Synthesis of 5-(Carboranylalkylmercapto)-2′-deoxyuridines and 3-(Carboranylalkyl)thymidines and Their Evaluation as Substrates for Human Thymidine Kinases 1 and 2

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Derivatives of thymidine containing *o*-carboranylalkyl groups at the N-3 position and derivatives of 2′-deoxyuridine containing *o*-carboranylalkylmercapto groups at the C-5 position were synthesized. The alkyl spacers consist of 4–8 methylene units. The synthesis of the former compounds required 3–4 reaction steps in up to 75% overall yield and that of the latter 9–10 reaction steps with significantly lower overall yield. Derivatives of thymidine substituted with carboranylalkyl substituents at the N-3 position and short spacers were phosphorylated by both recombinant and purified cytosolic thymidine kinase (TK1) to a relatively high degree. None of the tested 2′-deoxyuridine derivatives possessing carboranyl substituents at the C-5 position were phosphorylated by either recombinant or purified TK1. The amounts of phosphorylation product detected for some of the C-5-substituted nucleosides with recombinant mitochondrial thymidine kinase (TK2) were low but significant and decreased with increasing lengths of the alkyl spacer. The data obtained in this study do not seem to support the tether concept applied in the synthesis of the new C-5- and N-3-substituted carboranyl nucleosides intended to reduce possible steric interference in the binding of carboranyl nucleosides with deoxynucleoside kinases. Instead, it appeared that a closer proximity of the bulky carborane moiety to the nucleoside scaffold resulted in better substrate characteristics.

Introduction

Boron neutron capture therapy (BNCT) is a binary system for the treatment of cancer. In order for this therapy to be effective, the targeted cancer cells must attain a sufficient concentration of ^{10}B, a stable isotope comprising approximately 20% of natural elemental boron. At the time of treatment, this localized boron content is activated by a suitable flux of low-energy (thermal) neutrons. The nuclear reaction [^{10}B(n,α)^{7}Li] that is initiated by neutron capture yields high-linear-energy-transfer (LET) particles, $^{4}He^{2+}$ and $^{7}Li^{3+}$ ions. These particles have a range of 9 and 5 μm, respectively, in biological tissue and can cause cell death through various cytocidal effects. However, the damage is largely restricted to the tumor because of the limited range of these particles. Provided that ^{10}B is selectively taken up by the tumor, the amount of this nuclide required to sustain lethal tumor cell damage has been calculated to be in the range of 15–30 μg/g of tumor, assuming a homogeneous distribution throughout all intra- and extracellular compartments.[1]

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In order for BNCT to be successful, ^{10}B delivery systems have to be developed that selectively target tumor cells. The observation that a combination of disodium mercaptoundecahydrododecaborate (BSH) and 4-dihydroxyborylphenylalanine (BPA), the two agents used in clinical BNCT, proved to be more effective in rats with intracerebral F98 glioma than either compound alone lends strong support to the concept that a "cocktail" of different compounds will eventually be used in clinical BNCT, just as cancer chemotherapy is carried out using combinations of drugs.[2]

Nucleoside kinases play a pivotal role in the use of nucleosides for cancer and antiviral therapy.[3] For BNCT, the cytosolic thymidine kinase (TK1) may be a particularly important target enzyme. This enzyme of the pyrimidine salvage pathway catalyzes the phosphorylation of thymidine (Thd) and 2′-deoxyuridine (dUrd) to the corresponding monophosphates. TK1 activity is present in proliferating cells but is absent from all quiescent cells.[4] In actively proliferating cells, TK1 activity is not constant, increasing dramatically during the S-phase and then decreasing at cytokinesis.[5] Boron-containing Thd and dUrd derivatives that are good substrates for TK1 may be entrapped in proliferating neoplastic cells after conversion to the monophosphate due to the acquired negative charge. In addition, conversion of a boronated Thd/dUrd monophosphate to the di- and triphosphates and subsequent incorporation into tumor cell DNA could result in the relocation of

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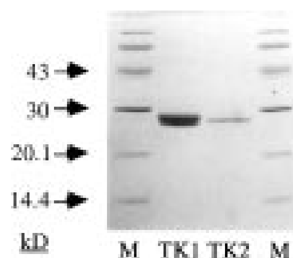


Figure 1. SDS–PAGE of recombinant TK1 and TK2 preparations. 50 ng of TK1 and 15 ng of TK2 were loaded on the gel; M = molecular weight markers.

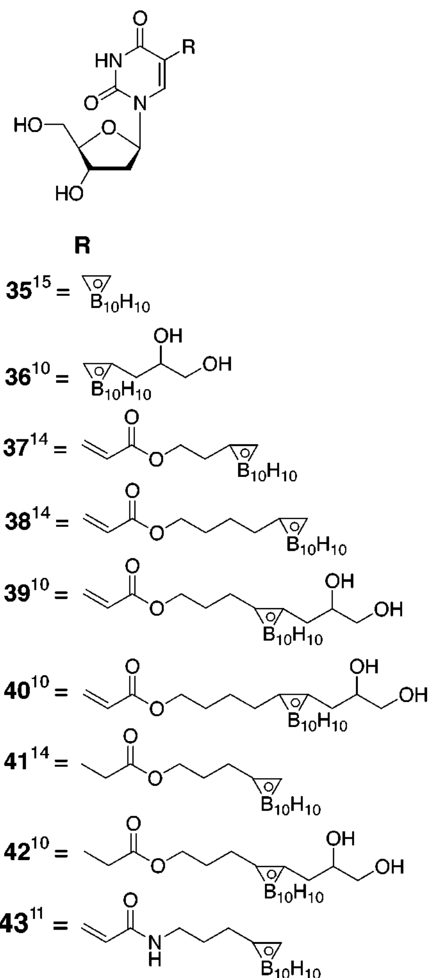
boron in closest proximity to DNA which is the most crucial target of the $^{4}He^{2+}$ and $^{7}Li^{3+}$ particles, thereby increasing the radiobiological effectiveness (RBE) of the boron neutron capture reaction significantly.[6] Therefore, Thd and dUrd analogues may be excellent vehicles for the selective delivery of ^{10}B to those parts of tumors consisting of viable cells and would certainly be ideal components of a "BNCT cocktail".

A large number of natural and unnatural nucleosides modified with various boron moieties at different positions of either the base or the sugar have been synthesized and evaluated biologically for use in BNCT.[1,7,8] Some dUrd derivatives were found to be phosphorylated in CEM cells in vitro[9] and in phosphoryl-transfer assays with human thymidine kinase.[10,11] However, the observed rates of phosphorylation were low compared to natural nucleosides, and the roles of cytosolic and mitochondrial thymidine kinase (TK2) in the phosphorylation were not investigated. At present, it is not known if certain types of tumors contain high levels of TK2 activity. This enzyme seems to be localized predominantly in mitochondria and appears to be equally active in proliferating and nonproliferating cells.[12] The substrate specificities of TK1 and TK2 differ significantly,[13] and therefore it is important to be able to determine the activities of boronated nucleoside analogues with defined preparations of TK1 and TK2.

This paper describes in detail the synthesis of new tethered carboranyl Thd and dUrd analogues and their evaluation in phosphoryl-transfer assays with recombinant human TK1 and TK2 (Figure 1), as well as with human TK1 purified from leukemia cells, in comparison with several known boronated nucleosides[10,11,14,15] which are listed in Figure 2.

Results and Discussion

Chemistry. *o*-Carborane was chosen as the boron moiety for Thd and dUrd analogues because it contains 10 boron atoms and is easily incorporated into organic structures.[1] In addition, there is a lack of appropriate alternative boron entities possessing a sufficient number of boron atoms that are suitable for attachment to nucleosides. The inherent drawbacks of *o*-carborane are its extreme lipophilicity, often rendering potentially bioactive structures containing this cluster water-insoluble,[1] and its dimensions, which are approximately 50% larger than the space occupied by the three-dimensional sweep of a phenyl group.[16] The possibility of steric hindrance prompted us to apply the concept of a hydrocarbon tether between the carborane and the nucleoside portion, unlike other boronated nucleosides having the carborane directly attached to the nucleoside



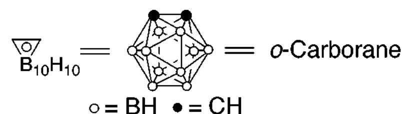
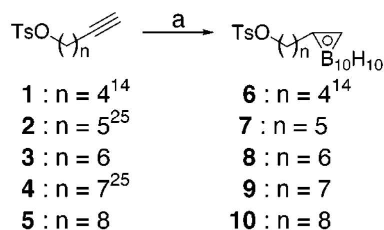


Figure 2. *C*-5-Carboranyl-2′-deoxyuridine analogues.

component.[7,8] The positioning of a bulky group such as the carborane cage in immediate proximity to the nucleoside may dramatically interfere with its binding to thymidine kinases, whereas addition of a tether propels the bulky boron moiety away from the nucleoside, thereby decreasing steric interference and allowing better binding to the enzyme. The concept of using a tether to overcome 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.[17,18] The results of these affinity chromatography studies indicated that a tether length of approximately 7 methylene units provided optimal binding.[17,18] Shorter tethers bound enzyme less efficiently because of possible steric interference by the nearby column matrix, and longer tethers may fold back on themselves thereby reducing the effective tether length. The tether concept has been applied previously by us[10,11,14] and others[19] in the synthesis of carboranyl nucleosides.

The C-5 position of dUrd and the N-3 position of Thd were chosen for the attachment of the *o*-carborane via a tether. Reports indicate that 5-(methylmercapto)-2′-

Scheme 1[a]



[a] (a) $B_{10}H_{12}(CH_3CN)_2$/toluene/reflux/4−8 h.

deoxyuridine was phosphorylated[20] and rapidly incorporated into DNA.[21] Because of this tolerance for 5-mercapto substituents by enzymes involved in DNA biosynthesis, the carboranylalkyl moieties in compounds **24**−**27** were also attached through a thioether linkage at the 5-position of dUrd. A brief description of the synthesis of 5-(carboranylalkylmercapto)-2′-deoxyuridines has been published previously.[22]

The carboranylalkyl substituents were also bound to N-3 of Thd (**29**−**32**) since in the affinity chromatography studies mentioned above, Thd was most likely linked through this position to the stationary phase.[23] Since thymidine kinases bound to the putative N-3-linked Thd ligand of the affinity columns, it is reasonable to assume that the same type of linkage might be tolerated for the tethered attachment of the bulky carboranes without significant loss of binding affinity. Nucleosides possessing a cyanoborane substituent at the N-3 position have been described previously by Sood et al.[24]

Tosylates **1**−**5** were prepared according to a procedure described by Kabalka et al.[25] from the corresponding alcohols (Scheme 1).[26−28] To the best of our knowledge, compounds **3** and **5** have not been described previously and were analyzed by ^{1}H NMR. The tosyl functions serve as electrophiles for the alkylation procedures described subsequently and as protective groups for the hydroxyl functions in the following boronation reaction, since the presence of a free hydroxyl group would prohibit carborane formation.[1] For the boronation of **1**−**5**, the bis(acetonitrile)decaborane complex, $B_{10}H_{12}(CH_3CN)_2$, was obtained by refluxing decaborane in acetonitrile for 4 h.[29] This complex was used to boronate compounds **1**−**5** to form the respective carboranyl derivatives **6**−**10** by refluxing in toluene for 4−8 h.[29]

S-Alkylation[30] of 5-mercaptouracil[31] (**11**) was accomplished under argon and in deoxygenated methanol to prevent the oxidation of **11** during the reaction (Scheme 2). 5-Mercaptouracil[31] (**11**) was alkylated with carboranyl tosylates **6**−**8** and **10** to yield the 5-S-alkylated mercaptouracils **12**−**15** using only 1 equiv of sodium methoxide to avoid the possible alkoxide degradation of the carboranes.[32]

The 5-(carboranylalkylmercapto)pyrimidines **12**−**15** were silylated with hexamethyldisilazane (HMDS) and a catalytic amount of trimethylsilyl chloride (TMSCl) in THF to yield the intermediate 2,4-bis-silylated pyrimidines which were reacted in situ with α-D-2-deoxy-3,5-di-*O*-*p*-toluoylribofuranosyl chloride[33] in carbon tetrachloride with $ZnCl_2$ as a catalyst, to yield nucleosides **16**−**23** (Scheme 2).[30] The labile TMS group at the 4-position was lost during the workup.

