Preparation and Biological Evaluation of ^{10}B-Enriched 3-[5-{2-(2,3-Dihydroxyprop-1-yl)-*o*-carboran-1-yl}pentan-1-yl]thymidine (N5-2OH), a New Boron Delivery Agent for Boron Neutron Capture Therapy of Brain Tumors

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3-[5-{2-(2,3-Dihydroxyprop-1-yl)-*o*-carboran-1-yl}pentan-1-yl]thymidine (compound **1**, N5-2OH) belongs to a novel class of boron delivery agents for neutron capture therapy, which was designated 3-carboranylthymidine analogue (3CTAs). Two shorter and more convenient synthetic routes were developed for the synthesis of **1** in the ^{10}B-enriched form, which is necessary for its preclinical and clinical evaluation in neutron irradiation studies. For more insight on structure−activity relationships, various stereochemical and geometrical isomers of **1** were synthesized and their specificities as substrate for human thymidine kinase 1 (hTK1) were evaluated. A computational model for the binding of various isomers of **1** to the active site of hTK1 was developed. Preliminary studies carried out in F98 glioma bearing rats that had received a ^{10}B-enriched form of **1** followed by neutron irradiation demonstrated a significant prolongation in survival times compared to control animals, suggesting that further studies are warranted to evaluate the therapeutic potential of **1**.

Introduction

Glioblastoma multiforme (GBM), the most malignant type of all primary brain tumors, remains resistant to conventional cancer therapies.[1] The average survival of patients diagnosed with GBM is about 1 year.[2,3] The failure of current treatments to cure GBM patients is primarily due to the lack of eradication of microinvasive tumors cells within the brain.[4,5] Therefore, there is an urgent need to develop novel therapeutic strategies for GBM. Boron neutron capture therapy (BNCT) has been considered as a promising modality for the treatment of malignant brain tumors.[6,7] BNCT is based on the irradiation of the boron-10 isotope (^{10}B) with thermal neutrons producing high linear energy transfer (LET) particles: $^{4}He^{2+}$ (α) and $^{7}Li^{3+}$ ions. These particles have destructive path lengths of only 5−9 μm, which approximate one cell diameter. Therefore, brain tumor cells can be destroyed by BNCT with minimal damage to the surrounding normal tissues provided they selectively accumulate ^{10}B-containing compounds. For BNCT to be effective, the minimum amount of the ^{10}B isotope that must be retained in the tumor is ∼20 μg/g tumor tissue combined with a tumor to blood ratio or normal tissue ratio of >4:1.[6,7]

Thymidine analogues substituted with a bulky carborane cluster at the N-3 position (3CTAs[a]) have been synthesized and evaluated as boron delivery agents for BNCT because of their potential metabolic accumulation in tumors cells by the action of human thymidine kinase 1 (hTK1).[8−14] Human TK1 is a cytosolic deoxyribonucleoside kinase in the salvage pathway of DNA synthesis, and its function is 5′-monophosphorylation of thymidine (Thd) and 2′-deoxyuridine (dUrd).[15] The activity of this kinase is very low in G_1 and G_0 cells, increases at G_1/early S boundary, and reaches maximum values in late S phase/G_2 during the cell cycle.[16,17] High hTK1 activity has been found in a variety of cancers, including brain tumors.[13,14,18,19]

Recently, we have demonstrated that 3CTAs were good substrates for hTK1, which apparently led to their selective accumulation in the tumor cells both in vitro and in vivo.[8−14] Among the 3CTAs that were evaluated as BNCT agents, 3-[5-(2-(2,3-dihydroxyprop-1-yl)-*o*-carboran-1-yl)pentan-1-yl]thymidine[13] [**1** (N5-2OH[13]) in Figure 1] was identified as a lead compound because of its overall superior biological activities compared with other 3CTAs, although it is poorly water-soluble.[13,14]

The isotopes of the element boron occur with a natural abundance of 19.8% for ^{10}B and 80.2% for ^{11}B. Only the former has the capacity to effectively capture thermal neutrons and thus can be utilized for BNCT.[7] To reduce their administered quantities by a factor of 4 and consequently decrease systemic toxicity, clinical BNCT agents, such as *p*-boranophenylalanine (BPA) and sodium borocaptate (BSH) (Figure 1), are usually synthesized in the ^{10}B-enriched form.[6,20]

Previously, we have reported a laborious 10-step procedure for the synthesis of **1** with natural boron isotope abundance and as a mixture of two stereochemical isomers, which have (*R*)- and (*S*)-configuration at the C-16, marked with an asterisk (∗) in the structure of **1** (Figure 1).[12] Here, we describe novel synthetic routes more pertinent for the synthesis of **1** in the ^{10}B-enriched form (compound **1B**) from commercially available ^{10}B-enriched *o*-carborane and decaborane. Both stereochemically pure forms of **1** (compounds **10A** and **10B**) as well as geometrical isomers derived from *m*- and *p*-carborane (compounds **11** and **12**, respectively) were prepared and evaluated to complete the structure−activity relationship (SAR) studies of this

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[a] Abbreviations: 3CTAs, 3-carboranyl thymidine analogue; CaTK, *Clostridium acetobutylicum* thymidine kinase; CED, convection-enhanced delivery; dUrd, 2′-deoxyuridine; hTK1, human thymidine kinase 1; Thd, thymidine; TTP, thymidine triphosphate; UuTK, *Ureaplasma urealyticum* thymidine kinase.

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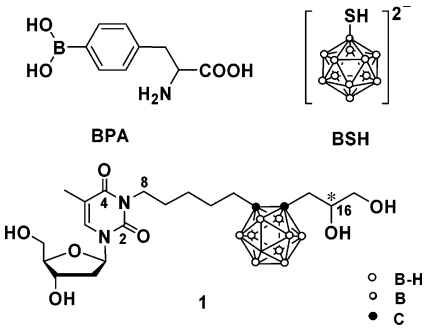


Figure 1. Structures of BPA, BSH, and **1**.

3CTA series. Computational models for the binding of **10A**, **11** ((*R*)-epimer), and **12** ((*R*)-epimer) to the active site of hTK1 were also developed. The results of pilot neutron irradiation studies with rats bearing intracerebral F98 glioma that were treated with **1B** prior to irradiation are reported.

Chemistry

The original synthesis of **1** in naturally occurring boron-isotope abundance was reported by Al-Madhoun and co-workers.[12] Compound **1** was produced in a lengthy 10-step synthetic sequence from 3-heptyn-1-ol. Although this procedure was suitable for the preparation of sufficient quantities of **1** and several its homologues for initial structure−activity relationship (SAR) studies, it was found to be inefficient and costly for the synthesis of **1** in ^{10}B-enriched form (**1B**). Another limitation of the original synthetic scheme was the fact that it cannot be applied for the synthesis of pure stereochemical (**10A**, **10B**) and geometrical isomers (**11**, **12**) of **1**.

The first improved synthetic route for **1B** from *o*-carborane is shown in Scheme 1. The target compound was prepared from ^{10}B-enriched *o*-carborane in four steps. Scheme 2 shows an alternative synthetic scheme for **1B** starting from ^{10}B-enriched decaborane. Prior to the use of two expensive starting materials, the natural-abundance form of **1B**, compound **1A**, was synthesized to optimize reaction conditions in each step. Briefly, the reaction of allyl bromide with the lithium salt of *o*-carborane, which was generated from *o*-carborane by treatment with *n*-butyllithium, provided compound **2A**. The homobifunctional reagent 1,5-pentanediol di-*p*-tosylate was used to connect compound **2A** with the N-3 position of the Thd scaffold according to a procedure previously developed by us.[10] To prevent disubstitution during the second step of Scheme 1, the lithium salt of **2A** was dissolved in benzene and the resulting solution was added very slowly to a solution of 1,5-pentanediol di-*p*-ditosylate in benzene at 5−10 °C. Under these conditions, compound **3A** was obtained in 70% yield. Compound **3A** was reacted with Thd in DMF/acetone (1:1) at 35 °C for 96 h to afford **4A** in a ratio of >30:1 with its olefinic isomer, which was not easily separated from **4A** by silica gel chromatography. Higher reaction temperatures (50 °C) and shorter reaction duration (48 h) produced **4A** and its olefinic isomer in 1:1 ratio. Previously, it was reported that compound **2A** isomerizes in the presence of potassium *tert*-butoxide in *tert*-butyl alcohol or benzene at 40−45 °C to produce 1-(*trans*-1-propenyl)-*o*-carborane.[21,22] When the mixture of **4A** and its olefinic isomer was treated with the osmium tetroxide and 4-methylmorpholine *N*-oxide (NMO), only **4A** was dihydroxylated to afford target compound **1A**. The olefinic isomer of **4A** appeared to be more resistant to the dihydroxylation reaction presumably because of steric hindrance by the bulky *o*-carborane cluster. By use of optimized reaction conditions, **1B** was obtained in four steps from ^{10}B-enriched *o*-carborane in 15% overall yield.

IR and MS analysis of **1A** and **1B** revealed interesting differences as a result of the isotopic composition of both compounds. The IR spectra exhibited minor differences in the B−H stretching band of 2580 cm^{-1} for **1A** vs 2586 cm^{-1} for **1B**. Mass spectra of **1A** and **1B**, which differ significantly in their isotope patterns, are shown in Supporting Information.

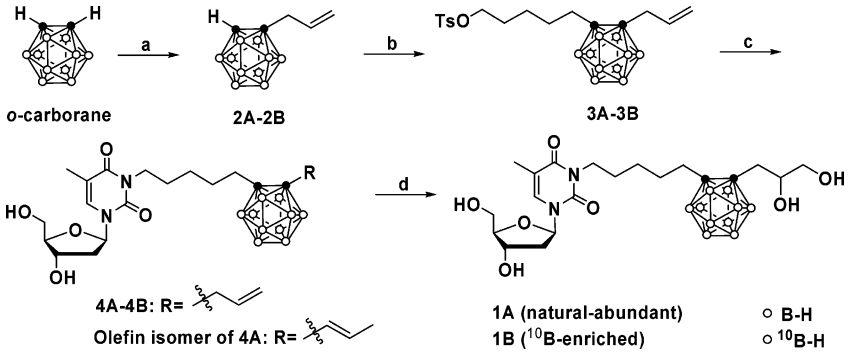
The synthetic route for **1B** from ^{10}B-enriched decaborane is shown in Scheme 2. By employment of biphasic reaction conditions reported by Sneddon and co-workers,[23] the reaction of decaborane with trimethylsilyl acetylene in a mixture of 1-butyl-3-methylimidiazolium (bmin) chloride and toluene afforded the compound **5** in 50% yield. In contrast, the conventional reaction of a decaborane−acetonitrile adduct with trimethylsilyl acetylene[24] gave compound **5** in only 23% yield. Lithium salt formation of compound **5** with n-BuLi, followed by addition of allyl bromide, provided compound **6** in 60% yield. Removal of the trimethylsilyl group of **6** using TBAF[25] gave compound **2B** (80% yield), which was further processed as described in Scheme 1 to yield target compound **1B**.

A previous analysis in our laboratory by 1D NMR confirmed N-3 alkylation in **1** only based on typical differences of ^{13}C chemical shifts between 4-OCH$_2$ and 3-NCH$_2$ groups.[12] Recently, however, it was reported that the reaction of Thd with 8-dioxane-3-cobalt bis(dicarbolide) in toluene using sodium hydride as a base produced a mixture of O-4 and N-3 alkylated products.[26] Re-evaluation of selective N-3 substitution in **1A** under reaction conditions described here was confirmed by 2D HMBC NMR. As expected for a N-3 alkylated Thd derivative, the hydrogens attached to C-8 of **1A** coupled with C-2 and C-4. In an O-4 alkylated Thd derivative, both hydrogens would couple with C-4 and C-5 (^{1}H NMR, ^{1}H−^{1}H COSY, ^{13}C NMR, ^{13}C-DEPT, ^{1}H−^{13}C HMBC, and ^{1}H−^{13}C HMQC are provided in Supporting Information).

