Synthesis and Antiviral Activity of Novel 5-(1-Azido-2-haloethyl) and 5-(1-Azido-, amino-, or methoxyethyl) Analogs of 2'-Deoxyuridine[1]

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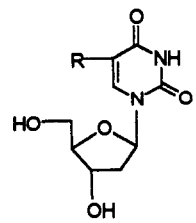
A new class of 5-(1-azido-2-haloethyl)-2'-deoxyuridines **3a–c** was synthesized by the regiospecific addition of XN_3 (X = I, Br, Cl) to the vinyl substituent of 5-vinyl-2'-deoxyuridine. Treatment of the 5-(1-azido-2-iodoethyl) compound (**3a**) with H_2 and 10% Pd/C yielded the 5-(1-azidoethyl) (**4**) and 5-(1-aminoethyl) (**5**) derivatives of 2'-deoxyuridine. A similar hydrogenation of 5-(1-methoxy-2-iodoethyl)-2'-deoxyuridine (**1f**) afforded the 5-(1-methoxyethyl) analog **6**. The 5-(1-azido-2-haloethyl)-2'-deoxyuridines **3a–c** exhibited *in vitro* antiviral activity against HSV-1, HSV-2, VZV, and EBV, but they were inactive against HCMV. In this group of compounds, the activity order was Cl ≥ I > Br against HSV-1 and Br ≥ Cl > I against HSV-2. A halogen atom in the 5-(1-azido-2-haloethyl) moiety **3a–c** is an essential requirement since the 5-(1-azidoethyl) analog **4** was inactive, except for weak antiviral activity against VZV. Although the 5-(1-aminoethyl)-2'-deoxyuridine·HI (**5**) was inactive against HSV-1 and HSV-2, the 5-(1-methoxyethyl) compound **6** was equiactive to 5-ethyl-2'-deoxyuridine (EDU) against both HSV-1 and HSV-2 and 7-fold and 12-fold less active against HCMV relative to EDU and ganciclovir, respectively. All compounds investigated (**3–6**) exhibited low host cell cytotoxicity (IC_{50} > 118 μM) and inhibited cell proliferation only at high concentrations (IC_{50} > 76 μM).

The development of new methods for the synthesis of 2'-deoxyuridines that possess novel 2-carbon substituents at the C-5 position, which exhibit potent and selective antiviral activity, represents an important area of antiviral drug design. Of the many 5-substituted pyrimidine nucleosides that have been investigated, (*E*)-5-(2-halovinyl)- (**1a**, IVDU; **1b**, BVDU; **1c**, CVDU)[2] and 5-(2-chloroethyl)-2'-deoxyuridine (**1d**, CEDU)[3] are among the most potent and selective in their action against herpes simplex virus type 1 (HSV-1). CEDU is effective against systemic HSV-1 infection and HSV-1 encephalitis in mice at a 5–15-fold lower dose than BVDU.[4] In contrast, the less potent 5-ethyl-2'-deoxyuridine (EDU, **1e**) is approximately equiactive against HSV-1 and HSV-2.[2] In earlier studies, we reported that the 5-(1-methoxy-2-iodoethyl) (**1f**)[5] and 5-(1-methoxy-2-bromoethyl) (**1g**)[6] derivatives of 2'-deoxyuridine exhibited appreciable *in vitro* anti-HSV-1 activity. Prusoff *et al.*[7] have reported the thymidine derivative, 5-(azidomethyl)-2'-deoxyuridine (AMDR, **1h**), which is a potent inhibitor of HSV-1 that is not specific for virally infected cells, and the 5-(aminoethyl) analog (AEDU, **1i**) which was inactive against HSV-1.[7] It was therefore of interest to investigate the hitherto unknown 5-(1-azido-2-haloethyl)-2'-deoxyuridines **3a–c** which can be considered to be hybrids of **1d** (R = CH_2CH_2Cl) and **1h** (R = CH_2N_3), the 5-(1-aminoethyl) analog **5** which is a hybrid of **1e** (R = CH_2CH_3) and **1i** (R = CH_2NH_2), and 5-(1-methoxyethyl)-2'-deoxyuridine (**6**) which is a hybrid of **1e** (R = CH_2CH_3) and **1f** [R = $CH(OMe)CH_2I$]. It was postulated that these 5-(1-azido-2-haloethyl)-, 5-(1-azidoethyl)-, 5-(1-aminoethyl)-, and 5-(1-methoxyethyl)-2'-deoxyuridines, in contrast to EDU (**1e**),[8] would be resistant to metabolic hydroxylation at the C-1 position of the 5-substituent due to obstructive substitution by the azido, amino, or methoxy substituent. Furthermore, it is conceivable that 5-(1-azido-2-haloethyl)-2'-deoxyuridines may serve as prodrugs, due to elimination of hydrogen azide, under physiological conditions to yield IVDU (**1a**), BVDU (**1b**), or CVDU (**1c**). There is precedent for this latter postulate since 5-(1-mesyloxy)uracil was spontaneously converted to 5-vinyluracil during its attempted synthesis.[9] We now report the synthesis and antiviral activities for a new class of 5-(1-azido-2-haloethyl)-2'-deoxyuridines **3a–c** and related 5-(1-azidoethyl) (**4**), 5-(1-aminoethyl) (**5**), and 5-(1-methoxyethyl) (**6**) analogs.



