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Review

The use of boron clusters in the rational design of boronated nucleosides for neutron capture therapy of cancer

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Dedicated to Professor Sheldon G. Shore on the occasion of his 70th birthday.

Abstract

Boron neutron capture therapy (BNCT) is a chemoradio–therapeutic method for the treatment of cancer. It depends on the selective targeting of tumor cells by boron-containing compounds. One category of BNCT agents that has received extensive attention during recent years is boronated nucleosides. Such structures may be converted to the corresponding 5′-monophosphates by phosphorylating enzymes and thereby entrapped in tumor cells by the virtue of the acquired negative charge. This review analyzes previous design strategies applied in the synthesis of boron cluster-containing nucleosides and discusses possible future developments in this field based on existing knowledge of enzyme tissue expressions, enzyme substrate specificities, and contemporary trends in rational drug design.

Keywords: Boron; Boranes; Carboranes; Nucleosides; Neutron capture therapy; Rational drug design

1. Introduction

The development of ^{10}B-containing compounds for the treatment of cancer by boron neutron capture therapy (BNCT) requires the synthesis and evaluation of non-toxic agents that selectively target malignant cells in contrast with adjacent normal tissue and are retained intracellulary. The boron content localized within the tumor cells is activated by an external flux of thermal neutrons at the time of treatment generating highly destructive $^{4}He^{2+}$ and $^{7}Li^{3+}$ ions by a neutron capture reaction [$^{10}B(1n,\alpha)^{7}Li$]. These ions have path lengths of < 10 μm in biological tissue. Because of this limited range, the caused lethal damage is largely restricted to the tumor [1,2].

Recent studies [3] have shown that hyperosmotic mannitol induced blood–brain barrier disruption (BBB-D) combined with intracarotid administration of BSH and BPA, the two agents currently being used in clinical BNCT, [1,3] resulted in a 25% cure rate in rats having intracerebral implants of the F98 glioma [3]. Heretofore, the F98 glioma was refractory to chemotherapy, radiotherapy, and BNCT [4]. These promising results give rise to the assumption that successful BNCT can be achieved by using a mixture or 'cocktail' of different tumor-targeting boronated agents. Different boron-containing agents would target different tumor cell subpopulations (e.g. proliferating vs. nonproliferating, oxic vs. hypoxic), various subcellular compartments (e.g. nucleus, mitochondria, and lysosomes) as well as tumor blood vessels.

Tumor-seeking compounds utilized for the preparation of boronated agents appropriate for 'BNCT cocktails' were e.g. nucleic acid precursor, amino acids, peptides, phospholipids, carbohydrates, lipoproteins, porphyrins, phthalocyanines, DNA alkylator, DNA intercalator, DNA groove binders, polyamines, oligonucleotides, monoclonal–bispecific antibodies, growth factors, hormones, radiation sensitizers, cyclic thioureas, amines, and CNS depressants [1,2]. In most cases boron entities were attached as pendant moieties to these structures [1,2]. The boron clusters applied

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were mainly the carboranes **12**, **13** and **14**, the borane anions **4** and **6**, and *nido*-carborane **16**. Also, entities containing only one boron atom, such as the dihydroxyboryl group (**1**), amine adducts of e.g. BH_2CN and BH_2COOH (**2**), and the boranophosphate group (**3**) have been used frequently. Derivatives of compound **7** have been studied extensively as components of liposomal formulations [1,2]. Other boron clusters (**5**, **9**, **11**, **15**, **17**) were utilized in very few described syntheses [1,2] and structures **8**, **10**, and **18** have been proposed as potential boron moieties–precursors for BNCT agents but have not been applied as yet [5–7].

The minimum proviso for boron moieties selected for the synthesis of BNCT compounds is their stability under physiological conditions. This must be the case for the moiety itself as well as for the linkage to any tumor-targeting carrier. They should also be easily accessible in ^{10}B-enriched form and contain a large number of boron atoms. Lack of boron atoms is the major disadvantage of structures such as **1**, **2**, and **3**. Ideally, methods for their incorporation into organic–biochemical structures should be well developed and they should not confer unacceptable toxic properties onto the entire molecule due to their own specific nature. The boron clusters displayed in Fig. 1 fulfill these requirements only partly. The physicochernical properties and dimensions of these polyhedrons become decisive factors when they are used in the synthesis of low molecular weight tumor-targeting agents. Most widely applied is the *ortho*-$C_2B_{10}H_{12}$ cage (**12**) [1,2]. The space occupied by this carborane is about 50% larger than that of the phenyl group rotating about its C1–C4 axis [8]. Roughly the same applies to boranes **4** and **6**. The neutral *ortho*-carborane cluster is extremely hydrophobic and comparable to the adamantyl group in lipophilicity [9]. Numerous reports address the adverse affects that its size and lipophilicity have on the tumor-targeting ability of various carriers [10–12]. Enzyme inhibition, [13] water-insolubility, [11] high accumulation levels in the liver [12] and unspecific binding to membrane lipids [10] are frequently observed. Linking of the polyhedral borane anions displayed in Fig. 1 to complex biomolecules is difficult [14,15] and large-scale purification methods for such derivatives are not well

