for 24–48 h have been infected with 100 PFU/mL of virus; after a 2-h adsorption, cultures are covered with the agar medium and a disk of blotting paper, embedded with 200 μg of the compound dissolved in ethanol and dried, is overlayed. After a 3–4-day incubation at 37 °C in 5% CO_2, cultures are stained with neutral red. The toxicity, as unstained halos, and the activity, as stained areas where lysis plaques produced by the virus are reduced or have disappeared, are evidenced at the same time.

Cytotoxicity gross evaluation firstly is assessed on the basis of toxicity halos in tests with agar medium, and as maximum tolerated dose (MxTD) in liquid medium, at the same time with the test of antiviral activity previously described. A more accurate evaluation of cytotoxicity is obtained by incubating Hep cells with serial doses of the compound for 3 days and by calculating the 50% inhibiting dose ($TCID_{50}$) after determination of the protein contents.[14]

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Antiviral Activity and Cytotoxicity in Fluid Medium. Determination of cytotoxicity and of herpes simplex, HRSV, and Coxsackie viruses inhibition in human carcinoma cells was performed as previously described.[15]

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Registry No. 4, 16019-33-3; **5**, 111209-66-6; **6**, 103298-51-7; **6** (oxalate), 111209-67-7; **7**, 602-03-9; **8**, 602-02-8; **9**, 111209-68-8; **10**, 111209-69-9; **11**, 103298-52-8; **11** (oxalate), 111209-70-2; *erythro*-3-amino-2-nonanol, 51714-10-4; adenosine deaminase, 9026-93-1.

Supplementary Material Available: Antiviral activity and cytotoxicity of EHNA deaza analogues in fluid medium (Table III) and antitumor activity of ara-A and 3-deaza-EHNA (**3**) alone and in combination on murine leukemia L1210 (Table IV) (1 page). Ordering information is given on any current masthead page.

Synthesis and Biological Activities of 4-*O*-(Difluoromethyl)-5-substituted-uracil Nucleoside Analogues

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Various 4-*O*-difluoromethyl analogues of 5-substituted uridine (Urd), 2′-deoxyuridine (dUrd), and arabinofuranosyluracil (araU) nucleosides were prepared via a CF_2-insertion reaction into 4-*O*-silylated nucleosides and evaluated for activity against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) and cytotoxicity in human embryonic lung fibroblast (HELF) cell cultures. The introduction of the 4-substituent led to a strong reduction of antiviral activity for dUrd but not for araU analogues. Three of the 4,5-disubstituted uracil nucleoside derivatives, 4-*O*-(difluoromethyl)-5-bromo-araU (**5c**), -5-methyl-araU (**5e**), and -(*E*)-5-(2-bromovinyl)-araU (**5g**), displayed a high and selective inhibitory effect against HSV-1, but only **5e** was effective against both HSV-1 and HSV-2 comparably with the antiherpes potential of the reference compounds 9-[(2-hydroxyethoxy)methyl]guanine (acyclovir) and 1-β-D-arabinofuranosylthymine (araT).

Analogues of thymidine (dThd; 5-methyl-2′-deoxyuridine), a natural precursor of deoxyribonucleic acid (DNA), were among the first compounds discovered to exert antiviral activity against DNA viruses.[1] During the past 10 years we have been engaged in the synthesis and biological evaluation of 5-substituted pyrimidine nucleoside analogues with antiviral potential.[2–4] In the search for compounds that surpass the antiviral effect of 5-iodo-2′-deoxyuridine (IdUrd)[1] with simultaneously reduced toxicity to uninfected host cells, a large number of 5-substituted dUrd and 2′-deoxycytidine (dCyd) derivatives have been synthesized.[3,5,6] In our pursuit of this approach, (*E*)-5-(2-bromovinyl)-dUrd (BrV-dUrd) emerged as a highly potent and selective antiherpes agent that inhibits the replication of herpes simplex virus type 1 (HSV-1), varicella zoster virus (VZV), and Epstein–Barr virus (EBV), but not of herpes simplex virus type 2 (HSV-2), at levels far lower than the cytotoxic concentrations.[5,7–10] Much effort has been invested in elucidating the ideal structural requirements of the 5-vinyl substituent for maximal antiherpes viral activity and low cytotoxicity;[11] but exchange of the olefinic hydrogen atoms by other halogens or by nonhalogen substituents as well as comparison of *E* and *Z* isomers did not improve the low anti-HSV-2 potential of BrV-dUrd.[12,13]

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The newly synthesized 5-substituted 1-β-D-arabinofuranosyluracil (araU) analogues[14,15] have attracted con-

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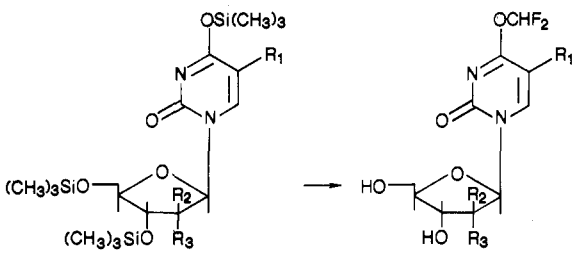
Scheme I[a]



