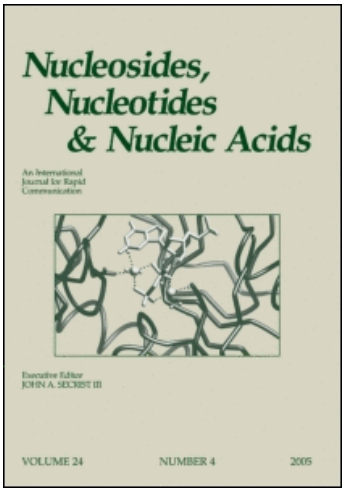

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A General Synthetic Method of 5-Carboranyluracil Nucleosides with Potential Antiviral Activity and use in Neutron Capture Therapy

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A GENERAL SYNTHETIC METHOD OF 5-CARBORANYLURACIL NUCLEOSIDES WITH POTENTIAL ANTIVIRAL ACTIVITY AND USE IN NEUTRON CAPTURE THERAPY[1]

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Abstract: Previous biochemical and pharmacological studies indicated that 5-*o*-carboranyl-2'-deoxyuridine is a lead candidate for boron neutron capture therapy. This prompted the development of a rapid and stereoselective N^1-glycosylation reaction of silylated 5-*o*-carboranyluracil with a variety of protected sugars. The key intermediate, 5-*o*-carboranyluracil (**6**), was prepared from 5-iodouracil in six steps. A novel coupling procedure of the 2,4-dimethoxy-5-ethynylpyrimidine (**4**) with decaborane without activator was used. Silylated **6** was coupled with a variety of carbohydrates under mild conditions to produce several carborane containing nucleosides. In each case, the stereochemistry and stereoselectivity of the glycosylation reaction was not affected by the presence of the carborane at the 5-position of the uracil and produced exclusively closo [*closo*-1,2-$C_2B_{10}H_{12}$ cage] nucleosides. This was confirmed by X-ray structure determination of racemic 5-carboranyl-2',3'-dideoxy-3'-thiauridine. This compound demonstrated an *anti*-conformation with the oxathiolane ring in a pseudo C-2'-endo conformation. The toxicity profile of the new compounds and their precursors was determined in three cell culture systems, two of human origin (PBM and CEM cells) and one of monkey origin (Vero cells). The compounds were also evaluated for their potential antiviral activity against human immunodeficiency virus and herpes simplex virus *in vitro*. 5-*o*-Carboranyl-xylofuranosyluracil (**12**) demonstrated low toxicity in culture and in mice.

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INTRODUCTION

There is a renewed interest in boron neutron capture therapy (BNCT) as a viable binary modality for the treatment of certain malignancies.[2-4] This combined modality is based on the interaction of boron 10 (^{10}B) and a low energy neutron, thermal (< 1 eV) or epithermal neutron (1-20 keV), each relatively innocuous, producing intense ionizing radiation that is confined to single or adjacent cells.

With the development of practical methods for production of improved neutron beams with a low γ ray component for BNCT,[5] the synthesis of new boron containing carriers has become a high priority. Previously, Schinazi et al.[6] and Yamamoto et al.[7] reported the synthesis of boron-nucleosides containing a single boron atom. The *o*-carborane moiety, one of the most stable boron clusters, provides an enhancement of boron content over the boronic acid function by a factor of 10. Recently, hydroxyalkylated carboranes, glycosyl carboranes, carboranylporphyrins,[8] carboranylpeptides[9] and carboranyl nucleosides[4,10a,b] have been prepared for their potential use in BNCT or as potential antiviral agents. The aim of our work was to synthesize additional boron containing nucleosides that are hydrolytically stable and that are substrates for nucleoside kinases found in tumor cells. The targeted molecules were chosen because they may possess the following desirable properties: (a) high lipophilicity so they can permeate certain protected pharmacological compartments such as the brain and prostate;[11] (b) high transportability into the cell and probably into the nuclei of tumors cells; (c) phosphorylation by cellular kinases leading to entrapment in the tumor cell and possibly incorporation into DNA; and (d) resistance to catabolism; for example, they should not be a substrate for nucleoside phosphorylases or dehydrogenases.

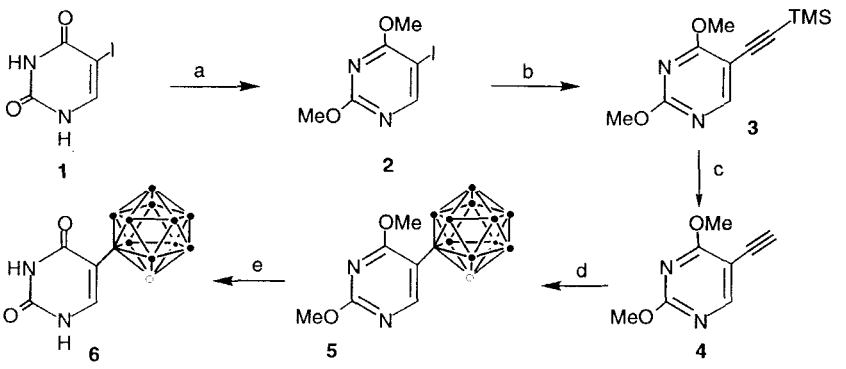
One of the compounds developed, 5-*o*-carboranyl-2'-deoxyuridine (CDU)[10] synthesized starting from the corresponding 5-iodopyrimidine nucleoside, was shown by our group to be phosphorylated intracellularly in normal and malignant human cells, thus preventing its rapid egress from cells.[12] This was the first time that a 5-*o*-carboranyl nucleoside has been conclusively shown to be a substrate for cellular kinases using radiolabeled compound. CDU was also shown to concentrate in prostate cancer tissue in mice.[11] The interesting biological results obtained with this carborane containing nucleoside, and the need for multigram quantities of this and related compounds for preclinical studies, prompted us to develop alternative chemical syntheses. New CDU analogs prepared were evaluated for anti-HIV and cytotoxicity *in vitro*, and

compound (**12**) was studied in mice. The crystal structure of a 5-carboranylpyrimidine nucleoside was determined in order to confirm the structure of the boron cluster.[13]

