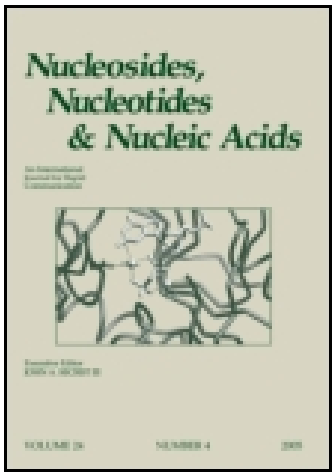

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SYNTHESIS OF 1-(2-DEOXY-β-D-RIBOFURANOSYL)-2,4-DIFLUORO-5-SUBSTITUTED-BENZENES*: "THYMINE REPLACEMENT" ANALOGS OF THYMIDINE FOR EVALUATION AS ANTICANCER AND ANTIVIRAL AGENTS

Zhi-Xian Wang [a], Weili Duan [a], Leonard I. Wiebe [a], Jan Balzarini [b], Erik De Clercq [b] & Edward E. Knaus [c]

[a] Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, T6G 2N8, Canada

[b] Rega Institute for Medical Research, Minderbroedersstraat 10, Leuven, B-3000, Belgium

[c] Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, T6G 2N8, Canada

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SYNTHESIS OF 1-(2-DEOXY-β-D-RIBOFURANOSYL)-2,4-DIFLUORO-5-SUBSTITUTED-BENZENES*: "THYMINE REPLACEMENT" ANALOGS OF THYMIDINE FOR EVALUATION AS ANTICANCER AND ANTIVIRAL AGENTS

Zhi-Xian Wang,[1] Weili Duan,[1] Leonard I. Wiebe,[1] Jan Balzarini,[2] Erik De Clercq,[2] and Edward E. Knaus,[1,†]

[1]Faculty of Pharmacy and Pharmaceutical Sciences,
University of Alberta, Edmonton, AB, Canada T6G 2N8
[2]Rega Institute for Medical Research, Minderbroedersstraat 10,
Leuven B-3000, Belgium

ABSTRACT

A group of unnatural 1-(2-deoxy-β-D-ribofuranosyl)-2,4-difluorobenzenes having a variety of C-5 two-carbon substituents [$-C\equiv C-X$, X = I, Br; $-C\equiv CH$; $(E)-CH=CH-X$, X = I, Br; $-CH=CH_2$; $-CH_2CH_3$; $-CH(N_3)CH_2Br$], designed as nucleoside mimics, were synthesized for evaluation as anticancer and antiviral agents. The 5-substituted $(E)-CH=CH-I$ and $-CH_2CH_3$ compounds exhibited negligible cytotoxicity in a MTT assay ($CC_{50} = 10^{-3}$ to $10^{-4}M$ range), relative to thymidine ($CC_{50} = 10^{-3}$ to $10^{-5}M$ range), against a variety of cancer cell lines. In contrast, the C-5 substituted $-C\equiv C-I$ and $-CH(N_3)CH_2Br$ compounds were more cytotoxic ($CC_{50} = 10^{-5}$ to $10^{-6}M$ range). The $-C\equiv C-I$ and $-CH_2CH_3$ compounds exhibited similar cytotoxicity against non-transfected (KBALB, 143B) and HSV-1 TK^+ gene transfected (KBALB-STK, 143B-LTK) cancer cell lines expressing the herpes simplex virus type 1 (HSV-1) thymidine kinase gene (TK^+). This observation indicates

*Nucleoside-like numbering is used by analogy to nucleoside nomenclature.

†Address correspondence to Edward E. Knaus. E-mail: eknaus@pharmacy.ualberta.ca

that expression of the viral TK enzyme did not provide a gene therapeutic effect. The parent group of 5-substituted compounds, that were evaluated using a wide variety of antiviral assay systems [HSV-1, HSV-2, varicella-zoster virus (VZV), vaccinia virus, vesicular stomatitis, cytomegalovirus (CMV), and human immunodeficiency (HIV-1, HIV-2) viruses], showed that this class of unnatural C-aryl nucleoside mimics are inactive and/or weakly active antiviral agents.

INTRODUCTION

The development of new methods for the synthesis of 2′-deoxyuridines possessing novel 2-carbon substituents at the C-5 position, which are potent and selective antivirals, represented an important area of antiviral drug design. Great progress in antiviral therapy was made with the discovery of (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU, Brivudin), (*E*)-5-(2-bromovinyl)arabinouridine (BVAU, Brovavir), and 5-(prop-1-ynyl)arabinouridine (PAU, Zonavir). Earlier antiherpetics, such as 5-iodo- and 5-trifluoromethyl-2′-deoxyuridine, exhibited little if any selectivity in their antiviral action. Conversely, BVDU, BVAU, and PAU are highly specific inhibitors of herpes simplex virus (HSV-1) and varicella-zoster virus (VZV) replication. Their selectivity is due to specific phosphorylation by virus-encoded thymidine kinase (TK) in virus-infected, but not in normal, host cells, (1–4). Of the many 5-substituted pyrimidine nucleosides investigated, (*E*)-5-[2-halo (iodo, IVDU; bromo, BVDU; chloro, CVDU]-vinyl (1,2) and 5-(2-chloroethyl)-2′-deoxyuridine (CEDU) (5) are among the most potent and selective in their action against HSV-1. CEDU is effective against systemic HSV-1 infection and HSV-1 encephalitis in mice at a 5–15-fold lower dose than BVDU (6). In contrast, the less potent 5-ethyl-2′-deoxyuridine (EDU) is approximately equiactive against HSV-1 and HSV-2 (1,2).

Modification of pyrimidine nucleoside bases can bestow new properties (oral bioavailability, metabolic stability, and pharmacokinetic) (7), which the natural bases lack, and, when incorporated into nucleic acids by enzymatic processes, can further alter the structure and/or function of these biopolymers. In this respect, 2′-deoxyuridine derivatives, which possess a C-5 substituent two-carbon atoms in length, generally exhibit antiviral, but not anticancer, activity. Structure–activity relationship correlations (SARCs) for 5-olefinic 2′-deoxyuridine analogs showed that optimum inhibition of HSV-1 in vitro occurred when the 5-substituent was unsaturated and conjugated with the uracil ring, was not longer than four carbon atoms, had the (*E*)-stereochemistry, and included a hydrophobic electronegative functionality such as (*E*)−CH=CH−I) (8). There has been considerable interest in 5-alkynyl pyrimidine nucleosides to treat VZV infections. Although 5-ethynyl-2′-deoxyuridine (5−C≡CH) was highly active against HSV-1, CMV, and VZV in vitro, it was also cytotoxic. In contrast, 5-(−C≡C−CH_3) derivatives of 2′-deoxyuridine and arabinouridine (Zonavir) exhibited highly selective anti-VZV activity and they were noncytotoxic. Thus, changing the C-5 substituent from −C≡CH to

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$-C\equiv C-CH_3$ greatly increased VZV selectivity and decreased cytotoxicity, effects attributed to an inability of the $-C\equiv C-CH_3$ analogs to act as substrates for cellular TK, or to decreased affinity of the monophosphate for thymidylate synthase (TS) (9). A number of *C*-nucleosides exhibit anticancer and antiviral activities, that is likely due to their structural resemblance to natural *N*-nucleosides (10–12). Accordingly, nonpolar hydrophobic isosteres of pyrimidine nucleosides that retain close structural, steric, and isoelectronic relationships to the natural base were recently designed by Schweitzer and Kool (13). Thus, the 2,4-difluoro-5-methylphenyl isostere **1** was designed as an unnatural mimic of thymidine **(2)**. The 5′-triphosphate of **1** (**1**-TP) was inserted into replicating DNA strands by the Klenow fragment (KF, exo$^-$ mutant) of *E. coli* DNA polymerase 1 (14). Steady-state measurements indicated that **1**-TP was inserted opposite adenine (A) with an efficacy (V_{max}/K_m) only 40-fold lower than the triphosphate of thymidine (dTTP). In addition, it was inserted opposite A, relative to C, G, or T, with a selectivity nearly as high as that for dTTP. Consequently, the 2,4-difluoro-5-methylphenyl moiety of **1** is a shape mimic utilized by KF polymerase similar to the natural thymine base that it replaces (15–17). It was, therefore, anticipated that nucleoside mimic derivatives of **1**, possessing a C-5 two-carbon substituent, may be cytotoxic to rapidly multiplying cancer cells (inhibit tumor growth) and/or act as antiviral agents due to their selective phosphorylation by virus-infected cells (18), and as radiopharmaceutical agents for imaging (19), or as chemotherapeutic agents for treating (20), herpes simplex virus type-1 thymidine kinase positive (HSV-1 TK$^+$) gene-transfected tumors (gene therapy of cancer) (21). We now report the synthesis, antiviral, and anticancer activities for a group of 1-(2-deoxy-β-D-ribofuranosyl)-2,4-difluoro-5-substituted-benzenes (**5**, **7**) designed as 5-substituted-2′-deoxyuridine (thymidine) mimics (Fig. 1).

