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Enantioselective Synthesis and Biological Evaluation of 5-*o*-Carboranyl Pyrimidine Nucleosides

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Abstract—Base-modified carborane-containing nucleosides such as 5-*o*-carboranyl-2′-deoxyuridine (CDU) when combined with neutrons have potential for the treatment of certain malignancies. Lack of toxicity in various cells, high accumulation in cancer cells and intracellular phosphorylation are desirable characteristics for modified nucleosides used in boron neutron capture therapy (BNCT) for brain tumors and other malignancies. The aim of this work was to synthesize the two β-enantiomers of several 5-*o*-carboranyl-containing nucleosides. These derivatives may possess favorable properties such as high lipophilicity, high transportability, the ability to be phosphorylated, and resistance to catabolism. β-Isomers of 2′,3′-dihydroxynucleosides and analogues containing a heteroatom in the sugar moiety were also synthesized. Carboranyl pyrimidine nucleosides were prepared either from the parent β-D-nucleoside, β-L-nucleoside, or by a coupling reaction. The dioxolane derivative **7** was prepared by a coupling reaction between protected 5-*o*-carboranyluracil (**8**, CU) and the corresponding protected heterocycle. Specific catalysts were used during the *N*-glycosylation process to favor the formation of the β-isomer. Biological evaluation of these new chiral 5-*o*-carboranyl pyrimidine derivatives indicated that most of these compounds have low toxicity in a variety of normal and malignant cells and achieved high cellular levels in a lymphoblastoid cell line. Increasing the number of hydroxyl groups on the sugar moiety decreased the cellular accumulation and serum binding to different extents. Five compounds were identified for further biological evaluation as potential agents for BNCT.

Introduction

High boron content molecules are desirable for the boron neutron capture therapy (BNCT) used for the treatment of gliomas, melanomas and malignancies.[1,2] This therapeutic modality combines the high accumulation of boron-containing compounds targeted to the tumor cells with neutron irradiation to produce micronuclear reactions within tumors.[3] The nuclear reaction occurs by irradiating stable non-radioactive ^{10}B with low energy thermal neutrons, leading to cell destruction by the high linear energy transfer radiation released in the neutron capture reaction. To be successful, this concept requires that a sufficient amount of a non-toxic ^{10}B carrier is delivered to the cancer cells to produce this nuclear reaction. It has been calculated that the effective dose of ^{10}B in the tumor should be in the range of 5 to 30 ppm.[4,5] In general, carborane-cluster-containing compounds should be more effective than organo-boronic acids due to their high boron content and lipophilic properties.

Several carboranyl compounds have already been synthesized such as 5-(*o*-carboran-1-yl)-2′-deoxyuridine (**1**, D-CDU, Fig. 1)[6,7] and 5-*o*-carboranyl-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)uracil (D-CFAU).[8] Our group demonstrated that D-CDU is taken up by human lymphocytic cells (CEM) and primary human peripheral blood mononuclear (PBM) cells and is phosphorylated intracellularly.[7] The finding that the 5′-monophosphate of D-CDU is formed intracellularly suggested that the 5-*o*-carboranyl moiety is well tolerated and can mimic a pyrimidine nucleoside analogue. The entrapment of this lipophilic molecule in cells offers the possibility to enhance the tumor-to-blood and tumor-to-normal tissue ratios for BNCT since malignant cells divide faster than normal tissues in compartments such as the brain where gliomas develop. The encouraging results obtained with D-CDU or D-CFAU prompted us to develop the enantiomeric synthesis of new 5-*o*-caboranyl

Key words: L-Nucleosides; boron neutron capture therapy; antiviral activity.

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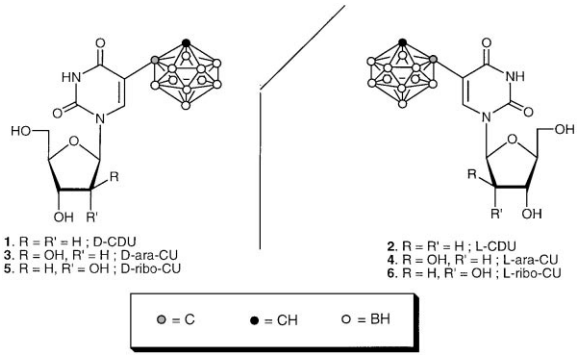


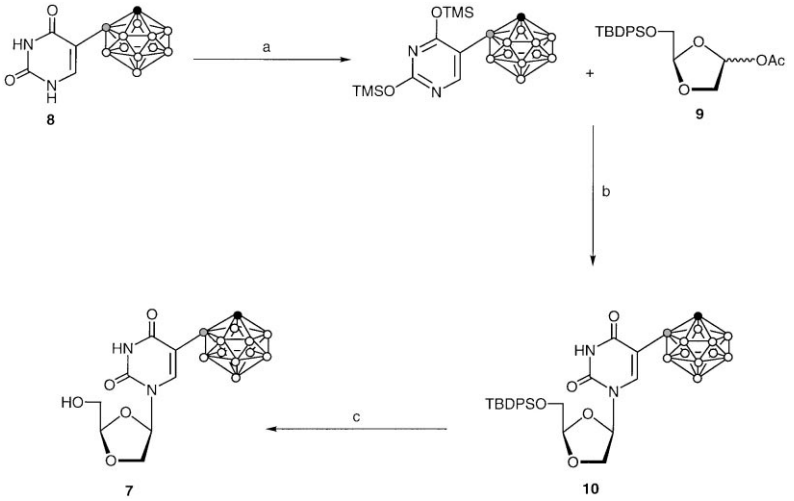
Figure 1. Structures of several enantiomers of 5-*o*-carboranyl pyrimidine nucleosides.

nucleoside derivatives with both the D- or L-configuration. Certain L-nucleosides can be phosphorylated as well as their corresponding D-enantiomers and their nucleotides have increased stability to phosphodiesterases.[9] In certain cases, L-enantiomers were found to be more potent and less toxic than their corresponding D-counterparts.[10,11] One of the most important examples is the (−)-enantiomer of β-2′,3′-dideoxy-3′-thiacytidine (3TC), which is at least 10-fold less toxic than the (+)-enantiomer and has potent and selective antiviral activity.[10,12] Based on these findings, several new β-L- and D-5-*o*-carboranyl pyrimidine nucleosides were synthesized with potentially improved physicochemical and biological properties.

