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The development of carboranyl nucleic acid precursors for use in neutron capture therapy of tumors

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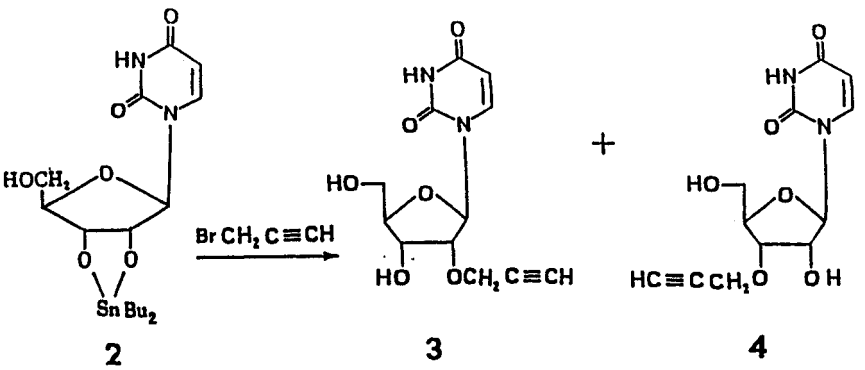
Abstract. The synthesis of a carboranyl nucleic acid precursor 2'-O-(o-carboran-1-yl methyl)uridine, 1, and a carboranyl nucleotide, 8, is described. Significantly greater incorporation and persistence of 1 in F98 glioma cells, compared with the clinically used $Na_2B_{12}H_{11}SH$ is observed.

The development of boron compounds for the treatment of cancer by boron neutron capture therapy (BNCT) is based upon the nuclear fission of nonradioactive boron-10 atoms by thermal neutrons, generating high linear energy transfer (LET) particles (1). These have a destructive range of approximately 10 μm. The biological effectivenes of BNCT is greater if boron compounds are localized intracellularly vis-a-vis on cell membrane or in extracellular spaces. It has been calculated that the effect will be at least twice as great if this capture reaction were to occur in the cell nucleus rather than in the cytoplasm (2). The closer proximity of the boron compound to the tumor cell nucleus, therefore, the greater its radiobiological effect. How can such localization be achieved has been an ongoing question? One of the early approaches was the attempted synthesis of boron heterocycles, which may be viewed as analogues of the purine and pyrimidine bases of the naturally-occurring nucleic acids (3-7). Many of these, however, were hydrolytically unstable and/or failed to mimic the bases as measured by cellular incorporation. One structure which more closely resembled these precursors was 5-dihydroxyboryluracil (8). This led to the synthesis of the first boron-containing pyrimidine nucleoside, 5-dihydroxyboryl-2'-deoxyuridine (9). Initial biological studies indicated that this compound was a competitive inhibitor in the phosphorylation of thymidine (10), was nontoxic to Vero (African Green Monkey) cells at a concentration of 1,600 μM and appeared to replace thymidine to the extent of 5-15% as determined by radiobiological studies with V79 Chinese hamster cells (11).

The nucleosides that had been initially prepared (9,12,13) contained a single boron atom which was inserted into the purine or pyrimidine base. Our objective was to incorporate a carboranyl moiety into the 2'-position on the carbohydrate portion of the nucleoside. The rationale was that such structures would possess: (1) a tenfold increase in boron content compared with the above boronic acid derivatives; (2) enhanced lipophilicity for cellular penetration due to the carborane moiety; (3) possible cellular entrapment and retention properties in proliferating tumor cells due to the action of kinases; and (4) a retained capacity when incorporated into an oligonucleotide to hybridize strongly with RNA and DNA sequences (14) since the base component would be naturally-occurring. As the first structure, we undertook to synthesize 2'-O-(o-carboran-1-ylmethyl) uridine, 1.

The method of synthesis involved the initial alkylation of 2',3'-O-(dibutylstannylene) uridine, 2, (15) with 3-bromopropyne to produce a mixture of the alkylated products, 2',(3')-O-(3-propynyl)uridine (3 and 4). The ratio of 3 to 4, as estimated by NMR, was 5:3 but attempts at chromatographic separation were unsuccessful. However, when the mixture was acetylated, the two isomers were readily separated chromatographically on silica gel and the desired one, 3',5'-di-O-acetyl-2'-O-(3-propynyl) uridine, 5, was obtained in 45% yield from the tin complex. Reaction of 5 with the bis(acetonitrile)decaborane complex furnished the corresponding carborane, 6, which was quantitatively deacetylated by catalytic amounts of sodium methoxide in methanol to yield the desired target compound 1 as shown by IR, ^1H NMR and FAB mass spectrometry (16). Degradation of the carborane cage to the corresponding *nido* structure, 7, was achieved by warming to 40-45° in methanolic piperidine; the product obtained was the piperidinium salt of 7. Work is currently underway to prepare the sodium salt of 7 through ion exchange chromatography of this piperidinium salt. Conversion of 1 to a 5'-nucleotide derivative, 8, was obtained by use of p-nitrophenylphosphorodichloridate in pyridine/acetonitrile (17). This and certain other phosphorylating agents have the capacity for generating 5'-phospates without phosphorylating unprotected secondary alcoholic functions in the carbohydrate portion of the nucleoside (18). Compound 8 is the first example of a carboranylnucleotide and removal of the p-nitrophenyl masking group to produce the free nucleotide is planned.