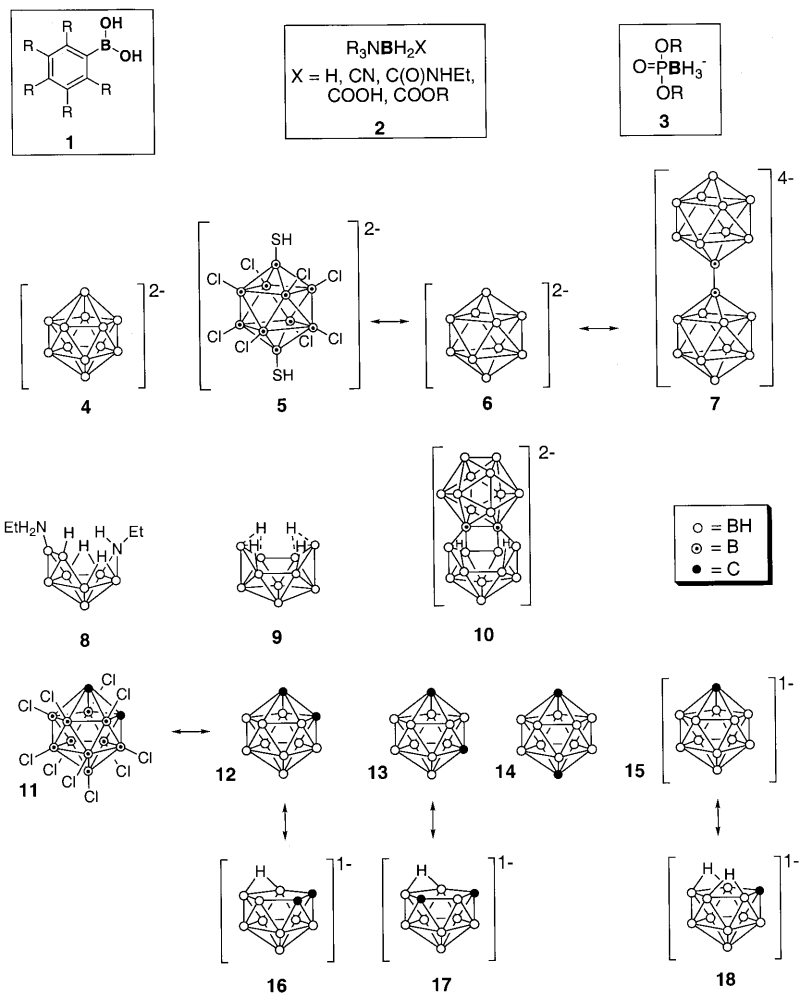

Fig. 1. Boron moieties used for the synthesis of BNCT agents.

developed [16]. The polyhedral borane moieties are charged species and their incorporation may significantly influence a compound's transport especially with respect to its ability to cross lipophilic barriers such as those in cell membranes or the blood–brain barrier.

In summary, there is a lack of variety among the boron entities that are accessible to the chemists involved in the design and synthesis of agents for BNCT. All frequently used boron clusters are comparable in their dimensions. They are either very hydrophobic or very hydrophilic. It is imperative that new boron pharmacophores be developed that provide a broader scope in size and physicochernical properties, not only for BNCT but also for pharmaceutical applications in general.

2. Boronated nucleosides

As will be outlined in detail in this manuscript, boronated nucleosides have the potential to be converted to the corresponding 5′-monophosphates by phosphorylating enzymes. Consequently, they may be entrapped in malignant cells due to a negatively charged phosphate group. As a result of their potential uptake by tumor cells, extensive efforts in the design and synthesis of BNCT agents have been focused on the development of boronated nucleosides. As for BNCT chemistry in general, a major dilemma in the development of boronated nucleosides has been the lack of appropriate boron moieties. By far most approaches have utilized the *ortho*-carborane cage (**12**) [17–20]. Only five pyrimidine nucleosides containing a *nido*- (**43**–**47**) [21–23] and one pyrimidine nucleoside with a *para*-carborane cage (**28**) [21] have been described. The syntheses and evaluations of several purine and pyrimidine nucleosides have been reported possessing amine adducts of cyanoborane (**2**), [24–31] the dihydroxyboryl group (**1**), [32] or a single boron atom incorporated into the pyrimidine ring structure [33]. In the following sections, a detailed description of enzymes that may be involved in the phosphorylation of carboranyl nucleosides and the design strategies used in the past for their development will be presented. A short section about nucleosides containing single boron atoms will also be included.

2.1. Nucleoside phosphorylating enzymes

For a rational design of boron-containing nucleosides, in-depth knowledge of the tissue expression and substrate specificity of their potential target enzymes are of crucial importance. There are three types of enzymes that phosphorylate nucleosides. One group, the nucleoside kinases, utilizes nucleoside triphosphates such as ATP as phosphate donors in a direct phosphorylation reaction. The second class, phosphotransferases or 5′nucleotidases, catalyzes the phosphorylation reaction via a phosphorylated enzyme intermediate using nucleoside monophosphates as donors. In rare cases, protein kinases have been implicated in the phosphorylation of nucleosides such as ganciclovir [34–37].

