evaporated under reduced pressure. The residue was extracted with hot ethanol several times; the extract was concentrated to dryness, and the residue was recrystallized from ethanol, giving 0.25 g (59%) of the oxime of **4**, mp 227–228 °C. Anal. ($C_{12}H_{19}N_2O_5P$), C, H.

[(4-Formyl-3-hydroxy-2-methyl-5-pyridyl)methyl]phosphonic Acid (2) and Ethyl [(4-Formyl-3-hydroxy-2-methyl-5-pyridyl)methyl]phosphonate (3). A solution of **4** (1 g, 3.45 mmol) in 20% HCl (50 mL) was refluxed, hydrolysis being monitored by removal of small portions, dilution into 0.1 N HCl, and measurement of the UV–visible spectrum. Refluxing was continued for about 5 h until the absorbance ratio A_{296nm}/A_{339nm} attained its minimal value of about 1.8. The solution was concentrated under vacuum and applied to a 1.2 × 50 cm column of Dowex 50W-X4 (100–200 mesh) ion-exchange resin in the H^+ form and well washed with water. The column was eluted under nitrogen with water at a flow rate of 36 mL/h. The eluate was monitored at 280 nm. Compound **3** appeared in the second peak and compound **2** in the third peak detected in this way; selected fractions were concentrated under vacuum and lyophilized: yield 51 mg (40%) of **2** and 26 mg (20%) of **3**. Both **2** and **3** were crystallized from water–acetone. NMR (D_2O) of **2**: δ 2.38 (s, 3 H), 3.29 (d, 2 H), 7.5 (s, 1 H, broad), 10.13 (s, 1 H, broad). NMR (D_2O) of **3**: δ 1.09 (t, 3 H), 2.31 (d, 3 H), 3.22 (d, 2 H), 3.71 (sextet, 2 H), 7.41 (s, 1 H, broad), 10.10 (s, 1 H, broad). Mass spectra by fission fragment ionization for **4**, m/z 230 [$(M-1)^-$], 212 [$(M-1-H_2O)^-$], 202 [$(M-1-CO)^-$], 80 [$(PO_3H)^-$], 63 [$(PO_2)^-$], 49 [$(POH_2)^-$]; for **3**, m/z 258 [$(M-1)^-$], 230 [$(M-C_2H_5)^-$], 228 [$(M-1-C_2H_6)^-$], 212 [$(M-1-C_2H_5OH)^-$], 79 [$(PO_3)^-$], 63 [$(PO_2)^-$], 49 [$(POH_2)^-$]. Electrophoresis: both **2** and **3** migrated toward the anode at about the same rate and a little slower than **1** at pH 6.4. A trace impurity, detectable by fluorescence, migrated ahead of **2** and **3**. The IR spectra (KBr) of **4** and **8** show the three special peaks of phosphate or phosphonate groups at ca. 1245, 1030 (P–O–C), and 1160 (P–O–C) cm^{-1},[20] the aldehyde peaks of both **2** and **4** appear at 1661 cm^{-1}.

Enzyme Assay. The enzymatic activity of aspartate aminotransferase was assayed by direct spectrophotometric observation of oxaloacetate formation as described by Furbish et al.[3] Glutamate decarboxylase activity was assayed manometrically[21] at pH 4.6.

Acknowledgment. This work was supported in part by Research Grant AM-01549 from the United States Public Health Service. We thank Kevin Kelly for assistance and Frank H. Field and the Rockefeller University Mass Spectrometric Biotechnology Research Resource for the fission fragment ionization mass spectra.

Registry No. **2**, 26210-18-4; **3**, 84521-12-0; **4**, 84521-13-1; **4** (oxime derivative), 84521-14-2; **6**, 14142-90-6; **7**, 84521-15-3; **8**, 84537-05-3.

(20) K. Nakanishi, "Infrared Absorption Spectroscopy—Practical"; Holden-Day: San Francisco; Nankodo: Tokyo, 1962; p 56.
(21) M. L. Fonda, *J. Biol. Chem.*, **246**, 2230 (1971).

