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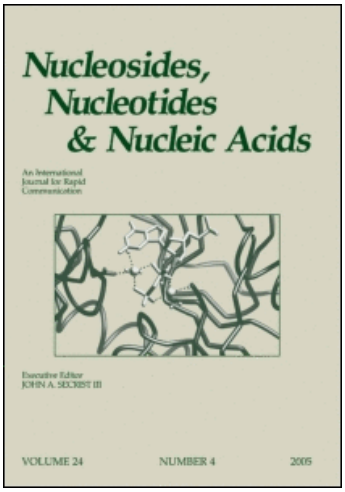

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Boron Containing Pyrimidines, Nucleosides, and Oligonucleotides for Neutron Capture Therapy

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NUCLEOSIDES & NUCLEOTIDES, 13(1-3), 849-880 (1994)

REVIEW

BORON CONTAINING PYRIMIDINES, NUCLEOSIDES, AND OLIGONUCLEOTIDES FOR NEUTRON CAPTURE THERAPY†

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Abstract: The synthesis and encouraging biological findings with boron-containing nucleosides, such as 5-dihydroxyboryl-2'-deoxyuridine, which could be used for boron neutron capture therapy (BNCT) for the treatment of various malignancies, has provided momentum to synthesize several boron containing nucleosides and oligomers. BNCT is based on the property of the non-radioactive boron-10 isotope to capture low energy neutrons, thereby producing a localized cell-destroying nuclear reaction. Thus, irradiation of tumor cells with neutrons, following incorporation of the boronated nucleoside, would result in the destruction of tumor tissue only. Intracellular phosphorylation by nucleoside kinases, and/or incorporation into the cancer cell DNA as a false nucleotide precursor, followed by irradiation by neutrons, would lead primarily to tumor cell death. The synthetic and biological approaches for boronated pyrimidines, nucleosides, and oligonucleotides for BNCT are reviewed.

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Introduction.

The pharmacological uses of boron compounds have been known for several decades.[1] Recently, there has been an increased interest in the preparation of boron compounds for their potential medicinal and biochemical applications.[2,3] Organoboron compounds are being investigated with respect to their possible use in cancer therapy by boron neutron capture therapy (BNCT).[4-6] This combined modality is based on the

†Dedicated to the memory of Drs. Ronald K. Robins and Ralph Fairchild, pioneers in nucleoside chemistry and neutron capture therapy, respectively.

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nuclear fission of nonradioactive boron-10 atoms by thermal neutrons, generating high linear energy transfer (LET) particles.[4,6]

$$^{10}B + {}^{1}n \longrightarrow [^{11}B] \longrightarrow {}^{4}He + {}^{7}Li + \gamma + 2.79\ MeV$$

An important advantage of neutron capture therapy (NCT) is that each component can be independently manipulated and combined when the timing is optimal for tumor destruction. The heavy particles from this nuclear fission reaction deposit their energy within 1 or 2 cell surface areas, thereby sparing nearby normal cells. Normal tissue constituents—such as hydrogen, nitrogen and carbon—have capture cross-section (σ) values that are more than three orders of magnitude less than boron-10. Other nuclides—such as ^{3}He, ^{113}Cd, and ^{235}U—with high σ values are excluded from potential use for BNCT, because they give rise to various types of radiation that cannot be confined and/or are radioactive. Natural boron consists of two non-radioactive isotopes; viz., boron-11 and boron-10 in a 4:1 ratio of which only the latter has a high cross-section for neutrons.[6] Thus, enrichment of boron-10 containing compounds is attractive for BNCT.

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Various boron containing heterocycles which resemble the bases of DNA have been tested for anti-cancer activity, but most of these have been found to be either hydrolytically and biologically unstable or too toxic.[7,8,10] The potential of nucleoside analogues for the chemotherapy of viruses and cancers is well recognized.[7] The rationale for the synthesis of boronated pyrimidines, purines, and other nucleosides, which closely resembles one of the naturally occurring nucleosides, could lead to intracellular entrapment. In order to be considered useful for BNCT, the compound must have the following properties: (1) The new agents must exhibit very low toxicity *in vitro* and *in vivo*. If this condition is not met, adverse effects that result from non-selective uptake of these agents should be minimized. (2) If the focus is to develop compounds to treat gliomas, the materials must be able to effectively permeate the blood-brain-barrier (BBB) even if some disruption of the BBB by the tumor occurs. Thus, modifications that optimize lipophilicity of nucleosides are beneficial. (3) In order to minimize the amount of time required for an effective dose of the drug to infuse into the tumor, high boron content nucleosides are desirable; thus, in addition to providing a high boron content, covalently linking polyboron containing carboranyl groups on nucleosides results in significant increased lipophilicity (R.F. Schinazi, unpublished). (4) In order to maximize the destructive power of these agents in tumor tissue during NCT, the boron-containing

nucleosides should ideally be transported into the nuclei of the tumor cells; *incorporation into nucleic acids is not essential.* This requires that the nucleosides must be substrates for both cellular kinases and, after triphosphate formation, DNA polymerases; alternatively, boron containing oligomers that target specific genes found in tumor cells could be used. In general, incorporation into nucleic acids, particularly mitochondrial or nuclear DNA, may result in increased cytotoxicity and mutagenicity; thus our more recent approaches have been to synthesize compounds that are not incorporated into DNA to a large extent and/or are DNA chain terminators. (5) In addition to the anabolic requirements stated above (*i.e.*, cell permeation, phosphorylation, and incorporation into the cellular DNA), it is also desirable that the agent be resistant to catabolism. In order to accomplish these goals, nucleosides that are known to be phosphorylated intracellularly at sites that tolerate moderately sized substituents (*i.e.*, the 5-position of pyrimidine nucleosides) should be modified. (6) Finally, to be successful for NCT, it is generally accepted that 5-30 ppm of boron-10 must be delivered to the tumor cells.[4,9] The impact would be greatest if the compound is delivered intracellularly, preferably to the nucleus of the cell. The synthetic and biological studies performed by our group and other workers in the area of boronated pyrimidines, nucleosides, and oligonucleotides, are reviewed.

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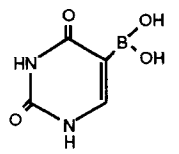
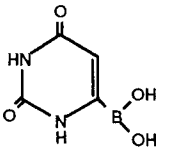
Boron Containing Pyrimidines.

(a) Boronic Acids and Esters: Liao et al.[11] prepared 5-(dihydroxyboryl)uracil (**1**) directly by a halogen-metal exchange reaction on 5-bromo-2,4-bis(benzyloxy)-pyrimidine followed by boronation and debenzylation. Schinazi and Prusoff[10,12] modified the lithiation and boronation reaction conditions to obtain the 5-(dihydroxyboryl)-2,4-bis(benzyloxy)pyrimidine in high yield. The reaction was conducted in anhydrous tetrahydrofuran (THF) at -85°C to -95°C under argon atmosphere. Deprotection of the benzyl groups by direct catalytic hydrogenation yielded 5-(dihydroxyboryl)uracil. This compound was further characterized by formation of the hydrolytically stable cyclic iminodiethanol derivative. 6-(Dihydroxyboryl)uracil (**2**), which is isostructural and isoelectronic with orotic acid, was prepared by utilizing the lithiation, boronation, and debenzylation reaction.[10,12]

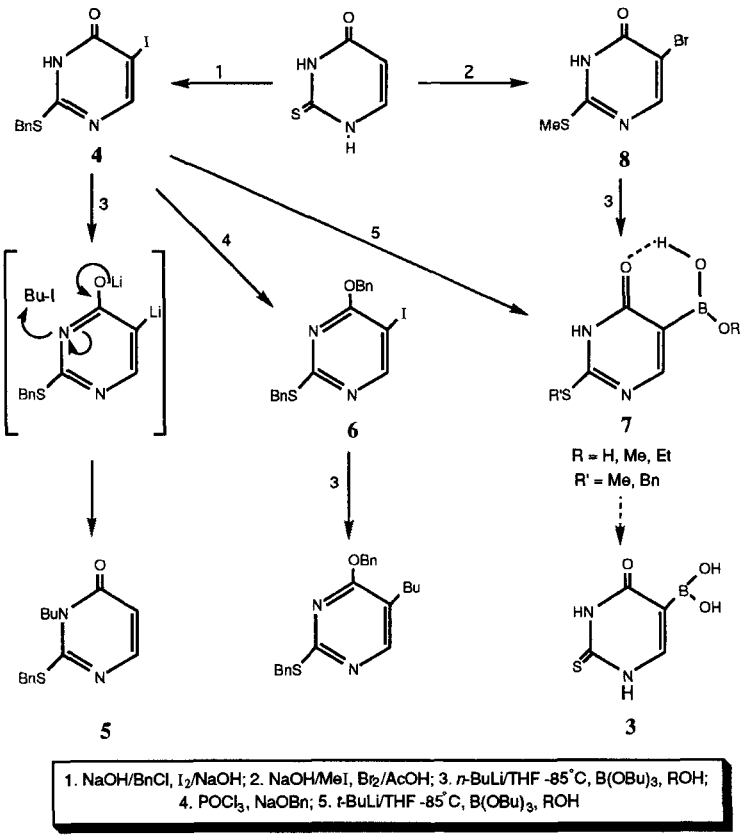


5-(Dihydroxyboryl)uracil (**1**)



6-(Dihydroxyboryl)uracil (**2**)