Enzymes of these three classes play pivotal roles in the activation of anticancer and antiviral drugs such as e.g. ara-C, gemcitabine, AZT, ddC, acyclovir, ganciclovir, and abacavir [34,35,38,39]. Irrespective to the fates and final targets of these agents, the key activation step is invariably the initial conversion of the nucleoside to the corresponding monophosphate by a phosphorylating enzyme. In some cases, both kinases and phosphotransferases have proven to initiate drug activation through phosphorylation [40].

It is for this reason that phosphorylating enzymes are also potentially very important targets for BNCT. Boronated derivatives of nucleosides may enter malignant and benign cells via facilitated transport or passive diffusion. Derivatives that are good substrates for phosphorylating enzymes may be retained intracellulary following their conversion to the monophosphates due to the acquired negative charge. The minimum proviso for a selective uptake of a boronated nucleoside in malignant versus benign cells is that one or more phosphorylating enzymes with substrate specificity for such a nucleoside have elevated activity levels in malignant cells. Further, conversion of a deoxyribonucleoside monophosphate to the di- and triphosphate and the subsequent incorporation of the latter into tumor cell DNA could result in the relocation of boron in close proximity to DNA, the most critical target of the $^{4}He^{2+}$ and $^{7}Li^{3+}$ ions. This would enhance the effectiveness of the [$^{10}B(n,\alpha)^{7}Li$] reaction significantly [41]. However, the initial intracellular entrapment due to phosphorylation may be sufficient, even if boronated deoxyribonucleosides were not incorporated into DNA. Boronated ribonucleosides may be phosphorylated by ribonucleoside kinase but DNA incorporation of the corresponding nucleotides should not be possible [35b].

Although high specific activity in proliferating cells has been reported for cytosolic phosphotransferase, [42] there is generally little information available about activity levels in tumor cells and substrate specificities for this class of enzymes. The same is true for protein kinases with respect to their nucleoside phosphorylation capacities [34,35]. However, in the case of the deoxyribonucleoside kinases, detailed information both about tissue expression and substrate specificity has become available in recent years [34,35,43]. There are four principle kinases of the deoxyribonucleoside salvage pathway: cytosolic thymidine kinase (TK1), mitochondrial thymidine kinase (TK2), 2′-deoxycytidine kinase (dCK), and 2′-deoxyguanosine kinase (dGK). The ex-

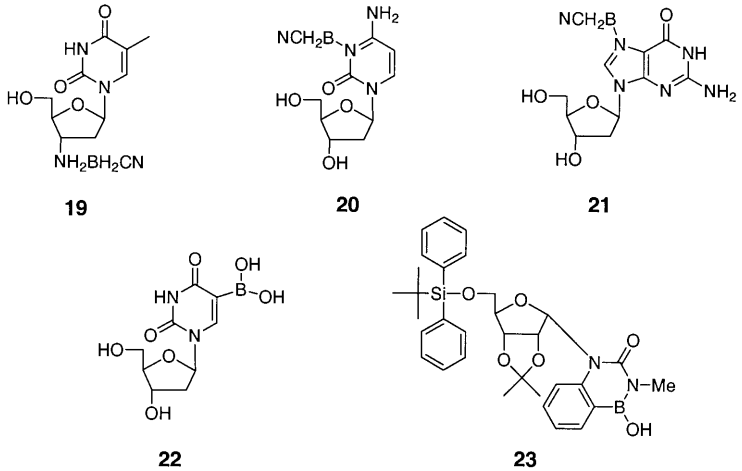

Fig. 2. Nucleosides containing a single boron atom.

pression of TK1 is tightly regulated during the cell cycle and the active enzyme is found only in S-phase cells [44–47]. TK1 is widely distributed and expressed in all proliferating neoplastic cells but it is virtually absent in normal tissue [48–52]. Unlike TK1, dCK is not cell cycle regulated. It is expressed by a wide variety of cell types [53–57]. Various malignant tumors showed a two- to five-fold increase of dCK levels compared to the corresponding normal tissues [35,57–60]. At present, it is not known if certain types of tumors contain high levels of TK2 or dGK activity. These enzymes are localized predominantly in mitochondria and appear to be equally active in proliferating and nonproliferating cells at low to moderate levels [35]. Thus, among all nucleoside–phosphorylating enzymes, TK1 and dCK appear to be primary targets of boronated nucleosides for BNCT and knowledge about the substrate specificity of both enzymes becomes indispensable for the rational design of such derivatives.

Previous studies have indicated that TK1 has the most stringent substrate specificity among all deoxyribonucleoside kinases allowing only phosphorylation of native thymidine–2′deoxyuridine and, to a limited extent, analogs with minor modifications at either the 5-position (Cl, Br, I) or the Y-position (N3, F) [34,35,43]. dCK phosphorylates numerous cytidine analogs with 2′- and Y-modifications, acyclic sugars, and minor changes at the 5-position of the cytosine base. 2′-Deoxyadenosine, 2′-deoxyguanosine, and 2-modified 2′-deoxyadenosines are also excellent substrates for dCK. Based on similarities in their gene sequences, deoxyribonucleoside kinases have been classified into two distinct categories: (a) The poxvirus, bacterial and cytosolic thymidine kinases (TK1), and (b) the herpes virus kinases, dCK, TK2 and dGK [61,62]. Human, chinese hamster, mouse, and chicken TK1s show a high degree of homology with only few substitutions in their amino acid sequences [62]. To some degree, the differences in amino acid sequences between the two types of kinases correlate also with differences in substrate specificities of the enzymes in both groups [34,35,43,63].