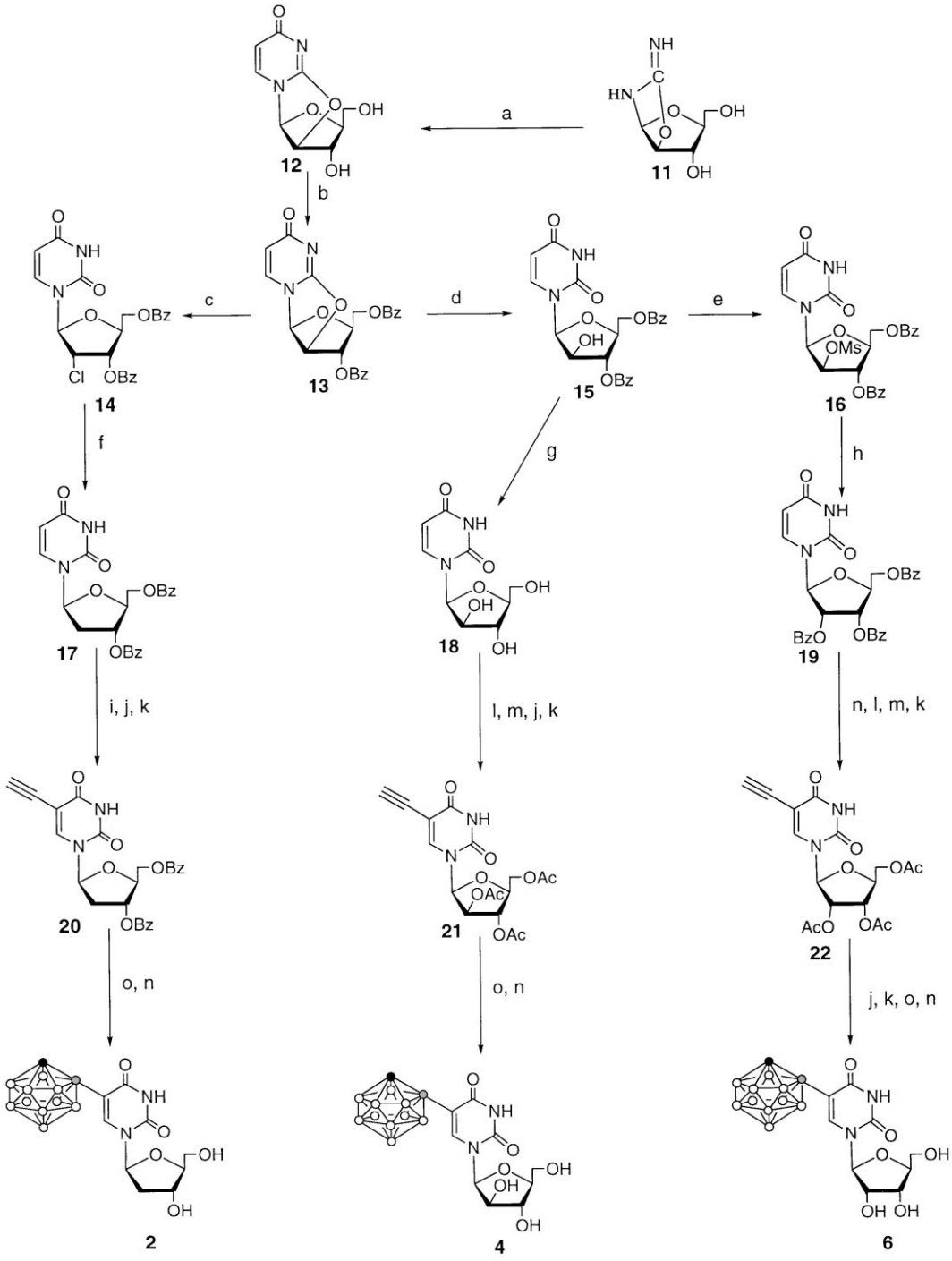
Results

5-*o*-Carboranyl derivatives were prepared either by glycosylation (compounds **5** and **7**) or directly from the parent β-D- or β-L-nucleoside (compounds **1**–**4** and **6**). Using the previously described synthesis of compound **5**,[13] the dioxolane analogue (**7**) was obtained by a coupling procedure. The key intermediate, 5-*o*-carboranyluracil (**8**), was prepared from 5-iodouracil in six steps.[13] Coupling of the silylated 5-*o*-carboranyl base with the protected dioxolane sugar (**9**)[14] using tin(IV) chloride ($SnCl_4$) yielded the β-protected nucleoside **7** in good yield (Scheme 1). The mechanism of this reaction involves the formation of an oxonium ion at the α-face of the sugar moiety which mainly allows attack of the silylated base on the β-face.[15–17] An equimolar mixture of the α and β forms was obtained when trimethylsilyl trifluoromethanesulfonate (TMSOTf) was used. Deprotection was performed with 1 M tetrabutylammonium fluoride solution in THF, leading to the desired 5-*o*-carboranyl derivative **7** which was characterized by NOE experiments.

In order to avoid α-analogue formation for the synthesis of other derivatives, the synthesis was initiated directly from the β-parent nucleoside. Compound **13** (Scheme 2) was a common intermediate for the synthesis of several L-derivatives. 3′,5′-Di-*O*-benzoyl-2,2′-anhydro-β-L-uridine (**13**) was obtained from L-arabinose in good overall yield according to the procedure described by Holý.[18] Opening of the 2,2′-anhydro linkage of compound **13** with anhydrous HCl in DMF provided the 2′-chloro-2′-deoxy derivative **14**. Reduction with tri-*n*-butyltin hydride in the presence of AIBN as a catalyst afforded 3′,5′-di-*O*-benzoyl-2′-deoxy-β-L-uridine (**17**) in quantitative yield. Iodination with *N*-iodosuccinimide and catalytic amounts of *n*-butyl disulfide afforded the 5-iodo derivative which was coupled with (trimethylsilyl)acetylene in the presence of bis(triphenylphosphine)palladium(II) chloride, copper iodide and triethylamine. Desilylation was performed before the carboranylation step. Decaborane was heated under reflux in toluene and propionitrile with the formed ethynyl derivative, yielding the 1-[β-L-(2-deoxyribofuranosyl)]-5-*o*-carboranyluracil (L-CDU, **2**) after deprotection with a 0.1 M sodium methoxide solution. 1-(β-D-Arabinofuranosyl)-5-*o*-carboranyluracil (**3**) was obtained following the same procedure as L-CDU from 2,2′-anhydro-1-(β-D-arabinofuranosyl)uracil, which was prepared by the method of Hampton and Nichol.[19] 2′-Hydroxyl group inversion involved the use of diphenyl carbonate which is safe and readily available.