RESULTS AND DISCUSSION

Chemistry. Our approach was to synthesize the 5-carboranyluracil (**6**) and then glycosylate the base with a variety of natural and modified sugars. 5-Ethynyluracil was first synthesized starting from 5-iodouracil (**1**) in 2 steps. The protected 5-ethynyl-pyrimidines (acetyl, benzoyl, benzyl, trimethylsilyl) were then coupled with decaborane using propionitrile as an activating agent in different solvents (THF, toluene, dichloromethane, dichloroethane). Unfortunately, this approach was ineffective in producing the desired product **6** either at room temperature or at the boiling point of the solvent used. We suspect that steric hindrance due to the relatively large protecting group on the pyrimidine base may be responsible.

The successful approach involved conversion of 5-iodouracil (**1**) to 2,4-dichloro-5-iodopyrimidine with phosphorus oxychloride, which on treatment with sodium methoxide yielded 2,4-dimethoxy-5-iodopyrimidine (**2**)[14] (Scheme 1). The latter was coupled with trimethylsilylacetylene in the presence of $(Ph_3P)_2PdCl_2/CuI$, Et_3N in CH_2Cl_2 followed by deprotection of the trimethylsilyl group with n-Bu_4NF to give 2,4-dimethoxy-5-ethynylpyrimidine (**4**).[15a,b] The coupling of decaborane with the alkyne **4** was first conducted at 110°C in toluene in the presence of proprionitrile as an activating agent for the decaborane. This previously reported procedure[16] is known to give very good yields (> 70%) with a variety of terminal alkynes. Unfortunately, when these conditions were applied to compound **4**, the formation of two products in equal proportion was observed by TLC in CH_2Cl_2. The less polar component corresponded to an unidentified product which had two methoxy groups, but no signal for the B-C-H proton by ^1H NMR. We suspect this product to be a ionic complex[17]—insoluble in the reaction mixture—between the decaborane and compound **4**. The second more polar product corresponded to the desired compound **5**. A kinetics study of this reaction monitored by TLC showed the initial exclusive formation of the desired product. However, when the reaction was maintained at 110°C, the formation of the less polar product occurred and increased if the reaction was maintained at the same temperature. These observations were confirmed by ^1H NMR after quenching the reaction with MeOH at different times. The temperature at which the reaction was performed appeared critical since it had been reported[16] that



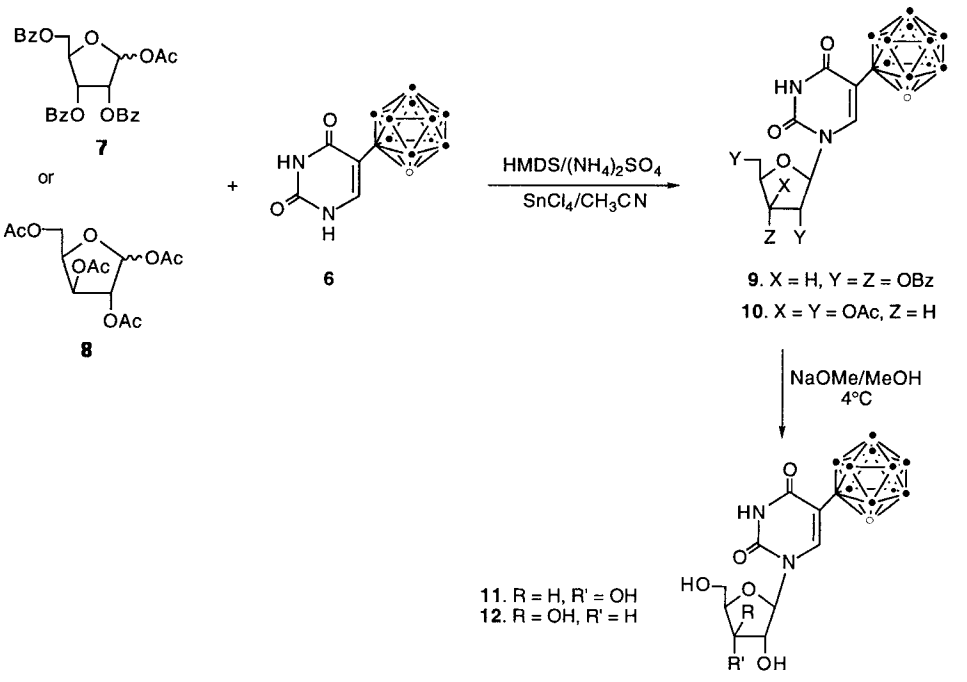
a. $POCl_3$, NaOMe/MeOH; b. HC≡C-TMS, $(Ph_3P)_2PdCl_2$/CuI, Et_3N;
c. *n*-Bu_4NF/THF; d. $B_{10}H_{14}$, Toluene; e. TMSI/CH_2Cl_2.
● : BH, ○ : CH

Scheme 1

formation of the decaborane-proprionitrile complex occurs only at high temperature.