2.2. Nucleosides containing a single boron atom

The synthesis of several cyanoborane nucleosides and the in vitro and in vivo evaluation of their cytoxic, anti-neoplastic, anti-inflammatory, anti-osteoporotic, and hypolipidemic activities were described in detail by Spielvogel and his associates [24–31,52]. A representative selection of this type of compounds is displayed Fig. 2 (**19**, **20**, **21**). In vivo biodistribution studies in tumor bearing mice showed tumor selectivity for compound **20** indicating that it could be a suitable candidate for BNCT [24]. Compound **22** is the most prominent representative of the nucleosides possessing dihydroxyboryl groups [32]. It's potential as a BNCT agent has been extensively studied. About 1% replacement of thymidine by **22** was observed in cellular DNA in vitro [20]. In vivo studies with a munine melanoma model revealed $\sim$1.8% thymidine replacement in the tumor [20]. Recently, Soloway and his associates have reported the synthesis of the first boron-containing nucleoside with a single boron atom incorporated into the pyrimidine ring structure (**23**) [33]. This benzoborauracil nucleoside is a model compound demonstrating that such structures can be prepared and are stable. The synthesis of borapyrimidine or borapurine nucleosides corresponding more closely to natural nucleosides remains a highly desirable but unachieved target since there is evidence that enzymes involved in DNA synthesis appear to operate mainly based on structural substrate recognition [64].

2.3. Nucleosides modified with a closo-carborane cage at the ribose portion

Initial in vitro experiments [23] using F98 glioma cell showed that uridines **24**–**26** were taken up to a significantly higher extend and persisted longer in these cells than the clinically used BSH. Unfortunately, more detailed studies [12] revealed that the observed uptake was based on a nonspecific interaction of the highly lipophilic carborane cage with the cell membrane rather than any type of specific tumor cell metabolism. The syntheses of thymidines **27** and **28** were recently reported [21]. Phosphoryl transfer assays using recombinant TK1 showed a low phosphorylation velocity for compound **27** indicating that the Y-position of thymidine is a preferred site for structural modifications that are tolerated by this important kinase of the nucleoside salvage passways. Compound **28**, the first nucleoside containing a *para*-carborane cage and structurally very similar to **27**, was not a substrate for TK1. This clearly demonstrates the narrow window for any type of modification that this enzyme can accept (Fig. 3).

2.4. Nucleosides modified with a closo-carborane cage at the base portion

Major efforts in the synthesis of boronated nucleosides by Schinazi and Yamamoto and their associates have focused on the attachment of an *ortho*-carborane cage to the 5-position of natural and unnatural uridines both with respect to an application in BNCT and antiviral therapy [18–20,22,65–69]. The motivation for this strategy certainly stems in part from the fact that 5-modified pyrimidine nucleosides such 5-iodo- and 5-bromo-2′deoxyuridine are excellent substrates for the enzymes involved in DNA synthesis. Indeed, both compounds are able to replace thymidine in DNA to a high extent [70].

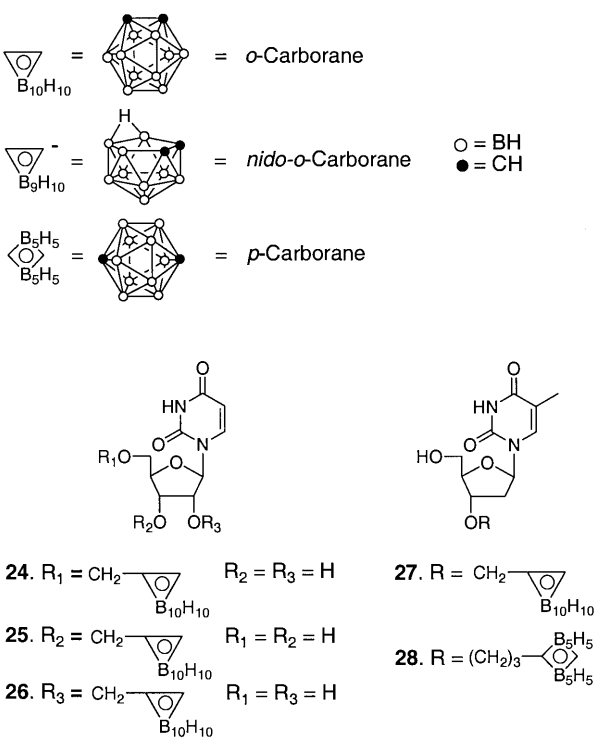

Fig. 3. Sugar-modified *closo*-carboranyl nucleosides.

