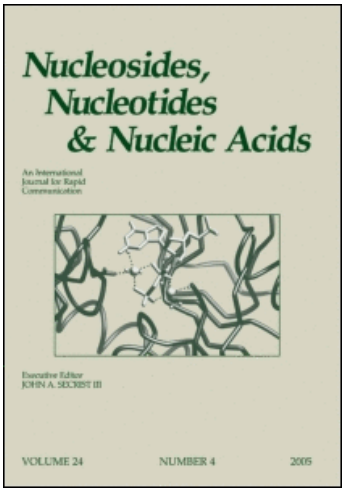

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SYNTHESIS AND BIOLOGICAL PROPERTIES OF THE FOUR OPTICAL ISOMERS OF 5-*o*-CARBORANYL-2',3'-DIDEHYDRO-2',3'-DIDEOXYURIDINE

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ABSTRACT: The four isomers of the 5-*o*-carboranyl-2',3'-didehydro-2',3'-dideoxyuridine (d4CU) were synthesized and their antiviral activity and cytotoxicity in normal and cancer human cells determined. Coupling of silylated 5-*o*-carboranyluracil with the protected D/L 2,3-dideoxy-2-phenylselenenylribosylacetates provided after oxidative elimination and deprotection, the desired compounds. The presence of the electron deficient 5-*o*-carboranyl moiety on uracil influenced the yield of the various isomers. In general, the compounds demonstrated weak anti-human immunodeficiency virus activity in primary human lymphocytes. No marked difference in the biological profile was noted for the various optical isomers, suggesting that the high lipophilicity of these nucleosides imparted by the carboranyl moiety overrides stereochemical considerations in the 2',3'-didehydro-2',3'-dideoxy-aglycon moiety.

INTRODUCTION

There is a renewed interest in organoboron molecules which are applicable to boron neutron capture therapy (BNCT), a binary procedure used in the treatment of malignancies such as brain gliomas.[1,2] With the development of practical methods for production of improved neutron fluxes for BNCT,[3] the synthesis of new organoboron compounds has become a high priority. Several carboranyl derivatives have been synthesized, such as 5-*o*-carboranyluracil (CU)[4-6] and its corresponding nucleoside 5-*o*-carboranyl-2',3'-dideoxyuridine (β-D-CDU).[4,6]

The interest in incorporating an *o*-carborane moiety into a nucleoside include: 1) it is one of the most stable boron clusters and may provide a sufficient quantity of fissionable ^{10}B to tumor cells; 2) the enhanced lipophilicity of these compounds compared with standard nucleosides and their derivatives may accentuate membrane permeability, such as the blood-brain-barrier or the prostate capsule;[7] and 3) certain nucleosides such as CDU can be phosphorylated in malignant human cells,[4] thus trapping the compound intracellularly.

Our goal was to synthesize compounds which are able to combine the properties of carboranyl nucleoside derivatives against tumor cells and those of the d4-nucleosides against viruses. Several 2',3'-didehydro-2',3'-dideoxynucleosides (d4N) like Stavudine (d4T) have been shown to have potent *in vitro* and *in vivo* activity against human immunodeficiency virus (HIV).[8] Their clinical efficacy has also been improved when used in combination with other nucleoside and non-nucleoside HIV reverse transcriptase, or protease inhibitors. Their 5'-triphosphate derivatives inhibit viral reverse transcriptase by competing with the natural substrates at the enzyme active site by acting as viral DNA chain terminators. The target chosen was the synthesis of the four isomers (α- and β-diastereoisomers of the D- and L-enantiomers) of 5-*o*-carboranyl-2',3'-didehydro-2',3'-dideoxyuridine (d4CU). The synthesis of the L-enantiomers could provide compounds with improved *in vitro* and *in vivo* pharmacological profiles compared to their D-counterparts.

CHEMISTRY AND DISCUSSION

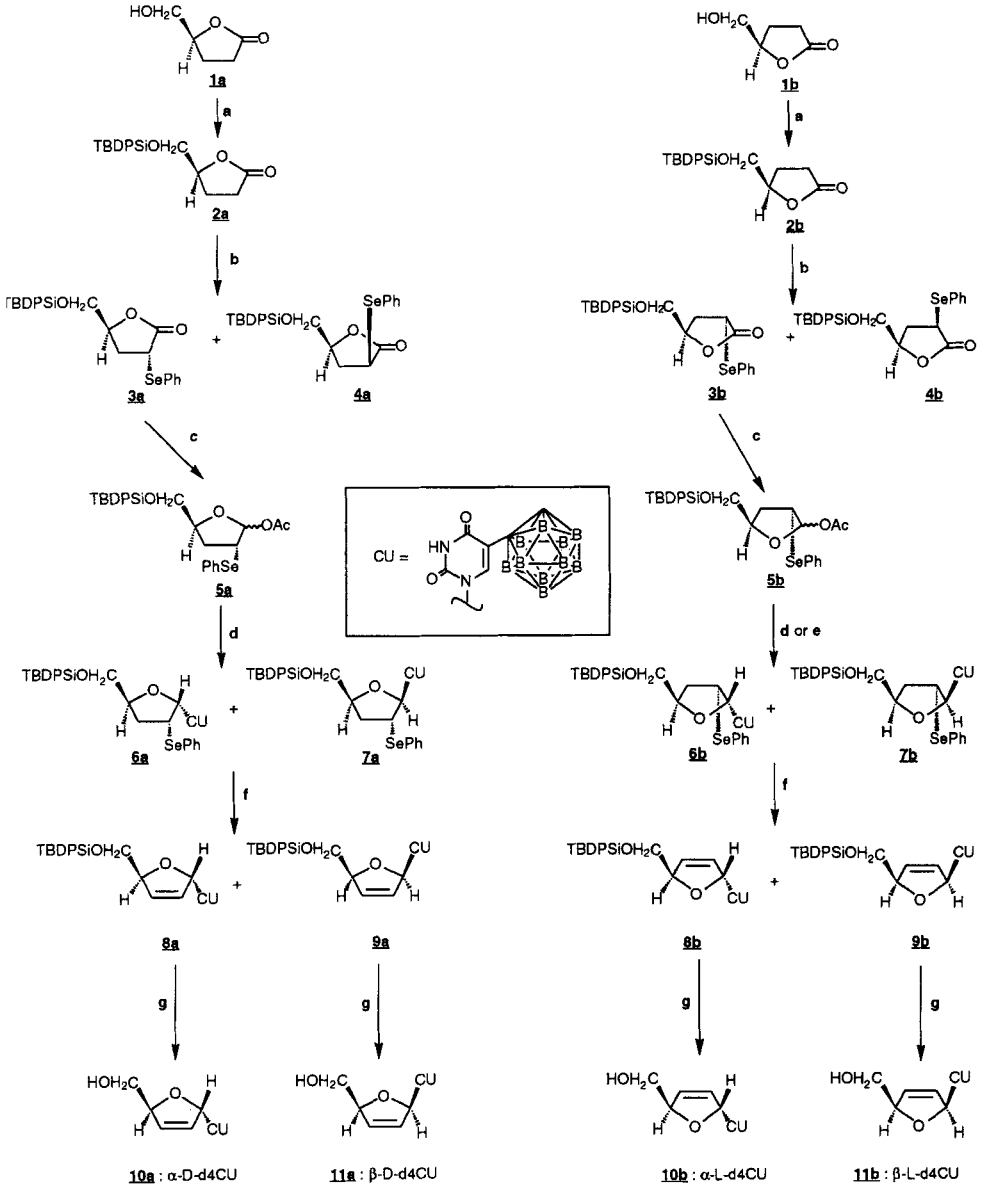
The approach selected was to synthesize 5-*o*-carboranyluracil, and then to glycosylate the modified base with a protected chiral sugar. Synthesis of the 5-*o*-carboranyluracil was achieved using a procedure described by our group,[5] starting from 5-iodouracil. The substituted chiral sugars (D- and L- isomers, **5a** and **5b**) desirable for the coupling step were obtained using the methodology developed by Beach *et al.* [9] for the synthesis of the β-D-enantiomer of d4T.