1: R_2 = H, R_3 = $OSi(CH_3)_3$
2: R_2 = $OSi(CH_3)_3$, R_3 = H
3: R_2 = R_3 = H
4: R_2 = H, R_3 = OH
5: R_2 = OH, R_3 = H
6: R_2 = R_3 = H

[a] Compounds 1–6: a, R_1 = H; **b**, R_1 = F, **c**, R_1 = Br; **d**, R_1 = I; e, R_1 = CH_3; **f**, R_1 = C_2H_5; **g**, R_1 = CH=CHBr.

siderable interest. In addition to their high in vitro and in vivo effect against HSV-1,[14,16a,b] (*E*)-5-(2-bromovinyl)-araU (BrV-araU) and 5-vinyl-araU (VaraU) possess superior antiviral activity against VZV (BrV-araU)[17] and HSV-2 (VaraU)[14] and cause considerably less toxicity to human embryonic lung fibroblasts (HELF) compared with BrV-dUrd.[16a] In addition, we have found that VaraU is also effective against EBV in cell culture and against experimental HSV-2 encephalitis in mice.[16c,d]

Progress has been made in achieving a broader antiviral spectrum by introduction of a fluoro substituent at the 2′-hydroxy "up" position of 5-substituted araU and aracytidine (araC) analogues.[18] The antiviral effects of 1-(2-fluoro-2-deoxy-β-D-arabinofuranosyl)-5-iodocytosine (FIAC) and -5-methyluracil (FMAU) against VZV[19] and HSV-2[19] have been found to be considerably higher than that of the clinical drug acyclovir (9-[(2-hydroxyethoxy)-methyl]guanine; Zovirax), and recent studies show that FIAC and FMAU clearly surpass acyclovir in its efficacy against EBV[10] and cytomegalovirus (CMV).[20] In contrast, all efforts were unsuccessful to find effective antiherpetics within a large number of 5-substituted "acyclic" pyrimidine nucleoside analogues.[21,22a] Recently, the synthesis and properties of 4-*O*-alkylthymidines were published, but without any biological data.[22b]

We now have synthesized a series of 5-substituted 4-*O*-(difluoromethyl)uracil nucleoside derivatives in order to investigate the influence of structural changes at the 4-position of the uracil moiety on the biological activity of 5-substituted pyrimidine nucleoside analogues.

Chemistry. 5-Substituted ribonucleosides, the starting materials for compounds **1b–d** in Scheme I, were synthesized from uridine according to well-known procedures.[23,24] The synthesis of the arabinofuranosyl nucleosides **2a–d** was carried out by a simple alkaline cleavage of 2,2′-anhydrouridine[25] followed by halogenation at C-5 of the isolated arabinofuranosyluridine and a subsequent silylation of the 5-substituted products. Compounds **2e** and **2f** could be isolated via a cleavage of the 2,2′-anhydro-1-β-D-arabinofuranosylthymine and 2,2′-anhydro-1-β-D-arabinofuranosyl-5-ethyluracil, which we have synthesized from 1-β-D-ribofuranosylthymine and 1-β-D-ribofuranosyl-5-ethyluracil, respectively. The treatment of these compounds with hexamethyldisilazane gave **2e** and **2f** in good yields. The synthesis of 1-β-D-ribofuranosylthymine and 1-β-D-ribofuranosyl-5-ethyluracil was carried out by the method of Vorbrüggen[26] by starting from thymine or 5-ethyluracil and 2,3,5-tri-*O*-benzoyl-1-*O*-acetylribose followed by alkaline deblocking with 0.1 M methanolic sodium methylate solution. The same procedure was used to obtain **3f**. Treatment of 5-ethyluracil with 2-deoxy-3,5-di-*O*-(*p*-chlorobenzoyl)-1-*O*-acetylribose according to Vorbrüggen[26] followed by deblocking and silylation gave 2′-deoxy-5-ethyluridine **3f** in satisfactory yields.

The synthesis of (*E*)-1-β-D-arabinofuranosyl-5-(2-bromovinyl)uracil, starting material for **2g**, was carried out as described by Bärwolff and Langen.[30]

For silylation the nucleosides were refluxed with a 10-fold excess of hexamethyldisilazane in the presence of traces of $(NH_4)_2SO_4$. After being stirred under reflux for about 1 h (at least up to the complete solution of the nucleoside), the solution was concentrated, and the resulting syrup could be used for the following synthesis without any further purification. Treatment of the silylated nucleosides **1–3** with difluorocarbene was carried out in dry tetrahydrofuran with exclusion of atmospheric moisture. Favorable difluorocarbene was generated from $Hg(CF_3)_2$ in the presence of sodium iodide in situ.[27] After the appropriate reaction was performed, the reaction mixture was acidically hydrolyzed to give the desired 4-*O*-difluoromethylated nucleosides **4–6** in yields of about 50–60%. A simultaneous difluoromethylation of a hydroxy function in the carbohydrate moiety could not be obtained, probably due to the different stabilities of the silyloxy groups in the carbohydrate to those of the base. These observations are consistent with our recent studies[28,29] on the difluoromethylation of different pyrimidine derivatives and pyrimidine nucleosides.

