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SYNTHESIS AND BIOLOGICAL PROPERTIES OF CARBORANYLAZIRIDINES BEARING CASCADE POLYOLS

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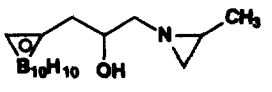
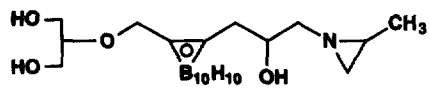
Abstract 1-(Carboranyl)-3-(2-methylaziridino)-2-propanols bearing cascade polyols, $MACB(OH)_2$ and $MACB(OH)_4$, were prepared in order to increase water solubility in comparison with MACB itself. By attaching cascade polyols as a water solubilizing element, cytotoxicity decreased and boron uptake by cancer cells increased.

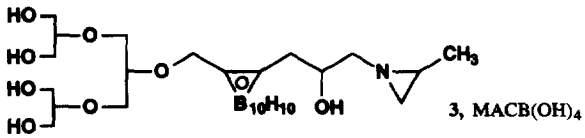
The application of the cytotoxic ^{10}B neutron-capture reaction [$^{10}B(n, \alpha)^7Li$] to the treatment of human tumors has received much attention in recent years. The interaction of boron-10 isotope and thermal neutron produces an α-particle and recoils a lithium-7 ion bearing approximately 2.4 MeV. The heavy, charged particles, ^4He and ^7Li, have ranges in tissue of only 9 and 5 μm, respectively. Thus ionizing radiation is deposited preferentially in and around the tumor. The destructive effect is, therefore, highly localized to boron loaded tissue. Boron neutron capture therapy (BNCT) is a binary therapy in which a substance labeled with ^{10}B preferentially accumulates in a tumor before the tumor area is irradiated by slow neutrons.[1-4] A key requirement of BNCT is the selective delivery of an adequate concentration of boron-10 to tumors (15-30 μg ^{10}B/g tumor).[5] Boronated analogues of compounds that are known to localize in various tumors have been the focus of compounds developed in this area.[6] We have reported recently that 1-carboranyl-3-(2-methylaziridino)-2-propanol (MACB) **1** exhibited relatively high growth inhibition toward some cancer cells and was incorporated selectively into B16 melanoma cells.[7] As was indicated in the case of α-(1-aziridinylmethyl)-2-nitro-1H-imidazole-1-ethanol (RSU 1069),[8] MACB may alkylate DNA at the phosphate and purine bases via the aziridine group, a process that leads to DNA strand breakage. However, poor solubility of MACB in aqueous or biological media has been an obstacle to the effective delivery of potentially useful MACB. We have developed