Scheme 1. Reagents and conditions: (a) 1,1,1,3,3,3-hexamethydisilazane, ammonium sulfate, reflux, 6 h; (b) tin(IV) chloride, CH_2Cl_2, 0°C, 2 h; (c) tetrabutylammonium fluoride, 1 M sol in THF, 0°C, 3 h.



Scheme 2. Reagents and conditions: (a) methyl propiolate, 50% aqueous EtOH, reflux, 5 h; (b) BzCl, pyridine, 5°C, 16 h; (c) HCl, 4 M sol in dioxane, DMF, 90°C, 90 min; (d) H_2SO_4, 0.2 M sol in DMF:H_2O (1:1 v/v), 100°C, 3 h; (e) CH_3SO_2Cl, pyridine, 5°C, 18 h, rt, 3 h; (f) $[CH_3(CH_2)_3]_3SnH$, AIBN, benzene, reflux, 1.5 h; (g) NH_3/MeOH, rt, 4 h; (h) sodium benzoate, HMPA, 120°C, 7 h; (i) *N*-iodosuccinimide, *n*-butyl disulfide, DMSO, rt, 18 h; (j) (trimethylsilyl)acetylene, bis(triphenylphosphine)palladium(II) chloride, CuI, Et_3N, THF, 40°C, 4 h; (k) TBAF, 1 M sol in THF, rt, 20 min; (l) I_2, $CHCl_3$, 1 N aqueous HNO_3, reflux, 2 h; (m) Ac_2O, pyridine, rt, 18 h; (n) 0.1 N methanolic NaOMe, 5°C, 18 h; (o) decaborane, propionitrile, toluene, reflux, 6 h.

The iodination step was performed with iodine in refluxing 0.1 M HNO_3 solution in $CHCl_3$ as described by Schinazi et al.[20] Although this reaction produced lower yields compared to the precedent method using *N*-iodosuccinimide, the advantages were that no DMSO was used and reaction times were shorter.

1-(β-L-Arabinofuranosyl)-5-*o*-carboranyluracil (**4**) and 5-*o*-carboranyl-β-L-uridine (**6**) were synthesized from the common intermediate 1-(β-L-arabinofuranosyl-3,5-di-*O*-benzoyl)uracil (**15**) obtained by hydrolysis of compound **13** under acidic conditions. Carboranylation steps were conducted as described for compound **3**. The

benzoyl protecting groups had to be removed prior to iodination to avoid incomplete transformation and the formation of byproducts. In addition, synthesis of 5-*o*-carboranyl-β-L-uridine required 2′-hydroxyl group inversion of compound **15**. This transformation was initiated from the mesyl protected derivative **16** with sodium benzoate in HMPA at 120°C. Under mild deprotection conditions less than 7% of *nido* derivatives were detected by HPLC.[13,21] These conversions to *nido* isomers occurred most likely during the deblocking of acetylated or benzoylated hydroxy functions of carboranyl analogues under basic conditions.[21] All carboranyl derivatives tested demonstrated low toxicity against the various cell lines used (Table 1). Accumulation of the compounds in CEM cells and their sensitivities to the presence of 10% serum in the medium decreased in the order: CU > CDU > ara-CU > ribo-CU (Fig. 2). The ratio of compound accumulated in the presence of 10% serum to that accumulated in the absence of serum ranged from 0.1 to 0.7 for CU and L-ribo-CU, respectively. The D-enantiomers of CDU and ara-CU were less sensitive to the presence of serum than their L-counterparts.

Discussion

Although compounds described were primarily developed for BNCT, they were also tested for cytotoxicity and antiviral activity in various normal and cancer cell systems and for anti-HIV activity in primary human lymphocytes. β-D-Dioxolane-CU (**7**) was the most potent anti-HIV-1 analogue ($EC_{50} = 4.6\,\mu M$, Table 1), but its therapeutic index was approximately 2 in PBM cells. It was also found to be moderately toxic in CEM and rapidly dividing Vero cells at higher concentrations. Low toxicity is a highly desirable property for compounds considered for BNCT. When evaluated in different cells, L-enantiomers exhibited the lowest toxicity in CEM cells. The relative toxicity in CEM cells was: β-D-ara-CU > β-D-ribo-CU > β-D-CDU ≈ β-D-dioxolane-CU > β-L-ribo-CU ≈ β-L-CDU > β-L-ara-CU. In addition, L-enantiomers gave similar cytotoxicity profiles in PBM and rapidly dividing Vero cells. Most of the compounds tested demonstrated low toxicity in melanoma, prostate and lung cancer cells. Accumulation experiments of the carboranyl derivatives in CEM cells (Fig. 2) demonstrated that the presence of a sugar moiety and the number and orientation of its substituent hydroxyl groups can alter cellular accumulation and serum binding. It was observed that the presence of an aglycon, and increasing the number of hydroxyl functions on this sugar moiety from two to three, decreased the cellular accumulation and serum binding to different extents. This was expected since both biological parameters are influenced by changes in lipophilicity. Further biological testing is warranted to determine how these structural changes alter the in vivo pharmacokinetics and tissue selectivity of these carboranyl-containing agents.

Conclusions

This paper contains the first report on the synthesis of L-enantiomers of boron nucleoside analogues for potential use in BNCT. Based on the relatively low in vitro cytotoxicity in primary human PBM cells and other cell lines (Table 1), and low protein-binding properties (Fig. 2), β-L-ribo-CU (**6**) and β-L-ara-CU (**4**) have desirable properties for BNCT. However, D- or L-CDU (**1** and **2**) and the nucleoside base CU (**8**) should also be considered since they accumulate in CEM cells to the greatest extent.

Table 1. In vitro antiviral activity and cytotoxicity of several 5-*o*-carboranyl pyrimidines in various normal and cancer cell lines

Compound	HIV-1, EC_{50} (μM)[a]	Cytotoxicity, IC_{50} (μM)[a]							
		PBM[b]	CEM[c]	Vero[d]	SK-MEL-28[e]	SK-MES-1[f]	LNCaP[g]	MCF-7[h]	9L[i]
β-D-CDU (**1**)	66.4	> 100	55.0	17.3	83.6	> 100	> 100	> 100	83.3
β-L-CDU (**2**)	> 100	> 100	68.5	65.9	64.8	> 100	44.4	51.8	81.2
β-D-ara-CU (**3**)	44.7	39.5	13.8	47.2	85.4	> 100	52.4	> 100	> 100
β-L-ara-CU (**4**)	> 100	> 100	81.1	103.0	112	> 100	> 100	> 100	> 100
β-D-ribo-CU (**5**)	49.6	81.9	31.6	90.1	> 100	> 100	> 100	> 100	81.4
β-L-ribo-CU (**6**)	77.9	71.6	68.1	51.0	> 100	> 100	> 100	> 100	> 100
β-D-dioxolane-CU (**7**)	4.6	9.6	58.7	44.0	101.0	> 100	> 100	ND[j]	97.0
Cycloheximide	—	2.8	0.14	2.1	1.3	2.6	2.4	1.5	0.2
5-Fluorouracil	—	0.5	90.5	6.8	6.1	13.1	30.0	43.7	0.8
AZT	0.002	> 100	14.0	29.0	> 100	> 100	> 100	> 100	> 100