In recent years, considerable attention has been focused on the synthesis of 5-(dihydroxyboryl)-2-thiouracil, because 5-iodo-2-thiouracil may selectively incorporate into murine melanomas during melanin synthesis.[13] In a study of biosynthesis of melanin, Whittaker[14] showed that 2-thiouracil was incorporated into growing melanin in melanomas as a false precursor. It was suggested that 2-thiouracil condenses with quinone intermediates in the melanin biosynthetic pathway which then incorporates into the melanin polymer. Any potential false precursor of melanin in the thiouracil series should have a free sulfur group.[14]



Based on these considerations, Schinazi et al.[15,16] conducted extensive studies to synthesize boron containing 2-thiouracil analogue, in particular 5-(dihydroxyboryl)-2-thiouracil (**3**). The synthesis of 2-thiouracil that contains a dihydroxyboryl group in the 5-

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position can be achieved by direct lithiation on 5-halo substituted 2-thiopyrimidines followed by boronation with trialkyl borate. Because of the inability to obtain the trimethylsilyl (TMS) protected 5-iodo-2-thiouracil, the 5-iodo-2-(benzythio)uracil (**4**) was used as precursor for the synthesis of boronated 2-thiouracil analogues. This compound on lithiation with *n*-butyllithium in THF at -85°C followed by boronation with tri-*n*-butyl borate led to the unexpected product N^3-*n*-butyl-(2-thio-benzyl)uracil (**5**). Furthermore, *O,S*-bis(benzyl)-5-iodouracil (**6**) prepared *via* the 4-chloro analogue on lithium-halogen exchange and boronation resulted in the formation of 5-*n*-butyl pyrimidine analogue (see scheme below). However, lithiation of 5-iodo-2-(benzylthio)uracil (**4**) with *tert*-butyllithium in THF at -85°C followed by boronation with tri-*n*-butyl borate yielded the desired 5-borapyrimidine analogues **7**. This type of 5-boronated 2-thiopyrimidine derivative can also be obtained by the lithiation of 5-bromo-(2-thiomethyl)uracil (**8**) with *n*-butyllithium and boronation. In both cases, the free boronic acid was obtained by hydrolysis, and crystallization of the product either from methanol or ethanol yielded the corresponding monoalkyl boronic ester. The structure of the monoester was confirmed by NMR and X-ray crystallography (R. Schinazi and P. Van Roey, unpublished data). The formation of a 6-membered ring is probably the thermodynamically preferred conformation. Hydrogen bonding of hydroxyl group of the boronic acid with the 4-lactam functional group of 2-thiouracil derivative confers this stability. However, attempts to deprotect either 2-thiobenzyl or 2-thiomethyl boronated pyrimidines **7** with a variety of dealkylating reagents failed to yield the target molecule 5-(dihydroxyboryl)-2-thiouracil (**3**).[15,16]

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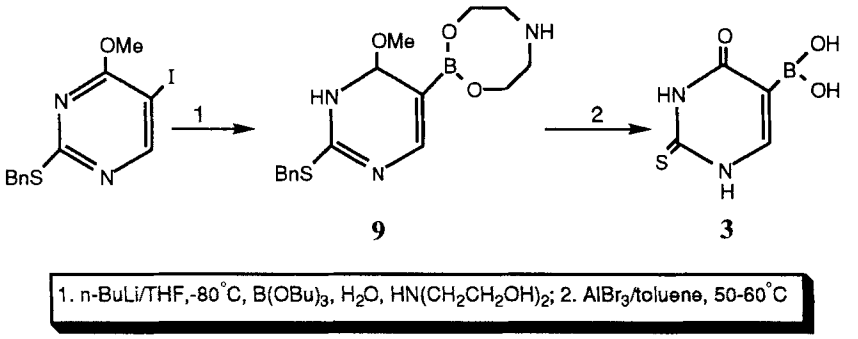
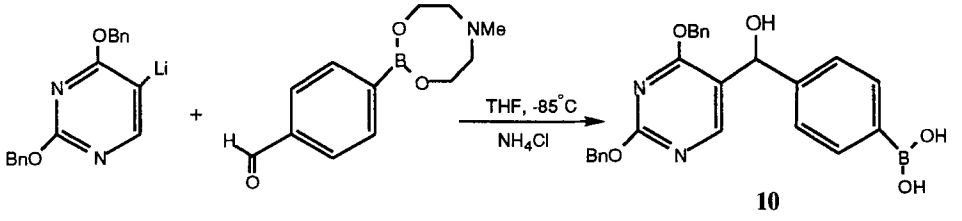
Tjarks and Gabel[17] attempted the reaction of *O,S*-bis(trimethylsilyl)-5-iodouracil with *n*-butyllithium and tri-*n*-butyl borate in THF at -80°C to -100°C followed by hydrolysis. The isolated product was characterized as 2-(*n*-butylthio)-5-trimethylsilyluracil presumably formed by the rearrangement of 5-lithiated pyrimidine intermediate. When *S*-benzyl and *O*-alkyl groups were used as protecting groups during the lithiation and boronation reaction, the desired 2-(benzylthio)-5-(dihydroxyboryl)-4-methoxypyrimidine was obtained. This compound was further converted to the stable cyclic iminodiethanol derivative **9** to facilitate isolation as well as provide greater solubility. Deprotection of all groups simultaneously with $AlBr_3$ in toluene at 50 to 60°C yielded 5-(dihydroxyboryl)-2-thiouracil (**3**).[17,18] 5-(Dihydroxyboryl)-2,4-dithiouracil, 5-(dihydroxyboryl)-6-propyl-2-thiouracil and 5-(dihydroxyboryl)-2,4-dithio-6-propyluracil were claimed to have been obtained by a similar route.[17]



Finally, Yamamoto et al.[19] reported the synthesis of boronated pyrimidines **10** by 1,2-addition of uracil lithiates with several protected 4-formylphenylboronic acids in anhydrous THF.



(b) o-Carborane Derivatives: Wilson[20] attempted to synthesize 5-o-carboranyl-2-thiouracil by the direct alkylation of *O,S*-bis(trimethylsilyl)-2-thiouracil with propargyl bromide followed by reaction of the resulting alkyne with decaborane. However, the alkylation of the TMS derivative with propargyl bromide in acetonitrile leads to the formation of 2-thio-*n*-propargyluracil. Nevertheless, 5-(Δ-4-o-carboranylpentynyl)-6-methyl-2-thiouracil (**13**) was prepared successfully in three steps. First, the 5-Δ-4-pentynyl-6-methyl-2-thiouracil (**12**) was made by the condensation of 2-Δ-4-pentynyl acetoacetic ester (**11**) with thiourea in the presence of one equivalent of sodium ethoxide in absolute ethanol at refluxing temperature. The triple bond in this 2-thiouracil analogue was sufficiently remote from the keto function to exclude the possibility of cyclization. The 2-thiouracil acetylenic derivative was then converted to the bis(trimethylsilyl) derivative which, on reaction with decaborane (as bisacetonitrile adduct) in refluxing benzene, yielded the o-carborane 2-thiouracil analogue.[20]

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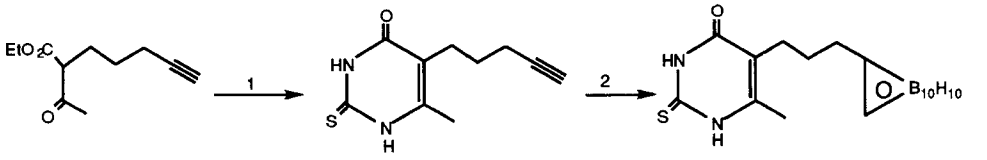
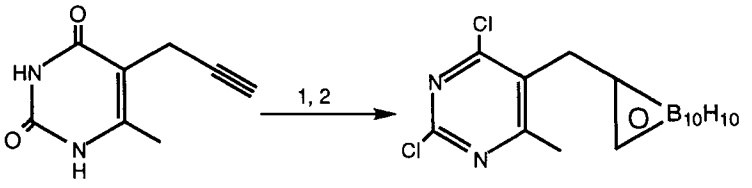


11 12 13

1. $SC(NH_2)_2$, NaOEt/EtOH; 2. HMDS, $B_{10}H_{10}(CH_3CN)_2$/Benzene, Reflux

Reynolds et al.[21] reported the synthesis of 2,4-dichloro-5-(1-o-carboranylmethyl)-6-methylpyrimidine (**15**), a potential synthon for the preparation of a variety of 2,4-substituted 5-(1-o-carboranylmethyl)-6-methylpyrimidines. Reaction of 6-methyl-5-(2-propynyl)uracil (**14**) with $POCl_3$, followed by addition of decaborane (as a bisacetonitrile adduct) to the dichloro intermediate in refluxing toluene yielded the 2,4-dichloro-o-carborane pyrimidine derivative.[21] This compound is a useful precursor for nucleophilic substitution reactions, allowing a great diversity of o-carboranyl pyrimidines.