The synthetic procedures reported by Al-Madhoun et al.[12] and described in Schemes 1 and 2 provided **1**, **1A**, and **1B**, respectively, as a mixture of two epimers with different configurations at the C-16 position. Compounds **10A** and **10B** were prepared, as described in Scheme 3, to explore whether they interact differently with the active site of hTK1 (see discussion below). Compound **7A** was prepared in 63% yield by reacting *o*-carborane with (*S*)-(+)-2,2-dimethyl-1,3-dioxolane-4-ylmethyl *p*-tosylate. Addition of a solution of the lithium salt of compound **7A** in benzene to a solution of 1,5-pentanediol di-*p*-tosylate in benzene gave compound **8A** in 58% yield. Condensation of compound **8A** with Thd, followed by the deprotection of the isopropylidene group under acidic conditions, afforded compound **10A**, with (*R*)-configuration at C-16. Compound **10B** and racemic **10C** were prepared from (*R*)- and (*rac*)-2,2-dimethyl-1,3-dioxolane-4-ylmethyl *p*-tosylate, respectively, in accordance with the procedures described for **10A**. This synthetic route is potentially also suitable for the preparation of **1B**.

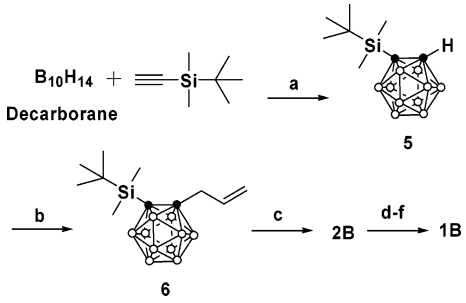
A chiral solvating agent was used to determine the ratio of (*R*)- and (*S*)-epimers of **1A**. When 1 equiv of (*S*)-(+)-2,2,2-trifluoro-1-(9-anthryl)ethanol (Pirkle alcohol)[27] was treated with **1A** in CDCl$_3$, the ^{1}H NMR spectrum showed a partial overlap of two triplets for H-1′ with different intensities (Supporting Information). In contrast, when the pure epimers **10A** and **10B** were treated with Pirkle alcohol under the same conditions, single triplets for H-1′ appeared at 6.13 and 6.10 ppm, respectively. Two signals were also observed for the hydroxyl proton at C-16 of **1A**. On the basis of this analysis, compound **1A** consisted of **10A** and **10B** approximately in a 2:3 ratio. The optical rotation values for **1A** (−5.1), **10A** (+17.5), and **10B** (−14.8) indicated a similar epimeric composition for **1A**. Efforts

Scheme 1. Synthetic Route for **1A** and **1B** from *o*-Carborane[a]



[a] Reagents and reaction conditions: (a) n-BuLi, allyl bromide, THF, room temp, 12 h; (b) n-BuLi, 1,5-pentanediol di-*p*-tosylate, benzene, 5−10 °C, 1 h; (c) thymidine, K_2CO_3, DMF/acetone (1:1), 35 °C, 4 days; (d) OsO_4, NMO, 1,4-dioxane.

Scheme 2. Synthesis of **1B** from Decaborane[a]



[a] Reagents and reaction conditions: (a) (bmin)Cl, toluene, reflux, 10 min; (b) n-BuLi, allyl bromide, THF, room temp, 12 h; (c) TBAF, THF; (d) n-BuLi, 1,5-pentanediol di-*p*-tosylate, benzene, 5−10 °C, 1 h; (e) thymidine, K_2CO_3, DMF/acetone (1:1), 35 °C, 4 days; (f) OsO_4, NMO, 1,4-dioxane.

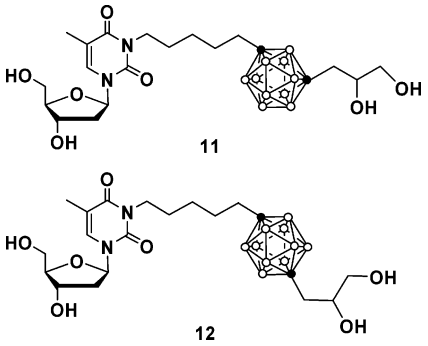


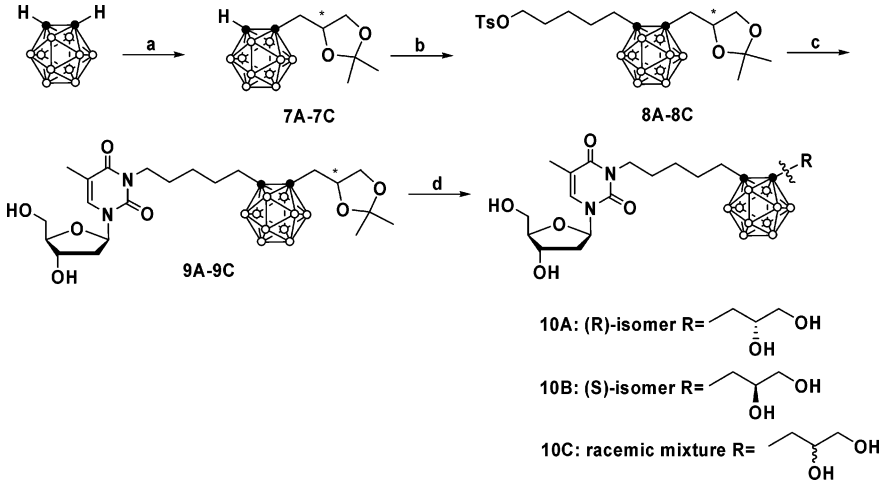
Figure 2. Compounds **11** and **12**.

to separate the epimers of **1A** by HPLC using either a reversed-phase (C18) column or a β-cyclodextrin-based chiral column (CYCLOBOND I, Advanced Separation Technologies Inc.) were not successful, and circular dichroism (CD) experiments did not show any difference between **10A** and **10B**.

The synthetic approach described in Scheme 3 was also used for the preparation of (*R*/*S*) mixtures of compounds **11** and **12** in four steps in overall 19% and 16% yields from *m*- and *p*-carborane, respectively (Figure 2; see Supporting Information for the synthetic procedures and analytical data). Similar to the epimers **10A** and **10B**, different orientations of the dihroxypropyl groups in **11** and **12** may result in different binding patterns with active site of hTK1.

Purity verification of all target compounds by analytical reversed-phase (C18) HPLC using methanol/water and acetonitrile/water gradient systems revealed interesting retention times in particular for **10C**, **11**, and **12** (Table 1 and Supporting Information). In general, the hydrophobicity of the three carborane isomers decreases in the order of *p*-carborane > *m*-carborane > *o*-carborane presumably because of the decreasing potential of C−H for hydrogen bond formation.[28,29] By use of an acetonitrile/water gradient system, however, the retention times of the **10C**, **11**, and **12** and thus their hydrophobicities decreased notably in the order of **10C** > **11** > **12** while differences among **1A**, **1B**, **10A**, **10B**, and **10C** were negligible. This trend was also observed when a methanol/water gradient was used. Accordingly, the ratios of apolar surface areas (APSA)

Scheme 3. Synthetic Route for **10A**−**C**[a]



[a] Reagents and reaction conditions: (a) n-BuLi, 2,2-dimethyl-1,3-dioxolane-4-ylmethyl *p*-tosylate, benzene, room temp, 14 h; (b) n-BuLi, 1,5-pentanediol di-*p*-tosylate, benzene, 5−10 °C, 1 h; (e) thymidine, K_2CO_3, DMF/acetone (1:1), 50 °C, 2 days; (f) 17% HCl, MeOH.

Table 1. Human TK1 Phosphorylation Rates, Retention Time, and Ratios of Apolar to Polar Surface Area (APSA/PSA) of **1A**, **1B**, **10A**−**C**, **11**, and **12**

compd	hTK1 phosphorylation,[b] %	retention time (RP-18),[c] min	APSA/PSA ratio[d]
1[a] (19.9%)	39 ± 8	26.9	
1A (19.9%)	39 ± 6	26.9	
1B (^{10}B-enriched)	36 ± 7	26.5	
10A (*R*, 19.9%)	34 ± 7	26.7	3.31 (461.4 Å^2/139.3 Å^2)
10B (*S*, 19.9%)	33 ± 6	26.6	
10C (*rac*, 19.9%)	32 ± 7	26.9	
11 (*rac*, 19.9%)	42 ± 4	25.7	3.16 (492.7 Å^2/156.1 Å^2)
12 (*rac*, 19.9%)	45 ± 6	23.7	3.02 (491.8 Å^2/162.9 Å^2)

[a] Compound **1** was synthesized using the method reported by Al-Madhoun et al.[12] [b] Compound concentrations were 40 μM, and the DMSO concentration was set to 1%. Enzyme activities were determined using a spectrophotometric assay, and the mean and SEM values of three independent determinations are shown using the activity with 20 μM Thd as 100%. [c] Water/acetonitrile gradient (from 100:0 to 70:30 over 5 min, from 70:30 to 40:60 over 25 min, from 40:60 to 0:100 over 20 min) with a flow rate of 1 mL/min was applied. [d] PSAs and APSAs of the energy-minimized structures of **10A** and the (*R*)-epimers of **11** and **12** were calculated using the VEGA ZZ 2.0.4 program.

to polar surface areas (PSA) for **10A**, the (*R*)-epimer of **11**, and the (*R*)-epimer of **12**, calculated with VEGA ZZ 2.0.4 software (Milano, Italy),[30] decreased in the order **10C** > (*R*)-**11** > (*R*)-**12** (Table 1). On the basis of their energy-minimized 3D structures (see Supporting Information), this pattern of hydrophobicities and APSA/PSA ratios is probably due to an amphiphilic nature of **10A**, which is less pronounced in (*R*)-**11** and absent in (*R*)-**12**.

Biology

The hTK1 substrate characteristics of all target compounds were evaluated in phosphoryl transfer assays using recombinant hTK1.[31] Phosphorylation of the tested compounds by hTK1 ranged from 32% to 45% relative to Thd (Table 1). Compound **1B** showed a relative hTK1 phosphorylation rate that was very similar to that of **1A**. There was also no significant difference among hTK1 phosphorylation rates of compounds **10A**−**C**, indicating that the stereochemistry at the C-16 position apparently did not affect their hTK1 substrate characteristics. If at all, the hTK1 phosphorylation rates of **11** and **12** appeared to be slightly higher than that of the more hydrophobic **10C**, all of which were synthesized according to the same procedure. Previously, we reported that the hydrophilicity in a series of zwitterionic *m-nido*-3CTAs correlated strongly with their hTK1 substrate charcteristics.[10] The hTK1 phosphorylation rates obtained in this study for **10A**, **11**, and **12** may be indicative of a similar pattern. Overall, however, differences in phosphorylation rates between all target compounds appeared to be insignificant. The obtained results indicated that the dihydroxylpropyl group and carborane cage might be located outside the active site of hTK1, which is also supported by the computational model for the binding of **10A** and **12** to the active site of hTK1 discussed in the following.

