Nucleosides. 133. Synthesis of 5-Alkenyl-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)cytosines and Related Pyrimidine Nucleosides as Potential Antiviral Agents[†]

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The synthesis of 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)cytosines with a halovinyl (8 and 9) or vinyl (10) substituent at C-5 was accomplished from the corresponding 5-iodo (FIAC, 1) and/or 5-chloromercuri (5) nucleoside analogues with use of Li_2PdCl_4- and $Pd(OAc)_2$-mediated coupling reactions. Thiation of the benzoylated derivative of the 5-ethyluracil nucleoside 3 followed by S-methylation and then ammonolysis provided 5-ethyl-2′-fluoro-ara-C (11). 5-Ethynyl-2′-fluoro-ara-C (19a) and 5-ethynyl-2′-fluoro-ara-U (19b) were also obtained from the persilylated 5-iodo nucleosides 1 and 16, respectively, by Pd^{II}/Cu^{I} catalyzed coupling with (trimethylsilyl)acetylene. With use of selective sugar deprotection of the initial coupling products with H_2O/Me_2SO, the corresponding 5-[2-(trimethylsilyl)ethynyl] derivatives 18a and 18b could be isolated. Most of the new compounds showed activity in vitro against both HSV-1 and HSV-2, as did the known corresponding 5-alkenyluracil nucleosides synthesized earlier. The 5-vinylcytosine and -uracil nucleosides 10 and 24, respectively, were highly effective against HSV-1 (ED_{90} = 0.40 and 0.043 μM, respectively) and HSV-2 (ED_{90} = 0.59 and 0.56 μM, respectively). Unlike BVDU, the 2′-fluoroarabinosyl derivatives of 5-(halovinyl)cytosine and -uracil showed activity against both types of herpes simplex virus. The therapeutic indices of these compounds are in some cases superior to those of 2′-fluoro-5-methyl-ara-U (FMAU, 2). Moderate antileukemic activity was observed in vitro for the 5-alkynyl and 5-vinyl compounds. The competition of these compounds with thymidine for viral-induced thymidine kinases was also studied.

A number of 5-substituted pyrimidine nucleosides containing the 2-deoxy-2-fluoro-β-D-arabinofuranosyl moiety have been synthesized in our laboratory as potential anticancer and/or antiviral agents.[1-3] Among these the 5-iodocytosine nucleoside 1 [2′-fluoro-5-iodo-ara-C or FIAC] has shown clinical efficacy in phase I[4] and phase II[5] studies with immunosuppressed cancer patients. The thymine analogue 2 [2′-fluoro-5-methyl-ara-U or FMAU] has demonstrated much more potent in vivo activity in mice infected with herpes simplex type 1[6] and type 2[7] (HSV-1 and HSV-2) without toxicity at effective dose levels. Furthermore, FMAU was shown[8] to be highly active in vivo against mouse leukemia P815 or L1210 made resistant to arabinosylcytosine (ara-C). The potent antiherpetic activity of 5(E)-(2-halovinyl)-2′-deoxyuridines reported by De Clercq et al.[9] prompted us to synthesize the 2′-fluoroarabinosyl analogues of these nucleosides.[3] These 2′-fluoro-5-substituted pyrimidine nucleosides, however, were found to be less active than 5(E)-(2-bromovinyl)-2′-deoxyuridine (BVDU) against HSV-1. Apparently, the presence of the 2′-fluoro substituent did not potentiate the in vitro antiherpetic activity as had been generally observed previously.[1,2,6,10] We have also synthesized the 5-ethyl analogue 3 [2′-fluoro-5-ethyl-ara-U or FEAU], which, although a log order less active than FIAC against HSV-1 in vitro, was found to be much less cytotoxic, resulting in a therapeutic index superior to that of FIAC.[3]

The synthesis of 2′-deoxycytidine derivatives containing the vinyl[11,12] or (E)-(2-halovinyl)[13] substituent at the C-5 position has been reported recently. These cytosine nucleosides were more selective in their anti-HSV-1 activity than their uracil counterparts.[12,14] We report herein the syntheses and antiviral activities of several 5-alkenylcytosine nucleosides containing the 2′-fluoro-β-D-arabinosyl

Scheme I
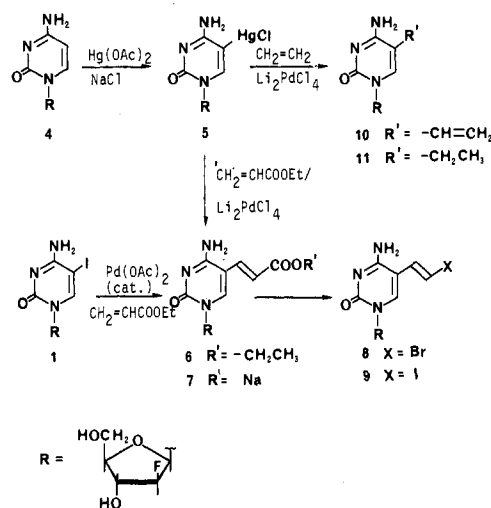
moiety as potential inhibitors of herpes virus replication with high selectivity. The 2′-fluoro-arabino nucleosides
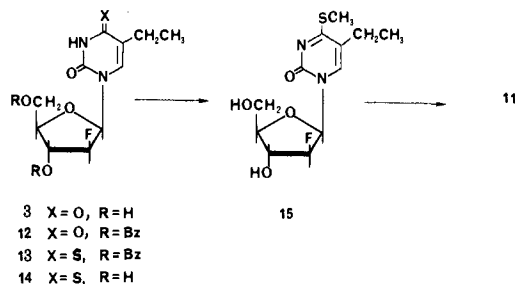
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containing 5-ethylcytosine, 5-ethynylcytosine, and 5-ethynyluracil were also synthesized since FEAU (3) was shown to have an extremely high therapeutic index[3] and the 5-ethynyl analogues of 2'-deoxyuridine[15,16] and 2'-deoxycytidine[14] were reported to exhibit antiherpetic and antitumor activity. Comparative activities of those new nucleosides along with some previously synthesized analogues in this series are also reported.
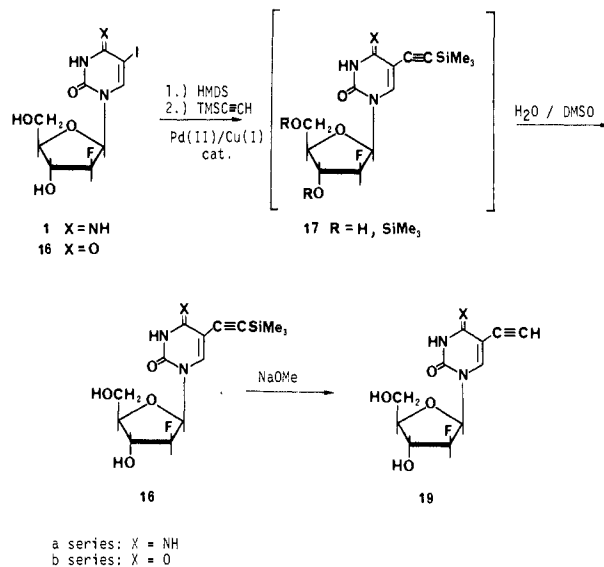
For the synthesis of these new 2'-fluoro-5-substituted pyrimidine nucleosides, we adapted the recently developed methods[17] for introduction of alkenyl or alkynyl groups at C-5 of preformed nucleosides with use of palladium catalysts. This approach avoids difficulties associated with the preparation of the corresponding cytosine bases, the separation of anomeric mixtures, and the deprotection of reactive products. Thus, 2'-fluoro-ara-C (4, FAC) was converted into the 5-chloromercuri nucleoside 5 according to the method of Bergstrom et al.[18] (Scheme I) and treated with ethyl acrylate in the presence of a stoichiometric amount of Li_2PdCl_4 to afford the ester 6. This product was very light sensitive and readily formed fluorescent material, as was reported for 5(E)-[2-(methoxycarbonyl)-vinyl]cytidine.[19]