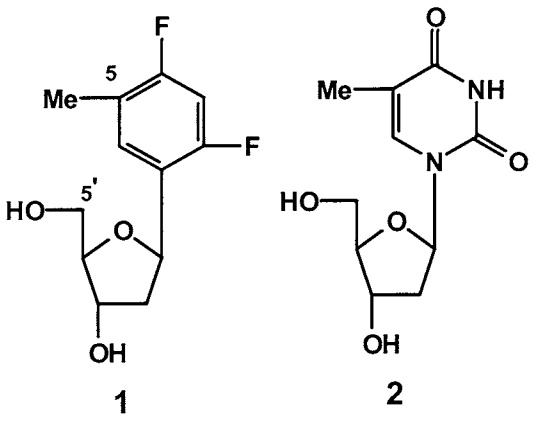

Figure 1. Structures of 1-(2-deoxy-β-D-ribofuranosyl)-2,4-difluoro-5-methylbenzene **(1)** and thymidine **(2)**.

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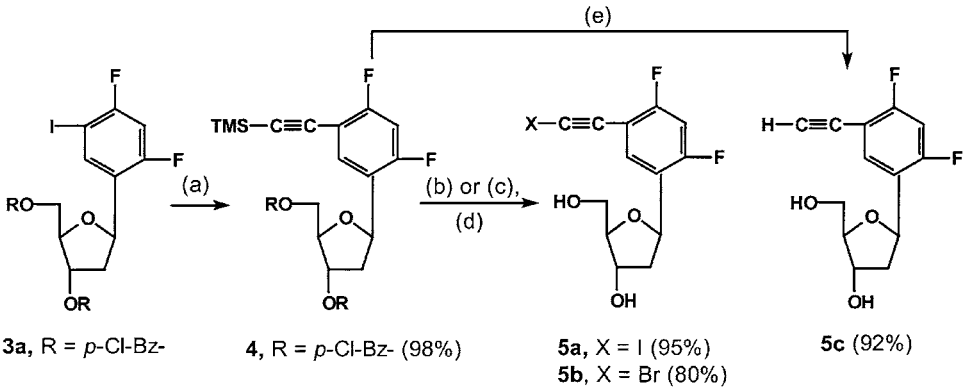
CHEMISTRY

Reaction of the 5-iodo-2,4-difluorophenyl compound **3a** with TMS−C≡C−H in the presence of $(Ph_3P)_2PdCl_2$ and CuI in Et_3N (22) afforded the corresponding 5-(trimethylsilyl)ethynyl derivative **4** (see Scheme 1). The 5-iodoethynyl **5a**, or 5-bromoethynyl **5b** derivatives were prepared by reaction of the 5-(trimethylsilyl) ethynyl compound **4** with either *N*-iodosuccinimide or *N*-bromosuccinimide, in the presence of $AgNO_3$ catalyst in acetone (23), and then removal of the *p*-chlorobenzoyl protecting groups in the sugar moiety, using NaOMe in MeOH. Treatment of the 5-(trimethylsilyl)ethynyl *p*-chlorobenzoyl protected compound **4** with NaOMe in MeOH simultaneously removed the trimethylsilyl and *p*-chlorobenzoyl groups, to afford the 5-ethynyl nucleoside mimic **5c**.

Synthesis of the (*E*)-5-(2-trimethylsilylvinyl) derivative **6b** was performed using a methodology similar to that reported previously (24) for the synthesis of (*E*)-5-(2-iodovinyl)-2′-fluoro-2′-deoxyuridine (IVFRU), as illustrated in Scheme 2. Accordingly, reaction of the protected 5-iodo compound **3a** with (*E*)-1-trimethylsilyl-2- tributylstannylethene (25) catalyzed by $(Ph_3P)_2PdCl_2$ in MeCN with subsequent removal of the *p*-chlorobenzoyl protecting groups, using NaOMe in MeOH afforded **6b**. The (*E*)-5-(2-trimethylsilylvinyl) group is stable under these reaction conditions. The proton NMR spectrum for **6b** showed a $J_{CH=CH} = 19.5$ Hz coupling constant that is indicative of the (*E*)-5-(−CH=CH−TMS) stereochemistry. Compound **6b** was also prepared directly from the unprotected 5-iodo compound **3b**, using the same procedure.

The (*E*)-5-(2-iodovinyl) compound **7a** was synthesized by the reaction of **6b** with ICl in MeCN at 25°C (24). Proton NMR spectral analysis of **7a** indicated that only the (*E*)-isomer was produced ($J_{CH=CH} = 15.0$ Hz). In contrast, reaction of the (*E*)-5-(2-trimethylsilylvinyl) compound **6b** with Br_2 or BrCl, under similar



Scheme 1. Reagents and conditions: (a) $(Ph_3P)_2PdCl_2$, H–C≡C–TMS, CuI, Et_3N, 25°C, 12 h; (b) *N*-iodosuccinimide, $AgNO_3$, acetone, 25°C, 2 h **(5a)**; (c) *N*-iodosuccinimide, $AgNO_3$, acetone, 25°C, 1.5 h **(5b)**; (d) NaOMe, MeOH, 25°C, 15 min **(5a)** or 20 min **(5b)**; and (e) NaOMe, MeOH, 25°C, 30 min.

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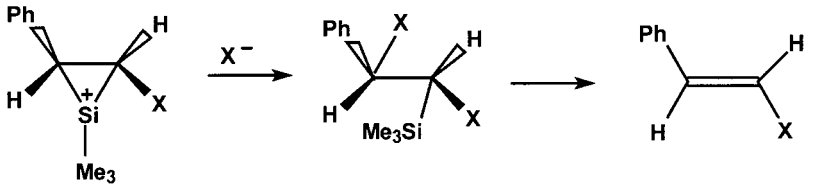
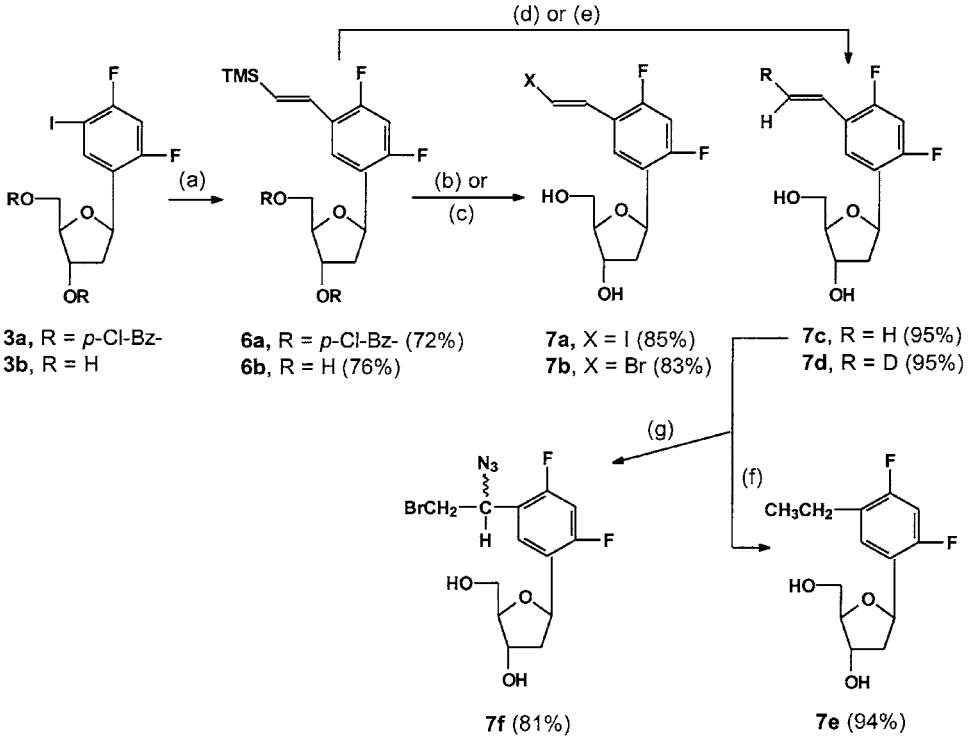