Since no significant difference in relative phosphorylation rates among **1A**, **1B**, **10A**−**C**, **11**, and **12** were observed, **1B** was chosen for pilot in vivo irradiation studies. For these studies, the F98 glioma cell line was selected to induce brain tumors in rats because the biological behavior of intracerebral tumors produced in this way simulates that of human GBM in several ways. First, they form progressively growing tumors with islands of cells at varying distances from the centrally growing mass.[32] Second, they do not spread outside the brain and are weakly immunogenic.[33] Finally, they are resistant to various forms of therapy including conventional chemotherapy, radiation therapy, gene therapy, and immunotherapy and invariably kill the host.[34] Also, it should be noted that rat TK1 and hTK1 have very similar substrate specificities.[15,35]

BNCT studies were carried at the Massachusetts Institute of Technology (MIT) Nuclear Research Laboratory (Cambridge, MA). F98 glioma bearing rats were divided into three groups of seven to nine animals each as follows: group I, 500 μg of **1B**, administered intracerebrally (ic) by convection enhanced delivery (CED); group II received 50% DMSO alone by CED, as per group I, followed by neutron irradiation; group III received 500 μg of **1B** by CED *without* neutron irradiation. Biodistribution of boron was determined in a group of four rats that had received **1A**, as per groups I and III, but these animals were euthanized 24 h later. Samples of tumor, blood, and brain were analyzed for boron content by means of direct current plasma atom emission spectroscopy (DCP-AES),[36] and the corresponding boron concentrations were 17.3 ± 4.3 μg/g for tumor and <0.5 μg/g for both normal brain and blood. Following BNCT all rats initially lost weight, but they regained it within a few days until they developed progressively growing tumors and eventually died or were euthanized. Survival data are summarized in Table 2. Animals that received **1B**, followed by BNCT, had a mean survival time (MST) of 37 ± 7 days compared to 31 ± 3 days for irradiated controls and 25 ± 2 days for untreated controls, and the differences between these groups were significant ($p < 0.02$). A more detailed description of the in vivo BNCT studies that we have carried out with **1B** will be reported elsewhere. These preliminary survival data are encouraging because they were carried out without optimizing the formulation, dosing, and delivery of **1B**. Furthermore, it should be noted that the plasma concentrations of Thd, which competes with **1B** at the active site of hTK1, are lower in humans than in rodents,[37] and therefore, it is possible that BNCT with **1B** could be more effective in man than in rodents.

Table 2. Survival of Rats Bearing F98 Glioma in Preclinical Neutron Irradiation Studies

	survival ratio[a]		
	group I (**1B** and neutron irradiation)	group II (DMSO and neutron irradiation)	group III (**1B** and no irradiation)
21 days	9/9	8/8	6/7
28 days	9/9	5/8	0/7
35 days	5/9	1/8	0/7
42 days	2/9	0/8	0/7
mean ± SD	37 ± 7 days	31 ± 3 days	25 ± 2 days

[a] The data represent the ratio of the number of surviving animals per group to the total number of animals per group.

In Silico Studies

Recently, crystal structures of hTK1,[38,39] *Ureaplasma urealyticum* TK (UuTK),[39,40] and *Clostridium acetobutylicum* TK (CaTK, PDB code 1XX6) were determined and are now available for computational structure-based drug design. The crystal

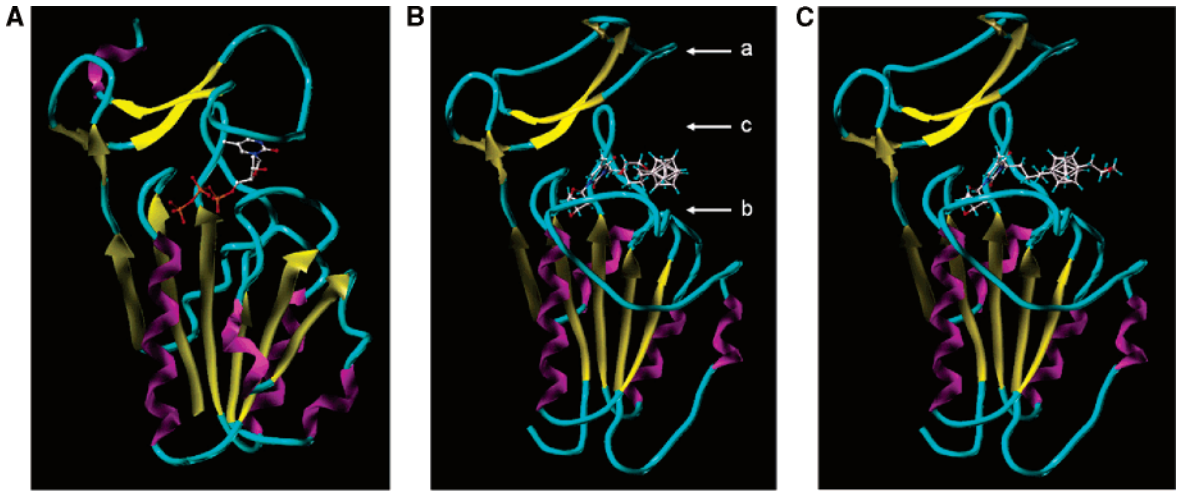


Figure 3. (A) hTK1 crystal structure (PDB code 1W4R) with TTP, (B) hTK1 homology model docked with **10A**, and (C) hTK1 homology model docked with the (*R*)-epimer of **12**. In part B are shown the (a) lasso domain, (b) loop connecting the β2 and β3 strands, and (c) extended cleft between the lasso domain and the loop connecting the β2 and β3 strands.

structures of hTK1 and UuTK were resolved as tetramers containing Thd or thymidine triphosphate (TTP), which is a feedback inhibitor of TK. However, CaTK was crystallized as a dimer complexed with adenosine diphosphate (ADP).

A visual inspection indicated that the hTK1s and UuTKs may represent "closed" TK forms, while CaTK may constitute an "open" or "semiopen" TK form. As in the case of many other nucleoside kinases and nucleoside monophosphate kinase, binding of the substrates and ATP appears to be associated with a conformational change from an open unoccupied form, over a partially closed form involving Thd or ATP binding, to a closed form binding both Thd and ATP.[41−45] The structures of **10A**, (*R*)-**11**, and (*R*)-**12** were minimized at the level of B3LYP/6-31G* using the Gaussian 03 program (Gaussian, Inc., Wallingford, CT). Their coordinates were then transferred into Sybyl 7.1 (Tripos Inc., St. Louis, MO), and the atom type of the boron atoms of the carborane cages were changed to the C.3 atom type because Sybyl does not provide default parameters for calculations involving molecules with hexavalent boron atoms. This manipulation of boron atom types was described previously by us and was found to be suitable for docking studies with other carborane-containing agents.[46,47] Compounds **10A**, (*R*)-**11**, and (*R*)-**12** were then docked into the active site of both hTK1 crystal structures[38,39] using the FlexX module of Sybyl 7.1, but neither compound was found to bind effectively.

The amino acid sequence identity of CaTK with hTK1 is 39% (http://www.ncbi.nlm.nih.gov/blast/bl2seq/). Thus, a homology model of hTK1, presumably representing an "open" or "semiopen" form of hTK1, was generated using CaTK as a template. As shown in parts A and B of Figure 3, the difference in the distance between the loop connecting the β2 and β3 strands (area b) and the "lasso" domain (area a) in the crystal structure of hTK1 and the homology model of hTK1 is substantial. We acknowledge the limitations of docking studies involving a homology model.[48] Eventually, only an in-depth X-ray crystallographic analysis can reveal realistic dimensions of open and closed forms of hTK1. Nevertheless, in contrast with the hTK1 crystal structures, docking of compounds **10A**, (*R*)-**11**, and (*R*)-**12** into the homology model of hTK1 revealed an interesting binding pattern. The bulky carboranyl side chain at the N-3 position of **10A** is oriented toward the extended gap (area c) between the loop connecting the β2 and β3 strands (area b) and the "lasso" domain (area a). We hypothesize that this extended gap allows effective binding of **10A** to the active site of the open form of hTK1 and that partial closure of the lasso due to binding of **10A** will eventually result in an orientation of the ribose portion that allows for an effective transfer of the γ-phosphate from ATP to the 5′- or 3′-hydroxyl group of **10A** while the carborane cluster of **10A** is relocated to the enzyme surface through unfolding and extension of the pentylene spacer. This model accounts for the efficient phosphorylation of **10A** by hTK1. It also provides an explanation for the apparent lack of steric factors on the binding of **1A**, **1B**, **10A**−**C**, **11**, and **12** to hTK1 because the carborane cage, which is the center of all steric alterations, is located toward the surface of the enzyme (Figure 3). The (*R*)-epimer **11** docked in a similar way to hTK1 as **10A** (see Supporting Information). However, in the case of the (*R*)-epimer of **12**, the dihydroxypropyl group attached to *p*-carborane projects away from the active site of hTK1 (Figure 3C) while the same group attached to *o*-carborane in **10A** apparently folds back the enzyme surface. The specific orientation of the dihydroxypropyl group in the (*R*)-epimer of **12** may cause less hindrance during the closure of the "lasso" portion of hTK1 compared with **10A**, which could explain the slightly higher relative phosphorylation rate (Table 1).

Summary and Conclusion

Two feasible synthetic routes for the preparation of **1B** were developed. This agent was for the first time evaluated as a boron delivery agent for BNCT in pilot neutron irradiation experiments. Treatment of rats, bearing intracerebral F98 glioma, with **1B** and neutron irradiation resulted in prolonged survival compared to control groups of animals, indicating that **1B** has a significant potential as a boron-delivery agent for BNCT of malignant brain tumors. Human TK1 phosphorylation rates and docking studies with **10A**, (*R*)-**11**, and (*R*)-**12** indicated that the dihydroxypropyl group attached to the carborane cluster may only function as a hydrophilicity-enhancing structural element but does not increase the binding affinity to the active site of hTK1. Overall, the studies described in this paper identify **1B** as one of the most promising novel BNCT agents.

Experimental Section

^{1}H NMR, ^{13}C NMR, and ^{11}B NMR spectra were obtained on Bruker 250, 300, or 400 MHz FT-NMR instruments. Chemical shifts are reported in parts per million (ppm). The coupling constants are reported in hertz (Hz). High-resolution electrospray ionization (HR-ESI) mass spectra were recorded on a Micromass QTOF-

electrospray mass spectrometer and a 3 T Finnigan FTMS-2000 Fourier Transform mass spectrometer at The Ohio State University Campus Chemical Instrumentation Center (OSU−CCIC). Electron-impact (EI) mass spectra were obtained with a Kratos MS-25 mass spectrometer using 70 eV ionization conditions and were generated at The Ohio State University Department of Chemistry. IR spectra were run on a Nicolet Protégé 460 Magna FT-IR spectrometer. Optical rotations were measured on a Perkin-Elmer 241 automatic polarimeter. Compound visualization on silica gel 60 F_{254} precoated TLC plates (0.25 mm layer thickness) (Merck, Darmstadt, Germany) was attained by UV light and $KMnO_4$ spray. Carborane-containing compounds were selectively visualized by spraying a solution of 0.06% $PdCl_2$/1% aqueous HCl on TLC plates and subsequent heating to ∼120 °C. Reagent grade solvents were used for column chromatography using silica gel 60, particle size 0.040−0.063 mm (Merck, NJ). Analytical HPLC data of the target compounds were obtained with reversed-phase C18 (RP-18) LiChrosphere 100 Å [5 μm] columns (Merck,) using a Rainin HPLC instrument equipped with a Dynamax DA controller, HPXL pumps, and a Dynamax UV-1 detector (Rainin Instrument Company Inc., Woburn, MA). HPLC grade water, methanol, and acetonitrile were used as solvents. A water/methanol gradient (from 100:0 to 30:70 over 10 min, from 30:70 to 10:90 over 20 min, and from 10:90 to 0:100 over 10 min) with a flow rate of 1 mL/min was applied for all target compounds. Alternatively, a water/acetonitrile gradient (from 100:0 to 70:30 over 5 min, from 70:30 to 40:60 over 25 min, from 40:60 to 0:100 over 20 min) was used. Reagent grade chemicals were obtained from commercial vendors and used as such. Decaborane (99.5% ^{10}B-enriched) and *o*-carborane (98.0% ^{10}B-enriched) were purchased from Katchem (Prague, Czech Republic), and osmium tetraoxide was purchased from Strem Chemicals (Newburyport, MA). Solvents were dried prior to use following standard procedures. All reactions were carried out under argon atmosphere. *Caution: Decaborane is a highly toxic, impact-sensitive compound, which forms explosive mixtures especially with halogenated materials. Osmium tetraoxide is a highly toxic and flammable liquid. A careful study of the MSDS is advisable before usage of both chemicals.*