1, MACB

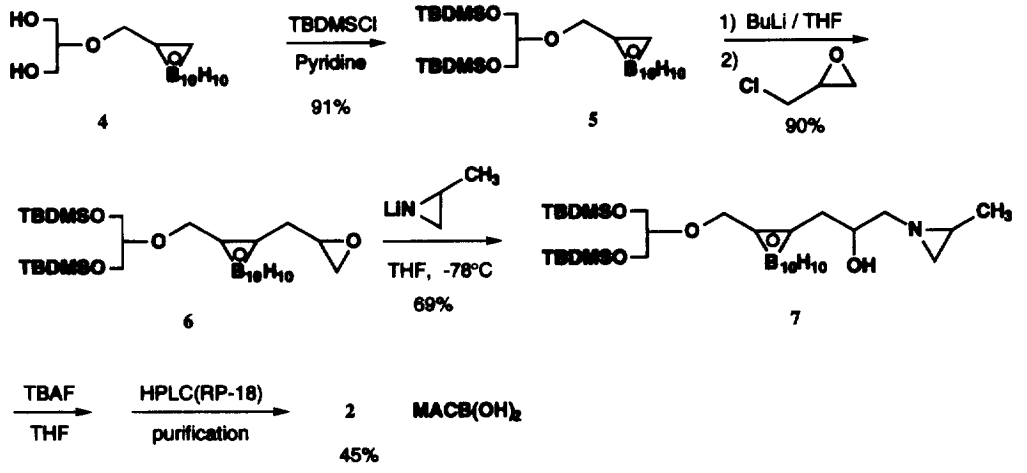


2, $MACB(OH)_2$

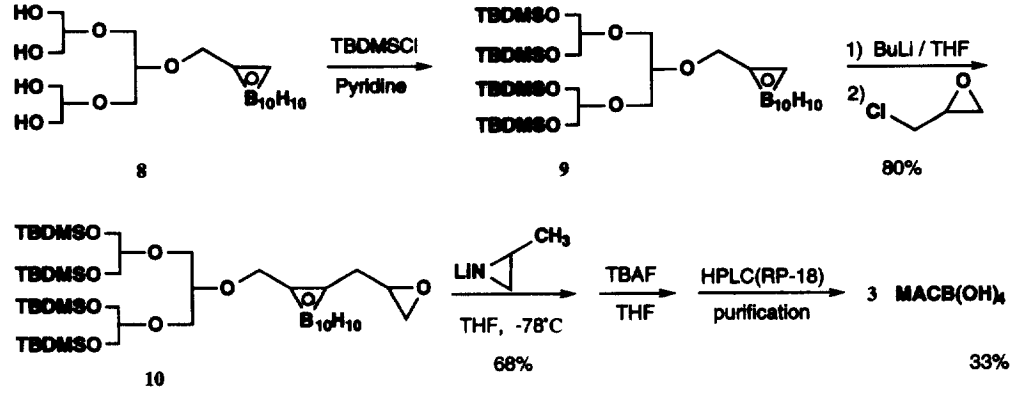


3, $MACB(OH)_4$

polyols of a cascade type as a water solubilizing element.[9] The number of hydroxyl groups can be managed at will, and thus a systematic change of water solubility is attained using the cascade polyols. It occurred to us that we may control the water solubility of MACB by attaching the cascade polyols, thereby elucidating relationship between the molecular structures, water solubilities, cytotoxicities, and cellular uptake. We now report that, by attaching cascade polyols to MACB (**2** and **3**), cytotoxicity to cancer (B16) and normal (TIG-1-20) cells decreases and boron uptake by B16 cells increases.



Scheme 1. Synthesis of $MACB(OH)_2$ (**2**)



Scheme 2. Synthesis of $MACB(OH)_4$ (**3**)

Synthetic Chemistry. The synthesis of 1-(2-glycerylmethyl)-2-(2-hydroxy-3-(*N*-2-methylaziridinyl) propylcarborane **2** is shown in Scheme 1. We initially used cascade polyols protected with benzyl ethers[9], but realized that debenzylation at the final stage using $H_2/Pd(OH)_2$-C or other methods was accompanied with decomposition of the compound presumably due to the presence of aziridine ring. Accordingly, two hydroxyl groups of **4**[9] were protected with t-butyldimethylsilyl ethers. Treatment of **5** with BuLi at -78°C followed by

addition of epichlorohydrin gave the epoxide **6** in 90% yield. The reaction of **6** with lithiated 1-methylaziridine at -78°C, which was prepared separately from BuLi and 1-methylaziridine at -78°C, afforded **7** as a mixture of diastereoisomers in 69% yield. Deprotection of TBDMS group was carried out using TBAF in THF, but purification of the product was difficult. The use of silica gel and alumina column chromatography, and the use of reversed phase HPLC using silica gel-based RP-18 column (neutral eluent) lead to decomposition of the desired product. Pure **2** was obtained in 45% yield by using alumina-based RP-18 column (basic eluent). The synthesis of 1-[(1,3-di-(2-glyceryl)-2-glyceryl)methyl]-2-[2-hydroxy-3-(*N*-2-methylaziridinyl) propyl]carborane **3** is shown in Scheme 2. The similar procedures as above were employed. TBDMS protection of four hydroxyl groups of **8**[9] gave **9** in 71% yield, which was converted to **10** in 80% yield. Treatment with the lithium amide followed by the usual deprotection and purification using HPLC gave **3** in 33% yield.

Biological Properties. (1) Water Solubility. A saturated solution of each boron compound was obtained, and the concentration of boron atom was measured by using ICP-AES.[7] The water solubilities of MACB, $MACB(OH)_2$, and $MACB(OH)_4$ are shown in Table 1.

Table 1. Concentration of Sat. Solution at 37°C

$MACB(OH)_n$		Concentration (M, mol dm^{-3})
MACB	1	$<< 1 \times 10^{-3}$
$MACB(OH)_2$	2	8.1×10^{-3}
$MACB(OH)_4$	3	$> 1 \times 10^{-2}$

Table 2. Growth Inhibition of B16 and TIG-1-20 Cell Lines

$MACB(OH)_n$		IC_{50} (M, mol dm^{-3}) B16	TIG-1-20
MACB	1	5.4×10^{-6}	1.8×10^{-5}
$MACB(OH)_2$	2	2.9×10^{-5}	4.1×10^{-5}
$MACB(OH)_4$	3	5.0×10^{-5}	6.8×10^{-5}