At present, little is known regarding the mechanisms for achieving selective tumor cell incorporation by various boron compounds. And, since there is no information as to how these new potential nucleic acid precursors will be handled biologically in vivo, it was of interest to determine the uptake and retention of these compounds in vitro using various tumor cell lines. After demonstrating uptake and persistence under such optimal conditions, it would be reasonable to carry out in vivo distribution studies of these nucleosides and nucleotides in tumor-bearing animals.



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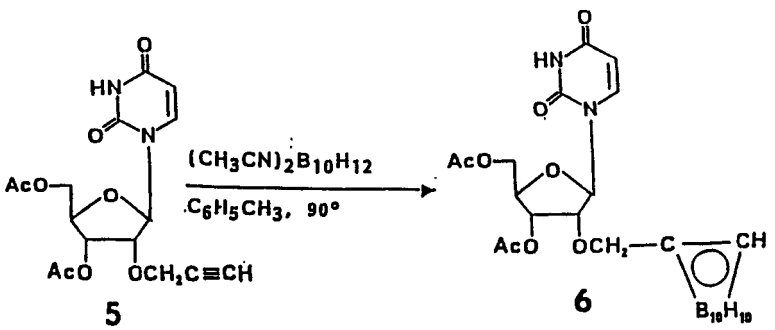

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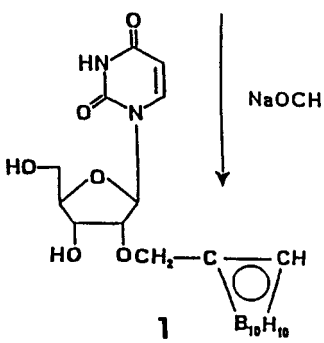

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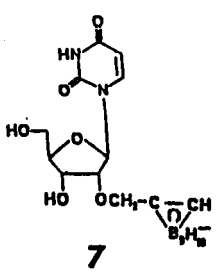

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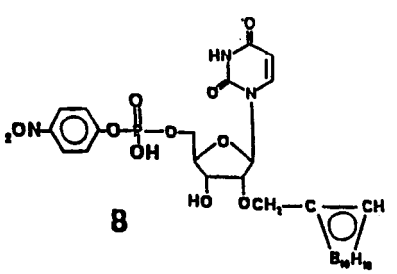

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The first compound which has been evaluated in F98 glioma cells is 1. In order to demonstrate whether there was cellular uptake and retention, the following procedure was used. Semiconfluent F98 glioma cells were incubated with the compound for 16 hours and after two washings with serum-free media, the cells were trypsinized, washed twice again and aliquots were analyzed for boron by Direct Current Plasma (DCP) Atomic Emission Spectroscopy (19). The results, as shown in Table 1, demonstrate significantly greater incorporation of the carboranyluridine, 1, compared with the clinically-used $Na_2B_{12}H_{11}SH$. This, in part, may be due to the greater lipophilicity of 1 in comparison with the mercaptopolyhedral borane anion.

TABLE 1.

Compound	Boron Conc. in Incubating Media µg/ml	F98 Glioma Cells Boron Conc. µ/g 16 hrs incubation	Persistence Studies 12 hrs µB/g	24 hrs µB/g	48 hrs µB/g
$Na_2B_{12}H_{11}SH$	14.6	4.3	3.1+	.4+	*
"	58.0	7.2	2.8+	.8+	*
"	116.0	26.7	3.4+	1.8+	*
Carboranyl Uridine	13.5	88.9	23.9	19.3	10.5
" "	40.5	98.2	24.1	22.0	12.7

*Not measured
+These approximate the blank value for cells--the average DCP reading being 1.3 (range .4 - 3.0)

Of great importance is whether the compound once taken up into the malignant cell, is retained, regardless of the biochemical mechanism involved, or whether it is washed out into the incubating media? In order to determine whether such cellular persistence occurred, cells which had demonstrated compound uptake were resuspended in boron-free media and incubated for 12, 24, and 48 hrs. Aliquots of cells were then analyzed for boron content and these are also presented in Table 1. It is apparent from these preliminary results that 1 persists for appreciable times once it is incorporated into F98 glioma cells and this is in marked contrast with $Na_2B_{12}H_{11}SH$, which is rapidly removed, attaining background levels within 6-12 hrs. It remains to be determined the precise biochemical mechanism by which such retention occurs and *in vivo* studies now are planned in order to assess the potential utility of 1 for BNCT. Also *in vitro* studies with 8 and the free nucleotide of 9 will be carried out prior to any *in vivo* examination in tumor-bearing animals.

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