Surprisingly, when the coupling reaction was conducted in the absence of proprionitrile, the formation of desired compound **5** was obtained in 60% yield. It is likely that two molecules of the base **4** removed two hydrogen atoms from the decaborane and played the role of activating agent usually attributed to propionitrile.[18] This allows the formation of the $B_{10}H_{12}$·2M species necessary for the insertion reaction starting from decaborane. Demethylation of the methoxy groups using iodotrimethylsilane gave, after crystallization in MeOH, the desired pure 5-carboranyluracil (**6**) exclusively in the *closo*-form as a white crystalline solid. The absence of the *nido*-compound [(*nido*-7,8-$C_2B_9H_{11}$)$^-$] was confirmed by mass spectroscopy and a stain test (spraying the the previously spotted TLC plate with 0.1 % $PdCl_2$ in concentrated HCl and heating, produces a gray color for the *closo*-form and a black color for the *nido*-form of carboranes). The availability of 5-carboranyluracil (**6**) provided a versatile intermediate for the synthesis of a number of nucleoside analogues described below.

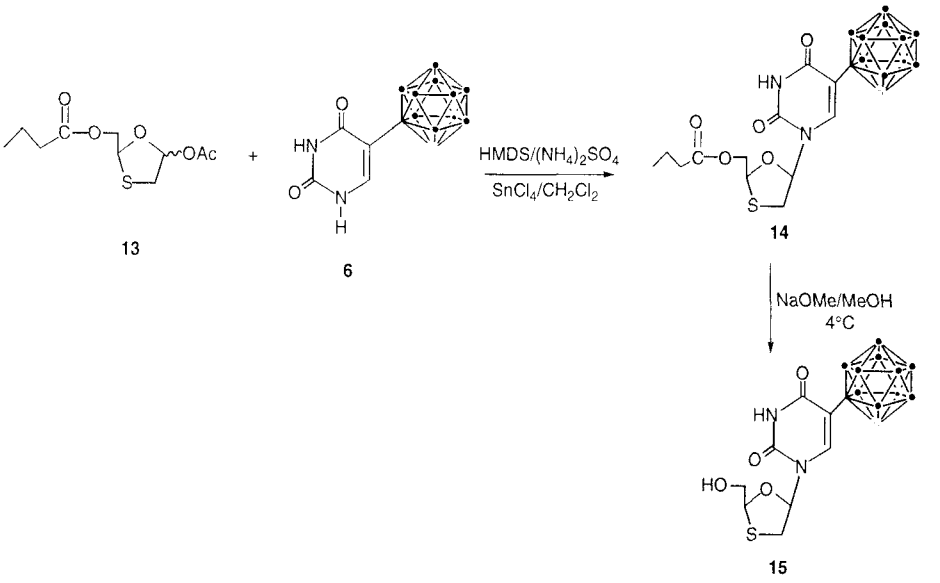
The next step consisted of coupling the silylated **6** with various protected sugars under different conditions. Coupling of silylated 5-carboranyluracil with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**7**) and 1,2,3,5-tetra-*O*-acetyl-xylofuranose (**8**), using the Vorbrüggen and Hoffle approach[19] was conducted in



Scheme 2

the presence of $SnCl_4$ (Scheme 2). The mechanism of the reaction involves the formation of an oxonium ion at the α-face of the sugar moiety[19] which allows attack of the silylated base on the β-face to give the protected nucleosides **9** and **10** in good yield. Deprotection was performed with NaOMe at 4°C to avoid the formation of the *nido*-cluster from the *closo* derivatives. This yielded 5-carboranyl-1-(β-D-ribofuranosyl)uracil (**11**) and 5-carboranyl-1-(β-D-xylofuranosyl)uracil (**12**).

The viability of 3'-heteranucleosides, such as 2',3'-dideoxy-5-fluoro-3'-thiacytidine (FTC) as both anti-human immunodeficiency virus type 1 (HIV-1) and anti-hepatitis B virus agents,[20a,b] was recently demonstrated by our group. Therefore, we wished to prepare a 3'-thianucleoside by coupling the base **6** with the 1-*O*-acetyl-2,3-dideoxy-3-thiaribofuranose (**13**). The oxathiolane, prepared in 5 steps starting from 1,2-butanediol and thioglycolic acid to give the acetate derivative **13**, was coupled with the silylated base **6** using the $SnCl_4$ method developed in our laboratories[21], yielding a 31:1 ratio of the β-anomer **14** in 82% yield (Scheme 3). After deprotection of the ester, the 5-*o*-carboranyl-2',3'-dideoxy-



Scheme 3

3'-thiauridine (**15**) was obtained in 85% yield. This compound had chemical characteristics identical to the compound synthesized starting from racemic 2',3'-dideoxy-5-iodo-3'-thiauridine.[10,12,22]

X-ray crystallography of the nucleoside analogue **15** showed that the base adopted an *anti*-conformation and the oxathiolane ring existed in an S3'-exo/C2'-endo envelope conformation (Figure 1 and Table 1), consistent with the previously reported structure of the antiviral agent (2*R*,5*S*)-(-)-1-[2-(hydroxymethyl)oxathiolan-5-yl]-5-fluorocytosine, also known as (-)-FTC.[23]

These studies provided conclusive evidence for the *closo*-configuration of the carboranyl moiety using this chemical approach, and demonstrated the similar planar size of the carboranyl moiety and the pyrimidine base.