Many of the compounds **29**–**40** have been synthesized in form of the D-, and L-enantiomers and/or the α- and β-anomers and were tested for cytotoxicity, anticancer, and antiviral activity in numerous cell lines. The results have been summarized in some recent reviews and publications [18–20,22,65–69]. Compound **33**, originally synthesized by Yamamoto et al. [71] has undergone a particularly thorough evaluation [18–20,65,66]. Previous in vitro studies have shown that 2′-deoxyuridine **33** has a low toxicity in human lymphoblastoid CEM cells, human U-251 glioma cells, and rat 9L glioma cells [18]. The compound is phosphorylated to a low degree in CEM and PBM cells [18]. Phosphoryl transfer assays carried out with recombinant TK1 and TK2 indicate a low phosphorylation rate for **33** with recombinant TK2 [8]. The compound is not a substrate for TK1 [8]. It was suggested that cellular uptake of **33** may be mediated by a nucleoside base transporter [65]. The treatment of 9L rat brain tumors by neutron capture therapy after i.p. application of **33** was also described [66]. The compound was not toxic to Fischer rats after i.p. administration of 150 mg Kg^{-1}. Two hours after i.p. administration of 30 mg Kg^{-1} and 150 mg Kg^{-1} boron, respectively, tumor boron concentrations of 2.3 μg (0.43 μg ^{10}B) boron/g tissue and 7.4 μg (1.38 μg ^{10}B) boron/g tissue, respectively, were found. Tumor to normal brain ratios were 11.5 and 6.8, respectively. Neutron irradiation was carried out 2 h after compound administration (i.p.). All animals of the untreated control group died within 28 days. Half of the animals survived for 32 days in the group receiving neutron radiation only, 55 days in the group receiving neutron irradiation and 30 mg Kg^{-1} compound, and 38 days in the group receiving neutron irradiation and 150 mg Kg^{-1} compound. Compounds **41** and **42** were never deprotected and their potentials as BNCT agents were therefore never tested (Fig. 4).

2.5. Nucleosides modified with a nido-carborane cage

The syntheses and biological evaluations of a limited number of *nido*-carboranyl pyrimidine nucleosides have been reported [21–23]. The disadvantage of attaching a negatively charged cluster to a nucleoside is obvious: It is questionable whether such a structure is a substrate for any membrane nucleoside transporter and it is most unlikely to pass cell membranes by passive diffusion. In

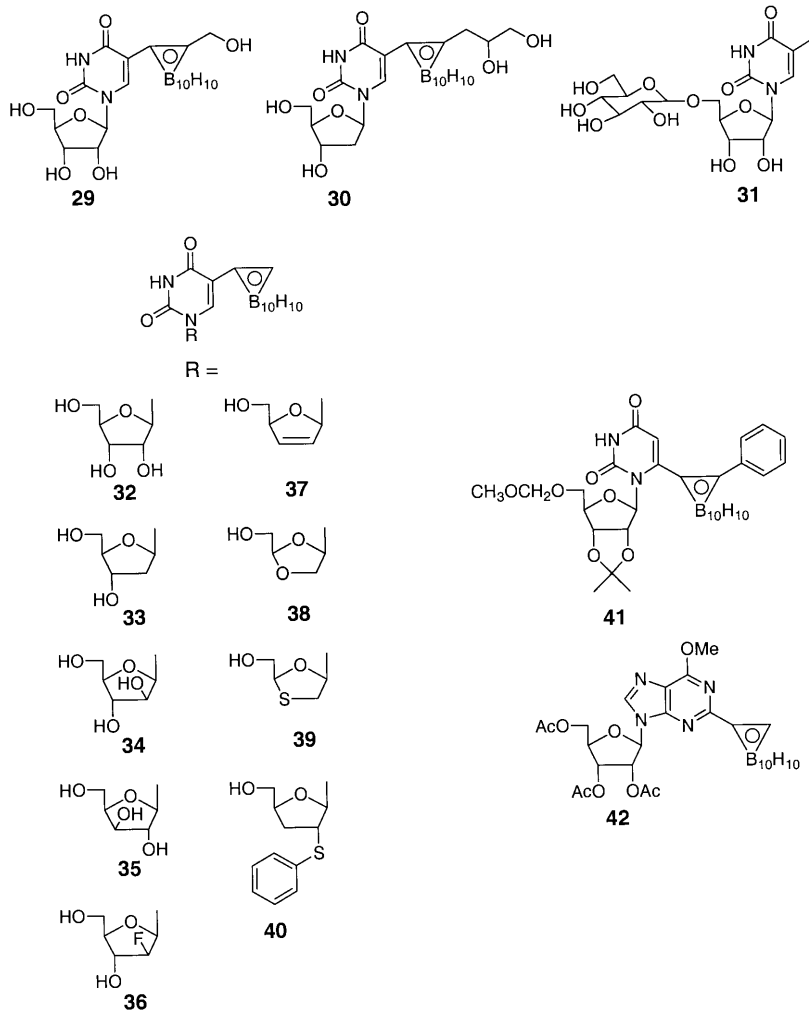

Fig. 4. Base-modified *closo*-carboranyl nucleosides.

contrast to its *closo*-counterpart **24**, F98 glioma cells did not take up compound **46** in vitro [23]. Consequently, it did not show any cytotoxicity in this cell line. A very similar uptake pattern was recently reported for the *nido*-form of compound **33**, which reached just 5% of the uptake of its *closo*-counterpart in CEM cells [65]. No synthetic procedure for *nido*-**33** has been described as yet.

Thymidine derivative **47** was not a substrate for recombinant human TK1 [21]. In vitro uptake and cytotoxicity of compounds **43**–**45** were studied in munine B16 melanoma and human TIG-1-20 fibroblast cells [22]. Uptake levels for these compounds were relatively high and comparable to those of the *closo*-carboranyl counterparts **32**, **39**, and **40** while the cytotoxicity was significantly lower (Fig. 5).