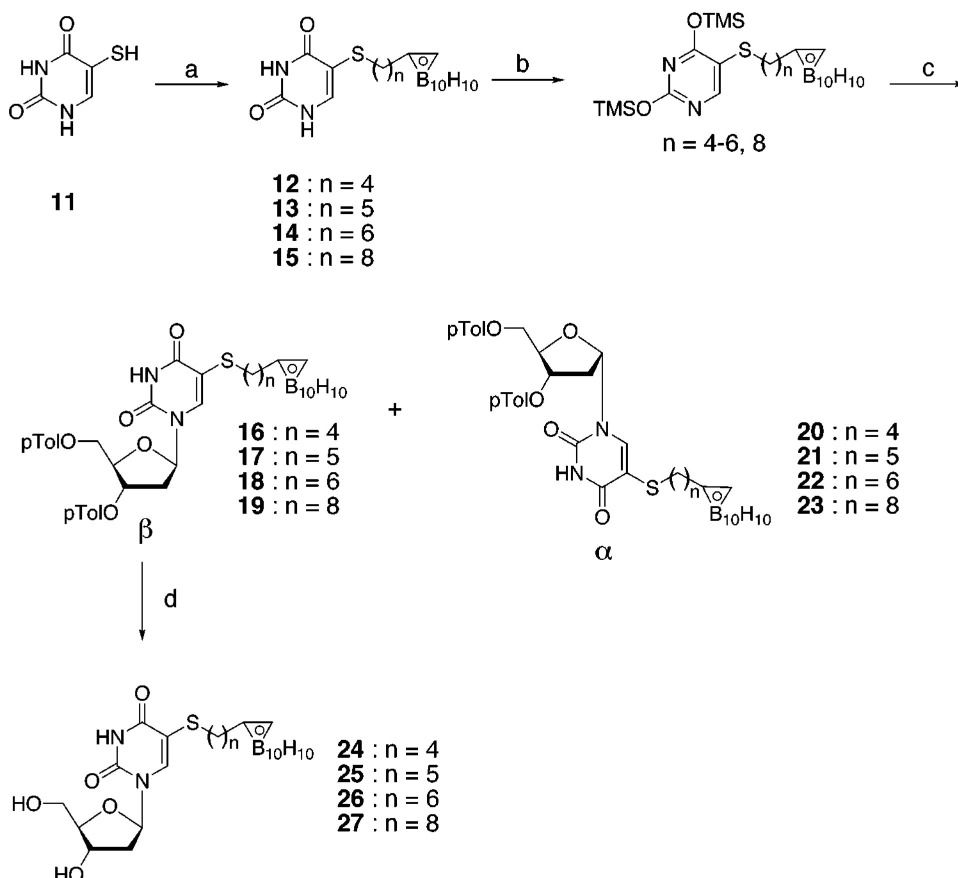
Nucleophilic displacement of the chloride in α-D-2-deoxy-3,5-di-*O*-*p*-toluoylribofuranosyl chloride[33] by an S_N2 mechanism led to the formation of nucleosides **16**−**19** with the desired β-configuration. The α-anomers **20**−**23** were also produced to a lesser extent, possibly from an S_N1 mechanism, but the use of $ZnCl_2$ catalyst was found to favor the desired β-anomer. The anomeric configurations were determined by ^{1}H NMR where the H-1 signals are characteristically a doublet of doublets ($J \approx 3$ and 7 Hz) for α-nucleosides and a pseudotriplet ($J \approx 7$ Hz) for β-nucleosides.[34] The anomeric ratios (β:α) averaged 3:1 with the use of $ZnCl_2$ in contrast to 1:1 without catalyst. The β-anomers were deprotected at 0 °C with sodium methoxide in methanol to yield the target compounds **24**−**27** (Scheme 2). As a result of the mild reaction conditions, base degradation of the carborane cage could be largely prevented.[35]

The 3-(carboranylalkyl)thymidines **29**−**32** (Scheme 3) were synthesized in a one-step reaction from Thd (**28**) by adapting procedures described previously.[36,37] Tosylates **6**−**9** were added to a solution of potassium carbonate and Thd in a DMF/acetone mixture at 50 °C to yield the target compounds **29**−**32** (Scheme 3). The basic conditions were mild enough not to degrade the carborane cage. While alkylation at other positions may generally be possible under these reaction conditions, it has not been reported in the literature,[36,37] and selective alkylation of the N-3 position was confirmed by a correlation of the ^{13}C NMR chemical shifts of **29**−**32** with those of 3-methylthymidine[38] and 4-*O*-methylthymidine.[38]

Compound **34**, the disodium salt of the 5′-monophosphate of nucleoside **29**, was prepared according to a procedure described previously[39] in order to function as a reference in phosphoryl-transfer assays described in the following section (Scheme 3). Bis(trichloroethyl)-phosphorochloridate and compound **29** were reacted in acetonitrile in the presence of pyridine to yield the protected phosphate **33**. Removal of the trichloroethyl groups using zinc powder in 90% acetic acid, conversion to the sodium salt by cation exchange, and subsequent purification by reversed-phase flash chromatography furnished compound **34**.

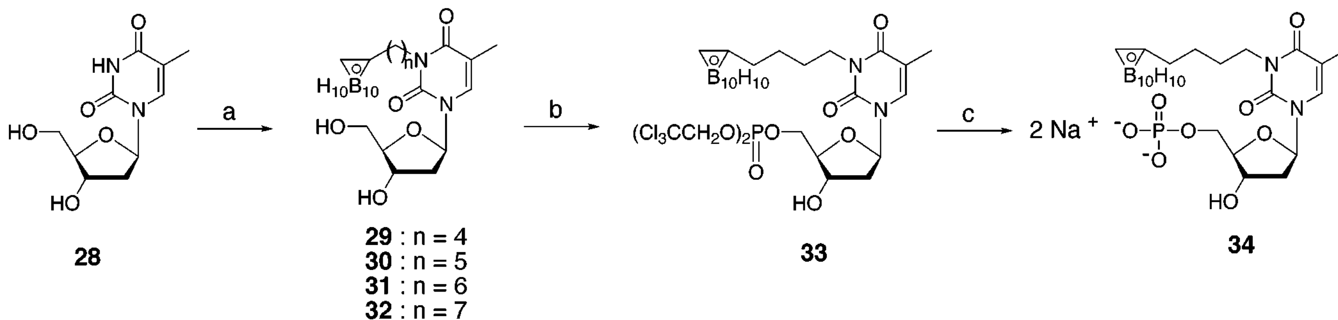
Biological Results. The results of the phosphoryl-transfer assays with recombinant human TK1 and TK2, as well as with purified human TK1, indicated that compounds **30**, **31**, **36**−**38**, and **40**−**43** are not phosphorylated by recombinant TK2 (data not shown). Low but significant levels of phosphorylation products with recombinant TK2 could be detected for all 5-S-substituted dUrd analogues (**24**−**27**), two N-3-substituted Thd analogues (**29**, **32**), compound **35** (previously designated as CDU[9]), and compound **39** (data not shown). Among the 5-S-substituted dUrd analogues, there was clearly higher activity for the compounds with a shorter 5-*S*-alkyl tether between nucleoside and carborane cage, in particular for **24** (Figure 3). Recombinant TK1 exhibited no activity with all C-5-substituted carboranyl dUrd analogues (**24**−**27**, **35**−**43**). In addition, compound **25** was not phosphorylated by purified TK1. However, all N-3-substituted carboranyl Thd analogues were phosphorylated with both recombinant and purified TK1 at relatively high rates ranging from 3−28% for recombinant TK1 and ∼10−20% for purified TK1 (in relation to Thd as the reference) (Table 1). The results in Table 1 are compiled from two independent sets of experiments,

Scheme 2[a]



[a] (a) **6**−**8**, **10**/$NaOCH_3$/CH_3OH/60 °C/4−6 h; (b) HMDS/TMSCl, THF/reflux/4−6 h; (c) 3,5-di-*O*-*p*-toluoyl-2-deoxy-α-D-ribofuranosyl chloride/$ZnCl_2$/CCl_4/rt/2−4 days; (d) $NaOCH_3$/CH_3OH/0 °C/3−5 days.

Scheme 3[a]



[a] (a) **6**−**9**/K_2CO_3/DMF−acetone (1:1)/50 °C/2 days; (b) **29**/bis(2,2,2-trichloroethyl)phosphorochloridate/pyridine/0 °C/3 days; (c) Zn/AcOH/rt/ 2 h/Dowex 50X8-100 (Na^+-form).

and there is an apparent discrepancy between the phosphorylation rates of compounds **31** and **32** with recombinant and purified TK1. This is most likely due to the different enzyme preparations and assay conditions. In the case of the assays with purified TK1, concentrations of protein, substrate, and solubilizing DMSO (2.5%) were high and that of ATP was low. In the assays with pure recombinant TK1, the concentrations of protein, substrate, and DMSO (~0.3%) were much lower while that of ATP was higher. Therefore, it is difficult to compare directly the activities since the varying solubilities of the nucleoside analogues may also have a significant effect on their phosphorylation rates. Nevertheless, no increase in activity for the N-3-substituted carboranyl Thd analogues with increasing spacer length could be observed with any of the TK1 preparations.

For compounds **29** and **30**, which appear to possess the most favorable phosphorylation rates in both TK1 assay systems, we have also determined rather high K_m values (105 ± 12 and 71 ± 3 μM, respectively, with recombinant TK1). For comparison, the K_m for Thd is ~1 μM.[12] These data translate into a relative efficiency (V_{max}/K_m) of ~0.3% for both **29** and **30**.

The obtained data for N-3-substituted carboranyl Thd analogues with purified TK1 do not support the tether concept, and the phosphorylation rates found for these nucleosides with recombinant TK1 decrease with increasing tether lengths. These results seem to contradict the tether concept. However, we do not know whether

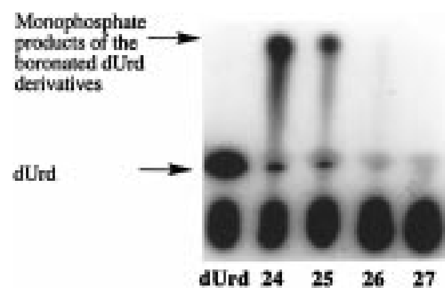


Figure 3. Phosphorylation of dUrd and 5-*S*-carboranyl-2′-deoxyuridine analogues **24**−**27** by recombinant TK2. The reaction products were separated by PEI-cellulose TLC. The concentrations of [γ-^{32}P]ATP and substrates were 100 μM. The assay conditions are described in detail in the Experimental Section. The spot for the 5′-monophosphate of **26** is very faint, and that of **27** can only be observed when higher substrate and ATP concentrations are exposed to TK2 for an extended period of time.

