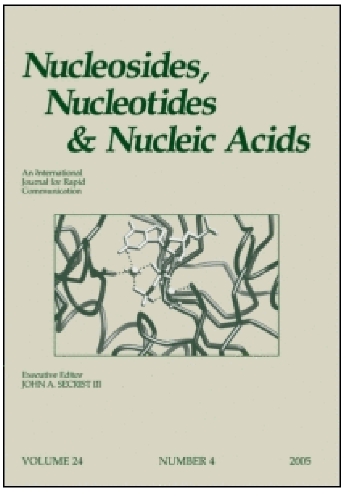


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis and Biochemical Activity of 5-Tethered Carborane-Containing Pyrimidine Nucleosides as Potential Agents for DNA Incorporation

Feng-Guang Rong[a]; Albert H. Soloway[a]; Seiichiro Ikeda[b]; David H. Ives[b]
[a] College of Pharmacy, The Ohio State University, Columbus, OH, U.S.A. [b] College of Biological Sciences, The Ohio State University, Columbus, OH, U.S.A.

To cite this Article Rong, Feng-Guang , Soloway, Albert H. , Ikeda, Seiichiro and Ives, David H.(1995) 'Synthesis and Biochemical Activity of 5-Tethered Carborane-Containing Pyrimidine Nucleosides as Potential Agents for DNA Incorporation', Nucleosides, Nucleotides and Nucleic Acids, 14: 9, 1873 — 1887

To link to this Article: DOI: 10.1080/15257779508010710

URL: http://dx.doi.org/10.1080/15257779508010710

NUCLEOSIDES & NUCLEOTIDES, 14(9&10), 1873-1887 (1995)

SYNTHESIS AND BIOCHEMICAL ACTIVITY OF 5-TETHERED CARBORANE-CONTAINING PYRIMIDINE NUCLEOSIDES AS POTENTIAL AGENTS FOR DNA INCORPORATION

Feng-Guang Rong†, Albert H. Soloway*†, Seiichiro Ikeda‡ and David H. Ives‡

†College of Pharmacy and ‡College of Biological Sciences, The Ohio State
University, 500 West 12th Avenue, Columbus, OH 43210, U.S.A.

Abstract. Synthesis and biochemical evaluation of a series of 5-tethered carborane-containing pyrimidine nucleosides have been undertaken. The biochemical studies with human TK demonstrated that these compounds can be phosphorylated to the corresponding 5'-monophosphate and that the most significant conversion occurred when seven atoms were inserted between the carborane cage and the pyrimidine base, and of these the best was the one with saturated ester.

Introduction

Boron-containing nucleosides that use a carrier-mediated transport process for penetrating the CNS and have the potential for becoming incorporated into proliferating cells may be useful agents for BNCT. Such carborane-containing nucleoside derivatives may achieve higher concentration differentials in rapidly proliferating malignant cells compared to the mitotically-less active normal tissues of the brain.[1]

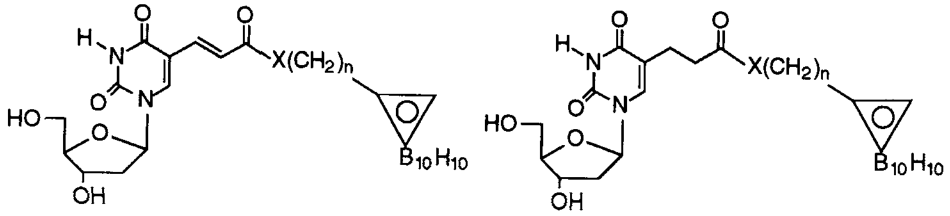
In recent years, numerous boron-containing nucleoside analogies have been synthesized by Yamamoto et al.,[2] Schinazi et al.,[3-5] Spielvogel et al.,[6] and in our laboratories.[7] Some of these were found to be either hydrolytically and biologically unstable, were too toxic, or failed to become incorporated selectively into tumor cells or their nucleic acids. With regard to potential clinical applications, the initial enzymatic step for cellular retention and incorporation is phosphorylation of the boronated nucleosides. Unfortunately, there is a paucity of data as to whether these structures are phosphorylated and, if so, the enzyme kinetics involved.

Several 5-substituted pyrimidine nucleoside derivatives have been synthesized, and some of these compounds have been shown to have anti herpes simplex virus activity.[8] Among them, 5-iodo-2'-deoxyuridine and 5-(trifluoromethyl)-2'-deoxyuridine have been in clinical use for years. E-5-(2-bromovinyl)-2'-deoxyuridine was converted to its mono- and diphosphate by the virus-encoded thymidine kinase and is further metabolized to the triphosphate by a cellular kinase.[9] The basis for the biological activity of the 5-substituted pyrimidine nucleosides is purported to be their conversion to the corresponding nucleotide and the latter's incorporation into viral DNA.

The effect of 5-substituents on the activity of human thymidine kinase has been only studied to a limited extent. Linear extension of the methyl by short aliphatic chains increase K_m or K_i values by an order of magnitude.[10] Bulk tolerance for more massive substituents at this position have not been studied systematically. Though intracellular phosphorylation of 5-carboranyldeoxyuridine has been shown,[11] it is not known whether nucleotide formation is catalyzed by thymidine kinase, nor has the rate of intracellular phosphorylation been determined. It seems possible that some steric interference with substrate binding may be produced by such a large substituent attached directly to 5-position on the pyrimidine nucleoside, but that remains to be demonstrated.