2.6. Tethered ortho-carboranyl nucleosides

Tethered *ortho*-carboranyl pyrimidine nucleosides were prepared and evaluated mainly by Soloway and

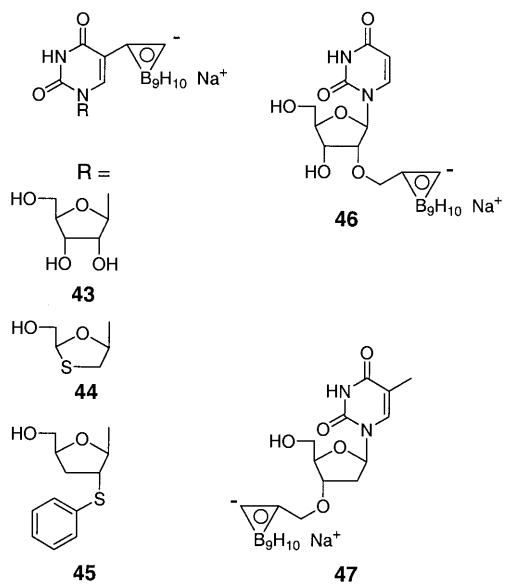

Fig. 5. *Nido*-carboranyl nucleosides.

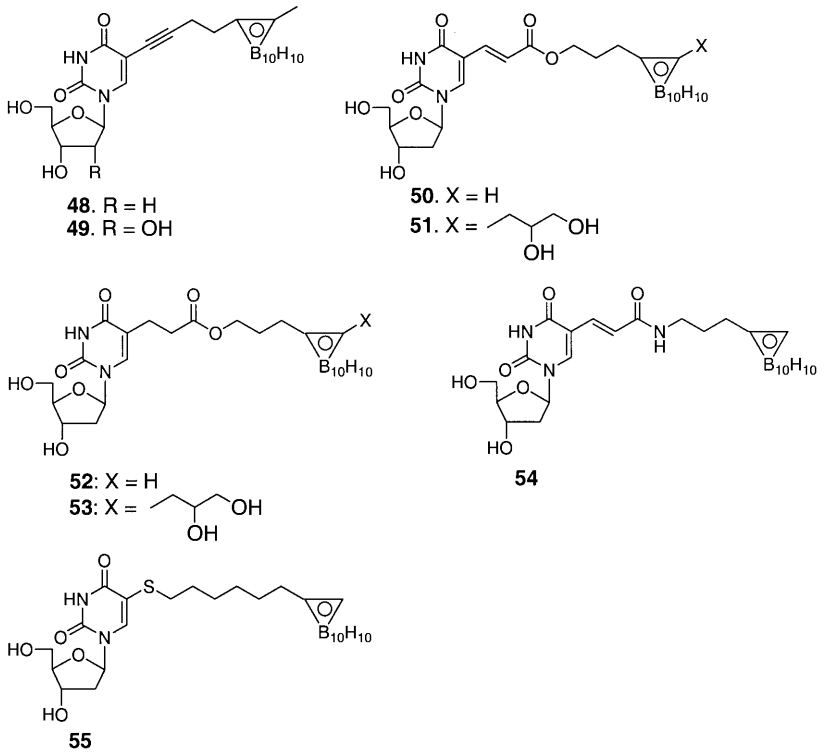

Fig. 6. C-5 tethered *ortho*-carboranyl nucleosides.

his associates [8,13,72,73]. A representative selection of this type of compounds is displayed in Fig. 6 Fig. 7. Compounds **48** and **49** were synthesized by Kabalka et al. [74]. Compared with nucleosides presented in the previous sections, there are two significant differences in the design and evaluation strategies applied for compounds **48**–**57**. (a) A linker was inserted between the carborane cage and nucleoside scaffold to reduce possible steric interference in the binding of carboranyl nucleosides with target enzymes because of the proximity of the bulky carborane moiety on the nucleoside. All base-substituted nucleosides described in the previous section possess the *ortho*-carborane cage directly attached to the base component. The concept of using a spacer to reduce steric hindrance has proven useful in the application of affinity chromatography that exploits the binding of enzymes to substrates covalently linked to a solid support matrix [75]. (b) The TK1 and TK2 substrate characteristics of compounds **50**–**57** were evaluated in phosphoryl transfer assays using recombinant and purified enzyme preparations. These compounds have not been tested in cell culture or tumor–animal models as of yet. The rationale for this approach stems from experiences obtained in earlier in vitro and in vivo studies with compounds **24**–**26** and **46** [23]. These experiments were expensive and very time consuming. Eventually, the obtained results revealed that nucleosides **24**–**26** and **46** were not suitable candidates for BNCT. However, there were many pitfalls to overcome. Interpretation of the results did not provide a direct answer to the single most important question in the design and evaluation of boronated nucleosides: Are these compounds substrates for phosphorylating enzymes involved in DNA synthesis that exhibit elevated activity levels in malignant cells?