Starting from either (*S*)-(+)-dihydro-5-(hydroxymethyl)-2(3*H*)-furanone (D-enantiomer, **1a**) or (*R*)-(-)-dihydro-5-(hydroxymethyl)-2(3*H*)-furanone (L-enantiomer, **1b**), the 5-hydroxymethyl group was protected using a *tert*-butyldiphenylsilylether (**2a** and **2b**), followed by the introduction of the phenylselenenyl group by reaction of LiHMDS and phenylselenenyl bromide

on the 2-position of the protected furanone providing after separation by column chromatography the *trans* isomers **3a** and **3b** and the *cis* isomers **4a** and **4b** (Scheme 1). Using the phenylselenenyl substituted *trans* isomers (**3a** and **3b**), the D- and L-enantiomers of the 1-*O*-acetyl-5-*O*-(*tert*-butyldiphenylsilyl)-3-deoxy-2-phenylselenenyl-α- and -β-pentofuranoses (**5a** and **5b**) were synthesized by reduction of the carbonyl function using DIBAL-H and acetylation by acetic anhydride (Scheme 1). The coupling of the silylated 5-carboranyluracil with the 1-*O*-acetyl-sugars **5a** and **5b** was achieved using a Lewis acid such as tin(IV) chloride ($SnCl_4$)[5,10-13] or trimethylsilyl trifluoromethanesulfonate (TMSOTf),[9,11-13] producing the desired nucleosides (**6a/7a** and **6b/7b**). The coupling reaction involving $SnCl_4$ was achieved using a precomplexation of the persilylated 5-*o*-carboranyluracil with the catalyst, followed by the addition of the sugar **5b** in 1,2-dichloroethane at room temperature (Scheme 1). The use of this catalyst usually provides a high β selectivity,[5,9-11,13] since the proposed mechanism involves the complexation of the catalyst on the α-face of the sugar moiety[11], allowing attack of the silylated base on the β-face to give the corresponding protected nucleoside with a high β stereoselectivity. Previous work from this laboratory[5] has shown that the coupling reaction involving 5-*o*-carboranyluracil and $SnCl_4$ as the catalyst resulted in high stereoselectivity producing predominantly the β-isomer in high yield, in the presence of benzoylester or *tert*-butyldiphenylsilylether as 5'-hydroxymethyl protecting group and benzoyloxy substituents on the α-face of the sugar moiety. In our case, the use of $SnCl_4$ provided the desired protected compounds **6b/7b** as an α/β isomeric mixture, but with a low β selectivity (1/2 α/β ratio, as determined by ^1H NMR integration of glycoside proton) and modest yield (46%) (Scheme 1).

This step was also performed on compounds **5a** and **5b** using TMSOTf (Scheme 1). The same desired nucleosides (**6a/7a** and **6b/7b**, Scheme 1) were obtained in higher yield than with $SnCl_4$ (57% *versus* 46%)[13] and with a lower β stereoselectivity (2/1 α/β isomeric ratio). These unexpected α/β ratios were contrary to the usually observed high β-selectivity with the above two catalysts.[9,11]

The reaction of an 1-*O*-acetyl-pentofuranose with TMSOTf is known to produce an oxonium ion which can be attacked by a nucleic base without selectivity if no participating group is existed in the 2-or 3-positions, resulting in a 1/1 α/β isomeric ratio.[11,12,14] We anticipated that the presence of a