4-*O*-(Difluoromethyl)uracil nucleosides are stable compounds in acidic and neutral aqueous solution (no decomposition in neutral aqueous solution was obtained after

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Table I. Antiviral and Anticellular Activity of 5-Substituted 4-O-(Difluoromethyl)uracil Nucleosides in Human Embryonic Lung Fibroblast Cell Cultures

no.	compound	ID_{50},[a] μM HSV-1	ID_{50},[a] μM HSV-2	toxicity,[b] μM
	1-β-D-Ribofuranosyluracil (Urd) Nucleosides			
4a	4-O-(difluoromethyl)-Urd	>1000	>1000	>1000
4b	4-O-(difluoromethyl)-5-fluoro-Urd	500	>500	>500
4c	4-O-(difluoromethyl)-5-bromo-Urd	>250	>250	>250
4d	4-O-(difluoromethyl)-5-iodo-Urd	>500	>500	>500
	1-β-D-Arabinofuranosyluracil (araU) Nucleosides			
5a	4-O-(difluoromethyl)-araU	430	>1000	>1000
5b	4-O-(difluoromethyl)-5-fluoro-araU	110	300	>500
5c	4-O-(difluoromethyl)-5-bromo-araU	3.6	17.6	>1000
5d	4-O-(difluoromethyl)-5-iodo-araU	29	82	>1000
5e	4-O-(difluoromethyl)-5-methyl-araU	0.8	3	>1000
5f	4-O-(difluoromethyl)-5-ethyl-araU	27	210	>500[d]
5g	(E)-4-O-(difluoromethyl)-5-(2-bromovinyl)-araU	1.1	>500[d]	>500[d]
	1-(2-Deoxy-β-D-ribofuranosyl)uracil (dUrd) Nucleosides			
6c	4-O-(difluoromethyl)-5-bromo-dUrd	130	>500	>500
6e	4-O-(difluoromethyl)-5-methyl-dUrd	>1000	>1000	>1000
6f	4-O-(difluoromethyl)-5-ethyl-dUrd	>1000	>1000	>1000
	Reference Compounds			
ACV	9-[(2-hydroxyethoxy)methyl]guanine (acyclovir)	0.48	1.6	360[c]
BrV-dUrd	(E)-5-(2-bromovinyl)-2′-deoxyuridine	0.05	27	170[c]
BrdUrd	5-bromo-2′-deoxyuridine	0.8	1.4	>1000
EthdUrd	5-ethyl-2′-deoxyuridine	10	14.5	>1000
BrV-araU	(E)-1-β-D-arabinofuranosyl-5-(2-bromovinyl)uracil	0.115	49	>1000[c]
araT	1-β-D-arabinofuranosylthymine	0.64	1.65	>1000
BraraU	1-β-D-arabinofuranosyl-5-bromouracil	1.35	7.9	>1000
IaraU	1-β-D-arabinofuranosyl-5-iodouracil	51	70	>500

[a] (Inhibitory dose)$_{50}$ = concentration required to reduce plaque formation of HSV-1 (strain 77) or HSV-2 (strain 74) by 50%. [b] Minimum concentration not causing a microscopically detectable cytotoxic alteration of normal HELF cell morphology. [c] ID_{50} for HELF cell proliferation during a 3-day treatment period.[16a] [d] Highest concentration tested.

6-month storage at room temperature). Under alkaline conditions (pH >7.5), hydrolyses of 4-O-difluoromethylated compounds are observed after a few minutes.

An examination of the ^1H NMR spectra of the 4-O-difluoromethylated nucleosides revealed a characteristic triplet for the CF_2H group at 7.5–7.9 ppm (J_{CHF_2} = 57.0 Hz) whereas the N-3–H signal disappeared. The chemical shifts and the magnitude of the coupling constants of the carbohydrate moiety are consistent with the assignments in the literature. Finally, the site of attachment of the base was confirmed by high-resolution mass spectrometry. The appearance of a M – OCF_2H fragment indicates a difluoromethylation at O-4.

