Synthesis and Biological Properties of 5-o-Carboranyl-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)uracil

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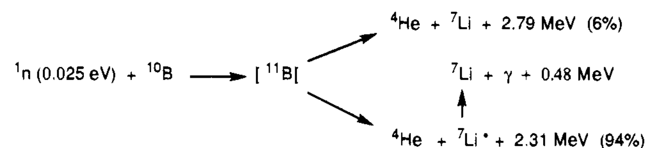
A novel 5-o-carboranyl-containing nucleoside, 5-o-carboranyl-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)uracil (**6**, CFAU), was synthesized as a potential intracellular neutron capture agent. This compound was prepared in five steps starting from 5-iodo-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)uracil (**1**). The desired carboranyl derivative was obtained by addition of decaborane [as the bis(propionitrile) adduct] to the protected acetylenic nucleoside precursor followed by debenzoylation. The synthesis of CFAU was also performed by glycosylation of the suitably protected 5-o-carboranyluracil with the appropriate 2-fluoroarabinosyl derivative. This compound was evaluated for its cytotoxicity in human lymphocytes, monkey cells, and rat and human gliomas cells, as well as for antiviral activity against human immunodeficiency virus and herpes simplex virus type 1. Its biological activity was compared to 5-o-carboranyl-1-(2-deoxyribofuranosyl)uracil in these cell culture systems, human bone marrow cells, and mice. The results obtained to date suggest that CFAU has suitable characteristics as a sensitizer for boron neutron capture therapy.

Introduction

High-boron-content molecules are desirable for the boron neutron capture therapy (BNCT) used for the treatment of gliomas, melanomas, and malignancies.[1] This cancer therapeutic modality combines the utilization of boron-containing compounds targeted to the tumor cells and neutron irradiation as an initiator to produce micronuclear reactions within tumors.[2] The nuclear reaction (Scheme 1) occurs by irradiating the stable nonradioactive isotope ^{10}B with low-energy thermal neutrons, leading to cell destruction by the high energy released in the neutron capture reaction.

To be successful, this concept requires that a sufficient amount of ^{10}B carriers be delivered to the cancer cells to sustain lethal reactions. It has been calculated that the effective dose of ^{10}B in the tumor should be in the range of 5–30 ppm.[3,4] Earlier, synthetic methods for the preparation of monoboron-containing molecules, like boronic acids, were reported.[5–10] However, it appears that using carborane-cluster-containing compounds should be more effective due to their high boron content and lipophilic properties. A variety of carboranyl compounds including amino acids,[11] peptides and immunoconjugates,[12,13] porphyrins,[14] and nucleosides have been synthesized.[15–24] Recently, Yamamoto et al.[24] and our group[21,22] described the synthesis of 5-o-carboranyl-2′-deoxyuridine (CDU). We demonstrated that CDU is taken up by human lymphoma (CEM) and primary human peripheral blood mononuclear (PBM) cells and is intracellularly phosphorylated.[22] The finding that CDU 5′-monophosphate is formed intracellularly suggests that the 5-o-carboranyl moiety is well tolerated

Scheme 1. Boron Neutron Capture Reaction



and can mimic a pyrimidine nucleoside analogue. The entrapment of this molecule in cells offers the possibility to enhance the tumor-to-blood ratio for BNCT. These encouraging results prompted us to synthesize 5-o-carboranyl-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)uracil (CFAU). The choice of this compound was based on the increased glycosidic bond stability conferred by the β-fluorine group positioned on the 2′-carbon of the nucleoside.[25,26] Furthermore, it is noteworthy that the pyrimidine 2′-fluoronucleoside analogues exhibit broad antiviral activity against herpes and hepadnaviruses *in vitro*.[27–30] We report herein the synthesis, antiviral properties, and cytotoxicity of CFAU.

