Identification, development, synthesis and evaluation of boron-containing nucleosides for neutron capture therapy

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Abstract

The development of boron compounds with the capacity for selectively targeting tumor cells would offer the potential for specifically destroying such cells using the capture reaction of the nonradioactive ^{10}B nuclide and thermal neutrons. The key problem is the development of compounds with the ability to discriminate between tumor cells and contiguous normal cells and to concentrate in the former at suitable concentration levels. One category of agents that has been explored is boron-containing nucleosides. Such recent structures have been biochemically converted in vitro to their corresponding nucleotides by the action of human thymidine kinase. These studies have attempted to correlate a compound's physiochemical properties with its biochemical attributes. Since only a fraction of cells are undergoing replication at any one time, requiring the need for nucleic acid precursors, such boron compounds must be only one component of a cocktail of agents that are targeting malignant cells. This presentation is selective, focusing on those boron-containing nucleosides that have been designed for studies with kinases. © 1999 Elsevier Science S.A. All rights reserved.

*Keywords:* Boron; Nucleosides; Neutron capture

1. Introduction

The chemistry of boron neutron capture therapy (BNCT) has been summarized in a recent review [1]. This potential use of boron compounds for the treatment of cancer is based upon the unique nuclear properties of the nonradioactive ^{10}B nucleus and its high capacity to absorb thermal neutrons. The resulting activated ^{11}B nucleus, following this capture reaction, undergoes prompt fission. The size and energy of the particles emitted are very large by nuclear standards and provide the basis for attempting to destroy malignant cells selectively without adversely affecting surrounding or nearby normal cells. In essence, this is a binary chemoradio-therapeutic procedure that is totally dependent upon the selective targeting of tumor cells by boron compounds.

One of the major classes of compounds that has been proposed as potential tumor-targeting agents is boron-containing nucleosides. The rationale for their synthesis resides in the fact that for cancer cells, there is, in general, an increased requirement for nucleic acid precursors. This need is based upon the higher proliferative rates of malignant cells by comparison with the normal cells from which they are derived and the involvement of salvage pathways designed to reutilize such important structures. The nucleosides may be viewed as precursors of the biochemical building blocks for which malignant cells may possess a requirement. An important question is whether boronated analogues of the nucleosides can be synthesized that would emulate the naturally-occurring counterparts and be taken up and metabolized by cancer cells?

Early work in this area of DNA precursors focused on the development and synthesis of boron-containing purine and pyrimidine bases [2]. A number of these compounds proved to be hydrolytically unstable and

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therefore were of little use as potential BNCT agents. This was especially the case when the boron atom was placed within the purine or pyrimidine nucleus and flanked by two nitrogen atoms. In those instances, where bulky and hydrophobic entities were incorporated onto the boron moiety, there was increased aqueous stability, but these compounds no longer possessed the biochemical properties of the naturally-occurring purine and pyrimidine bases. This result stimulated greater interest in attaching the boron moiety directly to a nucleic acid base, forming a boron–carbon linkage. In contrast with bases in which the boron was flanked by two nitrogen atoms, these derivatives should possess much greater hydrolytic stability. One of the early compounds that was synthesized was 5-(dihydroxyboryl)uracil (**1**) [3]. As expected, it did possess the needed aqueous stability, the first requirement. The second was to determine whether such a compound would get into cells and remain there, possible by emulating the natural bases and becoming inserted into nucleic acids. A more proximate derivative that may be better able to penetrate the cell membrane than the parent base is the corresponding nucleoside 5-(dihydroxyboryl)-2'-deoxyuridine (**2**). This was the first boron-containing nucleoside to be synthesized [4] and much of the subsequent research has focused upon nucleosides rather than upon boron-containing bases. The development of such analogues is based upon the use of various isosteric compounds in cancer chemotherapy and as antiviral agents. Additional support for the synthesis of such derivatives is found in the fact that thymidine isosteres, 5-bromo-2'-deoxyuridine (BUdR) and 5-iodo-2'-deoxyuridine (IUdR) possess K_m values for thymidine kinase that are comparable with that for thymidine itself [5]. These analogues and this ability have been the basis for assessing a tumor's proliferative activity, namely the fraction of cells that are in the S-phase [6]. In essence, this is a measure of the degree of a tumor's malignancy and BUdR has been used for this purpose. As may be expected, though tumors may have originated from a single cell clone, the cancer is no longer a monolithic entity and the percentage of cells that can be labeled by BUdR has shown great variability.