Figure 2. Mechanism for the iodination of silicon-stabilized cationic species.

reaction conditions gave a mixture of (*E*)- and (*Z*)-isomers in a ratio of about 2:1 with *J* values of 14.2 and 10.1 Hz, respectively. The production of both (*E*)- and (*Z*)-isomers is attributed to the fact that the *trans*-double bond generates a *trans*-dibromide adduct that converts to the (*Z*)-bromovinyl product via a *trans*-elimination reaction (26). Miller and McGarvey (27) reported that halogenation of *trans*-β-trimethylsilylstyrene in CH_2Cl_2 at low temperature proceeds via a silicon-stabilized cationic species, which upon attack by a halide anion would afford a dihalide adduct that is formally the product of *cis*-halogenation. *Trans*-elimination of this dihalide would then give a *trans*-β-halostyrene, an effective replacement of silicon by halide with retention of stereochemistry about the double bond (see Fig. 2) (27,28). In this respect, addition of Br_2 to **6b** at -78°C, followed by treatment of the reaction mixture with a cold saturated solution of KF in DMSO at 0°C, afforded the (*E*)-5-(2-bromovinyl) product **7b** ($J_{CH=CH} = 14.2$ Hz) as the sole product (see Scheme 2). Exclusive formation of the (*E*)$-$CH$=$CH$-$Br product **7b** is likely due to stabilization of the silicon-bridged cationic species at -78°C and the effect of the phenyl moiety (27). Desilylation of the (*E*)-5-(2-trimethylsilylvinyl) compound **6b** upon treatment with CF_3CO_2H (29,30) for 5–10 min gave the 5-vinyl product **7c**. A similar desilylation using CF_3CO_2D, in place of CF_3CO_2H, produced the (*E*)-5-(2-deuteriovinyl) product **7d** (70% D-labelled as determined by ^{1}H NMR). Hydrogenation of the 5-vinyl compound **7c**, using 10% Pd-on-C and H_2 gas at 15 psi in EtOH afforded the 5-ethyl derivative **7e**. Reaction of the 5-vinyl compound **7c** with BrN_3, generated in situ by the treatment of NaN_3 with Br_2 in CH_2Cl_2 in the presence of concentrated HCl (31), in $MeNO_2$ at 25°C afforded the 5-(1-azido-2-bromoethyl) product **7f**. Product **7f** exists as a mixture of two inseparable diastereomers due to the presence of an asymmetric center at the C-1 position of the 5-(1-azido-2-bromoethyl) moiety.

BIOLOGICAL RESULTS AND DISCUSSION

Nucleoside mimics, in which the natural thymine base in thymidine is replaced by an unnatural aryl isostere, could induce new properties that the natural nucleosides lack thereby providing a strategy to design a new class of *C*-aryl nucleoside mimics. *C*-aryl nucleoside mimics possess some potential advantages, relative to classical 5-substituted-2′-deoxyuridines, that include i) in vivo resistance to glycosyl bond cleavage by pyrimidine phosphorylases, ii) decreased host cell toxicity



Scheme 2. Reagents and conditions: (a) $(Ph_3P)_2PdCl_2$, (*E*)-TMS–CH=CHSnBu$_3$, MeCN, 60°C, 4 h **(3a → 6a, 3b → 6b)**, and then NaOMe, MeOH, 25°C, 30 min **(6a → 6b)**; (b) **6b**, ICl, MeCN, 25°C, 30 min **(7a)**; (c) **6b**, Br_2, CH_2Cl_2, −78°C, 2 h, and then KF/DMSO at 0°C for 1 h and at 25°C for 2 h **(7b)**; (d) **6b**, CF_3CO_2H, 25°C, 5 min **(7c)**; (e) **6a**, CF_3CO_2D, 25°C, 5 min **(7d)**; (f) 10% Pd-on-C, H_2 gas at 15 psi, EtOH, 60°C, 5 h; and (g) BrN_3, $MeNO_2$, CH_2Cl_2, 25°C, 10 min.

due to an inability to serve as substrates for thymidylate synthase (TS), and iii) increased lipophilicity that could enhance their ability to penetrate the blood-brain-barrier (BBB) to improve their efficacy for the treatment of brain viral infections and brain tumors. A group of 1-(2-deoxy-β-D-ribofuranosyl)-2,4-difluorobenzenes having a variety C-5 two-carbon substituents [−C≡C−X, X = I, Br; −C≡C−H; (*E*)−CH=CH−X, X = I, Br; −CH=CH$_2$; CH$_2$CH$_3$;−CH(N$_3$)CH$_2$Br] were investigated to probe the effect of size, electronic, hybridization [sp (−C≡C−), sp^2 (−CH=CH−), sp^3 (−CH−CH−)], and lipophilic parameters that are potential determinants of antiviral efficacy.

5-Substituted β-anomers **5a, 7a, 7e, 7f**, and the reference compounds 5-iodo-2′-deoxyuridine (IUDR), 5-fluoro-2′-deoxyuridine (FUDR), and thymidine, were evaluated using the MTT cytotoxicity assay (32) (see Tab. 1). The 5-substituted [(*E*)−CH=CH−I **7a**; −CH$_2$CH$_3$ **7e**] compounds exhibited negligible cytotoxicity ($CC_{50} = 10^{-3}$ to $10^{-4}M$ range), even when compared to thymidine ($CC_{50} = 10^{-3}$ to $10^{-5}M$ range), against KBALB, KBALB-STK, 143B, 143B-LTK, EMT-6, and R-970-5 cancer cell lines. In contrast, the C-5 substituted −C≡C−I **5a**

Table 1. In Vitro Cell Cytotoxicity of 1-(2-Deoxy-β-D-ribofuranosyl)-2,4-difluoro-5-substituted-benzenes Determined Using the 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium Bromide (MTT) Assay

	Cellular Toxicity (CC_{50}, M) Toward Various Cell Lines[a]					
Compounds	KBALB[b]	KBALB-STK[c]	143B[d]	143B-LTK[c]	EMT-6[e]	R-970-5[f]
5a	6.8×10^{-6}	4.0×10^{-5}	4.0×10^{-5}	6.0×10^{-6}	2.6×10^{-6}	3.0×10^{-5}
7a	1.7×10^{-4}	–	1.5×10^{-4}	–	1.7×10^{-4}	1.8×10^{-4}
7e	2.3×10^{-3}	2.6×10^{-3}	2.3×10^{-3}	8.9×10^{-4}	–	–
7f	1.3×10^{-6}	–	–	–	–	–
IUDR[g]	9.7×10^{-5}	1.0×10^{-5}	7.0×10^{-3}	7.4×10^{-3}	3.8×10^{-4}	6.2×10^{-4}
FudR[h]	6.0×10^{-11}	8.8×10^{-11}	9.0×10^{-5}	1.0×10^{-4}	9.0×10^{-12}	5.5×10^{-5}
Thymidine	9.5×10^{-5}	1.0×10^{-4}	–	–	1.3×10^{-4}	2.5×10^{-3}

[a]The molar concentration of the test compound that killed 50% of the cells (or 50% cell survival) upon incubation for 3–5 days at 37°C in a humidified atmosphere of 95% air and 5% CO_2 (mean value, $n = 6$).
[b]Transformed fibroblast sarcoma cell line.
[c]These cells were transfected by, and expressed, the herpes simplex virus type 1 thymidine kinase (HSV-1 TK) gene.
[d]Human osteosarcoma cell line.
[e]Mouse mammary carcinoma cell line.
[f]Human osteosarcoma cell line.
[g]IUdR = 5-iodo-2′-deoxyuridine.
[h]FUdR = 5-fluoro-2′-deoxyuridine.

and $-CH(N_3)CH_2Br$ **7f** compounds were more cytotoxic ($CC_{50} = 10^{-5}$ to $10^{-6} M$ range). The C-5 $-C{\equiv}C-I$ **5a** and $-CH_2CH_3$ **7e** compounds exhibited similar cytotoxicity against non-transfected (KBALB, 143B), and the corresponding transfected (KBALB-STK, 143B-LTK) cancer cell lines expressing the herpes simplex virus type 1 (HSV-1) thymidine kinase gene (TK^+).