Antiherpes Activity and Toxicity Studies. The antiviral potential and the toxicity of these 4-O-(difluoromethyl)-5-substituted-uracil nucleoside analogues were evaluated in human embryonic lung fibroblast (HELF) cell cultures infected with herpes simplex virus type 1 (HSV-1) and herpes simplex virus type 2 (HSV-2) by using assay systems that have proven useful for comparison of antiherpes efficacy of nucleoside analogues.[14,16a] 9-[(2-Hydroxyethoxy)methyl]guanine (ACV), (E)-5-(2-bromovinyl)-2′-deoxyuridine (BrV-dUrd), 5-bromo-2′-deoxyuridine (BrdUrd), 5-ethyl-2′-deoxyuridine (EthdUrd), and 1-β-D-arabinofuranosylthymine (araT) as well as 5-bromo-, 5-iodo- and (E)-1-β-D-arabinofuranosyl-5-(2-bromovinyl)uracil (BraraU, IaraU, BrV-araU) were used as reference compounds. Their inhibition data obtained in present investigations correspond to previously reported values.[14] ACV and araT exhibited a 50% inhibition of HSV-1 plaque formation (ID_{50}) at concentrations of 0.48 and 0.64 μM, respectively, whereas BrV-araU and BrV-dUrd were nearly 5–10 times more effective (Table I). In contrast to BrV-araU and BrV-dUrd, which displayed a nearly 500-fold lower inhibitory effect against HSV-2 compared with HSV-1, ACV and araT were only 2–3-fold less active against HSV-2. However, the four 4,5-disubstituted uracil ribonucleosides **4a–d** were devoid of any antiherpes activity even at concentrations of 250–1000 μM (Table I), except for the 5-fluoro analogue **4b**, which exhibited an ID_{50} of 500 μM. It was not possible to determine the ID_{50}'s for the other analogues **4a**, **4c**, and **4d** because higher concentrations were cytotoxic (Table I, right column). The seven 4-O-(difluoromethyl)-5-substituted-uracil arabino nucleosides **5a–g** exhibited partially significant antiviral activity against HSV-1 and HSV-2, with the following order of decreasing efficiency: **5e = 5g > 5c > 5f = 5d > 5b > 5a** (bearing methyl, 2-bromovinyl, bromo, ethyl, iodo, fluoro, and hydrogen at the C-5 position of uracil) against HSV-1, and **5e > 5c > 5d > 5f = 5b** against HSV-2. Compounds **5g** and **5a** did not suppress HSV-2 replication in concentrations as high as 500–1000 μM. The inhibitory effect of the 4,5-disubstituted araU derivatives **5a–g** was more pronounced against HSV-1 than against HSV-2, as is usual for 5-substituted araU analogues.[14] Only one of the new analogues (**5e**) had anti-HSV activity comparable to that of ACV and araT. BrV-dUrd was 15–20-fold more potent against HSV-1 than **5e** and **5g**. The other analogues **5a–d** and **5f** displayed anti-HSV activities at concentrations much higher than those of the reference compounds, but the bromo derivative **5c** was comparable with BrV-dUrd in its anti-HSV-2 effect (Table I). Compared with 5-substituted araU nucleosides previously described[14] (see also reference compounds in Table I), the substitution of the 4-hydroxy hydrogen atom by a difluoromethyl group led to a marginal reduction of the antiherpes potential for the 4,5-disubstituted araU derivatives with the exception of **5d** and **5e**, exhibiting activities in accord with 5-iodo-araU and araT, respectively. Only the new 5-(2-bromovinyl)derivative **5g** had a nearly 10 times lower anti-HSV activity. The 4-O-difluoromethyl analogues of araT and BrV-araU (**5e** and **5g**) showed the

Table II. Antiherpes Activity of Compound 5e and the Reference Drug araT in HELF Cell Cultures

	ID_{50},[a] μM				
compd	HSV-1[b]	HSV-2[b]	VZV[c]	HCMV[d]	HSV-1-(TK⁻)[e]
5e	0.8	3	1	>500	>500
araT	0.64	1.65	0.53	>500	>500

[a] (Inhibitory dose)$_{50}$ = concentration required to reduce plaque formation of herpes viruses by 50% (2 days p.i. for HSV-2; 3 days p.i. for HSV-1; 5–6 days p.i. for VZV; 8–12 days p.i. for HCMV). [b] Data from Table I. [c] Fresh clinical isolate of VZV, strain 3/S.H. [d] Laboratory strain HCMV Davis. [e] Thymidine kinase deficient strain of HSV-1 (B 2006).

largest margin between antiviral potency and cytotoxicity (Table I), indicating a high selectivity of the antiherpes viral effect, comparable with that of acyclovir, araT, and BrV-araU. Compounds **5a–g** were not cytotoxic for HELF cells at concentrations as high as 500–1000 μM.

Extending the antiviral activity test spectrum to varicella zoster virus (VZV), human cytomegalovirus (HCMV), and a thymidine kinase deficient (TK⁻) HSV-1 strain (B 2006), we found that **5e**, like araT, strongly inhibited VZV, but was ineffective against HCMV and the HSV-1 (B 2006) TK⁻ strain (see Table II). Obviously, as in the case of araT, phosphorylation by the virus-coded thymidine kinase is a prerequisite step to the antiherpes activity of **5e**. In terms of selectivity, no differences in toxicity of araT and **5e** for nonproliferating HELF cell cultures in concentrations as high as 1000 μM were observed. Preliminary data to the cytostatic effect on actively growing HELF cells (two new cell generations) indicate a more pronounced inhibitory potential of **5e** compared with araT in concentrations higher than 500 μM (data not shown).

Of the three 4,5-disubstituted 2′-deoxyuridine (dUrd) derivatives tested, the 5-bromo compound **6c** inhibited HSV-1 replication at more than 2000-fold higher concentrations than BrV-dUrd, whereas the methyl and ethyl derivatives **6e** and **6f** were devoid of any anti-HSV effect at high concentrations (Table I). It is noteworthy that the corresponding 5-substituted dUrd analogues, 5-bromo- and 5-ethyl-dUrd, displayed high antiherpes activity (see Table I), indicating that in contrast to 5-substituted araU's the introduction of a 4-*O*-difluoromethyl substituent into 5-substituted dUrd's led to a strong reduction or loss of the antiviral effect.