(2) Cytotoxicity. B16 melanoma cells were chosen as a model of cancer cells and TIG-1-20 fibrobrast cells (human fetal lung normal cells) as a model of normal cells.[10] The IC_{50} values of $MACB(OH)_n$ were obtained by the standard method,[7] and the results are summarized in Table 2. Obviously, $MACB(OH)_4$ and $MACB(OH)_2$ are less toxic than MACB itself. Very interestingly, the difference of IC_{50} values between **1-3** is larger in the case of B16 than that in TIG-1-20; the difference between **1** and **3** is ca. 9.3 times in B16, although it is ca. 3.8 times in TIG-1-20. As expected, the cytotoxicity decreased along with increase of water solubility.[11]

(3) Cellular Uptake. Incorporation of $MACB(OH)_n$ in the cells was measured by the standard procedure using ICP-AES.[7,11] The cells[$(4.5\text{-}5.0)\times10^6$] were incubated with Eagle-MEM medium containing $MACB(OH)_n$ with the concentration of IC_{50} values. At 3, 12, and 24h, the cells were washed three times with PBS-(-) (Ca-and Mg-free phpsphate-buffered saline, 5mL) and processed for boron measurement by ICP-AES. The results are summarized in Fig 1.

Since the cytotoxicities of $MACB(OH)_2$ and $MACB(OH)_4$ decreased in comparison with MACB, larger amounts of the boron compounds could be incubated into the cells and therefore the boron incorporation of the cascade polyol attached MACB increased significantly. At 12h, boron uptake of $MACB(OH)_4$ by B16 was almost same as that by TIG-1-20. However, at 24h the former decreased to ca. 1.25 μg B/10^6 cells although the latter increased to ca. 3.5 μg B/10^6 cells. The reason for this difference is not clear. Concerning the biological properties of $MACB(OH)_n$, it is concluded that increase of water solubility decreases their cytotoxicities, hereby boron incorporation into cell lines increases due to increase of possible dose of the boron compound.[12]

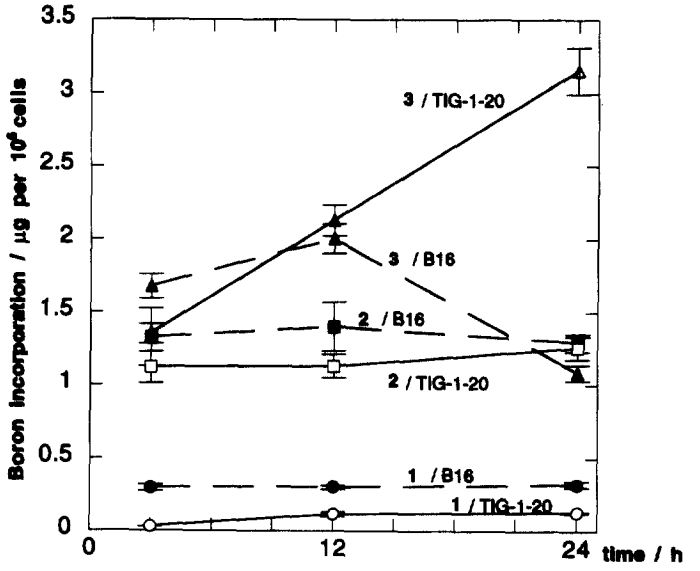


Fig. 1. Boron incorporation into B16 and TIG-1-20 cells preincubated with **1**-**3**.

MACB(OH)$_4$ / B16 MACB(OH)$_2$ / B16 MACB / B16
MACB(OH)$_4$ / TIG-1-20 MACB(OH)$_2$ / TIG-1-20 MACB / TIG-1-20

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(8) For example: Silver, A. R. J.; O'Neill, P. *Biochem. Pharmacol.* **1986**, *35*, 1107.

(9) Nemoto, H.; Wilson, J. G.; Nakamura, H.; Yamamoto, Y.; *J. Org. Chem.* **1992**, *57*, 435.

(10) In general, it is difficult for normal cells to bear up against *in vitro* experiments, although tumor cells are strong enough in such experiments. We previously used TIG-1-20 cells, for the purpose of comparison, as a model of normal cells: Yamamoto, Y.; Seko. T.; Nakamura, H.; Nemoto, H.; Hojo, H.; Mukai, N.; Hashimoto, Y. *J. Chem. Soc. Chem. Commun.* **1992**, 157, and see also ref 7.

(11) Nemoto, H.; Cai, J: Asao, N.; Iwamoto, S.; Yamamoto, Y.; *J. Med. Chem.* **1995**, *38*, 1673.

(12) A referee pointed out that boron uptake should be investigated at the same concentration of doses, instead of the concentration of each IC50 value. Scientifically, this is an important suggestion, but we are very much interested in how we can increase boron uptake practically.

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