The 1-(2-deoxy-β-D-ribofuranosyl)-2,4-difluoro-5-substituted-benzenes (**5a–c, 6b, 7a–c, 7f**) were evaluated for their antiviral activity in a wide variety of assay systems (33). Antiviral activities against herpes simplex virus type 1 (strains KOS, F, McIntyre), herpes simplex virus type 2 (strains G, 196, Lyons), thymidine kinase-deficient (TK^-) herpes simplex virus type 1 (strains KOS ACV^r, VMW-1837), vaccinia virus, and vesicular stomatitis virus in E_6SM cells were determined. This group of compounds were generally inactive, or exhibited negligible activity, as determined by reduction of virus-induced cytopathoicity, in these antiviral assay systems (at concentrations up to 400 μg/mL). In this regard, the $-C{\equiv}C-Br$ compound **5b** exhibited distinct ($IC_{50} = 9.6\,\mu$g/mL) and selective activity against HSV-1 (KOS, F, McIntyre), the $-C{\equiv}CH$ compound **5c** showed weak activity ($IC_{50} = 48\ \mu$g/mL) against HSV-1 (F), the $(E)-CH{=}CH-TMS$ compound **6b** exhibited weak activity ($IC_{50} = 48\ \mu$g/mL) against HSV-1 (McIntyre), HSV-2 (Lyons) and vaccinia virus, and the $(E)-CH{=}CH-I$ compound **7a** showed weak ($IC_{50} = 48\ \mu$g/mL), but selective activity against HSV-1 (KOS, F, McIntyre). In

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addition, the (*E*)−CH=CH−Br **7b** and −CH(N_3)CH_2Br **7f** compounds did not reduce cytopathogenicity induced by parainfluenza-3 virus, reovirus-1, Sindbis virus, Coxsackie B4 virus or Punta Toro virus in Vero cell cultures ($IC_{50} > 16$ μg/mL), or respiratory syncytial virus in HeLa cell cultures [$IC_{50} \geq 400$ μg/mL (**7b**) and >80 μg/mL (**7f**)].

Antiviral studies using VZV infected human embryonic lung cells [TK^+ VZV (YS and OKA strains), and TK deficient VZV/TK^- (07/1 and YS/R strains)] showed that the inhibitory concentration required to reduce virus plaque formation by 50% (IC_{50}) for a viral input of 20 plaque forming units (PFU) for the −C≡C−I **5a**, −C≡C−H **5c**, (*E*)−CH=CH−TMS **6b**, (*E*)−CH=CH−I **7a**, and −CH=CH_2 **7c** compounds was >20, >50, >20, >50, and >200 μM, respectively, whereas the −C≡C−Br compound **5b** showed IC_{50} values >50 μM except for TK^+ VZV (YS, $IC_{50} = 13$ μM; OKA, $IC_{50} = 29$ μM). The reference drug (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU) showed IC_{50} values of 0.008 and 0.009 μM against VZV TK^+ YS and OKA strains, and >60 and >150 μM against VZV TK^- 07/1 and YS/R strains, respectively. These results indicate that expression of the viral TK enzyme, which would be expected to enhance phosphorylation to the 5′-monophosphate and 5′-diphosphate (by the accompanying thymidylate kinase activity), did not increase the activity against VZV.

Antiviral activity against cytomegalovirus (CMV), strains AD-169, and Davis was determined using a cytopathicity (CPE) reduction assay (34) in confluent human embryonic lung (HEL) cells. All compounds tested were inactive at the highest concentration tested (−C≡C−I, $IC_{50} > 20$ μM; −C≡C−Br, $IC_{50} > 50$ μM; −C≡C−H, $IC_{50} > 200$ μM; (*E*)−CH=CH−I, $IC_{50} > 50$ μM; −CH=CH_2, $IC_{50} > 200$ μM) except for the (*E*)−CH=CH−TMS compound **6b**, which showed an IC_{50} value of 9 μM against AD-169 strain.

The (*E*)−CH=CH−Br **7b** and −CH(N_3)CH_2Br **7f** compounds were also evaluated for their activity against HIV-1 (strain III_B) and HIV-2 (strain ROD) in human T-lymphocytes (CEM cells). Neither **7b** nor **7f** inhibited replication of HIV-1 or HIV-2 ($IC_{50} > 50$ μM) at subtoxic concentrations ($CC_{50} \geq 78$ μM). Furthermore, **7b** ($IC_{50} > 100$ μM) and **7f** ($IC_{50} > 200$ μM) did not prevent Moloney murine sarcoma virus (MSV)-induced transformation of C3H/3T3 embryo murine fibroblasts in vitro.

The weak, or absence of, anticancer/antiviral efficacy for this novel class of 1-(2-deoxy-β-D-ribofuranosyl)-2,4-difluoro-5-substituted-benzenes could be due to a number of factors. For example, it is possible that the sugar moiety does not undergo phosphorylation by thymidine kinase (TK) to the 5′-monophosphate (5′-MP). Support for this explanation is based on the observations that there are generally negligible differences in anticancer/antiviral activities between non-transfected (KBALB, 143B) and viral TK-transfected (KBALB-STK, 143B-LTK) cell lines, or between herpes simplex virus-1 (KOS), and either herpes simplex virus-1 TK^- KOS or VMW-1837 (TK^-/TK^+) viral strains. These results do not unambiguously exclude the possibility that phosphorylation to the 5′-MP by non-transfected and transfected cells, and their subsequent transformation to the active 5′-triphosphate

(5′-TP) are equally effective. Accordingly, it has been shown that the 5′-TP of the thymidine mimic **1** is incorporated into replicating DNA stands by the Klenow fragment of *E. coli* DNA polymerase 1, and that further chain elongation occurs even after its incorporation (14). Therefore, it is possible that these unnatural mimics investigated may be incorporated into DNA without producing a cytotoxic or antiviral effect. Validation of this latter explanation could be determined by measuring incorporation of the parent thymidine mimic **1** into DNA. Alternatively, the group of nucleoside mimics evaluated may be devoid of anticancer/antiviral activity due to the fact that they are not inhibitors of thymidylate synthase (TS). Credence for this possibility is based on the belief that the anticancer activity exhibited by 5-fluoro-2′-deoxyuridine is primarily due to inhibition of DNA biosynthesis by blocking TS, the enzyme that catalyzes the methylation of 2′-deoxyuridine-5′-monophosphate to thymidine-5′-monophosphate (35,36). Furthermore, inhibition of TS is the mechanism by which certain nucleosides, such as (*E*)-5-(2-iodovinyl)-2′-deoxyuridine and 5-(1-azidovinyl)-2′-deoxyuridine, exhibit their cytostatic effect (37). These explanations are consistent with previous biological data that showed that a related class of thymidine mimics having a variety of C-5 substituents (H, Me, F, Cl, Br, I, CF_3, CN, NO_2, and NH_2) were also inactive anticancer and antiviral agents (38).

EXPERIMENTAL SECTION

General Methods. Melting points were determined with a Thomas Hoover capillary apparatus and are uncorrected. ^{1}H NMR, ^{13}C NMR, and ^{19}F NMR spectra were measured on a Bruker AM-300 spectrometer. Proton chemical shifts (δ) are given relative to internal TMS ($\delta = 0$). ^{13}C NMR spectra were acquired using the *J* modulated spin echo technique where methyl and methine carbon resonances appear as positive peaks and methylene and quaternary carbons appear as negative peaks, and carbon chemical shifts (δ) are given relative to $CDCl_3$ ($\delta = 77$). Fluorine chemical shifts (δ) are given relative to external C_6F_6 ($\delta = 0$). Infrared spectra were recorded on a Nicolet Magna 550 IR spectrometer, using air as reference. Elemental analyses, performed by the MicroAnalysis Service Laboratory, Department of Chemistry, University of Alberta. Silica gel 60 (E. Merck) was employed for all silica gel column flash chromatography separations. 1-(3,5-Bis-*O*-(*p*-chlorobenzoyl)-2′-deoxy-β-D-ribofuranosyl)-2,4-difluoro-5-iodobenzene (**3a**) and 1-(2-deoxy-β-D-ribofuranosyl)-2,4-difluoro-5-iodobenzene (**3b**) were prepared according to a previously reported procedure (38).