Further studies are in progress to evaluate the antiviral effect of the new 4-*O*-difluoromethyl analogues against varicella zoster virus, cytomegalovirus, and Epstein–Barr virus.

Experimental Section

Melting points were determined on a Boetius melting point apparatus and are uncorrected. Thin-layer chromatography (TLC) was done on Merck silica gel F_{254} plates (0.2 mm). Preparative separations were carried out by silica gel column chromatography followed by separation on Molselect Sephadex G 10. UV spectra were recorded with a Specord spectrophotometer from Carl Zeiss Jena and NMR spectra with a Tesla BS 487 C 80-MHz spectrometer. Mass spectral data were taken with a Hewlett-Packard GC/MS 5995 A instrument equipped with an EI source at 70 eV. High-resolution mass spectra were obtained with a MAT 212 spectrometer, Finnigan MAT Bremen. The elemental analyses were within ±0.4% of the theoretical values. Uridine, thymidine, and 5-bromo-2′-deoxyuridine were commercially available from SERVA.

Silylated 5-Substituted Uracil Nucleosides 1a–d, 2a–g, 3c,e,f. 5-Substituted uracil nucleosides (3 mmol) were stirred at 120 °C with hexamethyldisilazane (20 mmol) with exclusion of atmospheric moisture. After complete dissolution, the mixture was evaporated in vacuo to a syrup. Without further purification, the syrup was used for the following reactions.

4-*O*-(Difluoromethyl)uridine (4a). A stirred solution of 1a (3 mmol, 1.6 g) in dry tetrahydrofuran (THF) (10 mL) was refluxed in a nitrogen atmosphere. Sodium iodide (15 mmol, 2.25 g) was added. A solution of bis(trifluoromethyl)mercury in dry THF (2 g, 6 mmol in 10 mL) was slowly dropped to the stirred suspension. After being stirred for 1 h, the solution was concentrated in vacuo, and the residue was partitioned between water and chloroform. The aqueous layer was extracted with chloroform (2 × 50 mL), and to the filtered chloroform layer were added 50 mL of methanol and 0.5 mL of concentrated hydrochloric acid. After 1 h the solution was concentrated, and the residue was again partitioned between water and chloroform. The chloroform layer was extracted with water (2 × 50 mL). The aqueous layers were evaporated to dryness, and the resulting syrup was purified by column chromatography (silica gel, $CHCl_3$–MeOH, 95:5) followed by a further purification on Sephadex G 10. Crystallization from ethanol gave 4a (0.48 g, 54.4%): mp 152–153 °C; MS, *m/e* 294 (M^+); ^{1}H NMR (Me_2SO-d_6) δ 7.98 (d, 1, $^3J_{H\text{-}5,H\text{-}6}$ = 8.0 Hz, H-6), 7.51 (t, 1, $^2J_{H,F}$ = 57.0 Hz, OCF_2H), 5.73 (d, 1, $^3J_{H\text{-}5,H\text{-}6}$ = 8.0 Hz, H-5), 5.67 (d, 1, $^3J_{H\text{-}1',H\text{-}2'}$ = 4.5 Hz, H-1′), 5.38 (br d, 1, OH), 5.05 (m, 2, 2 × OH), 4.00–3.83 (m, 3, H-2′, H-3′, H-4′), 3.53 (m, 2, CH_2); UV λ_{max} (MeOH) 269 nm (ε 9600). Anal. ($C_{10}H_{12}N_2O_6F_2$) C, H, N.

4-*O*-(Difluoromethyl)-5-fluorouridine (4b). This compound was prepared by the same method as described above by treatment of 3 mmol (1.65 g) of **1b** and yielded 0.47 g (1.5 mmol) of 4b (50.1%): mp 174–175 °C; MS, *m/e* 312 (M^+); ^{1}H NMR (Me_2SO-d_6) δ 8.48 (d, 1, $^3J_{H\text{-}6,F\text{-}5}$ = 7.0 Hz, H-6), 7.63 (t, 1, $^2J_{H,F}$ = 57.0 Hz, OCF_2H), 5.67 (d, 1, $^3J_{H\text{-}1',H\text{-}2'}$ = 3.0 Hz, H-1′), 5.35 (m, 2, 2 × OH), 5.25 (s, 1, OH), 4.01–3.88 (m, 3, H-2′, H-3′, H-4′), 3.63 (m, 2, CH_2); UV λ_{max} (MeOH) 273 nm (ε 8850). Anal. ($C_{10}H_{11}N_2O_6F_3$) C, H, N.

4-*O*-(Difluoromethyl)-5-bromouridine (4c). The compound was prepared by the method as described for 4a: yield, 43.5%; white foam; MS, *m/e* 372/374 (M^+); ^{1}H NMR (Me_2SO-d_6) δ 8.59 (s, 1, H-6), 7.59 (t, 1, $^2J_{H,F}$ = 57.0 Hz, OCF_2H), 5.60 (d, 1, $^3J_{H\text{-}1',H\text{-}2'}$ = 3.0 Hz, H-1′), 5.45 (s, 1, OH), 5.35 (s, 1, OH), 5.04 (s, 1, OH), 3.99–3.74 (m, 3, H-2′, H-3′, H-4′), 3.59 (m, 2, CH_2); UV λ_{max} (MeOH) 287 nm (ε 7700). Anal. ($C_{10}H_{11}N_2O_6F_2Br$) C, H, N, Br.