As the above route requires the use of 1 equiv of palladium, we attempted to condense ethyl acrylate directly with FIAC (1) [which we have in hand] using catalytic quantities of $Pd(OAc)_2$ and Ph_3P in the presence of Et_3N according to the original method of Heck et al.[20] Although Bergstrom had reported the palladium-catalyzed reaction of 5-iodouridine with methyl acrylate in the absence of solvent,[21] application of such conditions to the reaction of FIAC (1) and ethyl acrylate afforded only small amounts of product. It was found, however, that the coupling reaction occurred smoothly when it was carried out in N,N-dimethylformamide (DMF) solution at 85 °C, affording compound 6 in crystalline form in 37% yield. Saponification of the ester 6 with 0.5 N NaOH at room temperature afforded the sodium salt 7, which was treated with excess N-bromosuccinimide (NBS) by the method of Jones et al.[13] to yield the 5(E)-(2-bromovinyl)cytosine nucleoside 8. Similar treatment of 7 with N-iodosuccinimide (NIS) afforded the 5(E)-(2-iodovinyl)cytosine derivative 9, which

Scheme II

3   X = O, R = H
12   X = O, R = Bz
13   X = S, R = Bz
14   X = S, R = H

15

11

Scheme III

1   X = NH
16   X = O

17   R = H, SiMe₃

16

19

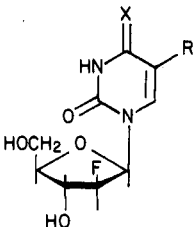a series: X = NH
b series: X = O

proved to be extremely hygroscopic and very difficult to filter without decomposition. Rapid filtration under a nitrogen flow was essential in order to obtain 9 in colorless crystalline form. This precaution was taken with all of the compounds prepared in this study, due to their tendency toward slight decomposition upon absorption of moisture. The pure, crystalline products could be handled without difficulty, however. The assignment of the E configuration to the 2-substituted-vinyl compounds 6–9 is supported by their ¹H NMR spectra, which are given in Table III.

The reaction of chloromercuri derivative 5 in dry DMF with ethylene gas under pressure in the presence of 1 equiv of Li_2PdCl_4 provided the 5-vinyl nucleoside 10 as a highly hygroscopic foam. This method was described briefly by Bergstrom[11] without experimental details for the first synthesis of 5-vinyl-2'-deoxycytidine. As expected, compound 10 was extremely labile and formed fluorescent material and polymer whenever any solution was concentrated to dryness and also during storage of the isolated product in the refrigerator. It could not be crystallized in our hands, and its HCl salt, though crystalline, was too unstable to be isolated. The sample of 10 that was prepared for biological testing was thus contaminated with about 10% of polymer. The elemental analysis indicated extensive hydration. Its identity was confirmed by the characteristic splitting pattern in the NMR spectrum for the vinyl group reported for the uracil analogue,[3] 5-vinyl-2'-deoxyuridine,[22] and 5-vinyl-2'-deoxycytidine[12] and by the mass spectrum which showed $(M + 1)^+$ (m/e 272) as well as free base $((B + 1)^+, m/e$ 138). The presence of both $(M + 1 - HF)^+$ and (sugar – HF), but little or no intact 2'-fluoroarabinose, demonstrates the ease of elimi-

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Table I. Antiviral and Cytotoxic Activity of 5-Substituted (2'-Fluoro-β-D-arabinofuranosyl)pyrimidines and Their Effect on Viral Thymidine Kinase Activities

compd	X	R	HSV-1 (F)[a] ED$_{50}$, μM	ED$_{90}$, μM	HSV-2 (G)[a] ED$_{50}$, μM	ED$_{90}$, μM	ID$_{50}$,[b] μM	therapeutic index (ID$_{50}$/ED$_{90}$) HSV-1	HSV-2	thymidine kinase activity (% of control[c]) HSV-1	HSV-2
6	NH	(E)-CH=CHCOOEt	>100	>100	>100	>100	126			99.1	107
7	NH	(E)-CH=CHCOONa	>100	>100	>100	>100	>200			95.7	111
8	NH	(E)-CH=CHBr	0.068	0.52	0.037	4.5	23	44	5	58.9	104
9	NH	(E)-CH=CHI	0.088	0.74	0.027	2.3	>200	>270	>87	63.6	101
10	NH	CH=CH$_2$	0.025	0.40	0.062	0.59	41	103	70	52.6	111
11	NH	CH$_2$CH$_3$	0.035	0.57	0.32	4.9	143	251	29	75.7	101
19a	NH	C≡CH	2.1	7.5	0.64	6.0	30	4	5	52.3	86.3
20 (FMAC)	NH	CH$_3$[d]	0.006	0.048	0.026	0.10	1.5	31	15	81.5	110
21	O	(E)-CH=CHCl[e]	0.059	0.70	0.52	5.5	159	227	29	41.4	106
22	O	(E)-CH=CHBr[e]	0.067	0.47	0.13	0.73	101	216	138	27.0	86.0
23	O	(E)-CH=CHI[e]	0.037	0.11	0.15	0.84	56	509	67	35.8	96.2
24	O	CH=CH$_2$[e]	0.009	0.043	0.09	0.56	10.8	251	19	45.0	109
3	O	CH$_2$CH$_3$[e]	0.024	0.26	0.24	0.91	>200	>769	>220	49.6	107
18b	O	C≡CSi(CH$_3$)$_3$	5.7	39.5	5.1	32.1	17.1	0.4	0.5	72.7	95.8
19b	O	C≡CH	1.9	46.6	3.0	7.9	62	1.3	8	49.2	90.8
2 (FMAU)	O	CH$_3$[d]	0.010	0.042	0.023	0.09	2.8	67	31	36.9	94.1
BVDU[f]	O		0.019	0.062	0.23	17.0	191	3080	11	14.4	110

[a] Tested in Vero cells by a plaque reduction assay; correlation coefficient ≥0.86. [b] Cytotoxic effect measured in rapidly dividing Vero cells. [c] [^{14}C]Thymidine phosphorylation activity. Control assays had no nucleoside analogues in the assay mixture. Final drug concentration was 200 μM. Variation between duplicate assays was less than 5%. The TK activity of the extracts was about 0.1 U/assay. [d] Reference 2. [e] Reference 3. [f] We thank Dr. E. DeClercq for the gift of BVDU.

nation of HF. A small impurity peak was detected at m/e 261.