1-(3,5-Bis-*O*-(*p*-chlorobenzoyl)-2′-deoxy-β-D-ribofuranosyl)-2,4-difluoro-5-(2-trimethylsilylethynyl)benzene (4). CuI (25 mg, 0.131 mmol), $(Ph_3P)_2PdCl_2$ (35 mg, 0.074 mmol) and (trimethylsilyl)acetylene (0.29 mL, 2 mmol) were added to a solution of **3a** (0.636 g, 1 mmol) in Et_3N (25 mL) with stirring under argon. The resulting pale yellow solution was stirred at 25°C for 12 h, the reaction mixture was diluted with Et_2O (100 mL) and filtered to remove solid material. Removal of the solvent from the filtrate gave a residue that was purified by silica

gel column flash chromatography (hexane/EtOAc, 20:1, v/v) to afford **4** (0.59 g, 98%) as white crystals after recrystallization from hexane; m.p. 92−94°C; ^{1}H NMR ($CDCl_3$) 8.02, 7.98, 7.46, 7.41 (d, $J = 8.5$ Hz, 2H each), 7.65 (t, $J = 7.9$ Hz, 1H), 6.81 (dd, $J = 10.1$, 8.8 Hz, 1H), 5.58 (br d, $J = 6.4$ Hz, 1H, H-3′), 5.40 (dd, $J = 10.7$, 5.2 Hz, 1H, H-1′), 4.68 (d, $J = 4.0$ Hz, 2H, H-5′), 4.52 (td, $J = 4.0$, 2.1 Hz, 1H, H-4′), 2.62 (br d, $J = 14.0$, 5.2 Hz, 1H, H-2′α), 2.12 (ddd, $J = 14.0$, 10.7, 6.4 Hz, 1H, H-2′β), 0.24 (s, 9H, Si(CH_3)$_3$); ^{13}C NMR ($CDCl_3$) 165.25, 165.0, 162.90 (dd), 159.39 (dd), 139.92, 139.60, 131.68 (dd, $J = 6.6$, 2.2 Hz), 130.96, 130.90, 128.78, 128.75, 128.02, 127.83, 124.13 (dd, $J = 14.3$, 4.4 Hz), 108.7 (dd), 103.93 (dd, $J = 26.4$, 25.3 Hz), 100.13, 96.53, 82.67, 77.19, 74.28, 64.62, 40.02, −0.23(3C); ^{19}F NMR ($CDCl_3$) 55.17 (m), 49.38 (q, $J = 9.1$ Hz). Anal. Calcd. for $C_{30}H_{26}Cl_2F_2O_5Si$: C, 59.70; H, 4.34. Found: C, 59.50; H, 4.05.

1-(2-Deoxy-β-D-ribofuranosyl)-2,4-difluoro-5-(2-iodoethynyl)benzene (5a). *N*-iodosuccinimide (0.09 g, 0.4 mmol) and ground $AgNO_3$ (0.014 g, 0.08 mmol) were added to a solution of **4** (0.2 g, 0.33 mmol) in acetone (4 mL) with stirring, and the reaction was allowed to proceed at 25°C for 2 h in the dark. Removal of the solvent in vacuo gave a residue that was purified via silica gel column flash chromatography (hexane/EtOAc, 10:1, v/v) to give white crystals (0.21 g, 97%), m.p. 161–162°C. This product (0.2 g, 0.304 mmol) was suspended in MeOH (10 mL), NaOMe (0.08 g, 1.48 mmol) was added, and the reaction was allowed to proceed with stirring at 25°C for 15 min at which time a clear solution was obtained. The reaction was quenched with solid NH_4Cl, and Et_2O (15 mL) was added. Filtration, and removal of the solvent from the filtrate in vacuo, gave a residue that was purified via silica gel column flash chromatography (hexane/EtOAc, 1:1.5, v/v) to afford **5a** (0.11 g, 95%) as white crystals; m.p. 125–126°C; ^{1}H NMR ($CDCl_3$) 7.54 (t, $J = 7.9$ Hz, 1H), 6.78 (dd, $J = 9.8$, 9.2 Hz, 1H), 5.26 (dd, $J = 10.1$, 5.8 Hz, 1H, H-1′), 4.36 (ddd, $J = 6.4$, 3.0, 2.1 Hz, 1H, H-3′), 3.96 (td, $J = 5.2$, 3.0 Hz, 1H, H-4′), 3.72 (d, $J = 5.2$ Hz, 2H, H-5′), 2.76 (s, 2H, OH), 2.29 (br dd, $J = 13.1$, 5.8 Hz, 1H, H-2′α), 1.90 (ddd, $J = 13.1$, 10.1, 6.4 Hz, 1H, H-2′β); ^{13}C NMR ($CDCl_3$) 163.08 (dd, $J = 253.8$, 12.1 Hz), 159.61 (dd, $J = 252.7$, 11.0 Hz), 132.23 (dd, $J = 6.6$, 2.2 Hz), 125.06 (dd, $J = 13.2$, 3.3 Hz), 108.10 (dd, $J = 15.2$, 4.4 Hz), 103.95 (t, $J = 25.3$ Hz), 87.10, 86.22, 73.67 (d, $J = 2.2$ Hz), 73.07, 65.77, 62.97, 42.24. Anal. Calcd. for $C_{13}H_{11}F_2IO_3$: C, 41.08; H, 2.92. Found: C, 41.51; H, 2.74.

1-(2-Deoxy-β-D-ribofuranosyl)-5-(2-bromoethynyl)-2,4-difluorobenzene (5b). *N*-bromosuccinimide (36 mg, 0.2 mmol) and ground $AgNO_3$ (0.01 g, 0.059 mmol) were added to a solution of **4** (0.11 g, 0.181 mmol) in acetone (2 mL) with stirring, and the reaction was allowed to proceed at 25°C in the dark for 1.5 h. Removal of the solvent in vacuo gave a residue that was purified via silica gel column flash chromatography (hexane/EtOAc, 10:1, v/v) to give white crystals (0.095 g), m.p. 124–125.5°C. This product was suspended in MeOH (5 mL), and NaOMe (0.03 g, 0.55 mmol) was added. The reaction was allowed to proceed at 25°C for 20 min during which time the solution turned clear. The reaction was quenched with solid NH_4Cl, and diluted with ether. Filtration, and removal of the

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solvent from the filtrate in vacuo, gave a residue that was purified via silica gel column flash chromatography (hexane/EtOAc, 1:1.5, v/v) to afford **5b** (0.048 g, 80% from **4**) as white crystals; m.p. 124–125°C; ^{1}H NMR ($CDCl_3$) 7.56 (t, $J = 7.9$ Hz, 1H), 6.78 (dd, $J = 10.1$, 9.2 Hz, 1H), 5.26 (dd, $J = 10.1$, 5.8 Hz, 1H, H-1′), 4.32 (ddd, $J = 6.4$, 3.0, 1.8 Hz, 1H, H-3′), 3.93 (td, $J = 4.9$, 3.0 Hz, 1H, H-4′), 3.70 (d, $J = 4.9$ Hz, 2H, H-5′), 2.95 (br s, 2H, O*H*), 2.28 (br dd, $J = 13.1$, 5.8 Hz, 1H, H-2′α), 1.89 (ddd, $J = 13.1$, 10.1, 6.4 Hz, 1H, H-2′β); ^{13}C NMR ($CDCl_3$) 162.70 (dd, $J = 253.8$, 11.4 Hz), 159.53 (dd, $J = 252.7$, 11.0 Hz), 131.92 (dd, $J = 6.6$, 2.2 Hz), 125.25 (dd, $J = 14.3$, 3.3 Hz), 107.41 (dd, $J = 15.4$, 3.3 Hz), 103.97 (t, $J = 25.3$ Hz), 87.14, 73.60, 72.94, 72.62, 62.88, 54.76, 42.21. Anal. Calcd. for $C_{13}H_{11}BrF_2O_3$: C, 46.87; H, 3.33. Found: C, 46.72; H, 3.13.