Synthesis and Biological Activity of 5-(Trifluoromethyl)- and 5-(Pentafluoroethyl)pyrimidine Nucleoside Analogues

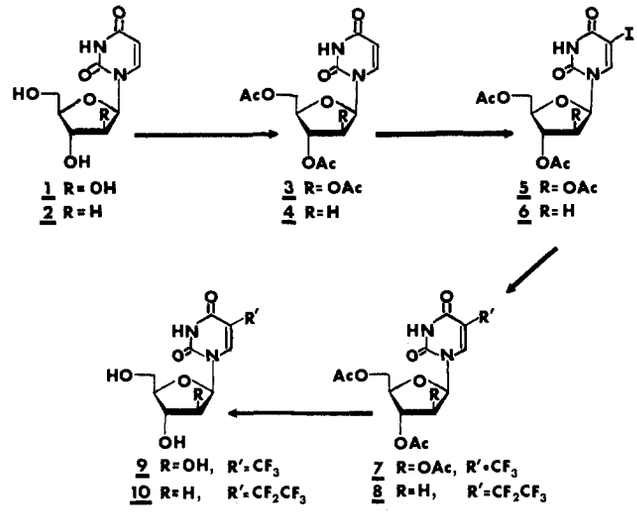
Tai-Shun Lin* and You-Song Gao[1]

Department of Pharmacology and Comprehensive Cancer Center, Yale University School of Medicine, New Haven, Connecticut 06510. *Received August 16, 1982*

Various 5-substituted perfluoroalkylpyrimidine nucleoside analogues have been synthesized, and their biological activity against L1210, S-180, Vero cells, and herpes simplex virus type 1 (HSV-1) was evaluated. The 5-trifluoromethyl derivatives, **7** and **9**, showed significant antiviral activity against HSV-1 with ED_{50} values of 7 and 5 μM, respectively. In addition, the unblocked nucleoside **9** was found to be about 64-fold less toxic to the host Vero cells and gave a favorable therapeutic index of 64 against HSV-1 in vitro.

Modifications of nucleosides in the sugar, the pyrimidine, or purine base, or both, have produced compounds with potent antiviral or anticancer activity. Arabinofuranosyl analogues of "normal" nucleoside with potent antiviral or anticancer activity include 1-β-D-arabinofuranosyladenine (*ara*-A), 1-β-D-arabinofuranosylcytosine (*ara*-C), and 1-β-D-arabinofuranosylthymine (*ara*-T).[2-5] In view of the biological activity of *ara*-T and other analogues of thymidine, a number of analogues of *ara*-T have been synthesized with varying substituents replacing the methyl moiety of *ara*-T, such as 1-β-D-arabinofuranosyl-5-ethyluracil,[6] 1-β-D-arabinofuranosyl-5-vinyluracil,[7] and 1-β-D-arabinofuranosyl-(*E*)-5-(2-bromovinyl)uracil.[8,9] Biological activity has also been obtained with arabinofuranosyluracil and arabinofuranosylcytosine substituted in the 5-position

Scheme I



(1) Visiting scientist from the Institute of Materia Medica, Chinese Academy of Medical Science, Beijing, The People's Republic of China.
(2) de Rudder, J.; Privat de Garilhe, M. *Pathol. Bacteriol.* **1966**, *14*, 369.
(3) Cohen, S. S. *Prog. Nucleic Acid Res. Mol. Biol.* **1966**, *5*, 1.
(4) North, T. W.; Cohen, S. S. *Pharmacol. Ther.* **1979**, *4*, 81.
(5) Gentry, G. A.; Aswell, J. F. *Virology* **1975**, *65*, 294.
(6) Machida, H.; Sakata, S.; Kuninaka, A.; Yoshino, H.; Nakayama, C.; Saneyoshi, M. *Antimicrob. Agents Chemother.* **1979**, *16*, 158.
(7) Machida, H.; Kuninaka, A.; Yoshino, H.; Ikeda, K.; Mizuno, Y. *Antimicrob. Agents Chemother.* **1980**, *17*, 1030.
(8) Machida, H.; Skata, S.; Kuninaka, A.; Yoshino, H. *Antimicrob. Agents Chemother.* **1981**, *18*, 47.
(9) Sakata, S.; Shibuya, S.; Machida, H.; Yoshino, H.; Hirota, K.; Senda, S.; Ikeda, K.; Mizuno, Y. *Nucleic Acids Res. Symp. Ser.* **1980**, *8*, s39.