The results from affinity chromatography studies with purine nucleotide-bound matrix demonstrated the importance of tethering nucleosides and nucleotides to a solid matrix support to achieve tight enzyme binding.[12] The optimal tether length was 8 to 10 Å. This same principle should be applicable in the design of new carborane-containing nucleosides for BNCT. Tethering the carboranyl moiety through a flexible chain at the 5-position may allow a compound's binding to kinases and its subsequent phosphorylation.

This approach led to the synthesis of new types of 5-substituted pyrimidine nucleosides (**1**-**3**)[13] and their biochemical evaluation. In order to increase the hydrophilicity of the tether, an ester or amide group has been inserted into the hydrocarbon chain. A vinyl group was also inserted at the



1a; X = O, n = 2; UB-6
1b; X = O, n = 3; UB-7
1c; X = O, n = 4; UB-8
2; X = NH, n = 3; UBN-7

3; X = O, n = 3; SUB-7

Scheme 1

5-position based upon the observation that 5-bromovinyl nucleosides were active antiviral agents.[9] Target compounds **1** and **2** are E-stereoisomers; and in compound **3**, the vinyl double bond is reduced (Scheme 1).

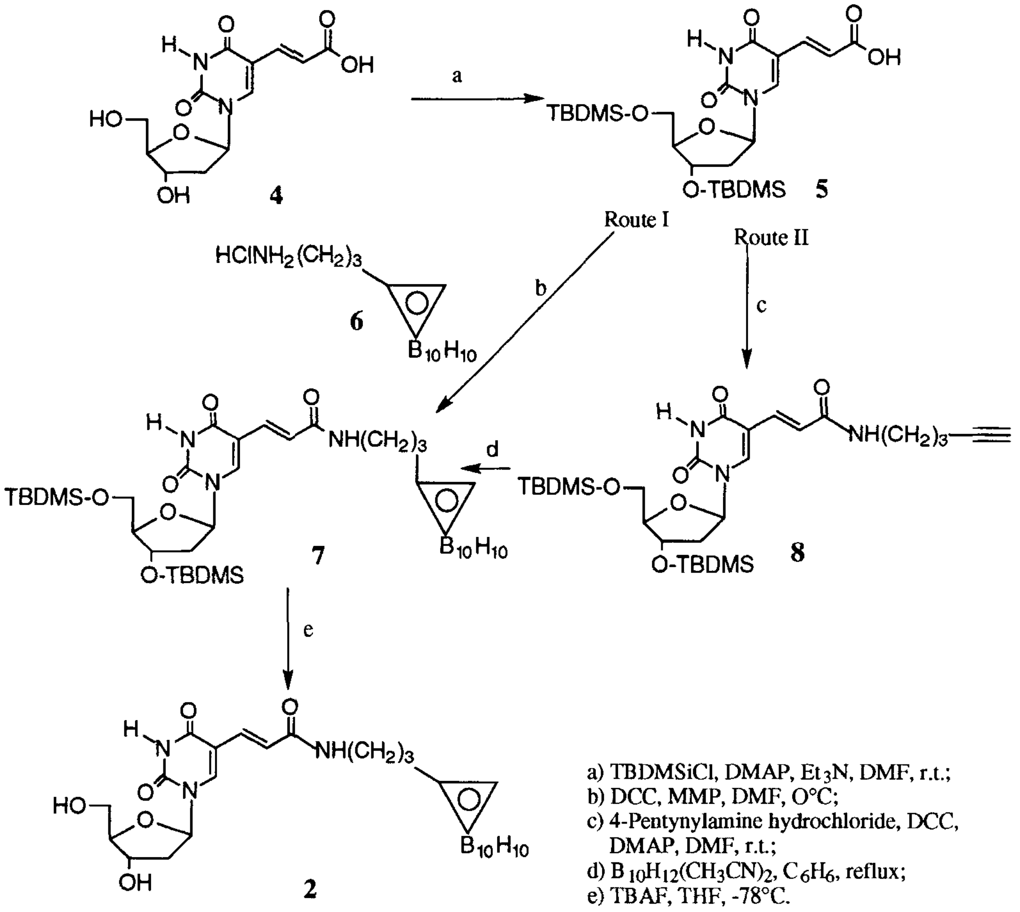
In the biochemical studies, compounds were screened for their inhibition of and rate of phosphorylation by human cytosolic thymidine kinase. Our objective was to correlate how chain length, unsaturation and functionality affected the biochemical activity of the 5-tethered carborane-containing pyrimidine nucleosides.

Synthetic Chemistry

The synthesis of target compounds, **1** and **3**, has been previously reported.[14] The intermediate, E-5-(2-carboxyvinyl)-2'-deoxyuridine, **4**, used for the preparation of target compounds **1**, **2** and **3** was generated by the Heck reaction from 5-iodo-2'-deoxyuridine.[14]

This is the first report of the preparation of 5-substituted long-chain boron-containing analogues in which an amide function is a linking group. Amino derivatives of various boron cluster compounds have been described, but these involved less basic aryl amines. The synthesis of related (aminoalkyl) carboranes is complicated by the fact that basic amines do degrade the carborane cluster generating the corresponding *nido*-undecaborate structure.[15] Based upon these facts, two approaches have been developed for the formation of amide linkage in target compound **2** (Scheme 2).