1-(2-Deoxy-β-D-ribofuranosyl)-2,4-difluoro-5-ethynylbenzene (5c). Compound **4** (0.30 g, 0.5 mmol) was added to a solution of NaOMe (0.11 g, 2 mmol) in MeOH (10 mL) with stirring, and the reaction was allowed to proceed at 25°C for 30 min prior to quenching with solid NH_4Cl. Removal of the solvent in vacuo gave a residue that was dissolved in EtOAc (20 mL), the resulting mixture was filtered, and washed with EtOAc (20 mL). Removal of the solvent from the filtrate gave a residue that was purified via silica gel column flash chromatography (hexane/EtOAc, 1:1.5, v/v) to afford **5c** (0.117 g, 92%) as white crystals after recrystallization from hexane–CH_2Cl_2; m.p. 143–144°C; ^{1}H NMR ($CDCl_3$) 7.57 (t, $J = 8.0$ Hz, 1H), 6.76 (dd, $J = 9.9$, 9.1 Hz, 1H), 5.23 (dd, $J = 9.9$, 5.9 Hz, 1H, H-1′), 4.28 (ddd, $J = 6.2$, 2.9, 1.8 Hz, 1H, H-3′), 3.90 (td, $J = 5.1$, 2.9 Hz, 1H, H-4′), 3.68 (dd, $J = 11.7$, 5.1 Hz, 1H, H-5′α), 3.62 (dd, $J = 11.7$, 5.1 Hz, 1H, H-5′β), 3.31 (br s, 2H, O*H*), 3.25 (s, 1H, –C≡C*H*), 2.25 (br dd, $J = 13.2$, 5.9 Hz, 1H, H-2'α), 1.86 (ddd, $J = 13.2$, 9.9, 6.2 Hz, 1H, H-2′β); ^{13}C NMR ($CDCl_3$) 162.59 (dd, $J = 253.8$, 12.1 Hz), 159.66 (dd, $J = 252.7$, 12.1 Hz), 132.08 (dd, $J = 6.6$, 2.2 Hz), 125.31 (dd, $J = 14.3$, 3.3 Hz), 106.77 (dd, $J = 15.4$, 3.3 Hz), 103.84 (dd, $J = 26.4$, 25.3 Hz), 87.17, 82.06, 73.52 (d, $J = 2.2$ Hz), 72.84, 62.80, 42.08; ^{19}F NMR ($CDCl_3$) 53.76 (q, $J = 9.1$ Hz), 50.20 (q, $J = 9.1$ Hz). Anal. Calcd. for $C_{13}H_{12}F_2O_3$: C, 61.42; H, 4.76. Found: C, 61.30; H, 4.69.

1-(3,5-Bis-*O*-(*p*-chlorobenzoyl)-2′-deoxy-β-D-ribofuranosyl)-2,4-difluoro-(*E*)-5-(2-trimethylsilylvinyl)benzene (6a). $(Ph_3P)_2PdCl_2$ (0.21 g, 0.3 mmol) was added to a mixture of **3a** (1.9 g, 3 mmol) and (*E*)-1-trimethylsilyl-2-tributylstannylethene (1.95 g, 5 mmol) in dry CH_3CN (40 mL), and the mixture was stirred vigorously at 60°C for 4 h under an argon atmosphere. Removal of the solvent in vacuo gave a residue that was purified via silica gel column flash chromatography (hexane/EtOAc, 20:1 to 15:1, v/v) to yield **6a** (1.3 g, 72%) as white crystals after recrystallization from hexane; m.p. 81–82°C; ^{1}H NMR ($CDCl_3$) 8.04, 7.97, 7.47, 7.34 (d, $J = 8.5$ Hz, 2H each), 7.72 (t, $J = 8.2$ Hz, 1H), 6.94 (d, $J = 19.5$ Hz, 1H, C*H*=CHTMS), 6.78 (dd, $J = 10.4$, 10.1 Hz, 1H), 6.43 (d, $J = 19.5$ Hz, 1H, CH=C*H*TMS), 5.61 (br d, $J = 6.1$ Hz, 1H, H-3′), 5.44 (dd, $J = 10.7$, 5.2 Hz, 1H, H-1′), 4.76 (dd, $J = 11.9$, 4.0 Hz, 1H, H-5′α), 4.68 (dd, $J = 11.9$, 4.0 Hz, 1H, H-5'β), 4.53 (td, $J = 4.0$, 2.1 Hz, 1H, H-4′), 2.65 (br dd, $J = 13.7$, 5.2 Hz, 1H, H-2′α), 2.16 (ddd, $J = 13.7$, 10.7, 6.1 Hz, 1H, H-2′β), 0.12 (s, 9H, Si(C*H*$_3$)$_3$); ^{13}C

NMR ($CDCl_3$) 165.24, 165.13, 159.44 (dd, $J = 251.6$, 12.1 Hz), 159.03 (dd, $J =$ 248.3, 9.9 Hz), 139.97, 139.69, 134.0 (d, $J = 3.3$ Hz), 132.67, 131.04, 130.92, 128.85, 128.79, 128.20, 128.03, 124.74 (t, $J = 5.5$ Hz), 123.95 (dd, $J = 13.2$, 3.3 Hz), 122.87 (dd, $J = 12.1$, 4.4 Hz), 103.76 (dd, $J = 26.4$, 25.3 Hz), 82.80, 77.42, 74.85 (d, $J = 2.2$ Hz), 64.78, 40.24, −1.33.

1-(2-Deoxy-β-D-ribofuranosyl)-2,4-difluoro-(*E*)-5-(2-trimethylsilylvinyl) benzene (6b). $(Ph_3P)_2PdCl_2$ (0.245 g, 0.35 mmol) was added to a mixture of **3b** (1.25 g, 3.5 mmol) and (*E*)-1-trimethylsilyl-2-tributylstannylethene (2.75 g, 7 mmol) in dry MeCN (50 mL), and the mixture was stirred vigorously under argon at 60°C for 4 h. Removal of the solvent in vacuo gave a residue that was purified via silica gel column flash chromatography (hexane/EtOAc, 1.5:1–1:1, v/v) to yield **6b** (1.0 g, 86%) as light yellow crystals, which were recrystallized from hexane to afford white crystals (0.88 g, 76%); m.p. 101–102°C; ^{1}H NMR ($CDCl_3$) 7.58 (t, $J = 10.4$ Hz, 1H), 6.97 (d, $J = 19.5$ Hz, 1H, C*H*=CHTMS), 6.76 (t, $J = 10.4$ Hz, 1H), 6.48 (d, $J = 19.5$ Hz, 1H, CH=C*H*TMS), 5.30 (dd, $J = 10.1$, 5.8 Hz, 1H, H-1′), 4.42 (ddd, $J = 6.4$, 3.4, 2.1 Hz, 1H, H-3′), 4.00 (td, $J = 4.9$, 3.4 Hz, 1H, H-4′), 3.79 (d, $J = 4.9$ Hz, 2H, H-5′), 2.90 (br s, 2H, O*H*), 2.30 (br dd, $J = 13.1$, 5.8 Hz, 1H, H-2′α), 2.04 (ddd, $J = 13.1$, 10.1, 6.4 Hz, 1H, H-2′β), 0.16 (s, 9H, Si(CH_3)$_3$); ^{13}C NMR ($CDCl_3$) 159.33 (dd, $J = 250.5$, 12.1 Hz, 2C), 134.07 (d, $J = 3.3$ Hz), 132.52, 125.21 (t, $J = 5.5$ Hz), 124.18 (dd, $J = 13.2$, 3.3 Hz), 122.52 (dd, $J = 11.0$, 3.3 Hz), 103.88 (t, $J = 26.4$ Hz), 87.04, 74.66, 73.45, 63.17, 42.35, −1.37; ^{19}F NMR ($CDCl_3$) 46.44 (m), 44.88 (m). Anal. Calcd. for $C_{16}H_{22}F_2O_3Si$: C, 58.51; H, 6.75. Found: C, 58.09; H, 6.75.

1-(2-Deoxy-β-D-ribofuranosyl)]-2,4-difluoro-(*E*)-5-(2-iodovinyl)benzene (7a). ICl (0.065 g, 0.4 mmol) was added to a solution of **6b** (0.132 g, 0.4 mmol) in dry MeCN (5 mL) with stirring at 25°C. The reaction was allowed to proceed at 25°C for 30 min with stirring, the solvent was removed in vacuo, and the residue obtained was purified via silica gel column flash chromatography (hexane/EtOAc, 1:1–1:1.5, v/v) to afford **7a** (0.147 g, 96%) as a light yellow syrup, which was recrystallized from hexane–CH_2Cl_2 to give slightly yellow crystals (0.130 g, 85%); m.p. 85–87°C; ^{1}H NMR ($CDCl_3$) 7.42 (d, $J = 15.0$ Hz, 1H, CH=C*H*I), 7.40 (t, $J = 8.2$ Hz, 1H), 6.94 (d, $J = 15.0$ Hz, 1H, C*H*=CHI), 6.74 (t, $J = 10.2$ Hz, 1H), 5.27 (dd, $J = 9.9$, 5.9 Hz, 1H, H–1′), 4.35–4.42 (m, 1H, H-3′), 3.99 (td, $J = 4.8$, 3.0 Hz, 1H, H-4′), 3.76 (d, $J = 4.8$ Hz, 2H, H-5′), 3.14 (br s, 2H, O*H*), 2.30 (br dd, $J = 13.2$, 5.9 Hz, 1H, H-2′α), 1.92 (m, 1H, H-2′β); ^{13}C NMR (CCl_3) 159.22 (dd, $J = 250.5$, 12.1 Hz), 158.76 (dd, $J = 252.7$, 12.1 Hz), 136.67, 125.85 (dd, $J = 12.1$, 5.5 Hz), 124.85 (dd, $J = 14.3$, 3.3 Hz), 121.77 (dd, $J = 13.2$, 3.3 Hz), 104.19 (dd, $J = 26.4$, 25.3 Hz), 87.02, 80.0 (d, $J = 6.6$ Hz), 74.18, 73.32, 63.12, 42.34. Anal. Calcd. for $C_{13}H_{13}F_2IO_3$: C, 40.86; H, 3.43. Found: C, 40.53; H, 3.15.