Reduction of **10** with Pd/C catalyst yielded the 5-ethyl analogue **11** as an extremely hygroscopic foam, which was shown by mass spectra to have trace amounts of contaminants. Pure, crystalline **11** was prepared, however, from readily available FEAU (**3**).[3] Benzoylation of **3** (Scheme II) yielded **12** and subsequent thiation[23-25] with P$_2$S$_5$ in dioxane provided the 4-thio nucleoside **13**. The latter was deprotected with NaOMe/MeOH to afford **14**, which was treated in situ with MeI to provide 4-MeS derivative **15** in 95% yield from **12**. This sequence was preferable to reaction of **13** with MeI and NaOMe in aqueous dioxane/MeOH, which in contrast with the case of the synthesis of the 4-MeS analogue of uridine[25] yielded primarily a monobenzoylated 4-MeS compound. Ammonolysis of **15** by heating in methanolic ammonia yielded crystalline **11**.

For the preparation of the 5-ethynylcytosine nucleoside **19a** (Scheme III), we utilized initially the method of Robins and Barr[26] for 5-alkynyl-2'-deoxyuridines, consisting of the reaction of toluoylated 5-iodo nucleosides with (trimethylsilyl)acetylene (Me$_3$SiC≡CH) in Et$_3$N at 50 °C using catalytic amounts of (Ph$_3$P)$_2$PdCl$_2$ and CuI. Silylation of the cytosine nucleoside **1** is more convenient than tritoluoylation, however, and after removal of the HMDS the coupling reaction in Et$_3$N was found to reach com-

pletion at room temperature. Schroeder et al.[27] have reported an analogous procedure for the synthesis of 6-ethynyluracil. The crystalline, partially sugar-silylated **17a** obtained in this manner was desilylated with sodium methoxide to provide **19a** in good yield. The synthesis of **19a** was recently reported[28] by condensation of 5-ethynylcytosine with the protected 2-fluoroarabinosyl bromide[29] in the presence of mercuric bromide, which proved less efficient due to the difficulty of separating the mixture of α and β anomers obtained in approximately equal amounts.

When 5-iodouracil nucleoside **16** was coupled with Me$_3$SiC≡CH in a similar manner, about 5% of the product after deprotection was found to be the deiodinated uracil nucleoside "FAU", which was not readily removed. It was possible, however, to selectively remove the O-silyl groups from **17b** in 10% H$_2$O in Me$_2$SO at room temperature to yield the 5-[2-(trimethylsilyl)ethynyl]uracil nucleoside **18b**, which was easily separated from FAU by silica gel column chromatography. Subsequent deprotection of **18b** yielded the 5-ethynyluracil nucleoside **19b** (25% yield from **16**). The ability to effect selective O-desilylation using H$_2$O in Me$_2$SO is an additional advantage of using trimethylsilyl groups for protecting 5-iodopyrimidine nucleosides, along with greater convenience and milder reaction conditions. It also may be of general utility in the preparation of other 5-[2-(trimethylsilyl)ethynyl]uracil nucleosides. O-Desily-

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lation of the mixture of silylated cytosine nucleosides **17a** with 10% H_2O in Me_2SO was considerably slower, however, and was completed only with difficulty. Silica gel chromatography of the reaction product provided **18a** with a trace of **19a**, and subsequent treatment with NaOMe/ MeOH affords pure **19a**.

Antiviral Activity. The compounds synthesized in this investigation as well as previously synthesized analogues[2,3] and BVDU were evaluated in parallel for cytotoxic and antiviral activities against HSV-1 (strain F) and HSV-2 (strain G) in Vero cells (Table I). The 5-(bromovinyl)- and 5-(iodovinyl)cytosine derivatives **8** and **9** exhibited potent antiviral activity against *both* viruses. The iodo compound **9** was at least 10 times less toxic to uninfected Vero cells than the bromo analogue. Compound **9** was also less toxic than the corresponding uracil analogue. However, the (bromovinyl)uracil compound **22** was less cytotoxic than the corresponding cytosine analogue **8**. The (halovinyl)uracil series of compounds (**21–23**) was also found to be effective against HSV-1 *and* HSV-2. The order of activity for the 2-(halovinyl)uracil compounds against HSV-1 was I > Br > Cl and for HSV-2, I = Br > Cl. These analogues were at least 20 times less cytotoxic than FMAU (**2**) and also exhibited better therapeutic indices than either FMAU or its cytosine analogue FMAC (**20**). In addition, the (iodovinyl)cytosine **9** was nearly 2 orders of magnitude less cytotoxic than the reference compound FMAU.

The 5-(2-bromovinyl)cytosine and -uracil nucleosides **8** and **22** were less effective than BVDU against HSV-1. Using a similar but not identical antiviral screening assay, we had previously reported data[3] for the uracil derivatives suggesting that the presence of a 2'-fluoro substituent did not confer selective activity against HSV-2. We were unable to confirm these preliminary findings in the present study. The (halovinyl)cytosine compounds **8** and **9** and the corresponding uracil derivatives **22** and **23** were about 4- to 20-fold more effective than BVDU against HSV-2. It is well appreciated that the 2'-fluoro function confers greater stability to the glycosyl bond,[30,31] and this may explain in part the greater activity noted against HSV-2 with these new analogues. [In humans, BVDU is highly susceptible to endogenous phosphorylases producing 5-(2-bromovinyl)uracil as the main metabolite.[32]] It is also conceivable that the presence of a 2'-fluoro substituent in these 5-(halovinyl)pyrimidine nucleosides enables them to be better substrates for the HSV-2-specified thymidine-thymidylate kinases than BVDU, the latter of which is a poor substrate of HSV-2 thymidylate kinase.[33]

5-Alkyl-substituted cytosine nucleosides FMAC and **11** had lower therapeutic indices than the corresponding uracil analogues FMAU and **3** (Table I). The 5-methyl-substituted nucleosides FMAC and FMAU were more effective but also more toxic than the 5-ethyl analogues **11** and **3**. However, we recently found that, if the 5-substituent in (2'-fluoroarabinosyl)uracil derivatives was longer than three carbon–carbon bonds, the antiviral and cytotoxic activity was completely abolished.[34] The 4-methylthio compound **15** (an analogue of **3** and **11**) was found to be devoid of

Table II. Comparison of in Vitro Tumor Growth Inhibitory Effects of Selected 5-Substituted (2-Fluoro-β-D-arabinofuranosyl)pyrimidines[a]

| compd | ID$_{50}$, μM | | |
	L1210/0	P815/0	P815/ara-C[b]
10	10.7	23.8	>300
18b	17.8	9.8	107
19a	10.1	52.1	$\gg$300
19b	23.5	20.4	84.7
24[c]	29.0	20.8	32.8
2[d]	2.95	0.98	12.4

[a] Provided by Dr. J. H. Burchenal. [b] Ara-C-resistant cell line; ID$_{50}$ for ara-C = 181 μM. [c] Reference 3. [d] Reference 2.

antiviral and cytotoxic activity.