One, involves the formation of the carboranyl amide by coupling a carboxylic acid nucleoside and the appropiate amino substituted carborane.



a) TBDMSiCl, DMAP, Et_3N, DMF, r.t.;
b) DCC, MMP, DMF, 0°C;
c) 4-Pentynylamine hydrochloride, DCC, DMAP, DMF, r.t.;
d) $B_{10}H_{12}(CH_3CN)_2$, C_6H_6, reflux;
e) TBAF, THF, -78°C.

Scheme 2

The second involves the formation of the appropiate acetylenic amide linked nucleoside and its subsequent boronation. Both methods have been used.

Compound **5** was the first component in both procedures. This compound was prepared from **4** in high yield (Scheme 2) using TBDMSCl in DMF in the presence of 4-dimethylaminopyridine (DMAP) and triethyl amine.[16] The second component in the first method was (aminopropyl)-*o*-carborane as the hydrochloride, **6**. The instability of the free bases related to **6** required their isolation, characterization and use as salts.[15] Compound **7** was produced by coupling **5** with (aminopropyl)-*o*-carborane hydrochloride, **6**, in the presence of

1,4-dicyclohexylcarbodiimide (DCC) and 4-methyl morpholine. The *nido* compound as a by-product was also found.

In order to increase the yield of **7** and avoid degradation of the carborane moiety by free amine function, the second method was developed. Compound **8** was generated in good yield by coupling **5** with 4-pentynylamine hydrochloride[17] in DMF in the presence of DCC and DMAP. Compound **7** was formed by refluxing **8** with decaborane bisacetonitrile complex[18] in benzene. No *nido* by-product was found. The target compound **2** was prepared from **7** by deprotecting the TBDMS group with *tetra*-butyl ammonium floride (TBAF) in THF at low temperature. Though some degradation of the *o*-carborane moiety occurred at longer reaction times, the second route is the one of choice.

Biochemical Studies

Human cytosolic thymidine kinase (TK) is responsible for the phosphorylation of diketopyrimidine deoxynucleosides (dThd and dUrd) and various analogs to the corresponding nucleotides.[19,20] If boronated deoxyuridine derivatives are to be trapped intracellularly, they must be phosphorylated to their monophosphates by TK; this is the first step in their potential metabolism into precursors of tumor DNA.

TK normally has limited capacity to phosphorylate 5-modified 2'-deoxyuridine analogs even though these compounds show anti-viral activity against herpes simplex.[21] However, our results have demonstrated that 5-tethered carborane-containing 2'-deoxyuridine derivatives can be phosphorylated at a significant rate to the corresponding 5'-monophosphate by human TK. Due to their limited aqueous solubility, a 3.2% methanol solution of the nucleosides at a concentration of 0.1 mM was necessary to carry out the enzymatic studies. The phosphorylation reaction was carried out at room temperature, using [γ-^{33}P]-ATP as a phosphate donor. In most cases, a conversion of up to 12% was observed after two hours of incubation during which time TK activity was minimally affected. The phosphorylation products were confirmed by I_2 reactivity, UV absorption, the color reaction with Pd/HCl and radioactivity on two TLC systems (PEI-Cellulose and Silica Gel 60F-254). The R_f value is 0.43 for monophosphorylated UB-7, and 0.67 for UB-7, using PEI-Cellulose plates developed by isobutyric acid/ammonium hydroxide/water system. The radioactivity of monophosphorylation products were detected by autoradiography, and quantitated by β-scanning. The phosphorylation velocity is shown in Figure 1. The results indicate: 1) All the boronated 2'-deoxyuridine

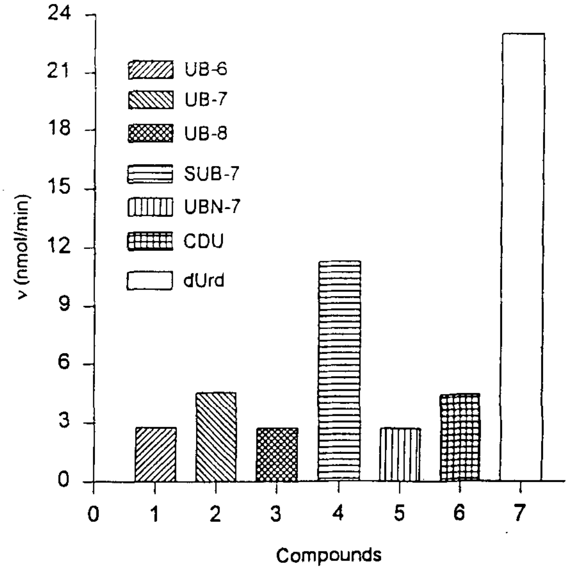


Figure 1. Velocity of phosphorylation of boronated 2'-deoxyuridine derivatives by human TK. The reaction mixture of phosphorylation contained 0.098 units of human TK. CDU represents 5-(1,2-dicarbo-*closo*-dodecaboranyl)-2'-deoxyuridine.

derivatives tested were phosphorylated at considerable rates, but slower than for dUrd and dThd. 2) The order of phosphorylation reactivity was SUB-7 > UB-7 > CDU > UB-6 > UB-8 > UBN-7. 3) Among the series of compounds (**1**) with a varying number of methylene groups in the vinylester linkage, UB-7 showed the maximum reactivity; SUB-7, which does not possess vinyl double bond between the carborane cage and the pyrimidine base, showed even higher reactivity than UB-7. The flexibility of the tethered chain seems to be an important factor in the reactivity of these compounds. The structure of UBN-7 differs from UB-7 only at its amide linkage. The low phosphorylation rate for UBN-7, which differs from UB-7 only its amide linkage, is not well understood at this stage.