(2,3-Propen-1-yl)-*o*-carborane (2A). To a solution of *o*-carborane (4.26 g, 30 mmol) in tetrahydrofuran (250 mL) was added a solution of *n*-butyllithium (12.6 mL, 31.5 mmol, 2.5 M solution in *n*-hexanes) at −78 °C. The reaction mixture was gradually warmed to room temperature and stirred for 1 h. Subsequently, the reaction mixture was cooled to −78 °C and allyl bromide (2.85 mL, 33 mmol) was added slowly. The mixture was warmed to room temperature and stirred for an additional 12 h. Distilled water (50 mL) was added, and excess tetrahydrofuran was removed under reduced pressure. The residue was extracted with ethyl acetate (150 mL × 2). The combined organic layers were washed with *d*-HCl solution (100 mL) and brine (100 mL) and dried over magnesium sulfate. After filtration and evaporation, the residue was purified by silica gel column chromatography using hexanes as the eluent to give compound **2A** (3.23 g, 59%). R_f = 0.50; ^{1}H NMR ($CDCl_3$) δ 2.94 (d, 2H, allyl-CH_2, J = 7.5 Hz), 3.56 (br s, 1H, H−$C_{carborane}$), 5.06−5.16 (dq, 1H, C═CH_2, J = 16.8, 1.3 Hz), 5.18−5.24 (dq, 1H, C═CH_2, J = 10.0, 1.3 Hz), 5.59−5.77 (m, 1H, CH═C); ^{13}C NMR ($CDCl_3$) δ 41.65 (CH_2), 59.44 ($C_{carborane}$−H), 73.50 ($C_{carborane}$−C), 121.02 (C═C), 131.19 (C═C); MS (HR-ESI) $C_5H_{16}B_{10}Na$ $(M + Na)^+$ calcd 209.2080, found 209.2067.

(2,3-Propen-1-yl)-*o*-carborane (^{10}B-Enriched 2B). Compound **2B** was prepared in 75% yield adapting the procedure described for compound **2A**. R_f = 0.50 (only hexanes); ^{1}H NMR ($CDCl_3$) δ 2.93 (d, 2H, allyl-CH_2, J = 7.5 Hz), 3.55 (br s, 1H, H−$C_{carborane}$), 5.11 (dd, 1H, C═CH_2, J = 16.8, 1.2 Hz), 5.20 (d, 1H, C═CH_2, J = 9.9 Hz), 5.62−5.77 (m, 1H, CH═C); ^{13}C NMR ($CDCl_3$) δ 41.66 (CH_2), 59.43 ($C_{carborane}$−H), 73.50 ($C_{carborane}$−C), 121.01 (C═C), 131.21 (C═C); MS (HR-EI) $C_5H_{16}B_{10}$ (M^+) calcd 176.2569, found 176.2567.

5-[2-(2,3-Propen-1-yl)-*o*-carboran-1-yl]pentyl Tosylate (3A). To a solution of compound **2A** (1.20 g, 6.57 mmol) in benzene (30 mL) was added a solution of *n*-butyllithium (2.89 mL, 7.23 mmol, 2.5 M in *n*-hexanes) at 10 °C over a period of 20 min. The solution was stirred at room temperature for 1 h and added dropwise to a solution of 1,5-pentanediol di-*p*-tosylate (3.25 g, 7.87 mmol) in benzene (20 mL) at 10 °C. The reaction mixture was stirred for 1 h at the same temperature, quenched with distilled water (20 mL), and extracted with ethyl acetate (40 mL × 3). The combined organic layers were washed with brine (20 mL) and dried over magnesium sulfate. After filtration and evaporation, the residue was purified by silica gel column chromatography using hexanes/ethyl acetate (4:1) as the eluent to give compound **3A** (2.08 g, 70%). R_f = 0.25; ^{1}H NMR ($CDCl_3$) δ 1.29−1.49 (m, 4H, CH_2), 1.52−1.69 (m, 2H, CH_2), 2.09−2.12 (m, 2H, CH_2−$C_{carborane,}$), 2.43 (s, 3H, CH_3), 2.89 (dt, 2H, allyl-CH_2, J = 7.2, 1.2 Hz), 3.98 (t, 2H, CH_2OTs, J = 6.2 Hz), 5.07 (dq, 1H, C═CH_2, J = 16.8, 1.3 Hz), 5.16 (dq, 1H, C═CH_2, J = 10.0, 1.3 Hz), 5.64−5.81 (m, 1H, CH═C), 7.33 (d, 2H, ArH, J = 13.7 Hz), 7.76 (d, 2H, ArH, J = 13.7 Hz); ^{13}C NMR ($CDCl_3$) δ 21.60 (CH_2), 25.04 (CH_2), 28.33 (CH_2), 28.83 (CH_2), 34.60 (CH_2), 39.28 (CH_2), 69.85 (O−CH_2), 77.97 ($C_{carborane}$−C), 78.89 ($C_{carborane}$−C), 119.45 (C═C), 127.78 (ArC), 129.86 (ArC), 132.50 (C═C), 132.93 (ArC), 144.87 (ArC); MS (HR-ESI) $C_{17}H_{32}B_{10}O_3S_1Na$ $(M + Na)^+$ calcd 447.2973, found 447.2983.

5-[2-(2,3-Propen-1-yl)-*o*-carboran-1-yl]pentyl Tosylate (^{10}B-Enriched 3B). Compound **3B** was prepared in 65% yield adapting the procedure described for compound **3A**: R_f = 0.24 (hexanes/ethyl acetate, 4:1); ^{1}H NMR ($CDCl_3$) δ 1.23−1.36 (m, 2H, CH_2), 1.42−1.50 (m, 2H, CH_2), 1.60−1.67 (m, 2H, CH_2), 2.07−2.15 (m, 2H, CH_2−$C_{carborane,}$), 2.43 (s, 3H, CH_3), 2.89 (d, 2H, allyl-CH_2, J = 7.1 Hz), 3.99 (t, 2H, CH_2OTs, J = 6.1 Hz), 5.07 (d, 1H, C═CH_2, J = 16.9 Hz), 5.16 (d, 1H, C═CH_2, J = 10.0 Hz), 5.68−5.77 (m, 1H, CH═C), 7.33 (d, 2H, ArH, J = 8.0 Hz), 7.76 (d, 2H, ArH, J = 8.0 Hz); ^{13}C NMR ($CDCl_3$) δ 21.66 (CH_2), 25.11 (CH_2), 28.39 (CH_2), 28.88 (CH_2), 34.67 (CH_2), 39.36 (CH_2), 69.84 (O−CH_2), 77.97 ($C_{carborane}$−C), 78.85 ($C_{carborane}$−C), 119.47 (C═C), 127.85 (ArC), 129.89 (ArC), 132.58 (C═C), 132.96 (ArC), 144.90 (ArC); MS (HR-ESI) $C_{17}H_{32}B_{10}O_3S_1Na$ $(M + Na)^+$ calcd 439.3264, found 439.3271.

3-{5-[2-(2,3-Propen-1-yl)-*o*-carboran-1-yl]pentan-1-yl}thymidine (4A). To a solution of compound **3A** (1.98 g, 4.66 mmol) in dimethylformamide/acetone (1:1, 40 mL) were added thymidine (2.82 g, 11.64 mmol) and potassium carbonate (2.57 g, 18.59 mmol). The solution was stirred for 4 days at 35 °C and filtered, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography using dichloromethane/acetone (4:1) as the eluent to give compound **4A** (1.21 g, 52%). R_f = 0.21; ^{1}H NMR (CD_3OD) δ 1.31−1.40 (m, 2H, CH_2), 1.52−1.67 (m, 4H, CH_2), 1.89 (d, 3H, CH_3, J = 1.2 Hz), 2.11−2.31 (m, 4H, H-2′ and CH_2−$C_{carborane}$), 3.05 (dt, 2H, allyl-CH_2, J = 7.2 Hz, 1.2 Hz), 3.71 (dd, 1H, H-5′, J = 12.0, 3.1 Hz), 3.79 (dd, 1H, H-5′, J = 12.00, 3.7 Hz), 3.87−3.93 (m, 3H, H-4′ and CH_2N), 4.36−4.41 (m, 1H, H-3′), 5.12−5.16 (m, 1H, C═CH_2), 5.19−5.20 (m, 1H, C═CH_2), 5.72−5.86 (m, 1H, CH═C), 6.29 (t, 1H, H-1′, J = 6.9 Hz), 7.82 (d, 1H, H-6, J = 1.2 Hz); ^{13}C NMR (CD_3OD) δ 13.23 (CH_3), 27.21 (CH_2), 27.88 (CH_2), 30.32 (CH_2), 35.62 (CH_2), 40.08 (CH_2), 41.34 (CH_2), 41.85 (CH_2), 62.75 (C-5′), 72.09 (C-3′), 80.03 ($C_{carborane}$−C), 81.28 ($C_{carborane}$−C), 87.07 (C-1′), 88.85 (C-4′), 110.68 (C-5), 119.92 (C═C), 134.31(C═C), 136.43 (C-6), 152.28 (C-2), 165.34 (C-4); MS (HR-ESI) $C_{20}H_{38}B_{10}N_2O_5Na$ $(M + Na)^+$ calcd 517.3676, found 517.3651.

3-{5-[2-(2,3-Propen-1-yl)-*o*-carboran-1-yl]pentan-1-yl}thymidine (^{10}B-Enriched 4B). Compound **4B** was prepared in 79% yield adapting the procedure described for compound **4A**. R_f = 0.21 (dichloromethane/acetone, 4:1); ^{1}H NMR (CD_3OD) δ 1.31−1.38 (m, 2H, CH_2), 1.54−1.65 (m, 4H, CH_2), 1.89 (d, 3H, CH_3, J = 1.1 Hz), 2.12−2.30 (m, 4H, H-2′ and CH_2−$C_{carborane}$), 3.05 (d, 2H, allyl-CH_2, J = 7.3 Hz), 3.71 (dd, 1H, H-5′, J = 12.0, 3.1 Hz), 3.79 (dd, 1H, H-5′, J = 12.00, 3.6 Hz), 3.88−3.92 (m, 3H, H-4′ and CH_2N), 4.37−4.40 (m, 1H, H-3′), 5.14−5.19 (m, 2H, C═CH_2), 5.76−5.86 (m, 1H, CH═C), 6.29 (t, 1H, H-1′, J = 6.9 Hz), 7.83 (d, 1H, H-6, J = 1.1 Hz); ^{13}C NMR (CD_3OD) δ 13.22 (CH_3), 27.21 (CH_2), 27.88 (CH_2), 30.33 (CH_2), 35.63 (CH_2), 40.09 (CH_2), 41.34 (CH_2), 41.84 (CH_2), 62.76 (C-5′), 72.11 (C-3′), 80.08 ($C_{carborane}$−C), 81.32 ($C_{carborane}$−C), 87.08 (C-1′), 88.87 (C-4′), 110.70 (C-5), 119.90

(C═C), 134.35 (C═C), 136.45 (C-6), 152.31 (C-2), 165.38 (C-4); MS (HR-ESI) $C_{20}H_{38}B_{10}N_2O_5Na$ $(M + Na)^+$ calcd 509.3972, found 509.3969.