1   **2**
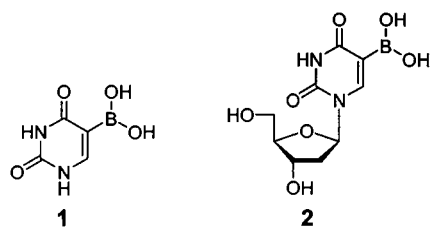
In the case of **2**, there is no biochemical evidence that it is phosphorylated. Such phosphorylation generating the corresponding negatively-charged nucleotide would

prevent simple diffusion from the cell. However, in radiobiological studies using the [10]B-enriched analogue, it has been estimated that thymidine in DNA was replaced by this analogue to the extent of approximately 5–15% [7]. This research provided the basis for the synthesis of subsequent boron-containing nucleosides. The rationale was that even if such structures were not incorporated into nucleic acids, per se, but were simply phosphorylated intracellularly into the corresponding nucleotide, that, by itself, may be sufficient. The requirement is to attain an adequate concentration and a suitable boron differential between tumor and contiguous normal cells. The important proviso is that the latter were not in a rapid proliferative stage. Since the objective in tumor targeting is not necessarily nucleic acid incorporation but intracellular entrapment and retention by the tumor, this may be achievable by intracellular nucleotide formation. For certain nucleosides, there is a carrier-mediated transport system operating independently of the compound's lipophilic properties, that is responsible for its ability to penetrate a cell's membrane [8]. The key question is whether any boronated nucleosides would be metabolized by intracellular kinases to their corresponding nucleotides in a manner analogous to what is observed with naturally-occurring nucleosides?

2. Experimental

2.1. Synthesis

One of our major efforts in developing boron-containing nucleosides has focused on the incorporation of stable boron clusters into various nucleosides. The rationale was that higher boron percentages in such nucleosides may be beneficial in achieving the needed cellular concentration without adversely affecting the compound's toxicity. The first cluster that has been used for this purpose is the *o*-carboranyl moiety, which contains 10 boron atoms. In order to demonstrate whether such compounds could be synthesized, to assess their hydrolytic stability and to characterize their other physiochemical properties, the *o*-carborane group was first attached to the 2' position of uridine, 2'-*O*-[(*o*-carboranyl)methyl]uridine (**3**) [9]. This was the first carborane-containing nucleoside that was described.

3   **4**

5

6

7

○ = BH
◑ = C
● = CH

10

11 n = 4, 5, 6, 8


12 n = 4, 5, 6, 7

○ = BH
◑ = C
● = CH
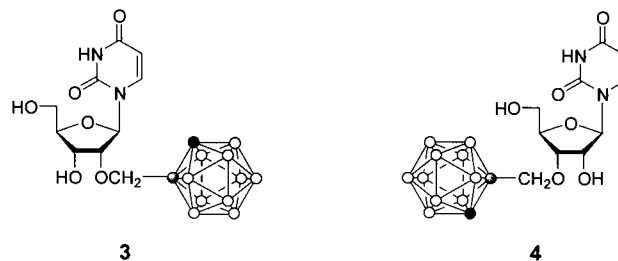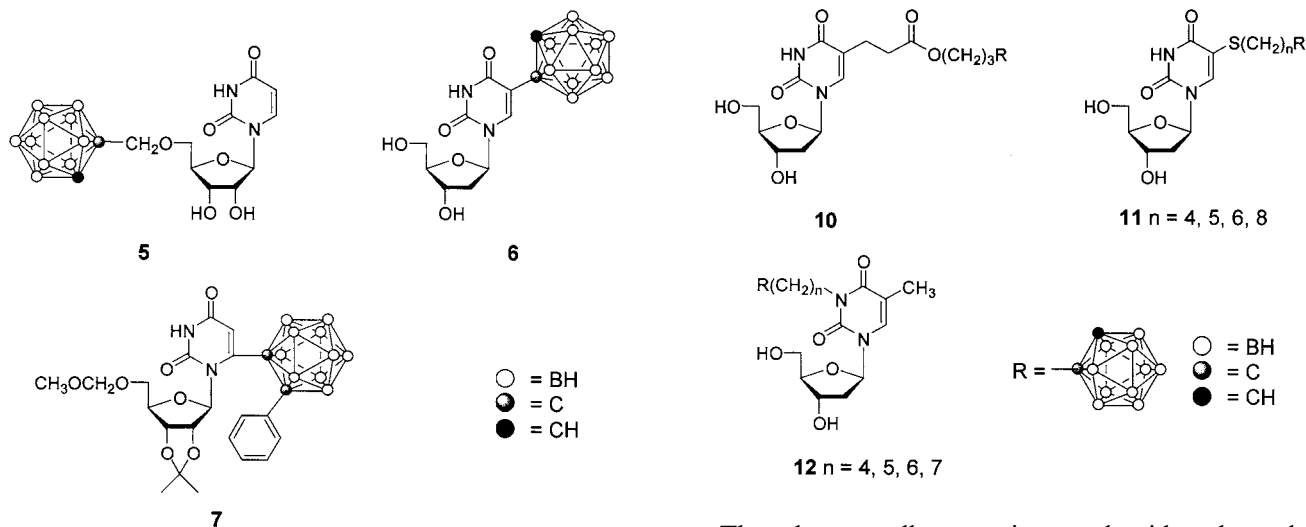
The chemical and hydrolytic stability of this compound has led to the synthesis of other carborane-containing nucleosides by us and others in which the carborane group is attached directly on or through a single methylene to the nucleoside. Among these that have been prepared are those in which this cluster was attached to the 3′,5′ carbons in the sugar moiety (**4** and **5**, respectively) [10], 5 and 6 carbons in the base (**6** and **7**, respectively) [11–13] of uridine and 2′-deoxyuridine.