Biological Activity. Although compounds described in this paper were primarily developed for BNCT, they were tested for cytotoxicity and antiviral activity in different cell culture systems. Low toxicity is a highly desirable property for any compound to be considered useful for BNCT. Compounds were first evaluated in primary human peripheral blood mononuclear (PBM) cells acutely infected with HIV-1_{LAI} as previously described.[28] Compounds **2** and **11** were inactive against HIV-1 in culture; the other compounds listed in Table 2

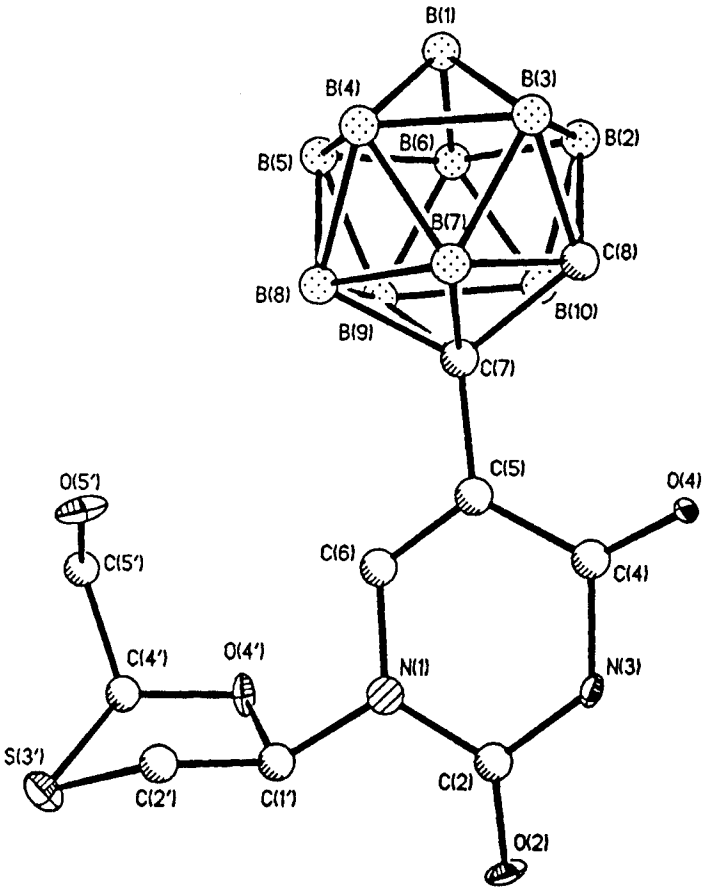


Figure 1 : ORTEP diagram of 5-*o*-carboranyl-2',3'-dideoxy-3'-thiauridine.

demonstrated low to modest potency. Interestingly, 2,4-di-*O*-benzyl-5-ethynylpyrimidine, and 2,4-dimethoxy-5-(trimethylsilyl)ethynylpyrimidine **3** had a median effective concentration (EC_{50}) against HIV-1 of 1.3 and 1.2 μM, respectively. These two compounds demonstrated low toxicity in various cell cultures. Similarly 5-ethynyl-2'-deoxyuridine had an EC_{50} of 0.61 μM. Although, 5-ethynyl-2'-deoxyuridine was a potent inhibitor of HIV-1 with a selectivity index > 164 in PBM cells, it was also one of the most toxic compounds when tested in rapidly dividing cells with a median inhibitory concentration (IC_{50}) of 0.12 μM. Conversion of the 5-ethynyl-2'-deoxyuridine to the 5-carboranyl moiety markedly decreased (> 100 fold) both the anti-HIV-1 activity in acutely infected PBM cells and toxicity in Vero cells. Modification in the sugar moiety in CDU

Table 1. Selected bond lengths (Å) and angles (deg) for $B_{10}C_{10}H_{20}N_2O_4S$ (**15**)

Bonds	Distances	Bonds	Distances
S(3')-C(4')	1.809(7)	C(7)-B(10)	1.746(9)
S(3')-C(2')	1.744(8)	C(7)-B(9)	1.718(10)
C(7)-C(8)	1.663(8)	C(8)-B(7)	1.722(10)
C(7)-B(7)	1.761(11)	C(8)-B(2)	1.700(10)
C(7)-B(8)	1.728(10)	C(8)-B(3)	1.675(12)
C(8)-B(10)	1.723(11)		

Bonds	Angles	Bonds	Angles
C(4')-S(3')-C(2')	89.1(3)	B(7)-C(7)-B(10)	112.6(5)
C(5)-C(7)-C(8)	121.2(5)	B(8)-C(7)-B(10)	112.9(5)
C(5)-C(7)-B(7)	120.4(5)	B(9)-C(7)-B(10)	62.4(4)
C(8)-C(7)-B(7)	60.3(4)	C(7)-C(8)-B(7)	62.7(4)
C(5)-C(7)-B(8)	121.9(5)	C(7)-C(8)-B(2)	111.3(5)
C(8)-C(7)-B(8)	108.8(5)	B(7)-C(8)-B(2)	115.7(5)
B(7)-C(7)-B(8)	61.7(4)	C(7)-C(8)-B(3)	113.1(5)
C(5)-C(7)-B(9)	118.9(6)	B(7)-C(8)-B(3)	63.0(4)
C(8)-C(7)-B(9)	109.6(5)	B(2)-C(8)-B(3)	63.9(5)
B(7)-C(7)-B(9)	112.9(5)	C(7)-C(8)-B(10)	62.1(4)
B(8)-C(7)-B(9)	62.0(4)	B(7)-C(8)-B(10)	115.8(5)
C(5)-C(7)-B(10)	116.1(6)	B(2)-C(8)-B(10)	61.0(5)
C(8)-C(7)-B(10)	60.7(4)	B(3)-C(8)-B(10)	114.8(5)

from 2'-deoxyribose to the ribonucleoside **11** resulted in a compound devoid of anti-HIV-1 activity and moderate toxicity in Vero and CEM cells.