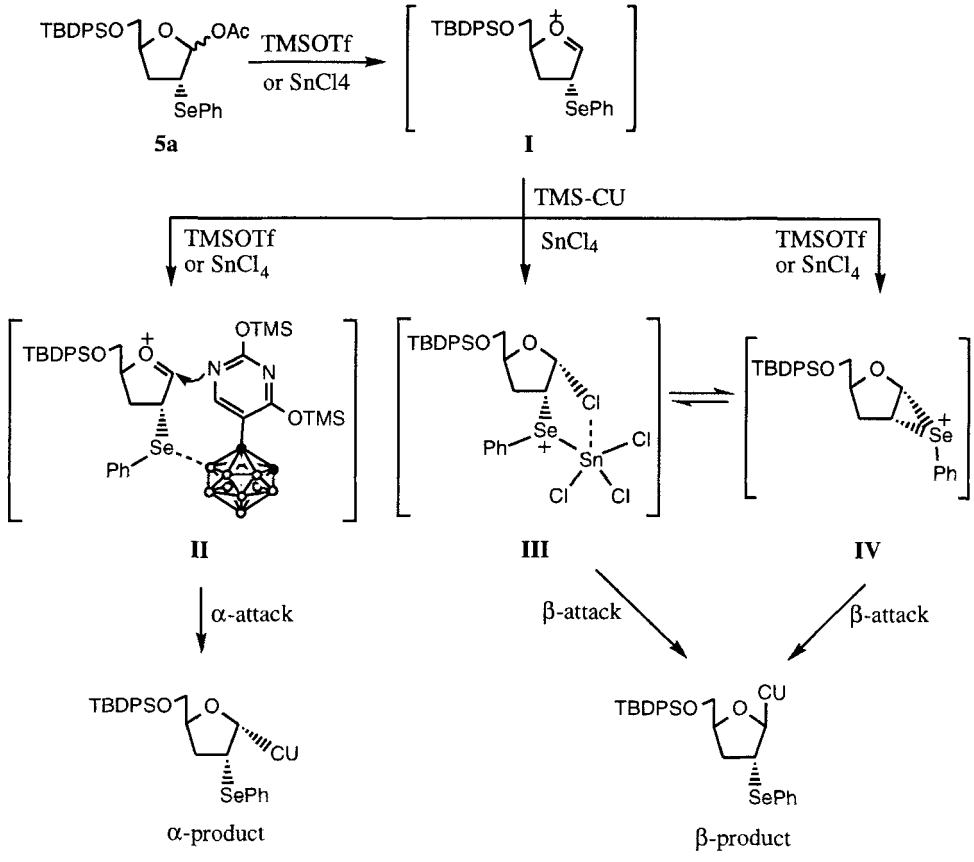


a: TBDPSiCl, imidazole, DMF; **b:** i- LiHMDS, THF, -78 °C, ii- TMSCl, -78 °C to 25 °C, iii- PhSeBr, -78 °C to 25 °C; **c:** i- DIBAL-H, PhMe, -78 °C, ii- Ac_2O, pyridine, DMAP, 0 °C; **d:** Persilylated 5-carboranyluracil (HMDS, $(NH_4)_2SO_4$), TMSOTf, 1,2-dichloroethane, 0 °C to 25 °C; **e:** Persilylated 5-carboranyluracil (HMDS, $(NH_4)_2SO_4$), $SnCl_4$, 1,2-dichloroethane, 0 °C to 25 °C; **f:** i- H_2O_2, pyridine, CH_2Cl_2, ii- Separation; **g:** TBAF, THF.

SCHEME 1. Synthetic route to D- and L-d4CU.

participating group such as the phenylselenenyl group at the 2-position on the α-face of the sugar would have formed a selenonium ion or a complex of selenium-$SnCl_4$ as an intermediate which blocked the α-face of the sugar, producing the corresponding β-nucleoside as the predominant product.[5,9,11,13]

We had previously shown that for the silylated 5-*o*-carboranyluracil coupling reaction, the presence of a sulfur substituent on the α-face of the sugar resulted the predominant β-isomer (β/α ratio = 20:1).[5] Surprisingly, the coupling reaction between silylated 5-o-carboranyluracil and 2α-phenylselenenyl substituted sugar resulted in no selectivity in our case either with $SnCl_4$ or TMSOTf. The existing known mechanisms for the coupling reaction catalyzed by $SnCl_4$ or TMSOTf can not explain this unusual β/α ratio. For example, any similar mechanisms involving an intermediate of a sulfonium ion,[11] or a complex between the sulfur substituent of the sugar and $SnCl_4$,[10] would lead to the predominant β-isomers. There must be some novel factors which caused this unusual ratio of the products. We believe that the phenylselenenyl and carboranyl moieties are responsible for the results, since the coupling reactions without these two substituents or only with one of them proceed as expected. We hypothesize that interaction occurs between the electron rich selenium on the phenylselenenyl substituent and the carboranyl moiety which is electron-deficient due to the presence of boron. This interaction must be specific to the carboranyl moiety since it has not been observed with non carboranyl containing bases in the presence of the same selenenyl substituent on the sugar.[9,15] The unusually high amount of α-isomer found when $SnCl_4$ is used, can be explained by the formation of an intermediate **II** (Scheme 2) resulting from the selenium-carboranyl moiety interaction. Because of the favorable distance of the base and the oxonium ion, the coupling step is achieved by an intramolecular addition, forming the α-isomer. The presence of $SnCl_4$ in the reaction can result in the formation of a selenium-$SnCl_4$ complex on the α-face of the sugar (**III**, Scheme 2) by interaction of the catalyst with the selenium atom, similar to the sulfur-$SnCl_4$ complex in the oxathiolane case.[10] Another possible intermediate involved in the reaction could be the selenonium ion **IV** (Scheme 2) which could be in an equilibrium with the intermediate **III**. The later two intermediates, blocking the α-face of the sugar, would result in the β-isomer, which is indeed the major product (**7b**).



SCHEME 2. Possible mechanism of glycosylation.

In the case of TMSOTf, the oxonium ion **I** (Scheme 2) could also lead to the same intermediates **II** and **IV** (Scheme 2). The favorable selenium-carborane interaction, which is counterbalanced by a weak steric repulsion between SePh and 5-*o*-carboranyluracil, favors intermediate **II**. Because of the close proximity between the nucleic base and the oxonium ion in the intermediate **II**, the intramolecular addition in the intermediate **II** is faster than the intermolecular addition in the intermediate **IV**, favoring a larger yield of α–isomer. The proposed mechanism depicted in scheme 2 shows the D-forms. For the L-isomers, the mechanism is the same except for the opposite configurations.

The elimination of phenylselenenyl substituent was achieved using H_2O_2 and a catalytic amount of pyridine first at 0 °C and then at room temperature overnight,[9,15] providing compounds **8a/8b** (α-diastereoisomers of the D- and L-enantiomers) and the corresponding β-isomers **9a/9b** (Scheme 1). The separation of the α and β isomers for the D- and L-compounds was achieved by column chromatography either on the phenylselenenyl carboranyl derivatives (**6a/7a** and **6b/7b**) or on the protected 5-*o*-carboranyl-2',3'-dideoxy-2',3'-didehydronucleosides (**8a/9a** and **8b/9b**). The final step consisted in the removal of the silyl protecting group using TBAF (1.0 M in THF) in THF, providing the desired compounds **10a** (α-D-d4CU, 94%), **11a** (β-D-d4CU, 67%), **10b** (α-L-d4CU, 60%) and **11b** (β-L-d4CU, 50%) (Scheme 1).