3-{5-[2-(2,3-Dihydroxyprop-1-yl)-*o*-carboran-1-yl]pentan-1-yl}thymidine (1A). To a solution of compound **4A** (980 mg, 1.98 mmol) and 4-methylmorpholine *N*-oxide (500 mg, 4.26 mmol) in 1,4-dioxane (20 mL) was added an aqueous solution of osmium tetraoxide (0.1 g in 10 mL of water). The reaction mixture was protected from light by covering the reaction flask with aluminum foil, stirred at room temperature for 6 h, and quenched with a concentrated aqueous solution of $Na_2S_2O_3$ (500 mg, 3.16 mmol). Excess solvents were removed under reduced pressure. The residue was purified by silica gel column chromatography using dichloromethane/acetone (1:1) as the eluent to give compound **1A** (400 mg, 40%). $R_f = 0.30$; $[\alpha]^{25}_D$ −5.1 (*c* 0.10, MeOH); ^{1}H NMR (CD_3OD) δ 1.28−1.39 (m, 2H, CH_2), 1.52−1.67 (m, 4H, CH_2), 1.89 (d, 3H, CH_3, $J = 1.2$ Hz), 2.12−2.36 (m, 5H, H-2′, CH_2−$C_{carborane}$, and CH(OH)−CH_2−$C_{carborane}$), 2.56 (dd, 1H, CH(OH)−CH_2−$C_{carborane}$, $J = 15.8$, 1.7 Hz), 3.33 (dd, 1H, CH_2OH, $J = 11.0$, 6.5 Hz), 3.47 (dd, 1H, CH_2OH, $J = 11.0$, 5.3 Hz), 3.71 (dd, 1H, H-5′, $J = 12.0$, 3.7 Hz), 3.74−3.80 (m, 1H, C*H*(OH)−CH_2OH), 3.80 (dd, 1H, H-5′, $J = 12.0$, 3.1 Hz), 3.87−3.93 (m, 3H, H-4′ and CH_2N), 4.36−4.41 (m, 1H, H-3′), 6.29 (t, 1H, H-1′, $J = 6.9$ Hz), 7.83 (d, 1H, H-6, $J = 1.2$ Hz); ^{13}C NMR (CD_3OD) δ 13.23 (CH_3), 27.29 (CH_2), 27.90 (CH_2), 30.38 (CH_2), 35.80 (*C*−$C_{carborane}$), 39.89 (*C*−$C_{carborane}$), 41.34 (C-2′), 41.92 (CH_2N), 62.76 (C-5′), 66.91 (O−CH_2), 72.11 (O−CH), 72.21 (C-3′), 80.44 ($C_{carborane}$−C), 81.89 ($C_{carborane}$−C), 87.10 (C-1′), 88.87 (C-4′), 110.71 (C-5), 136.47 (C-6), 152.33 (C-2), 165.43 (C-4); IR 1054, 1099, 1269, 1472, 1627, 1666, 1694, 2580 (B−H), 2935, 3405; MS (HR-ESI) $C_{20}H_{40}B_{10}N_2O_7Na$ $(M + Na)^+$ calcd 551.3747, found 551.3728; reversed-phase-18 HPLC retention time, 26.9 min (water/acetonitrile), 21.1 min (water/methanol).

3-{5-[2-(2,3-Dihydroxyprop-1-yl)-*o*-carboran-1-yl]pentan-1-yl}thymidine (^{10}B-Enriched 1B). Compound **1B** was prepared in 40% yield adapting the procedure described for compound **1A**. $R_f = 0.30$ (dichloromethane/acetone, 1:1); ^{1}H NMR (CD_3OD) δ 1.28−1.39 (m, 2H, CH_2), 1.52−1.67 (m, 4H, CH_2), 1.89 (d, 3H, CH_3, $J = 1.1$ Hz), 2.17−2.39 (m, 5H, H-2′ and CH_2−$C_{carborane}$, and CH-(OH)−CH_2−$C_{carborane}$), 2.56 (dd, 1H, CH(OH)−CH_2−$C_{carborane}$, $J = 15.8$, 1.6 Hz), 3.33 (dd, 1H, CH_2OH, $J = 11.0$, 6.5 Hz), 3.46 (dd, 1H, CH_2OH, $J = 11.0$, 5.3 Hz), 3.72 (dd, 1H, H-5′, $J = 12.0$, 3.7 Hz), 3.76−3.80 (m, 1H, C*H*(OH)−CH_2OH), 3.79 (dd, 1H, H-5′, $J = 12.0$, 3.1 Hz), 3.81−3.92 (m, 3H, H-4′ and CH_2N), 4.37−4.40 (m, 1H, H-3′), 6.30 (t, 1H, H-1′, $J = 6.7$ Hz), 7.83 (d, 1H, H-6, $J = 1.1$ Hz); ^{13}C NMR (CD_3OD) δ 13.21 (CH_3), 27.29 (CH_2), 27.91 (CH_2), 30.39 (CH_2), 35.83 (CH_2), 39.92 (CH_2), 41.34 (CH_2), 41.92 (CH_2), 62.77 (C-5′), 66.92 (O−CH_2), 72.13 (O−CH_2), 72.23 (C-3′), 80.49 ($C_{carborane}$−C), 81.94 ($C_{carborane}$−C), 87.11 (C-1′), 88.89 (C-4′), 110.72 (C-5), 136.49 (C-6), 152.34 (C-2), 165.45 (C-4); IR 1054, 1097, 1267, 1470, 1625, 1664, 1690, 2587 (B−H), 2926, 3385; MS (HR-ESI) $C_{20}H_{40}B_{10}N_2O_7Na$ $(M + Na)^+$ calcd 543.4027, found 543.4003; reversed-phase-18 HPLC retention time, 26.5 min (water/acetonitrile), 21.0 min (water/methanol).

***tert*-Butyldimethylsilyl-*o*-carborane (^{10}B-Enriched 5). Method A.** Decaborane (635 mg, 5.21 mmol) was dissolved in acetonitrile (10 mL) and refluxed at 80 °C for 30 min. The solution turned yellow because of the formation of the decaborane−acetonitrile adduct. To this solution was added *tert*-butylsilylacetylene (0.75 mL, 3.85 mmol) in toluene (10 mL), and the resulting reaction mixture was refluxed for 24 h. The dark-brown solution was cooled to room temperature, and methanol (1 mL) was added. After evaporation, the residue was purified by silica gel column chromatography using hexanes as the eluent to give the compound **5** (299 mg, 23%).

Method B. To a solution of 1-butyl-3-methylimidiazolium chloride (990 mg) in toluene was added decaborane (122 mg, 1 mmol) and *tert*-butylsilylacetylene (0.56 mL, 3 mmol) at 120 °C under argon atmosphere. The mixture was stirred vigorously at the same temperature for 10 min. After removal of toluene, the residue was purified by silica gel column chromatography using hexane as the eluent to give the compound **5** (125 mg, 50%). $R_f = 0.50$ (100% pentane); ^{1}H NMR ($CDCl_3$) δ 0.91 (s, 6H, −Si(CH_3)$_2$), 1.22 (s, 9H, C(CH_3)$_3$), 3.56 (br s, 1H, *H*−$C_{carborane}$); ^{13}C NMR ($CDCl_3$) δ −5.34 (CH_3), 13.21 (CH_3), 28.87, 59.44 ($C_{carborane}$−H), 73.50 ($C_{carborane}$−C); MS (HR-EI) $C_8H_{26}B_{10}Si$ (M^+) calcd 250.8765, found 250.8654.

1-(*tert*-Butyldimethylsilyl)-2-(2,3-propen-1-yl)-*o*-carborane (^{10}B-Enriched 6). To a solution of **5** (290 mg, 1.16 mmol) in tetrahydrofuran (50 mL) was added *n*-butyllithium (0.464 mL, 1.16 mmol, 2.5 M solution in hexanes) at −78 °C over a period of 10 min. The solution was gradually warmed to room temperature and was stirred for 1 h. Allyl bromide (0.10 mL, 1.2 mmol) was added to the solution at −78 °C, and the reaction mixture was stirred at room temperature for 12 h. Distilled water (50 mL) was added, and the tetrahydrofuran was removed under reduced pressure. The residue was extracted with ethyl acetate (30 mL × 4). The combined organic layers were washed with *d*-HCl solution (100 mL) and brine (100 mL) and dried over $MgSO_4$. After filtration and evaporation, the residue was purified by silica gel column chromatography using hexanes as the eluent to give compound **6** (201 mg, 60%). $R_f = 0.60$ (100% hexanes); ^{1}H NMR ($CDCl_3$) δ 0.92 (s, 6H, −Si(CH_3)$_2$), 1.23 (s, 9H, C(CH_3)$_3$), 2.93 (d, 2H, allyl-CH_2, $J = 7.5$ Hz), 5.06−5.16 (m, 1H, C═CH_2), 5.18−5.24 (m, 1H, C═CH_2), 5.59−5.77 (m, 1H, CH═C); ^{13}C NMR ($CDCl_3$) δ −5.28 (CH_3), 13.21 (CH_3), 21.90 (CH_3), 28.87, 41.65 (CH_2), 72.76 ($C_{carborane}$−C), 73.50 ($C_{carborane}$−C), 121.02 (C═C), 131.19 (C═C); MS (HR-EI) $C_{11}H_{30}B_{10}Si$ (M^+) calcd 290.2080, found 290.2067.

(2,3-Propen-1-yl)-*o*-carborane (^{10}B-Enriched 2B). To a solution of compound **6** (170 mg, 0.96 mmol) in THF (10 mL) was added TBAF (1.5 mL, 1.0 M in THF) at −78 °C. The mixture was stirred at room temperature for 2 h and then poured into ice−water (30 mL) and extracted with diethyl ether (30 mL × 3). The combined organic layers were washed with brine and dried over $MgSO_4$. After filtration and evaporation, crude compound **2B** (135 mg, 80%) was obtained. Analytical data were identical to those for the **2B** preparation described above.

(*R*)-4-(*o*-Carboran-1-yl)methyl-2,2-dimethyl-1,3-dioxolane (7A). To a solution of *o*-carborane (213 mg, 1.5 mmol) in benzene (5 mL) was added a solution of *n*-butylithium (0.66 mL, 1.65 mmol, 2.5 M solution in hexanes) at 0 °C. The solution was first stirred for 30 min at 0 °C and subsequently for 30 min at room temperature. The reaction mixture was again cooled to 0 °C, and (*S*)-(+)-2,2-dimethyl-1,3-dioxolane-4-ylmethyl *p*-tosylate (429 mg, 1.5 mmol) in benzene (10 mL) was added dropwise. The reaction mixture was stirred at room temperature for 14 h. Subsequently, distilled water (10 mL) was added and excess benzene was removed under reduced pressure. The residue was extracted with ethyl acetate (50 mL). The organic layer was washed with HCl-*d* solution (20 mL) and brine (20 mL) and dried over $MgSO_4$. After filtration and evaporation, the residue was purified by silica gel column chromatography using hexanes/ethyl acetate (25:1) as the eluent to give compound **7A** (242 mg, 63%). $R_f = 0.12$; ^{1}H NMR ($CDCl_3$) δ 1.31 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 2.39 (dd, 1H, CH_2, $J = 15.1$, 3.4 Hz), 2.47 (dd, 1H, CH_2, $J = 15.1$, 9.1 Hz), 3.47 (dd, 1H, CH_2, $J = 8.4$, 5.9 Hz), 4.02 (br s, 1H, *H*−$C_{carborane}$), 4.05 (dd, 1H, CH_2, $J = 8.4$, 6.2 Hz), 4.17−4.23 (m, 1H, CH); ^{13}C NMR ($CDCl_3$) δ 25.4 (*C*H_3), 26.9 (*C*H_3), 42.0 (*C*H_2−$C_{carborane}$), 59.8 (H−*C*$_{carborane}$), 68.7 (*C*H_2), 72.4 (CH_2−*C*$_{carborane}$), 73.8 (*C*H), 110.2 [*C*(CH_3)$_2$].