[a] EC_{50} and IC_{50} are the median effective (antiviral) and inhibitory (cytotoxic) concentrations, respectively.
[b] PBM: human peripheral blood mononuclear cells, cell count endpoint on day 5; MTT dye uptake endpoint on day 5.
[c] CEM: human T-cell lymphoma, cell count endpoint on day 5; MTT dye uptake endpoint on day 4.
[d] Vero: African green monkey kidney, cell count endpoint on day 3; MTT dye uptake endpoint on day 3.
[e] SK-MEL-28: human lung squamous carcinoma, MTT dye uptake endpoint on day 4.
[f] SK-MES-1: human lung squamous carcinoma, MTT dye uptake endpoint on day 5.
[g] LNCaP: human prostate adenocarcinoma, MTT dye uptake endpoint on day 5.
[h] MCF-7: human breast carcinoma, MTT dye uptake endpoint on day 4.
[i] 9L: rat glioma, MTT dye uptake endpoint on day 5.
[j] ND: not determined.

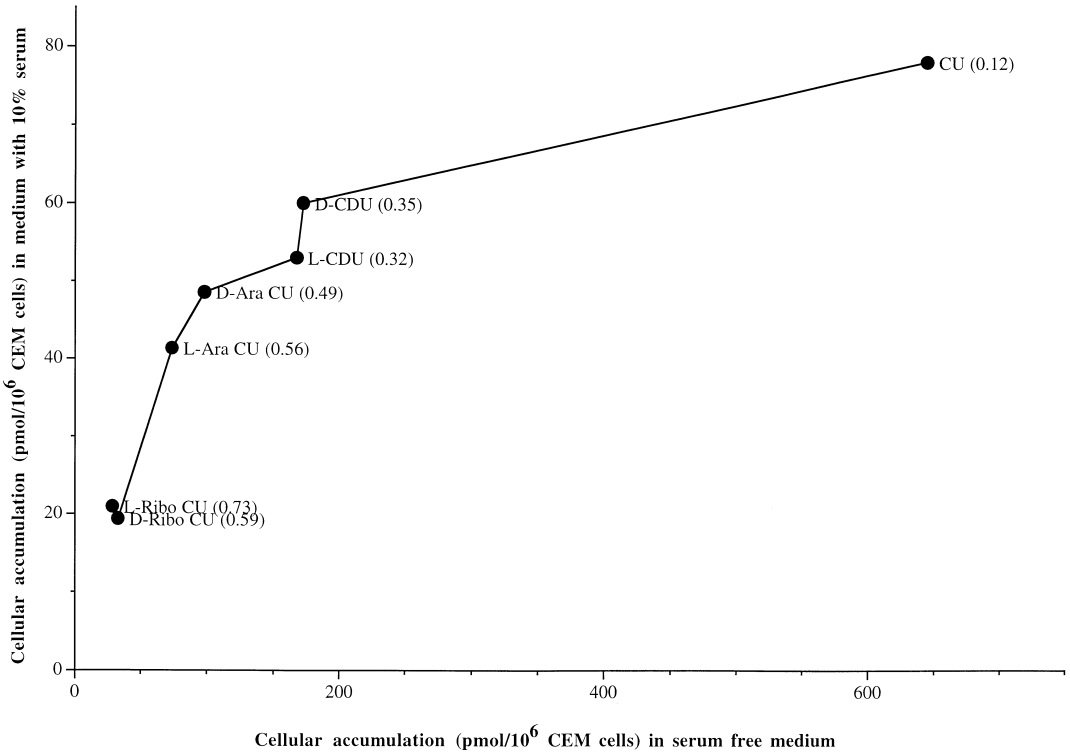


Figure 2. Accumulation of 5-*o*-carboranyl pyrimidines in CEM cells for 2.5 h. Numbers in parentheses represent the ratio of accumulation in 10% serum to accumulation without serum.

Experimental

Chemistry

Melting points (mp) were measured on an Electrothermal melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity Plus 400 spectrometer at 400 and 100.6 MHz, respectively. Tetramethylsilane was used as an internal standard and *J* values are given in Hz. Microanalyses were performed by Atlantic Microlab, Norcross, Georgia. Thin layer chromatography was performed on Whatman silica gel HP-KF glass-backed plates. Merck silica gel (grade 10184) was used for flash column chromatography. Anhydrous solvents were purchased from the Aldrich Chemical Company, Milwaukee, WI, and used without further purification or drying. Triethylamine and propionitrile were dried by heating at 70–80°C with calcium hydride, then distilled at atmospheric pressure. HPLC analysis were performed on a Hewlett–Packard 1050 instrument. Decaborane (purity > 99%) was purchased from Boron Biologicals Inc. (Raleigh, NC). *o*-Carboranyluracil (CU, **8**) was obtained as described previously.[13]

General procedure A: synthesis of iodinated nucleosides. The unprotected nucleoside (1.0 equiv) and iodine (1.8 equiv) were suspended in a mixture of $CHCl_3$ and 1 M aqueous HNO_3 (1:4/v:v). The resulting biphasic reaction mixture was refluxed for 2 h, then refrigerated overnight. The iodinated derivative was collected as a crystalline solid by filtration and repeated washes with Et_2O in order to remove the excess iodine.

General procedure B: reaction with trimethylsilyacetylene. To a solution of the corresponding iodinated derivative (1.0 equiv), bis(triphenylphosphine)palladium(II) chloride (0.1 equiv) and copper iodide (0.1 equiv) in THF were added triethylamine (2.0 equiv) and (trimethylsilyl)acetylene (1.4 equiv). The reaction mixture was heated at 40°C for 4 h. The solvent was removed under reduced pressure and the residue purified by flash chromatography on silica gel using a gradient of hexane–ethyl acetate as eluent.

General procedure C: deprotection of silylated hydroxy functions. The protected nucleoside (1.0 equiv) was dissolved in THF and tetrabutylammonium fluoride, 1 M solution in THF (2.0 equiv), was added. The resulting solution was stirred at room temperature for 1–2 h and then concentrated under reduced pressure. A solution of the residue in CH_2Cl_2 (50 mL) was washed with H_2O (2×50 mL) and saturated brine (50 mL), dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by flash chromatography on silica gel. Combination and evaporation of the fractions eluted with hexane–ethyl acetate gradient afforded the corresponding unprotected derivative.