Another approach in the use of boron clusters is based upon the observation that kinase binding to immobilized nucleic acid precursors was increased significantly by using a series of linked spacer atoms, which both insulate and project this precursor from the surface of the solid matrix. When this tether approximates 10 Å, by incorporating 6–8 methylene groups or a comparable chain containing isosteres of these methylenes, kinase binding was very appreciably increased. We applied this approach to the development of *o*-carborane-containing nucleosides [14–16]. The basis for using this approach, in part, is the fact that the *o*-carboranyl moiety is a bulky entity, being somewhat larger than the three-dimensional sweep of a phenyl group. This fact plus the observation that 5-carboranyl-2′-deoxyuridine (**6** = CDU) is not a very good substrate for human thymidine kinase [17], led to the placement of the *o*-carborane group at the end of a flexible chain attached to either the N3 or C5 position of deoxyuridine or thymidine. Examples of such structures that have been synthesized are **8**–**12**, as shown in the following [14–16]:

8 n = 2, 3, 4

9
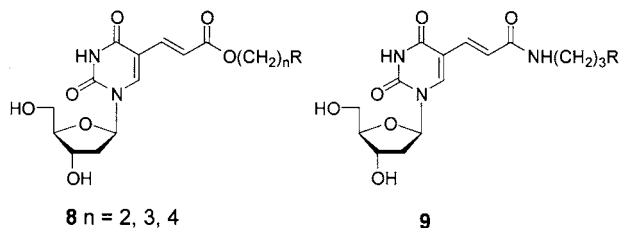
Though naturally-occurring nucleosides themselves are hydrophilic, incorporation of the carborane group greatly enhanced the compound's lipophilicity, to the point that evaluating the biochemical properties of these nucleosides was severely compromised due to their low aqueous solubility. In order to ameliorate this situation, a dihydroxypropyl group was attached to the non-tethered carbon atom of the *o*-carborane group. Among the examples of such structures that we have prepared are compounds **13** and **14** [17]:

13 X = CH$_2$CH$_2$ ○ = BH
**14** X = HC≡CH   ◑ = C
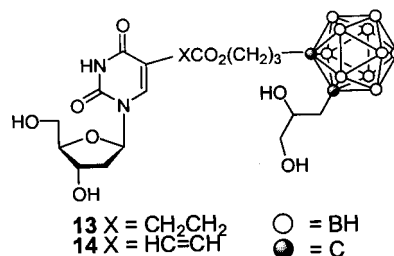
Though a higher percentage of boron in a nucleoside would appear desirable, of greater importance is that these boron analogues simulate more closely the naturally-occurring nucleosides in their biochemical attributes. In order to achieve this objective, we and others have attempted to replace the carbonyl function at the 4-position in the pyrimidine nucleus with the B–OH group. In essence, these may be viewed as being isosteres of naturally-occurring pyrimidines. In contrast with the earlier boron-containing bases where the boron atom was flanked by two nitrogen atoms [18,19], the aqueous stability of these compounds may well be enhanced since the boron atom has one carbon–boron bond. As a model for the parent structures, which to date have not been synthesized, and as the basis for determining the inherent stability of such novel heterocyclic rings, we and others have prepared and fully characterized the benzo bases **15** and **16** [20–23]:
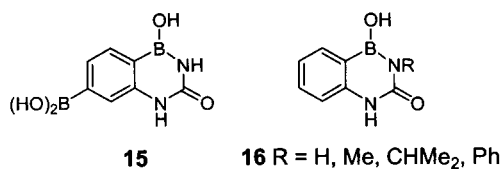
These results lend credence to the hypothesis that the boron analogues of the naturally-occurring bases may well be stable under physiological conditions, but their syntheses still remain to be accomplished.