(*S*)-4-(*o*-Carboran-1-yl)methyl-2,2-dimethyl-1,3-dioxolane (7B). Compound **7B** was prepared in 60% yield adapting the procedure described for compound **7A**. $R_f = 0.12$ (hexanes/ethyl acetate, 25:1); ^{1}H NMR ($CDCl_3$) δ 1.31 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 2.39 (dd, 1H, CH_2, $J = 15.1$, 3.5 Hz), 2.47 (dd, 1H, CH_2, $J = 15.1$, 9.1 Hz), 3.47 (dd, 1H, CH_2, $J = 8.4$, 5.9 Hz), 4.02 (br s, 1H, *H*−$C_{carborane}$), 4.05 (dd, 1H, CH_2, $J = 8.4$, 6.2 Hz), 4.18−4.23 (m, 1H, CH); ^{13}C NMR ($CDCl_3$) δ 25.4 (*C*H_3), 26.9 (*C*H_3), 42.0 (*C*H_2−$C_{carborane}$), 59.8 (H−*C*$_{carborane}$), 68.7 (*C*H_2), 72.4 (CH_2−*C*$_{carborane}$), 73.8 (*C*H), 110.2 [*C*(CH_3)$_2$].

4-(*o*-Carboran-1-yl)methyl-2,2-dimethyl-1,3-dioxolane (7C). Compound **7C** was prepared in 65% yield adapting the procedure described for compound **7A**. $R_f = 0.12$ (hexanes/ethyl acetate, 25:1); ^{1}H NMR ($CDCl_3$) δ 1.31 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 2.38 (dd, 1H, CH_2, $J = 15.2$, 3.9 Hz), 2.47 (dd, 1H, CH_2, $J = 15.2$,

8.8 Hz), 3.47 (dd, 1H, CH_2, J = 8.4, 5.9 Hz), 4.02 (br s, 1H, $H-C_{carborane}$), 4.05 (dd, 1H, CH_2, J = 8.4, 6.1 Hz), 4.18−4.23 (m, 1H, CH); ^{13}C NMR ($CDCl_3$) δ 25.8 (CH_3), 27.3 (CH_3), 42.4 ($CH_2-C_{carborane}$), 60.3 ($H-C_{carborane}$), 69.1 (CH_2), 72.8 ($CH_2-C_{carborane}$), 74.2 (*C*H), 110.6 [$C(CH_3)_2$].

(*R*)-5-[2-(2,3-Isopropylidenedioxyprop-1-yl)-*o*-carboran-1-yl]-pentyl Tosylate (8A). To a solution of compound **7A** (129 mg, 0.5 mmol) in benzene (5 mL) was added *n*-butyllithium (0.22 mL, 0.55 mmol, 2.5 M solution in hexanes) at 5 °C over a period of 20 min. The solution was first stirred at the same temperature for 30 min and then at room temperature for 30 min. The solution was then slowly added to a solution of 1,5-pentanediol di-*p*-tosylate (330 mg, 0.80 mmol) in benzene (10 mL) at 5 °C (ice bath). The reaction mixture was stirred for 1 h at the same temperature. Distilled water (5 mL) was added, and the reaction mixture was extracted with ethyl acetate (15 mL × 3). The combined organic layers were washed with brine (20 mL) and dried over magnesium sulfate. After filtration and evaporation, the residue was purified by silica gel column chromatography using hexanes/ethyl acetate (4:1) as the eluent to give compound **8A** (144 mg, 58%). R_f = 0.21; ^{1}H NMR ($CDCl_3$) δ 1.31 (s, 3H, CH_3), 1.35 (s, 3H, CH_3), 1.41−1.51 (m, 2H, CH_2), 1.60−1.67 (m, 2H, CH_2), 2.06−2.23 (m, 4H, CH_2 and $CH_2-C_{carborane}$), 2.34 (dd, 1H, $CH_2-C_{carborane}$, J = 15.5, 5.1 Hz), 2.41 (dd, J = 15.5, 6.5 Hz), 2.43 (s, 3H, CH_3), 3.54 (dd, 1H, CH_2, J = 8.3, 6.5 Hz), 3.99 (t, 2H, OCH_2, J = 6.2 Hz), 4.11 (dd, 1H, CH_2, J = 8.3, 6.0 Hz), 4.19−4.25 (m, 1H, CH), 7.32 (d, 2H, ArH, J = 8.1 Hz), 7.75 (d, 2H, ArH, J = 8.1 Hz); ^{13}C NMR ($CDCl_3$) δ 21.62 (CH_3), 25.05 (CH_3), 25.25 (CH_3), 26.79 (CH_2), 28.35 (CH_2), 28.87 (CH_2), 34.81 ($CH_2-C_{carborane}$), 39.40 ($CH_2-C_{carborane}$), 69.04 (CH_2), 69.81 (CH_2OTs), 74.39 (*C*H), 76.86 ($C-C_{carborane}$), 79.62 ($C-C_{carborane}$), 109.54 [$C(CH_3)_2$], 127.80 (ArC), 129.87 (ArC), 132.90 (ArC), 144.86 (ArC); MS (HR-ESI) $C_{20}H_{38}B_{10}O_5S_1Na$ $(M + Na)^+$ calcd 521.3352, found 521.3347.

(*S*)-5-[2-(2,3-Isopropylidenedioxyprop-1-yl)-*o*-carboran-1-yl]-pentyl Tosylate (8B). Compound **8B** was prepared in 62% yield adapting the procedure described for compound **8A**. R_f = 0.21 (hexanes/ethyl acetate, 4:1); ^{1}H NMR ($CDCl_3$) δ 1.32 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.42−1.51 (m, 2H, CH_2), 1.61−1.68 (m, 2H, CH_2), 2.02−2.23 (m, 4H, CH_2 and $CH_2-C_{carborane}$), 2.34 (dd, 1H, $CH_2-C_{carborane}$, J = 15.4, 5.2 Hz), 2.42 (dd, 1H, $CH_2-C_{carborane}$, J = 15.4, 6.5 Hz), 2.45 (s, 3H, CH_3), 3.54 (dd, 1H, CH_2, J = 8.3, 6.4 Hz), 3.99 (t, 2H, OCH_2, J = 6.2 Hz), 4.12 (dd, 1H, CH_2, J = 8.3, 6.0 Hz), 4.19−4.25 (m, 1H, CH), 7.32 (d, 2H, ArH, J = 8.1 Hz), 7.75 (d, 2H, ArH, J = 8.1 Hz); ^{13}C NMR ($CDCl_3$) δ 21.64 (CH_3), 25.07 (CH_3), 25.26 (CH_3), 26.81 (CH_2), 28.37 (CH_2), 28.89 (CH_2), 34.83 ($CH_2-C_{carborane}$), 39.42 ($CH_2-C_{carborane}$), 69.07 (CH_2), 69.81 (CH_2OTs), 74.41 (*C*H), 76.84 ($C-C_{carborane}$), 79.62 ($C-C_{carborane}$), 109.55 [$C(CH_3)_2$], 127.82 (ArC), 129.87 (ArC), 132.92 (ArC), 144.87 (ArC); MS (HR-ESI) $C_{20}H_{38}B_{10}O_5S_1Na$ $(M + Na)^+$ calcd 521.3352, found 521.3345.

5-[2-(2,3-Isopropylidenedioxyprop-1-yl)-*o*-carboran-1-yl]-pentyl Tosylate (8C). Compound **8C** was prepared in 60% yield following the procedure described for compound **8A**. R_f = 0.21 (hexanes/ethyl acetate, 4:1); ^{1}H NMR ($CDCl_3$) δ 1.32 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.42−1.51 (m, 2H, CH_2), 1.61−1.68 (m, 2H, CH_2), 2.02−2.23 (m, 4H, CH_2 and $CH_2-C_{carborane}$), 2.34 (dd, 1H, $CH_2-C_{carborane}$, J = 15.4, 5.2 Hz), 2.42 (dd, 1H, $CH_2-C_{carborane}$, J = 15.4, 6.4 Hz), 2.44 (s, 3H, CH_3), 3.55 (dd, 1H, CH_2, J = 8.2, 6.5 Hz), 3.99 (t, 2H, OCH_2, J = 6.1 Hz), 4.12 (dd, 1H, CH_2, J = 8.2, 6.1 Hz), 4.20−4.26 (m, 1H, CH), 7.33 (d, 2H, ArH, J = 8.1 Hz), 7.76 (d, 2H, ArH, J = 8.1 Hz); ^{13}C NMR ($CDCl_3$) δ 21.7 (CH_3), 25.0 (CH_3), 25.3 (CH_3), 26.8 (CH_2), 28.7 (CH_2), 30.9 (CH_2), 34.8 ($CH_2-C_{carborane}$), 39.5 ($CH_2-C_{carborane}$), 69.1 (CH_2), 70.8 (CH_2-OTs), 74.5 (*C*H), 109.6 [$C(CH_3)_2$], 127.8 (ArC), 129.9 (ArC), 133.0 (ArC), 144.9 (ArC); MS (HR-ESI) $C_{20}H_{38}B_{10}O_5S_1Na$ $(M + Na)^+$ calcd 521.3352, found 521.3365.

(*R*)-3-{5-[2-(2,3-Isopropylidenedioxyprop-1-yl)-*o*-carboran-1-yl]pentan-1-yl}thymidine (9A). To a solution of compound **8A** (125 mg, 0.25 mmol) in a mixture of dimethylformamide and acetone (10 mL, 1:1) was added thymidine (150 mg, 0.62 mmol) and potassium carbonate (150 mg, 1.13 mmol). The reaction mixture was stirred at 50 °C for 48 h. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography using ethyl acetate/methanol (20:1) as the eluent to give compound **9A** (88 mg, 62%). R_f = 0.29; ^{1}H NMR (MeOH-d_4) δ 1.30−1.35 (m, 2H, CH_2), 1.32 (s, 3H, CH_3), 1.34 (s, 3H, CH_3), 1.54−1.65 (m, 4H, CH_2), 1.89 (d, 3H, CH_3, J = 1.1 Hz), 2.15−2.28 (m, 2H, H-2′), 2.28−2.34 (m, 2H, $CH_2-C_{carborane}$), 2.51 (dd, 1H, $CH_2-C_{carborane}$, J = 15.9, 7.9 Hz), 2.58 (dd, 1H, $CH_2-C_{carborane}$, J = 15.9, 3.5 Hz), 3.53 (dd, 1H, CH_2, J = 8.2, 7.2 Hz), 3.72 (dd, 1H, H-5′, J = 12.0, 3.7 Hz), 3.79 (dd, 1H, H-5′, J = 12.0, 3.7 Hz), 3.88−3.92 (m, 3H, H-4′ and CH_2N), 4.10 (dd, 1H, CH_2, J = 8.3, 6.1 Hz), 4.23−4.29 (m, 1H, CH), 4.37−4.40 (m, 1H, H-3′), 6.30 (t, 1H, H-1′, J = 6.8 Hz), 7.83 (d, 1H, H-6, J = 1.1 Hz); ^{13}C NMR (MeOH-d_4) δ 13.24 (CH_3), 25.85 (CH_3), 27.20 (CH_3), 27.26 (CH_2), 27.92 (CH_2), 30.34 (CH_2), 35.73 ($CH_2-C_{carborane}$), 40.25 ($CH_2-C_{carborane}$), 41.35 (C-2′), 41.87 (CH_2-N), 62.76 (C-5′), 69.99 (CH_2), 72.11 (C-3′), 75.95 (*C*H), 79.53 ($CH_2-C_{carborane}$), 81.77 ($CH_2-C_{carborane}$), 87.05 (C-1′), 88.86 (C-4′), 110.70 (C-5), 110.89 [$C(CH_3)_2$], 136.44 (C-6), 152.30 (C-2), 165.36 (C-4); MS (HR-ESI) $C_{23}H_{44}B_{10}N_2O_7Na$ $(M + Na)^+$ calcd 591.4061, found 591.4063.