The 5-vinylcytosine and -uracil nucleosides **10** and **24** are highly effective against both HSV-1 and HSV-2. The 5-vinylcytosine derivative **10** was at least 10-fold more active than the corresponding ethynyl derivative **19a**. A similar order of antiviral activity was noted for the corresponding uracil analogues (**24** and **19b**). The lower activity for the ethynyl derivatives (**19a** and **19b**) than for compounds with even larger 5-substituents (e.g., vinyl, halovinyl, or ethyl) may be due to the linearity and conformational rigidity of the ethynyl moiety. The 5-[2-(trimethylsilyl)ethynyl]uracil nucleoside **18b** was less active than the deprotected analogue **19b**, but the difference is not nearly as great as one might have expected considering the added steric bulk and the dramatic decrease in activity created by large aliphatic substituents in 5-alkynyl-2'-deoxyuridines.[35]

Thymidine Kinase (TK) Studies. The phosphorylating behavior of HSV-1- and HSV-2-induced thymidine kinases in the presence of the nucleoside analogues are shown in Table I. Most of the compounds tested did not affect the phosphorylation of thymidine by the TK present in extracts from HSV-2-infected cells and yet some of these drugs had ED$_{90}$'s against HSV-2 in cell culture below 1 μM. These compounds probably do not compete effectively for the HSV-2 TK in the presence of thymidine in a cell-free system. The K_i values for inhibition by (2'-fluoro-arabinosyl)pyrimidines of HSV-1-induced thymidine kinase were reported to be significantly lower than those for the HSV-2 enzyme in a study by Cheng et al.[36] As previously observed with other antivirals,[37] some of the 2'-fluoro analogues in Table I caused activation of the TK enzymes. The reasons for this unusual phenomenon are not clear.

Most of the compounds in Table I with potent HSV-1 activity also competed effectively with thymidine for HSV-1 TK. The uracil nucleosides showed a greater affinity for HSV-1 TK than the corresponding cytosine nucleosides. Consistent with this observation is the study by Cheng et al.[36] in which lower K_i values for inhibition of both HSV-1 and HSV-2 TK were found for FMAU than for FMAC and for FIAU (**16**) than for FIAC (**1**). It was suggested that the much lower K_m values reported[38] for thymidine than for 2'-deoxycytidine as substrates of these enzymes might be responsible for this relationship, and this could apply as well to the results for the newly synthesized analogues. However, little correlation between

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antiviral activity and thymidine kinase inhibition can be discerned from the present data, implying that although phosphorylation by viral TK is probably required for antiviral activity, other factors may also be of importance in the expression of activity.

Antileukemic Activity. Preliminary results of in vitro screening of selected (2'-fluoroarabinosyl)pyrimidines against mouse leukemia cell lines are shown in Table II. Only those compounds with values of ID_{50} less than 30 μM for at least one cell line are shown. Both vinylcytosine compound **10** and vinyluracil compound **24** exhibit tumor growth inhibitory activity but not of the magnitude observed for FMAU (**2**). 5-Vinyl-2'-fluoro-ara-U (**24**) does, however, have an effect on the ara-C-resistant P815 cell line, although again somewhat less than that of FMAU.

The 5-alkynyl-substituted nucleosides also show moderate antileukemic activity. As there is evidence[15,39,40] that inhibition of thymidylate synthetase is the primary mode of action of 5-ethynyl-2'-deoxyuridine (possibly by a "mechanism-based" process), then it could perhaps also play a role in the antitumor effect of the 5-ethynyl compounds **19a** and **19b**. In light of the poor activity of 2'-deoxyuridines with bulky 5-alkynyl substituents,[35] the trimethylsilyl substituent on **18b** would be expected to preclude significant antitumor and/or antiviral activity as mentioned earlier. Thus **18b** may very well be a latent form of **19b** due to facile cleavage in vitro of the trimethylsilyl moiety from the ethynyl group.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. ^{1}H NMR spectra (Table III) were recorded on a JEOL PFT-100 spectrometer using Me$_4$Si as an internal standard except when D$_2$O is cited as the solvent, where DSS was the internal standard. Ultraviolet spectra were obtained on a Cary 15 spectrophotometer, in EtOH unless otherwise stated. Mass spectra were measured in the chemical-ionization mode (isobutane) at the Mass Spectrometric Biotechnology Research Resource of Rockefeller University. Silica gel TLC was performed on Analtech Uniplates with short-wavelength ultraviolet light for visualization. Column chromatography was conducted on flash grade silica gel (Merck 9385, 0.040–0.063 μm). Analytical HPLC was performed on a Waters C$_{18}$ μ-Bondapak column (10 μm, 3.9 mm i.d. × 30 cm) with a Waters Model 6000A solvent delivery system, Model U6K injector, and Model 440 UV detector (254 nm), and preparative HPLC was conducted on a C$_{18}$ μ-Bondapak column (10 μm, 7.8 mm i.d. × 30 cm) or on a Waters Prep LC 500A using a Prep Pak-500 C$_{18}$ column. Elemental analyses were performed by MHW Laboratories or by Spang Microanalytical Laboratory.

All compounds in the final stages were filtered under a nitrogen flow. All palladium catalysts were obtained from Aldrich Chemical Co. unless otherwise indicated. (Trimethylsilyl)acetylene was obtained from Petrarch Systems, Inc.

5-(Chloromercuri)-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)cytosine (5). Compound **4**[29] (6.13 g, 25.0 mmol) was dissolved in 30 mL of H$_2$O at 60 °C, Hg(OAc)$_2$ (8.05 g, 25.25 mmol) was added, and the mixture was stirred at 60 °C for 16 h. Water (15 mL) was added and stirring was continued with heating for 3 h. After the mixture cooled at room temperature, NaCl (3.16 g) in water (12 mL) was added, and the thick suspension was agitated (with shaker) for 1 h, filtered, and washed successively with 0.1 M aqueous NaCl (75 mL), H$_2$O, EtOH, and ether. Drying over P$_2$O$_5$ in vacuo at 80 °C gave 9.54 g of **5** as a white powder (80%): mp 189–193 °C dec; ^{1}H NMR (0.1 M KCN in D$_2$O) δ 3.87 (m, 2, H-5',5''), 4.07 (m, 1, H-4'), 4.39 (dm, 1, H-3', $J_{3',F}$ = 20.1