It should be noted that the use of basic conditions such as pyridine in the elimination of phenylselenenyl group can partially convert the *closo*-carboranyl nucleosides into their negatively charged *nido*-isomers.[16] However, HPLC analysis using a reverse phase C18 column with MeOH/H_2O (70 : 30) as the mobile phase and mass spectrometry of **10a-b** and **11a-b** indicated that the *closo*-carboranyl nucleosides were obtained predominantly, if not exclusively. Reverse phase HPLC retention times and optical rotations for the four optical isomers of d4CU are given in Table 1. The HPLC and optical rotation procedures are described in the general experimental section.

BIOLOGY AND CONCLUSIONS

The purpose of our research is to synthesize essentially non-toxic boron-containing nucleosides that are phosphorylated in cell culture. A lipophilic boron nucleoside analog that is phosphorylated selectively in cells may result in trapping significant quantities of the boron containing compound, thus increasing its intracellular residence time. We had previously demonstrated that β-D-CDU is phosphorylated intracellularly, despite the bulkiness of the carboranyl moiety at the 5-position of 2'-deoxyuridine.[4] The determination of some biological effect for the synthesized compounds such as antiviral activity or cytotoxicity even at high concentration, can provide indirect evidence of intracellular phosphorylation, although other mechanisms could be involved.

The synthesized d4CU compounds were evaluated for anti-HIV-1 activity in human lymphocytes; and for cytotoxicity in various cells such as the lymphoblastoid CEM cells, human peripheral blood mononuclear (PBM) cells, in rapidly dividing Vero cells, and in various cancer human cells such

TABLE 1. Retention times and optical rotations of the four isomers of d4CU.

d4CU isomer	Retention time (min)[a]	$[\alpha]_D^{25}$
α-D (**10a**)	9.9	-143° (c 0.1, MeOH)
α-L (**10b**)	9.3	+ 140° (c 0.1, MeOH)
β-D (**11a**)	7.5	+ 80° (c 0.1, MeOH)
β-L (**11b**)	7.2	- 85° (c 0.1, MeOH)

[a] HPLC conditions are described in the general experimental section.

as lung carcinoma cells (SK-MES-1), breast adenocarcinoma cells (MCF-7) and prostate carcinoma cells (LNCaP). The results are reported in Table 2.

The d4CU compounds showed weak but selective activity against HIV-1 in human PBM cells. They were more potent than β-D-CDU, but less potent than 5-*o*-carboranyl-1-(2-deoxy-2-fluoro-β-D-arabinosyl)uracil (β-D-CFAU).[17] All the d4-compounds showed modest cytotoxicity in Vero cells. Compound **11b** had a weak selective inhibitory activity on HIV-1 in human lymphocytes comparable to β-D-CFAU. All the synthesized compounds showed an essentially non-toxic profile in human anchored cancer cells with an IC_{50} greater than 50 μM. In particular, α-D- (**10a**) and α-L-d4CU (**10b**) showed no demonstrable cytotoxicity up to 80 μM in these cells.

It was surprising to note that the different enantiomers and diastereoisomers have similar biological profiles; their EC_{50} or IC_{50} values were within a 3-fold range or less. This suggests that the biologically important structural feature for these nucleosides is the presence of a carboranyl function at the 5-position which imparts great lipophilicity to the nucleoside analogs.

EXPERIMENTAL SECTION

Melting points (mp) were determined on an Electrothermal IA 8100 digital melting point apparatus and are uncorrected. ^1H and ^{13}C NMR were recorded on a Varian Unity Plus 400 spectrometer at 400 MHz and 100.6 MHz, respectively. Tetramethylsilane was used as an internal standard and J values are given in Hz. Thin layer chromatography was performed on Whatman silica gel HP-KF glass-backed plates. Fisher silica gel (200-425 Mesh, grade 633) was used for flash column chromatography. Anhydrous solvents and other chemicals were purchased from Aldrich Chemical Company (Milwaukee,

TABLE 2. *In vitro* antiviral activity and cytotoxicity in various human cells (normal and cancer cells) of four isomers of d4CU[a]

Compound	Anti-HIV-1 Activity in human PBM cells		Cytotoxicity (IC_{50}, μM)					
	EC_{50}, μM	EC_{90}, μM	PBM	CEM	Vero	SK-MES-1	MCF-7	LNCaP
α-D-d4CU (**10a**)	39.0	75.0	50.6	8.8	52.9	>100	~131	89.2
α-L-d4CU (**10b**)	32.0	67.0	55.1	21.7	35.9	>100	>100	83.9
β-D-d4CU (**11a**)	21.0	84.0	55.4	37.5	16.5	~100	53.8	52.2
β-L-d4CU (**11b**)	9.0	50.0	47.1	37.1	31.8	>100	77.4	72
β-D-CDU	73.2	189.0	>100	>100	17.3	>100	>100	~115
β-D-CFAU	7.3	130.0	>100	56.5	41.1	ND	ND	ND
β-D-AZT	0.004	0.02	>100	13.0	29.0	ND	ND	ND
Cycloheximide	ND	ND	0.57	0.08	0.2	0.85	1.4	0.55

[a] EC_{50} and EC_{90} are the median and 90% effective antiviral concentrations; IC_{50} is the median inhibitory (cytotoxic) concentration; ND = not determined; cycloheximide was used as a positive cytotoxic control.

WI) and were used without further purification or drying. Microanalyses were performed at Atlantic Microlab (Norcross, GA). Mass Spectrometry was performed at the Emory University Mass Spectrometry Center (Atlanta, GA). HPLC analyses were performed on a Waters 600 series instrument, using the following conditions: reverse phase RP-18 column; isocratic 70:30 MeOH/H_2O; flow rate 1 mL/min; detection at 254 nm. Optical rotations were determined in methanol on a Perkin-Elmer MC 241 polarimeter. Decaborane (purity >99%) was purchased from Boron Biologicals, Inc. (Raleigh, NC). β-D-CDU and β-D-CFAU were prepared as previously reported.[4,17]