0022-2623/83/1826-0598$01.50/0

Table I. Effect of Various 5-(Perfluoroalkyl)deoxyribopyrimidine Nucleoside Analogues on the Replication of L1210 Cells, Sarcoma 180 Cells, Vero Cells, and Herpes Simplex Virus Type 1 in Vitro[a]

				% inhibition			
compd	R	R′	concn, μM	L1210	S-180	vero cells	HSV-1
7	OAc	CF_3	5			0	17
			10				61
			20				92
			25	13	47	8	
			40				97
			50	58	60	57	
			80				98
			100	95	95	100	
8	H	CF_2CF_3	1.25				26
			12.5	14	37		51
9	OH	CF_3	10				74
			25	19	41		
			50	33	46	0	88
			75				99
			100	66	59	4	99
			200	84	71	14	
			400	87		66	
10	H	CF_2CF_3	100	17	28		15
			400				30
5-(trifluoromethyl)-2′-deoxyuridine[b]			50				99
			10			100	
			1			63	
			0.1		100		

[a] Assays were carried out in triplicate with appropriate controls. [b] Data from Lin, T. S.; Chai, C.; Prusoff, W. H. *J. Med. Chem.* 1976, *19*, 915.

Table II. ED_{50} Values of Various 5-(Perfluoroalkyl)deoxyribopyrimidine Nucleoside Analogues on the Replication of L1210 Cells, Sarcoma 180 Cells, Vero Cells, and Herpes Simplex Virus Type 1 in Vitro

			ED_{50},[a] μM				therapeutic index: ED_{50} (Vero)/ED_{50} (HSV-1)
compd	R	R′	L1210	S-180	Vero	HSV-1	
7	OAc	CF_3	45	25	45	7	6.4
8	H	CF_2CF_3				12.5	
9	OH	CF_3	70	65	320	5	64
10	H	CF_2CF_3				>400	

[a] The ED_{50} values were estimated from dose-response curves compiled from at least two independent experiments and represent the drug concentration required to inhibit replication of L1210 cells, S-180 cells, Vero cells, and HSV-1 by 50%.

with various halogens.[10-12] Modification of the arabinose moiety, as well as the pyrimidine, has resulted, in some instances, in very potent antiviral compounds, such as 1-(2-fluoro-2-deoxy-β-D-arabinofuranosyl)-5-iodocytosine (FIAC)[13,14] and its metabolic derivative, 1-(2-fluoro-2-deoxy-β-D-arabinofuranosyl)-5-methyluracil (FMAU).[13,14] The replacement of the 5′-hydroxy group of 5-iodo-2′-deoxyuridine (IdUrd) with an amino moiety produced 5-iodo-5′-amino-2′,5′-dideoxyuridine (AIdUrd), which retains significant antiviral activity and has a remarkable lack of toxicity.[15-17]

Because of the potent antiviral activity of 5-(trifluoromethyl)-[18] and 5-ethyl-2′-deoxyuridine,[19-21] various 5-(perfluoroalkyl)pyrimidine arabinofuranosyl and 2′-deoxyribofuranosyl nucleoside analogues have been synthesized and their biological activity evaluated.

Chemistry. Starting compound, *ara*-U (1), was prepared from uridine by the method of Hampton and Nichol.[22] Acetylation[23] of 1 and 2 with acetyl anhydride in pyridine at 4 °C for 24 h gave the acetates 3 and 4. Iodination[24] of 3 with iodine and CF_3COOAg afforded 5. Another 5-iodo derivative, 6, was obtained by two methods: (1) treatment of 4 with mercuric acetate and iodine in sodium acetate buffer;[15] (2) direct acetylation[25] of 5-iodo-2′-deoxyuridine (IUdR) in a mixture of glacial acetic acid,