Table 1. Relative Phosphorylation of Thd, dUrd, and **29**−**32** by Recombinant and Purified TK1

compd	recombinant TK1	purified TK1
Thd	1[a]	nm[c]
dUrd	0.55 ± 0.18	1[b]
29	0.28 ± 0.03	0.20 ± 0.05
30	0.19 ± 0.10	0.35 ± 0.01
31	0.04 ± 0.02	0.32 ± 0.01
32	0.03 ± 0.03	0.34 ± 0.06

[a] For recombinant TK1, the obtained value for Thd was set to 1. [b] For purified TK1, the obtained value for dUrd was set to 1. [c] nm, not measured. Mean ± SD values are based on 4 experiments for recombinant TK1 and 3 experiments for purified TK1. The assay conditions are described in detail in the Experimental Section.

this contradiction is due to a flaw in the concept or to the water solubility of N-3-substituted carboranyl nucleosides, which decreases with increasing tether length thus probably limiting the number of substrates that find access to the enzyme. Extended studies are currently underway in our laboratories, which address in greater detail the influence of tether length and water solubility on the phosphorylation rates of N-3-substituted carboranyl nucleosides with recombinant TK1.

TK2 generally possesses much broader substrate specificity for pyrimidine nucleosides than TK1 and is even known to tolerate relatively large substituents at the 5-position of the base.[12,13] However, the attachment of any type of bulky carboranyl substituent at either N-3 of Thd or C-5 of dUrd seems to prevent an efficient interaction of recombinant TK2 with the substrates. As in the case of compounds **29**−**32** with recombinant TK1, the phosphorylation rates observed for the 5-S-substituted carboranyl dUrd analogues (**24**−**27**) with recombinant TK2 do not seem to support the tether concept. However, the relationship between water solubilities and TK2 phosphorylation velocities of compounds **24**−**27** also remains to be determined.

Two different methods were employed to prove unequivocally that the product spots observed on PEI cellulose TLC plates with the phosphoryl-transfer reaction mixtures were indeed caused by phosphorylation of the carboranyl nucleosides: (1) a R_f value comparison of the phosphorylation product of **29** with synthetic 5′-monophosphate **34** and (2) enzymatic removal of the phosphate moiety from **34** as well as from the phosphorylation product of **29**. It is known that alkaline phosphatase catalyzes the release of phosphate from several phosphorylated compounds,[40] and this procedure has also been applied previously to prove indirectly the in vitro phosphorylation of compound **35**.[9] Both experiments were visualized by a combination of β- and α-radiography[41] which allows the detection of ^{32}P (β-particles) and ^{10}B (α-particles from the [^{10}B(n,α)^{7}Li] reaction) on TLC plates (Figure 4, see Experimental Section for details). The pattern of the β/α-radiogram displayed in Figure 4 shows that the phosphorylation products of **29** obtained with TK1 and both [γ-^{32}P]ATP (lane c) and [^{31}P]ATP (lane g) as well as compound **34** (lane i) have the same migrating ratio. Mixing of enzymatic phosphorylation products with synthetic **34** does not change this ratio (lanes e and f). When products from lanes a (Thd-5′-monophosphate), c, g, and i (compound **34**) were exposed to alkaline phosphatase, the phosphorylated products disappear (lanes b, d, h, and j), and in the case of lane j, a new product becomes clearly visible at the position of compound **29** (lane k). Very faint spots are also visible at the position of **29** on lanes g and h because of incomplete conversion of **29** to **34** by TK1 (g) and/or reconversion of **34** to **29** by alkaline phosphatase (h). All products at the positions of **29** and **34** are also UV-active. There are some additional product spots that cannot be explained (lanes c, e, f, g, and h). Particularly interesting are those at the Thd-5′-monophosphate position on lanes c, e, f, and g. The product causing these spots contains obviously both ^{32}P and ^{10}B and is exclusively produced when compound **29** is exposed to TK1 and ATP. Therefore, it cannot be Thd-5′-monophosphate. Since this product seems to be significantly more polar than **34**, it could be the diphosphate of **29** or the *nido*-carboranyl analogue of **34**. However, neither is TK1 known to catalyze the formation of diphosphates, nor are the experimental conditions of the phosphoryl-transfer assay sufficiently basic to cause degradation of the *o*-carborane cage in **34**.

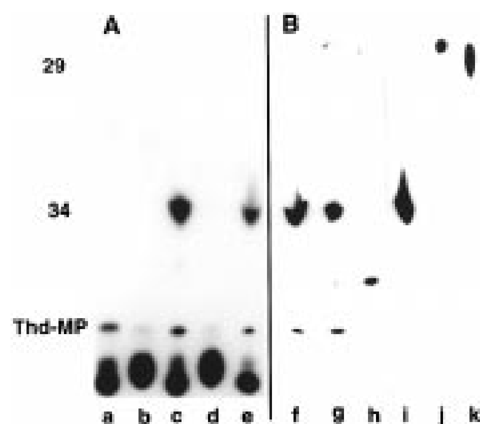


Figure 4. Comparative β/α-radiogram of enzymatically (TK1) produced phosphorylation products of compound **29** and synthetic 5′-monophosphate **34** as well as the results of alkaline phosphatase treatment of these products: (A) β-radiogram; (B) α-radiogram. Lanes: a = TK1 + Thd + [γ-^{32}P]-ATP; b = a + alkaline phosphatase; c = TK1 + **29** + [γ-^{32}P]ATP; d = c + alkaline phosphatase; e = c + i; f = g + i; g = TK1 + **29** + [^{31}P]ATP; h = g + alkaline phosphatase; i = **34**; j = i + alkaline phosphatase; k = **29**; MP = 5′-monophosphate.

We have recently found evidence that TK1 purified from human leukemic cells previously used in our laboratories for the evaluation of compounds **35**−**43** as

substrates for TK1[10,11] may have been contaminated with TK2.[42] The results of the present study indicate that the phosphorylation found for compounds **35**−**43** in these earlier studies[10,11] may have been caused by this TK2 contamination rather than by TK1.

Summary and Conclusion

The reaction sequences for the syntheses of 3-(carboranylalkyl)thymidines **29**−**32** involve 3−4 steps from readily available starting materials in up to ~75% overall yield (**29**). Complicated reaction and purification procedures are not required. Thus, the synthesis of N-3-substituted carboranyl Thd analogues applying the methodology described herein should generally be suitable for large-scale production. The syntheses of 5-(carboranylalkylmercapto)-2′-deoxyuridines **24**−**27** require 9−10 steps and involve the separations of anomeric mixtures. The overall yields are very low. In particular, the necessary synthesis of 5-mercaptouracil starting from 5-aminouracil proved to be a very tedious procedure in our hands with yields not exceeding 6%.[42]

Both recombinant and purified TK1, the therapeutically relevant thymidine kinase isoform, tolerate bulky carboranylalkyl substituents at the N-3 position of Thd but do not tolerate bulky carboranyl groups at the C-5 position of dUrd. In the case of recombinant TK1, the phosphorylation rates for the tested N-3-substituted nucleosides decrease with increasing tether lengths between nucleoside and carborane cage. To the best of our knowledge, substantial phosphorylation velocities for Thd derivatives with bulky groups at the N-3 position with TK1 have not been reported as yet. Our findings are supported by previous studies demonstrating that Thd analogues substituted at the N-3 position with various groups are potent inhibitors of Thd phosphorylation by TK1.[43,44] Low amounts of phosphorylation product were detected for 8 out of 17 nucleosides tested with recombinant TK2. Nine nucleosides were not phosphorylated by recombinant TK2. The compounds with a shorter 5-*S*-alkyl tether between nucleoside and carborane cage seem to be better substrates for recombinant TK2.

These discoveries lend support to the assumption that previous design strategies for the synthesis of boronated nucleosides for BNCT, which have mainly focused on the attachment of various types of carboranyl moieties to the C-5 position of uridines, were based on biochemical considerations that may not be applicable. The results of our experiments may open new avenues in the future design of boronated Thd and dUrd analogues for BNCT and may also have impact in compound development for traditional diagnosis and therapy of cancer and viral diseases.

Experimental Section

Chemistry. Proton, carbon-13, and phosphorus-31 NMR spectra were obtained on Bruker 250 and 270 MHz FT-NMRs at The Ohio State University College of Pharmacy as well as Bruker 300 and 500 MHz FT-NMRs at The Ohio State University Campus Chemical Instrumentation Center. NMR spectra on the latter two instruments were produced by Dr. Charles E. Cottrell. Chemical shifts (δ) are reported in ppm from an internal tetramethylsilane or an external phosphoric acid standard. Coupling constants are reported in Hz. All mass spectra were obtained at The Ohio State University Campus Chemical Instrumentation Center by Dr. David Chang on a VG 70-250S, Nicolet FTMS-2000, or Finnigan MAT-900 mass spectrometer. Ionization was achieved by electron impact for high-resolution mass spectra (HR-EI) and fast atom bombardment (FAB) with Xe using *m*-nitrobenzyl alcohol (3-NBA) as a matrix. For all carborane-containing compounds, the mass of the most intensive peak of the isotopic pattern was reported; measured patterns agreed with calculated patterns, and the mass was calculated for a 80% boron-11 and 20% boron-10 distribution in the case of HR-EI. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN, Atlantic Microlab, Inc., Norcross, GA, or Robertson Microlit Laboratories, Inc., Madison, NJ. IR spectra were recorded on a Laser Precision Corp. RFX-40 FT-IR spectrometer. Melting points were determined on a Fisher-Johns or Thomas-Hoover melting point apparatus and are reported uncorrected. Precoated glass-backed TLC plates with silica gel 60 F_{254} (0.25-mm layer thickness) and silica gel 60 (70−230 mesh) from Merck were used for TLC and column chromatography, respectively. General compound visualization for TLC was achieved by UV light and I_2 vapor. Carbohydrate-containing compounds were selectively visualized by spraying the plate with 1% H_2SO_4 and heating at 120 °C. Carborane-containing compounds were selectively visualized by spraying the plate with a 0.06% $PdCl_2$/1% HCl solution and heating at 120 °C which caused the slow (15−45 s) formation of a gray spot due to reduction of Pd^{2+} to Pd^0. Dynamax C_{18} and C_8 columns (10.0 × 250 mm, 8-μm particle size, 60 Å pore size) were used for analytical reversed-phase chromatography on an analytical/semipreparative Rainin HPLC system with Macintosh-based data acquisition and Rainin Dynamax model UV-1 detector. Merck C_8 silica gel (particle size = 40 μm, pore size = 60 Å) was used for preparative reversed-phase flash chromatography. Reagent-grade solvents were used for column chromatography. Pyridine, acetonitrile, and dimethylformamide (DMF) were dried over 4 Å molecular sieves. Tetrahydrofuran (THF) was dried by distillation from sodium and benzophenone indicator under argon. Toluene was dried over sodium. Other chemicals were purchased from commercial suppliers.

General Procedure for the Tosylation of Acetylenic Alcohols. Following a procedure developed by Kalbalka,[25] alkyn-1-ols were dissolved in 35 mL of dichloromethane and cooled to −10 °C. Pyridine (3 equiv) and *p*-toluenesulfonyl chloride (2 equiv) were added, and the solution was stirred for 2 h during which time the reaction reached room temperature. Equal volumes of water and diethyl ether were added. The aqueous layers were extracted three times with diethyl ether and the combined ether layers were washed with 2 N HCl, 5% $NaHCO_3$, and saturated NaCl solutions. The solutions were dried over $MgSO_4$, filtered, and evaporated to crude oils which were purified by silica gel column chromatography.

