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Synthesis of a New Amphiphilic *ortho*-Carborane as a Potential BNCT Agent: 4-[*N,N*-Formyl-(*ortho*-carboranylmethyl)-amino]benzoyl-L-glutamic Acid.

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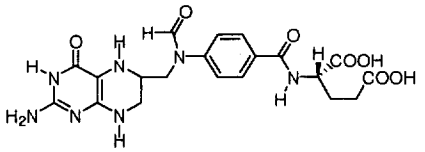
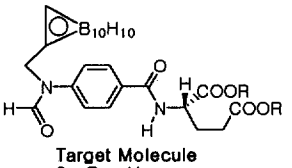
Abstract: A 1,2-dicarba-*closo*-dodecaboranyl derivative (**8**) and its disodium salt (**9**) were prepared from 4-aminobenzoic acid (**1**). The water solubility and the biological data (toxicity and cell-uptake) of the disodium salt (**9**) were identified to be enough for clinical trial.

Boron neutron capture therapy (BNCT) has received an attention as a more attractive binary therapy than other radio and chemotherapeutic methods for cancer therapy, since a practical method producing highly purified thermal neutron has been developed.[1,2] BNCT is based on the intense ionizing radiation, which is produced by the nuclear reaction of boron-10 with thermal neutrons. The requirement for this method is the localization of 10-30 ppm ^{10}B in cancer cells. To meet this requirement, the design and synthesis of boron-rich molecule (boron cluster) linked with proper biological carriers are one of the very important and challenging fields. The best candidate as a carrier seems to be natural



10-formyl-tetrahydrofolic acid
(10-formyl-THF)



Target Molecule
8 : R = H
9 : R = Na

compounds due to the low toxicity and the possibility of existence of cell-uptake mechanism

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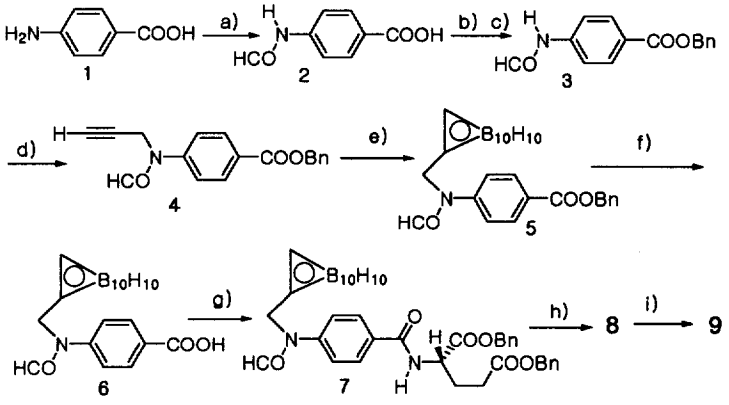
in the biological system.[3]

The folic acid is one of coenzymes and has its own cell-uptake mechanism.[4] The 4-aminobenzoylglutamate moiety of folic acid was identified as very important for the solubility in water and the cell-uptake mechanism of this coenzyme. The design of our target molecule (**8**) was based on the structure of this folic acid. The target molecule (**8**) has 4-formylaminobenzoyl-L-glutamic acid moiety like 10-formyl-THF, which is one of the active forms of tetrahydrofolic acid. The hydrophilic moiety of amphiphilic target molecule (**8**) might be very important to change the solubility of hydrophobic and cell-uptake (or nesting in phospholipid cell membrane due to amphiphilic character of this target molecule). This amphiphilic character might be essential as an ideal carrier.



a) Ac_2O in HCOOH at rt b) Cs_2CO_3 in MeOH c) BnBr in DMF at rt d) propargyl bromide, $(nBu)_4NBr$, K_2CO_3 in acetone e) $B_{10}H_{14}$ in acetonitrile and toluene f) 40psi H_2, 10% Pd/C in EtOH g) dibenzyl glutamate, EEDQ in $CHCl_3$ at rt h) 40psi H_2, 10% Pd/C in EtOH i) $NaHCO_3$