Results and Discussion

Chemistry. CFAU was synthesized adopting the methodology developed by Yamamoto et al.[24] (Scheme 2). 5-Iodo-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)uracil (FIAU)[31] (**1**) was first converted to its 3,3′,5′-tri-N,O,O-benzoylated derivative **2** with benzoyl chloride in pyridine. The fully protected 5-iodo nucleoside in dry THF was then coupled with (trimethylsilyl)acetylene in the presence of triethylamine at 50 °C using copper iodide and $(Ph_3P)_2PdCl_2$ as catalysts to give the 5-(trimethylsilyl)ethynyl-protected nucleoside **3**. Removal of the TMS group was performed in THF using n-tetrabutylammonium fluoride. Under these conditions, cleavage of the N^3-benzoyl group occurred, leading to 5-ethynyl-1-(3,5-di-O-benzoyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)uracil (**4b**). Furthermore, TLC analysis showed

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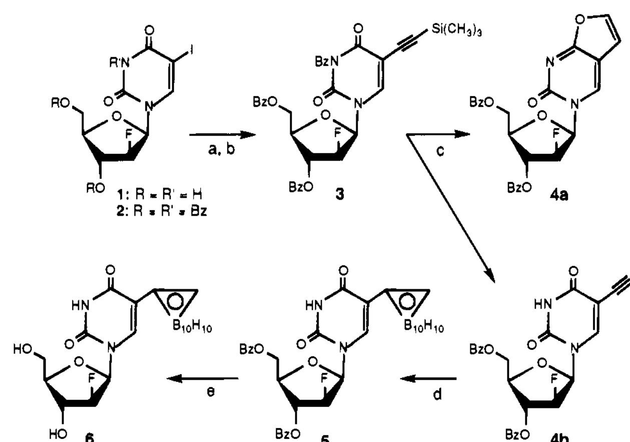
Scheme 2. Synthesis of CFAU[a]

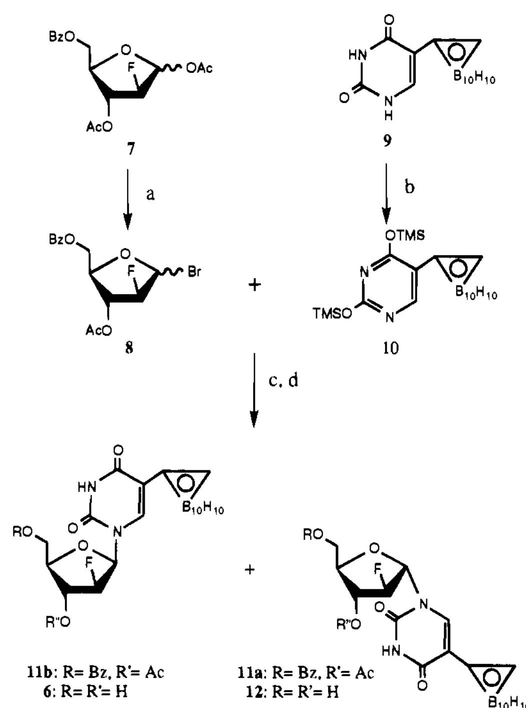


[a] (a) BzCl/pyridine; (b) $HC{\equiv}CSi(Me)_3$, $PdCl_2(PPh_3)_2$, Et_3N/THF; (c) TBAF/THF; (d) $B_{10}H_{12}(CH_3CH_2CN)_2$/toluene; (e) MeONa/MeOH.

the formation of a fluorescent byproduct presumed to be the furanopyrimidinone **4a**. The formation of a similar byproduct has been reported to occur during alkynyl addition to the C-5 position of a nucleoside derivative by Robins and Barr.[32] In addition, a Japanese group[24] observed the formation of a cyclized furano[2,3-*d*]pyrimidin-2-one compound during the alkynyl deprotection step using similar conditions to those described herein. The 5-alkynyl nucleoside **4b** was reacted with decaborane, as a bis(propionitrile) adduct, in refluxing toluene to yield the 5-*o*-carboranyl derivative **5**. Deprotection of hydroxyl functions with methanolic sodium methoxide led to the desired compound CFAU (**6**). However, it should be noted that, under such basic conditions, it is possible to partially convert the *closo*-carboranyl nucleoside into its negatively charged *nido*-isomers. HPLC analysis of CFAU using a reverse phase C18 column and a gradient of acetonitrile in triethylammonium acetate buffer as the mobile phase indicated the presence of less than 1% of the *nido*-CFAU derivatives, which are characteristically more polar than the *closo*-isomer.