Certainly relevant to this work is the question of whether nucleosides of such structures can be prepared in which the carbohydrate portion of the molecule can be attached to the appropriate nitrogen atom in the presence of the hydoxyboryl moiety. We have now succeeded [24] in synthesizing the nucleoside **17**:
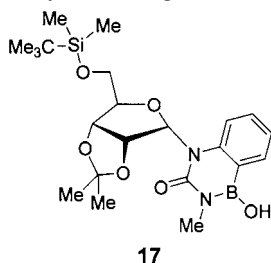
This result demonstrates the feasibility and stability of such boron-containing nucleosides.

2.2. Biochemical studies

Before evaluating boronated nucleosides for their in vivo properties in tumor-bearing animals, it is most pertinent to examine their function in vitro as substrates for kinases. In the case of those nucleosides that purportedly would mimic thymidine, the enzyme thymidine kinase (TK) is appropriate. Since this enzyme is responsible for the first phosphorylation of diketopyrimidine deoxynucleosides and their various analogues to the corresponding nucleotide, its use in screening these boronated nucleosides appeared compelling and a necessary prelude to any in vivo studies. Human TK was purified by affinity chromatography from an extract of human acute lymphoblastic leukemic T cells. The assay of enzymatic activity was based on a described radiometric procedure [25].


Thymidine kinase phosphorylation of tritiated deoxythymidine
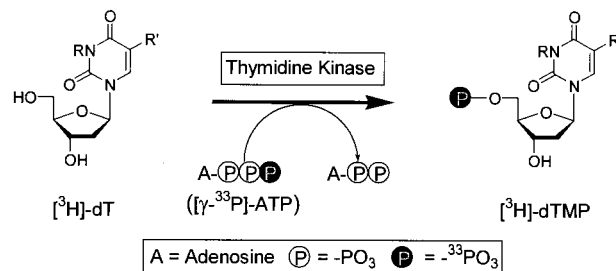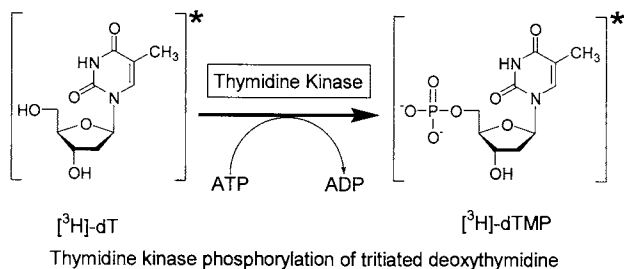
The use of $[\gamma\text{-}^{33}P]$-ATP in the thymidine kinase phosphorylation assays of carboranyl nucleosides

The rates of phosphorylation of the boronated nucleosides were compared against thymidine and deoxyuridine using $[\Upsilon\text{-}^{33}P]$-ATP as the phosphate donor [17]. The nucleotides produced were detected by their radioactivity and quantitated by β-scanning. Although the rates of phosphorylation of some tethered nucleosides appeared to be enhanced over CDU, they were not fully predictable. One of the major limitations with the initial compounds was their extremely low aqueous solubility requiring the use of methanol or DMSO. The more water soluble analogues were, in general, more readily phosphorylated than their more lipophilic counterparts. Of those substituted at the 5 position, 5-[2-[3-[2-(2′,3′-dihydroxypropan-1′-yl)-1,2-dicarbo-*closo*-dodecaboranyl]propoxycarbonyl]ethyl]-2′-deoxyuridine (compound **13**) more closely approximated the rate of conversion that was observed for 2′-deoxyuridine than any of the other analogues.

3. Discussion

Although flexibility of the side chain at the 5-position and increased aqueous solubility appeared to be translated into an increase in the rate of nucleotide formation for certain analogues, this approach is a very simplistic and empirical one. It did not take into account the percentage of the mitochondrial isozyme of TK present and certainly did not consider the shape and the physicochemical parameters of the active site within the cytosolic thymidine kinase molecule where phosphorylation occurs. It is well known that the substrate specificities of cytosolic TK and mitochondrial TK differ significantly [26]. Until these are delineated by obtaining a crystal structure of the mammalian enzyme docked to thymidine, there remain clear limitations in new drug design.