The phosphorylation reaction by TK on these boronated 2'-deoxyuridine derivatives was further analyzed by the kinetics of their inhibitory effect vs. dThd or dUrd as substrates. The assay mixture contained a fixed amount of each compound (final 0.5 - 3.2 mM) and methanol (final 0.5 - 3.2%, accordingly) as

Table 1. Inhibitory Effect of Boronated Nucleosides on dThd Phosphorylation by Human TK[a]

Compounds	K_i (mM)	Relative Inhibition
UB-6	1.8	0.18
UB-7	1.1	0.30
UB-8	1.5	0.22
SUB-7	5.2	0.06
UBN-7	16.9	0.02
dUrd	0.33	1.00
dThd	0.0029 (K_m)	
CDU	25.9	0.013

[a] The Inhibition experiment was carried out at 37 °C for 30 min as described in Experimental Section.

described in Experimental Section. In the case of UB-6, UB-7 or UB-8, each compound remained in solution, while a portion of SUB-7, CDU and UBN-7 became insoluble after mixing. We did not attempt to measure the exact concentration of each compound remaining in solution, and the assay was carried out, ignoring the insoluble part. As expected, however, in all cases, competitive inhibition against dThd or dUrd was clearly observed. The apparent K_i calculated for each compound is listed in Table 1. However, the values for SUB-7, CDU and UBN-7 should be regarded as preliminary results because of their partial solubility. Among the analogous compounds of varying chain length, UB-7 showed the maximum affinity for TK and is consistent with the results obtained from the phosphorylation experiments. CDU, in which the bulky carborane moiety is attached directly to the 5-position of pyrimidine, showed significantly poorer affinity than UB-7 (23 - fold larger K_i), but that difference may be somewhat overestimated because of CDU's partial solubility. This solubility problem may also explain the high Ki observed for SUB-7.

From these biochemical studies, it has been shown that a flexible linkage of appropriate length between the carborane cage and the 5-position of the pyrimidine base of the nucleoside allows these nucleosides to undergo phosphorylation by TK. In preliminary attempts towards geometric optimization by molecular modeling using HyperChem, it has been found that the tether lengths of UB-7 and SUB-7 are about 8.5 Å and 8.9 Å, respectively, within the range established to give the most effective enzyme binding.[12]

The lack of sufficient aqueous solubility has been a limiting factor in measuring both rates of phosphorylation and K_i values for these compounds. We are in the process of making more water soluble analogues by attaching non-ionic, hydrophilic groups on the carborane moiety. Such a location should not interfer sterically with enzyme binding.

Experimental Section

Synthetic Chemistry

General. All the experiments were carried out under an argon atmosphere. Melting points were determined on a Fisher-Johns apparatus and reported uncorrected. ^1H and ^{13}C NMR spectra were recorded on a AF-250 FT-NMR spectrometer and the chemical shifts are indicated in ppm with the values relative to internal tetramethylsilane standard unless otherwise noted. Coupling constants (J) are reported in Hz. The signals of H-B in ^1H NMR are very broad and range from 1.2 to 4.2 ppm. Infrared spectra were carried out on a RFX40 FT-IR spectrometer (Laser precision Co.) with samples prepared as KBr disks or neat. FAB$^+$ mass spectra were obtained on a Finnigan MAT-900 mass spectrometer through ionization with Xe using a 3-nitrobenzyl alcohol (3-NBA) as the matrix compound. High resolution mass spectra (EI) were measured with a VG 70-250S spectrometer. For all boron-containing compounds, the mass of the most intense peak of the isotope pattern is indicated. The measured patterns agree with the theoretical ones. Elemental analyses were performed at Atlantic Microlab Inc., Norcross, Georgia. TLC plates with Silica Gel 60F-254 and Silica Gel 60 (70 - 230 mesh) from E. Merck were used for TLC and column chromatography. Compound visualization was achieved with UV light (254 nm), and spraying with 0.06% $PdCl_2$/1% HCl and subsequent heating at 120 °C for 2 - 5 min. Reagent-grade solvents were used for column chromatography. Pyridine, acetonitrile and dimethyl formamide were dried with molecular sieves (4 Å). Benzene was dried over sodium. THF and ether were distilled over sodium and benzophenone under argon prior to use. Other chemicals were purchased from commercial suppliers.