CFAU was also synthesized *de novo* by performing a coupling reaction between 5-*o*-carboranyluracil and suitably protected 2-deoxy-2-fluoro-D-arabinofuranose.[33] The rationale for this new synthesis is based upon the potentially broad accessibility of 5-*o*-carboranyl-modified nucleosides available by glycosylation of 5-*o*-carboranyluracil with natural or modified protected carbohydrates.[34] 5-*o*-Carboranyluracil was synthesized in five steps from commercial 5-iodouracil, as described recently by our group.[34] After conversion of 5-iodouracil to 2,4-dimethoxy-5-iodouracil, the procedure utilized for the carboranylation of the base was the same as that described for the corresponding nucleoside and involved three main steps: i.e., alkynylation, deprotection, and decaborane insertion as the bis(propionitrile) adduct. The synthesis of 5-*O*-benzoyl-3-*O*-acetyl-2-deoxy-2-D-fluoroarabinofuranose **7** was performed according to the known procedure.[35] The sugar derivative was then converted to its 1-bromo reactive form by bromination with HBr gas in CH_2Cl_2 at 0 °C. The first coupling reaction was conducted in CH_2Cl_2 at room temperature between the silylated base and the 1-bromo carbohydrate derivative (Scheme 3). After 5 days, only traces of α,β-nucleosides were observed by TLC. When the

Scheme 3. Synthesis of CFAU by Coupling Reaction[a]



[a] (a) HBr(g)/CH_2Cl_2; (b) HMDS, $(NH_4)_2SO_4$; (c) $ZnBr_2/CH_2Cl_2$; (d) NaOMe/MeOH.

same reaction was then performed using $ZnBr_2$ as catalyst, substantial amounts of two nucleosides were detectable after an overnight reaction. The separation of the anomeric mixture of protected nucleosides into the two pure diastereoisomers by column chromatography failed. Therefore, the mixture of compounds was deblocked using NaOMe in MeOH at 0 °C, and then, α-CFAU (**12**) and β-CFAU (**6**) were separated by preparative thin-layer chromatography.

We also attempted the condensation of protected 5-*o*-carboranyluracil with the 2-deoxy-2-fluoroarabinose derivative **8** under a variety of other conditions, which failed to provide the desired compounds. For example, a mixture of the silylated 5-*o*-carboranyluracil (prepared from 40 mg of carboranyl base) and 2-fluoroarabinofuranosyl bromide in methylene chloride (40 mL) was stirred at room temperature for 2 days. TLC ($CHCl_3$–MeOH, 9:1, v/v) showed that no reaction took place. The reaction mixture was then heated under reflux for 2 days to no avail (TLC showed no reaction). The reaction mixture was then cooled, tin(II) chloride was added, and the mixture was stirred at room temperature for 2 days. Again, TLC showed no reaction. When the mixture was heated under reflux for 4 h, the reaction mixture became dark and TLC showed several products which fluoresced under UV light. The reaction was quenched by addition of methanol (ca. 2 mL), and the insoluble precipitates were removed by filtration through a Celite pad. The filtrate was washed with water and cold saturated $NaHCO_3$, dried Na_2SO_4, and evaporated in vacuum. The residue was dissolved in methylene chloride, and the solution was chromatographed over a silica gel column using $CHCl_3$–MeOH (from 19:1 to 4:1, v/v). No nucleosidic product was eluted from the column. It appears from this work that the use of $ZnBr_2$, as described above, was essential for obtaining the desired nucleosides.