7-Octynyl Tosylate (3). A quantity of 2.1 g (16.7 mmol) of 7-octyn-1-ol[27] yielded 3.6 g (77%) of product isolated as a clear, colorless oil: R_f 0.92 (CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 1.19−1.39 (m, 4H, alkane), 1.45 (m, 2H, CH_2−CH$_2$C≡CH), 1.62 (q, J = 6, 2H, CH_2−CH$_2$O), 1.88 (t, J = 3, 1H, HC≡C), 2.13 (m, 2H, CH$_2$C≡C), 2.43 (s, 3H, CH$_3$), 3.97 (t, J = 6, 2H, CH$_2$−O), 7.55 (dd, J = 8, 4H, ArH).

9-Decynyl Tosylate (5). A quantity of 1.8 g (12 mmol) of 9-decyn-1-ol[28] yielded 3.0 g (84%) of product isolated as a white, waxlike solid: mp 29 °C; R_f 0.75 (2:1 dichloromethane:hexane); ^1H NMR ($CDCl_3$) δ 1.10−1.37 (m, 8H, alkane), 1.48 (q, J = 7, 2H, CH_2−CH$_2$C≡CH), 1.62 (q, J = 7, 2H, CH_2−CH$_2$O), 1.91 (t, J = 3, 1H, HC≡C), 2.14 (m, 2H, CH$_2$C≡C), 2.41 (s, 3H, CH$_3$), 4.00 (t, J = 7, 2H, CH$_2$−O), 7.55 (dd, J = 8 Hz, 4H, ArH).

General Procedure for the Boronation of Acetylenic Tosylates. Following a standard procedure described previously,[29] a bis(acetonitrile)decaborane complex was prepared by refluxing decaborane in 45 mL of anhydrous acetonitrile for 4 h. ***Decaborane is a highly toxic, impact-sensitive compound which forms explosive mixtures especially with halogenated materials. A careful study of the MSDS is advisable before usage.*** On cooling to room temperature, the complex precipitated out of solution, was filtered, and was washed with diisopropyl ether. The complex was obtained as

a white powder (85%). The acetylenic tosylates (1 equiv) and the complex (1.5 equiv) were dissolved in 75 mL of toluene and refluxed for 2−6 h. The solvent was evaporated leaving thick red syrups which were extracted several times with diethyl ether. Upon near complete extraction of the products, the syrups yielded yellow solids which were removed by filtration. The ether extracts were combined and evaporated to crude oils which were purified by silica gel column chromatography.

5-(*o*-Carboran-1-yl)pentyl Tosylate (7). Utilizing the above general procedure, 3.3 g (12.4 mmol) of 6-heptynyl tosylate[26] yielded the corresponding carboranyl analogue. After purification by silica gel column chromatography and recrystallization from hexane containing a minimal amount of ethyl acetate, 3.0 g (62%) of product was isolated as a white crystalline solid: mp 109−110 °C; R_f 0.59 (2:1 hexane:ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 1.00−3.61 (br m, 10H, BH), 1.19−1.51 (m, 4H, alkane), 1.62 (q, $J = 7$, 2H, C*H*$_2$−CH_2O), 2.14 (m, 2H, CH_2−$C_{Carborane}$), 2.45 (s, 3H, CH_3), 3.52 (br s, 1H, $C_{Carborane}$-H), 3.99 (t, $J = 6$, 2H, CH_2−O), 7.55 (dd, $J = 8$, 4H, ArH), ^{13}C NMR ($CDCl_3$) δ 21.59 (CH_3), 24.87 (CH_2), 28.35 (CH_2), 28.44 (CH_2), 37.87 (CH_2), 61.12 ($C_{Carborane}$-H), 69.77 (CH_2−O), 74.98 ($C_{Carborane}$-C), 127.84 (Ar), 129.88 (Ar), 133.28 (Ar), 144.86 (Ar); MS (HR-EI) $C_{14}H_{28}O_3SB_{10}$ calcd 384.2762, found 384.2735.

6-(*o*-Carboran-1-yl)hexyl Tosylate (8). Utilizing the above general procedure, 2.6 g (9.2 mmol) of 7-octynyl tosylate (**3**) yielded the corresponding carboranyl analogue. After purification by silica gel column chromatography and recrystallization from hexane containing a minimal amount of ethyl acetate, 1.7 g (45%) of product was isolated as a white crystalline solid: mp 52−53 °C; R_f 0.56 (2:1 hexane:ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 1.10−3.75 (br m, 10H, BH), 1.10−1.50 (m, 6H, alkane), 1.61 (q, $J = 6$, 2H, C*H*$_2$−CH_2O), 2.12 (m, 2H, CH_2−$C_{Carborane}$), 2.42 (s, 3H, CH_3), 3.55 (br s, 1H, $C_{Carborane}$-H), 4.00 (t, $J = 6$, 2H, CH_2−O), 7.55 (dd, $J = 8$, 4H, ArH); ^{13}C NMR ($CDCl_3$) δ 21.58 (CH_3), 24.96 (CH_2), 28.21 (CH_2), 28.61 (CH_2), 28.90 (CH_2), 37.94 (CH_2), 61.09 ($C_{Carborane}$-H), 70.16 (CH_2−O), 75.23 ($C_{Carborane}$-C), 127.84 (Ar), 129.83 (Ar), 133.41 (Ar), 144.74 (Ar); MS (HR-EI) $C_{15}H_{30}O_3SB_{10}$ calcd 398.2919, found 398.2915.

7-(*o*-Carboran-1-yl)heptyl Tosylate (9). Utilizing the above general procedure, 3.1 g (10.5 mmol) of 8-nonynyl tosylate[26] yielded the corresponding carboranyl analogue. After purification by silica gel column chromatography and recrystallization from hexane containing a minimal amount of ethyl acetate, 2.5 g (59%) of product was isolated as a white crystalline solid: mp 62−65 °C; R_f 0.52 (2:1 hexane:ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 1.10−3.55 (br m, 10H, BH), 1.10−1.47 (m, 8H, alkane), 1.62 (q, $J = 6$, 2H, C*H*$_2$−CH_2O), 2.14 (m, 2H, CH_2−$C_{Carborane}$), 2.44 (s, 3H, CH_3), 3.55 (br s, 1H, $C_{Carborane}$-H), 4.00 (t, $J = 6$, 2H, CH_2−O), 7.55 (dd, $J = 8$, 4H, ArH); ^{13}C NMR ($CDCl_3$) δ 21.55 (CH_3), 25.11 (CH_2), 28.33 (CH_2), 28.60 (CH_2), 28.69 (CH_2), 28.95 (CH_2), 37.98 (CH_2), 61.08 ($C_{Carborane}$-H), 70.35 (CH_2−O), 75.38 ($C_{Carborane}$-C), 127.80 (Ar), 129.80 (Ar), 133.36 (Ar), 144.69 (Ar); MS (HR-EI) $C_{16}H_{30}O_3SB_{10}$ calcd 412.3075, found 412.3067.

8-(*o*-Carboran-1-yl)octyl Tosylate (10). Utilizing the above general procedure, 3.0 g (9.7 mmol) of 9-decynyl tosylate (**5**) yielded the corresponding carboranyl analogue. After purification by silica gel column chromatography and recrystallization from hexane containing a minimal amount of ethyl acetate, 2.1 g (50%) of product was isolated as a white crystalline solid: mp 37−40 °C; R_f 0.58 (2:1 hexane:ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 1.10−3.55 (br m, 10H, BH), 1.10−1.50 (m, 10H, alkane), 1.60 (q, $J = 6$, 2H, C*H*$_2$−CH_2O), 2.15 (m, 2H, CH_2−$C_{Carborane}$), 2.41 (s, 3H, CH_3), 3.55 (br s, 1H, $C_{Carborane}$-H), 3.95 (t, $J = 6$, 2H, CH_2−O), 7.55 (dd, $J = 8$, 4H, ArH); ^{13}C NMR ($CDCl_3$) δ 21.52 (CH_3), 25.12 (CH_2), 28.52 (CH_2), 28.59 (CH_2), 28.64 (CH_2), 28.71 (CH_2), 29.00 (CH_2), 37.90 (CH_2), 61.02 ($C_{Carborane}$-H), 70.51 (CH_2−O), 75.43 ($C_{Carborane}$-C), 127.72 (Ar), 129.76 (Ar), 133.10 (Ar), 144.66 (Ar); MS (HR-EI) $C_{17}H_{34}O_3SB_{10}$ calcd 426.3232, found 426.3224.

General Procedure for the Alkylation of 5-Mercaptouracil. Following a procedure similar to the one used by Dinan,[45,46] 5-mercaptouracil[31] (1 equiv) and NaOMe (1 equiv) were added to 50 mL of anhydrous MeOH previously degassed with argon. The corresponding carboranylalkyl tosylates (1 equiv) were added immediately, and the mixtures were stirred under argon for 4−6 h at 60 °C. The reactions were cooled and then neutralized with HCl/MeOH. Solvents were evaporated and the solid residues taken up in a minimum amount of THF and adsorbed to a minimal amount of silica gel by careful evaporation of the THF under reduced pressure. The mixtures were chromatographed on silica gel columns to obtain both the products and the unreacted tosylates.

5-[4-(*o*-Carboran-1-yl)butylmercapto]uracil (12). Following the procedure outlined above, 1.1 g (7.6 mmol) of 5-mercaptouracil (**11**) and 2.8 g (7.6 mmol) of 4-(*o*-carboran-1-yl)butyl tosylate[14] yielded 0.89 g (35%) of product as a white amorphous solid: mp 255−256 °C; R_f 0.71 (9:1 ethyl acetate: hexane); ^{1}H NMR (DMSO-d_6) δ 1.10−3.15 (br m, 10H, BH), 1.30−1.55 (m, 4H, alkane), 2.25 (m, 2H, CH_2−$C_{Carborane}$), 2.62 (t, $J = 7$, 2H, CH_2−S), 5.14 (br s, 1H, $C_{Carborane}$-H), 7.58 (d, $J = 6$, 1H, H-6), 11.10 (br d, 1H, H-1), 11.28 (br s, 1H, H-3); ^{13}C NMR (DMSO-d_6) δ 27.54 (CH_2), 27.64 (CH_2), 31.75 (CH_2), 35.95 (CH_2), 62.95 ($C_{Carborane}$-H), 76.38 ($C_{Carborane}$-C), 104.46 (C-5), 144.40 (C-6), 151.00 (C-2), 162.57 (C-4); MS (HR-EI) $C_{10}H_{22}O_2N_2SB_{10}$ calcd 342.2405, found 342.2391.

5-[5-(*o*-Carboran-1-yl)pentylmercapto]uracil (13). Following the procedure outlined above, 0.40 g (2.8 mmol) of 5-mercaptouracil (**11**) and 1.1 g (2.8 mmol) of 5-(*o*-carboran-1-yl)pentyl tosylate (**7**) yielded 0.40 g (40%) of product as a white amorphous solid: mp 261−262 °C; R_f 0.75 (9:1 ethyl acetate:hexane); ^{1}H NMR (DMSO-d_6) δ 1.10−3.20 (br m, 10H, BH), 1.10−1.50 (m, 6H, alkane), 2.22 (m, 2H, CH_2−$C_{Carborane}$), 2.61 (t, $J = 7$, 2H, CH_2−S), 5.14 (br s, 1H, $C_{Carborane}$-H), 7.56 (s, 1H, H-6), 11.08 (br s, 1H, H-1), 11.27 (br s, 1H, H-3); ^{13}C NMR (DMSO-d_6) δ 26.89 (CH_2), 27.82 (CH_2), 28.14 (CH_2), 32.14 (CH_2), 36.38 (CH_2), 62.82 ($C_{Carborane}$-H), 76.40 ($C_{Carborane}$-C), 104.72 (C-5), 144.11 (C-6), 150.95 (C-2), 162.49 (C-4); MS (HR-EI) $C_{11}H_{24}O_2N_2SB_{10}$ calcd 356.2562, found 356.2560.