Preparation of E-5-(2-carboxyvinyl)-3',5'-di(*tert*-butyldimethyl silyloxy)-2'-deoxyuridine (5) To a solution of **4** (0.477 g, 1.60 mmol), *tert*-butyldimethylsilyl chloride (0.965 g, 6.40 mmol) and 4-dimethylaminopyridine

(78 mg, 0.64 mmol) in 30 mL of DMF was added 0.89 mL of triethyl amine (6.4 mmol), and the mixture was stirred for 27 hours at room temperature. After filtration of the triethylamine hydrochloride and 4-dimethylaminopyridine hydrochloride precipitate, the solvent was evaporated under reduced pressure. The residue was poured into ice-water, and extracted three times with 40 mL aliquots of ether. The combined ether extracts were washed successively with 1 : 1 hydrochloric acid solution, with brine and dried over anhydrous magnesium sulfate. After solvent evaporation, the residue was purified by column chromatography on silica gel eluted with ethyl acetate/methanol (10 : 1) to give the product as a light yellow solid in 77.4% yield (0.65 g, 1.23 mmol). TLC (ethyl acetate/methanol, 10 : 1): $R_f = 0.57$; mp 180 - 182 °C; FT-IR (KBr): 3600-3186, 3062, 2956, 2931, 2897, 2887, 2858, 1690, 1618, 1471, 1464, 1417, 1385, 1292, 1255, 1106 cm^{-1}; ^1H NMR (CD_3OD) δ 8.05 (s, 1H,H_6), 7.33 (d, J = 15.8, 1H, H-vinyl), 6.90 (d, J = 15.8, 1H, H-vinyl), 6.22 (t, J = 6.7, 1H, $H_{1'}$), 4.49-4.46 (m, 1H, $H_{4'}$), 3.99-3.97 (m, 1H, $H_{3'}$), 3.95-3.80 (m, 2H, $H_{5'}$), 2.37-2.28 (m, 1H, $H_{2'}$), 2.25-2.19 (m, 1H, $H_{2'}$), 0.93 (s, 9H, $C(CH_3)_3$), 0.92 (s, 9H, $C(CH_3)_3$), 0.14 (s, 6H, $Si(CH_3)_2$), 0.12 (s, 6H, $Si(CH_3)_2$); ^{13}C NMR (acetone-d^6) δ 168.26, 162.15, 150.05, 143.70, 138.46, 119.27, 110.07, 89.03, 86.51, 73.39, 63.97, 41.75, 26.43 ($C(CH_3)_3$), 26.18 ($C(CH_3)_3$), 19.06, 18.56, -4.46, -4.58, -5.08, -5.13; EI-HRMS calcd for $C_{24}H_{43}N_2Si_2O_7$ (M+H): 527.2609, found: 527.2302. Anal. Calcd for $C_{24}H_{42}N_2Si_2O_7$: C, 54.72; H, 8.04; N, 5.32. Found: C, 54.66; H, 8.10; N, 5.30.

Preparation of E-5-[2-(4-pentynylaminocarbonyl)vinyl]-3',5'-di(*tert*-butyldimethylsilyloxy)-2'-deoxyuridine (8) To a solution of **5** (148 mg, 0.28 mmol), 4-dimethylaminopyridine (41 mg, 0.34 mmol), and 4-pentynylamine hydrochloride (47 mg, 0.39 mmol) in 20 mL of DMF was added at 0 °C with stirring a solution of 1,4-dicyclohexyl carbodiimide (70 mg, 0.34 mmol) in 5 mL of DMF, and then the mixture was stirred for 2 hours at room temperature. The reaction was monitored by TLC for the loss of starting material **5**. After evaporation of the solvent, the product was obtained in 79% yield by column chromatography on silica gel eluted with hexane/ethyl acetate/methanol (1 : 1 : 0.5). TLC (hexane/ethyl acetate/methanol, 1 : 1 : 0.5): $R_f = 0.60$; mp 81 - 83 °C; FT-IR (KBr): 3378, 3315, 3050, 2954, 2931, 2897, 2858, 1716, 1687, 1620, 1471, 1464, 1385, 1292, 1255, 1105 cm^{-1}; ^1H NMR (CD_3OD) δ 7.87 (s, 1H, H_6), 7.11 (d, J = 15.4, 1H, H-vinyl), 7.02 (d, J = 15.4, 1H, H-vinyl), 6.14 (t, J = 6.7, 1H, $H_{1'}$), 4.41-4.38 (m, 1H, $H_{4'}$), 3.91-3.89 (m, 1H, $H_{3'}$), 3.80-3.75 (m, 2H, $H_{5'}$), 3.30-3.23 (m, 2H, N-CH_2), 2.23-2.11 (m, 5H, $H_{2'}$, CH_2-alkynyl and Halkynyl), 1.68-1.62 (m, 2H, CH_2-*CH_2*-CH_2), 0.85 (s, 9H, $C(CH_3)_3$), 0.84 (s, 9H, $C(CH_3)_3$), 0.05 (s, 6H, $Si(CH_3)_2$), 0.04 (s, 6H, $Si(CH_3)_2$); ^{13}C NMR (CD_3OD) δ 169.11, 163.67, 151.15, 143.32, 134.15, 122.98, 111.12, 89.78, 87.39, 84.19, 74.06, 69.90, 64.31, 42.15, 39.66, 29.53, 26.49 (3C, C(**C**$H_3)_3$), 26.24 (3C, C(**C**$H_3)_3$),19.28, 18.83, 16.71, -4.52, -4.63, -5.12, -5.21; EI-HRMS calcd for $C_{29}H_{49}N_3Si_2O_6$: 591.3160, found: 591.3165. Anal. Calcd for $C_{29}H_{49}N_3Si_2O_6$: C, 58.85; H, 8.34; N, 7.11. Found: C, 58.96; H, 8.30; N, 7.15.