Table 1. Cytotoxicity and Antiviral Activity of CFAU Compared to Those of CDU

	IC_{50}, μM[a]					EC_{50}, μM[a]	
compd	CEM on day 6	Vero on day 3	PBM on day 6	9L on day 3	U251 on day 3	HSV-1[b] on day 2	HIV-1[c] on day 6
CFAU	56.5	41.1	≥100	24.9	54.3	>10	7.3
CDU	>100	17.3	>100	>100	>100	11.9	73.2
AZT[d]	13.0	29.0	>100	ND	ND	>50	0.004

[a] EC_{50} and IC_{50} are the median effective (antiviral) and inhibitory (cytotoxic) concentrations, respectively. The variability for the data from replicate assays was less than 15%. [b] In Vero cells using HSV-1 strain F. [c] In human PBM cells using HIV-1 strain LAI. [d] 3′-Azido-3′-deoxythymidine (AZT) was used as a control for the HIV-1 assays.

Biology. CFAU exhibited anti-HIV activity 10-fold greater than its non-fluorinated analogue CDU. CFAU had a therapeutic index greater than 14 in human PBM cells acutely infected with HIV-1. No apparent effect on herpes simplex virus type 1 (HSV-1) was noted in a plaque reduction assay when CFAU was tested up to 10 μM. Its cytotoxicity was slightly higher in all cells examined, except in Vero cells (Table 1). Reversal cytotoxicity assays of CFAU and CDU were performed with thymidine in Vero cells to ascertain if a cellular thymidine kinase was involved in their phosphorylation. Surprisingly, reversal of the toxic effect was not observed for either carboranyl compounds, suggesting that another metabolic pathway than thymidine kinase is responsible for their cytotoxicity (data not shown). It is likely that CFAU and CDU are phosphorylated by other cellular kinases such as adenosine kinase. The toxicity of these compounds in various kinase-deficient cells will be determined. Rat 9L[36,37] and human U251[38] glioma cells were not affected to a marked extent by either CDU or CFAU, although the latter was more toxic. Acyclovir and 3′-azido-3′-deoxythymidine (AZT), used as positive controls for the anti-HSV-1 and anti-HIV-1 assays, respectively, were active at submicromolar concentrations. Initial uptake studies with 10 μM [^{3}H]carboranyl-radiolabeled CFAU (1000 dpm/pmol) in human U251 glioma cells indicated that substantial amounts (≥20% of total radioactivity at 6 h) of CFAU 5′-monophosphate was formed intracellularly.[39]

Bone marrow toxicity has been demonstrated to be the limiting toxicity of certain nucleosides; therefore, the effect of CDU and CFAU on these cells was determined (Table 2). CDU and CFAU were significantly less toxic than AZT to human granulocyte macrophage precursor cells; whereas AZT displayed a 50% suppression of colony formation at concentrations of 1.5 μM, CDU and CFAU required a 13-fold or greater concentration to produce the same effect. Similar results were obtained in erythroid precursor cells (Table 2). It appears that CDU and CFAU were more toxic to granulocyte macrophage precursor cells than to erythroid precursor cells.

Mice Toxicity. A toxicity study was conducted on 6-week-old Swiss female mice treated with CDU and CFAU at 30 mg/kg/day. The compounds were prepared in sterile DMSO and injected intraperitoneally (0.1 mL). DMSO and water were used as controls, with the same injection volume as that used for the boronated nucleosides. The dosing schedule used was once a day for 6 days, and the mice were weighed biweekly for 28 days. Results shown in Table 3 somewhat correlate with the cytotoxicity assays *in vitro*. Although CFAU and CDU produced comparable weight loss after 1 week, CFAU appeared to be more toxic than CDU as monitored by survival outcome. At 4 weeks, all the surviving animals recovered their weight loss to control untreated levels.