5-[6-(*o*-Carboran-1-yl)hexylmercapto]uracil (14). Following the procedure outlined above, 0.29 g (2.0 mmol) of 5-mercaptouracil (**11**) and 0.80 g (2.0 mmol) of 6-(*o*-carboran-1-yl)hexyl tosylate (**8**) yielded 0.29 g (39%) of product as a white amorphous solid: mp 232−233 °C; R_f 0.77 (9:1 ethyl acetate:hexane); ^{1}H NMR (DMSO-d_6) δ 1.09−3.25 (br m, 10H, BH), 1.10−1.50 (m, 8H, alkane), 2.21 (m, 2H, CH_2−$C_{Carborane}$), 2.62 (t, $J = 7$, 2H, CH_2−S), 5.14 (br s, 1H, $C_{Carborane}$-H), 7.55 (s, 1H, H-6), 11.08 (br s, 1H, H-1), 11.25 (br s, 1H, H-3); ^{13}C NMR (DMSO-d_6) δ 27.16 (CH_2), 27.53 (CH_2), 28.11 (CH_2), 28.51 (CH_2), 32.25 (CH_2), 36.42 (CH_2), 62.85 ($C_{Carborane}$-H), 76.43 ($C_{Carborane}$-C), 104.90 (C-5), 143.91 (C-6), 150.92 (C-2), 162.49 (C-4); MS (HR-EI) $C_{12}H_{26}O_2N_2SB_{10}$ calcd 370.2718, found 370.2713.

5-[8-(*o*-Carboran-1-yl)octylmercapto]uracil (15). Following the procedure outlined above, 0.21 g (1.5 mmol) of 5-mercaptouracil (**11**) and 0.62 g (1.5 mmol) of 8-(*o*-carboran-1-yl)octyl tosylate (**10**) yielded 0.16 g (28%) of product as a white amorphous solid: mp 222−223 °C; R_f 0.69 (9:1 ethyl acetate:hexane); ^{1}H NMR (DMSO-d_6) δ 1.00−3.25 (br m, 10H, BH), 1.10−1.50 (m, 12H, alkane), 2.22 (m, 2H, CH_2−$C_{Carborane}$), 2.62 (t, $J = 7$, 2H, CH_2−S), 5.14 (br s, 1H, $C_{Carborane}$-H), 7.55 (d, $J = 5$, 1H, H-6), 11.08 (br d, $J = 5$, 1H, H-1), 11.26 (br s, 1H, H-3); ^{13}C NMR (DMSO-d_6) δ 27.60 (CH_2), 27.96 (CH_2), 28.09 (CH_2), 28.18 (CH_2), 28.28 (CH_2), 28.60 (CH_2), 32.28 (CH_2), 36.47 (CH_2), 62.88 ($C_{Carborane}$-H), 76.50 ($C_{Carborane}$-C), 104.94 (C-5), 143.87 (C-6), 150.94 (C-2), 162.49 (C-4); MS (HR-EI) $C_{14}H_{30}O_2N_2SB_{10}$ calcd 398.3031, found 398.3040.

General Procedure for the Preparation of Carboranyl Nucleosides. The silyl−Hilbert−Johnson reaction[47−50] was employed to synthesize the following blocked carboranyl nucleosides. 5-*S*-(Carboranylalkyl)uracils (1 equiv) were dissolved in 30 mL of anhydrous THF. Hexamethyldisilazane (HMDS) (2 equiv) and catalytic amounts of TMSCl (0.01 equiv) were added and the solutions refluxed under argon atmosphere. The byproduct, NH_4Cl, sublimed and was periodically removed from the condenser tips. The cessation of NH_4Cl

sublimation indicated that the reactions were complete (4−6 h). Removal of THF and excess HMDS by evaporation left clear oil residues which were immediately used for the condensation step. The oils were dissolved in 25 mL of anhydrous CCl_4, and freshly prepared α-D-2-deoxy-3,5-di-*O*-*p*-toluoylribofuranosyl chloride[33] (1.25 equiv) and $ZnCl_2$ (0.01 equiv) were added all at once. The reactions were stirred at room temperature for 2−4 days, and then CCl_4 was removed, leaving crude oils which were purified by silica column chromatography. In all cases the β-anomers eluted before the α-anomers. Fractions containing both anomers were analyzed by ^{1}H NMR to determine their anomeric ratios. The overall anomeric ratios were determined by weight.

5-[4-(*o*-Carboran-1-yl)butylmercapto]-3′,5′-di-*O*-*p*-toluoyl-β-D-2′-deoxyuridine (16). Performing the above general procedure, 0.86 g (2.5 mmol) of 5-[4-(*o*-carboran-1-yl)butylmercapto]uracil (**12**) yielded 0.90 g (51%) of the β-anomer as a white foam: mp 92−97 °C; R_f 0.35 (3:2 hexane:ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 1.10−3.40 (br m, 10H, BH), 1.34−1.63 (m, 4H, alkane), 2.17 (m, 2H, CH_2−$C_{Carborane}$), 2.42 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 2.49 (m, 2H, H-2′), 2.74 (m, 2H, CH_2−S), 3.72 (br s, 1H, $C_{Carborane}$-H), 4.57 (m, 1H, H-4′), 4.72 (m, 2H, H-5′), 5.60 (br d, 1H, H-3′), 6.37 (dd, J = 8.5, J = 5.5, 1H, H-1′), 7.61 (dd, J = 8 Hz, ArH), 7.83 (s, 1H, H-6), 9.51 (br s, 1H, H-3); ^{13}C NMR ($CDCl_3$) δ 21.86 (CH_3), 27.83 (CH_2), 28.36 (CH_2), 33.00 (CH_2), 37.31 (CH_2), 38.44 (C-2′), 61.23 ($C_{Carborane}$-H), 64.04 (C-5′), 74.70 (C-3′), 75.03 ($C_{Carborane}$-C), 83.23 (C-1′), 85.72 (C-4′), 108.78 (C-5), 126.15 (Ar), 126.41 (Ar), 129.28 (Ar), 129.40 (Ar), 129.64 (Ar), 129.80 (Ar), 144.47 (Ar), 144.61 (Ar), 141.61 (C-6), 149.88 (C-2), 161.83 (C-4), 166.00 (C=O), 166.07 (C=O); MS (FAB$^+$, 3-NBA) 695 (M + 1). The total nucleoside yield was 78% with a β:α ratio of 2:1.

5-[4-(*o*-Carboran-1-yl)butylmercapto]-3′,5′-di-*O*-*p*-toluoyl-α-D-2′-deoxyuridine (20). The α-anomer, 0.46 g (26%), was isolated as a white foam: R_f 0.30 (3:2 hexane:ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 1.15−3.35 (br m, 10H, BH), 1.32−1.64 (m, 4H, alkane), 2.16 (m, 2H, CH_2−$C_{Carborane}$), 2.42 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 2.74 (m, 2H, CH_2−S), 2.79 (m, 2H, H-2′), 3.65 (br s, 1H, $C_{Carborane}$-H), 4.54 (m, 2H, H-5′), 4.94 (t, 1H, H-4′), 5.63 (br d, 1H, H-3′), 6.31 (br d, J = 6, 1H, H-1′), 7.52 (dd, J = 8, 4H, ArH), 7.61 (dd, J = 8, 4H, ArH), 7.93 (s, 1H, H-6), 9.42 (br s, 1H, H-3).

5-[5-(*o*-Carboran-1-yl)pentylmercapto]-3′,5′-di-*O*-*p*-toluoyl-β-D-2′-deoxyuridine (17). Performing the general procedure above, 0.42 g (1.2 mmol) of 5-[5-(*o*-carboran-1-yl)pentylmercapto]uracil (**13**) yielded 0.50 g (58%) of the β-anomer as a white foam: mp 99−102 °C; R_f 0.35 (3:2 hexane:ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 1.18−3.15 (br m, 10H, BH), 1.18−1.34 (m, 2H, alkane), 1.34−1.53 (m, 4H, alkane), 2.16 (m, 2H, CH_2−$C_{Carborane}$), 2.42 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 2.49 (m, 2H, H-2′), 2.73 (m, 2H, CH_2−S), 3.67 (br s, 1H, $C_{Carborane}$-H), 4.57 (m, 1H, H-4′), 4.71 (m, 2H, H-5′), 5.61 (br d, 1H, H-3′), 6.39 (dd, J = 8.5, J = 5.5, 1H, H-1′), 7.61 (dd, J = 8, 8H, ArH), 7.81 (s, 1H, H-6), 9.42 (br s, 1H, H-3); ^{13}C NMR ($CDCl_3$) δ 21.76 (CH_3), 27.65 (CH_2), 28.57 (CH_2), 28.70 (CH_2), 33.43 (CH_2), 37.86 (CH_2), 38.48 (C-2′), 61.21 ($C_{Carborane}$-H), 64.19 (C-5′), 74.79 (C-3′), 75.31 ($C_{Carborane}$-C), 83.25 (C-1′), 85.69 (C-4′), 109.23 (C-5), 126.29 (Ar), 126.53 (Ar), 129.41 (Ar), 129.54 (Ar), 129.77 (Ar), 129.93 (Ar), 144.59 (Ar), 144.75 (Ar), 141.50 (C-6), 150.03 (C-2), 161.95 (C-4), 166.15 (C=O), 166.23 (C=O); MS (FAB$^+$, 3-NBA) 709 (M + 1). The total nucleoside yield was 90% with a β:α ratio of 1.8:1.

5-[5-(*o*-Carboran-1-yl)pentylmercapto]-3′,5′-di-*O*-*p*-toluoyl-α-D-2′-deoxyuridine (21). The α-anomer, 0.27 g (32%), was isolated as a white foam: R_f 0.26 (3:2 hexane:ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 1.15−3.10 (br m, 10H, BH), 1.15−1.35 (m, 2H, alkane), 1.35−1.53 (m, 4H, alkane), 2.16 (m, 2H, CH_2−$C_{Carborane}$), 2.40 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 2.72 (m, 2H, CH_2−S), 2.79 (m, 2H, H-2′), 3.65 (br s, 1H, $C_{Carborane}$-H), 4.54 (m, 2H, H-5′), 4.93 (t, 1H, H-4′), 5.63 (br d, 1H, H-3′), 6.33 (br d, J = 6, 1H, H-1′), 7.50 (dd, J = 8, 4H, ArH), 7.62 (dd, J = 8, 4H, ArH), 7.92 (s, 1H, H-6), 9.66 (br s, 1H, H-3).

5-[6-(*o*-Carboran-1-yl)hexylmercapto]-3′,5′-di-*O*-*p*-toluoyl-β-D-2′-deoxyuridine (18). Performing the general procedure above, 0.36 g (1.0 mmol) of 5-[6-(*o*-carboran-1-yl)hexylmercapto]uracil (**14**) yielded 0.43 g (59%) of the β-anomer as a white foam: mp 94−97 °C; R_f 0.38 (3:2 hexane:ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 1.10−3.14 (br m, 10H, BH), 1.10−1.34 (m, 4H, alkane), 1.34−1.52 (m, 4H, alkane), 2.17 (m, 2H, CH_2−$C_{Carborane}$), 2.42 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 2.49 (m, 2H, H-2′), 2.72 (m, 2H, CH_2−S), 3.59 (br s, 1H, $C_{Carborane}$-H), 4.56 (m, 1H, H-4′), 4.71 (m, 2H, H-5′), 5.60 (br d, 1H, H-3′), 6.40 (dd, J = 8, J = 5, 1H, H-1′), 7.60 (dd, J = 8, 8H, ArH), 7.80 (s, 1H, H-6), 9.18 (br s, 1H, H-3); ^{13}C NMR ($CDCl_3$) δ 21.74 (CH_3), 27.85 (CH_2), 28.40 (CH_2), 28.76 (CH_2), 29.04 (CH_2), 33.46 (CH_2), 37.94 (CH_2), 38.42 (C-2′), 61.11 ($C_{Carborane}$-H), 64.17 (C-5′), 74.76 (C-3′), 75.38 ($C_{Carborane}$-C), 83.17 (C-1′), 85.63 (C-4′), 109.39 (C-5), 126.24 (Ar), 126.48 (Ar), 129.33 (Ar), 129.46 (Ar), 129.71 (Ar), 129.87 (Ar), 144.47 (Ar), 144.66 (Ar), 141.17 (C-6), 150.00 (C-2), 161.61 (C-4), 166.04 (C=O), 166.14 (C=O); MS (FAB$^+$, 3-NBA) 723 (M + 1). The total nucleoside yield was 85% with a β:α ratio of 2.3:1.