(10) Fox, J. J.; Miller, N.; Wempen, I. *J. Med. Chem.* **1966**, *9*, 101.
(11) Underwood, G. E.; Wisner, C. A.; Wood, S. P. *Arch. Ophthal.* **1964**, *72*, 505.
(12) Renis, H. E.; Underwood, G. E.; Hunter, J. H. *Antimicrob. Agents Chemother.* **1967**, *4*, 675.
(13) Watanabe, K. A.; Reichmann, U.; Hirota, K.; Lopez, C.; Fox, J. J. *J. Med. Chem.* **1979**, *22*, 21.
(14) Fox, J. J.; Watanabe, K. A.; Lopez, C.; Philips, F. S.; Leyland Jones, B. "Herpesvirus—Clinical, Pharmacological and Basic Aspects"; Shiota, H.; Cheng, Y. C.; Prusoff, W. H., Eds.; Excerpta Medica: Amsterdam, Oxford, Princeton, 1982; p 135.
(15) Lin, T. S.; Neenan, J. P.; Cheng, Y. C.; Prusoff, W. H.; Ward, D. C. *J. Med. Chem.* **1976**, *19*, 495.
(16) Lin, T. S.; Prusoff, W. H. *J. Med. Chem.* **1978**, *21*, 106.
(17) Cheng, Y. C.; Goz, B.; Neenan, J. P.; Ward, D. C.; Prusoff, W. H. *J. Virology* **1975**, *15*, 1284.
(18) Heidelberger, C. "Antineoplastic and Immunosuppressive Agents"; Sartorelli, A. C.; Johns, D. G., Eds.; Springer: Berlin, 1975; Vol. 2, p 193.
(19) Walter, R. D.; Gauri, K. K. *Biochem. Pharmacol.* **1975**, *24*, 1025.
(20) Shugar, D. *FEBS Lett.* **1974**, *40*, 548.
(21) Kulikowski, T.; Shugar, D. *J. Med. Chem.* **1974**, *17*, 269.
(22) Hampton, A.; Nichol, A. W. *Biochemistry* **1966**, *5*, 2076.
(23) Brown, D. M.; Todd, A. R.; Varadarajan, S. *J. Chem. Soc.* **1956**, 2388.
(24) Kobayashi, Y.; Yamamoto, K.; Asai, T.; Nakano, M.; Kumadaki, I. *J. Chem. Soc., Perkin Trans.* 1, **1980**, 2755.
(25) Chang, P. K.; Welch, A. D. *J. Med. Chem.* **1963**, *6*, 428.

acetic anhydride, and acetyl chloride. Treatment of the 5-iodo derivatives **5** and **6** with the corresponding perfluoroalkyl iodide–copper complex[24] in hexamethylphosphoramide (HMPA) at 45–50 °C yielded the 5-trifluoromethyl- and 5-pentafluoroethyl-substituted analogues **7** and **8**, respectively. The perfluoroalkyl–copper complexes were prepared by the procedure of Kobayashi et al.[24] Deacetylation[26] of **7** and **8** by MeOH–HCl at 0 °C for 4 h gave the unblocked nucleosides **9** and **10**. The structure of these compounds were characterized by ^{1}H NMR, UV, and IR spectra and elemental analysis data.

Biological Activity. The antiviral activity of various compounds listed in Table I was determined. The effect of these compounds on the replication of mouse Sarcoma 180 neoplastic cells in culture was also investigated. The ED_{50} values (the drug concentration required to inhibit replication of L1210, S-180, Vero cells, and HSV-1 by 50%) that were estimated from dose–response curves are summarized in Table II.

The 5-trifluoromethyl-substituted derivatives, **7** and **9**, demonstrate significant antiviral activity against herpes simplex virus type 1, with ED_{50} values of 7 and 5 μM respectively. The acetate **7** is about equally cytotoxic to L1210, S-180, and Vero cells. At 100 μM, **7** inhibits the growth of L1210, S-180, and Vero cells by 95 to 100%; however, at the same concentration, the unblocked nucleoside **9** is about 25-fold less toxic to Vero cells (4% inhibition). Compound **9** gives a favorable therapeutic index of 64 against HSV-1 in vitro (Table II). Thus, **9** merits further investigation as an antiviral agent.

Since the antiviral activities of 5-(trifluoromethyl)-2′-deoxyuridine[18] and 5-ethyl-2′-deoxyuridine[19–21] are well established, it was unexpected that the 5-pentafluoroethyl analogues, **8** and **10**, are devoid of significant cytotoxicity against HSV-1 as well as other neoplastic cells in vitro.