1-(2-Deoxy-β-D-ribofuranosyl)-(*E*)-5-(2-bromovinyl)-2,4-difluorobenzene (7b). A solution of Br_2 (0.085 g, 0.53 mmol) in CH_2Cl_2 (0.2 mL) was added drop wise to a solution of **6b** (0.164 g, 0.5 mmol) in CH_2Cl_2 (10 mL) with stirring at −78°C. The reaction was allowed to proceed at −78°C for 2 h, a saturated solution of dry KF in DMSO (5 mL) was added, and the reaction mixture was allowed to

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warm to 0°C with subsequent stirring at 0°C for 1 h and then at 25°C for 2 h. The reaction mixture was diluted with water (10 mL) and extracted with EtOAc (3 × 20 mL). The combined organic extracts were washed with water (10 mL) and brine, and the organic fraction was dried (Na_2SO_4). After filtration, the solvent was removed in vacuo, and the residue was purified via silica gel column flash chromatography (hexane/EtOAc, 1:1.5, v/v) to afford **7b** (0.14 g, 83%) as white crystals after recrystallization from hexane–CH_2Cl_2; m.p. 116–117.5°C; ^{1}H NMR ($CDCl_3$) 7.44 (t, $J = 8.2$ Hz, 1H), 7.09 (d, $J = 14.2$ Hz, 1H, CH=C*H*Br), 6.89 (d, $J = 14.2$ Hz, 1H, C*H*=CHBr), 6.75 (t, $J = 10.2$ Hz, 1H), 5.28 (dd, $J = 10.1$, 5.8 Hz, 1H, H-1′), 4.33 (ddd, $J = 6.4$, 3.0, 2.1 Hz, 1H, H-3′), 3.94 (td, $J = 4.9$, 3.0 Hz, 1H, H-4′), 3.71 (d, $J = 4.9$ Hz, 2H, H-5′), 3.20 (br s, 2H, O*H*), 2.29 (br dd, $J = 13.1$, 5.8 Hz, 1H, H-2′α), 1.91 (ddd, $J = 13.1$, 10.1, 6.4 Hz, 1H, H-2′β); ^{13}C NMR ($CDCl_3$) 159.06 (dd, $J = 252.7$, 13.2 Hz), 158.8 (dd, $J = 252.7$, 12.1 Hz), 129.24, 126.01 (t, $J = 5.5$ Hz), 125.19 (dd, $J = 13.2$, 4.4 Hz), 119.94 (dd, $J = 13.2$, 4.4 Hz), 109.38 (dd, $J = 7.7$, 2.2 Hz), 104.06 (dd, $J = 26.4$, 25.3 Hz), 87.10, 73.89, 72.89, 62.81, 42.21. Anal. Calcd. for $C_{13}H_{13}BrF_2O_3$: C, 46.59; H, 3.91. Found: C, 46.62; H, 3.61.

1-(2-Deoxy-β-D-ribofuranosyl)-2,4-difluoro-5-vinylbenzene (7c). A solution of **6b** (0.81 g, 2.47 mmol) in CF_3CO_2H (5 mL) was stirred at 25°C for 5 min. Excess CF_3CO_2H was removed in vacuo, the residue was dissolved in MeOH (10 mL), and this solution was treated with K_2CO_3 powder (0.5 g) for 10 min with stirring. Removal of the MeOH in vacuo gave a residue, which was extracted with EtOAc (2 × 10 mL). Removal of the solvent from the extracts in vacuo gave a residue, which was purified via silica gel column flash chromatography (hexane/EtOAc, 1:1.5, v/v) to afford **7c** (0.6 g, 95%) as white needles after recrystallization from hexane–CH_2Cl_2; m.p. 116–118°C; ^{1}H NMR ($CDCl_3$) 7.55 (t, $J = 8.4$ Hz, 1H), 6.79 (t, $J = 10.4$ Hz, 1H), 6.79 (dd, $J = 18.0$, 11.3 Hz, 1H, C*H*=CH_2), 5.77 [d, $J = 18.0$ Hz, 1H, (E)–CH=CH*H*], 5.36 [d, $J = 11.3$ Hz, 1H, (Z)–CH=CH*H*], 5.32 (ddd, $J = 10.1$, 5.8 Hz, 1H, H-1′), 4.47 (ddd, $J = 6.4$, 3.4, 1.8 Hz, 1H, H-3′), 4.03 (ddd, $J = 4.9$, 4.3, 3.4 Hz, 1H, H-4′), 3.86 (dd, $J = 11.6$, 4.3 Hz, 1H, H-5′a), 3.78 (dd, $J = 11.6$, 4.9 Hz, 1H, H-5′b), 2.33 (ddt, $J = 13.1$, 5.8, 1.8 Hz, 1H, H-2′α), 2.07 (ddd, $J = 13.1$, 10.1, 6.4 Hz, 1H, H-2′β), 1.99 (br s, 2H, O*H*); ^{13}C NMR ($CDCl_3$) 159.28 (dd, $J = 252.7$, 13.2 Hz), 159.05 (dd, $J = 250.5$, 12.1 Hz), 128.27, 125.43 (d, $J = 5.5$ Hz), 124.56 (dd, $J = 13.2$, 3.3 Hz), 121.51 (dd, $J = 12.1$, 3.3 Hz), 116.19 (d, $J = 3.3$ Hz), 103.69 (t, $J = 26.4$ Hz), 87.10, 74.18, 72.90, 62.82, 42.0; ^{19}F NMR ($CDCl_3$) 46.31 (m), 45.87 (q, $J = 9.2$ Hz). Anal. Calcd. for $C_{13}H_{14}F_2O_3$: C, 60.93; H, 5.51. Found: C, 60.83; H, 5.37.

The deutero analog **7d** was prepared by a similar procedure, using CF_3CO_2D in place of CF_3CO_2H as described above. The atom percent deuterium was determined from the ^{1}H NMR integral for the (E)–CH=CH*H* resonance at $\delta = 5.77$.

1-(2-Deoxy-β-D-ribofuranosyl)-2,4-difluoro-5-ethylbenzene (7e). 10% Pd-on-C (0.05 g) was added to a solution of **7c** (0.128 g, 0.5 mmol) in EtOH (5 mL), and the resultant mixture was stirred under 1 atm of hydrogen gas at 60°C for 5 h. After cooling to 25°C, the reaction mixture was filtered and washed with

EtOH (5 mL). Removal of the solvent from the filtrate in vacuo gave a residue, which was recrystallized from hexane–Et_2O to afford **7e** (0.121 g, 94%) as white crystals; m.p. 66–67.5°C; ^{1}H NMR ($CDCl_3$) 7.24 (dd, $J = 8.5$, 8.2 Hz, 1H), 6.74 (t, $J = 10.1$ Hz, 1H), 5.30 (dd, $J = 10.1$, 5.8 Hz, 1H, H-1′), 4.44 (ddd, $J = 6.4$, 3.4, 2.1 Hz, 1H, H-3′), 4.0 (td, $J = 4.6$, 3.4 Hz, 1H, H-4′), 3.81 (dd, $J = 11.6$, 4.6 Hz, 1H, H-5′α), 3.76 (dd, $J = 11.6$, 4.6 Hz, 1H, H-5′β), 2.62 (q, $J = 7.6$ Hz, 2H, CH_2CH$_3$), 2.29 (dd, $J = 13.1$, 5.8 Hz, 1H, H-2′α), 2.06 (ddd, $J = 13.1$, 10.1, 6.4 Hz, 1H, H-2′β), 1.20 (t, $J = 7.7$, 3H, CH$_2$CH_3); ^{13}C NMR ($CDCl_3$) 160.14 (dd, $J = 247.2$, 12.1 Hz), 158.26 (dd, $J = 247.2$, 12.1 Hz), 128.02 (dd, $J = 6.6$, 4.6 Hz), 126.79 (dd, $J = 16.5$, 3.3 Hz), 123.69 (dd, $J = 13.2$, 4.4 Hz), 103.47 (dd, $J = 26.4$, 25.3 Hz), 87.10, 74.77, 73.55, 63.27, 42.38, 21.82 (d, $J = 2.2$ Hz), 14.41. Anal. Calcd. for $C_{13}H_{16}F_2O_3$: C, 60.46; H, 6.24. Found: C, 60.10; H, 6.62.