Preparation of E-5-[2-(1,2-dicarbo-*closo*-dodecaboranyl propylaminocarbonyl)vinyl)-3',5'-di(*tert*-butyldimethylsilyloxy)-2'-deoxyuridine (7) from 8 A solution of **8** (223 mg, 0.37 mmol) and bis(acetonitrile)decaborane complex (127 mg, 0.63 mmol) in 15 mL of dry benzene was refluxed with stirring for 2 hours under argon. After evaporation of the solvent, the residue was purified in the same manner as described above to give the product in yield of 46%. TLC (hexane/ethyl acetate/methanol, 1 : 1 : 0.05): R_f = 0.53; mp 114 - 117 °C; FT-IR (KBr): 3438, 3050, 2956, 2931, 2858, 2588, 1712, 1685, 1618, 1541, 1464, 1385, 1290, 1255, 1105 cm^{-1}; ^1H NMR (CD_3OD) δ 7.86 (s, 1H, H_6), 7.07 (d, J = 15.4, 1H, H-vinyl), 6.99 (d, J = 15.4, 1H, H-vinyl), 6.11 (t, J = 6.7, 1H, $H_{1'}$), 4.45 (bs, 1H, C-H carborane), 4.38-4.35 (m, 1H, $H_{4'}$), 3.88-3.87 (m, 1H, $H_{3'}$), 3.78-3.72 (m, 2H, $H_{5'}$), 3.11 (t, J = 6.7, 2H, N-CH_2), 2.22-2.08 (m, 4H, $H_{2'}$ and CH_2-carborane), 1.63-1.55 (m, 2H, CH_2-*CH_2*-CH_2), 0.82 (s, 9H, $C(CH_3)_3$), 0.81 (s, 9H, $C(CH_3)_3$), 0.28 (s, 6H, $Si(CH_3)_2$), 0.01 (s, 6H, $Si(CH_3)_2$); ^{13}C NMR (CD_3OD) δ 169.11, 163.56, 151.03, 143.45, 134.41, 122.76, 111.03, 89.79, 87.39, 74.01, 64.28, 63.63, 42.24, 39.51, 36.32, 34.74, 30.48, 26.76, 26.53 (2C, C(**C**$H_3)_3$), 26.27 (2C, C(**C**$H_3)_3$), 26.02, 19.30, 18.83, -4.48, -4.58, -5.06, -5.14; EI-HRMS calcd for $C_{29}H_{60}B_{10}N_3Si_2O_6$ (M+H): 712.4953, found: 712.4960.

Preparation of 7 from 5 To a solution of **5** (87 mg, 0.16 mmol), 1,3-dicyclohexylcarbodiimide (41 mg, 0.20 mmol) and 4-methylmorpholine (0.04 mL, 0.40 mmol) in 20 mL of DMF was added dropwise at 0 °C with vigorous stirring a solution of 1-(aminopropyl)-1,2-dicarbo-*closo*-dodecaborane hydrochloride (47 mg, 0.20 mmol) in 5 mL of DMF. The mixture was then stirred for an additional 3 hours. After evaporation of the solvent, the residue was purified by column chromatography on silica gel eluted with hexane/ethyl acetate/methanol (1 : 1 : 0.05) to give the product as a light yellow solid in 23% yield (26 mg, 0.04 mmol).

Preparation of E-5-[2-(1,2-dicarbo-*closo*-dodecaboranyl propylaminocarbonyl)vinyl)-2'-deoxyuridine (2) To a solution of **7** (150 mg, 0.21 mmol) in 20 mL of THF was slowly added dropwise at -78 °C 1.25 mL of 1.0 M *tetra-n*-butylammonium fluoride in THF (1.25 mmol). The mixture was stirred an additional 2 hours, and then allowed warm to room temperature. The mixture was then poured into 30 mL of water, and extracted three times with 40 mL aliquots of ether. The combined ether extracts were washed with brine and dried over anhydrous magnesium sulfate. After solvent evaporation, the residue was purified by column chromatography on silica gel eluted with ethyl acetate/methanol (4 : 1) to give the product as an off-white solid in 45% yield. TLC (chloroform/methanol, 5 : 1): R_f = 0.80; mp 250 °C dec; FT-IR (KBr): 3700-3100, 3050, 2939, 2875, 2588, 1701, 1653, 1531, 1456, 1385, 1356, 1333, 1286, 1090 cm^{-1}; ^1H NMR (CD_3OD) δ 8.37 (s, 1H, H_6), 7.21 (d, J = 15.5, 1H, H-vinyl), 7.04 (d, J = 15.5, 1H, H-vinyl), 6.27 (t, J = 6.3, 1H, $H_{1'}$), 4.55 (bs, 1H, C-H carborane), 4.43-4.38 (m, 1H, $H_{4'}$), 3.95-3.87 (m, 1H, $H_{3'}$), 3.86-3.71 (m, 2H, $H_{5'}$), 3.22 (t, J = 6.7, 2H, N-CH_2), 2.35-2.24 (m, 4H, $H_{2'}$ and CH_2-carborane), 1.74-1.67 (m, 2H, CH_2-CH_2-CH_2); ^{13}C NMR (CD_3OD) δ 169.33, 164.01, 143.93, 134.46, 122.00, 110.94, 89.21, 87.09, 71.92, 63.73, 62.60, 49.34, 48.32, 41.83, 39.53, 36.34, 30.47; EI-HRMS calcd for $C_{17}H_{31}B_{10}N_3O_6$: 483.3143, found: 483.3149. Anal. Calcd for $C_{17}H_{31}B_{10}N_3O_6$: C, 42.40; H, 6.49; N, 8.73. Found: C, 42.36; H, 6.53; N, 8.76.