Scheme

The target molecule (**8**) has been synthesized as follows.(Scheme) The 4-formylamino-benzoic acid (**2**) was synthesized by the reaction of 4-aminobenzoic acid in a solution of acetic anhydride and formic acid at rt in 92% yield.[5] Compound (**2**) was esterified by treating the salt with benzyl bromide in DMF at rt after the preparation of cesium salt by stirring **2** and cesium carbonate in methanol at rt (yield: 88%).[6] The reaction of compound (**3**) with propargyl bromide in acetone in the presence of tetra-n-butylammonium bromide at rt for 48 h gave compound (**4**) in 87 % yield.[7] The reaction of ester (**4**) with $B_{10}H_{14}$ in refluxing toluene and acetonitrile gave an *ortho*-carborane (**5**) in 77% yield after column chromatography on silica gel.[8] This compound (**5**) was debenzylated under 45 psi hydrogen

pressure using 10% Pd/C in ethanol to give a benzoic acid (**6**) in 94% yield[9] and coupled with dibenzyl L-glutamate in chloroform using EEDQ (2-ethoxy-1-ethoxy-carbonyl-1,2-dihydroxyquinoline) to give an amide (**7**) in 76% yield. Other coupling reagents such as DCC and ethyl chloroformate did not work for this reaction. Hydrogenolysis of ester (**7**) using 10% Pd/C in ethanol under 45 psi hydrogen pressure for 26 h gave the benzoic acid (**8**)[10] in 97% yield. Hydrogenolysis of **7** was slower than that of **5**. Excess amount of Pd/C was used for this hydrogenolysis.

The compound (**8**) was not soluble in organic solvents, such as chloroform and ethyl acetate, and water. To increase the water solubility for the bilogical test, we made the disodium salt (**9**) by the reaction of compound (**8**) with sodium bicarbonate. According to the *in vitro* test, it is clear that disodium salt (**9**) is accumulated into B-16 melanoma cells with significaltly high level although it is highly water soluble and its cytotoxicity is significantly low as shown in Table 1.

Table 1. Cytotoxicity toward B-16 Melanoma and Boron Incorporation

compound	cytotoxicity IC_{50} (M)	administration (M),	(μgB/mL)	boron incorporation (μgB/10^6cells)[a]
9	6.9×10^{-4}	6.9×10^{-4}	74.5[b]	0.37 ± 0.095
		1.0×10^{-4}	10.8	0.074 ± 0.013
BPA	8.6×10^{-3}	1.0×10^{-3}	10.8	0.31 ± 0.031

[a]Boron incorporated into the cells after 24 h incubation is shown in μgB / 10^6 cells.
Values are mean ± standard deviation of an average of three experiments.
[b]Concentration was based on the IC_{50} value.

The synthesis of other analogs (homologs) and the biological evaluation of this new *ortho*-carborane cluster compound will be studied together with physical properties concerning the probability of lethal reaction in cancer cells.

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10. **4-*N,N*-formyl-(o-dicarbaboranylmethyl)aminobenzoyl-L-glutamic acid (8):** mp: 123-124 ℃; IR (KBr pellet): 3364, 3063-2929, 2596, 1722, 1678, 1607 cm^{-1}; ^1H NMR (CD_3OD, 200 MHz): 8.54 (s, 1H, CHO) 7.99 (d, aromatic 2H, J = 8.4 Hz) 7.47 (d, aromatic 2H, J = 8.4 Hz), 4.72 (s, 2H, CH_2), 4.58 (s, br, 1H, CH in carborane cage), 4.37-4.42 (m, 1H, CH in glutamate), 2.32-2.47 (m, 4H, 2 x CH_2 in glutamate). HRMS (FAB) Calcd for $C_{16}H_{26}N_2O_6B_{10}$ $(M + H)^+$ 453.2799 Found: 453.2773