14 15

1. $POCl_3$/DMF; 2. $B_{10}H_{12}(CH_3CN)_2$/Toluene, Reflux

Goudgaon et al.[22] prepared 5-carboranyluracil (**19**) as a versatile intermediate for the synthesis of various substituted o-carboranyl nucleoside analogues starting from commercially available 5-iodouracil. Reaction of 5-iodouracil (**16**) with excess $POCl_3$ generate 2,4-dichloro-5-iodopyrimidine, which on treatment with NaOMe in MeOH yielded 2,4-dimethoxy-5-iodopyrimidine (**17**). The latter was coupled with trimethylsilyl acetylene in the presence of $(Ph_3P)_2PdCl_2$/CuI in triethylamine followed by deprotection of the acetylenic group with *n*-Bu_4NF to give 2,4-dimethoxy-5-ethynylpyrimidine (**18**). Reaction of the acetylenic pyrimidine derivative with decaborane (as the bispropionitrile adduct) in refluxing toluene, followed by demethylation using iodotrimethylsilane, yielded 5-carboranyluracil.[22] The utility of 5-carboranyluracil for the synthesis of a

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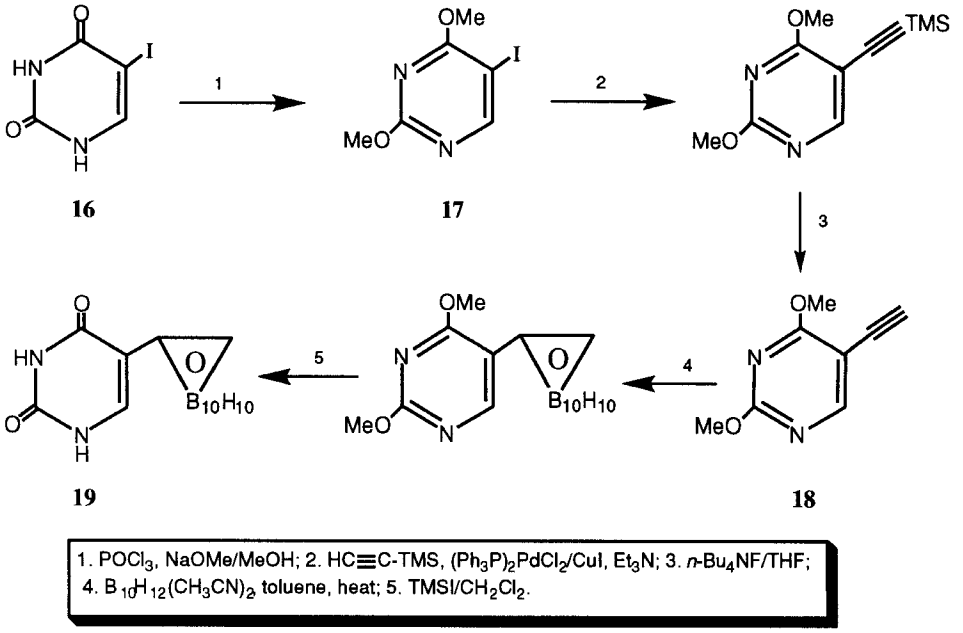
variety of 5-carboranyl pyrimidine nucleosides has been demonstrated by our group.[23]



1. $POCl_3$, NaOMe/MeOH; 2. HC≡C-TMS, $(Ph_3P)_2PdCl_2$/CuI, Et_3N; 3. *n*-Bu_4NF/THF; 4. $B_{10}H_{12}(CH_3CN)_2$ toluene, heat; 5. TMSI/CH_2Cl_2.

Boron Containing Nucleosides.

(a) Boronic Acids and Esters: The first boron-substituted nucleoside, 5-(dihydroxyboryl)-2'-deoxyuridine (DBDU, **20**) was prepared as an analogue of thymidine (**21**) by Schinazi and Prusoff[10,12] by a metal-halogen exchange on 5-bromo-3',5'-bis(O-trimethylsilyl)-2'-deoxyuridine in anhydrous THF followed by boronation and hydrolysis. The optimum yield was obtained by conducting lithiation at -40°C and the boronation at -60°C in the presence of 8% hexamethylphosphoric triamide (HMPT) under anhydrous conditions. The major by-product in this synthesis of DBDU from the protected 5-bromo-2'-deoxyuridine was 2'-deoxyuridine (dUrd). The ratio of dUrd:DBDU produced in the reaction mixture can be easily monitored by high-pressure liquid chromatography. Attempts to increase the yield of DBDU by varying the lithiating reagent, temperature and/or cosolvent (i.e., HMPT) conditions have so far failed. An important advantage of the route described above was that it was easily modified to prepare boron-10-enriched DBDU starting from boron-10-tri-*n*-butyl borate for radiation and biochemical studies.[9]

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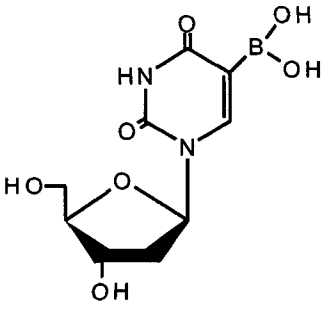
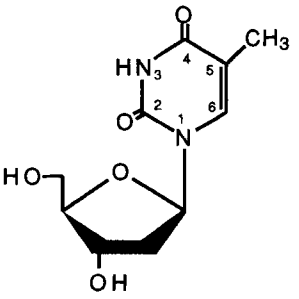


5-Dihydroxyboryl-2'-deoxyuridine (DBDU, **20**)



Thymidine (**21**)

Yamamoto et al.[19] synthesized boronated pyrimidine as well as purine nucleosides adopting the organolithium-aldehyde condensation methodology described for the synthesis of pyrimidines.

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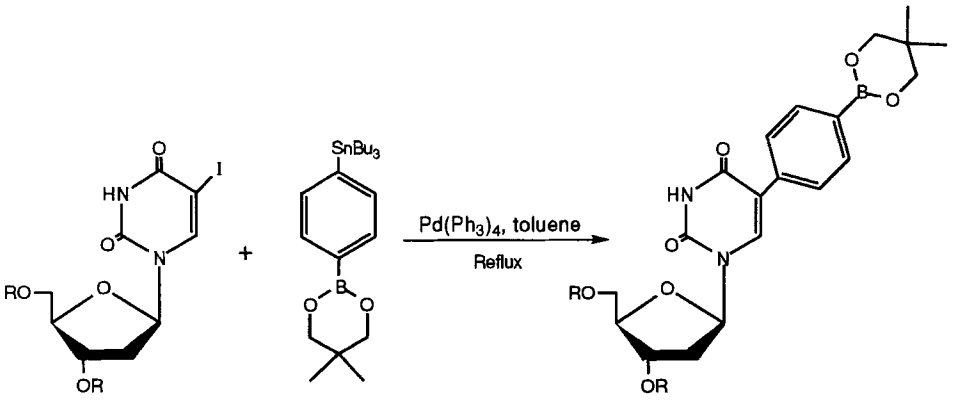
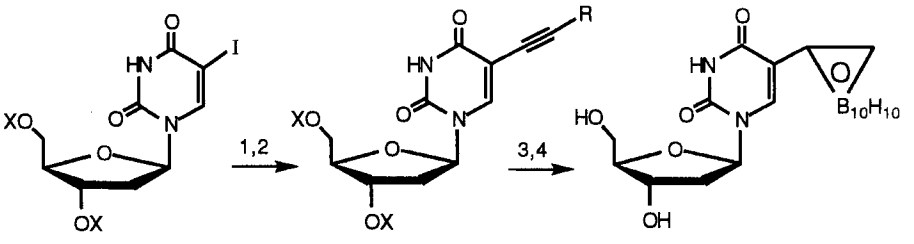


22

Another novel method developed by the same group for synthesizing boronated nucleoside analogues **22** consisted of a palladium catalyzed coupling reaction of an halogenated nucleoside with aryltin compound having a boronic moiety in refluxing toluene. The boronic acid group must be protected as the cyclic ester for the coupling reaction in order to proceed. $Pd(PPh_3)_4$ was the most effective catalyst in this coupling reaction.[19]

(b) o-Carborane Derivatives: Yamamoto et al.[24] and our group[25-28] synthesized o-carborane substituted pyrimidine and purine nucleosides, since these compounds have a high boron content and an intact 3'- and 5'-hydroxyl function. These compounds are

lipophilic, readily phosphorylated by cellular kinases (see below), and in certain cells could incorporate into DNA as analogues of natural 2'-deoxypyrimidine nucleosides. For example, 5-carboranyl-2'-deoxyuridine (CDU, **25**) was synthesized starting from 5-iodo-2'-deoxyuridine. The protected nucleoside 5-iodo-3',5'-bis-*O*-benzoyl-2'-deoxyuridine (**23**) was coupled with trimethylsilyl acetylene in the presence of $(Ph_3P)_2PdCl_2$/CuI in triethylamine followed by deprotection of the acetylenic group with *n*-Bu_4NF gave 3',5'-bis-*O*-benzoyl-5-ethynyl-2'-deoxyuridine (**24**). Reaction of this alkyne with decaborane (as bispropionitrile adduct) in refluxing toluene, followed by deprotection using methanolic sodium methoxide, yielded CDU (**25**).[24-29] Similarly, 5-carboranyluridine (CU), 5-(1-hydroxymethyl)carboranyluridine (HMCU) and 5-(1-hydroxymethyl)-carboranyl-2'-deoxyuridine (HMCDU) can be obtained.