General procedure D: carboranylation reactions. A solution of the corresponding unprotected ethynyl derivative (1.0 equiv), decaborane (2.0 equiv) and propionitrile (20.0 equiv) in toluene (20 mL) was refluxed for 4–6 h. After cooling to room temperature, the solvents were removed under reduced pressure. The residue was purified by flash chromatography on silica gel, and combination of the fractions eluted with hexane–ethyl acetate

gradient afforded the carboranyl derivative as colorless solid.

General procedure E: specific deprotections of acetyl or benzoyl groups under mild conditions. Protected carboranyl analogues (1.0 equiv) and 0.1 M sodium methoxide (3.3 equiv) in MeOH were stirred at 5°C for 18 h. The ion exchange resin DOWEX 50WX8-100 H^+ form was added until the pH was 7. The solution was filtered and the resin washed with MeOH. The residue was purified by flash chromatography on silica gel. Concentration and evaporation of the fractions eluted with CH_2Cl_2–MeOH gradient afforded the desired compound.

1-[β-L-(2-Deoxyribofuranosyl)]-5-*o*-carboranyluracil (2). Use of 3′,5′-di-*O*-benzoyl-5-ethynyl-2′-deoxy-L-uridine (**20**) afforded **2** by the application of general procedures D and E. Mp 223–224°C; ^{1}H NMR (DMSO-d_6) δ 1.6–2.8 (br, 10H, $B_{10}\underline{H}_{10}$), 2.09 (m, 1H, H-2′), 2.2 (m, 1H, H-2′), 3.60 (m, 2H, H-5′), 3.87 (m, 1H, H-4′), 4.25 (m, 1H, H-3′), 5.15 (br, 1H, OH, ex.), 5.29 (br, 1H, OH, ex.), 6.10 (s, 1H, $B_{10}H_{10}$-C$\underline{H}$), 6.15 (m, 1H, H-1′), 8.33 (s, 1H, H-1′), 11.83 (s, 1H, N$\underline{H}$, ex.); ^{13}C NMR (DMSO-d_6) δ 59.29, 60.99, 70.34, 71.45, 85.45, 87.94, 105.06, 142.75, 148.76, 160.66. Anal. calcd for $C_{11}H_{22}B_{10}N_2O_5$: C, 35.67; H, 5.99; N, 7.56. Found: C, 35.53; H, 5.90; N, 7.47.

1-(β-D-Arabinofuranosyl)-5-*o*-carboranyluracil (3). Use of 2,2′-anhydro-1-(β-D-arabinofuranosyl)uracil[20] afforded **3** by the application of general procedures A, B, C, D and E. Mp 241°C; ^{1}H NMR (DMSO-d_6) δ 1.2–3.0 (br, 10H, $B_{10}\underline{H}_{10}$), 3.60 (m, 2H, H-5′), 3.80 (m, 1H, H-4′), 3.98 (m, 2H, H-3′, H-2′), 5.15 (br, 1H, OH, ex.), 5.58 (br, 1H, OH, ex.), 5.73 (br, 1H, OH, ex.), 6.03 (m, 2H, H-1′, $B_{10}H_{10}C\underline{H}$), 7.94 (s, 1H, H-6), 11.87 (s, 1H, NH, ex.). Anal. calcd for $C_{11}H_{22}B_{10}N_2O_6$: C, 34.19; H, 5.74; N, 7.25. Found: C, 34.17; H, 5.77; N, 7.28.

1-(β-L-Arabinofuranosyl)-5-*o*-carboranyluracil (4). Use of compound **21** afforded **4** by the application of general procedures D and E. Mp 260–261°C; ^{1}H NMR (DMSO-d_6) δ 1.6–2.8 (br, 10H, $B_{10}\underline{H}_{10}$), 3.55–3.67 (m, 2H, H-5′), 3.80 (m, 1H, H-4′), 3.99 (m, 2H, H-3′, H-2′), 5.15 (br, 1H, OH, ex.), 5.55 (br, 1H, OH, ex.), 5.73 (m, 1H, OH, ex.), 6.00 (s, 1H, $B_{10}H_{10}C\underline{H}$), 6.04 (d, 1H, J = 4.4 Hz, H-1′), 7.95 (s, 1H, H-6), 11.87 (s, 1H, NH, ex.); ^{13}C NMR (DMSO-d_6) δ 54.79, 59.11, 60.44, 71.55, 74.85, 75.37, 85.21, 85.57, 103.36, 144.51, 148.82, 160.66. Anal. calcd for $C_{11}H_{22}B_{10}N_2O_6$: C, 34.19; H, 5.74; N, 7.25. Found: C, 34.22; H, 5.66; N, 7.18.

1-(β-L-Ribofuranosyl)-5-*o*-carboranyluracil (6). Use of compound **22** afforded **6** by the application of general procedures D and E. Mp 250–251°C; ^{1}H NMR (DMSO-d_6) δ 1.6–2.8 (br, 10H, $B_{10}\underline{H}_{10}$), 3.63 (m, 2H, H-5′), 3.93 (m, 1H, H-4′), 3.97 (m, 1H, H-3′), 4.05 (m, 1H, H-2′), 5.14 (br, 1H, OH, ex.), 5.26 (br, 1H, OH, ex.), 5.45 (br, 1H, OH, ex.), 5.78 (m, 1H, H-1′), 6.03 (s, 1H, $B_{10}H_{10}C\underline{H}$), 8.39 (s, 1H, H-6), 11.86 (s, 1H, NH, ex.); ^{13}C NMR (DMSO-d_6) δ 59.29, 60.32, 69.79, 71.43, 74.46, 85.02, 88.61, 105.36, 142.87, 149.00, 160.59. Anal. calcd for $C_{11}H_{22}B_{10}N_2O_6$: C, 34.19; H, 5.74; N, 7.25. Found: C, 34.23; H, 5.77; N, 7.20.