5-*O*-(*tert*-Butyldiphenylsilyl)-2,3-dideoxy-L-glyceropentonic acid-γ-lactone (2b). (*R*)-(-)-Dihydro-5-hydroxymethyl-2(3*H*)-furanone (2.0 g, 17 mmol, 1 eq) (**1b**) and imidazole (2.32 g, 34 mmol, 2 eq) were dissolved in anhydrous DMF (15 mL) under an argon atmosphere at room temperature. *tert*-Butyldiphenylsilyl chloride (5.6 g, 21 mmol, 1.2 eq) was slowly added and the mixture was stirred for 1 hr at room temperature and then the solvent was

removed under reduced pressure. The residue was dissolved in $CHCl_3$ (20 mL), washed with water and brine (2 x 15 mL) and the organic phase was dried over Na_2SO_4, filtered and concentrated under *vacuum* to give a colored oil. Compound **2b** was obtained by crystallization from hot hexane as a white solid (4.0 g, 66%). ^1H NMR ($CDCl_3$) δ: 1.10 (s, 9H, *t*-Bu), 2.25 (m, 2H, CH_2-3), 2.60 (dd, 2H, CH_2-2), 3.80 (dd, 2H, CH_2-5), 4.50 (m, 1H, H-4), 7.40-7.80 (m, 10H, 2 x C_6H_5).

5-*O*-(*tert*-Butyldiphenylsilyl)-2,3-dideoxy-2-phenylselenenyl-L-*threo*-pentonic acid-γ-lactone (3b) and 5-*O*-(*tert*-butyldiphenylsilyl)-2,3-dideoxy-2-phenylselenenyl-L-*erythro*-pentonic acid-γ-lactone (4b). To a solution of **2b** (4.0 g, 12 mmol) in dry THF (40 mL) stirred at -78 °C under argon was added lithium hexamethyldisilazide (LiHMDS, 1 M in THF) (14 mL, 14 mmol, 1.2 eq) over a period of 5 min. After 1 hr at -78 °C, chlorotrimethylsilane (1.54 g, 1.42 mmol) was added dropwise and the reaction mixture was allowed to warm to room temperature. After stirring at room temperature for 30 min, the mixture was again cooled to -78 °C and a solution of phenylselenenyl bromide (4.0 g, 17 mmol) in dry THF (20 mL) was rapidly added. The mixture was then diluted with diethyl ether (40 mL) and washed with H_2O until the ether layer was almost colorless. The organic layer was separated, dried over Na_2SO_4, filtered and concentrated. The oily residue was purified by flash chromatography over silica gel providing the desired $C_{2\alpha}$ isomer **3b** (2.45 g, 42%) using EtOAc (0-7%) in hexane as eluent: R_f (EtOAc/Hex: 7/93) = 0.38; ^1H NMR ($CDCl_3$) δ: 1.10 (s, 9H, *t*-Bu), 2.30 (m, 1H, H_a-3), 2.70 (m, 1H, H_b-3), 3.60 (dd, $J_{4,5a}$ = 2.6 Hz, $J_{5a,5b}$ = 11.9 Hz, 1H, H_a-5), 3.83 (dd, $J_{4,5b}$ = 2.6 Hz, $J_{5a,5b}$ = 11.9 Hz, 1H, H_b-5), 4.10 (dd, J = 5.1 Hz and J = 9.4 Hz, 1H, H-2), 4.33-4.37 (m, 1H, H-4), 7.30-7.70 (m, 15H, 3 x C_6H_5) and the $C_{2\beta}$ isomer **4b** (1.4 g, 25%) using 10% EtOAc in hexane as eluent: R_f (EtOAc/Hex: 7/93) = 0.29; ^1H NMR ($CDCl_3$) δ: 1.05 (s, 9H, *t*-Bu), 2.20 (m, 1H, H_a-3), 2.65 (m, 1H, H_b-3), 3.60 (m, 2H, H-5), 4.05 (t, J = 9.4 Hz, 1H, H-2), 4.45-4.60 (m, 1H, H-4), 7.30-7.70 (m, 15H, 3 x C_6H_5).

1-*O*-Acetyl-5-*O*-(*tert*-butyldiphenylsilyl)-2,3-dideoxy-2-phenylselenenyl-α- and β-L-*threo*-pentofuranose (5b). To a stirred solution of **3b** (1.0 g, 2 mmol) in dry toluene (15 mL) was added DIBAL-H (3.7 mL of a 1.0 M solution in toluene, 37 mmol, 1.6 eq) over a 5 min period at -78 °C under argon. After stirring for 2 hr at -78 °C, the reaction was quenched with MeOH (5 mL), allowed to warm to -20 °C and stirred at this temperature for 30 min. The

mixture was then diluted with EtOAc (15 mL), washed with a sat. $NaHCO_3$ aqueous solution, water and brine. The organic phase was separated, dried over Na_2SO_4, filtered and concentrated. The resulting crude lactol (0.9 g, 90%) was acetylated by treatment with acetic anhydride (0.48 mL, 5.1 mmol, 2.5 eq), pyridine (1.02 mL, 1 mmol, 7 eq) and DMAP (4 mg, cat. amount) in CH_2Cl_2 (20 mL) at 0°C for 2 hr. After removal of the solvent under reduced pressure, compound **5b** (0.990 g, 90%) was obtained as a clear yellow liquid (mixture of α- and β-anomers): ^1H NMR ($CDCl_3$) δ: 1.00 and 1.05 (s, 9H, *t*-Bu), 2.10 (s, 3H, Ac), 2.20-2.65 (m, 2H, CH_2-3), 3.50-3.85 (m, 3H, H-2 and CH_2-5), 4.45 (m, 1H, H-4), 6.28 (s, 0.66H, H-1), 6.50 (s, 0.33H, H-1), 7.30-7.70 (m, 15H, 3 x C_6H_5).

General procedure for the coupling of 5-*o*-carboranyl uracil. A suspension of 5-carboranyluracil[4-6] (1 eq) and ammonium sulfate (catalytic amount) in hexamethyldisilazane (HMDS, 5 mL) was heated under reflux for 2 hr under argon. After removal of the solvent by evaporation under reduced pressure, the residue was treated with a solution of the acetate **5a** or **5b** (1 eq) in dry 1,2-dichloroethane (6 mL) under argon. The reaction mixture was cooled to 5 °C, and TMSOTf (1 eq) was added. After stirring at 5 °C for 10 min, the reaction mixture was stirred at room temperature for another 30 min. Then the mixture was poured into a mixture of EtOAc and sat. $NaHCO_3$ solution with stirring. The organic layer was separated, washed with sat. $NaHCO_3$ solution, water, and brine, dried (Na_2SO_4), filtered, and concentrated. The residue was purified by flash chromatography over silica gel eluting with CH_2Cl_2 to provide the desired compound as an α/β mixture.