Experimental Section

Melting points were taken on a Thomas-Hoover Unimelt apparatus and are not corrected. Thin-layer chromatography was performed on EM silica gel 60 F_{254} sheets (0.2 mm). IR spectra were recorded on a Perkin-Elmer-21 spectrophotometer. The UV spectra were recorded on a Beckman-25 spectrophotometer, and the NMR spectra were taken on a Bruker 270 HX and/or a Varian T-60 spectrophotometer at 270 and 60 MHz, respectively, with Me_4Si as internal reference. The elemental analyses were carried out by Baron Consulting Co., Analytical Services, Orange, CT. Where analyses are indicated only by symbols of the elements, the analytical results obtained for those elements were within ±0.4% of the theoretical values.

1-(2,3,5-Tri-*O*-acetyl-β-D-arabinofuranosyl)uracil (3). Acetic anhydride (37.6 g, 368 mmol) was added to an ice-cooled solution of 1-β-D-arabinofuranosyluracil (**1**; 9.00 g, 36.85 mmol) in 90 mL of pyridine. The reaction mixture was kept at 4 °C for 24 h. The solvent and excess Ac_2O were removed under reduced pressure, and the residue was coevaporated several times with EtOH. The product crystallized out from EtOH in the refrigerator overnight. The crystals were then collected by filtration, washed with cooled EtOH and Et_2O, and dried to yield 12.4 g (91%): mp 128–130 °C, (lit.[23] 129–130 °C); NMR (Me_2SO-d_6) δ 1.96, 2.02, and 2.04 (s, 9 H, $OCOCH_3$), 4.30 (m, 3 H, 4′-H and 5′-H), 5.25 (m, 2 H, 2′-H and 3′-H), 5.62 (d, 1 H, 5-H), 6.20 (d, 1 H, 1′-H, $J_{1',2'}$ = 4.0 Hz), 7.52 (d, 1 H, 6-H), 11.20 (br s, 1 H, 3-NH D_2O exchangeable).

3′,5′-Di-*O*-acetyl-2′-deoxyuridine (4). Compound **4** was prepared by the same procedure as described for the synthesis of **3**. Acetic anhydride (6.70 g, 65 mmol) was added slowly to an ice-cooled solution of **2** (3.0 g, 13 mmol) in 30 mL of pyridine with stirring. Upon isolation and crystallization from EtOH, 3.80 g (94%) of **4** was obtained: mp 102–104 °C; NMR (Me_2SO-d_6) δ 2.05 and 2.07 (d, 6 H, $OCOCH_3$), 2.26–2.51 (m, 2 H, 2′-H), 4.14–4.23 (m, 3 H, 4′-H and 5′-H), 5.18 (m, 1 H, 3′-H), 5.70 (d, 1 H, 5-H), 6.15 (t, 1 H, 1′-H, $J_{1',2'}$ = 6.34 Hz), 7.66 (d, 1 H, 6-H), 11.38 (s, 1 H, 3-NH, D_2O exchangeable).

1-(2,3,5-Tri-*O*-acetyl-β-D-arabinofuranosyl)-5-iodouracil (5). Compound **3** (4.00 g, 10.8 mmol) and CF_3COOAg (4.72 g, 21.4 mmol) were suspended and stirred in dry CH_2Cl_2 (200 mL) at 0 °C (ice–H_2O bath). A solution of iodine (8.24 g, 32.5 mmol) in dry CH_2Cl_2 (200 mL) was added to the suspension. Upon completion of the addition, the reaction mixture was then stirred for an additional 5 h at room temperature. Saturated aqueous $NaHCO_3$ solution (800 mL) was added, and the mixture was filtered through Celite. The organic layer was separated, washed with H_2O, saturated $Na_2S_2O_3$ solution, H_2O, and dried (Na_2SO_4). The solvent was evaporated to dryness, and the solid residue was recrystallized from EtOH–CH_2Cl_2 (10:1) to yield 5.0 g (93%): mp 184–186 °C; R_f 0.64 ($CHCl_3$–EtOH, 4:1): UV (MeOH) λ_{max} 283 nm (ε 8888),[35] λ_{min} 244 nm; NMR (Me_2SO-d_6) δ 1.98 and 2.18 (s, 9 H, $OCOCH_3$), 4.38 (m, 3 H, 4′-H and 5′-H), 5.30 (m, 2 H, 2′-H and 3′-H), 6.22 (d, 1 H, 1′-H, $J_{1',2'}$ = 4.0 Hz), 7.90 (s, 1 H, 6-H), 11.80 (br s, 1 H, 3-NH, D_2O exchangeable).