1-(β-D-1,3-Dioxolane-4-yl)-5-*o*-carboranyluracil (7). A mixture of **10** (18.0 mg, 0.03 mmol) in THF (3 mL) and 1 M tetra-*n*-butylammonium fluoride solution in THF (30 μL, 0.06 mmol) was stirred at room temperature for 2 h. After the mixture was concentrated, the residue was purified by PLC ($CHCl_3$:MeOH, 95:5) to yield **7** (6.1 mg, 56%) as a white solid. Mp 219–220°C; ^{1}H NMR (DMSO-d_6) δ 1.20–3.00 (br, 10H, $B_{10}\underline{H}_{10}$), 3.65 (br, 2H, H-5′), 4.08 (m, 1H, H-2′), 4.27 (m, 1H, H-2′), 4.95 (m, 1H, H-4′), 5.25 (br, 1H, OH, ex.), 6.14 (br, 1H, $B_{10}H_{10}C\underline{H}$), 6.16 (m, 1H, H-1′), 8.09 (s, 1H, H-6). Anal. calcd for $C_{10}H_{20}B_{10}N_2O_5$: C, 33.70; H, 5.65; N, 7.86. Found: C, 33.87; H, 5.98; N, 7.54.

1-[2-[(*tert*-Butyldiphenylsilyloxy)methyl]-β-D-1,3-dioxolan-4-yl]-5-*o*-carboranyluracil (10). A mixture of 5-*o*-carboranyluracil (**8**) (0.1 g, 0.39 mmol) in hexamethyldisilazane (15 mL) and catalytic amount of ammonium sulfate was refluxed for 3 h. The resulting clear solution was concentrated in vacuo under anhydrous conditions to yield silylated-5-*o*-carboranyluracil as colorless oil. To a solution of silylated base in dry dichloromethane (5 mL) were added a solution of **9**[14] (0.157 g, 0.39 mmol) in dry dichloromethane and $SnCl_4$ (0.47 mL, 0.47 mmol) at 5°C, and the reaction mixture was stirred at room temperature for 2 h under argon atmosphere. The reaction mixture was quenched by saturated aqueous $NaHCO_3$ solution (10 mL) and stirred for an additional 30 min at room temperature. The organic layer was separated and the aqueous layer was extracted with methylene chloride (3×30 mL). The combined organic layer was washed with saturated aqueous $NaHCO_3$ solution and water and dried (anhydrous $MgSO_4$). After filtration, the filtrate was concentrated and the residue was purified by silica gel column chromatography ($CHCl_3$:MeOH, 100:1) to yield **10** (19.3 mg, 10%) as white foam. ^{1}H NMR ($CDCl_3$) δ 1.06 (s, 9H, *t*Bu), 1.60–2.80 (br, 10H, $B_{10}\underline{H}_{10}$), 3.89 (m, 2H, H-5′), 4.16–4.24 (m, 2H, H-2′), 5.17 (t, 1H, J = 4.6 Hz, H-4′), 5.64 (br, 1H, $B_{10}H_{10}C\underline{H}$), 6.20 (d, 1H, J = 4.8 Hz, H-1′), 7.37–7.74 (m, 11H, aromatic, H-6), 8.92 (s, 1H, NH, ex.).

2-Amino-β-L-arabinofurano[1′,2′:4,5]oxazoline (11). The title compound was synthesized according to the procedure of Holý and isolated in similar yield.[18] Mp 180°C; ^{1}H NMR (DMSO-d_6 + D_2O) δ 3.25 (m, 2H, H-5′), 3.63 (m, 1H, H-4′), 4.01 (m, 1H, H-3′), 4.54 (m, 1H, H-2′), 5.65 (d, 1H, J = 6.0 Hz, H-1′); ^{13}C NMR (DMSO-d_6) δ 61.47, 75.55, 84.48, 87.99, 99.77, 162.11.

2,2′-Anhydro-L-uridine (12). The title compound was synthesized according to the procedure of Holý and isolated in similar yield.[18] Mp 239°C; ^{1}H NMR (DMSO-d_6) δ 3.18–3.26 (m, 2H, H-5′), 4.07 (m, 1H, H-4′), 4.38 (m, 1H, H-3′), 4.98 (t, 1H, J = 5.2 Hz, 5′OH, ex.), 5.20 (m, 1H, H-2′), 5.85 (d, 1H, J = 7.6 Hz, H-5), 5.88 (d, 1H, J = 4.4 Hz, 3′OH, ex.), 6.30 (d, 1H, J = 6.0 Hz, H-1′), 7.84 (d, 1H, J = 7.6 Hz, H-6).

3′,5′-Di-*O*-benzoyl-2,2′-anhydro-L-uridine (13). The title compound was synthesized according to the procedure of Holý and isolated in similar yield.[18] ^{1}H NMR (DMSO-d_6) δ 4.37 (m, 2H, H-5′), 4.85 (m, 1H, H-4′),

5.72 (m, 1H, H-3′), 5.78 (m, 1H, H-2′), 5.90 (d, 1H, J = 7.6 Hz, H-5), 6.50 (d, 1H, J = 6.0 Hz, H-1′), 7.85–8.07 (m, 11H, aromatic, H-6); ^{13}C NMR (DMSO-d_6) δ 62.99, 77.13, 82.11, 86.06, 89.76, 108.88, 128.61, 128.91, 133.34, 133.77, 136.38, 159.20, 164.78, 165.09, 170.43.

3′,5′-Di-*O*-benzoyl-2′-chloro-2′-deoxy-L-uridine (14). The title compound was synthesized according to the procedure of Holý and isolated in similar yield.[18] Mp 166–167°C; ^{1}H NMR (DMSO-d_6) δ 4.60–4.68 (m, 3H, H-5′, H-4′), 5.27 (m, 1H, H-3′), 5.69 (d, 1H, J = 8.0 Hz, H-5), 5.74 (m, 1H, H-2′), 6.17 (d, 1H, J = 8.0 Hz, H-1′), 7.52-8.07 (m, 11H, aromatic, H-6), 11.56 (s, 1H, NH, ex.).

1-(β-L-Arabinofuranosyl-3,5-di-*O*-benzoyl)uracil (15). The title compound was synthesized according to the procedure of Holý and isolated in similar yield.[18] ^{1}H NMR (DMSO-d_6) δ 4.45 (m, 1H, H-4′), 4.52 (m, 1H, H-3′), 4.68 (m, 2H, H-5′), 5.39 (m, 1H, H-2′), 5.58 (d, 1H, J = 8.4 Hz, H-5), 6.20 (d, 1H, J = 4.4 Hz, H-1′), 6.28 (br, 1H, OH, ex.), 7.53–8.06 (m, 11H, aromatic, H-6), 11.20 (br, 1H, NH, ex.).