α- and β-D-5'-*O*-(*tert*-Butyldiphenylsilyl)-5-*o*-carboranyl-2',3'-dideoxy-2'-phenylselenenyluridine (6a/7a). Following the described procedure, reaction of 5-carboranyluracil (0.240 g, 0.94 mmol), ammonium sulfate (0.013 g, 0.1 mmol) and acetate **5a**[9] (0.542 g, 0.98 mmol) provided after purification with CH_2Cl_2 the α-isomer **6a** (0.100 g, 14%) as a colorless foam: ^1H NMR ($CDCl_3$) δ: 1.08 (s, 9H, *t*-Bu), 1.80-2.80 (m, 12H, CH_2-3' and $B_{10}H_{10}$), 3.50-3.80 (m, 3H, CH_2-5' and H-2'), 4.40-4.45 (m, 1H, H-4'), 5.63 (bs, 1H, $B_{10}H_{10}CH$), 6.07 (d, J = 7.2 Hz, 1H, H-1'), 7.22-7.67 (m, 16H, H-6 and 3 x C_6H_5); and an α/β 2/1 isomeric mixture (**6a/7a**, 0.222 g, 31%) as a white foam: ^1H NMR ($CDCl_3$) δ: 1.08 and 1.11 (2s, 9H, *t*-Bu), 1.80-2.80 (m, 12H, CH_2-3' and $B_{10}H_{10}$), 3.50-3.95 (m, 3H, CH_2-5' and H-2'), 4.40-4.45 and 4.50-4.60 (m, 1H, H-4'), 5.59 and 5.73 (2 bs, 1H, $B_{10}H_{10}CH$), 6.02-6.08 (m, 1H, H-1'), 7.24-7.67 (m, 16H, H-6 and 3 x C_6H_5).

α- and β-L-5'-*O*-(*tert*-Butyldiphenylsilyl)-5-*o*-carboranyl-2',3'-dideoxy-2'-phenylselenenyluridine (6b/7b). As described above for **6a/7a**, reaction of 5-carboranyluracil (0.220 g, 0.88 mmol), ammonium sulfate (0.01 g, 0.08 mmol) and acetate **5b** (0.440 g, 0.80 mmol) led to compounds **6b/7b** as a white foam, in a 2/1 ratio of an α/β isomeric mixture (0.340 g, 57%): R_f (CH_2Cl_2) = 0.38; ^1H NMR ($CDCl_3$) δ: 1.05 and 1.00 (2s, 9H, *t*-Bu), 1.90-2.90 (m, 24H, 2 CH_2-3' and 2 $B_{10}H_{10}$), 3.60-4.00 (m, 6H, 2 H-2' and 2 CH_2-5'), 4.40 (m, 1H, H-4'), 4.65 (m, 1H, H-4'), 5.75 (bs, 1H, $B_{10}H_{10}CH$), 5.90 (bs, 1H, $B_{10}H_{10}CH$), 6.20 (d, 1H, H-1'), 6.25 (bs, 1H, H-1'), 7.30-7.90 (m, 32H, 2 H-6 and 6 x C_6H_5), 9.30 (s, 1H, NH), 9.70 (s, 1H, NH).

In an analogous manner, compounds **6b/7b** were also obtained using $SnCl_4$. 5-*O*-Carboranyluracil (0.220 g, 1.2 eq, 0.88 mmol) was persilylated as described above and then dissolved in anhydrous CH_2Cl_2. $SnCl_4$ (1.0 M in CH_2Cl_2, 1.13 mL) was added to this solution at 0 °C and the mixture was stirred for 20 min at this temperature. Compound **5b** (0.400 g, 1 eq, 0.72 mmol) in anhydrous CH_2Cl_2 (15 mL) was added. After 2.5 hr at 0 °C, an aqueous solution of NH_4OH (5 mL) was added. The mixture was stirred at room temperature for 15 min and filtered through Celite which was washed with warm $CHCl_3$ and warm EtOAc/EtOH (9/1 v/v). The organic layers were combined and evaporated to dryness to give a colored foam. Column chromatography provided the corresponding α/β mixture as a white foam (0.250 g, 46%, 1/2 α/β isomeric mixture) which was identical to the one obtained using TMSOTf as catalyst as determined by TLC and ^1H NMR spectra analysis.

General procedure for the preparation of the 2',3'-didehydro-2',3'-dideoxy moiety. To the solution of the above α/β-isomeric mixture (**6a/7a** or **6b/7b**, 1 eq) in CH_2Cl_2 (3 mL) containing a catalytic amount of pyridine (1 drop) at 0 °C was added H_2O_2 (30%, 8 eq) over a period of 5 min. The reaction mixture was stirred from 0 °C to room temperature overnight. The mixture was diluted with CH_2Cl_2 (30 mL), and the organic layer was separated, washed with water, dried (Na_2SO_4), filtered, and concentrated. The residue was purified by flash chromatography using CH_2Cl_2 as eluent providing first the α-isomer (**8a/8b**) and then the β-isomer (**9a/9b**).

α-D-5'-*O*-(*tert*-Butyldiphenylsilyl)-5-*o*-carboranyl-2',3'-didehydro-2',3'-dideoxyuridine (8a) and β-D-5'-*O*-(*tert*-butyldiphenylsilyl)-5-*o*-carboranyl-2',3'-