Table 2. Relative Effect of CDU and CFAU on Human Myeloid (CFU-GM) and Erythroid (BFU-E) Progenitor Cells Using Clonogenic Assays

		% of control (±SD)[a] at				
compd	cells	0.1 μM	1.0 μM	10 μM	100 μM	IC_{50} ± SD, μM
CDU	CFU-GM	88.1 (10.8)	90.4 (8.6)	79.5 (10.7)	7.4 (2.4)	25.6 ± 6.0
	BFU-E	95.2 (8.3)	93.6 (8.4)	85.4 (12.0)	38.6 (12.1)	57.1 ± 46.4
CFAU	CFU-GM	94.1 (12.4)	85.3 (8.5)	67.0 (10.0)	6.5 (2.8)	19.1 ± 6.0
	BFU-E	94.7 (10.9)	93.1 (11.6)	82.5 (14.2)	68.3 (11.6)	>100
AZT	CFU-GM					1.5 ± 1.2[b]
	BFU-E					0.6 ± 0.5[b]

[a] Results represent mean values of three separate experiments using different donors performed in triplicate. The correlation coefficient for the IC_{50} values was at least 0.93. [b] Data obtained from ref 42.

Table 3. Effect of CDU and CFAU on Survival of Normal Swiss Mice (30 mg/kg/day, QD × 6d)

	% of weight gained or lost on week				survival at week 4,
compd	1	2	3	4	alive/total (%)
CDU	−7.4	0.1	4.8	11.2	5/5 (100)
CFAU	−6.8	−0.4	5.0	9.6	4/5 (80)[a]
DMSO	−0.9	3.0	6.3	10.0	5/5 (100)
H_2O	6.1	7.8	10.2	13.1	5/5 (100)

[a] Death of mouse occurred on day 7.

Experimental Procedures

Melting points were determined on an Electrothermal IA 8100 digital melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a General Electric QE-300 (300 MHz) spectrometer. Mass spectrum analyses were performed using a PE-Sciex AP1 III LCMS with an electrospray interface (University of Alabama at Birmingham, Birmingham, AL) and on a VG Instruments 70-SE spectrometer (Emory University, Atlanta, GA). Experiments were monitored using TLC analysis performed on Kodak chromatogram sheets precoated with silica gel containing a fluorescent indicator, while column chromatography, employing silica gel (60−200 mesh; Fisher Scientific, Fair Lawn, NJ), was used for the purification of products. Microanalyses were performed at Atlantic Microlabs Inc. (Atlanta, GA). Decaborane (purity >99%) was purchased from Boron Biologicals, Inc. (Raleigh, NC).

1-(3,5-Di-*O*-benzoyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-*N*³-benzoyl-5-iodouracil (2). To a stirred solution of FIAU[31] (2.23 g, 6 mmol) (1) in anhydrous pyridine (25 mL) was added benzoyl chloride (1.8 mL, 15 mmol) with stirring under a N_2 atmosphere. The reaction mixture was heated overnight at 50 °C. The solution was cooled to room temperature and poured onto ice−water and the precipitate filtered. The crude product was chromatographed on a silica gel column using CH_2Cl_2 as eluant to yield the fully protected nucleoside **2** as a white powder (2.8 g, 69%): mp 186−189 °C; ^{1}H NMR ($CDCl_3$) δ 4.56 (m, 1H, 4′-H), 4.85 (2 dd, 2H, 5′-H, 5″-H), 5.29 (apparent dd, 1H, 2′-H, $J_{H2',F}$ = 50 Hz), 5.60 (apparent dd, 1H, 3′-H, $J_{H3',F}$ = 19.4 Hz), 6.27 (dd, 1H, 1′-H, $J_{H1',F}$ = 23.8 Hz), 7.20−8.18 (m, 16H, aromatic protons, 6-H). Anal. ($C_{30}FH_{22}IN_2O_8$) C, H, N.

1-(3,5-Di-*O*-benzoyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-*N*³-benzoyl-5-[2-(trimethylsilyl)ethynyl]uracil (3). The protected fluoronucleoside **2** (2.6 g, 3.78 mmol), $(PPh_3)_2PdCl_2$ (265 mg, 0.38 mmol), and CuI (144 mg,