1-(β-L-Arabinofuranosyl-2-*O*-methanesulfonyl-3,5-di-*O*-benzoyl)uracil (16). To a cooled (0°C; ice/water bath) solution of 1-(β-L-arabinofuranosyl-3,5-di-*O*-benzoyl)-uracil (**15**) (3.04 g, 6.72 mmol) in pyridine (35 mL) methanesulfonyl chloride (0.78 mL, 10.08 mmol) was added dropwise. The solution was stirred at 5°C for 18 h and a further 0.3 mL (3.36 mmol) of methanesulfonyl chloride added. Stirring was continued for 3 h at room temperature, water (5 mL) was added and the solvent removed under reduced pressure. The residue was dissolved in $CHCl_3$ (200 mL), washed with H_2O (200 mL), 10% HCl in water (200 mL) and saturated aqueous $NaHCO_3$ (200 mL), dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was triturated with EtOH (100 mL) and kept at room temperature overnight. The title compound, a colorless solid, homogeneous by TLC, was collected by filtration (3.27 g, 6.16 mmol, 98%). ^{1}H NMR (DMSO-d_6) δ 3.28 (s, 3H, SO_2CH_3), 4.60 (m, 1H, H-4′), 4.68 (m, 2H, H-5′), 5.62 (m, 3H, H-5, H-3′), 5.77 (m, 1H, H-2′), 6.36 (d, 1H, J = 5.2 Hz, H-1′), 7.50–8.08 (m, 11H, aromatic, H-6), 11.49 (s, 1H, NH, ex.).

3′,5′-Di-*O*-benzoyl-2′-deoxy-L-uridine (17). 3′,5′-Di-*O*-benzoyl-2′-chloro-2′-deoxy-L-uridine (**14**) (6.09 g, 12.9 mmol), tri-*n*-butyltin hydride (14.28 mL, 53.3 mmol) and AIBN (0.05 g, 0.3 mmol) were refluxed in benzene (130 mL) for 1.5 h. The solution was allowed to cool to room temperature and the title compound, a colorless solid, was collected by filtration (5.36 g, 12.28 mmol, 95%). The compound was recrystallized from boiling EtOH. Mp 218–219°C; ^{1}H NMR (DMSO-d_6) δ 2.62 (m, 2H, H-2′), 4.52 (m, 1H, H-4′), 4.61 (m, 2H, H-5′), 5.62 (m, 2H, H-5, H-3′), 6.29 (m, 1H, H-1′), 7.51–8.04 (m, 11H, aromatic, H-6), 11.42 (s, 1H, NH, ex.).

1-β-L-Arabinofuranosyluracil (18). Use of 1-(β-L-arabinofuranosyl-3,5-di-*O*-benzoyl)uracil (**15**) (2.2 g, 4.86 mmol) by the application of general procedure E afforded **18**. Mp 234–235°C; ^{1}H NMR (DMSO-d_6) δ 3.59 (m, 2H, H-5′), 3.73 (m, 1H, H-4′), 3.89 (m, 1H, H-3′), 3.95 (m, 1H, H-2′), 5.04 (t, 1H, J = 5.2 Hz, 5′OH, ex.), 5.46 (d, 1H, J = 4.8 Hz, OH, ex.), 5.59 (m, 2H, H-5, OH, ex.), 5.99 (d, 1H, J = 4.4 Hz, H-1′), 7.63 (d, 1H, J = 8.4 Hz, H-6), 11.23 (s, 1H, NH, ex.).

1-(β-L-Ribofuranosyl-2,3,5-tri-*O*-benzoyl)uracil (19). 1-(β-L-Arabinofuranosyl-2-*O*-methanesulfonyl-3,5-di-*O*-benzoyl)uracil (**16**) (1 g, 1.88 mmol) and sodium benzoate (2.71 g, 18.8 mmol) were heated at 120°C in HMPA (20 mL) for 7 h. The products were allowed to cool to room temperature and ethyl acetate (200 mL) was added. The solution was washed with saturated brine (3×100 mL) and H_2O (2×100 mL), dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by flash chromatography on silica gel, combination and evaporation of the fractions eluted with CH_2Cl_2:MeOH (9:1 v/v) afforded the title compound as a colorless solid (0.65 g, 1.17 mmol, 62%). ^{1}H NMR (DMSO-d_6) δ 4.70–4.77 (m, 3H, H-5′, H-4′), 5.69 (d, 1H, J = 7.6 Hz, H-5), 5.93 (m, 2H, H-3′, H-2′), 6.16 (d, 1H, J = 3.6 Hz, H-1′), 7.38–8.02 (m, 11 H, aromatic, H-6), 11.54 (s, 1H, NH, ex.).

3′,5′-Di-*O*-benzoyl-5-ethynyl-2′-deoxy-L-uridine (20). Use of compound **17** afforded **20** by the application of general procedures A, B and C. ^{1}H NMR ($CDCl_3$) δ 2.32 (m, 1H, H-2′), 2.80 (m, 1H, H-2′), 3.02 (s, 1H, ethynyl), 4.59 (m, 1H, H-4′), 4.71 (m, 1H, H-5′), 4.80 (m, 1H, H-5′), 5.63 (m, 1H, H-3′), 6.38 (m, 1H, H-1′), 7.44–8.07 (m, 11H, aromatic, H-6).

1-(β-L-Arabinofuranosyl-2,3,5-tri-*O*-acetyl)-5-ethynyluracil (21). Use of compound **18** afforded **21** as a colorless glass by the application of general procedures A, B and C. ^{1}H NMR (DMSO-d_6) δ 1.97 (s, 3H, acetyl), 2.09 (s, 6H, acetyl), 4.18 (s, 1H, ethynyl), 4.26 (m, 1H, H-4′), 4.35 (m, 2H, H-5′), 5.27 (m, 1H, H-3′), 5.40 (m, 1H, H-2′), 6.23 (d, 1H, J = 4.8 Hz, H-1′), 7.94 (s, 1H, H-6), 11.81 (s, 1H, NH, ex.).

1-(β-L-Ribofuranosyl-2,3,5-tri-*O*-acetyl)-5-ethynyluracil (22). Use of unprotected compound **19** afforded **22** by the application of general procedures A, B and C. ^{1}H NMR (DMSO-d_6) δ 2.06 (s, 6H, acetyl), 4.21 (s, 1H, ethynyl), 4.20–4.36 (m, 3H, H-5′, H-4′), 5.34 (m, 1H, H-3′), 5.47 (m, 1H, H-2′), 5.89 (d, 1H, J = 4.8 Hz, H-1′), 8.15 (s, 1H, H-6), 11.82 (s,1H, NH, ex.).