didehydro-2',3'-dideoxyuridine (9a). Following the above procedure, the α/β-isomeric mixture (**6a/7a**, 0.222 g, 0.30 mmol) when treated with H_2O_2 (30%, 0.19 mL) provided after purification the α-isomer **8a** as a white foam (0.090 g, 51%): mp 90-92 °C; R_f (CH_2Cl_2/EtOAc: 9/1) = 0.54 ; ^1H NMR ($CDCl_3$) δ: 1.07 (s, 9H, *t*-Bu), 1.60-3.00 (m, 10H, $B_{10}H_{10}$), 3.80 (d, J = 4.0 Hz, 2H, H-5'), 5.12-5.13 (m, 1H, H-4'), 5.71 (bs, 1H, $B_{10}H_{10}CH$), 5.95 (d, J = 5.2 Hz, 1H, H-2'), 6.45 (d, J = 6.0 Hz, 1H, H-3'), 6.98 (d, J = 5.2 Hz, 1H, H-1'), 7.39-7.48 (m, 6H, arom), 7.49 (s, 1H, H-6), 7.66-7.67 (m, 4H, arom), 8.12 (bs, 1H, NH); and the β-isomer **9a** as a white foam (0.040 g, 22%): mp 133-135 °C; R_f (CH_2Cl_2/EtOAc: 9/1) = 0.35; ^1H NMR ($CDCl_3$) δ:1.09 (s, 9H, *t*-Bu), 1.60-3.00 (m, 10H, $B_{10}H_{10}$), 3.71 (dd, J = 6.8 Hz and 10.0 Hz, 1H, H-5'b), 3.81 (dd, J = 6.8 Hz and 10.4 Hz, 1H, H-5'a), 5.00-5.01 (m, 1H, H-4'), 5.64 (bs, 1H, $B_{10}H_{10}CH$), 5.86 (dd, J = 1.6 Hz and 6.0 Hz, 1H, H-2'), 6.50-6.53 (dt, J = 1.6 Hz and 4.0 Hz, 1H, H-3'), 6.92-6.93 (m, 1H, H-1'), 7.36-7.48 (m, 6H, arom.), 7.51 (s, 1H, H-6), 7.65-7.67 (m, 4H, arom.), 8.16 (bs, 1H, NH).

In an analogous manner, the α-isomeric product **8a** (0.050 g, 65%) was also obtained from compound **6a** (0.100 g, 0.13 mmol). This compound was identical to that obtained from the α/β-isomeric mixture by comparing their mp, TLC, and ^1H NMR spectra.

α-L-5'-*O*-(*tert*-Butyldiphenylsilyl)-5-*o*-carboranyl-2',3'-didehydro-2',3'-dideoxyuridine (8b) and β-L-5'-*O*-(*tert*-butyldiphenylsilyl)-5-*o*-carboranyl-2',3'-didehydro-2',3'-dideoxyuridine (9b). When a mixture of compounds **6b/7b** (0.300 g, 0.40 mmol) was reacted with H_2O_2 (30%, 0.35 mL), the α-isomer **8b** (0.100 g, 42%) was obtained as a white foam: mp 90-92 °C; R_f (CH_2Cl_2) = 0.22; ^1H NMR ($CDCl_3$) δ: 1.05 (s, 9H, *t*-Bu), 1.90-2.80 (b, 10H, $B_{10}H_{10}$), 3.80 (d, J = 4.5 Hz, 2H, CH_2-5'), 5.15 (bs, 1H, H-4'), 5.75 (bs, 1H, $B_{10}H_{10}CH$), 5.90 (d, J = 5.6 Hz, 1H, H-2'), 6.45 (d, J = 6.0 Hz, 1H, H-3'), 6.97 (d, J = 5.0 Hz, 1H, H-1'), 7.40-7.70 (m, 11H, H-6 and 2 x C_6H_5), 9.40 (s, 1H, NH). Similarly, the β-isomer **9b** was obtained as a white foam (0.06 g, 21%): mp 122-124 °C; R_f (CH_2Cl_2) = 0.05; ^1H NMR ($CDCl_3$) d: 1.05 (s, 9H, *t*-Bu), 1.90-2.80 (b, 10H, $B_{10}H_{10}$), 3.65 (dd, J = 6.8 Hz and 10.3 Hz, 1H, H-5'$_b$), 3.80 (dd, J = 6.0 Hz and 10.3 Hz, 1H, H-5'$_a$), 5.00 (bs, 1H, H-4'), 5.65 (bs, 1H, $B_{10}H_{10}CH$), 5.85 (d, J = 6.0 Hz, 1H, H-2'), 6.50 (d, J = 6.0 Hz, 1H, H-3'), 6.95 (s, 1H, H-1'), 7.30-7.70 (m, 11H, H-6 and 2 x C_6H_5), 9.10 (s, 1H, NH).

α-D-5-*o*-Carboranyl-2',3'-didehydro-2',3'-dideoxyuridine (10a). To a solution of **8a** (0.130 g, 0.22 mmol) in THF (3 mL), TBAF (1.0 M soln. in THF,

0.22 mL, 0.22 mmol) was added. The reaction mixture was stirred at room temperature for 2 hr and then the solvent was removed by evaporation. After purification by flash chromatography eluting first with CH_2Cl_2 and then CH_2Cl_2/MeOH (99/1), **10a** (0.073 g, 94%) was obtained as a white solid: mp > 240 °C (dec.); R_f ($CHCl_3$/MeOH: 9/1) = 0.59; ^1H NMR ($CDCl_3$) δ: 1.60-3.05 (m, 10H, $B_{10}H_{10}$), 3.71 (dd, J = 4.0 Hz and 12.0 Hz, 1H, H-5'$_b$), 3.89 (dd, J = 3.6 Hz and 12.0 Hz, 1H, H-5'$_a$), 5.19-5.21 (m, 1H, H-4'), 5.69 (s, 1H, $B_{10}H_{10}CH$), 5.99 (dd, J = 1.6 Hz and 6.0 Hz, 1H, H-2'), 6.49 (dd, J = 2.0 Hz and 6.0 Hz, 1H, H-3'), 7.05 (d, J = 4.8 Hz, 1H, H-1'), 7.51 (s, 1H, H-6), 8.42 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ: 57.8, 63.9, 69.4, 88.2, 91.1, 107.6, 126.1, 135.1, 141.7, 148.7, 160.0; HRMS/FAB calcd for $C_{11}H_{19}N_2O_4B_{10}$ $(M-H)^-$: 353.2275, found: 353.2289; Anal. Calcd for $C_{11}H_{20}N_2O_4B_{10}$: C, 37.49; H, 5.72; N, 7.95. Found: C, 36.74; H, 5.58; N, 7.55; $[\alpha]_D^{25}$ = -143° (c 0.1, MeOH).