The xylofuranosyl derivative **12** was essentially inactive against HIV-1. In contrast to the other carboranyl nucleosides, compound **12** had no toxicity in PBM or Vero cells suggesting that certain modifications in the sugar moiety produce boron containing compounds which have no toxicity in several cell culture systems. Because of the favorable *in vitro* property of the xylofuranosyl nucleoside **12,** additional preclinical studies were initiated (see below). The 3'-thia derivative **15** had a similar anti-HIV and cytotoxicity profile when compared to compound **6** and CDU. The toxicity profile of the 2 possible enantiomers of **15** will be determined once they have been separated by chiral column chroma-

tography or enzymatically. 5-*o*-Carboranyluracil (**6**) had an EC_{50} of 19.8 μM against HIV-1 and demonstrated modest toxicity in CEM and Vero cells, but no toxicity to human PBM cells up to 100 μM (Table 2). It should be noted that none of the carboranyl compounds synthesized had greater potency than 3'-azido-3'-deoxythymidine (AZT) against HIV-1 in human PBM cells, although the majority were less toxic than AZT.

The spectrum of antiviral activity of the compounds described in Table 2 was extended to herpes simplex virus type 1 (HSV-1) using plaque assays in Vero cells. Compound **5**, **6**, 5-ethynyl-2'-deoxyuridine and CDU had an EC_{50} of 21.5, 16.2, 11.6, and 11.9 μM respectively. However, the selectivity index (ratio of IC_{50}/EC_{50} in Vero cells) for these compounds was low compared to the positive control acyclovir.

Carboranyl nucleoside **12** was evaluated in mice for toxicity by administering the compound for 6 days and monitoring death, weight loss or failure to gain weight for 9 weeks. The toxicity profile for the drug treated group as measured by weight loss was identical to the control DMSO/PBS treated group; except that one out of the six animals in the treated group died on day 8. No further death in any group occurred for the duration of the experiment (65 days). These results suggest that compound **12** was well tolerated in mice at 30 mg/kg/day and had a comparable profile to 5-*o*-carboranyl-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)uracil.[10b]

CONCLUSION

The versatility of 5-carboranyluracil (**6**) to synthesize various nucleoside analogues for BNCT was demonstrated. Different conditions for the coupling reaction were applied using this new 5-*o*-carboranylpyrimidine analogue. This approach constitutes a facile and efficient way to synthesize several carboranyl nucleoside analogues bearing modifications in the sugar moiety. Some of these compounds could be useful for BNCT, as building blocks for oligonucleotide synthesis,[24a,b,c] or as chain-terminators for biochemical studies. The crystal structure of a 5-carboranyl pyrimidine nucleoside was determined. This work represents the first such determination for a boron containing nucleoside. Low cell culture and mammalian toxicity was noted for compound **12**. The intracellular metabolism, uptake and egress in relevant human cells of some of these novel compounds is planned. The hope is that one or more of these novel 5-carboranylpyrimidine nucleosides may offer pharmacological advantages over currently available BNCT agents.

Table 2. Biological evaluation of various 5-carboranylpyrimidines against HIV-1, HSV-1, and for cytotoxicity in PBM, CEM, and Vero cells compared to AZT.

Compound	Anti-HIV-1 in PBMC EC_{50}, μM	Toxicity in PBM cells[a] IC_{50}, μM	Toxicity in CEM cells[a] IC_{50}, μM	Toxicity in Vero cells[a] IC_{50}, μM	Anti-HSV-1 in Vero cells EC_{50}, μM[b]
2,4-Diacetyl-5-ethynylpyrimidine	>100	>100	>100	>100	>100
2,4-Dibenzoyl-5-ethynylpyrimidine	2.1	≥100	>100	>100	>100
2,4-Di-*O*-benzyl-5-ethynylpyrimidine	1.3	>100	>100	>100	>100
2,4-Dimethoxy-5-iodopyrimidine (**2**)	>100	>100	>100	>100	>100
2,4-Dimethoxy-5-(trimethylsilyl)-ethynylpyrimidine (**3**)	1.2	≥100	63.1	>100	>100
2,4-Dimethoxy-5-ethynylpyrimidine (**4**)	65.2	>100	>100	>100	>100
5-Ethynyluracil	37.7	>100[c]	>100	>100	>100
5-Ethynyl-2'-deoxyuridine	0.61	>100	20.3	0.12	11.6
2,4-Dimethoxy-5-carboranylpyrimidine (**5**)	25.5	91.4	>100	97.7	21.5
5-Carboranyluracil (**6**)	19.8	>100	16.4	40.7	15.2
5-Carboranyl-ribofuranosyluracil (**11**)	>100	>100	76.1	26.4	>100
5-Carboranyl-xylofuranosyluracil (**12**)	97.6	> 100	>100	>100	≥100
2',3'-Dideoxy-3'-thia-5-carboranyluridine (**15**)	12.7	>100	37.9	34.2	>100
5-Carboranyl-2'-deoxyuridine (CDU)	69.8	>100	70.9	26.6	11.9
3'-Azido-3'-deoxythymidine (AZT)	0.004	>100	13.0	26.0	>100

[a] By cell proliferation or trypan blue exclusion.
[b] Acyclovir as apositive control had an EC_{50} of 0.04 μM.
[c] By ^{3}H-thymidine uptake.

EXPERIMENTAL SECTION

Melting points were determined on an Electrothermal IA 8100 digital melting point apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on a General Electric QE-300 (300 MHz) spectrometer. UV spectrum were recorded on Shimadzu UV-2101PC spectrophotometer and FTIR spectra were measured on a Nicolet Impact 400 spectrometer. Mass spectroscopy was performed with JEOL [JMS-SX102/SX102A/E] spectrometer. Experiments were monitored using TLC analysis performed on Kodak chromatogram sheets precoated with silica gel and a fluorescent indicator. Column chromatography, employing silica gel (60-200 mesh; Fisher Scientific, Fair Lawn, NJ), was used for the purification of products. Tetrahydrofuran (THF) was freshly dried and distilled in the presence of sodium benzophenone. Trimethylsilyl iodide and other chemicals, including the carbohydrates, were purchased from Aldrich Chemical Company (Milwaukee, WI). Microanalyses were performed at Atlantic Microlab, Inc. (Norcross, GA).