23 **24** **25**

1. $HC{\equiv}C\text{-}SiMe_3$, $(Ph_3P)_2PdCl_2$/CuI, Et_3N, 50°C; 2. Bu_4NF/THF; 3. $B_{10}H_{12}(CH_3CN)_2$, toluene,Δ; 4. NaOMe/MeOH

More recently, our group in collaboration with Dr. D. C. Liotta (Emory University) synthesized 5-carboranyl substituted 3'-thianucleoside analogues modified in the sugar moiety, since 3'-heteranucleosides, such as racemic 2',3'-dideoxy-3'-thiacytidine (BCH-189) and 2',3'-dideoxy-5-fluoro-3'-thiacytidine (FTC), exhibited potent antiviral activity.[30-34] These compounds, which readily permeate cell membranes, are excellent substrates for cellular kinases, and resist many other catabolic processes that are commonly observed with other 2'-deoxycytidine derivatives. Racemic 5-carboranyl-2',3'-dideoxy-3'-thiauridine (**26**, **27**) was prepared, adopting the methodology described in CDU synthesis, using the protected racemic 2',3'-dideoxy-5-iodo-3'-thiauridine. The key intermediate was obtained by coupling silylated 5-iodouracil with the appropriately protected 1,3-thioxolane moiety using the methodology developed by Choi et al.[35]

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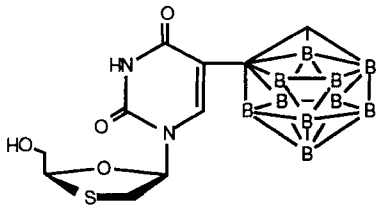

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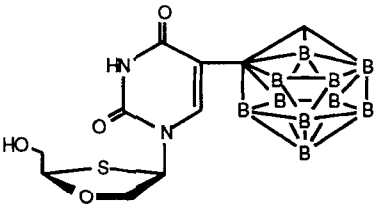

27

The racemates of 3'-thianucleosides can be resolved using lipase-mediated hydrolyses of appropriate 5'-acyl derivatives.[33] Specifically, the extreme low toxicity of the (-)-enantiomers of BCH-189 and FTC was attributed to the fact that these compounds are "unnatural" L-enantiomers. Thus, these materials are accepted as substrates by cellular kinases, but are not recognized by most other key cellular enzymes and, therefore, may not interfere with normal cell functions. The use of "unnatural" enantiomers could represent an important new approach for designing a variety of chemotherapeutic agents that exhibit low toxicity and which could be useful for treating gliomas, and other tumors. Attempts are ongoing in our laboratories to resolve the 5-carboranyl-2',3'-dideoxy-3'-thianucleosides and characterizing the biological properties of the 2 enantiomers.

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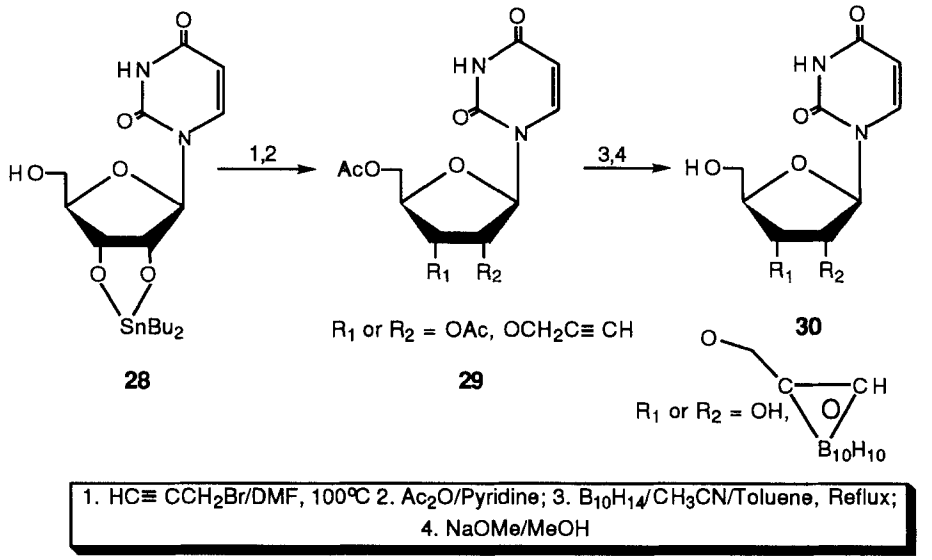
Tjarks et al.[36] reported that boron containing nucleosides with a carboranyl moiety linked to one of the ribosyl hydroxyl groups. The starting material is 2',3'-*O*-(dibutylstannylene)uridine (**28**), which was readily prepared by heating a methanolic solution of uridine with dibutyltin oxide. This material on alkylation with propargyl bromide in DMF at 100°C gave an inseparable mixture of 2'-*O*-(3-prop-1-ynyl) and 3'-*O*-(3-prop-1-ynyl)uridines (**29**) in a 3:2 ratio. The mixture was acetylated with acetic anhydride in the presence of pyridine, and the acetylated isomers were readily separated by silica gel chromatography. The straightforward addition of decaborane (as bis-acetonitrile adduct) to the alkyne in refluxing toluene and deacetylation with methanolic NaOMe yielded the corresponding carboranyl substituted uridine **30**.[36-38] Similarly, 5'-O-(o-carboran-1-ylmethyl)uridine was prepared starting from 2',3'-O-isopropylideneuridine using the methodology described above.

Yamamoto and co-workers[19] explored the methodology for the synthesis of several boronated nucleosides by reacting the organolithium protected nucleoside



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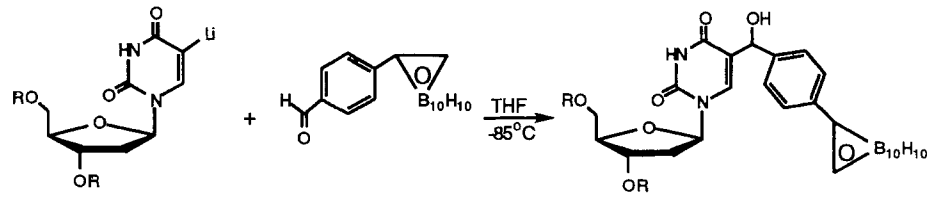
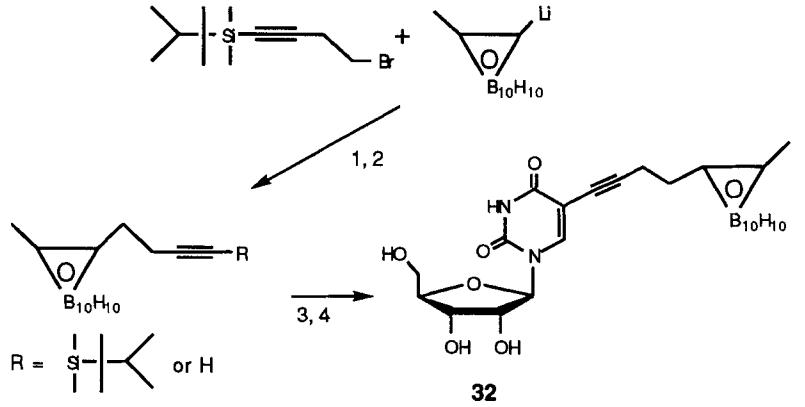
aldehyde with phenyl bearing a p-carborane moiety. The o-carborane derivative of formylbenzaldehyde was prepared by the reaction of acetylenic formylbenzaldehyde with decaborane (as bispropionitrile adduct) in refluxing toluene. Coupling of the protected uridine lithiate in THF at -85°C with the o-carborane derivative of formylbenzaldehyde readily yielded the boronated nucleoside **31**.[39]



Recently, Kabalka et al.[40] reported the synthesis of 5-[4-(methyl-o-carboranyl)-1-butynyl]uridine (**32**) having a spacer introduced between the carboranyl moiety and the pyrimidine base. This compound was synthesized by coupling the 2',3',5'-O-trimethoxymethyl-5-iodouridine with methyl o-carboranyl residue using the standard palladium catalyzed cross-coupling methodology. The precursor, methyl o-carboranyl substituted derivative was prepared by reacting methyl o-carboranyllithium with 4-bromo-dimethylthexylsilylbutyne in the presence of lithium iodide, followed by deprotection of the acetylenic group.



32

1: LiI; 2: *n*-Bu_4NF; 3: 2',3',5'-tri-O-methoxymethyl-5-iodouridine, $[Ph_3P]_4Pd$, CuI, NEt_3; 4; 50% TFA

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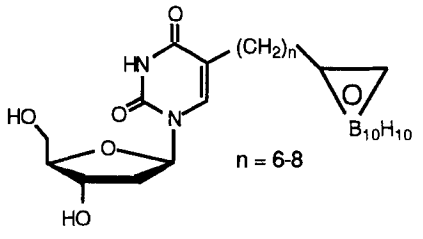
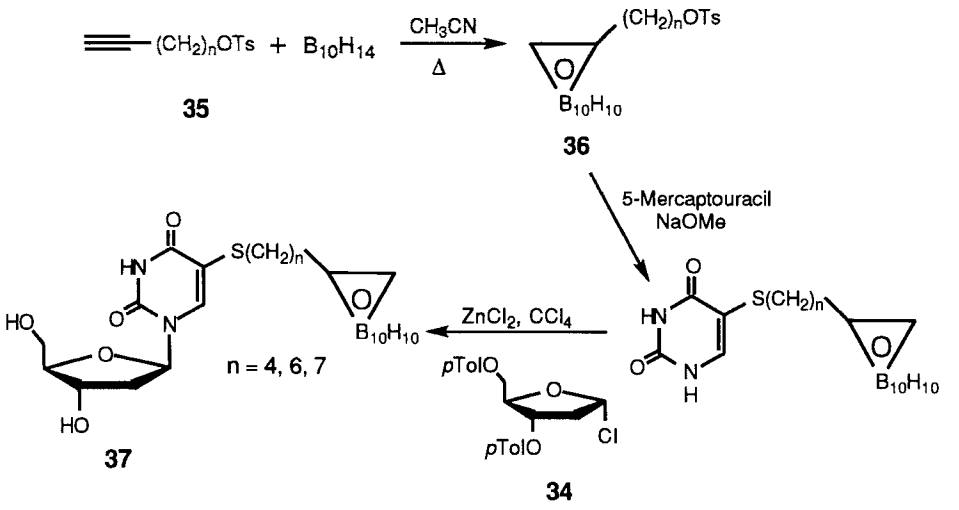
Similar type of modified 5-o-carboranyl-2'-deoxyuridine containing a polymethylene linker between carborane cluster and the pyrimidine base were synthesized by Rong and Soloway.[41] The key step in the synthesis of this class of compounds was to link a hydrocarbon chain directly at the 5-position of 2'-deoxyuridine. In this case, 5-bromo-2'-deoxyuridine was reacted with various organoboron substituted derivatives, such as B-alkyl(carboranyl)-9-borabicyclo[3.3.1]nonanes (B-R-9-BBN) *via* the palladium-catalyzed cross coupling reaction to yield the desired compounds **33**.