3′,5′-Di-*O*-acetyl-2′-deoxy-5-iodouridine (6). Method A. A solution of **4** (1.00 g, 3.24 mmol), in 100 mL of 0.5 M NaOAc buffer (pH 6.0) and 25 mL of EtOH, and mercuric acetate (4.00 g, 12.5 mmol) was heated at 50 °C with stirring overnight. The solution was cooled to room temperature, and a 0.2 M solution of iodine in EtOH (100 mL) was added. The reaction mixture was stirred for an additional 90 min and filtered. The filtrate was concentrated to a smaller volume (~20 mL) and then extracted with $CHCl_3$ (5 × 60 mL). The combined $CHCl_3$ solution was clarified with charcoal, dried ($MgSO_4$), and passed through a short silica gel column ($CHCl_3$–EtOH, 4:1). The solvent was evaporated to dryness in vacuo, and the solid residue was recrystallized from EtOH to afford 0.75 g (54%) of **6**: mp 158–160 °C (lit.[25] 158–160 °C).

Method B. Compound **6** was also prepared by a modification of a method reported by Chang and Welch.[25] A solution of 5-iododeoxyuridine (5.0 g, 0.014 mol), glacial acetic acid (50 mL), acetic anhydride (75 mL), and acetyl chloride (75 mL) was stirred at 0 °C for 1 h and then at room temperature for 24 h. The clear solution was concentrated in vacuo at 30 °C to remove the excess solvents. The residue was then dissolved and coevaporated 3 times with 25 mL of methanol. The crystalline residue that resulted was recrystallized from ethanol (120 mL) to yield fine, white, needle-like crystals of the diacetyl derivative (5.8 g, 95%), mp 163–164 °C (lit.[25] 158–160 °C).

The products synthesized from both methods A and B gave identical NMR spectra: NMR (Me_2SO-d_6) δ 2.06 and 2.10 (s, 6 H, $OCOCH_3$), 2.25–2.54 (m, 2 H, 2′-H), 4.18–4.26 (m, 3 H, 4′-H and 5′-H), 5.18 (m, 1 H, 3′-H), 6.11 (t, 1 H, 1′-H, $J_{1',2'}$ = 6.35 Hz), 8.05 (s, 1 H, 6-H), 11.76 (s, 1 H, 3-NH, D_2O exchangeable).

1-(2,3,5-Tri-*O*-acetyl-β-D-arabinofuranosyl)-5-(trifluoromethyl)uracil (7). Trifluoromethyl iodide (27 g, 138 mmol) and copper powder[27] (16.5 g, 260 mmol) in HMPA (100 mL) were stirred in a stainless-steel bomb at 110–120 °C for 3 h. The bomb was cooled to room temperature, and the excess copper powder was removed by filtration through Celite in a glove box with the exclusion of air (with argon). The dark green trifluoromethyl–copper complex solution was added to the 5- iodo nucleoside **5** (5.0 g, 10.1 mmol), and the reaction mixture was stirred in a glass pressure bottle under argon at 45–50 °C overnight (~19 h). The resulting solution was then added to ice–H_2O (700 mL) and extracted with EtOAc (6 × 300 mL). The combined EtOAc solution was washed with H_2O (2 × 200 mL), dried (Na_2SO_4), and evaporated to dryness under reduced pressure. The residue (brown gum) was purified by silica gel column (4 × 60 cm) chromatography (EtOAc–hexane, 8:1) to give 0.8 g (18%) of product: mp 178–180 °C; R_f 0.56 (EtOAc–hexane, 8:1), UV (MeOH) λ_{max} 260 nm (ε 12100), λ_{min} 227 nm; UV (0.01N HCl) λ_{max} 260 nm (ε 10350), λ_{min} 222 nm; UV (0.1 N NaOH) λ_{max} 260 nm (ε 7368), λ_{min} 238 nm; NMR (Me_2SO-d_6) δ 1.94, 2.04, and 2.09 (s, 9 H, $OCOCH_3$), 4.29 (m, 1 H, 4′-H), 4.34 (m, 2 H, 5′-H), 5.18 (m, 1 H, 3′-H), 5.33 (m, 1 H, 2′-H), 6.22 (d, 1 H, 1′-H, $J_{1',2'}$ = 4.59 Hz), 8.00 (s, 1 H,

(26) Watanabe, K. A.; Reichman, U.; Chu, C. K.; Hollenberg, D. H.; Fox, J. J. *J. Med. Chem.* **1980**, *23*, 1088.