5-(Trimethylsilyl)ethynyluracil. The title compound was prepared as previously described· m.p. 187-189°C (lit. m.p.[25] 189°C); ^1H NMR ($CDCl_3$) δ 0.23 (s, 9H, 3 CH_3), 7.9 (s, 1H, 6-H), 11.55 (m, 2H, 2 NH).

5-Ethynyluracil. This compound was prepared according to the methodology described by Spector et al.[26] The physical data were in agreement with the structure of the compound. m.p. 168-170°C (lit. m.p.[26] 167°C); ^1H NMR (DMSO-d_6) δ 3.3 (s, 1H, acetylenic proton), 7.5 (s, 1H, 6-H), 11.6 (m, 2H, 2 NH).

2,4-Di-*O*-acetyl-5-ethynylpyrimidine. A suspension of 5-ethynyluracil (0.5 g, 3.65 mmol) in anhydrous pyridine (30 ml) containing acetic anhydride (18.25 mmol, 1.8 ml) was stirred at room temperature for 18 h. The resulting clear solution was evaporated to dryness, dissolved in CH_2Cl_2 and then poured into saturated aqueous $NaHCO_3$. The organic fraction was separated, washed with H_2O, dried over $MgSO_4$, filtered and evaporated to dryness under reduced pressure several times with toluene. The crude product was purified by silica gel column chromatography using CH_2Cl_2/MeOH (9:1) as eluent to yield the title compound (0.63 g, 78%). m.p. 212-215°C; ^1H NMR ($CDCl_3$) δ 1.8 (2s, 3H each, 2 $COCH_3$), 3.26 (s, 1H, acetylenic proton), 8.25 (s, 1H, 6-H); Anal. Calcd for $C_{10}H_8N_2O_4$: C, 54.54; H, 3.63; N, 12.72. Found: C, 54.63; H, 3.80; N, 12.66.

2,4-Di-*O*-benzoyl-5-ethynylpyrimidine. A suspension of 5-ethynyluracil (0.5 g, 3.65 mmol) in anhydrous pyridine (30 ml) containing benzoic anhydride (18.25 mmol, 3.35 ml) was stirred at room temperature for 18 h. The solution was worked up as described above for the di-*O*-acyl derivative. (1.02 g, 81%). m.p.

235-238°C; ^{1}H NMR ($CDCl_3$) δ 3.29 (s, 1H, acetylenic proton), 6.90-7.45 (m, 10H, aromatic proton), 8.2 (s, 1H, 6-H).

2,4-Di-*O*-benzyl-5-ethynylpyrimidine. A suspension of 5-ethynyluracil (0.5 g, 3.65 mmol) in anhydrous THF (30 ml) containing benzyl chloride (18.3 mmol, 2 ml) and sodium hydride (20 mmol; 480 mg) was stirred at room temperature for 18 h. The excess hydride was destroyed by the slow addition of MeOH. The resulting mixture was processed as described above to yield the desired compound (0.82 g, 75%). m.p. 212-216°C; ^{1}H NMR ($CDCl_3$) δ 3.25 (s, 2H, CH_2-Ph), 3.3 (m, 3H, acetylenic proton and CH_2-Ph), 7.0-7.4 (m, 10H, aromatic proton), 8.3 (s, 1H, 6-H); Anal. Calcd for $C_{20}H_{16}N_2O_2$: C, 75.94; H, 5.06; N, 8.86. Found: C, 76.00; H, 5.11; N, 8.92.

2,4-Di(trimethylsilyl)-5-ethynylpyrimidine. A suspension of 5-ethynyluracil (0.5 g, 3.65 mmol) in anhydrous 1,1,1,3,3,3-hexamethyldisilazane (50 ml) was stirred at 120°C for 5 h. The solvent was removed under vacuum and kept under argon and used without purification for the next reaction.

2,4-Dimethoxy-5-iodopyrimidine (2). The title compound was prepared as previously described.[14] m.p. 65-67°C (lit. m.p.[14] 69°C); ^{1}H NMR ($CDCl_3$) δ 3.98 and 4.06 (2 s, 6H, 2 OCH_3), 8.42 (s, 1H, 6-H); ^{13}C NMR ($CDCl_3$) δ 168.77 (C-4), 165.44 (C-2), 164.44 (C-6), 68.91 (C-5), 55.04 (OCH_3), 55.14 (OCH_3).

2,4-Dimethoxy-5-(trimethylsilyl)ethynylpyrimidine (3). The title compound was prepared according to the method of Coe and Walker.[15 a,b] m.p. 72-74°C (lit. m.p.[15 a,b] 76°C); ^{1}H NMR ($CDCl_3$) δ 0.22 (s, 9H, $SiMe_3$), 3.96 and 4.05 (2 s, 6H, 2 OCH_3), 8.36 (s, 1H, 6-H); ^{13}C NMR (DMSO-d_6) δ 172.24 (C-4), 165.48 (C-2), 162.83 (C-6), 101.59 (C-5), 101.00 (C acetylenic), 97.09 (C acetylenic), 55.69 (OCH_3), 55.14 (OCH_3), 0.09 (Si-CH_3).

2,4-Dimethoxy-5-ethynylpyrimidine (4). This compound was synthesized according to the method of Coe and Walker.[15 a,b] m.p. 75-77°C (lit. m.p.[15a] 74°C; lit. m.p.[15b] 83-84°C) ; ^{1}H NMR ($CDCl_3$) δ 3.35 (s, 1H, acetylenic proton), 3.98 and 4.08 (2 s, 6H, 2 OCH_3), 8.38 (s, 1H, 6-H); ^{13}C NMR ($CDCl_3$) δ 170.77 (C-4), 164.15 (C-2), 162.02 (C-6), 98.71 (C-5), 83.39 (C-acetylenic), 75.16 (C acetylenic), 54.94 (OCH_3), 54.38 (OCH_3).