Biochemistry

Materials. [γ-^{33}P]-ATP was purchased from Du Pont Co. BSA, DTT, ATP, dTMP, dThd, dUrd and other chemicals were obtained from Sigma Chemical Co.

Enzyme purification. dThd-Sepharose was prepared by coupling dThd directly to the Sepharose activated by bifunctional oxirane, 1,4-butanediol diglycidyl ether.[22, 23] Human cytosolic thymidine kinase was purified (1560 - fold) by affinity chromatography on dThd-Sepharose from an extract of human acute lymphoblastic leukemic T-cells. These cells, collected from patients by leukophoresis, were provided by the Tissue Procurement Service of The Ohio State University Comprehensive Cancer Center. Frozen cellular extracts were stored until needed.

Thymidine kinase assay. Thymidine kinase assay was carried out radiometrically based on the procedure described by Ives.[24] The standard assay mixture[19] contained 0.19 M Tris-HCl, pH 8.0, 1.9 mM ATP, 1.9 mM $MgCl_2$, 20 μM dThd, 0.5 μCi [^{3}H]-dThd, 10 mM DTT, 1% BSA, 3.0 mM phosphocreatine and 0.54 units creatine kinase in a final volume of 80 µl. The enzyme reaction began by adding 4.85 X 10^{-3} units of purified human TK, and carried out at 37 °C for 30 min. For inhibition kinetics, the boronated nucleoside derivatives were dissolved in methanol (100 mM), then diluted with water and added to the assay mixture.

Phosphorylation of boronated nucleoside derivatives were performed using [γ-^{33}P]-ATP as a phosphate donor. The reaction mixture contained 0.10 M Tris-HCl, pH 8.0, 0.25 µM [γ-^{33}P]-ATP (5 µCi), 25.0 µM $MgCl_2$, 10.0 mM DTT, 1.0% BSA, 3.2% methanol, 100 µM boronated pyrimidine nucleoside and 0.098 units of purified TK in a final volume of 20 µl. The reaction was carried out at room temperature for 0.5 to 24 hours. The reaction products were monitored on PEI-Cellulose TLC plates developed with isobutyric acid/ammonium hydroxide/water (v/v, 66/1/33), or on Silica Gel TLC plates developed by *n*-butanol/methanol (v/v, 1 : 1). The developed TLC plate was exposed to Biomax MR Kodak Scientific Imaging Film for 2 to 4 hours at -78 °C, and then the film was developed on CWP 14-plus Automatic Film Processor. Alternatively, the radioactivity (cpm) on developed TLC plates was counted directly using BETASCOPE 603 Blot Analyzer.

Acknowledgments This research has been supported by the U.S. Department of Energy Grant DE-FG02-90ER60972 and contract DE-AC02-76CH000616 and the National Cancer Institute of the U.S. Public Health Service Grant R01 CA-53896. The authors thank Dr. Joseph Barendt of Callery Chemical Company for providing decaborane and Dr. David H.S. Chang of the Campus Chemical Instrument Center of The Ohio State University for providing the mass spectra.

REFERENCES

(1) Wyzlic, I. M.; Tjarks, W.; Soloway, A. H.; Anisuzzaman, A. K. M.; Rong, F.-G.; Barth, R. F. Strategies for the design and synthesis of boronated nucleic acid and protein components as potential delivery agents for neutron capture therapy. *Int. J. Radiation Oncology Biol. Phys.* **1994**, *28*, 1203-1213.

(2) Yamamoto, Y. Molecular design and synthesis of B-10 carriers for neutron capture therapy. *Pure Appl. Chem.* **1991**, *63*, 423-426.

(3) Goudgaon, N. M.; Fulcrand-El Kattan, G.; Schinazi, R. F. Boron containing pyrimidines, nucleosides, and oligonucleotides for neutron capture therapy. *Nucleosides & Nucleotides.* **1994**, *13,* 849-880.

(4) Fulcrand-El Kattan, G.; Lesnikowski, Z. J.; Yao, S.; Tanious, F.; Wilson, W. D.; Schinazi, R. F. Carboranyl oligonucleotides. 2. Synthesis and physicochemical properties of dodecathymidylate containing 5-(*o*-carboran-1-yl)-2'-deoxyuridine. *J. Am. Chem. Soc.* **1994**, *116*, 7494-7501.

(5) Fulcrand-El Kattan, G.; Goudgaon, N. M.; Ilksoy, N.; Huang, J.-T.; Watanabe, K. A.; Sommadossi, J.-P.; Schinazi, R. F. Synthesis and biological properties of 5-*o*-carboranyl-1-(2-deoxy-2-fluoro-ß-D-arabinofuranosyl)uracil. *J. Med. Chem.* **1994**, *37*, 2583-2588.