(27) Brewster, R. O.; Groening, T. *Org. Synth.* **1948**, *2*, 445.

6-H), 12.06 (br s, 1 H, 3-NH, D_2O exchangeable). Anal. ($C_{16}H_{17}F_3N_2O_9$) C, H, F, N.

3',5'-Di-*O*-acetyl-2'-deoxy-5-(pentafluoroethyl)uridine (8). The synthesis of 8 followed the same method as described previously for the preparation of 7. Pentafluoroethyl iodide (21.6 g, 87.8 mmol) and copper powder[27] (18.0 g, 283.5 mmol) in HMPA (60 mL) were stirred and heated at 110–120 °C in a bomb for 3 h. The pentafluoroethyl–copper complex was then added to 6 (3.0 g, 6.9 mmol). The crude product was purified by silica gel column (4 × 60 cm) chromatography (Et_2O–EtOAC, 2:1) to afford 0.54 g (17%): mp 166–167 °C; UV (MeOH) λ_{max} 263 nm (ϵ 11 250), λ_{min} 232 nm; NMR (Me_2SO-d_6) δ 2.02 and 2.07 (s, 6 H, $OCOCH_3$), 2.38–2.59 (m, 2 H, 2'-H), 4.22 (m, 2 H, 5'-H), 4.30 (m, 1 H, 4'-H), 5.19 (m, 1 H, 3'-H), 6.06 (t, 1 H, $J_{1',2'}$ = 6.83 Hz), 8.06 (s, 1 H, 6-H), 11.96 (s, 1 H, 3-NH, D_2O exchangeable). Anal. ($C_{15}H_{15}F_5N_2O_7$) C, H, F, N.

1-β-D-Arabinofuranosyl-5-(trifluoromethyl)uracil (9). A solution of 7 (0.6 g, 13.7 mmol) in saturated HCl–MeOH (6 mL) was stirred at 0 °C (ice–H_2O bath) for 4 h. The solvent was removed in vacuo at room temperature. The residue was coevaporated several times with MeOH. The crude product was redissolved in MeOH, clairified with charcoal, and filtered. The filtrate was evaporated to dryness under reduced pressure. The residue was then triturated with Et_2O–MeOH (10 mL/0.1 mL). The solid product was collected by filtration to yield 0.34 g (80%): mp 220–222 °C; UV (MeOH) λ_{max} 263 nm (ϵ 12 750), λ_{min} 230 nm; UV (0.1N HCl) λ_{max} 263 nm (ϵ 12 250), λ_{min} 227 nm; UV (0.01 N NaOH) λ_{max} 263 nm (ϵ 8500), λ_{min} 238; NMR (Me_2SO-d_6) δ 3.60 (m, 2 H, 5'-H), 3.76 (m, 1 H, 4'-H), 3.92 (m, 1 H, 3'-H), 4.07 (m, 1 H, 2'-H), 5.18 (m, 1 H, 5'-OH, D_2O exchangeable), 5.51 (d, 1 H, 3'-OH, D_2O exchangeable), 5.71 (d, 1 H, 2'-OH, D_2O exchangeable), 6.02 (d, 1 H, 1'-H, $J_{1',2'}$ = 4.67 Hz), 8.32 (s, 1 H, 6-H), 11.89 (s, 1 H, 3-NH, D_2O exchangeable). Anal. ($C_{10}H_{11}F_3N_2O_6$) C, H, F, N.