All of the structures, which have been discussed, are related to the diketopyrimidine deoxynucleosides. The very novel and highly intriguing research of Kool, et al. [27] in which the thymine base in thymidine is replaced by a difluorotolyl moiety may be worth examining in designing potential BNCT agents if such structures are phosphorylated in vitro to the corresponding nucleotide.

18
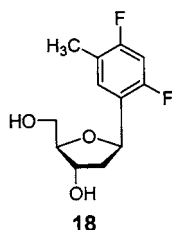
His results with **18** indicate that this nucleoside has a remarkable ability to promote nucleotide insertion into DNA that is comparable to that observed for thymidine itself. Apparently, it mimics the shape and conformation of the naturally-occurring nucleoside, if not its chemical composition. This observation may offer the possibility of synthesizing boron-containing purine nucleosides that may emulate naturally-occurring structures in their biochemical properties. This possibility is based in part upon the very great hydrolytic stability of the boron heterocycle, **19** [28].

19
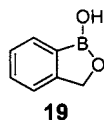
It may be useful to attach such a moiety to deoxyribose of a purine nucleoside. The following analogues and related compounds (**20–22**) are possibilities:

20   **21**   **22**
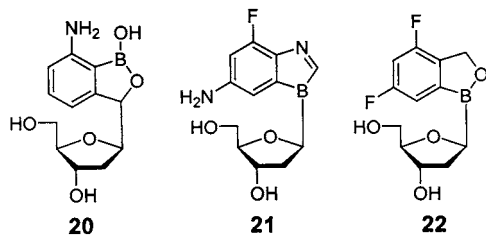
Obviously, before undertaking any synthetic approaches, it is essential that in computer-modeling studies that any structure simulates the shape of the naturally-occurring purine deoxynucleosides that it is attempting to mimic.

However, even if these boronated nucleosides are capable of being rapidly converted to their corresponding nucleotides by cytosolic thymidine or deoxycytidine kinase in vitro, that provides no assurance that these compounds will be useful as in vivo tumor-targeting agents for BNCT. In order to meet that objective, the compounds must be able to: be transported effectively through the vascular system that is supplying nutrients to the tumor; penetrate the tumor cell's membrane; and, at the very least, be metabolized within the cytosol to a structure that will not be freely diffusible, resulting in intracellular retention. That is no small task since there are many barriers that may impede the achievement of such a result. Among these, are the need to

attain a suitable lipophilic/hydrophilic balance within the compound. Compounds that are too hydrophilic may lack the ability to penetrate the tumor cell's membrane; while those that are too hydrophobic may be retained in the vascular system through nonspecific lipophilic binding. Even if a suitable balance is achieved within the molecule, there is no guarantee that a hydrophilic/lipophilic dipole would not compromise a compound's potential utility. Also, there is no assurance that the compound will resist being metabolically transformed within the vascular system and thereby never reach its tumor target. Another impediment may be its rapid extraction by nontumor structures such as the spleen, kidney and liver where metabolic transformation can occur. These organs have been instrumental in the past for the lack of success of other boron-containing compounds. Also, a contributing factor is competition from de novo synthesized precursors.

Probably the most critical limitation in the development and possible use of boron-containing nucleosides is not the compound per se but the very significant heterogeneity among the tumor cells themselves. Those which require DNA precursors in preparation for cell division are in S-phase and measuring the percentage of cells undergoing mitosis is a crude estimate of the proliferative activity within the tumor. The incorporation of bromodeoxyuridine (BUdR) has been the basis for determining the labeling index and even for the most highly malignant brain tumors, the range is from 5 to 20% [29]. Clearly, one cannot expect to label every single malignant cell by boron-containing nucleosides even under optimal conditions. Therefore at the start, one must view the development of such agents as merely one component to be used in concert with other boron compounds for targeting tumor cells. The objective is to attain 10^9 boron-10 atoms per cell using any combination of boron agents and among these, boronated nucleosides may well be important in achieving that goal.

The studies described above to identify, design and synthesize boron-containing nucleosides that will be effective substrates for human thymidine kinases is merely the first step in the drug discovery process. Subsequent steps, as a prelude to any animal irradiation tests, must include assessing in vitro uptake by tumor cells and in vivo pharmacokinetic studies in appropriate tumor-bearing animal models. These, together with an evaluation of the most promising compound's toxicity profile, will provide the basis for determining whether or not such a boron-containing nucleoside warrants further evaluation in clinical studies. The steps are many, with numerous possible pitfalls, in this overall process of identifying a potential new BNCT agent that will lead to its ultimate use in a clinical trial.

Acknowledgements

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