(*S*)-3-{5-[2-(2,3-Isopropylidenedioxyprop-1-yl)-*o*-carboran-1-yl]pentan-1-yl}thymidine (9B). Compound **9B** was prepared in 65% yield adapting the procedure described for compound **9A**. R_f = 0.29 (ethyl acetate/methanol, 20:1); ^{1}H NMR (MeOH-d_4) δ 1.30−1.35 (m, 2H, CH_2) 1.32 (s, 3H, CH_3), 1.34 (s, 3H, CH_3), 1.54−1.65 (m, 4H, CH_2), 1.89 (d, 3H, CH_3, J = 1.1 Hz), 2.14−2.28 (m, 2H, H-2′), 2.28−2.34 (m, 2H, $CH_2-C_{carborane}$), 2.51 (dd, 1H, $CH_2-C_{carborane}$, J = 15.9, 7.9 Hz), 2.58 (dd, 1H, $CH_2-C_{carborane}$, J = 15.9, 3.5 Hz), 3.53 (dd, 1H, CH_2, J = 8.2, 7.2 Hz), 3.71 (dd, 1H, H-5′, J = 12.0, 3.7 Hz), 3.79 (dd, 1H, H-5′, J = 12.0, 3.7 Hz), 3.88−3.92 (m, 3H, H-4′ and CH_2N), 4.10 (dd, 1H, CH_2, J = 8.3, 5.9 Hz), 4.23−4.29 (m, 1H, CH), 4.37−4.40 (m, 1H, H-3′), 6.30 (t, 1H, H-1′, J = 6.8 Hz), 7.83 (d, 1H, H-6, J = 1.1 Hz); ^{13}C NMR (MeOH-d_4) δ 13.23 (CH_3), 25.85 (CH_3), 27.20 (CH_3), 27.26 (CH_2), 27.93 (CH_2), 30.35 (CH_2), 35.74 ($CH_2-C_{carborane}$), 40.25 ($CH_2-C_{carborane}$), 41.35 (C-2′), 41.87 (CH_2-N), 62.76 (C-5′), 69.99 (CH_2), 72.11 (C-3′), 75.96 (*C*H), 79.54 ($CH_2-C_{carborane}$), 81.78 ($CH_2-C_{carborane}$), 87.06 (C-1′), 88.87 (C-4′), 110.70 (C-5), 110.90 [$C(CH_3)_2$], 136.45 (C-6), 152.31 (C-2), 165.37 (C-4); MS (HR-ESI) $C_{23}H_{44}B_{10}N_2O_7Na$ $(M + Na)^+$ calcd 591.4061, found 591.4067.

3-{5-[2-(2,3-Isopropylidenedioxyprop-1-yl)-*o*-carboran-1-yl]-pentan-1-yl}thymidine (9C). Compound **9C** was prepared in 60% yield adapting the procedure described for compound **9A**. R_f = 0.29 (ethyl acetate/methanol, 20:1); ^{1}H NMR (MeOH-d_4) δ 1.30−1.35 (m, 2H, CH_2), 1.32 (s, 3H, CH_3), 1.34 (s, 3H, CH_3), 1.54−1.65 (m, 4H, CH_2), 1.89 (d, 3H, CH_3, J = 1.2 Hz), 2.14−2.28 (m, 2H, H-2′), 2.28−2.34 (m, 2H, $CH_2-C_{carborane}$), 2.51 (dd, 1H, $CH_2-C_{carborane}$, J = 15.9, 7.9 Hz), 2.58 (dd, 1H, $CH_2-C_{carborane}$, J = 15.9, 3.4 Hz), 3.54 (dd, 1H, CH_2, J = 8.2, 6.5 Hz), 3.71 (dd, 1H, H-5′, J = 12.0, 3.7 Hz), 3.79 (dd, 1H, H-5′, J = 12.0,3.7 Hz), 3.88−3.91 (m, 3H, H-4′ and CH_2N), 4.09 (dd, 1H, CH_2, J = 8.1, 6.1 Hz), 4.23−4.29 (m, 1H, CH), 4.37−4.40 (m, 1H, H-3′), 6.30 (t, 1H, H-1′, J = 6.8 Hz), 7.83 (d, 1H, H-6, J = 1.2 Hz); ^{13}C NMR (MeOH-d_4) δ 13.22 (CH_3), 25.84 (CH_3), 27.19 (CH_3), 27.26 (CH_2), 27.92 (CH_2), 30.35 (CH_2), 35.74 ($CH_2-C_{carborane}$), 40.25 ($CH_2-C_{carborane}$), 41.35 (C-2′), 41.87 (CH_2-N), 62.76 (C-5′), 70.00 (CH_2), 72.12 (C-3′), 75.97 (*C*H), 79.54 ($CH_2-C_{carborane}$), 81.78 ($CH_2-C_{carborane}$), 87.06 (C-1′), 88.88 (C-4′), 110.70 (C-5), 110.90 ($C(CH_3)_2$), 136.49 (C-6), 152.32 (C-2), 165.38 (C-4); MS (HR-ESI) $C_{23}H_{44}B_{10}N_2O_7Na$ $(M + Na)^+$ calcd 591.4061, found 591.4061.

(*R*)-3-{5-[2-(2,3-Dihydroxyprop-1-yl)-*o*-carboran-1-yl]pentan-1-yl}thymidine (10A). To a solution of compound **9A** (40 mg, 0.07 mmol) in methanol (5 mL) was added a mixture of 3 N HCl and ethanol (1 mL, 1:1). The reaction mixture was stirred at room temperature for 20 h. Potassium carbonate (17 mg) was added to the reaction mixture, which was then stirred for 30 min at room temperature. The reaction mixture was filtered using a Buchner funnel to remove the solids, and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel columns chromatography using ethyl acetate/methanol (15:1) as the eluent

to give compound **10A** (30 mg, 81%). R_f = 0.25; $[\alpha]^{25}_D$ +17.5 (*c* 0.15, MeOH); ^{1}H NMR (MeOH-d_4) δ 1.30−1.38 (m, 2H, CH_2), 1.55−1.65 (m, 4H, CH_2), 1.89 (d, 3H, CH_3, J = 1.1 Hz), 2.15−2.39 (m, 5H, H-2′, CH_2−$C_{carborane}$, and CH(OH)−CH_2−$C_{carborane}$), 2.56 (dd, 1H, CH_2−$C_{carborane}$, J = 15.8, 1.5 Hz), 3.34 (dd, 1H, CH_2OH, J = 11.0, 6.5 Hz), 3.47 (dd, 1H, CH_2OH, J = 11.0, 5.3 Hz), 3.72 (dd, 1H, H-5′, J = 12.1, 3.7 Hz), 3.75−3.79 (m, 1H, CH(OH)−CH_2OH), 3.79 (dd, 1H, H-5′, J = 12.1, 3.2 Hz), 3.88−3.91 (m, 3H, H-4′ and CH_2N), 4.37−4.40 (m, 1H, H-3′), 6.29 (t, 1H, H-1′, J = 6.8 Hz), 7.83 (d, 1H, H-6, J = 1.1 Hz); ^{13}C NMR (CD_3-OD) δ 13.26 (CH_3), 27.27 (CH_2), 27.88 (CH_2), 30.36 (CH_2), 35.77 (CH_2), 39.84 (CH_2), 41.32 (CH_2), 41.92 (CH_2), 62.73 (O−CH_2), 66.89 (O−CH_2), 72.07 (O−CH_2), 72.17 (O−CH_2), 80.39 ($C_{carborane}$−C), 81.85 ($C_{carborane}$−C), 87.06 (O−CH), 88.81 (O−CH), 110.67 (C-5), 136.44 (C-6), 152.26 (C-2), 165.37 (C-4); MS (HR-ESI) $C_{20}H_{40}B_{10}N_2O_7Na$ $(M + Na)^+$ calcd 551.3747, found 551.3755; reversed-phase-18 HPLC retention time, 26.7 min (water/acetonitrile), 21.1 min (water/methanol).

(*S*)-3-{5-[2-(2,3-Dihydroxyprop-1-yl)-*o*-carboran-1-yl]pentan-1-yl}thymidine (10B). Compound **10B** was prepared in 85% yield adapting the procedure described for compound **10A**. R_f = 0.25 (ethyl acetate/methanol, 15:1); $[\alpha]^{25}_D$ −14.8 (*c* 0.1, MeOH); ^{1}H NMR (MeOH-d_4) δ 1.30−1.38 (m, 2H, CH_2), 1.54−1.65 (m, 4H, CH_2), 1.89 (d, 3H, CH_3, J = 1.1 Hz), 2.15−2.39 (m, 5H, H-2′, CH_2−$C_{carborane}$, and CH(OH)−CH_2−$C_{carborane}$), 2.56 (dd, 1H, CH_2−$C_{carborane}$, J = 15.8, 1.6 Hz), 3.33 (dd, 1H, CH_2OH, J = 11.0, 6.5 Hz), 3.47 (dd, 1H, CH_2OH, J = 11.0, 5.3 Hz), 3.72 (dd, 1H, H-5′, J = 12.1, 3.7 Hz), 3.75−3.79 (m, 1H, CH(OH)−CH_2OH), 3.79 (dd, 1H, H-5′, J = 12.1, 3.2 Hz), 3.88−3.92 (m, 3H, H-4′ and CH_2N), 4.37−4.40 (m, 1H, H-3′), 6.29 (t, 1H, H-1′, J = 6.8 Hz), 7.83 (d, 1H, H-6, J = 1.1 Hz); ^{13}C NMR (MeOH-d_4) δ 13.24 (CH_3), 27.28 (CH_2), 27.90 (CH_2), 30.37 (CH_2), 35.79 (CH_2), 39.87 (CH_2), 41.33 (CH_2), 41.92 (CH_2), 62.75 (O−CH_2), 66.90 (O−CH_2), 72.09 (O−CH_2), 72.20 (O−CH_2), 80.42 ($C_{carborane}$−C), 81.87 ($C_{carborane}$−C), 87.09 (O−CH), 88.85 (O−CH), 110.69 (C-5), 136.46 (C-6), 152.30 (C-2), 165.41 (C-4); MS (HR-ESI) $C_{20}H_{40}B_{10}N_2O_7$-Na $(M + Na)^+$ calcd 551.3747, found 551.3749; reversed-phase-18 HPLC retention time, 26.6 min (water/acetonitrile), 21.1 min (water/methanol).