0.76 mmol) were dissolved in anhydrous THF (38 mL); then, triethylamine (1.6 mL, 11.3 mmol) and (trimethylsilyl)acetylene (1.1 mL, 7.56 mmol) were added under an argon atmosphere. The mixture was stirred at room temperature, and after 15 min, the solution turned dark. The mixture was kept at room temperature overnight and the solvent removed under vacuum. The crude product was chromatographed on a silica gel column using a gradient of cyclohexane–CH_2Cl_2 (1:1–0:1) as eluant to yield the desired compound **3** (1.94 g, 78%): mp 126–128 °C; ^{1}H NMR ($CDCl_3$) δ 0.21 (s, 9H, Si(CH_3)$_3$), 4.57 (m, 1H, 4'-H), 4.83 (2 dd, 2H, 5'-H, 5''-H), 5.35 (apparent dd, 1H, 2'-H, $J_{H2',F}$ = 52.1 Hz), 5.63 (apparent dd, 1H, 3'-H, $J_{H3',F}$ = 18.4 Hz), 6.30 (dd, 1H, 1'-H, $J_{H1',F}$ = 20.8 Hz), 7.27–8.13 (m, 16H, aromatic protons, 6-H). Anal. ($C_{35}FH_{31}N_2O_8Si$) C, H, N.

1-(3,5-Di-*O*-benzoyl-2-deoxy-2-fluoro-*β*-D-arabinofuranosyl)-5-ethynyluracil (4b). To an anhydrous THF (30 mL) solution of **3** (1.94 g, 2.96 mmol) was added tetra-*n*-butylammonium fluoride (3.6 mL, 3.6 mmol). The reaction was followed by TLC and reached completion after 6 h at room temperature. However, formation of a fluorescent byproduct, most probably corresponding to **4a**, was also detected by TLC. To the mixture was added CH_2Cl_2 (30 mL) which was then poured into water (50 mL). The organic layer was extracted and washed twice with water (2 × 50 mL), dried over Na_2SO_4, filtered, and evaporated under vacuum. Purification of the crude product by silica gel column chromatography, using CH_2Cl_2–EtOAc (9:1) as eluant, gave the desired compound **4b** (fast eluting) as a white foam (647 mg, 46%): ^{1}H NMR ($CDCl_3$) δ 3.10 (br s, 1H, acetylenic proton), 4.55 (m, 1H, 4'-H), 4.83 (2 dd, 2H, 5'-H, 5''-H), 5.28 (apparent dd, 1H, 2'-H, $J_{H2',F}$ = 50.2 Hz), 5.63 (apparent dd, 1H, 3'-H, $J_{H3',F}$ = 17.2 Hz), 6.31 (dd, 1H, 1'-H, $J_{H1',F}$ = 20.9 Hz), 7.44–8.13 (m, 10H, aromatic protons), 7.92 (s, 1H, 6-H), 8.22 (br s, 1H, NH). Anal. ($C_{25}FH_{19}N_2O_7$·$0.5H_2O$) C, H, N.

1-(3,5-Di-*O*-benzoyl-2-deoxy-2-fluoro-*β*-D-arabinofuranosyl)-5-*o*-carboranyluracil (5). The 5-alkynyl nucleoside **4b** (600 mg, 1.25 mmol) and decaborane (184 mg, 1.50 mmol) were dissolved in anhydrous toluene (50 mL). To this mixture was added freshly distilled propionitrile (1.8 mL, 25 mmol) under an argon atmosphere. The mixture was heated under reflux for 18 h and then cooled to room temperature. The solvent was evaporated under reduced pressure, and the residue was chromatographed on a silica gel column using CH_2Cl_2 as eluant to yield the desired 5-*o*-carboranyl compound **5** as a white powder (318 mg, 43%): mp 242–244 °C; ^{1}H NMR ($CDCl_3$) δ 1.2–3 (br, 10H, carborane protons), 4.59 (m, 1H, 4'-H), 4.79 (2 dd, 2H, 5'-H, 5''-H), 5.34 (apparent dd, 1H, 2'-H, $J_{H2',F}$ = 49.9 Hz), 5.59 (br s, 1H, carborane proton), 5.65 (apparent dd, 1H, 3'-H, $J_{H3',F}$ = 17.1 Hz), 6.26 (dd, 1H, 1'-H, $J_{H1',F}$ = 21.2 Hz), 7.36–8.10 (m, 10H, aromatic protons), 7.99 (s, 1H, 6-H), 8.44 (br s, 1H, NH). Anal. ($B_{10}C_{25}FH_{29}N_2O_7$·$0.25CH_2Cl_2$) C, H, N.

