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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. © 1999 Elsevier Science Ltd. All rights reserved.

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.¹² 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 ¹⁰B 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



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

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) Ac₂O in HCOOH at rt b) Cs₂CO₃ in MeOH c) BnBr in DMF at rt d) propargyl bromide, (nBu)₄NBr, K₂CO₃ in acetone e) B₁₀H₁₄ in acetonitrile and toluene f) 40psi H₂, 10% Pd/C in EtOH g) dibenzyl glutamate, EEDQ in CHCl₃ at rt h) 40psi H₂, 10% Pd/C in EtOH i) NaHCO₃

Scheme

The target molecule (8) has been synthesized as follows.(Scheme) The 4-formylaminobenzoic 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 B₁₀H₁₄ 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% yield⁹ and coupled with dibenzyl L-glutamate in chloroform using EEDQ (2-ethoxy-1-ethoxy-carbonyl-1,2-di-hydroxyquinoline) 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 significally high level although it is highly water soluble and its cytotoxicity is significantly low as shown in Table 1.

compound	cytotoxicity	administration		boron incorporation
	IC ₅₀ (M)	(M), ($\mu \text{gB/mL})$	$(\mu gB/10^6 cells)^a$
9	6.9 x 10 ⁻⁴	6.9 x 10 ⁻⁴	74.5°	0.37 ± 0.095
		1.0×10^{-4}	10.8	0.074 ± 0.013
BPA	8.6 x 10 ⁻³	1.0×10^{-3}	10.8	0.31 ± 0.031

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

^aBoron incorporated into the cells after 24 h incubation is shown in μ gB / 10⁶ cells. Values are mean \pm standard deviation of an average of three experiments. ^bConcentration was based on the IC₅₀ 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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- 4-N,N-formyl-(o-dicarbaboranylmethyl)aminobenzoyl-L-glutamic acid (8): mp: 123-124 °C; IR (KBr pellet): 3364, 3063-2929, 2596, 1722, 1678, 1607 cm⁻¹; ¹H NMR (CD₃OD, 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, CH₂), 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 CH₂ in glutamate). HRMS (FAB) Calcd for C₁₆H₂₆N₂O₆B₁₀ (M + H)⁺ 453.2799 Found: 453.2773