α-L-5-*o*-Carboranyl-2',3'-didehydro-2',3'-dideoxyuridine (10b). To a solution of **8b** (0.100 g, 0.17 mmol) in dry THF (5 mL) was added a solution of TBAF in THF (0.17 mL of 1.0 M, 0.17 mmol) and the reaction mixture was stirred at room temperature under argon for 5.5 hr. The solvent was then removed under reduced pressure and the residue was purified by chromatography using first CH_2Cl_2 and then CH_2Cl_2/EtOAc (9:1) as eluent to provide **10b** as a white solid (0.040 g, 60%): mp > 240 °C (dec.); R_f ($CHCl_3$/MeOH: 9/1) = 0.60; ^1H NMR ($CDCl_3$) δ: 1.90-2.80 (b, 10H, $B_{10}H_{10}$), 3.67 (dd, J = 4.5 Hz and 12.2 Hz, 1H, H-5'$_b$), 3.87 (dd, J = 3.3 Hz and 12.2 Hz, 1H, H-5'$_a$), 5.20 (bt, 1H, H-4'), 5.70 (bs, 1H, $B_{10}H_{10}CH$), 5.95 (d, J = 7.5 Hz, 1H, H-2'), 6.45 (d, J = 7.2 Hz, 1H, H-3'), 7.05 (d, J = 4.7 Hz, 1H, H-1'), 7.45 (s, 1H, H-6), 9.60 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ: 59.6, 66.6, 70.2, 89.0, 91.8, 108.3, 128.6, 136.3, 142.5, 149.6, 160.9; LSIMS/FAB calcd for $C_{11}H_{19}N_2O_4B_{10}$ $(M-H)^-$: 353.2, found: 353.2; Anal. Calcd for $C_{11}H_{20}N_2O_4B_{10}$: C, 37.49; H, 5.72; N, 7.95. Found: C, 37.35; H, 5.69; N, 7.70; $[\alpha]_D^{25}$ = +140° (c 0.1, MeOH).

β-D-5-*o*-Carboranyl-2',3'-didehydro-2',3'-dideoxyuridine (11a). In an analogous manner to compounds **10a** and **10b**, compound **11a** (0.016 g, 67%) was obtained from compound **9a** (0.040 g, 0.07 mmol) as a white solid: mp > 240 °C (dec.); R_f ($CHCl_3$/MeOH: 9/1) = 0.64; ^1H NMR ($CDCl_3$) δ: 1.60-3.00 (m, 10H, $B_{10}H_{10}$), 3.89 (dd, J = 2.8 Hz and 12.0 Hz, 1H, H-5'$_b$), 4.00 (dd, J = 2.8 Hz and 12.0 Hz, 1H, H-5'$_a$), 5.02 (bs, 1H, H-4'), 5.73 (bs, 1H, $B_{10}H_{10}CH$), 5.91 (d, J = 6.0 Hz, 1H, H-2'), 6.42 (dd, J = 1.6 Hz and 6.0 Hz, 1H, H-3'), 6.99-7.00 (m, 1H, H-1'),

8.16 (s, 1H, NH), 8.20 (s, 1H, H-6); ^{13}C NMR ($CDCl_3$) δ: 58.2, 63.0, 69.9, 87.4, 90.6, 107.1, 125.9, 135.2, 143.5, 148.8, 160.1; HRMS/FAB calcd for $C_{11}H_{19}N_2O_4B_{10}$ $(M-H)^-$: 353.2275, found: 353.2265; Anal. Calcd for $C_{11}H_{20}N_2O_4B_{10}$: C, 37.49; H, 5.72; N, 7.95. Found: C, 37.20; H, 5.64; N, 7.44; $[\alpha]_D^{25}$ = +80° (c 0.1, MeOH).

β-L-5-*o*-Carboranyl-2',3'-didehydro-2',3'-dideoxyuridine (11b). Compound **9b** (0.06 g, 0.10 mmol) was treated as described above for compounds **10a**, **10b** and **11a** to yield compound **11b** as a white solid (0.015 g, 50%): mp > 240 °C (dec.); R_f ($CHCl_3$/MeOH: 9/1) = 0.65; ^1H NMR ($CDCl_3$) δ: 1.90-2.80 (b, 10H, $B_{10}H_{10}$), 3.87 (dd, J = 2.4 Hz and 12.5 Hz, 1H, H-5'$_b$), 4.00 (dd, J = 2.5 Hz and 12.5 Hz, 1H, H-5'$_a$), 5.05 (bs, 1H, H-4'), 5.74 (bs, 1H, $B_{10}H_{10}CH$), 5.90 (d, J = 7.3 Hz, 1H, H-2'), 6.42 (d, J = 7.3 Hz, 1H, H-3'), 7.00 (m, 1H, H-1'), 8.20 (s, 1H, NH), 8.60 (s, 1H, H-6); ^{13}C NMR ($CDCl_3$) δ: 59.6, 63.8, 65.5, 88.1, 90.9, 107.8, 128.3, 136.2, 151.4, 163.7; HRMS/FAB calcd for $C_{11}H_{19}N_2O_4B_{10}$ $(M-H)^-$: 353.2275, found: 353.2270; Anal. Calcd for $C_{11}H_{20}N_2O_4B_{10}$: C, 37.49; H, 5.72; N, 7.95. Found: C, 37.27; H, 5.60; N, 7.44; $[\alpha]_D^{25}$ = -85° (c 0.1, MeOH).

Biological assays. Antiviral and Cytotoxicity Assays. Anti-HIV-1 activity of the compounds was determined in human PBM cells as described previously.[18] Stock solutions (40 mM) of the nucleosides analogs were prepared in sterile DMSO and then diluted to the desired concentration. Cells were infected with the prototype HIV-1_{LAI} at a multiplicity of infection of 0.01. Virus obtained from the cell supernatant was quantified on day 6 after infection by a reverse transcriptase assay using $(rA)_n.(dT)_{12-18}$ as a 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.[18] The EC_{50}, EC_{90} and median inhibitory concentration (IC_{50}) were obtained from the concentration-response curve using the median effective method as described previously.[19,20] The compounds were also evaluated for their potential toxic effects on LNCaP (metastatic prostate adenocarcinoma, human), MCF7 (breast adenocarcinoma, human), and SK-MES-1 (lung carcinoma, human) cells. Appropriate numbers of cells were cultured with the drug for a specific number of days in 96 well plates (LNCap: 5 days; MCF7: 4 days; SK-MES-1: 5 days). Cycloheximide was included as a positive cytotoxic control and untreated cells exposed to solvent were included as negative controls. After incubation, actively metabolizing cells were quantified using the CellTiter 96 Cell Proliferation Assay (MTT, Promega, Madison, WI), as described by the manufacturer.

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