2'-Deoxy-5-(pentafluoroethyl)uridine (10). The preparation of 10 followed the same procedure as that depicted for 9. Compound 8 (0.24 g, 0.55 mmol) was treated with saturated HCl–MeOH (10 mL) for 4 h to give 0.16 g (83%) of product: mp 160–162 °C; UV (MeOH) λ_{max} 263 nm (ϵ 12 244), λ_{min} 230 nm; UV (0.01 N HCl) λ_{max} 263 nm (ϵ 12 040), λ_{min} 228 nm; UV (0.01 N NaOH λ_{max} 262 nm (ϵ 8673), λ_{min} 235 nm; NMR (Me_2SO-d_6) δ 2.18 (m, 2 H, 2'-H), 3.52–3.72 (m, 2 H, 5'-H), 3.83 (m, 1 H, 4'-H), 4.23 (m, 1 H, 3'-H), 5.22 (t, 1 H, 5'-OH, D_2O exchangeable), 5.32 (d, 1 H, 3'-OH, D_2O exchangeable), 6.06 (t, 1 H, 1'-H, $J_{1',2'a}$ = 5.59 Hz, $J_{1',2'b}$ = 6.02 Hz), 8.71 (s, 1 H, 6-H), 11.81 (s, 1 H, 3-NH, D_2O exchangeable). Anal. ($C_{11}H_{11}F_5N_2O_5$) C, H, F, N.

Biological Test Procedures. Mouse L1210 and S-180 cells were maintaned as suspension cultures in Fischer's medium supplemented with 10% horse serum at 37 °C in a humidified atmosphere of 5% CO_2–95% air. Under these conditions the generation time for L1210 and S-180 cells is approximately 12 and 18 h, respectively. Each compound at the given concentration was added to L1210 and S-180 cells (2×10^4 cells/mL), which were in their exponential phase of growth. The increase in cell number of the drug-free culture (control), as well as that of the cultures supplemented with the tested compounds, was determined after 24, 48, and 72 h of growth.

The antiviral activity of various compounds listed in Table I was determined. Vero cells were grown to confluency in 25-cm^2 Falcon flasks with Dulbecco's medium supplemented with 10% fetal calf serum. The cells were then infected with herpes simplex virus, type 1 (CL-101, obtained from Dr. Wilma Summers who originally received the virus from Dr. Saul Kit), at a multiplicity of infection (MOI) of 10. After a 1-h absorption period at 37 °C, the viral inoculum was removed, and the flask was washed once with phosphate-buffered saline. The test compounds indicated in Table I were dissolved in Dulbecco's medium supplemented with serum and then added to the flask. The infected cultures were incubated at 37 °C for 40 h and then frozen until virus titrations were performed. Virus was released by freezing and thawing the media–cell suspension one time. The cell lysates were diluted directly, and the virus yield was assayed by plaque formation on Vero cells. The number of plaque-forming units (pfu) of virus in the drug-treated cultures relative to that found in the drug-free condition was determined and expressed as percent inhibition in Table I.

The cytotoxicity of the various test compounds on the uninfected host Vero cells was determined (Table I). Vero cells in Dulbecco's medium (2.5 mL) supplemented with 10% fetal calf serum were added to eight 25-cm^2 Falcon flasks at a concentration equivalent to 0.1 confluency for each compound under assay. After incubation at 37 °C in 5% CO_2–95% air for 1 day, the test compound, dissolved in 2.5 mL of the above growth medium, was added, and two flasks were harvested immediately by decanting the medium, washing once with 5 mL of buffered saline, and then incubating at 37 °C for 15 min with a 5 mL solution of trypsin (0.125%) and EDTA (0.02%). The cells dislodged from the flask by this latter procedure were generally in clumps and were dispersed by repeated forceful pipetting of the suspension against the surface of the flask. To 1 mL of the well-dispersed cell suspension was added 0.2 mL of Trypan blue solution, and the number of cells was counted with a hemocytometer. Each day for the next 3 days, two of the remaining flasks were harvested in the manner just described for determination of cell number.

Acknowledgment. This research was supported by Grant CH-227 from the American Cancer Society and USPHS Grant CA-05262 from the National Cancer Institute, DHHS. We express our appreciation to Diane Mozdziesz and J. J. Lee for their excellent technical assistance. We also acknowledge the support of the Southern New England High Field NMR Facility, made possible by a grant from the Biotechnology Resources Program of the National Institutes of Health (RR-798).

Registry No. 1, 3083-77-0; 2, 951-78-0; 3, 14057-18-2; 4, 13030-62-1; 5, 84500-33-4; 6, 1956-30-5; 7, 84500-34-5; 8, 84500-35-6; 9, 3574-08-1; 10, 84500-36-7; 5-iododeoxyuridine, 54-42-2; trifluoromethyl iodide, 2314-97-8; pentafluoroethyl iodide, 354-64-3.