medical School of Kobe University. Other cells were obtained from Cancer Cell Repository, Research Institute for Tuberculosis and Cancer, Tohoku University.

Synthesis of 1-Carboranyl-3-(2-methylaziridino)-2-propanol (3). To a THF solution (3 mL) of 2-methylaziridine (0.07 mL, 10 mmol) was added nBuLi in hexane (0.8 mL, 1.6 M, 1.2 mmol) at –78 °C under Ar. The mixture was stirred for 30 min. Copper cyanide (53.7 mg, 0.6 mmol) was added, and the mixture was allowed to warm to –38 °C. At this temperature, CuCN dissolved and the color of the solution turned clear dark yellow. The mixture was again cooled to –78 °C. Epoxy-1-allylcarborane[9] (50 mg, 0.25 mmol) in THF (2 mL) was added, and the mixture was allowed to warm to room temperature over 1 h. The reaction was quenched with a 1:1 mixture of aqueous saturated NH_4Cl and 28% NH_4OH solution (5 mL). The usual workup and purification by silica gel column chromatography gave 3 in 50% yield: mp 103 °C; IR ($CHCl_3$) 3600–3150, 3025 (OH), 2600 (B-H), 1760, 1660, 1400, 1300, 1200, 1110, 1090, 1050 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.30 (bs, 1 H, $CB_{10}H_{10}CH$), 3.88 (m, 1 H, –CH(OH)–), 1.46 (m, 1 H, $NCHCH_3$), the following signals of two diastereomers of 3 appeared at different chemical shift (major isomer) 2.34 (m, 3 H, $CH_2CH(OH)CH_2N$), 2.02 (dd, 1 H, J = 11.5, 4.0 Hz, CH_2-CH(OH)), 1.58 (d, 1 H, J = 3.5 Hz, NCH_2CH), 1.37 (d, 1 H, J =

6.5 Hz, NCH_2CH), 1.18 (d, 3 H, J = 5.0 Hz, CH_3); (minor isomer) 2.34 (m, 3 H, $CH_2CH(OH)CH_2N$), 2.01 (d, 1 H, J = 11.5, 4.0 Hz, $CH_2CH(OH)$), 1.51 (d, 1 H, J = 4.0 Hz, NCH_2CH) 1.29 (d, 1 H, J = 6.5 Hz, NCH_2CH), 1.15 (d, 3 H, J = 4.0 Hz, CH_3), ^{13}C NMR ($CDCl_3$) δ 65.73, 60.08, 42.52, 34.86, 34.71; (major isomer) 69.61, 35.29, 18.53; (minor isomer) 69.13, 35.23, 18.30; HRMS (EI) calcd for $C_8H_{23}ONB_{10}$ m/z 259.2710, found m/z 259.2706. Anal. ($C_8H_{23}ONB_{10}$) C, H, N, B.

Determination of IC_{50}. The boron compound **3** (10 mg) was dissolved in 1 mL of dimethyl sulfoxide (DMSO), and the resulting solution was diluted with Eagle-MEM medium (10% FCS). In Falcon 3002 culture dish (60-mm diameter), the cells (1×10^5 cells/dish) were cultured with the medium containing **3** at various concentrations (1, 2.5, 5, 10, 25, 50, and 100 ppm) and incubated for 3 days at 37 °C in CO_2 incubator. It is known that DMSO is nontoxic at the 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 cell count. The cell number of the control experiment was assigned to be 100%, and the average values of the cell number at each concentration were indicated by percentage. Three replications of each experiment were carried out. The concentration of 3 was plotted as the abscissa and the cell number (%) as the ordinate. The IC_{50} values were obtained from these curves. In the case of BPA, BPA·HCl salt was dissolved in the medium without using DMSO.

Kinetics of the Growth Inhibition. **3** exhibited the highest killing effect toward B-16 melanoma cells among the cells examined in Table I. Accordingly, the kinetics of the growth inhibition were investigated (Figure 1). The experiment was carried out in a similar manner as shown above, except for the culture time (5 days). As evident, the cell growth was completely inhibited by adding 3 ppm (1.17×10^{-5} M) of **3**.

Boron Incorporation into B-16 and TIG-1-20 cells. B-16 melanoma cells were cultured in Falcon 3025 dishes (150-mm diameter). When the cells were grown to fill up the dish, the cell number was counted (5.0×10^6 cells/dish). One dish was for control experiment. **3** (1.5 ppm) and BPA·HCl (200 ppm) were added to dishes. The cells were incubated for 1–24 h at 37 °C in 20 mL of the medium (Eagle-MEM, 10% FCS). The cells were washed 3 times with Ca–Mg-free phosphate-buffered saline [BPS-(−)], 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 (Milipore, 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. The results are shown in Figure 2. In the case of TIG-1-20 cells, a similar procedure was used. Two replications of each experiment were carried out and the standard deviations are shown in Table III and Figure 2.

Acknowledgment. We thank Mr. Takeyoshi Kondo for the ICP-AES spectra.

Supplementary Material Available: ^1H and ^{13}C NMR spectra for **3** (2 pages). Ordering information is given on any current masthead page.

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