33

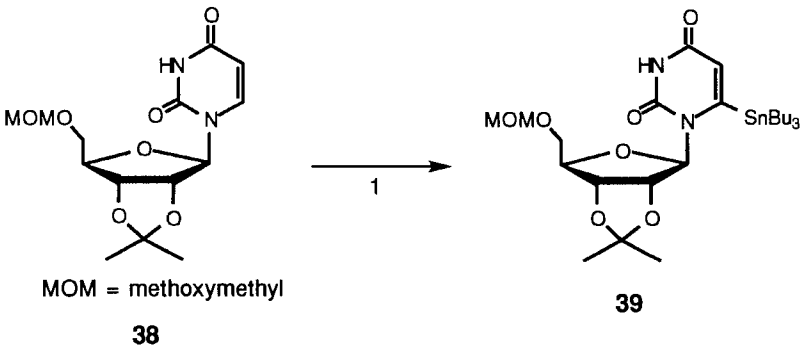
Another class of compounds containing 5-S-polymethylenecarboranyl-2'-deoxyuridine was reported by the same group.[42] The synthesis of these new carboranyl nucleoside analogs involved the preparation of a suitable boronated uracil derivative, which was then condensed with 3,5-di-O-*p*-toluoyl-2-deoxy-α-D-ribofuranosyl chloride (**34**) in CCl_4 using zinc chloride as a catalyst. The spacers **36** were synthesized by reacting the corresponding terminal acetylenic aliphatic tosylate **35** with decaborane using the standard methodology. Then, the 5-mercaptouracil was S-alkylated by

displacement of the tosylate group using sodium methoxide to produce nucleoside **37**. The anomeric ratio for the α and β nucleosides was not reported.



Recently, protected 6-(o-carboran-1-yl-phenyl)uridine (**41**) was synthesized by Palmisano and Santagostino[43] using Sn-Pd transmetallation-coupling reaction. Initially, 6-stannylated protected uridine was obtained by regiospecific C-6 lithiation of 2',3'-O-isopropylidene-5'-O-methoxymethyluridine (**38**) with LDA followed by electrophilic *in situ* addition of *n*-tributyltinchloride.

This stannylated intermediate **39** which is stable at low temperature was then coupled with 2-iodo-phenylacetylene using $Pd(Ph_3P)_4$ catalyst in the presence of CuI. This phenylalkynyl uridine **40** was subsequently boronated with decaborane as bisacetonitrile adduct.



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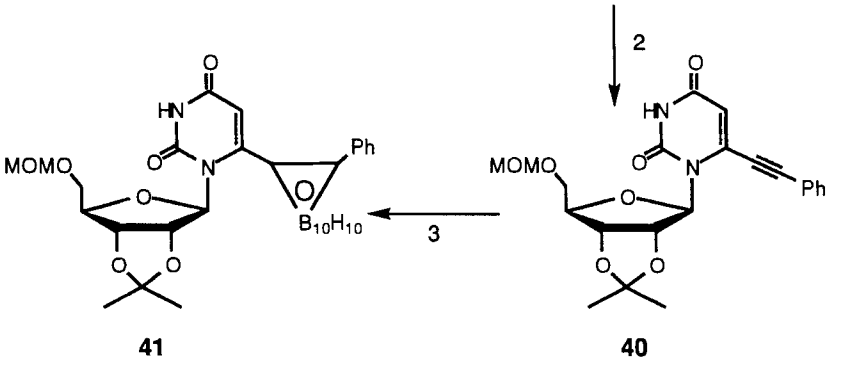

41 40

1: LDA, THF, -78°C, *n*-Bu_3SnCl; 2: PhC≡CI, $Pd(PPh_3)_4$, CuI, DMF, 80°C; 3: $B_{10}H_{12}(CH_3CN)_2$, Δ

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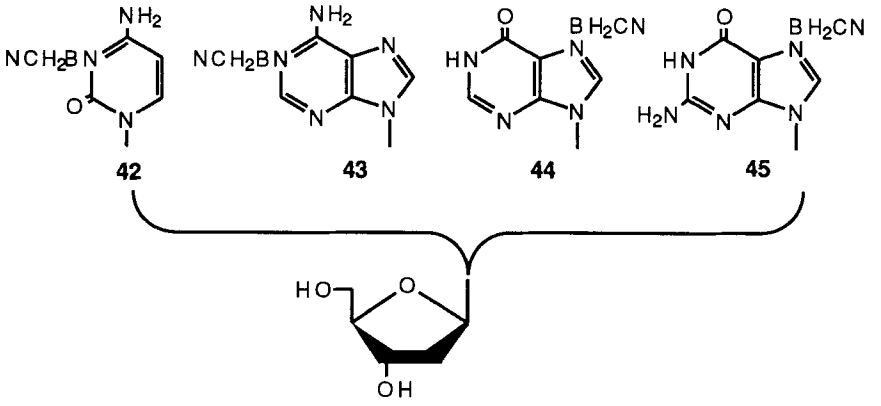
(c) Miscellaneous Boronated Nucleosides and Nucleotides: Sood et al.[44] reported the synthesis of a series of cyanoborane adducts of 2'-deoxynucleosides, specifically 2'-deoxycytidine-N^3-cyanoborane (**42**), 2'-deoxyadenosine-N^1-cyanoborane (**43**), 2'-deoxyinosine-N^7-cyanoborane (**44**), and 2'-deoxyguanosine-N^7-cyanoborane (**45**).



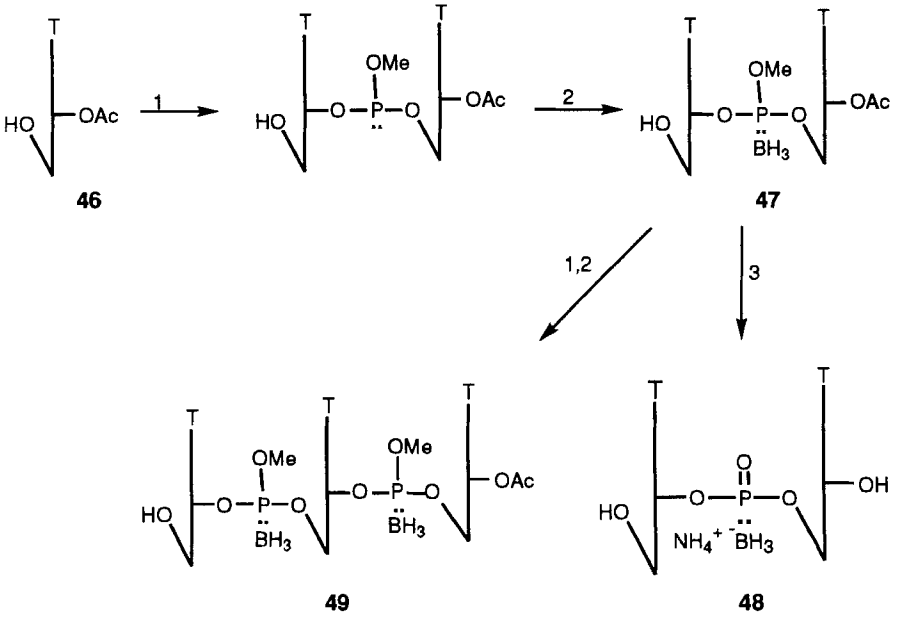
42 43 44 45

These compounds were prepared by an exchange reaction of triphenylphosphine-cyanoborane (PH_3PBH_2CN) with 3',5'-O-protected nucleosides in anhydrous THF at reflux temperature followed by deprotection. The site of boron coordination was determined by ^{15}N NMR spectroscopy. Similarly, [2-^{14}C]-2'-deoxycytidine-N^3-cyanoborane was prepared for biodistribution and pharmacokinetic studies starting from [2-^{14}C]-2'-deoxycytidine using the methodology as described above.[45]

Tomasz et al reported the synthesis of 5'-P-borane-substituted thymidine mono- and triphosphate in which a BH_3 group replaces one non-bridging oxygen of the α-phosphate.[46] The authors suggest that these boranophosphates may have similar utility in biochemistry and molecular biology as phosphorothioates. The 5'-P-borane mononucleotide was unstable to low pH and was shown to be a substrate for acid phosphatases and 5'-nucleotidases. The mixture of diastereoisomers of 5'-P-borane-nucleotide triphosphate were successfully used as substrates for extension of a 17-mer primer using sequenase. However, no evidence was provided for the ability of the 5'-P-borane nucleoside monophosphate to serve a substrate for any nucleoside kinase.