4-*O*-(Difluoromethyl)-5-iodouridine (4d). For the preparation was used the method of preparation of **4a** by starting from 3 mmol (2.0 g) of **1d**: yield, 0.59 g (1.4 mmol, 46.7%); mp 177–178 °C; MS, *m/e* 420 (M^+); ^{1}H NMR (Me_2SO-d_6) δ 8.57 (s, 1, H-6), 7.54 (t, 1, $^2J_{H,F}$ = 57.0 Hz, OCF_2H), 5.57 (d, 1, $^3J_{H\text{-}1',H\text{-}2'}$ = 3.5 Hz, H-1′), 3.91–3.83 (m, 3, H-2′, H-3′, H-4′), 5.36 (s, 1, OH), 5.23 (s, 1, OH), 4.97 (s, 1, OH), 3.55 (m, 2, CH_2); UV λ_{max} (MeOH) 296 nm (ε 7600). Anal. ($C_{10}H_{11}N_2O_6F_2I$) C, H, N, I.

4-*O*-(Difluoromethyl)-5-bromo-2′-deoxyuridine (6c). Compound **6c** was obtained by the same method as described for 4a by starting from **3c** (1.2 g, 2 mmol): yield, 0.33 g (0.92 mmol), 46.2%); white foam; MS, *m/e* 356/358; ^{1}H NMR (Me_2SO-d_6) δ 8.34 (s, 1, H-6), 7.58 (t, 1, $^2J_{H,F}$ = 57.0 Hz, OCF_2H), 6.03 (t, 1, $^3J_{H\text{-}1',H\text{-}2',2''}$ = 6.6 Hz, H-1′), 4.17 (m, 1, H-3′), 3.68 (m, 1, H-4′), 3.50 (m, 2, CH_2 C-5′), 2.05 (dd, 2, CH_2 C-2′); UV λ_{max} (MeOH 287 nm (ε 7800). Anal. ($C_{10}H_{11}N_2O_5F_2Br$).

4-*O*-(Difluoromethyl)thymidine (6e). The method as described for 4a was used by starting from 3 mmol of **3e** (1.4 g): yield, 0.35 g (1.2 mmol, 39.8%); white foam; MS, *m/e* 292 (M^+); ^{1}H NMR (Me_2SO-d_6) δ 7.78 (s, 1, H-6), 7.57 (t, 1, $^2J_{H,F}$ = 57.0 Hz, OCF_2M), 6.03 (t, 1, $^3J_{H\text{-}1',H\text{-}2',2''}$ = 6.6 Hz, H-1′), 5.13 (s, 1, OH), 4.97 (s, 1, OH), 4.15 (m, 1, H-3′), 3.67 (m, 1, H-4′), 3.49 (m, 2, CH_2 C-5′), 2.03 (dd, 2, $^3J_{H\text{-}1',H\text{-}2',2''}$ = 6.6 Hz, CH_2C-2′), 1.73 (s, 3, CH_3); UV λ_{max} (MeOH) 275 nm (ε 9200). Anal. ($C_{11}H_{14}N_2O_5F_2$) C, H, N.

4-*O*-(Difluoromethyl)-5-ethyl-2′-deoxyuridine (6f). Compound **6f** can be isolated as a white foam by following the general procedure as described above, starting from 2.5 mmol (1.2 g) of **3f**: yield, 0.29 g (0.94 mmol, 37.5%); white foam; MS, *m/e* 306 (M^+); ^{1}H NMR (Me_2SO-d_6) δ 7.78 (s, 1, H-6), 7.57 (t, 1, $^2J_{H,F}$ = 57.0 Hz, OCF_2H), 6.06 (t, 1, $^3J_{H\text{-}1',H\text{-}2',2''}$ = 6.5 Hz, H-1′), 5.18 (s, 1, OH), 4.97 (s, 1, OH), 4.15 (m, 1, H-3′), 3.70 (m, 1, H-4′), 3.52 (m, 2, CH_2 C-5′), 2.05 (dd, 2, $^3J_{H\text{-}1',H\text{-}2',2''}$ = 6.5 Hz, CH_2 C-2′), 2.15 (q, 2, ^3J = 7.0 Hz, CH_2-ethyl), 0.96 (t, 3, ^3J = 7.0 Hz, CH_3); UV λ_{max} (MeOH) 271.5 nm (ε 10 400). Anal. ($C_{12}H_{16}N_2O_5F_2$) C, H, N.