5-[6-(*o*-Carboran-1-yl)hexylmercapto]-3′,5′-di-*O*-*p*-toluoyl-α-D-2′-deoxyuridine (22). The α-anomer, 0.19 g (26%), was isolated as a white foam: R_f 0.29 (3:2 hexane:ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 1.03−3.10 (br m, 10H, BH), 1.03−1.33 (m, 4H, alkane), 1.33−1.53 (m, 4H, alkane), 2.16 (m, 2H, CH_2−$C_{Carborane}$), 2.40 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 2.72 (m, 2H, CH_2−S), 2.80 (m, 2H, H-2′), 3.65 (br s, 1H, $C_{Carborane}$-H), 4.53 (m, 2H, H-5′), 4.93 (t, 1H, H-4′), 5.64 (br d, 1H, H-3′), 6.33 (br d, J = 6, 1H, H-1′), 7.52 (dd, J = 9, 4H, ArH), 7.62 (dd, J = 9, 4H, ArH), 7.92 (s, 1H, H-6), 10.00 (br s, 1H, H-3).

5-[8-(*o*-Carboran-1-yl)octylmercapto]-3′,5′-di-*O*-*p*-toluoyl-β-D-2′-deoxyuridine (19). Performing the general procedure above, 1.1 g (2.75 mmol) of 5-[8-(*o*-carboran-1-yl)octylmercapto]uracil (**15**) yielded 0.46 g (60%) of the β-anomer as a white foam: mp 87−92 °C; R_f 0.39 (3:2 hexane:ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 0.80−3.30 (br m, 10H, BH), 1.10−1.35 (m, 8H, alkane), 1.35−1.53 (m, 4H, alkane), 2.16 (m, 2H, CH_2−$C_{Carborane}$), 2.42 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 2.49 (m, 2H, H-2′), 2.72 (m, 2H, CH_2−S), 3.59 (br s, 1H, $C_{Carborane}$-H), 4.56 (m, 1H, H-4′), 4.71 (m, 2H, H-5′), 5.60 (br d, 1H, H-3′), 6.40 (dd, J = 8, J = 5, 1H, H-1′), 7.60 (dd, J = 8, 8H, ArH), 7.80 (s, 1H, H-6), 8.81 (br s, 1H, H-3); ^{13}C NMR ($CDCl_3$) δ 21.75 (CH_3), 28.35 (CH_2), 28.86 (CH_2), 28.96 (CH_2), 29.01 (CH_2), 29.15 (CH_2), 33.53 (CH_2), 38.09 (CH_2), 38.44 (C-2′), 61.02 ($C_{Carborane}$-H), 64.18 (C-5′), 74.81 (C-3′), 75.49 ($C_{Carborane}$-C), 83.19 (C-1′), 85.61 (C-4′), 109.67 (C-5), 126.31 (Ar), 126.56 (Ar), 129.40 (Ar), 129.52 (Ar), 129.78 (Ar), 129.93 (Ar), 144.51 (Ar), 144.73 (Ar), 140.99 (C-6) 149.96 (C-2), 161.39 (C-4), 166.12 (C=O), 166.20 (C=O); MS (FAB$^+$, 3-NBA) 751 (M + 1). The total nucleoside yield was 82% with a β:α ratio of 2.7:1.

5-[8-(*o*-Carboran-1-yl)octylmercapto]-3′,5′-di-*O*-*p*-toluoyl-α-D-2′-deoxyuridine (23). The α-anomer, 0.17 g (22%), was isolated as a white foam: R_f 0.30 (3:2 hexane:ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 0.90−3.30 (br m, 10H, BH), 1.10−1.35 (m, 8H, alkane), 1.35−1.58 (m, 4H, alkane), 2.17 (m, 2H, CH_2−$C_{Carborane}$), 2.40 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 2.73 (m, 2H, CH_2−S), 2.79 (m, 2H, H-2′), 3.57 (br s, 1H, $C_{Carborane}$-H), 4.54 (AB m, 2H, H-5′), 4.92 (t, 1H, H-4′), 5.64 (br d, 1H, H-3′), 6.33 (br d, J = 6, 1H, H-1′), 7.53 (dd, J = 8, 4H, ArH), 7.61 (dd, J = 8, 4H, ArH), 7.91 (s, 1H, H-6), 8.97 (br s, 1H, H-3).

General Procedure for the Deprotection of the *p*-Toluoyl-Protected Nucleosides. The blocked nucleosides were dissolved in 10 mL of anhydrous MeOH, and a solution of 40% NaOMe/MeOH was added dropwise to raise the pH to 9. The reaction mixtures were kept in a desiccator at 0 °C for 3−5 days. Periodically, it was necessary to add several drops of the 40% NaOMe/MeOH solution to the reaction mixtures to maintain a pH of 9. The reactions were neutralized with Dowex 50WX2-200 ion-exchange resin (H^+-form), filtered, and evaporated to oils which were purified by silica gel column chromatography. If clear glasses were obtained after evaporation of the solvent, the glasses were dissolved in diethyl ether and the ether was evaporated to obtain white foams.

5-[4-(*o*-Carboran-1-yl)butylmercapto]-β-D-2′-deoxyuridine (24). Following the above general procedure, 40 mg (0.06 mmol) 5-[4-(*o*-carboran-1-yl)butylmercapto]-3′,5′-di-*O*-*p*-tolu-

oyl-β-D-2′-deoxyuridine (**16**) yielded 23 mg (86%) of product as a white foam. The product was recrystallized from diethyl ether to give a crystalline white solid: mp 145−146 °C; R_f 0.25 (4:1 $CHCl_3$:acetone); ^{1}H NMR (CD_3OD) δ 1.30−3.17 (br m, 10H, BH), 1.35−1.62 (m, 4H, alkane), 2.29 (m, 4H, H-2′ and $CH_2-C_{Carborane}$), 2.65 (br t, J = 6.5, 2H, CH_2−S), 3.71 (m, 2H, H-5′), 3.89 (dd, J = 6, J = 3, 1H, H-3′), 4.35 (m, 1H, H-4′), 4.44 (br s, 1H, $C_{Carborane}$-H), 6.19 (t, J = 6.5, 1H, H-1′), 8.23 (s, 1H, H-6); ^{13}C NMR (CD_3OD) δ 29.13 (CH_2), 29.46 (CH_2), 33.87 (CH_2), 38.33 (CH_2), 41.72 (C-2′), 62.74 ($C_{Carborane}$-H), 63.59 (C-5′), 72.22 (C-3′), 77.19 ($C_{Carborane}$-C), 87.07 (C-1′), 89.22 (C-4′), 108.84 (C-5), 144.64 (C-6), 151.94 (C-2), 164.42 (C-4); MS (FAB$^+$, 3-NBA) 459 (M + 1). Anal. ($C_{15}H_{30}O_5N_2SB_{10}$) C, H, N.

5-[5-(*o*-Carboran-1-yl)pentylmercapto]-β-D-2′-deoxyuridine (25). Following the above general procedure, 100 mg (0.15 mmol) of 5-[5-(*o*-carboran-1-yl)pentylmercapto]-3′,5′-di-*O*-*p*-toluoyl-β-D-2′-deoxyuridine (**17**) yielded 57 mg (86%) of product as a white foam: mp 88−93 °C; R_f 0.25 (4:1 $CHCl_3$:acetone); ^{1}H NMR (CD_3OD) δ 1.18−3.25 (br m, 10H, BH), 1.31−1.62 (m, 6H, alkane), 2.26 (m, 4H, H-2′ and $CH_2-C_{Carborane}$), 2.68 (br t, J = 7, 2H, CH_2−S), 3.76 (m, 2H, H-5′), 3.94 (dd, J = 6, J = 3, 1H, H-3′), 4.40 (m, 1H, H-4′), 4.50 (br s, 1H, $C_{Carborane}$-H), 6.26 (t, J = 6.5, 1H, H-1′), 8.30 (s, 1H, H-6); ^{13}C NMR (CD_3OD) δ 28.66 (CH_2), 29.52 (CH_2), 29.89 (CH_2), 34.19 (CH_2), 38.70 (CH_2), 41.71 (C-2′), 62.76 ($C_{Carborane}$-H), 63.57 (C-5′), 72.26 (C-3′), 77.30 ($C_{Carborane}$-C), 87.01 (C-1′), 89.18 (C-4′), 109.00 (C-5), 144.59 (C-6), 151.94 (C-2), 164.42 (C-4); MS (FAB$^+$, 3-NBA) 473 (M + 1). Anal. ($C_{16}H_{32}O_5N_2SB_{10}$) C, H, N.

5-[6-(*o*-Carboran-1-yl)hexylmercapto]-β-D-2′-deoxyuridine (26). Following the above general procedure, 100 mg (0.14 mmol) of 5-[6-(*o*-carboran-1-yl)hexylmercapto]-3′,5′-di-*O*-*p*-toluoyl-β-D-2′-deoxyuridine (**18**) yielded 55 mg (81%) of product as a white foam: mp 84−86 °C; R_f 0.29 (4:1 $CHCl_3$:acetone); ^{1}H NMR (CD_3OD) δ 1.19−3.19 (br m, 10H, BH), 1.19−1.62 (m, 8H, alkane), 2.25 (m, 4H, H-2′ and $CH_2-C_{Carborane}$), 2.70 (br t, J = 7, 2H, CH_2−S), 3.77 (m, 2H, H-5′), 3.95 (dd, J = 6, J = 3, 1H, H-3′), 4.41 (m, 1H, H-4′), 4.50 (br s, 1H, $C_{Carborane}$-H), 6.26 (t, J = 6.5, 1H, H-1′), 8.29 (s, 1H, H-6); ^{13}C NMR (CD_3OD) δ 28.84 (CH_2), 29.43 (CH_2), 29.80 (CH_2), 30.17 (CH_2), 34.25 (CH_2), 38.74 (CH_2), 41.64 (C-2′), 62.74 ($C_{Carborane}$-H), 63.52 (C-5′), 72.21 (C-3′), 77.34 ($C_{Carborane}$-C), 86.97 (C-1′), 89.105 (C-4′), 109.22 (C-5), 144.18 (C-6), 151.87 (C-2), 164.32 (C-4); MS (FAB$^+$, 3-NBA) 487 (M + 1). Anal. ($C_{17}H_{34}O_5N_2SB_{10}$) C, H, N.