Boron Containing Oligonucleotides.

Sood et al.[47] and Shaw et al.[48] reported the synthesis of oligonucleotides with a boronated internucleotide backbone; viz., boranophosphates and boranophosphate methyl esters. The borane (BH_3) group in these boronated oligonucleotides is isoelectronic and isostructural with normal O-oligonucleotides and oligonucleotide methylphosphonates.



1. 5'-DMT-thymidine phosphoramidite, tetrazole, CH_3CN; 2. $BH_3.SMe_2$; 3. conc. NH_4OH

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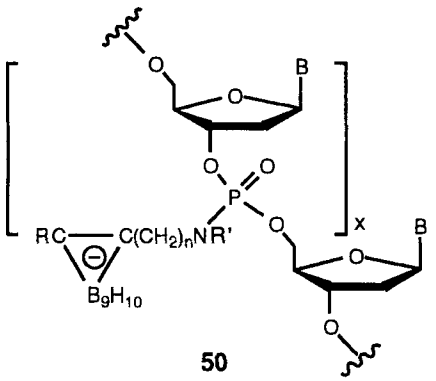
The boronated oligonucleotides were prepared by the reaction of 5'-O-dimethoxytritylthymidine-3'-O-phosphoramidite with 3'-O-acetylthymidine (**46**) in the presence of tetrazole in THF followed by boronation with borane-dimethyl sulfide yielded the dimer dithymidilyl boranophosphate methyl ester **47**. An added advantage of this reaction was the removal of the DMT protecting group during boronation which is required for chain elongation. The reaction of dimer with 5'-DMT-thymidine phosphoramidite in CH_3CN followed by boronation yielded the trimer **48**. Hydrolysis of the dimer with concentrated NH_4OH gave the unprotected dinucleoside boranophosphate **49**.[47]

More recently, Powell et al.[49] reported the synthesis of boronated oligonucleotides which could localize in the cell nucleus and by this way increase the efficacy of these potential BNCT agents. The oligonucleotides were obtained as *nido*-o-carboranyl-phosphoramidate derivatives **50**. However, the phosphoramidate bond is acid labile which could be then degraded under certain conditions.



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Recently our group has developed new methodologies for the preparation of two types of oligonucleotide modifications: a) oligonucleotides (**51**) bearing uncharged 3',5'-O,O-[(o-carboran-1-yl-methyl)phosphonate] internucleotide linkage instead of the natural 3',5'-O,O-phosphate residue, and b) oligonucleotides (**52**) bearing a modified base, such as 5-(o-carboranyl)uracil.[50,51] As molecular models, tetra[thymidine(o-carboran-1-yl-methyl)phosphonate], tetra[5-(o-carboran-1-yl)-2'-deoxyuridine phosphate] and dodeca-(thymidine phosphate) bearing one or more 3',5'-O,O-[(o-carboran-1-yl-methyl)-phosphonate] or 5-(o-carboran-1-yl)-2'-deoxyuridine modifications have been synthesized.

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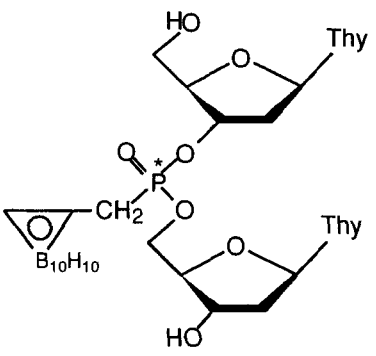

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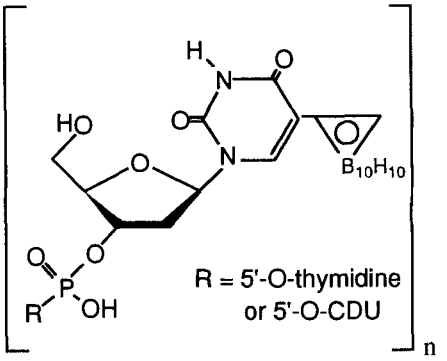
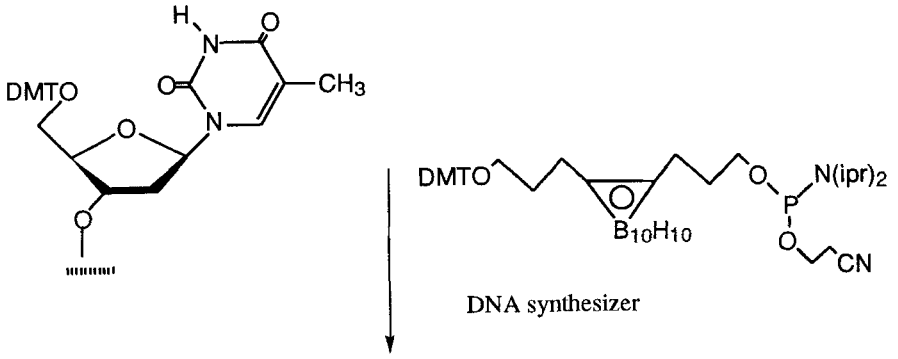


52

Their physicochemical and biological properties, such as T_m, partition coefficient, resistance against nucleases, and effect of modification upon cellular uptake and egress, are currently being studied and compared with unmodified and methylphosphonate modified oligonucleotides. These initial studies will serve as the foundation for developing boron-containing therapeutic oligonucleotides that target specific genes that are overexpressed in tumor cells, especially gliomas. This novel strategy based on AOT for synthesis of improved tumor specific boron carrier molecules has implications beyond BNCT and AOT, such as in the area of tumor diagnosis and virology.

Kane et al. recently reported on the automated synthesis carborane-derived homogeneous oligophosphates **53** starting from o-carborane.[52] As a model, this group prepared thymidine-3'-monophosphate coupled to ligands containing, fluorescein and biotin which could be used for transport studies, diagnosis, or for immunoprotein-mediated BNCT. The water-soluble boron-rich oligophosphates were quantitatively converted to the *nido* form on cleavage from the support used for the automated synthesis by NH_4OH. These novel compounds have vast potential as boron trailer molecules that could be useful for modern immunological and molecular approaches.



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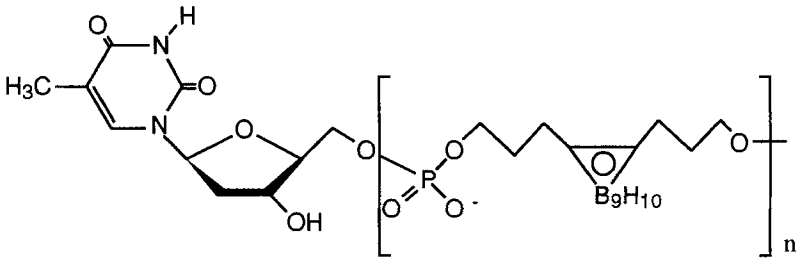

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Biological Activity.

Boron-Containing Pyrimidines.

Few biological studies have been performed with the bases containing boron, since these are poor substrates for cell-free enzymes involved in nucleoside biosynthesis or in mammalian cells.[10] For example, 6-(dihydroxyboryl)uracil was found to be a poor inhibitor of orotate phosphoribosyl transferase with a K_i of 22.1 ± 7.1 mM.[10] However, these compounds were modest inhibitors of dihydrouracil dehydrogenase obtained from mouse liver cytosol pyrimidine nucleoside phosphorylases with K_i values of 22.0 ± 2.8 μM and 26.6 ± 1.8 μM, respectively.[53] In contrast, orotic acid had a K_i greater than 1,000 μM. Schinazi and Prusoff[10] evaluated 5- and 6-(dihydroxyboryl)uracils for anti-herpes simplex virus type 1 (HSV-1) activity in Vero cells and found no significant activity at 100 μM. Yamamoto[39] demonstrated that 5-(dihydroxyboryl)uracil and a p-(dihydroxyboryl)phenyl analogue were not toxic to HeLa-S3 cells at 70 μM. Compounds containing a ribose sugar were also not toxic to these cells even at higher concentrations.

Tjarks and Gabel[17] found that 5-(dihydroxyboryl)-2-thiouracil, its 2,4-dithio analogue, and the 6-propyl analogues accumulated in B16 melanoma in mice with tumor:blood ratios of 4-to-31. Apparently, the compounds were orally bioavailable and were well tolerated in mice, although no specific dose or route was provided. Boron levels were determined by quantitative neutron capture radiography. Unfortunately, that method does not discriminate between intact base containing boron and boric acid which may have dissociated from the molecule *in vivo*.

Boron Containing Nucleosides.

(a) Boronic Acids: DBDU was not toxic to several mammalian cells when tested up to 1,600 μM. DBDU exhibited weak activity against HSV-1 which was only prevented

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by thymidine but not other 2'-deoxy- and ribonucleosides.[9,10] DBDU has no effect on human peripheral blood mononuclear (PBM) cells acutely infected with human immunodeficiency virus type 1 (HIV-1), although a modest activity was noted against HIV-2 ($EC_{50} \approx 12$ μM) (R. Schinazi, unpublished data).

Cells exposed to DBDU for 1 cell cycle incorporated an amount of boron such that the resultant biological effect was equivalent to a boron concentration of 6 μg boron-10/g cell (with an epithermal neutron beam; thermal beams would require more). Thus, it would appear that DBDU was capable of delivering boron concentrations approaching those which should be useful for NCT.[54] Similar to halogenated nucleosides, DBDU sensitizes cells to high and low LET radiation.