1-(2-Deoxy-2-fluoro-*β*-D-arabinofuranosyl)-5-*o*-carboranyluracil (6). The carboranyl derivative **5** (300 mg, 0.50 mmol) was dissolved in NaOMe/MeOH (0.1 M, 10 mL), and the mixture was stirred at room temperature for 3 h. Ion exchange resin (Dowex 50-X2 H^+ form) was added to neutralize the solution to pH 6–7. The mixture was filtered, the resin washed with MeOH, and the filtrate concentrated under vacuum. A first crop of carboranyl compound **6** was obtained from CH_2Cl_2 (138 mg), and after flash silica gel column chromatography of the filtrate and crystallization from EtOH/CH_2Cl_2, a second crop (36 mg) of the desired compound was obtained as white crystals (174 mg, 90%): mp 246–249 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.2–3 (br, 10H, carborane protons), 3.56 (2 dd, 2H, 5'-H, 5''-H), 3.82 (m, 1H, 4'-H), 4.22 (dm, 1H, 3'-H, $J_{H3',F}$ = 18.7 Hz), 5.07 (dt, 1H, 2'-H, $J_{H2',F}$ = 52.7 Hz), 5.93 (br s, 1H, carborane proton), 6.12 (dd, 1H, 1'-H, $J_{H1',F}$ = 13.9 Hz), 8.01 (s, 1H, 6-H), 12.1 (br s, 1H, NH); MS negative ion *m/e* 387 (M – H). Anal. ($B_{10}C_{11}FH_{21}N_2O_5$) C, H, N.

5-*o*-Carboranyluracil (9). The title compound was synthesized according to our previously described methodology:[34] mp >280 °C dec; ^{1}H NMR (DMSO-d_6) δ 11.28 (br s, 2H, NH, D_2O exchangeable), 7.64 (s, 1H, 6-H), 5.90 (s, 1H, carborane proton), 1.2–3.0 (br, 10H, carborane protons). Anal. ($B_{10}C_6H_{14}N_2O_2$) C, H, N.

1-Bromo-3-*O*-acetyl-5-*O*-benzoyl-2-deoxy-2-fluoro-D-arabinofuranose (8). The method followed for the bromination of **7**[36] has been earlier reported.[33] Briefly, a solution of **7** (1 g, 2.94 mmol) in dry CH_2Cl_2 (20 mL) was cooled in an ice bath, and HBr(g) was introduced into the solution for 20 min. The mixture was then maintained at 4 °C overnight, after which the excess of HBr was then removed by flushing the reaction mixture with N_2. The solvent was evaporated under vacuum, and traces of AcOH were removed by several coevaporations with dry toluene. This material was used in the next reaction without further purification.

1-(3-*O*-Acetyl-5-*O*-benzoyl-2-deoxy-2-fluoro-*α*-D-arabinofuranosyl)-5-*o*-carboranyluracil (11a) and 1-(3-*O*-Acetyl-5-*O*-benzoyl-2-deoxy-2-fluoro-*β*-D-arabinofuranosyl)-5-*o*-carboranyluracil (11b). 2,4-Bis[(trimethylsilyl)oxy]-5-*o*-carboranyluracil (**10**) was prepared by heating under reflux 5-*o*-carboranyluracil (**9**; 190 mg, 0.75 mmol) in $(Me_3Si)_2NH$ (HMDS, 5 mL) in the presence of $(NH_4)_2SO_4$ (~2 mg) until a clear solution was obtained. Excess of silylating agent (HMDS) was removed by distillation under vacuum, and a solution of **8** (273 mg, 0.76 mmol) in anhydrous CH_2Cl_2 (20 mL) was added. To this solution was added $ZnBr_2$ (170 mg, 0.76 mmol), and the reaction mixture was stirred overnight at room temperature. The mixture was diluted with MeOH (5 mL) and the suspension filtered through a Celite pad which was thoroughly washed with CH_2Cl_2 (3 × 50 mL). The combined filtrates were concentrated and washed with H_2O (2 × 25 mL). The organic layer was evaporated under reduced pressure and the residue chromatographed on a silica gel column using a gradient of CH_2Cl_2/EtOAc from 100% to 90% of CH_2Cl_2. The CFAU derivatives were obtained as a mixture of α- (**11a**) and β- (**11b**) nucleosides (218 mg, 41% yield).