(6) Spielvogel, B. F.; Sood, A.; Shaw, B. R.; Hall, I. H.; Fairchild, R. G.; Laster, B. H.; Gordon, C. Boron containing nucleic acids. *In Progress in neutron capture therapy for cancer*, Allen, B. J., Moore, D. E., eds.; Plenum Press: New York, 1992; pp 211-213.

(7) Tjaks, W.; Anisuzzaman, A. K. M.; Liu, L.; Soloway, A. H.; Barth, R. F.; Perkins, D. J.; Adams, D. M. Synthesis and in vitro evaluation of boronated uridine and glucose derivatives for boron neutron capture therapy. *J. Med. Chem.* **1992**, *35*, 1628-1633.

(8) Wigerinck, P.; Pannecouque, C.; Snoeck, R.; Claes, P.; De Clercq, E.; Herdewijn, P. 5-(5-Bromothien-2-yl)-2'-deoxyuridine and 5-(5-chlorothien-2-yl)-2'-deoxyuridine are equipotent to (*E*)-5-(2-bromovinyl)-2'-deoxyuridine in the inhibition of herpes simplex virus type I Replication. *J. Med. Chem.* **1991**, *34*, 2383-2389.

(9) De Clercq, E. *Approaches to Antiviral Agents*, Harnden, M. R., Ed.; McMillan: New York, 1985; pp 57-99.

(10) Lee, L.-S.; Cheng, Y.-C. Human deoxythymidine kinase II: Substrate specificity and kinetic behavior of the cytoplasmic and mitochondrial

isozymes derived from blast cells of acute myelocytic leukemia. *Biochem.* **1976**, *15*, 3686-3690.

(11) Schinazi, R. F.; Goudgaon, N. M.; Fulcrand, G.; El Kattan, Y.; Lesnikowski, Z.; Ullas, G.; Moravek, J.; Liotta, D. C. Cellular pharmacology and biological activity of 5-carboranyl-2'-deoxyuridine. *Int. J. Radiation Oncology Biol. Phys.* **1994**, *28*, 1113-1120.

(12) Lowe, C. R.; Dean, P. D. G. Spacer molecules and steric considerations. In *Affinity Chromatography*; Wiley-Interscience, John Wiley & Sons: London, 1974; pp 23-32.

(13) Rong, F.-G.; Soloway, A. H. Synthesis of 5-tethered dihydroxyboryl and carborane-containing pyrimidine nucleosides for BNCT. BUSA IV, Syracuse, NY, 1994; pp 63.

(14) Rong, F.-G.; Soloway, A. H. Synthesis of 5-tethered carborane-containing pyrimidine nucleosides as potential agents for DNA incorporation. *Nucleosides & Nucleotides.* **1994**, *13*, 2021-2034.

(15) Wilson, J. G.; Anisuzzaman, A. K. M.; Alam, F.; Soloway, A. H. Development of Carborane synthons: Synthesis and chemistry of (aminoalkyl)carboranes. *Inorg. Chem.* **1992**, *31*, 1955-1958.

(16) Corey, E. J.; Venkateswarlu, A. Protection of hydroxyl groups as *tert*-butyldimethylsilyl derivatives. *J. Am. Chem. Soc.* **1972**, *94*, 6190-6191.

(17) Sheehan, J. C.; Bolhofer, W. A. An improved procedure for the condensation of potassium phthalimide with organic halides. *J. Am. Chem. Soc.* **1950**, *72*, 2786-2788.

(18) Leukart, O.; Caviezel, M.; Eberle, A.; Escher, E.; Tun-Kyi, A.; Schwyzer, R. 225. L-*o*-Carboranylalanine, a boron analogue of phenylalanine. *Helv. Chim. Acta* **1976**, *59*, 2184-2187.

(19) Cheng, Y.-C. Thymidine kinase from blast cells of myelocytic leukemia. *Methods Enzymol.* **1991**, *51*, 346-354.

(20) Eriksson, S., Kierdaszuk, B., Munch-Petersen, B., Öberg, B., Johansson, N. G. Comparison of the substrate specificities of human thymidine kinase 1 and 2 and deoxycytidine kinase toward antiviral and cytostatic nucleoside analogs. *Biochem. Biophys. Res. Comm.* **1991**, *176*, 586-592.

(21) Su, T.-L.; Watanabe, K. A.; Schinazi, R. F.; Fox, J. J. Nucleosides. 136. Synthesis and antiviral effects of several 1-(2-deoxy-2-fluoro-ß-D-arabinofuranosyl)-5-alkyluracils. Some structure activity relationships. *J. Med. Chem.* **1986**, *29*, 151-154.

(22) Sundberg, L.; Porath, J. Preparation of adsorbents for biospecific affinity chromatography I. attachment of group-containing ligands to insoluble polymers by means of bifunctional oxiranes. *J. Chromatography,* **1974**, *90*, 87-98.

(23) Gröbner, P.; Loidl, P. Thymidine kinase. A novel affinity chromatography of the enzyme and its regulation by phosphorylation in physarum polycephalum. *J. Biol. Chem.* **1984**, *259*, 8012-8014.

(24) Ives, D. H. A new system for washing ion-exchange paper disks used in radiochemical enzyme assays. *Anal. Biochem.* **1984**, *136*, 416-420.

Received April 27, 1995
Accepted August 8, 1995