To study the incorporation of DBDU in cultured cells, ^{3}H-DBDU was prepared starting with (6-^{3}H)-5-bromo-2'-deoxyuridine (BrdUrd). V-79 Chinese hamster cells were incubated with 10 μM labeled DBDU and 1 μM thymidine over one cell cycle, and the amount of radioactivity incorporated into macromolecular components (insoluble in 5% trichloroacetic acid) was determined by liquid scintillation counting. At the Brookhaven Medical Research Reactor (BMRR), a method was developed for determining the boron content of similarly treated cells based on neutron irradiation and counting the tracks produced in a plastic film by the alpha-particles emitted in the decomposition of boron-10. The tracks were made visible by etching the film with alkali. Initially, *de novo* thymidine synthesis was blocked with aminopterin. On the assumption that the analogue replaced thymidine in DNA, both the radioactivity incorporated and the boron analyses indicated a replacement level of about 2%. Sensitivity of the cells to neutron irradiation after DBDU incorporation was determined. Irradiations were conducted at the BMRR, and cell survival was determined by a colony assay. A dose enhancement factor of ~ 1.5 due to DBDU (unenriched in boron-10) incorporation was found. In further experiments, thymidine synthesis was blocked by 5-fluoro-2'-deoxyuridine (FdUrd; a thymidylate synthase inhibitor) in the hope that it would provide increased uptake of DBDU. However, no advantage was observed.[54]

Survival curves obtained with the 95% boron-10 enriched DBDU produced a dose enhancement factor (DEF) of about 2.3, with a nucleoside ratio (DBDU/dThd) of 10:1.[54] Such a ratio would have produced an average replacement of 40-50% with IdUrd or BrdUrd. It was found that no significant advantage was gained by loading the pulsing medium with DBDU (DBDU/dThd = 50:1 to 200:1),[54] although some increase in plating efficiency was obtained. Lowering the DBDU/dThd ratio to 1:1 significantly reduced the

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DEF (to ≤ 1.3). Maintaining the DBDU/dThd ratio at 10:1, but increasing the availability to thymidine (dThd), reduced the DEF (1.6 to 2.2). From these data, it appears that DBDU was bound during DNA synthesis, but it did not compete with dThd as effectively as IdUrd and BrdUrd. At a DBDU/dThd ratio of 10:1, the DEF of 2.2 indicates a nuclear boron concentration of ≈ 10 μg boron-10/g (2.5% replacement). From work with IdUrd, such a nucleoside ratio should have resulted in a replacement of about 40%, or approximately 96 μg B/g. Thus, it appears that the relative efficiency for replacement of DBDU for dThd is about 12.5%. From these data, one would infer that DBDU must be incorporated in DNA in order to have sensitizing effects analogous to the halogenated analogues, and that replacement of dThd by DBDU was between 5% and 15% at a DBDU/dThd ratio of 10:1.

In vivo studies with a murine melanoma model have not demonstrated any toxicity from DBDU following multi-day intravenous infusions (up to 60 mg/kg per day for 1 week). Thus, the possibility exists that incorporation in DNA may be built up over a period of time in animals. In addition, DBDU might be used as one of several boronated compounds employed simultaneously to build up boron levels for NCT. Such an approach could also be used to minimize heterogeneity in the distribution of boron-10 within tumors. *In vivo* incorporation studies introducing the DBDU by means of a continuous intravenous infusion over 3 days were performed. Preliminary studies indicated a 0.5-2.5% incorporation (replacement of dThd) of the radiolabel in gut and tumor. This analysis was accomplished through liquid scintillation analysis of the trichloroacetic acid insoluble fraction of tissue fragments (R. F. Schinazi, unpublished).

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In summary the data from all survival assays, ^{3}H counting, and track etching techniques were in agreement in that at least 1% replacement (DBDU for dThd) was obtained with cell culture, with blocking techniques. Perhaps most encouraging, prompt γ analysis revealed ~1.8% exchange following 3 days of intravenous infusion of mice, suggesting that *in vivo* administration, even without blocking, can provide significant sensitization. Experiments have shown that DBDU was incorporated into DNA with an efficacy smaller than IdUrd or BrdUrd but that, even at the lower percent incorporation obtained, the sensitizing properties were significant and should amplify the effects of other boron compounds in BNCT. It would appear quite likely that DBDU could also find use as a significant contributor to boron content if a "cocktail" of various compounds is found necessary to develop therapeutic levels of boron-10 in brain tumors. Some of these studies were performed in collaboration with Drs. Ralph Fairchild and Brenda Laster at Brookhaven National Laboratory.

5-Dihydroxyboryl-2-thiomethyluracil was administered intraperitoneally to 5 mice over 2 days, and the following mean boron levels were obtained by prompt gamma analysis of the boron-10 component in various tissues: intestine 15 μg B/g; tumor 1.8 μg B/g; liver, 2.8 μg B/g; and blood, 8.9 μg B/g.

Cyanoborane nucleosides. The boronated 2'-deoxycytidine-N^3-cyanoborane (dC·BH_2CN) has been shown to inhibit inflammation in mice, whereas 2'-deoxyguanosine-N^7-cyanoborane (dG·BH_2CN) had antilipidemic activity.[55,56] The mechanism of action of these boronated nucleosides in reducing inflammation and lowering cholesterol levels *in vivo* was not explained. In mice transplanted with Ehrlich ascites carcinoma, radiolabeled dC·BH_2CN had a tumor:blood ratio of 4.2 and 8.5 at 2 h and 4 h, respectively.[55] Surprisingly, some of these cyanoborane adducts are hydrolytically stable for 1 week with < 6% decomposition as determined by HPLC.[55] The inosine derivative was the most unstable compound with more than 50% decomposition after 1 week. dC·BH_2CN was shown to possess antineoplastic activity in murine and human cell culture systems at concentrations between 1 and 10 μg/ml.[57] The potency of these compounds was similar to 5-fluorouracil, 6-mercaptopurine, and other anticancer agents. Results suggest that these boron-containing purines affect primarily DNA synthesis, although protein and RNA synthesis was enhanced or suppressed for certain compounds. The compounds apparently bind to these macromolecules and are not incorporated into nucleic acids of the tumor cells. It is surprising that the authors did not evaluate the compounds in normal cells, such as human PBM cells or human bone marrow cells to determine their selectivity for tumor cells. Clearly more studies on the uptake and metabolism of dC·BH_2CN and related compounds is warranted. The synthesis and antineoplastic activity of N^2-isobutyryl-2'-deoxyguanosine-N^7-cyanoborane derivatives and related compounds was recently described.[58,59]

(b) o-Carboranyl Nucleosides:

Cellular uptake and phosphorylation of CDU. Studies were undertaken in our laboratory using unlabeled CDU in order to follow the intracellular profiles of the parent drug and metabolites detected within CEM cells. Quantitation of intracellular parent drug and metabolic derivatives were conducted by HPLC.[25,27,28] Significant amounts of CDU were found intracellularly at 12 h after treatment, which we estimate to be 137.5 ± 9.3 μM (average of 3 different determinations). *A new more polar metabolite than CDU was noted, which was not seen in the control untreated cell extract.* When this new metabolite was collected and pooled, and then treated with affinity purified bovine kidney alkaline phosphatase (EC3.1.3.1), it disappeared and a new peak corresponding to CDU appeared

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(as determined by HPLC). Furthermore, snake venom phosphodiesterase I (EC3.1.4.1) had no effect on the new metabolite, even when the cell-treated extract was incubated for 4 h with this enzyme. Interestingly, the new metabolite was not found in the medium in cells that were treated with CDU, suggesting that it is formed intracellularly. The metabolite had the same retention time as authentic synthetic CDU-5'-monophosphate. Based on these experiments, we conclude that the new metabolite is probably the 5'-monophosphate of CDU. Studies with radiolabeled material described below confirmed these findings. In addition, we have demonstrated that CDU and 5-carboranyl-2',3'-dideoxy-3'-thiauridine (CTU) are not substrates for *E. Coli* thymidine phosphorylase (dThdPase).

Cytotoxicity and other biological activity of CDU and analogues. An indirect way which suggests that the nucleosides synthesized by our group are phosphorylated is to determine if they exhibit, even at high concentration, some biological effect such as antiviral activity or cytotoxicity. This reasoning is not absolute since other mechanisms may be responsible for the biological activity observed. Nevertheless, CDU and related analogues and CTU were evaluated in CEM, in PBM cells and in rapidly dividing Vero cells for cytotoxicity using a trypan blue exclusion method. These compounds and related analogues were also evaluated against HIV-1 (strain LAI) in primary human lymphocytes (Table 1).

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It is of interest that the least toxic compound evaluated in all 3 cell systems was HMCU. The hydroxyl function on the carboranyl function may be readily ionized resulting in a molecule that is less likely to penetrate cells. CDU and 5-carboranyluridine (CU) exhibited significant toxicity in Vero cells. CTU was a weak, but selective inhibitor of HIV-1 in human PBM cells. Further studies with the resolved enantiomers should indicate which form has the greatest antiviral activity.

The finding that some of these novel nucleosides have biological activity suggests that they may be phosphorylated in various cells, although definite proof can only be obtained from careful cellular metabolism studies using radiolabeled compound. In effect, uptake studies in CEM and human PBM cells using radiolabeled CDU have confirmed our finding (see below).[25,28] CDU-MP had an intracellular half life of about 2 h. The ability of these compounds to sensitize cells to neutrons is currently under investigation at Brookhaven National Laboratory.