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Table III. ^{1}H NMR Parameters for 5-Substituted 1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)pyrimidines^a

compd	chemical shifts, δ								coupling constants,^{b} Hz				
	H-1'	H-2'	H-3'	H-4'	H-5',5''	H-6	R	exchangeable	R	$J_{1',2'}$	$J_{1',F}$	$J_{2',F}$	$J_{3',F}$	R
6	6.12 (dd)	5.05 (dt^c)	d	3.83 (m)	3.64 (m)	8.30 (s)	6.23 (d)^e	7.60 (NH), 5.86 (d), 5.26 (t)	4.3	14.6	53.4	d	$J_{1'',2''}$ = 15.9, $J_{H,H}$(ethyl) = 7.0
7	6.14 (dd)	5.05 (dt^c)	4.25 (dm)	3.66–3.80		8.05 (s)	6.06 (d) 7.00 (d)	7.38 (NH), 3.5–3.8	4.3	14.9	52.8	19.8	$J_{1'',2''}$ = 15.9
8	6.09 (dd)	5.02 (dt^c)	4.23 (dm)	3.78 (m)	3.64 (m)	7.91 (s)	6.72 (d) 7.06 (d)	7.41 (NH), 5.85 (br s), 5.18 (br s)	4.6	15.0	52.5	19.5	$J_{1'',2''}$ = 13.5
9	6.09 (dd)	5.02 (dt^c)	4.21 (dm)	3.78 (m)	3.65 (m)	7.92 (s)	6.70 (d) 7.31 (d)	7.30 (NH), 5.84 (br s), 5.20 (br s)	4.3	15.3	52.5	21.0	$J_{1'',2''}$ = 14.5
10	6.13 (dd)	5.05 (dt^c)	4.22 (dm)	3.81 (m)	3.60 (m)	7.93 (s)	f	7.37 (NH), 5.90 (d), 5.20 (t)	4.3	15.6	53.4	17.7	J_{trans} = 17.0, J_{cis} = 11.3, J_{gem} = 0.9
11	6.11 (dd)	4.97 (dt^c)	4.20 (dm)	3.76 (m)	3.61 (m)	7.44 (s)	1.03 (t) 2.26 (q)	7.22 (NH), 5.88 (d), 5.10 (t)	4.2	17.2	53.1	16.8	$J_{H,H}$ = 7.3
18b	6.09 (dd)	5.08 (dt^c)	4.24 (dm)	3.82 (m)	3.65 (m)	8.11 (d)	0.21 (s)	5.94 (d), 5.25 (t)	4.3	14.6	53.4	21.1	
19a	6.06 (dd)	5.00 (dt^c)	4.20 (dm)	3.81 (m)	3.61 (m)	8.04 (s)	4.38 (s)	7.85, 6.97 (NH), 5.85 (d), 5.15 (t)	3.8	16.9	52.5	21.1	
19b	6.09 (dd)	5.03 (dt^c)	4.22 (dm)	3.77 (m)	3.63 (m)	8.02 (d)	4.03 (s)	2.92–3.79	4.3	15.6	52.8	21.1	

^{a} Measured in Me$_2$SO-d_6. ^{b} Values given are first order; $J_{2',3'} = J_{3',4'} \cong 4$ Hz. ^{c} Apparent, probably ddd. ^{d} Obscured by ethyl signal. ^{e} 1.25 (CH$_3$, t), 4.16 (CH$_2$, q). ^{f} Vinyl: 6.59 (H-1'', dd), 5.41 (H-2'', trans to H-1'', dd), 5.10 (H-2'', cis to H-1'', dd).

Hz), 5.11 (appar dt, 1, H-2', $J_{2',F}$ = 52.2 Hz), 6.26 (dd, 1, H-1', $J_{1',2'}$ = 3.8 Hz, $J_{1',F}$ = 17.5 Hz), 7.80 (d, 1, H-6, $J_{6,F}$ = 1.2 Hz); UV (5 × 10⁻⁴ M KCN, pH 9.75) λ_{max} 273 nm (ϵ 8290), λ_{min} 255 (5800). Anal. ($C_9H_{11}ClFHgN_3O_4$) C, H, N.

1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-5(E)-[2-(ethoxycarbonyl)vinyl]cytosine (6). Due to the light sensitivity of **6**, all reactions and columns must be protected from light.

Method A. To a solution of Li_2PdCl_4[41] (4.2 mmol) in MeOH (40 mL) were added **5** (2.07 g, 4.3 mmol) and ethyl acrylate (4.76 mL, 44 mmol), and the mixture was stirred for 7 h at room temperature in the dark. After filtration of the mixture through Celite-545, the filtrate was stirred under H_2S for 5 min. The dark precipitate was removed by filtration and the filtrate was concentrated in vacuo to an oil, which was dissolved in MeOH, evaporated, triturated with EtOH, and cooled to yield crude **6** as the white, crystalline HCl salt (0.817 g). The salt was dissolved in H_2O/EtOH (1:1) and stirred with IR-45 C.P. resin (OH⁻). The resin was washed with EtOH and the solution filtered through Celite, concentrated, dissolved in EtOH, and evaporated. Chromatography (twice) on silica gel (20:1 to 6:1, CH_2Cl_2/EtOH) with protection from light yielded 0.336 g of **6** as white, chromatographically homogeneous crystals (23%), which was spectrally and chromatographically identical with **6** prepared by the following procedure.

Method B. Into a heavy-walled, pear-shaped glass bottle with a rubber-lined glass stopper were placed **1**[1] (1.85 g, 5.0 mmol), Ph_3P (0.131 g, 0.5 mmol), $Pd(OAc)_2$ (0.056 g, 0.25 mmol), dry DMF (10 mL), Et_3N (2.8 mL, 20 mmol), and ethyl acrylate (2.2 mL, 20 mmol). The vessel was flushed with argon and sealed and the mixture stirred at 85 °C (protected from light) for 24 h. The resulting clear brown solution was concentrated in vacuo with added EtOH at 30 °C to an oil, which was chromatographed on silica gel (20:1 to 6:1, CH_2Cl_2/EtOH). From the pure product fractions an oil was obtained, which crystallized from acetone to give **6** as white crystals. The mother liquor and the other fractions were further purified by reverse-phase HPLC using 2:1 H_2O/MeOH (with 1 mL of HOAc/gallon) as the mobile phase (on semipreparative or preparative columns). The desired fractions were evaporated, and the residue was crystallized from EtOH/MeOH to provide additional **6** (total 0.606 g, 37%): mp 208–212 °C; UV λ_{max} 312 nm (ϵ 12 500), 275 (16 000), λ_{min} 296 (10 700). Anal. ($C_{14}H_{18}FN_3O_6\cdot1/6H_2O$) C, H, N.

5(E)-(2-Carboxyvinyl)-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)cytosine Sodium Salt (7). The ester **6** (0.325 g, 0.95 mmol) was dissolved in 0.5 N NaOH (2.5 mL) and the solution stirred at room temperature for 3 h and then neutralized with IRC-50 (H⁺) resin. The resin was filtered and washed with water. The filtrate and washings were concentrated in vacuo and the residue was crystallized from EtOH/MeOH to give **7** as white crystals (0.292 g, 91%): mp 189 °C dec; UV λ_{max} 297 nm (sh, ϵ 7340), 265 (14 200). Anal. ($C_{12}H_{13}FN_3NaO_6\cdot1/4H_2O$) C, H, N.