1-(2-Deoxy-2-fluoro-*α*-D-arabinofuranosyl)-5-*o*-carboranyluracil (12) and 1-(2-Deoxy-2-fluoro-*β*-D-arabinofuranosyl)-5-*o*-carboranyluracil (6). The anomeric mixture of **11a,b** (100 mg, 0.18 mmol) was dissolved in NaOMe/MeOH (0.05 M, 3 mL), and the mixture was stirred at 0 °C for 1 h and then left at 4 °C overnight. Ion exchange resin (Dowex 50-X2 H^+ form) was added to neutralize the solution to pH 6–7. The mixture was filtered and the resin washed with MeOH, and then, the filtrate was concentrated under vacuum. The crude mixture (68 mg) was then purified by preparative TLC using an isocratic acetone/CH_2Cl_2 (17/83, v/v) as eluant. After 4-fold developments of the plate, the nucleosides α (**12**; 8.7 mg, R_f = 0.54 in MeOH/CH_2Cl_2, 1/9) and β (**6**; 10.7 mg, R_f = 0.48 in MeOH/CH_2Cl_2, 1/9) were resolved. The ^{1}H NMR analysis for the β nucleoside was identical to that obtained by the synthesis from FIAU. The characterization of α nucleoside **12** was also performed by ^{1}H NMR: (Me_2SO-d_6) δ 1.2–2.4 (br, 10H, carborane protons), 3.24–3.44 (2 m, 2H, 5'-H, 5''-H), 4.20 (m, 2H, 4'-H, 3'-H), 4.96 (t, 1H exch., 5'-OH), 5.10 (d, 1H, 2'H, J = 16.6 Hz), 5.95 (1 d overlapped with br s, 3H, 1'-H, 3'-OH exch., carborane proton), 7.90 (s, 1H, 6-H), 11.9 (br s, 1H, NH); MS (FAB/LSIMS) *m/e* 397.2881 (M^+ + Li); calcd 397.2525.

Biological Assays. Antiviral and Cytotoxicity Assays. Anti-HIV-1 activity of the compounds was determined in PBM cells as described previously.[40] Stock solutions (40 mM) of the new compounds were prepared in sterile DMSO and then diluted to the desired concentration. Other compounds such as AZT were dissolved 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 $(rA)_n$·$(dT)_{12-18}$ 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.[40] Antiviral activity against HSV-1 in Vero cells was performed by a plaque assay as described previously.[29] The EC_{50} and median inhibitory concentration (IC_{50}) were obtained from the concentration–response curve using the median effective method described by Chou and Talalay.[41] For the reversal cytotoxicity assay, Vero cells were treated with CFAU (50 and 100 μM) or CDU at the same concentrations in the presence or absence of

thymidine (from 10 to 400 μM). Cell proliferation was measured on day 3.

Assay of Colony-Forming Unit Granulocyte Macrophage (CFU-GM) or Burst-Forming Unit Erythroid (BFU-E) for Drug Cytotoxicity Studies. Human bone marrow cells were collected by aspiration from the posterior iliac crest of normal healthy volunteers, treated with heparin, and the mononuclear population was separated by Ficoll–Hypaque gradient centrifugation as described previously.[42] Cells were washed twice in Hanks balanced salt solution and counted with a hemacytometer, and their viability was >98% as assessed by trypan blue exclusion. The culture assays were performed using a bilayer soft agar or methyl cellulose method as previously described.[42] After incubation for 14 days at 37 °C in a humidified atmosphere of 5% CO_2 in air, colonies ($\geq$50 cells) were counted using an inverted microscope.

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