5-*o*-Carboranyl-2,4-dimethoxypyrimidine (5). To a refluxing solution of decaborane (2.52 g, 20.63 mmol) in toluene (70 ml) was added dropwise 2,4-dimethoxy-5-ethynylpyrimidine (3.55 g, 21.62 mmol) in toluene (100 ml). After the addition was completed, the resulting solution was heated under reflux for 30 min. The reaction product was cooled and concentrated to dryness *in vacuo*, and the residue purified by silica gel column chromatography using hexane/EtOAc

(9:1) as eluent to yield compound **5** (3.435 g; 59%) as a white solid. m.p. 140-143°C; FTIR (neat, cm^{-1}) 2602, 2560, 1594, 1558, 1474, 1404, 1229; ^1H NMR ($CDCl_3$) δ 1.2-3.0 (br, 10H, carborane protons), 4.05 and 4.15 (2 s, 6H, 2 OCH_3), 5.38 (s, 1H, carborane proton), 8.56 (s, 1H, 6-H); MS [FAB (Fast atom bombardment)] *m/z* 284 $(NBA+Li)^+$; Anal. Cald for $B_{10}C_8H_{18}N_2O_2$: B, 38.32; C, 34.03; H, 6.42; N, 9.92. Found: B, 38.45; C, 34.31; H, 6.49; N, 10.01.

5-*o*-Carboranyluracil (6). To a solution of 5-carboranyl-2,4-dimethoxypyrimidine (**5**) (285 mg, 1 mmol) in CH_2Cl_2 (10 ml), trimethylsilyl iodide (520 mg, 2.6 mmol) was added under anhydrous conditions at room temperature. The resulting yellow solution was stirred for 3 h. The excess trimethylsilyl iodide and the intermediate trimethylsilyl ethers formed during the reaction were hydrolyzed by addition of MeOH (5 ml). The solution was concentrated to dryness *in vacuo*, dissolved in CH_2Cl_2, filtered, and then redissolved in MeOH to give on crystallization the desired product as a white solid, (180 mg, 71%). m.p. > 280°C (dec); UV (MeOH) λ_{max} = 268 nm (ε = 9500), λ_{max} = 216 nm (ε = 10020), λ_{max} = 236 nm (ε = 2500); FTIR (KBr, cm^{-1}) 3222, 3082, 2839, 2572, 1715, 1685, 1448; ^1H NMR (DMSO-d_6) δ 1.2-3.0 (br, 10H, carborane protons), 5.90 (s, 1H, carborane proton), 7.64 (s, 1H, 6-H), 11.28 (br s, 2H, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6) δ 162.82 (C-4), 150.49 (C-2), 144.84 (C-6), 105.84 (C-5), 72.40 (C-C-5), 59.61(C-H). MS [EI (Electronic impact)], *m/e* 254 $(M)^+$; Anal. Cald for $B_{10}C_6H_{14}N_2O_2$: B, 42.54; C, 28.35; H, 5.54; N, 11.02 Found: B, 42.66; C, 28.49; H, 5.58; N, 10.96.

5-*o*-Carboranyluridine (11). To a suspension of 5-carboranyluracil **6** (127 mg, 0.5 mmol) in dry CH_3CN (10 ml) was added sequentially 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (265 mg, 0.525 mmol), hexamethyldisilazane (HMDS, 85 μl), and chlorotrimethylsilane (TMSCl, 51 μL) and the mixture was stirred under a N_2 atmosphere at room temperature. After 5 min, $SnCl_4$ (71 μl, 0.6 mmol) was added and the mixture was stirred for 1.5 h and then saturated $NaHCO_3$ was added while stirring. The resulting suspension was filtered through Celite, and washed with warm $CHCl_3$. The combined organic fractions were dried over Na_2SO_4 and concentrated to dryness under vacuum. The residue was dissolved in dry MeOH, NaOMe (100 mg) was added, and the reaction mixture was stirred for 6 h at 4°C. Dowex H^+ resin was added and the reaction mixture was filtered, evaporated to dryness, then chromatographed using CH_2Cl_2/MeOH, (9/1) as eluent to give pure 5-carboranyluridine (182 mg, 72%). m.p. 277-279°C (lit. m.p.[27] 279-280°C).

5-*o*-Carboranyl-xylofuranosyluracil (12). A suspension of 5-*o*-carboranyl-uracil (**6**) (127 mg, 0.5 mmol) in dry CH_3CN (10 mL) was added sequentially tetra-*O*-acetyl-β-D-xylofuranose (265 mg, 0.525 mmol), HMDS (85 µl), and TMSCl (51 µL) while stirring under a N_2 atmosphere at room temperature. The mixture was treated as described for compound **11** to yield pure 5-carboranylxylo-furanosyluracil (172 mg, 68 %). m.p. 274-278°C; ^1H NMR ($CDCl_3$) δ 1.3-2.8 (bm, 10H, -BH), 2.9 (m, 1H, 2'-H), 3.4-4.1 (m, 3H, 3'-H, 5'-H and 5''-H), 4.6 (m, 1H, 4'-H), 4.8 (m, 2H, 2'-OH and 3'-OH), 5.25 (t, 1H, 5'-OH), 5.68 (d, 1H, 1'-H; $J_{1',2'}$ = 6.02 Hz), 5.77 (bs, 1H, -B-CH), 7.98 (s, 1H, 6-H), 8.82 (s, 1H, NH); MS [LSIMS (Liquid secondary ion mass spectroscopy)] *m/z* 387 $(M+H)^+$; Anal. Cald for $B_{10}C_{11}H_{22}N_2O_6$: B, 28.89;.C, 32.07; H, 5.88; N, 7.48. Found: B, 29.01; C, 32.12; H, 6.00; N, 7.51.