2.6.1. C-5 tethered ortho-carboranyl nucleosides

The *ortho*-carborane cage was linked to the 5-position of 2′-deoxyuridine and uridine via propynyl- (**48**, **49**), [74] vinylester- (**50**, **51**), [13,72,73] propylester- (**52**, **53**), [13,72,73] vinylamide- (**54**), [13,72,73] and thioether

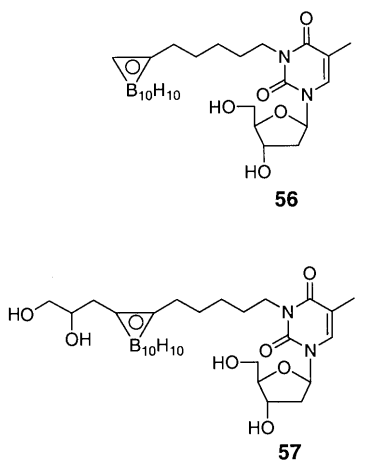

Fig. 7. N-3 tethered *ortho*-carboranyl nucleosides.

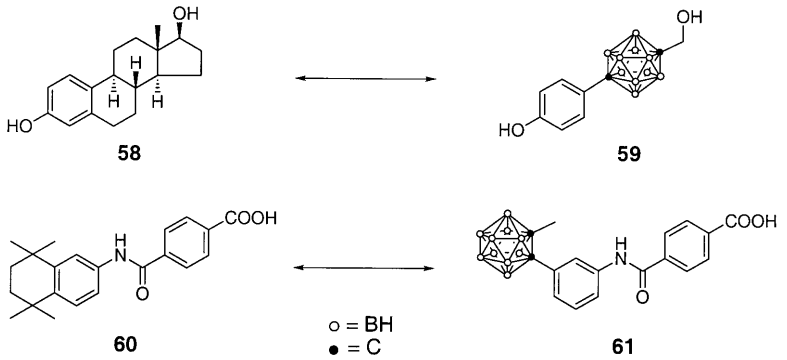

Fig. 8. 17β-Estradiol, retinobenzoic acid Am80, and their *ortho*-carboranyl analogs.

linkers (**55**) [8]. In some cases, a dihydroxypropyl group (**51**, **53**) was attached to the second carbon atom of the carborane cage to moderate the extreme lipophilicity of this cage structure. The phosphorylation velocities of nucleosides **50**–**55** were evaluated in comparison with **30** and **33** using recombinant TK1 and TK2. Some compounds (including **33**) showed low phosphorylation rates with TK2. The best values were obtained for compounds of the 'thioether linker' type (**55**) approaching 2% of the thymidine phosphorylation velocity in TK2 [8,76]. The results were inconclusive with respect to the validity of the tether concept [8]. None of the tested compounds were substrates for TK1 indicating that it does not tolerate bulky carboranyl groups at the 5-position of 2′-deoxyuridine, neither tethered nor directly bound [8].

2.6.2. N-3 tethered ortho-carboranyl nucleosides

Carboranylalkyl substituents of various lengths were also bound to the *N*-3 position of thymidine since in the affinity chromatography studies mentioned above, thymidine was most likely linked through this position to the stationary phase [75]. Two representative structures are displayed in Fig. 7. As in the case of the 5-substituted carboranyl 2′deoxyuridines (**50**–**55**), some compounds tested with TK2 showed low phosphorylation rates indicating that it may not be susceptible to this type of thymidine modification [8]. However, in the case of TK1, phosphorylation velocities measured approached 40% for thymidine [8,76]. These values are comparable with those found for AZT with TK1 under comparable experimental conditions [43]. The described phosphoryl transfer assays with N3 tethered carboranyl thymidine derivatives using human TK1 have for the first time shown that substantial modification at the N-3 position of thymidine is well tolerated by TK1. Previous studies demonstrated that N-3 substituted thymidine derivatives are potent inhibitors of thymidine phosphorylation by TK1 [77,78]. As in the case of the 5-substituted 2′deoxyuridines and TK2, the results obtained for N-3 substituted thymidines with TK1 were inconclusive with respect to the validity of the tether concept [8].

3. Summary and discussion

Results obtained in phosphoryl transfer assays with human TK1 and TK2 indicated that 5-substituted *ortho*-carboranyl pyrimidine deoxyribonucleosides, whether or not tethered, are not substrates for TK1 and, if at all, appear to be poor substrates for TK2. Considering the substrate specificities of the two categories of kinases (Section 2.1), 5-substituted *ortho*-carboranyl pyrimidine deoxyribonucleosides may not be appropriate substrates for any type of kinase that plays an important role in cancer or viral chemotherapy. Phosphotransferases or protein kinases may phosphorylate these compounds. A differential uptake in malignant versus normal cells could be the result of increased nucleoside transporter activity in tumor cell membranes provided the compounds are substrates for such proteins and ribonucleoside kinase may phosphorylate 5-substituted *ortho*-carboranyl pyrimidine ribonucleosides. However, considering presently available information, there seems to be no obvious biochemical cellular or viral target for 5-substituted *ortho*-carboranyl pyrimidine deoxyribonucleosides that would make them candidates for BNCT or antiviral therapy.

Based on existing knowledge of enzyme substrate specificities, future design strategies involving the attachment of polyhedral boranes, carboranes, and other boron moieties to a nucleoside scaffold should explore more intensively various possible binding sites at nucleosides. N-3 substituted thymidines are obviously good substrates for TK1, a prime target for BNCT as well as conventional chemotherapy of cancer and viral diseases. The evaluation of the potential of N-3 substituted carboranyl thymidines as BNCT agents has just begun. dCK is also a promising target for BNCT nucleosides and increased efforts in the rational design, synthesis, and evaluation of potential dCK substrates, in particular boronated cytidines and purines, are highly desirable.