3-{5-[2-(2,3-Dihydroxyprop-1-yl)-*o*-carboran-1-yl]pentan-1-yl}thymidine (10C). Compound **10C** was prepared in 80% yield following the procedure described for compound **10A**. R_f = 0.25 (ethyl acetate/methanol, 15:1); ^{1}H NMR (MeOH-d_4) δ 1.30−1.37 (m, 2H, CH_2), 1.55−1.65 (m, 4H, CH_2), 1.89 (d, 3H, CH_3, J = 1.1 Hz), 2.18−2.42 (m, 5H, H-2′, CH_2−$C_{carborane}$, and CH(OH)−CH_2−$C_{carborane}$), 2.55 (dd, 1H, CH_2−$C_{carborane}$, J = 15.8, 1.5 Hz), 3.33 (dd, 1H, CH_2OH, J = 11.0, 6.5 Hz), 3.46 (dd, 1H, CH_2OH, J = 11.0, 5.3 Hz), 3.71 (dd, 1H, H-5′, J = 12.1, 3.7 Hz), 3.74−3.79 (m, 1H, CH(OH)−CH_2OH), 3.79 (dd, 1H, H-5′, J = 12.1, 3.2 Hz), 3.88−3.92 (m, 3H, H-4′ and CH_2N), 4.37−4.40 (m, 1H, H-3′), 6.29 (t, 1H, H-1′, J = 6.8 Hz), 7.83 (d, 1H, H-6, J = 1.1 Hz); ^{13}C NMR (MeOH-d_4) δ 13.22 (CH_3), 27.29 (CH_2), 27.90 (CH_2), 30.38 (CH_2), 35.81 (CH_2), 39.90 (CH_2), 41.33 (CH_2), 41.92 (CH_2), 62.76 (O−CH_2), 66.91 (O−CH_2), 72.12 (O−CH_2), 72.21 (O−CH_2), 80.45 ($C_{carborane}$−C), 81.90 ($C_{carborane}$−C), 87.10 (O−CH), 88.88 (O−CH), 110.71 (C-5), 136.48 (C-6), 152.33 (C-2), 165.44 (C-4); MS (HR-ESI) $C_{20}H_{40}B_{10}N_2O_7Na$ $(M + Na)^+$ calcd 551.3747, found 551.3753; reversed-phase-18 HPLC retention time, 26.9 min (water/acetonitrile), 21.0 min (water/methanol).

3-{5-[7-(2,3-Dihydroxyprop-1-yl)-*m*-carboran-1-yl]pentan-1-yl}thymidine (11). See Supporting Information for a detailed description of experimental procedures for compound **11**. R_f = 0.25 (ethyl acetate/methanol, 15:1); ^{1}H NMR (MeOH-d_4) δ 1.21−1.28 (m, 2H, CH_2), 1.38−1.44 (m, 2H, CH_2), 1.53−1.60 (m, 2H, CH_2), 1.89 (d, 3H, CH_3, J = 1.1 Hz), 1.92−1.98 (m, 2H, CH_2−$C_{carborane}$), 2.16−2.30 (m, 4H, H-2′ and CH_2−$C_{carborane}$), 3.26 (dd, 1H, CH_2-OH, J = 11.1, 6.1 Hz), 3.36 (dd, 1H, CH_2OH, J = 11.1, 5.5 Hz), 3.55−3.60 (m, 1H, CH(OH)−CH_2OH), 3.72 (dd, 1H, H-5′, J = 12.1, 3.7 Hz), 3.79 (dd, 1H, H-5′, J = 12.1, 3.2 Hz), 3.86−3.92 (m, 3H, H-4′ and CH_2N), 4.37−4.40 (m, 1H, H-3′), 6.29 (t, 1H, H-1′, J = 6.8 Hz), 7.83 (d, 1H, H-6, J = 1.1 Hz); ^{13}C NMR (MeOH-d_4) δ 13.22 (CH_3), 27.40 (CH_2), 28.05 (CH_2), 30.74 (CH_2), 37.98 (CH_2), 41.34 (CH_2), 41.80 (CH_2), 42.01 (CH_2), 62.75 (O−CH_2), 66.94 (O−CH_2), 72.10 (O−CH_2), 72.35 (O−CH_2), 75.21 ($C_{carborane}$−C), 77.68 ($C_{carborane}$−C), 87.11 (O−CH), 88.86 (O−CH), 110.69 (C-5), 136.47 (C-6), 152.29 (C-2), 165.40 (C-4); MS (HR-ESI) $C_{20}H_{40}B_{10}N_2O_7Na$ $(M + Na)^+$ calcd 551.3747, found 551.3740; reversed-phase-18 HPLC retention time, 25.7 min (water/acetonitrile), 21.0 min (water/methanol).

3-{5-[12-(2,3-Dihydroxyprop-1-yl)-*p*-carboran-1-yl]pentan-1-yl}thymidine (12). See Supporting Information for a detailed description of experimental procedures for compound **12**. R_f = 0.25 (ethyl acetate/methanol, 15:1); ^{1}H NMR (MeOH-d_4) δ 1.10−1.20 (m, 4H, CH_2), 1.47−1.53 (m, 2H, CH_2), 1.60−1.66 (m, 3H, CH_2−$C_{carborane}$), 1.89 (d, 3H, CH_3, J = 1.1 Hz), 1.89−1.92 (m, 1H, CH_2−$C_{carborane}$), 2.14−2.29 (m, 2H, H-2′), 3.16 (dd, 1H, CH_2OH, J = 11.0, 6.0 Hz), 3.24 (dd, 1H, CH_2OH, J = 11.0, 5.6 Hz), 3.32−3.38 (m, 1H, CH), 3.71 (dd, 1H, H-5′, J = 12.1, 3.7 Hz), 3.79 (dd, 1H, CH_2OH, J = 12.1, 3.1 Hz), 3.83 (t, 2H, CH_2N, J = 7.6 Hz), 3.88−3.91 (m, 1H, H-4′), 4.36−4.40 (m, 1H, H-3′), 6.28 (t, 1H, H-1′, J = 6.8 Hz), 7.82 (d, 1H, H-6, J = 1.1 Hz); ^{13}C NMR (MeOH-d_4) δ 13.17 (CH_3), 27.33 (CH_2), 28.01 (CH_2), 30.27 (CH_2), 38.71 (CH_2), 41.34 (CH_2), 42.00 (CH_2), 42.52 (CH_2), 62.76 (O−CH_2), 66.87 (O−CH_2), 72.07 (O−CH_2), 72.16 (O−CH_2), 78.74 ($C_{carborane}$−C), 81.23 ($C_{carborane}$−C), 87.12 (O−CH), 88.86 (O−CH), 110.69 (C-5), 136.43 (C-6), 152.28 (C-2), 165.38 (C-4); MS (HR-ESI) $C_{20}H_{40}B_{10}N_2O_7Na$ $(M + Na)^+$ calcd 551.3747, found 551.3746; reversed-phase-18 HPLC retention time, 23.7 min (water/acetonitrile), 19.9 min (water/methanol).

Molecular Modeling. Calculations at the B3LYP 6-31G* level using the Gaussian 03 program (Gaussian Inc., Wallingford, CT)[49] running on a workstation at Ohio Super Computer Center were used for the energy minimization of conformations for **10A** and (*R*)-epimers of **11** and **12**. The (*R*)-epimers of compounds **11** and **12** were arbitrarily selected because molecular modeling with racemic mixtures is not possible. The energy-minimized structures were saved in mol2 file format and transferred to Sybyl 7.1 (Tripos Inc, St. Louis, MO). Atom and bond types of the compounds were manually adjusted for docking studies in Sybyl 7.1. Specifically, the atom type of the 10 boron atoms in the carborane cage was modified to the C.3 atom type because parameters for hexavalent boron atoms are not available in Sybyl 7.1, as has been discussed by us previously.[46,47] The initial coordinates of the hTK1 homology model were constructed with SWISS-MODEL (version 36.0003) using *Clostridium acetobutylicum* thymidine kinase (PDB code 1XX6) as the template. The obtained coordinates were then transferred to Sybyl 7.1, and hydrogen atoms were added. The hydrogen atom positions were minimized until an rms of 0.005 kcal mol^{-1} Å^{-1} was reached using the Powell method. This homology model was solvated with water molecules using the solvent/solvate option.[50] The solvated homology model was minimized until the gradient reached 0.05 kcal mol^{-1} Å^{-1} using Tripos force field. For docking studies, the active site was generated by selecting the same amino acid residues that are located within a radius of 12.0 Å from TTP in the hTK1 crystal structure (PDB code 1W4R).[38] Docking of **10A** and (*R*)-epimers of **11** and **12** into the active site was performed with the FlexX module in Sybyl 7.1.

Calculation of Polar Surface Areas (PSAs) and Apolar Surface Areas (APSAs). Compounds **10A** and (*R*)-epimers of **11** and **12** were minimized as described above with the Gaussian 03 program, and parameters were transferred to HyperChem 7.51 for windows. Boron atom type of the carborane cage was changed into the C.3 carbon atom type. For (A)PSA calculations, structure parameters were transferred to the VEGA ZZ 2.0.4 program (Milano, Italy). A probe radius of 0.5 was used for the calculation of (A)-PSAs.

Human TK1 Phosphorylation Assay. Recombinant hTK1 was expressed and purified as described previously.[8] Phosphorylation rates were determined by measuring the change of ADP production in absorbance at 340 nm, caused by NADH oxidation in a coupled enzyme system with pyruvate kinase and lactate dehydrogenase. The standard reaction mixture contained 40 μM of **1A**, **1B**, **10A**−

C, **11**, or **12**, 20 mM Tris-HCl, pH 7.6, 50 mM KCl, 2 mM $MgCl_2$, 0.5 mM ATP, 5 mM DTT, 1 mM phosphoenolpyruvate, 0.5 units/mL pyruvate kinase, 0.5 units/mL lactate dehydrogenase, 0.1 mM NADH, and 0.6 μg of enzyme in a total volume of 0.25 mL. The reaction was performed at 24 °C with a Cary 3 spectrophotometer (Varian Techtron, Mulgrave, Australia) and started by the addition of Thd, **1A**, **1B**, **10A**−**C**, **11**, or **12**. The enzyme activity values were calculated from the slope of the absorbance graph. The activity of hTK1 with 20 μM Thd was 640 nmol of TMP formed per minute and milligram of hTK1 protein.

Therapy Experiments. BNCT was performed 14 days following intracebrebral stereotactic implantation of 10^3 F98 glioma cells. Rats were transported to the Nuclear Reactor Laboratory at the Massachusetts Institute of Technology (MIT) and then randomized on the basis of weight into experimental groups of seven to nine animals each as follows: group I, 500 μg of **1B** in 200 μL of 50% DMSO, administered intracerebrally (ic) over a period of 24 h by convection-enhanced delivery (CED) using ALZET minipumps (model 2001D) at a flow rate of 8 μL/h, following which they were irradiated with a collimated beam of thermal neutrons at a reactor power of 4.8 MW. Group II received 50% DMSO alone by CED, as per group I, followed by neutron irradiation, and group III received 500 μg of **1B** in 50% DMSO via CED without neutron irradiation. Group IV was treated and processed for boron determinations by DCP-AES as described previously.[36] All irradiated rats were anesthetized with a mixture of ketamine and xylazine. BNCT was carried out at the MITR-II reactor in the MIT irradiation facility, which produces a beam of high-intensity thermal neutrons without any contaminating fast neutrons. After completion of BNCT, the animals were held at MIT for ~3 days to allow induced radioactivity to decay before they were returned to The Ohio State University for clinical monitoring.

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Supporting Information Available: MS data of **1A** and **1B**; NMR spectra of **1A**; methods for the synthesis **11** and **12**; partial ^{1}H NMR spectra of **1A** and **10A**−**C** with (*S*)-(+)-2,2,2-trifluoro-1-(9-anthryl)-ethanol; HPLC spectra/purity data for compounds **1A**, **1B**, **10A**−**C**, **11**, and **12**; results from Gaussian 03 calculations for **10A**, (*R*)-**11**, and (*R*)-**12**; and human TK1 homology model with docked (*R*)-**11**. This material is available free of charge via the Internet at http://pubs.acs.org.

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