5-*o*-Carboranyl-2',3'-dideoxy-3'-thiauridine (15). To the silylated 5-carboranyluracil (0.2 g, 0.78 mmol) prepared from 5-carboranyluracil **6** and HMDS (100 µl) in dry CH_2Cl_2 (20 ml), a $SnCl_4$ solution (1.05 mmol, 1.05 ml, 1 M solution in CH_2Cl_2) was added under a N_2 atmosphere. The mixture was stirred for 30 min at room temperature and then added to the acetate **13** (0.23 g, 0.94 mmol) in CH_2Cl_2 (20 ml). After 2 h, the reaction was quenched with a mixture of NH_4OH/CH_2Cl_2 (1:20, 50 ml) resulting in the formation of a white precipitate (tin salt). The mixture was allowed to stir for another 30 min, and then it was partially purified using a short silica gel column which was eluted sequentially with CH_2Cl_2, EtOAc, and EtOAc:EtOH (9:1; 100 mL). The eluates were combined and evaporated under reduced pressure and dissolved in dry MeOH. To this solution, NaOMe (150 mg) was added and the mixture was maintained at 4°C for 6 h. Water (5 ml) was then added and the solution was neutralized using Dowex H^+ resin and then filtered. The filtrate was evaporated and the residual solid was then column chromatographed on silica gel using CH_2Cl_2/MeOH (9:1) as eluent to give the title compound (238 mg, 82%). m.p. 256-259°C (lit. m.p.[10] 259°C).

Antiviral and cytotoxicity assays. Anti-HIV-1 activity of the compounds was determined in human PBM cells as described previously.[28] Stock solutions (10-40 mM) of the compounds were prepared in sterile DMSO and then diluted to the desired concentration in complete medium. AZT stock solutions were made in water. Cells were infected with the prototype HIV-1_{LAI} at a multiplicity of infection of 0.01. Virus obtained from the cell supernatant was quantitated on day 6 after infection by a reverse transcriptase assay using poly(rA)$_n$.oligo(dT)$_{12-18}$ as template-primer. The DMSO present in the diluted solution (< 0.1%) had no

effect on the virus yield. The toxicity of the compounds was assessed in human PBM, CEM, and Vero cells, as described previously.[28] Antiviral activity against HSV-1 in Vero cells was performed by a plaque assay as described previously.[29] The antiviral EC_{50} and cytotoxicity IC_{50} was obtained from the concentration-response curve using the median effective method described by Chou and Talalay.[30]

Toxicity in mice. 5-*o*-Carboranyl-xylofuranosyluracil (**12**) was given to six week old SCID mice (3 male and 3 female in each group) intraperitoneally once a day for 6 days at a dose of 30 mg/kg per day. The compound was prepared in sterile DMSO and injected intraperitoneally (0.2 ml). A phosphate buffered saline (PBS) and a DMSO/PBS (2%) control group was also included (6 mice each). The mice were weighed individually on day 0, 1, 2, 4, 8, and weekly thereafter for 8 weeks.

X-ray crystallography. Crystals of compound **15** ($B_{10}C_{10}H_{20}N_2O_4S$, MW = 372.4) were grown from a 1:1 mixture of $CHCl_3$:hexanes. A colorless crystal of dimensions 0.20 x 0.20 x 0.20 mm was selected, mounted and placed on a Siemens P4/RA diffractometer using CuKa radiation (l = 1.54178 Å) at -100°C for data collection. The orientation matrices and unit cell parameters were determined by least squares treatment of 35 machine centered reflections between 21° and 76° in 2q. The compound crystallized in the monoclinic space group P21/c, with a = 11.442(2) Å, b = 12.846(3) Å, c = 13.021(3) Å, b = 107.33(3)°, V = 1827(1) $Å^3$, Z = 4, and d_{calcd} = 1.354 g/cm^3. A total of 2367 reflections were collected between $2.0° \leq 2q \leq 97.6°$, yielding 1770 independent reflections (R_{int} = 2.37%), with 1435 [F > 4s(F)] observed reflections. The intensities of three reflections (1 1 3, 1 1 -4 and 1 2 2) were monitored every 100 reflections, at approximately 1 h intervals, and exhibited little decay. All data were corrected for Lp effects and no correction for absorption was applied. The structure was solved by standard direct and difference Fourier methods and refined by full matrix least squares using the program SHELXTL IRIS. The hydrogens on the boron cage were located from difference Fourier maps and their positional parameters refined with thermal parameters set to 0.05 times that of the parent boron. All other hydrogen atoms were included, but not refined in calculated positions (C-H 0.96 Å) with isotropic thermal parameters set at 0.05 times that of the parent carbon. All heteroatoms were refined with anisotropic thermal parameters, and all other atoms were refined isotropically to reduce the number of least squares parameters. The maximum shift/ESD for the final cycle of refinement was 0.001 and the maximum and minimum peaks in the difference

electron density map were 0.92 and -0.44 e/Å^3. Least squares refinement converged to R factors of R = 7.31% (obs) and R = 8.64% (all). Final non-hydrogen atom positional parameters have been deposited with the Cambridge Crystallographic Data Center and selected interatomic distances and angles are provided in Table 1. The scattering factors for all atoms, the anomalous-dispersion corrections, as well as the linear absorption coefficients are from the International Tables for X-ray Crystallography Volume C. Full tables of final fractional coordinates and isothermal parameters, bond distances, bond angles and anisotropic temperature factors have been deposited with the Cambridge Crystallography Data Center.

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