5-[8-(*o*-Carboran-1-yl)octylmercapto]-β-D-2′-deoxyuridine (27). Following the above general procedure, 100 mg (0.14 mmol) 5-[8-(*o*-carboran-1-yl)octylmercapto]-3′,5′-di-*O*-*p*-toluoyl-β-D-2′-deoxyuridine (**19**) yielded 63 mg (92%) of product as a white foam: mp 77−81 °C; R_f 0.35 (4:1 $CHCl_3$:acetone); ^{1}H NMR (CD_3OD) δ 1.10−3.26 (br m, 10H, BH), 1.10−1.62 (m, 12H, alkane), 2.21 (m, 4H, H-2′ and $CH_2-C_{Carborane}$), 2.70 (br t, J = 7, 2H, CH_2−S), 3.76 (m, 2H, H-5′), 3.95 (dd, J = 6, J = 3, 1H, H-3′), 4.41 (m, 1H, H-4′), 4.50 (br s, 1H, $C_{Carborane}$-H), 6.27 (t, J = 6.5, 1H, H-1′), 8.27 (s, 1H, H-6); ^{13}C NMR (CD_3OD) δ 29.34 (CH_2), 29.92 (CH_2), 29.94 (CH_2), 29.99 (CH_2), 30.03 (CH_2), 30.35 (CH_2), 34.35 (CH_2), 38.87 (CH_2), 41.66 (C-2′), 62.78 ($C_{Carborane}$-H), 63.60 (C-5′), 72.27 (C-3′), 77.42 ($C_{Carborane}$-C), 86.98 (C-1′), 89.17 (C-4′), 109.38 (C-5), 144.13 (C-6), 151.94 (C-2), 164.38 (C-4); MS (FAB$^+$, 3-NBA) 515 (M + 1). Anal. ($C_{19}H_{38}O_5N_2SB_{10}$) C, H, N.

General Procedure for the N-3 Alkylation of Thd. Adapting a procedure described by Sasaki et al. and Yamamoto et al.,[36,37] Thd (1 equiv), K_2CO_3 (2 equiv), and the corresponding alkylcarboranyl tosylates (1.1 equiv) were combined in 10 mL of anhydrous DMF/acetone (1/1). The mixtures were heated to 50 °C and stirred for 1−2 days. The solvents were evaporated, and the products were isolated by silica gel column chromatography. To remove trace amounts of DMF after isolation, the products were taken up in diethyl ether and washed with small amounts of water. This was repeated until evaporation of ether gave a white foam. The products were hygroscopic and were stored in a desiccator.

3-[4-(*o*-Carboran-1-yl)butyl]thymidine (29). Following the above-described procedure, 40 mg (0.32 mmol) of Thd (**28**) and 129 mg (0.35 mmol) of 4-(*o*-carboran-1-yl)butyl tosylate[14] yielded 68 mg (93%) of product as a white foam: mp 91−97 °C; R_f 0.50 (1:1 $CHCl_3$:acetone); ^{1}H NMR (DMSO-d_6) δ 1.20−2.63 (br m, 10H, BH), 1.30−1.58 (m, 4H, alkane), 1.82 (s, 3H, CH_3), 2.12 (t, J = 6, 2H, H-2′), 2.27 (m, 2H, $CH_2-C_{Carborane}$), 3.57 (m, 2H, H-5′), 3.77 (m, 3H, CH_2−N and H-3′), 4.23 (br d, 1H, H-4′), 5.15 (br s, 1H, $C_{Carborane}$-H), 6.20 (t, J = 6, 1H, H-1′), 7.78 (s, 1H, H-6); ^{13}C NMR (DMSO-d_6) δ 12.78 (CH_3), 26.09 (CH_2), 26.18 (CH_2), 35.97 (CH_2), 39.47 (CH_2), 40.28 (C-2′), 61.12 ($C_{Carborane}$-H), 62.89 (C-5′), 70.17 (C-3′), 76.39 ($C_{Carborane}$-C), 84.77 (C-1′), 87.32 (C-4′), 108.34 (C-5), 134.70 (C-6), 150.28 (C-2), 162.54 (C-4); MS (HR-EI) $C_{16}H_{32}O_5N_2B_{10}$ calcd 440.3314, found 440.3313. Anal. ($C_{16}H_{32}O_5N_2B_{10}$) C, H, N.

3-[5-(*o*-Carboran-1-yl)pentyl]thymidine (30). Following the above-described procedure, 40 mg (0.32 mmol) of Thd (**28**) and 134 mg (0.35 mmol) of 5-(*o*-carboran-1-yl)pentyl tosylate (**7**) yielded 37 mg (49%) of product as a white foam: mp 84−87 °C; R_f 0.55 (1:1 $CHCl_3$:acetone); ^{1}H NMR (DMSO-d_6) δ 1.20−3.10 (br m, 10H, BH), 1.21 (q, 2H, alkane), 1.33−1.62 (m, 4H, alkane), 1.82 (s, 3H, CH_3), 2.09 (dd, J = 6.5, ^3J = 5, 2H, H-2′), 2.27 (m, 2H, $CH_2-C_{Carborane}$), 3.57 (m, 2H, H-5′), 3.77 (m, 3H, CH_2−N and H-3′), 4.23 (br d, 1H, H-4′), 5.15 (br s, 1H, $C_{Carborane}$-H), 6.20 (t, J = 6, 1H, H-1′), 7.76 (s, 1H, H-6); ^{13}C NMR (DMSO-d_6) δ 12.76 (CH_3), 25.43 (CH_2), 26.47 (CH_2), 28.36 (CH_2), 36.29 (CH_2), 39.47 (CH_2), 40.28 (C-2′), 61.14 ($C_{Carborane}$-H), 62.95 (C-5′), 70.19 (C-3′), 76.47 ($C_{Carborane}$-C), 84.66 (C-1′), 87.27 (C-4′), 108.35 (C-5), 134.61 (C-6), 150.26 (C-2), 162.50 (C4); MS (HR-EI) $C_{17}H_{34}O_5N_2B_{10}$ calcd 454.3471, found 454.3455). Anal. ($C_{17}H_{34}O_5N_2B_{10}$) C, H, N.

3-[6-(*o*-Carboran-1-yl)hexyl]thymidine (31). Following the above-described procedure, 40 mg (0.32 mmol) of Thd (**28**) and 139 mg (0.35 mmol) of 6-(*o*-carboran-1-yl)hexyl tosylate (**8**) yielded 59 mg (77%) of product as a white foam: mp 73−79 °C; R_f 0.60 (1:1 $CHCl_3$:acetone); ^{1}H NMR (DMSO-d_6) δ 1.15−2.60 (br m, 10H, BH), 1.17−1.31 (m, 4H, alkane), 1.31−1.57 (m, 4H, alkane), 1.82 (s, 3H, CH_3), 2.09 (t, J = 6, 2H, H-2′), 2.24 (m, 2H, $CH_2-C_{Carborane}$), 3.58 (m, 2H, H-5′), 3.77 (m, 3H, CH_2−N and H-3′), 4.24 (m, 1H, H-4′), 5.05 (t, J = 5, 1H, 5′ OH), 5.17 (br s, 1H, $C_{Carborane}$-H), 5.25 (d, J = 4, 1H, 3′ OH), 6.21 (t, J = 7, 1H, H-1′), 7.76 (s, 1H, H-6); ^{13}C NMR (DMSO-d_6) δ 12.78 (CH_3), 25.73 (CH_2), 26.75 (CH_2), 27.77 (CH_2), 28.55 (CH_2), 36.46 (CH_2), 39.47 (CH_2), 40.28 (C-2′), 61.17 ($C_{Carborane}$-H), 62.93 (C-5′), 70.21 (C-3′), 76.53 ($C_{Carborane}$-C), 84.68 (C-1′), 87.29 (C-4′), 108.35 (C-5), 134.60 (C-6), 150.26 (C-2), 162.50 (C-4); MS (HR-EI) $C_{18}H_{36}O_5N_2B_{10}$ calcd 468.3627, found 468.3648. Anal. ($C_{18}H_{36}O_5N_2B_{10}$) C, H, N.

3-[7-(*o*-Carboran-1-yl)heptyl]thymidine (32). Following the above-described procedure, 40 mg (0.32 mmol) of Thd (**28**) and 144 mg (0.35 mmol) of 7-(*o*-carboran-1-yl)heptyl tosylate (**8**) yielded 58 mg (73%) of product as a white foam: mp 64−69 °C; R_f 0.57 (1:1 $CHCl_3$:acetone); ^{1}H NMR (DMSO-d_6) δ 1.09−2.70 (br m, 10H, BH), 1.09−1.31 (m, 6H, alkane), 1.31−1.57 (m, 4H, alkane), 1.82 (s, 3H, CH_3), 2.09 (t, J = 6, 2H, H-2′), 2.24 (m, 2H, $CH_2-C_{Carborane}$), 3.57 (m, 2H, H-5′), 3.77 (m, 3H, CH_2−N and H-3′), 4.24 (m, 1H, H-4′), 5.04 (t, J = 5, 1H, 5′ OH), 5.17 (br s, 1H, $C_{Carborane}$-H), 5.25 (d, J = 4, 1H, 3′ OH), 6.21 (t, J = 7, 1H, H-1′), 7.77 (s, 1H, H-6); ^{13}C NMR (DMSO-d_6) δ 12.82 (CH_3), 26.00 (CH_2), 26.85 (CH_2), 27.95 (CH_2), 28.00 (CH_2), 28.66 (CH_2), 36.45 (CH_2), 39.47 (CH_2), 40.28 (C-2′), 61.15 ($C_{Carborane}$-H), 62.97 (C-5′), 70.21 (C-3′), 76.55 ($C_{Carborane}$-C), 84.64 (C-1′), 87.28 (C-4′), 108.37 (C-5), 134.61 (C-6), 150.26 (C-2), 162.50 (C-4); MS (HR-EI) $C_{19}H_{38}O_5N_2B_{10}$ calcd 482.3784, found 482.3826. Anal. ($C_{19}H_{38}O_5N_2B_{10}$) C, H, N.

3-[4-(*o*-Carboran-1-yl)butyl]-5′-[bis(2,2,2-trichloroethyl)phosphoro]thymidine (33). A 330-mg (0.87 mmol) quantity of bis(2,2,2-trichloroethyl)phosphorochloridate was added to an ice-cooled solution of 330 mg (0.75 mmol) 3-[4-(*o*-carboran-1-yl)butyl]thymidine and 50 μL (0.95 mmol) of pyridine in 10 mL of acetonitrile. The solution was left in the refrigerator for 3−5 days. The reaction was terminated by adding 5 mL of methanol and stirring this solution for 30 min at room temperature. Subsequently, the solvents were evaporated, and the resulting residue was dissolved in 20 mL of diethyl ether and extracted two times with 10 mL of 0.1 M

aqueous HCl. The organic layer was dried with anhydrous magnesium sulfate, filtered, and evaporated to dryness. The residue was purified by column chromatography: clear glass; yield 317 mg (54%); R_f 0.77 (1:9 hexane:ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 1.30–3.00 (br m, 10H, B–H); 1.37–1.65 (m, 4H, $CH_2CH_2CH_2CH_2$), 1.95 (s, 3H, CH_3), 2.13–2.48 (m, 4H, CH_2–$C_{Carborane}$, 2 H-1′), 3.61 (br s, 1H, $C_{carborane}$-H); 3.90 (t, 2H, N–CH_2), 4.11 (m, 1H, H-3′), 4.59–4.78 (m, 6H, 2 O–CH_2, 2 H-5′, H-4′, C–OH), 6.34 (t, 1H, H-1′, J = 7.1), 7.30 (d, 1H, H-6); ^{13}C NMR ($CDCl_3$) δ 13. 43 (CH_3), 26.35 (CH_2), 26.72 (CH_2), 37.40 (CH_2), 40.33 (CH_2), 40.33 (C-2′), 61.37 ($C_{Carborane}$-H), 65.95 (C-5′), 71.20 (C-3′), 75.25 ($C_{Carborane}$-C), 77.40 (CH_2-CCl_3), 84.22 (C-1′), 85.85 (C-4′), 94.52 (CH_2CCl_3), 110.81 (C-5), 133.63 (C-6), 150.86 (C-2), 163.34 (C-4); MS (FAB$^+$, 3-NBA) 784 (M + 1).