1-β-D-**Arabinofuranosyl-4-*O*-(difluoromethyl)uracil (5a)**. The compound was prepared by the method as described for **4a** by starting from **2a** (3 mmol, 1.6 g): yield, 0.42 g (1.43 mmol, 47.8%); mp 155–156 °C; MS, *m/e* 294 (M^+); ^1H NMR (Me_2SO-d_6) δ 7.65 (d, 1, $^3J_{H\text{-}5,H\text{-}6}$ = 8.2 Hz, H-6), 7.56 (t, 1, $^2J_{H,F}$ = 57 Hz, OCF_2H), 5.86 (d, 1, $^3J_{H\text{-}1',H\text{-}2'}$ = 4 Hz, H-1′), 5.63 (d, 1, $^3J_{H\text{-}5,H\text{-}6}$ = 8.2 Hz, H-5), 5.38 (s, 2, OH 2×), 4.90 (s, 1, OH), 3.91 (dd, 1, H-2′), 3.76–3.63 (m, 2, H-3′, H-4′), 3.51 (m, 2, CH_2 C-5′); UV λ_{max} (MeOH) 271 nm (ε 9800). Anal. ($C_{10}H_{12}N_2O_6F_2$) C, H, N.

1-β-D-**Arabinofuranosyl-4-*O*-(difluoromethyl)-5-fluorouracil (5b)**. The compound was prepared as described above by starting from **2b** (2 mmol, 0.52 g): yield, 0.35 g (1.12 mmol, 56.0%); white foam; MS, *m/e* 312 (M^+); ^1H NMR (Me_2SO-d_6) δ 8.37 (d, 1, $^3J_{F\text{-}5,H\text{-}6}$ = 7.8 Hz, H-6), 7.91 (t, 1, $^2J_{H,F}$ = 57 Hz, OCF_2H), 6.18 (d, 1, $^3J_{H\text{-}1',H\text{-}2'}$ = 4 Hz, H-1′), 5.86 (s, 1, OH), 5.73 (s, 1, OH), 5.4 (s, 1, OH), 4.28–3.86 (m, 3, H-2′, H-3′, H-4′), 3.63 (m, 2, CH_2 C-5′); UV λ_{max} (MeOH) 277.5 nm (ε 7800). Anal. ($C_{10}H_{11}N_2O_6F_3$) C, H, N.

1-β-D-**Arabinofuranosyl-4-*O*-(difluoromethyl)-5-bromouracil (5c)**. Starting from 2 mmol (0.65 g) of **2c**, we used the same procedure as described for **4a**: yield, 0.39 g (1.05 mmol, 52.7%); white foam; MS, *m/e* = 372/374 (M^+); ^1H NMR (Me_2SO-d_6) δ 8.14 (s, 1, H-6), 7.59 (t, 1, $^2J_{H,F}$ = 57 Hz, OCF_2H), 5.88 (d, 1, $^3J_{H\text{-}1',H\text{-}2'}$ = 4.4 Hz, H-1′), 5.60 (s, 2, OH 2×), 5.00 (s, 1, OH), 4.00 (dd, 1, H-2′), 3.82 (m, 1, H-3′), 3.70 (m, 1, H-4′), 3.53 (m, 1, CH_2 C-5′); UV λ_{max} (MeOH) 287.5 nm (ε 7500). Anal. ($C_{10}H_{11}N_2O_6BrF_2$) C, H, N, Br.

1-β-D-**Arabinofuranosyl-4-*O*-(difluoromethyl)-5-iodouracil (5d)**. Compound **2d** (3 mmol, 1.11 g) was treated with CF_2 as described for 4a to yield 0.72 g (1.71 mmol, 57.0%) of **5d**: mp 207–208 °C; MS, *m/e* 420 (M^+); ^1H NMR (Me_2SO-d_6) δ 8.15 (s, 1, H-6), 7.57 (t, 1, $^2J_{H,F}$ = 57.0 Hz, OCF_2H), 5.85 (d, 1, $^3J_{H\text{-}1',H\text{-}2'}$ = 4.2 Hz, H-1′), 5.60 (s, 2, OH 2×), 4.72 (d, 1, OH), 3.98 (dd, 1, H-2′), 3.80–3.67 (m, 2, H-3′, H-4′), 3.55 (m, 2, CH_2 H-5′); UV λ_{max} (MeOH) 296.5 nm (ε 7400). Anal. ($C_{10}H_{11}N_2O_6F_2I$) C, H, N, F, I.

1-β-D-**Arabinofuranosyl-4-*O*-(difluoromethyl)thymine (5e)**. Compound **2e** (5 mmol, 1.30 g) yielded 0.96 g (3.1 mmol, 62.1%) of **5e** following the above-mentioned procedure: mp 139–140 °C; MS, *m/e* 308 (M^+); ^1H NMR (Me_2SO-d_6) δ 7.58 (s, 1, H-6), 7.58 (t, 1, $^2J_{H,F}$ = 57 Hz, OCF_2H), 5.87 (d, 1, $^3J_{H\text{-}1',H\text{-}2',2''}$ = 4.0 Hz, H-1′), 5.51 (d, 1, $^3J_{H\text{-}2',OH\text{-}2'}$ = 5 Hz, OH-2′), 5.39 (d, 1, $^3J_{H\text{-}3',OH\text{-}3'}$ = 4.5 Hz, OH-3′), 5.03 (t, 1, $^3J_{H\text{-}5',5'',OH\text{-}5'}$ = 5 Hz, OH-5′), 3.95 (dd, 1, H-2′), 3.83 (m, 1, H-3′), 3.67 (m, 1, H-4′), 3.57 (m, 2, CH_2-5′), 1.75 (s, 3, CH_3); UV λ_{max} (MeOH) 276.5 nm (ε 9000). Anal. ($C_{11}H_{14}N_2O_6F_2$) C, H, N.