5(E)-(2-Bromovinyl)-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)cytosine (8). A solution of **7** (101 mg, 0.3 mmol) and KOAc (29 mg, 0.3 mmol) in H_2O (5 mL) was heated to 65 °C and NBS (71 mg, 0.4 mmol) was added in portions over 20 min with stirring. The solution was then stirred for 2 h at room temperature. Another 0.1 mmol of NBS was added with heating over 15 min and the reaction again stirred at room temperature for 2 h. The solvent was removed in vacuo and the residue chromatographed on silica gel thick-layer plates, developed with 4:1 CH_2Cl_2/MeOH. The product was extracted with EtOH, and the extracts were evaporated and triturated with ether to yield 36 mg (34%) of **8** as an off-white powder. Recrystallization from EtOH yielded analytically pure material: mp 202–203 °C dec; UV λ_{max} 293 nm (ϵ 4620), 252 (12 000), λ_{min} 282 (4290). Anal. ($C_{11}H_{13}BrFN_3O_4$) C, H, N, Br.

1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-5(E)-(2-iodovinyl)cytosine (9). To a mixture of **7** (101 mg, 0.3 mmol) and KOAc (29 mg, 0.3 mmol) in dry DMF (15 mL) was added NIS (135 mg, 0.6 mmol) over 6 h with stirring under a nitrogen atmosphere and with protection from light. After stirring for an additional 24 h, the mixture was concentrated in vacuo and the residue was immediately chromatographed on silica gel (50:1 to

8:1, CH_2Cl_2/MeOH) with protection from light. The oil obtained was crystallized from MeOH and filtration using positive nitrogen pressure yielded 52 mg of **9** as a white powder (44%): mp 214–216 °C dec; UV λ_{max} 295 nm (sh, ϵ 7550), 257 (18 300). Anal. ($C_{11}H_{13}FIN_3O_4$) C, H, N, I.

1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-5-vinylcytosine (10). A solution of Li_2PdCl_4[41] (6.15 mmol) in dry DMF (65 mL) was placed in a 250-mL Parr bottle, **5** (3.10 g, 6.45 mmol) added, and the mixture shaken with ethylene at 20 psi for 7 h. The bottle was then evacuated, air allowed to enter, and the bottle sealed overnight. The mixture was filtered, the Pd(0) was washed with DMF, and the filtrates were flash evaporated to a dark oil, which was then dissolved in MeOH. After cooling the solution to 0 °C, it was stirred under H_2S for 3 min and filtered. Evaporation and coevaporation with EtOH yielded a yellow syrup. This was dissolved in EtOH (150 mL), 7.5 g of $NaHCO_3$ added, and the mixture stirred for $^1/_2$ h and filtered. The filtrate was concentrated and the residue chromatographed on silica gel (200 g), using CH_2Cl_2/MeOH (8:1) as eluent (the product fractions were not flash evaporated to dryness so as to minimize polymerization) to yield a chromatographically pure solution. The MeOH was removed by coevaporation with water, and the turbid aqueous solution was then filtered through a Millipore 0.45-μm filter to yield a clear solution, which was lyophilized to yield **10** as an off-white foam (0.95 g, 57%). TLC (8:1 CH_2Cl_2/MeOH) showed a small amount of fluorescent material at the origin, and a trace was insoluble in water. The ¹H NMR in Me_2SO, in which the foam is completely soluble, indicates that about 10% had decomposed: UV λ_{max} 292 nm (ϵ 6070), 244 (15 700), λ_{min} 273 (4420). MS, m/e 272 (M + 1), 261, 252, 138, 115. Anal. ($C_{11}H_{14}FN_3O_4\cdot5^1/_4H_2O$) C, H, N.

1-(2-Deoxy-3,5-di-O-benzoyl-2-fluoro-β-D-arabinofuranosyl)-5-ethyluracil (12). To a stirred solution of **3**[3] (5.0 g, 18.2 mmol) in anhydrous pyridine (25 mL) at 0 °C was added dropwise benzoyl chloride (4.22 mL, 36.4 mmol) in pyridine and the reaction stirred overnight at room temperature. Another 2.1 mL of benzoyl chloride was added in the same manner, in four portions during the course of 8 h, and the reaction sitrred overnight. The mixture was taken up into $CHCl_3$ (250 mL) and the solution extracted with water (2 × 250 mL), the aqueous layers were extracted with $CHCl_3$ (75 mL), and the $CHCl_3$ solutions were combined and evaporated in vacuo. The residue was dissolved in toluene and the solution reevaporated. After two more evaporations with toluene, the solid residue was crystallized from hot EtOH to yield 7.55 g (86%) of **12** as white crystals (mp 151–152 °C) nearly homogeneous by TLC (20:1 $CHCl_3$/EtOH).

1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-4-(methylthio)-5-ethyl-2(1H)-pyrimidinone (15). To a solution of **12** (7.24 g, 15.0 mmol) in dioxane (300 mL) was added P_2S_5 (6.67 g, 30.0 mmol). After stirring at reflux for 3 h, the hot mixture was filtered and the residue washed with hot dioxane. The filtrates were evaporated in vacuo to an oil, which was taken up into $CHCl_3$ (300 mL), extracted with H_2O (150 mL), saturated $NaHCO_3$ (2 × 150 mL), and H_2O (150 mL), and dried (Na_2SO_4). Evaporation in vacuo and storage in the cold yielded the 4-thio nucleoside **13** as yellow crystals (9.0 g). The crude **13** was partially dissolved in dry MeOH (325 mL), NaOMe added (18.0 mmol in 60 mL of MeOH), and the solution stirred at reflux for $1^1/_4$ h to yield the fully debenzoylated nucleoside **14**. This was not isolated, but after the solution had reached room temperature H_2O (75 mL) and MeI (3.3 mL, 54 mmol) were added and the reaction was allowed to stir overnight. Water (100 mL) was added and the solution concentrated to half volume. Water addition and evaporation was performed another two times, finally concentrating the solution to about 100 mL. The mixture was cooled and filtered to yield 4.33 g of **15** as tan crystals (95% from **12**), which was recrystallized from water and then EtOH to yield the analytical sample: mp 160–161 °C; ¹H NMR (Me_2SO-d_6) δ 1.13 (t, 3, CH_3, J = 7.3 Hz), 2.41 (q, 2, CH_2), 2.46 (s, 3, SCH_3), 3.62 (m, 2, H-5',5''), 3.88 (m, 1, H-4'), 4.24 (dm 1, H-3', $J_{3',F}$ = 20.4 Hz), 5.12 (appar dt, 1, H-2', $J_{2',F}$ = 52.2 Hz, $J_{1',2'}$ = 4.2 Hz), 5.16 (t, 1, 5-OH, exch), 5.88 (d, 1, 3'-OH, exch), 6.11 (dd, 1, H-1', $J_{1',F}$ = 15.3 Hz), 7.83 (s, 1, H-6); UV λ_{max} 310 (ϵ 27 500), 274 (23 600), λ_{min} 290 (20 800). Anal. ($C_{12}H_{17}FN_2O_4S$) C, H, N, S.