3-[4-(*o*-Carboran-1-yl)butyl]thymidine-5′-monophosphate (34). A 250-mg (0.32 mmol) quantity of 3-[4-(*o*-carboran-1-yl)butyl]-5′-[bis(2,2,2-trichloroethyl)phosphoro]thymidine and 300 mg (0.46 mmol) of zinc powder (100 mesh) were stirred at room temperature in 20 mL of 90% acidic acid for 2 h. Subsequently, the zinc powder was filtered off and the filtrate evaporated at room temperature (0.5 mm). The remaining residue was suspended in 10 mL of water, and 10% of formic acid was added in small aliquots to this suspension under vigorous stirring until all material was dissolved (pH 2–3). The solution was passed through a 2.5-cm × 50-cm column containing Dowex 50X8-100 (Na^+-form). Fractions of 10 mL were collected, and those showing UV absorption (254 nm) were combined and evaporated at room temperature (0.5 mm). The residue was purified by reversed-phase flash chromatography (water/methanol, 11:9). Fractions (5 mL) showing UV absorption (254 nm) were checked for purity by analytical HPLC, combined, and evaporated at room temperature (0.5 mm). The residue was dissolved in 5 mL of double-distilled water and freeze-dried: fluffy white powder; mp 269–273 °C; yield 82 mg (49%); HPLC retention time 6.1 min (broad peak); column Dynamax-60 Å 8 μm C_{18}; flow rate 0.75 mL/min; solvent system 80% water/20% methanol; ^{1}H NMR (CD_3OD) δ 1.30–3.00 (br m, 10H, B–H); 1.41–1.68 (m, 4H, $CH_2CH_2CH_2$-CH_2), 1.96 (s, 3H, CH_3), 2.17–2.38 (m, 4H, CH_2–$C_{Carborane}$, 2 H-1′), 3.91 (t, 2H, N–CH_2), 4.04 (m, 3H, H-3′, 2 H-5′), 4.59 (m, 2H, $C_{carborane}$-H, H-4′), 6.37 (t, 1H, H-1′, J = 7.3); 7.93 (s, 1H, H-6); ^{31}P NMR (CD_3OD, H_3PO_4) δ 2.11; ^{13}C NMR (CD_3-OD) δ 11.42 (CH_3), 25.70 (CH_2), 25.96 (CH_2), 36.36 (CH_2), 39.16 (CH_2), 39.46 (C-2′), 61.76 ($C_{Carborane}$-H), 63.85 (C-5′), 70.95 (C-3′), 75.34 ($C_{Carborane}$-C), 85.21 (C-1′), 86.20 (C-4′), 109.29 (C-5), 134.90 (C-6), 150.59 (C-2), 163.70 (C-4); MS (FAB$^-$, 3-NBA) 519 $(M + 1)^-$.

Expression and Purification of Recombinant Human TK1 and TK2. The cDNA for human TK1 was PCR-amplified with plasmid pTK11 as template[51] and a pair of primers (5′-sense primer, 5′CCATATGAGCTGCATTAACCTG, and 3′-reverse complement primer, 5′CG*GGATCC*CTCAGTTGGCAG) to create a *Nde*I site (underlined) at the 5′-end and a *Bam*HI site (italic) at the 3′-end of the fragment. After being treated with *Nde*I and *Bam*HI, the TK1 fragment was ligated to plasmid pET-14b (Novagen), which has been digested previously with the same restriction enzymes, to yield the expression plasmid pETKW3. After being sequenced to confirm the correct insertion, pETKW3 was transfected into *E. coli* BL21 (DE3) pLys host cells. The expression of recombinant TK1 was induced with 1 mM isopropyl β-D-thiogalactopyranoside, and the protein was purified by one-step affinity chromatography on chelated His•Bind resin (Novagen). The recombinant human TK2 was expressed and purified according to a procedure described previously.[52] The calculated molecular weights are 28 kDa for both recombinant human TK1 and TK2, and SDS–PAGE showed that both preparations were more than 95% pure (Figure 1). The double band visible in the case of TK1 is most likely due to the effects of heterogeneous properties of the protein from the bacterial expression and the strong charge of the tag, as well as the overloading of the gel. In the present study, the histidine tags at the N-terminal of the recombinant proteins were not removed, since kinetic characterization showed that the presence of the tag sequence appeared not to significantly affect the properties of the enzymes.

Purification of Thymidine Kinase from Human Leukemic Cells. TK1 was purified to near-homogeneity from chronic myelocytic leukemic cells concentrated by leukophoresis and provided by the Tissue Procurement Service of The Ohio State University Comprehensive Cancer Center. After extraction by freeze–thaw, preliminary purification of the supernatant enzyme entailed precipitation of nucleic acids with streptomycin sulfate (equal in weight to the total protein and added as a 10% solution, pH 7) and collection of the protein which precipitated between 20% and 50% saturation by ammonium sulfate. This precipitate was resuspended in buffer A (pH 7.5, 0.01 M Tris-HCl, 10% glycerol, 2 mM dithiothreitol (DTT), 0.5 mM EDTA) and mixed with an equal volume of glycerol for storage at −20 °C.

A 50-mL (2.5 cm diameter × 6.5 cm) affinity chromatography column was prepared with Thd-Sepharose matrix made by coupling Thd directly to epoxy-activated Sepharose-6B (Sigma) as described by Gröbner and Loidl[23] and equilibrated with buffer B (15 mM K_2PO_4, 20% glycerol, 2 mM DTT, pH 8). About 220 units of partially purified TK1 was applied and allowed to equilibrate overnight. The column was then washed successively with buffer B, buffer C (buffer B containing 0.1 M KCl), buffer B, buffer D (buffer C plus 0.01% Triton X-100). Elution of TK1 was achieved with 100 mL of buffer E (buffer D + 0.1 mM ThdTP) at 1.5 mL/min. Active fractions (ca. 160 units) were combined and concentrated by centrifugal ultrafiltration in a Centriplus-30 filter unit (Millipore Co.). The concentration of ThdTP was reduced to less than 0.001 mM by repeated dilution with buffer D followed by reconcentration. The final preparation, which was stabilized with 3% bovine serum albumin (final concentration), contained 58 units of TK1 in 0.75 mL. The TK1 activities throughout the purification procedure were assayed as described previously,[53,54] 1 unit of activity being defined as the activity converting 1 nmol of Thd to ThdMP per minute at 37 °C. Possible TK2 contamination of this TK1 preparation was evaluated by measuring the rate of dCyd phosphorylation[55] and found to be below the limits of detection.

Phosphoryl-Transfer Assay with Recombinant TK1 and TK2. Nucleosides **24–27**, **29–32**, and **35–43** were dissolved in DMSO to make stock solutions of various concentrations (4–120 mM). Thd and dUrd were dissolved in water prior to addition to the assay mixtures. The assays were carried out in reaction mixtures of 100 μM nucleoside, ~0.3% DMSO (except **36**, **37**, dUrd, and Thd where final DMSO concentrations were 2.5, 1.4, 0.0, and 0.0%, respectively), 100 μM [γ-^{32}P]ATP (Amersham), 50 mM Tris-HCl (pH 7.6), 5 mM $MgCl_2$, 15 mM NaF, 125 mM KCl, 10 mM DTT, and 0.5% bovine serum albumin (BSA). The reaction was initiated by the addition of 50 ng of enzyme, and the reaction mixture was incubated for 15–30 min at 37 °C. After being heated to 95 °C for 2 min to stop the reaction, the mixture was centrifuged, and 2-μL portions of the samples were applied to PEI-cellulose TLC plates (Merck). The TLC plates were developed for 10–15 h with isobutyric acid:ammonium hydroxide:water (66:1:33) followed by exposure at room temperature to X-ray films (Kodak). The β-radiograms were scanned with an LKB ultrascan XL laser densitometer and quantified with the software GelScan XL 2.1 (Pharmacia).

Phosphoryl-Transfer Assays with Purified TK1. Nucleosides **25** and **29–32** and dUrd were dissolved in DMSO to make 100 mM stock solutions. The assays were carried out in reaction mixtures of 2.5 mM nucleoside, 2.5% DMSO, 0.375 μM [γ-^{33}P]ATP (10–15 Ci/mmol; DuPont Co.), 100 mM Tris-HCl (pH 8), 0.2 mM $MgCl_2$, 10 mM DTT, and 0.5% BSA. Units of TK were adjusted (0.09–0.12 unit) to maintain conversion factors of 20%, or less, overall. Assay mixtures were incubated at 25 °C for 15–60 min, 4-μL aliquots were withdrawn and mixed with 10 μL of 0.1 N formic acid, and 2 μL of this mixture was spotted on PEI-cellulose TLC plates (Merck). These plates were developed with isobutyric acid:ammonium hydroxide: water (66:1:33) followed by exposure at room temperature to

a X-ray films (Kodak). These were quantitatively imaged on an AMBIS 1000 radioimaging instrument.

Double-Labeling Experiments Utilizing β- and α-Radiography. TK1 phosphoryl-transfer assays with 100 μM Thd/100 μM [γ-^{32}P]ATP, 5 mM **29**/5 mM [γ-^{32}P]ATP, and 5 mM **29**/5 mM [^{31}P]ATP were carried out as described above, and small aliquots of these mixtures were applied to a PEI-cellulose TLC plate on lanes a, c, and g, respectively (Figure 4). Aliquots of 3 mM solutions of compounds **29** and **34** in phosphoryl-transfer assay buffer without ATP and TK1 were applied on lanes k and i, respectively. Solutions (12.5 μL) applied to lanes a, c, g, and i were also subjected to alkaline phosphatase treatment by mixing with 1.5 μL of 10× alkaline phosphatase reaction buffer (100 mM Tris-HCl, pH 8.4, 10 mM $MgCl_2$) and 1 μL (20 U) of alkaline phosphatase from calf intestine (Sigma). These mixtures were incubated at 37 °C for 1 h, and the reaction was stopped by heating to 95 °C for 2 min.[40] Aliquots of these mixtures were applied to lanes b, d, h, and j. Finally, a mixture of solutions applied to lanes c and i was put on lane e, and a mixture of solutions applied to lanes g and i was put on lane f. This TLC plate was developed as described above and subsequently cut in two pieces between lanes e and f. Portion A (Figure 4), containing lanes a−e, was exposed at room temperature to a X-ray film (Kodak). A slightly modified version of a α-radiographic technique reported previously was used to produce the α-radiogram of the portion B (Figure 4) containing lanes f−k.[41] This modified technique, like the original technique, uses the solid-state nuclear track detector (SSNTD) CR-39.[56] The TLC plate was placed on similarly shaped pieces of CR-39 so that the TLC material was in contact with the CR-39. The TLC plate/CR-39 sandwich was irradiated in the thermal column of The Ohio State University Research Reactor (OSURR) to a thermal neutron fluence of approximately 3×10^{12} neutrons/cm^2. After irradiation, the TLC plate/CR-39 sandwich was separated and the CR-39 was etched in a 6.25 M NaOH solution at 60 °C for 130 min. After etching, the tracks from the passage of α-particles (from [^{10}B(n,α)^{7}Li] reactions) through the CR-39 are visible as small pits in the surface of the CR-39. Portion B in Figure 4 displays a black/white-inverse photographic image of this CR-39.

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