TABLE 1. Biological activity of various boron-containing compounds in cell culture.[26,27]

Compound	CEM cells IC_{50}, μM On day 6	Vero cells IC_{50}, μM On day 3	PBM cells IC_{50}, μM On day 6	Anti-HIV-1[1] EC_{50}, μM On day 6
CDU	70.9	17.1	> 100	73.2
CU	76.1	26.4	> 100	> 100
HMCU	> 100	> 100	> 100	> 100
CTU	37.9	34.2	> 100	12.1
BuCTU	18.8	4.6	> 100	6.4
AZT (control)	13.0	29.0	> 100	0.004

[1] In PBM cells.
5-Carboranyl-2'-deoxyuridine (CDU);
5-Carboranyluridine (CU);
5-Hydroxymethylcarboranyluridine (HMCU);
5-Carboranyl-2',3'-dideoxy-3'-thiauridine (CTU)
5'-O-Butyryl-5-carboranyl-2',3'-dideoxy-3'-thiauridine (BuCTU)
3'-Azido-3'-deoxythymidine (AZT)

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We have demonstrated for the first time that certain 5-carboranylpyrimidine nucleoside were phosphorylated intracellularly.[25,27,28] These results suggest that it is possible to deliver and trap for a limited time in cells large amounts of boron-containing nucleoside. CDU-5'-MP also appears to be phosphorylated further to the diphosphate in certain cells.[27,28] It is probably a poor substrate for thymidylate kinase, which may explain its low cytotoxicity in lymphocytes since it does not seem to incorporate into DNA.

Yamamoto et al.[29] evaluated CDU, CU, HMCU, and 5-(dihydroxyboryl)uridine for cytotoxicity in various cancer cells. Whereas 5-(dihydroxyboryl)uridine was not toxic to P-388, L1210, B-16, MBL-2, and MethA cells up to 30 μM, CDU and CU were uniformly toxic at 2-4 μM in these cells. Interestingly, HMCDU was not toxic in L1210 or MethA cells up to 30 μM. These results demonstrate that 5-carboranyl pyrimidine nucleosides are generally more toxic than their 5-(dihydroxyboryl) counterparts. In addition, the cytotoxicity of 5-carboranyl nucleosides varies markedly and is dependent on the cell culture system used (see Table 1 above). We believe that nucleosides that are planned to be used for BNCT should have negligible toxicity in culture or *in vivo*.

Toxicological and pharmacological studies in mice and rats with CDU are ongoing in our laboratory.

The quantitative determination of boron at the intracellular level in cell cultures can also be measured by using imaging secondary ion mass spectroscopy (ion microscopy).[60] The distribution of 5-carboranyl nucleosides in F-98 glioma cells was found to be preferentially localized in the cytoplasm, although significant amounts (≥ 121 μg B/g *versus* ≥ 222 μg B/g in the cytoplasm) of CDU, HMCDU, and CTU could be detected in the nucleus (G. H. Morrison, I., Gay, B. D. Bennett, and R. F. Schinazi, unpublished data). It appears that boronated nucleoside uptake was primarily a function of the lipophilic carboranyl moiety, although other transport mechanisms may be involved.

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2'-O-Carboranyluridine (CBU) and related analogue. The cellular uptake of CBU was determined in F98 rat glioma cells using plasma atomic emission spectroscopy (DCP) and ion microscopy (IM).[36,38,61] Persistent (≤ 48 h) boron levels were detected in the cytoplasm and nuclei of these tumor cells. However, these levels were lower than those found with CDU (see above). In mice bearing B16 melanoma, the boron levels achieved were inadequate. Unfortunately, the precise disposition and phosphorylation of CBU *in vitro* and *in vivo* was not determined using radiolabeled material combined with an analytical methodology such as HPLC and/or mass spectroscopy. Furthermore, although the 2'-O-carboranyl moiety should have little effect on the conformation of the base, it could interfere with the ability of CBU to be phosphorylated. In addition, ribonucleosides are easily susceptible to glycosidic cleavage by phosphorylases. Unfortunately, no enzyme stability studies were performed. Interestingly, there seems to be little difference in the uptake of CBU and its 3'- and 5'-O-carboranyluracil analogues,[36] suggesting that entrapment of these compounds in F98 glioma cells is perhaps achieved by mechanisms other than phosphorylation.

Boron Containing Oligonucleotides.

Oligonucleotides with modified backbones may be used as "antisense" agents to inhibit or control growth of viruses as well as to specifically control the expression of oncogenes or other genes associated with various genetic disorders.[62-65] The boranophosphate species is very closely related to the normal oligonucleotides and the oligonucleotide methylphosphonates. On the other hand, boranophosphate methyl esters closely resemble oligonucleotide phosphotriesters. It has been shown that the

boranophosphate internucleotide linkage was very stable toward acidic or basic hydrolysis as well as calf spleen phosphodiesterase and snake venom phosphodiesterase.[47,55] Although several papers and patents have disclosed chemical information on these boron-containing oligomers,[44,55,66,67] there has been a paucity of information on the biological activity of this class of compounds as potential therapeutic agents. For example, Spielvogel et al.[55] reported that a nucleotide dimer had activity against human colorectal adenocarcinoma in cells culture with a median effective concentration of 0.88 μg/ml compared to 3.1 μg/ml for 5-fluorouracil. No studies with oligomers targeted to tumor cells for BNCT have been reported. More recently, Hall et al. reported on the *in vitro* and *in vivo* antineoplastic activity of 5'-O-[(triphenylphosphine-boryl)carbonyl]-3'-O-acetylthymidine and 5'-(diethylphosphite-cyanoborane)-3'-acetylthymidine.[68] The compounds were more effective against Ehrlich ascites carcinoma than L1210 or Lewis lung cancer in mice when given for 9 days at 8 mg/kg per day. Although it is stated that doses as high as 40 mg/kg were given to the mice, no data on the effectiveness or toxicity of the compounds at that dose were provided, especially on prolonged treatment. 5'-(diethylphosphite-cyanoborane)-3'-acetylthymidine was a potent inhibitor of DNA polymerase α and other polymerases, suggesting that the inhibition was non-specific.

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Prospects.

There has been a renaissance in the area of BNCT as attested by the increasing number of publications on boron chemistry and biology. Considerable progress has been made in the last 8 years in optimizing neutron beam penetration, dosimetry, boron analytical methods, and animal models for NCT. The development of relevant animal models for evaluating nucleosides is essential since in general these compounds need to be activated to the nucleotide by specific kinases. The use of transplanted *human* tumors in immunodeficient mice may prove more useful than currently available models in rats and dogs since their kinases may not recognize the nucleoside. Development of new organoboron compounds which may accumulate selectively in tumors is in progress in our laboratories as well as several centers with expertise in boron chemistry. In addition to BNCT, these compounds are being investigated with respect to their possible use as antibacterial, hypolipidemic, anti-cancer, and antiviral agents.[1,28,29,55,69-71] In recent years, these efforts have focused on the most promising classes of compounds and approaches which include boron-containing amino acids, nucleic acid bases, nucleosides, oligonucleotides, porphyrins, steroids, liposome encapsulated boron compounds, and tumor specific antibodies. Clinical trials in Japan are still being conducted with an improved sulfhydryl borane monomer $Na_2B_{12}H_{11}SH$ (Boracaptate) and studies in the

Europe and US are planned. Relevant small animal models to evaluate the levels of boron in tumor cells have been developed and improved.[70,72,73] Modern analytical methods for detecting boron in these models are becoming a reality.[74,75] Although other approaches such as gene therapy are being considered to treat tumors, especially gliomas,[76] these may not produce complete cures. Therefore, it is likely that BNCT will continue to be an attractive binary modality for the treatment of various malignancies.

The main requirements for therapeutic selectivity for BNCT are: (a) a large concentration of boron-10 in the targeted cell, estimated to be 5-30 μg boron-10/g of tissue, and (b) a large enough ratio of localized boron-10 in tumor cells/supporting normal tissue in order to avoid adverse effects on normal tissue surrounding the tumor. Detailed studies have shown that a minimum of ≈15 μg ^{10}B/g tumor is needed when an epithermal neutron beam is used (30 μg with a thermal beam) with a tumor/normal tissue concentration ratio of about 10. In addition, since such radiation can be delivered selectively to tumor cells, major advantages should accrue; protection of competing normal cell pools can be achieved by excluding them from the treatment volume. For example, the dose in bone marrow can be obviated as long as these tissues are excluded from the treatment volume. However, there are still numerous difficulties to overcome before clinical trials of NCT to be implemented. Among these, the following need to be determined: (a) the factors critical in effecting the successful application of NCT; (b) the amount and fractionation of infused compound; (c) the time of infusion before irradiation; (d) the amount of irradiation; and (e) an in depth understanding of the pharmacological action of the boron-containing compound.

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Although significant advances have been made in NCT, because of the inherent difficulty in synthesizing rationally designed hydrolytically stable boron compounds, it is clear that more emphasis should be placed on the chemical, biochemical, pharmacological, and animal model aspects. The exploitation of BNCT by utilizing boronated nucleosides, pyrimidines, purines, and oligonucleotides which show preferential uptake and localization in tumors will undoubtedly continue. The hope is that sufficient information will be developed on these compounds so as to select one or more compounds for advanced preclinical studies prior to evaluation for BNCT in patients with diagnosed gliomas, melanomas, breast cancers, and other tumors.

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