Also important for a successful application of boronated nucleosides in clinical BNCT seems to be the utilization of alternative design strategies and alternative boron moieties. Recently, Endo and his associates have

described the synthesis of carboranyl derivatives of retinoids as well as estrogen agonists and antagonists [79–83]. Fig. 8 displays examples of the synthesized structures. Obvious is the structural resemblance between the carboranyl derivatives **59** and **61** their parental counterparts 17β-estradiol (**58**) and the retinobenzoic acid Am80 (**60**). Both **59** and **61** exhibit significant retinoidal and estrogenic activities, respectively. This is the first time that carborane cages have been used as hydrophobic pharmacophores to replace entire structural segments in biologically active molecules rather than as pendant groups (Fig. 9).

Boron clusters **62** and **63** have been proposed for similar use in drug design based on their steric properties which approximate those of the C_{60} cluster [84,85]. There is similarity between Endo's strategy and that used by Soloway in the synthesis of borauracil nucleosides where a single carbon atom is replaced as a structural element by a boron atom [17,33]. It is however, difficult to imagine how a carborane cage or any other polyhedral boron cluster could effectively replace structural elements within nucleoside scaffolds. There is some evidence [86] that smaller boron moieties than the *ortho*-carborane may be superior as pendant groups for N-3 substituted thymidines. The situation may be similar in the case of other nucleosides. Some smaller boron clusters with potential as BNCT moieties containing several boron atoms are depicted in Fig. 10.

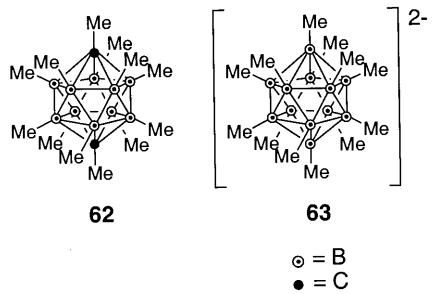

Fig. 9. Methylated boron cluster.

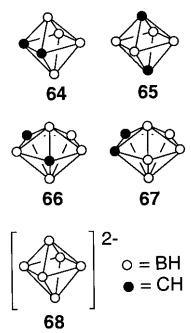

Fig. 10. 'Small' boron cluster with potential as boron moieties for BNCT.

The derivative chemistry of compounds **64**–**67** appears to be similar to that of the larger $C_2B_{10}H_{12}$-carboranes [87–90] and their steric properties are closer to those of structural elements usually found in naturally occurring molecules. Unfortunately, many of the smaller carboranes are hydrolytically unstable. Silylated derivatives of 2,3 dicarbahexaborane (**64**) proved to be unstable under physiological conditions as demonstrated by NMR studies [91] and 2,4–dicarbaheptaborane (**66**) reacted rapidly with water at room temperature [92]. However, 1,6-dicarbahexaborane (**65**) [92,93] and hexaborane (2-) (**68**) [94] were found to be sufficiently stable under aqueous conditions and the same may be true for 2,3-dicarbaheptaborane (**67**) [95]. Presently, it is not certain if compounds **65**, **67**, and **68** will be useful boron moieties for BNCT. Indeed, it can be anticipated that the development of small boron clusters that are suitable as pendant entities for nucleosides will be very difficult but, in the event of success, highly rewarding.

Computer-aided molecular design will certainly become a decisive tool in the development of boronated nucleosides. Unfortunately, neither NMR nor X-ray data of the three-dimensional structures for human TK1 and dCK are available as yet. However, low-resolution X-ray structures of herpes simplex virus 1 thymidine kinase (HSVI TK) with various substrates are available [96–100]. Studies have shown that HSVI TK, dCK, TK2, and dGK contain five out of six regions of strong sequence homology including the substrate-binding pocket [61,101–104]. TK1 is genetically and biochemical very different from HSVI TK, dCK, TK2, and dGK and it was concluded that a better understanding of its unusual substrate specificity would depend on the availability of crystallographic information [61,103]. Thus, the active site of HSVI TK may serve as an appropriate model for the structure of dCK and for the development of homology models of this kinase but not for TKl.

Important factors for the future design of boronated nucleosides are test systems that will be employed for their biological evaluation. Cell culture experiments focusing on e.g. uptake, cytotoxicity, and subcellular distribution remain to be essential in the selection of those compounds that are suitable for clinical studies as will be in vivo toxicity, uptake, persistence, and pharmacokinetic experiments with various animal–tumor models. However, biochemically-oriented high capacity screening systems will gain more importance. Relatively inexpensive phosphoryl transfer assays using recombinant preparations of human TK1 and TK2 have proven to be highly efficient in the selection of boronated nucleosides for further evaluation or from the exclusion thereof [8]. More traditional in vitro and in vivo evaluation methods used in BNCT research

cannot provide a comparable efficacy in an early stage of compound development and it appears to be desirable to develop and apply more biological screening systems that operate on the level of the most fundamental biochemical mechanisms for a selective uptake of BNCT agents in tumor cells.

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