Antiviral and cytotoxicity assays

Anti-HIV-1 activity of the compounds was determined in human peripheral blood mononuclear (PBM) cells as described previously.[22] Briefly, the antiviral EC_{50} and cytotoxicities were obtained from the concentration–response curve using the median effective method.[23,24] The anti-cancer activity of the compounds was determined in CEM (human lymphoblastoid), LNCaP (human metastatic prostate adenocarcinoma), SK-MES-1 (human lung carcinoma), SK-MEL-28 (human skin melanoma), MCF-7 (human breast carcinoma) and 9L (rat glioma) cells. Appropriate numbers of cells were cultured with the drug for a specific number of days in

96-well plates (PBM, LnCaP and SK-MES-1: 5 days; CEM, SK-MEL-28, MCF-7 and 9L: 4 days; Vero: 3 days). Positive cytotoxic controls included 5-fluorouracil, an inhibitor of thymidylate synthetase, cycloheximide, an inhibitor of rRNA transfer, and the antiretroviral nucleoside AZT. Untreated cells exposed to solvent were included as negative controls. After incubation, actively metabolizing cells were quantified using the Cell Titer 96 Cell Proliferation Assay (MTT, Promega, Madison, WI), as described by the manufacturer.

Accumulation in CEM cells

All radiolabeled nucleosides were custom synthesized by Moravek Biochemicals Inc. (Brea, CA), and were labeled on the acidic proton of the carboranyl group.[7] The specific activities of CU, β-D-ara-CU, β-D-ribo-CU, β-D-CDU, β-L-ara-CU, β-L-ribo-CU and β-L-CDU were 59.2, 6.7, 1.2, 1.8, 1.0, 2.3 and 23.1 Ci/mmol, respectively. CEM cells (10^6/mL) were incubated at 37°C in the presence of ^{3}H-radiolabeled compound (1 μM) in RPMI 1640 medium containing 0 and 10% FBS for 2.5 h. The accumulation of carboranyl derivatives was then measured by centrifuging quadruplicate samples of the cell suspensions (200 μL) through silicone oil, lysing the cells using formic acid and scintillation counting.[25] Counts per minute (cpm) were converted to pmol/10^6 cells using linear regressions of pmol compound versus cpm.

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References

1. Hawthorne, F. M. *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 950.
2. Soloway, A. H.; Tjarks, W.; Barnum, B. A.; Rong, F.-G.; Barth, R. F.; Codogni, I. M.; Wilson, J. G. *Chem. Rev.* **1998**, *98*, 1515.
3. Barth, R. F.; Soloway, A. H.; Fairchild, R. G.; Brugger, R. M. *Cancer* **1992**, *70*, 2995.
4. Fairchild, R. G.; Bond, V. P. *Int. J. Radiat. Oncol. Biol. Phys.* **1985**, *21*, 831.
5. Zamenhof, R. G.; Kalend, A. M.; Bloomer, W. D. *J. Natl. Cancer Inst.* **1992**, *84*, 1290.
6. Yamamoto, Y.; Seko, T.; Nakamura, H.; Nemoto, H.; Hojo, H.; Mukaoi, N.; Hashimoto, Y. *J. Chem. Soc., Chem. Commun.* **1992**, *157*.
7. Schinazi, R. F.; Goudgaon, N. M.; Fulcrand, G.; El Kattan, Y.; Lesnikowski, Z.; Ullas, G.; Moravek, J.; Liotta, D. C. *Int. J. Radiat. Oncol. Biol. Phys.* **1994**, *28*, 1113.
8. Fulcrand-El Kattan, G.; Goudgaon, N. M.; Ilksoy, N.; Huang, J. T.; Watanabe, K. A.; Sommadossi, J.-P.; Schinazi, R. F. *J. Med. Chem.* **1994**, *37*, 2583.
9. Shewach, D. S.; Liotta, D. C.; Schinazi, R. F. *Biochem. Pharmacol.* **1993**, *45*, 1540.
10. Schinazi, R. F.; Chu, C. K.; Peck, A.; McMillan, A.; Mathis, R.; Cannon, D.; Jeong, L.-S.; Beach, J. W.; Choi, W.-B.; Yeola, S.; Liotta, D. C. *Antimicrob. Agents Chemother.* **1992**, *36*, 672.
11. Schinazi, R. F.; McMillan, A.; Cannon, D.; Mathis, R.; Lloyd, R. M.; Peck, A.; Sommadossi, J.-P.; St Clair, M.; Wilson, J.; Furman, P. A.; Painter, G.; Choi, W.-B.; Liotta, D. C. *Antimicrob. Agents Chemother.* **1992**, *36*, 2423.
12. Sommadossi, J.-P.; Schinazi, R. F.; Chu, C. K.; Xie, M.-Y. *Biochem. Pharmacol.* **1992**, *44*, 1921.
13. Goudgaon, N. M.; El Kattan, Y.; Xia, X.; McAtee, J.; Soria, J.; Wey, S.-J.; Liotta, D. C.; Schinazi, R. F. *Nucleosides and Nucleotides* **1997**, *16*, 2133.
14. Kim, H. O.; Ahn, A. J.; Alves, S. K.; Beach, J. W.; Jeong, L. S.; Choi, B. G.; Van Roey, P.; Schinazi, R. F.; Chu, C. K. *J. Med. Chem.* **1992**, *35*, 1987.
15. Vorbrüggen, H.; Hofle, G. *Chem. Ber.* **1981**, *114*, 1256.
16. Graciet, J.-C.; Shi, J.; Schinazi, R. F. *Nucleosides and Nucleotides* **1998**, *17*, 711.
17. Shi, J.; McAtee, J. J.; Wirtz, S. S.; Tharnish, P. M.; Juodawlkis, A.; Liotta, D. C.; Schinazi, R. F. *J. Med. Chem.* **1999**, *42*, 859.
18. Holý, A. *Tet. Lett.* **1971**, *2*, 189.
19. Hampton, A.; Nichol, A. W. *Biochemistry* **1966**, *5*, 2076.
20. Schinazi, R. F.; Chen, M. S.; Prusoff, W. H. *J. Med. Chem.* **1979**, *22*, 1273.
21. Fulcrand-El Kattan, G.; Lesnikowski, Z. J.; Yao, S.; Tanious, F.; Wilson, W. D.; Schinazi, R. F. *J. Am. Chem. Soc.* **1994**, *116*, 7494.
22. Schinazi, R. F.; Sommadossi, J.-P.; Saalman, V.; Cannon, D. L.; Xie, M.-Y.; Hart, G. C.; Smith, G. A.; Hahn, E. F. *Antimicrob. Agents Chemother.* **1990**, *34*, 1061.
23. Chou, T. C.; Talalay, P. *Adv. Enzyme Regul.* **1984**, *22*, 27.
24. Belen'kii, M. S.; Schinazi, R. F. *Antiviral Res.* **1994**, *25*,1.
25. Hurwitz, S. J.; Terashima, M.; Mizunuma, N.; Slapak, C. A. *Blood* **1997**, *89*, 3745.