1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-5-ethylcytosine (11). Method A. A solution of **15** (0.304 g, 1.0 mmol) in MeOH

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(15 mL) was saturated with NH_3 at 0 °C and then heated for 29 h at 90 °C in a sealed tube. Evaporation to dryness at reduced pressure, and two subsequent coevaporations with MeOH/EtOH yielded a solid, which was then chromatographed on silica gel with 8:1 and then 6:1 CH_2Cl_2/MeOH as eluents. The desired fractions were evaporated, and the product was recrystallized from MeOH/EtOH to yield 0.212 g of **11** as white crystals (78%): mp 200–202.5 °C; UV λ_{max} 279 nm (ϵ 7430), 240 (sh, 7300), λ_{min} 260 (5350). Anal. ($C_{11}H_{16}FN_3O_4$) C, H, N.

Method B. A mixture of **10** (0.100 g, ca. 0.27 mmol, compound partially decomposed in storage according to ¹H NMR) and 10% Pd/C (50 mg, Alfa) in MeOH (15 mL) was shaken under 20 psi of H_2 for $4^1/_2$ h. The catalyst was removed by filtration through Celite and washed with methanol. The filtrate and washings were evaporated and then chromatographed on silica gel with 4:1 CH_2Cl_2/MeOH as eluent. The material was then dissolved in 1 mL of MeOH and 1 mL of water added to yield a turbid solution, which was subsequently filtered through a 0.5-μm Millipore filter. After flash evaporation, the residue was dissolved in water and then lyophilized to yield 45 mg of **11** as a white foam (ca. 61%): UV and NMR spectra very close to those of the material prepared by method A; MS, m/e 547 (2M + 1), 274 (M + 1), 254, 179, 164, 140.

1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-5-ethynyl-cytosine (19a). FIAC (1;¹ 1.48, 4.0 mmol) and $(NH_4)_2SO_4$ (60 mg) in hexamethyldisilazane (HMDS, 30 mL) was refluxed for 3 h, and the clear solution was evaporated in vacuo to a glass, which was dissolved in dry toluene and reevaporated. It was then taken up into dry Et_3N (30 mL, small amount insoluble), and CuI (62 mg, 0.32 mmol), $(Ph_3P)_2PdCl_2$ (62 mg, 0.088 mmol), and (trimethylsilyl)acetylene (2.8 mL, 20 mmol) were added. The vessel was flushed with nitrogen and sealed and the mixture stirred at room temperature for 24 h. The brown mixture was then evaporated to dryness, dissolved in $CHCl_3$ (100 mL), extracted with 5% aqueous EDTA (3 × 50 mL) and water (3 × 50 mL), dried (Na_2SO_4), and evaporated to a brown powder. Recrystallization from EtOAc (Norit) yielded 1.23 g of off-white needles: mp 213–220 °C. The TLC and NMR indicated that this was a mixture of O-silylated derivatives (**17a**) of 5-[2-(trimethylsilyl)-ethynyl]-2'-fluoro-ara-C (**18a**). A portion of this product (0.377 g, 1.4 mmol) was dissolved in 40 mL of hot MeOH. Then 1.0 N NaOH (2.1 mL) was added and the solution refluxed for 15 min. Neutralization was effected with IRC-50 (H⁺) resin, the resin was washed with MeOH, and the combined filtrate and washings were filtered through Celite and evaporated to an oil. Trituration with acetone provided 0.20 g (68% from FIAC, 99.6% purity by HPLC) of **19a** as flakes. Recrystallization from EtOH afforded white crystals: mp 196–200 °C dec (lit.²⁸ mp 184–188 °C dec); C_{18}-HPLC (3:1 H_2O/MeOH, trace AcOH) homogeneous; UV λ_{max} 293 nm (ϵ 8250), 235 (16 500), λ_{min} 263 (3630). Anal. ($C_{11}H_{12}FN_3O_4$) C, H, N.

1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-5-[2-(trimethylsilyl)ethynyl]cytosine (18a). To a stirred solution of **17a** (100 mg) in Me_2SO (10 mL) at 65 °C was slowly added H_2O (1 mL) dropwise (over 3–5 min). After stirring for $1^1/_4$ h at this temperature, the solution was flash evaporated to yield an oil, which was chromatographed on preparative TLC (8:1 CH_2Cl_2/MeOH). Extraction of the desired band with EtOH, evaporation, and trituration with ether yielded 53 mg (48% from 1) of **18a** as white crystals with a trace of **19a** according to analytical TLC (same solvent): mp 117–121 °C; ¹H NMR (Me_2SO-d_6) δ 0.23 (s, 9, $Si(CH_3)_3$), 3.61 (m, 2, H-5′,5″), 3.82 (m, 1, H-4′), 4.21 (dm, 1, H-3′, $J_{3',F'}$ = 19.8 Hz), 5.00 (appar dt, 1, H-2′, $J_{1',2'}$ = 3.7 Hz, $J_{2',F}$ = 53.7 Hz), 5.15 (t, 1, 5′-OH, exch), 5.87 (d, 1, 3′-OH, exch), 6.07 (dd, 1, H-1′, $J_{1',F}$ = 17.4 Hz), 6.76, 7.93 (2 s, 2, NH_2, exch), 8.00 (s, 1, H-6); UV λ_{max} 288 nm (ϵ 9080), 233 (18 600), λ_{min} 260 (4110). Anal. ($C_{14}H_{20}FN_3O_4Si·^1/_4H_2O$) C, H, N.

To a solution of **18a** (17.5 mg, 0.05 mmol) in MeOH (1 mL) was added 1.0 N NaOH (0.075 mL). After stirring at reflux for 15 min, the solution was neutralized with IRC-50 resin (H⁺), which was then washed with MeOH. The combined filtrates and washings were filtered through Celite to yield a solution, which was 99.9% **19a** by HPLC (85:15 H_2O/MeOH, trace AcOH).

1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-5-[2-(trimethylsilyl)ethynyl]uracil (18b). The crude C- and O-silylated nucleoside mixture **17b** was prepared from **16** in a manner similar to that used above to obtain **17a**, except that after silylation of **16** the syrup obtained was dissolved in 10 mL of dry DMF (thus adding only 20 mL of Et_3N) and the reaction with $Me_3SiC\equiv CH$ was conducted in the dark. After extraction to remove the Cu salt, the residue was recrystallized from EtOAc/heptane (with only brief heating to minimize decomposition) to provide a mixture of **17b** (major) and 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)uracil (FAU) (about 5% by HPLC).

A portion of this material (0.400 g) was dissolved in Me_2SO (15 mL) and then H_2O (1.5 mL) was added in a slow dropwise manner (to avoid precipitation). After stirring for 2 h at room temperature, the solution was evaporated in vacuo at 40 °C to a syrup, which was then chromatographed on silica gel (30 g) with CH_2Cl_2 as eluent, with a stepwise gradient to 5% MeOH in CH_2Cl_2. Evaporation and crystallization from ether/petroleum ether yielded **18b** as white crystals (0.163 g, 41% from **16**): mp 210–215 °C; UV λ_{max} 291 nm (ϵ 13 200), 233 (11 400), λ_{min} 256 (2960). Anal. ($C_{14}H_{19}FN_2O_5Si$) C, H, N.