1-(2-Deoxy-β-D-ribofuranosyl)-5-(1-azido-2-bromoethyl)-2,4-difluorobenzene (7f). Concentrated HCl (2 mL) and water (0.5 mL) were added to a suspension of NaN_3 (3.25 g, 50 mmol) in CH_2Cl_2 (10 mL) cooled to 0°C. This heterogeneous mixture was stirred for 10 min at 0°C, then Br_2 (0.8 g, 2.5 mmol) was added dropwise, and the resulting mixture was stirred at 0°C for 30 min. Separation of the CH_2Cl_2 layer yielded a brown solution of BrN_3, which was used directly in the subsequent reaction. A solution of BrN_3 (2.4 mL of 0.5 M, 1.2 mmol) obtained above was added to a suspension of compound **7c** (0.2 g, 0.78 mmol) in $MeNO_2$ (5 mL), and the resulting solution was stirred at 25°C for 10 min. Removal of the solvent in vacuo gave a residue, which was purified via silica gel column flash chromatography (hexane/EtOAc, 1:1, v/v) to give **7f** (0.24 g, 81%) as a colorless syrup. ^{19}F NMR analysis showed this product consisted of a mixture of two diastereomers (ratio A:B = 5:6) due to the chiral center at C-1 position of the 5-(1-azido-2-bromoethyl) moiety. ^{1}H NMR ($CDCl_3$) 7.53 (dd, $J = 8.2$, 7.6 Hz, 1H), 6.87 (t, $J = 9.9$ Hz, 1H), 5.38 (dd, $J = 9.8$, 5.8 Hz, 1H, H-1′), 5.08 (dd, $J = 6.7$, 3.4 Hz, 1H, H-3′), 4.42–4.52 [m, 1H, –CH(N$_3$)CH$_2$Br], 4.04 (ddd, $J = 4.6$, 4.0, 3.4 Hz, 1H, H-4′), 3.88 (dd, $J = 11.6$, 4.0 Hz, 1H, H-5′a), 3.81 and 3.80 (two dd, $J = 11.6$, 4.6 Hz, 1H total, H-5′b), 3.48–3.70 [complex m, 2H total, –CH(N$_3$)CH_2Br], 2.39 (m, 1H, H-2′α), 2.05 (m, 1H, H-2′β), 1.95 (br s, 2H, OH); ^{13}C NMR ($CDCl_3$) 159.83 (dm, $J = 251.6$ Hz), 159.21 (dm, $J = 251.6$ Hz), 126.74 (dd, $J = 6.6$, 5.5 Hz) and 126.42 (t, $J = 5.5$ Hz), 125.60 (m), 120.42 (dd, $J = 4.4$, 3.3 Hz) and 120.25 (t, $J = 3.3$ Hz), 104.26 (dd, $J = 26.4$, 25.3 Hz) and 104.22 (dd, $J = 26.4$, 25.3 Hz), 87.08, 74.22 and 74.09, 73.32, 63.09, 59.09, 59.96, 42.31, 33.71; ^{19}F NMR ($CDCl_3$) 49.02 (m, A) and 48.90 (m, B), 46.32 (m, A) and 46.16 (m, B). Anal. Calcd. for $C_{13}H_{14}BrF_2N_3O_3$: C, 41.29; H, 3.73. Found: C, 41.18; H, 3.69.

In Vitro Cell Cytotoxicity (MTT Assay). KBALB, KBALB-STK, human 143B, human 14B-LTK, and R-970-5 cells were cultured in complete DMEM medium supplemented with 10% fetal bovine serum (FBS), and EMT-6 cells were cultured in complete MAYMOUTH medium in 10% FBS. Exponentially growing cells were trypsinized, centrifuged, suspended in growth medium, and the cell number was readjusted to 8×10^3 cells/mL. Cells were seeded into 96-well plates

at 8×10^2 cells/well, and incubated at 37°C in a humidified 5% CO_2 atmosphere for 24 h.

The test compounds were dissolved in DMEM medium, and 100 μL of these solutions was added to cells in 96-well plates to produce the preselected test compound concentration. DMEM medium (100 μL) was added to control wells. The plates were incubated for 3–5 days at 37°C in a humidified atmosphere consisting of 95% air and 5% CO_2. At the end of the incubation, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT, Sigma) was dissolved in phosphate-buffered saline (PBS) to produce a concentration of 5 mg/mL, filtered through a 0.45 μm membrane filter, and diluted (1:5) with prewarmed DMEM medium. A 50 μL aliquot of this solution was added to each well, and the plates were incubated at 37°C for 4 h. The medium was removed from the wells, dimethyl sulfoxide (150 μL) was added to each well, and the plates were placed on a shaker for 15 min to dissolve the formazan crystals. The absorbance at 540 nm (A_{540}) was measured immediately in each well, using a scanning multi-well spectrophotometer (ELISA reader). A_{540} values, corrected for the absorbance in medium blanks, reflected the concentration of viable cells. The CC_{50} values reported are the test drug concentration that reduced the A_{540} to 50% of the control value (mean value, $n = 6$). This assay (32), which depends on the metabolic reduction of MTT to colored formazan, measures cytostatic and cytotoxic effects of the test drugs.

Antiviral Activity Assays. The antiviral assays, other than the anti-HIV assays, were based on an inhibition of virus-induced cytopathicity in either E_6SM, HeLa, Vero or HEL cell cultures, following previously established procedures (33,39–41). Herpes simplex virus type 1 (HSV-1) (KOS, F, McIntyre), HSV-2 (G, 196. Lyons), vaccinia virus, vesicular stomatitis virus (VSV), the thymidine kinase-deficient HSV-1 TK^- KOS (ACV^r), and HSV-1 TK^-/TK^+ (VMW 1837) strains were exposed to E_6SM cell cultures, parainfluenza-3 virus, reovirus-1, Sindbis virus, Coxsackie B4 virus and Punta Toro virus to Vero cell cultures, respiratory syncytial virus to HeLa cell cultures, and cytomegalovirus (CMV) (AD-169, Davis) and varicella-zoster virus (VZV) (YS, OKA), and the thymidine kinase-deficient VZV (07/1, YS/R) strains to HEL cell cultures. Briefly, confluent cell cultures in microtitre trays were incubated with 100 $CCID_{50}$ of virus, 1 $CCID_{50}$ being the virus dose required to infect 50% of the cell cultures. After a 1 h virus adsorption period, residual virus was removed, and the cell cultures were incubated in the presence of various concentrations (400, 100, .. μg/mL) of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures.

Inhibition of HIV-Induced Giant Cell Formation. CEM cell cultures were suspended at 250,000–300,000 cells/mL of culture medium, and infected with HIV-1(III_B) or HIV-2(ROD) at 100 $CCID_{50}$/mL. Then, 100 μL of the infected cell suspension were transferred to 200 μL microtiter plate wells containing 100 μL of serial dilutions of the test compound solutions. After four days of incubation at 37°C, cell cultures were examined for syncytium formation as previously described (42).

Cytostatic Activity Assays. The cytostatic assays were performed as previously described (43). Briefly, 100 μL aliquots of the cell suspensions (7.5×10^5 human T-lymphocyte CEM cells/mL) were added to the wells of a microtiter plate containing 100 μL of varying concentrations of the test compounds. After a three-day incubation period at 37°C in a humidified CO_2-controlled incubator, the number of viable cells was determined using a Coulter Counter. Cytostatic activity is expressed as the compound concentration that reduced the number of viable cells by 50% (CC_{50}). The cytotoxicity measurements were based on microscopically visible alteration of normal cell morphology (E_6SM, HeLa, Vero) or inhibition of cell growth (HEL), as previously described (41).

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