1-β-D-**Arabinofuranosyl-4-*O*-(difluoromethyl)-5-ethyluracil (5f)**. Compound **2f** (4 mmol, 1.3 g) yielded 0.67 g (2.1 mmol, 52.0%) of **5f** as a white foam by the procedure described for 4a: MS, *m/e* = 322 (M^+); ^1H NMR (Me_2SO-d_6) δ 7.60 (s, 1, H-6), 7.57 (t, 1, $^2J_{H,F}$ = 57 Hz, OCF_2H), 5.81 (d, 1, $^3J_{H\text{-}1',H\text{-}2'}$ = 4.5 Hz, H-1′), 3.97 (m, 1, H-2′), 3.77 (m, 1, H-3′), 3.65 (m, 1, H-4′), 3.55 (m, 2, CH_2 C-5′), 2.14 (q, 2, $^3J_{CH_2,CH_3}$ = 7.0 Hz, CH_2, ethyl), 0.97 (t, 3, $^3J_{CH_2,CH_2}$ = 7.0 Hz, CH_3, ethyl); UV λ_{max} (H_2O) = 277 nm (ε 10 600). Anal. ($C_{12}H_{16}N_2O_6F_2$) C, H, N.

(*E*)-1-β-D-**Arabinofuranosyl-4-*O*-(difluoromethyl)-5-(2-bromovinyl)uracil (5g)**. Compound **2g** (3 mmol, 1.04 g) yielded 0.55 g (1.4 mmol, 46.2%) of **5g** following the above-mentioned procedure: mp 199–203 °C dec; MS, *m/e* 396/398 (M^+); ^1H NMR (Me_2SO-d_6) δ 8.25 (s, 1, H-6), 7.70 (t, 1, $^2J_{H,F}$ = 57 Hz, OCF_2H), 7.42 (d, 1, $^3J_{CH,CHBr}$ = 14 Hz, CH bromovinyl group), 7.05 (d, 1, $^3J_{CHBr,CH}$ = 14 Hz, CHBr), 6.43 (d, 1, $^3J_{H\text{-}1',H\text{-}2}$ = 4.5 Hz, H-1′), 4.01–3.7 (m, 3, H-2′, H-3′, H-4′), 3.54 (m, 2, CH_2C-5′); UV λ_{max} (H_2O) 305 nm (ε 5360). Anal. ($C_{12}H_{13}N_2O_6f_2Br$) C, H, N, Br.

In Vitro Antiviral Assay. Inhibition of Virus Plaque Formation. Confluent monolayers of HELF cells were incubated for 1 h at 37 °C with virus suspensions of the clinical isolates HSV-1 strain 77 and HSV-2 strain 74[7] yielding 50–100 plaques per bottle. After the virus adsorption period, a methocel (0.5% W/V) overlay medium containing 10% fetal calf serum and appropriate substance solutions were added to each culture. Following further incubation at 37 °C (3 days for HSV-1 and 2 days for HSV-2), monolayers were stained with neutral red dye for plaque visualization. The ID_{50} was determined graphically as the drug concentration in micromoles/liter required to reduce virus plaque numbers by 50%.[14,16a]

In Vitro Toxicity Studies. Semiconfluent monolayers of HELF cells, 24 h after seeding, were treated for 3 days at 37 °C with concentrations of the nucleoside analogues as high as 1 mmol/L. After this treatment period, the cell cultures were evaluated microscopically for cytotoxic alterations of normal HELF cell morphology compared with untreated control cell cultures. Typical signs of toxicity were taken to be detachment of cells, destruction of the cell monolayers, or maintenance of semiconfluency. The minimal concentration not causing a microscopically detectable cytotoxic alteration of cell morphology was determined.

Registry No. **1a**, 51432-30-5; **1b**, 111582-83-3; **1c**, 111582-94-6; **1d**, 111582-84-4; **2a**, 53294-28-3; **2b**, 111582-86-6; **2c**, 111582-87-7; **2d**, 111582-88-8; **2e**, 111582-89-9; **2f**, 111582-90-2; **2g**, 111582-92-4; **3c**, 34279-87-3; **3e**, 34279-88-4; **3f**, 111582-85-5; **4a**, 102302-59-0; **4b**, 102302-60-3; **4c**, 102302-61-4; **4d**, 102302-62-5; **5a**, 102302-66-9; **5b**, 102302-67-0; **5c**, 102302-68-1; **5d**, 102302-69-2; **5e**, 102302-70-5; **5f**, 111582-91-3; **5g**, 111582-93-5; **6c**, 102302-65-8; **6e**, 102302-63-6; **6f**, 102302-64-7; $Hg(CF_3)_2$, 371-76-6.