1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-5-ethynyluracil (19b). A solution of **18b** (0.103 g, 0.3 mmol) in dry MeOH (3 mL) containing 0.6 mmol of NaOMe was stirred at room temperature for 4 h and then neutralized with IR-50 (H⁺) resin. The resin was filtered and washed with methanol, and the combined filtrate and washings were filtered through Celite and evaporated. Trituration with ethanol yielded **19b** as light tan crystals (50 mg, 62%). Recrystallization from MeOH/EtOH gave off-white crystals: mp 188–206 °C dec; UV λ_{max} 285 nm (ϵ 11 700), 225 (10 800), λ_{min} 249 (2500). Anal. ($C_{11}H_{11}FN_2O_5·^1/_4H_2O$) C, H, N.

Antiviral Activity. Antiviral activity was determined by a plaque-reduction assay in Vero cells.⁴³ Six well plates (Costar, Cambridge, MA; 3.5-cm diameter) containing mycoplasma-free confluent Vero cells (Flow Laboratories, McLean, VA) were infected (100 μL) with strain F of HSV-1 or strain G of HSV-2⁴² to give about 100 plaques/well. The plates were intermittently rocked at 15-min intervals for 1 h. The inoculum was then removed by suction, and the cells were washed with phosphate-buffered saline (pH 7.2). The compounds at five or more different concentrations [freshly dissolved in maintenance medium containing 0.1% pooled human globulin (Cutter Biological, Berkeley, CA)] were then added in replicate (3 mL/well). The plates were placed in a 5% CO_2/95% air incubator, and the plaques were allowed to develop for 48 h before fixation (10% buffered Formalin), staining (0.5% crystal violet in 20% EtOH in water), and enumeration with a dissecting stereomicroscope. The degree of inhibition (percent plaques of control) was calculated by counting the mean plaque counts for the different dilutions. The antiviral potency of the drugs was determined by estimating the values of ED_{50} and ED_{90}, the drug concentrations necessary to reduce the number of plaques by 50% and 90%, respectively, of those in the virus control cultures. These were derived from linear regression analyses of the plots of percent plaque reduction vs. log drug concentration (μM). Only the linear portion of the raw datum titration curve was used in the analysis. The linear regression statistical program used was obtained from Basic Business Software, Las Vegas, NV.

Cytotoxicity. The drug toxicity assays were determined in rapidly dividing Vero cells over a period of 3 days, as described previously.⁴³ The 50% inhibitory dose (ID_{50}) for each drug was determined by linear regression analysis as described above.

Thymidine Kinase Assay. The enzyme extract preparation used in the TK assays was obtained as previously described³⁷ from HeLa Bu 25 cells (deficient in cytocellular TK) that had been infected with HSV-1 or HSV-2 (the F and G strains, respectively). The TK assay was similar to that reported by Lee and Cheng.⁴⁴ Briefly, the enzyme extracts (10 μL) were added to duplicate tubes containing the antiviral drug (200 μM final concentration) and the assay mixture (75 μL), which included thymidine (97 μM) and ¹⁴C-labeled thymidine. After incubation for 1 h at 37 °C, the reaction mixture was spotted (50 μL) onto Whatman DE 81 disks (2.3 cm). The disks that retained phosphorylated nucleosides were

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washed three times in 95% EtOH, dried, and analyzed in a scintillation counter. The percent TK activity of control was determined by dividing the corrected counts per minute obtained in the presence of drug by those obtained in the absence of the drug ($\times$ 100).

Tumor Growth Inhibition Studies (by Dr. J. H. Burchenal). The technique of Fisher[45] was employed with modifications.[8] Mouse cell lines L1210/0, P815/0, and P815/Ara-C were incubated in McCoy's medium 5A with 15% fetal calf serum. The initial inoculum was 40 000–60 000 leukemia cells/mL. For growth inhibition studies, 0.1 mL of a 20-fold concentration of the nucleoside in question was added to 2 mL of media containing 4×10^4 cells/mL in Linbro tissue culture multiwell plates and allowed to incubate at 37 °C in 5% CO_2 for 96 h. Growth to approximately 10^6 cells/mL occurred in the control wells. The contents of each well were counted on a Coulter Counter and the percentage of inhibition of growth and the concentrations inhibiting cell growth by 50% were calculated.

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# Isoxazoles with Antipicornavirus Activity[†]

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The synthesis and evaluation of a series of 3,5-disubstituted isoxazoles as antipicornavirus agents have led to the discovery of several compounds effective in vitro against rhinovirus type 2 and poliovirus type 2. Compound **32** was found more effective than 4′,6-dichloroflavan against both viruses and was evaluated orally in mice infected intracerebrally with polio-2. At 31 mg/kg bid, compound **32** showed a 53% survival rate as compared to 22% for the nonmedicated animals.

Picornavirus infections are among the most common viral infections in man. This family of viruses consists of the rhinoviruses, of which there are over 120 serotypes, and the enteroviruses comprised of polio, Coxsackie A and B, ECHO, and five unclassified enteroviruses, including hepatitis A. Rhinoviruses are responsible for approximately 50% of the common cold infections and cause mild localized infections of the upper respiratory tract. The enteroviruses cause a broad spectrum of clinical illnesses ranging from mild upper respiratory ailments to more severe diseases such as aseptic meningitis, myocarditis, and poliomyelitis. Generally, enterovirus infections of the pediatric population result in greater morbidity and mortality.[1] Recent studies have shown that relatively mild pediatric infections can result in long-term neurological sequelae.[2]

In view of the lack of chemotherapeutic agents available for the treatment of picornavirus infections, we initiated a program directed towards the discovery of compounds active against this class of viruses. We initially examined compounds related to arildone, since this compound, in

CH₃O—⟨ring, Cl⟩—O(CH₂)₆CH(COC₂H₅)₂

arildone
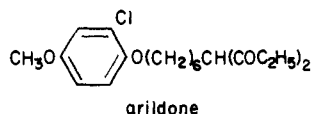
addition to exhibiting in vitro activity against herpesvirus,[3] was effective against poliovirus[4] and had demonstrated efficacy when administered orally to mice infected intra-

Scheme I. Synthesis of 3,4,5-Trisubstituted Isoxazoles
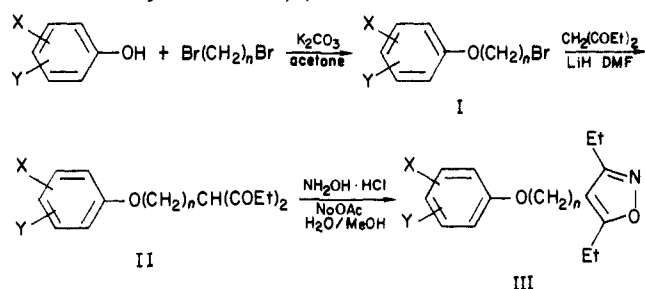
cerebrally with polio-2.[5] Arildone, however, was only marginally effective against the rhinoviruses, and since the objective was to synthesize an agent with broad spectrum activity against the picornaviruses, we began screening related compounds. As a result of this screening, the isoxazoles III possessing in vitro activity against both

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