1a, R= (E)-CH=CHI
1b, R= (E)-CH=CHBr
1c, R= (E)-CH=CHCl
1d, R= $-CH_2CH_2Cl$
1e, R= $-CH_2CH_3$
1f, R= $-CH(OMe)CH_2I$
1g, R= $-CH(OMe)CH_2Br$
1h, R= $-CH_2N_3$
1i, R= $-CH_2NH_2$

Chemistry

The target 5-(1-azido-2-haloethyl)-2'-deoxyuridines **3a–c** were synthesized by reaction of 5-vinyl-2'-deoxyuridine (**2**) with either ICl, *N*-bromosuccinimide, or *N*-chlorosuccinimide and sodium azide in 58, 51, and 43% yields, respectively, as illustrated in Scheme I. The ^{13}C NMR (*J*-modulated spin echo) spectra provided conclusive evidence for the regiospecific addition of XN_3 (X = I, Br, Cl) across the C-5 vinyl substituent of **2**. For example, the iodine atom of **3a** is attached to a methylene carbon that exhibited dual resonances at δ 6.37 and 7.03, whereas the azido substituent is attached to a chiral methine carbon that exhibited dual resonances at δ 60.71 and 61.29. Compounds **3a–c** are therefore a mixture of two diastereomers (1:1 ratio) which differ in configuration (*R* and *S*)

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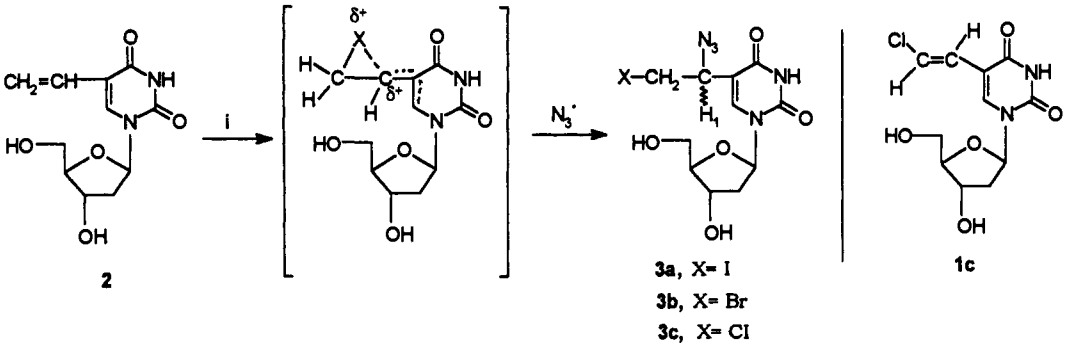
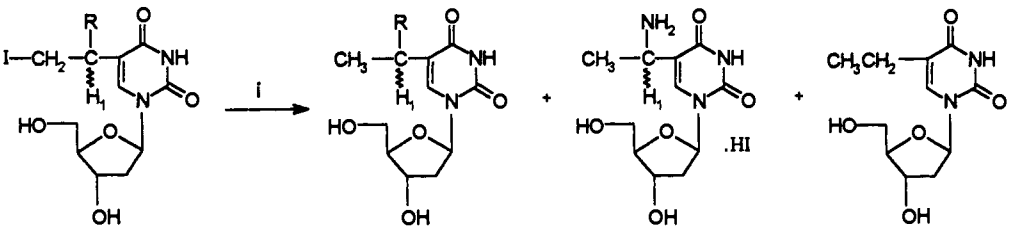
Scheme I[a]



[a] Reagents: (i) ICl, NaN_3, MeCN (3a); *N*-bromosuccinimide (3b) or *N*-chlorosuccinimide (3c), NaN_3, DME, H_2O.

Scheme II[a]



[a] Reagents: (i) H_2, 10% PdC/EtOH, 25 °C.

at the 1-carbon atom of the 5-(1-azido-2-haloethyl) substituent. This regiospecific addition is consistent with reports that unsymmetrical olefins, capable of halonium ion formation, were found to favor an unsymmetrical bridged intermediate of the type illustrated in Scheme I even in solvents having a high dipole moment.[10,11] Attempts to separate the diastereomers of 3a–c by flash column chromatography, or the multiple development TLC technique, were unsuccessful.

In the reaction of 2 with *N*-chlorosuccinimide and NaN_3 in aqueous DME, (*E*)-5-(2-chlorovinyl)-2'-deoxyuridine (1c, 4%) was also produced in addition to the target 5-(1-azido-2-chloroethyl) product 3c. The most plausible mechanism for the formation of 1c is an E_2 elimination reaction involving expulsion of HN_3 from 3c. In contrast, an E_1 elimination reaction would have been expected to also yield 5-(1-hydroxy-2-chloroethyl)-2'-deoxyuridine resulting from reaction of the carbonium ion intermediate produced with water.[12]

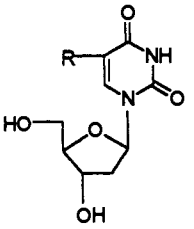
Reaction of 5-(1-azido-2-iodoethyl)-2'-deoxyuridine (3a) with hydrogen gas at 35 psi in the presence of 10% Pd/C in ethanol at 25 °C afforded 5-(1-azidoethyl)-2'-deoxyuridine (4, 9%), 5-(1-aminoethyl)-2'-deoxyuridine·HI (5, 38%), and 5-ethyl-2'-deoxyuridine (1e, 7%) as illustrated in Scheme II. A similar reaction employing 5-(1-methoxy-2-iodoethyl)-2'-deoxyuridine (1f) yielded 5-(1-methoxyethyl)- (6, 26%) and 5-ethyl-2'-deoxyuridine (1e, 13%).

Results and Discussion

The antiviral activities for this new class of 5-(1-azido-2-haloethyl)-2'-deoxyuridines 3a–c and related 5-(1-azido-, amino-, methoxyethyl) analogs 4–6 were determined against five viruses (HSV-1, HSV-2, VZV, EBV, HCMV), and the results are summarized in Table I. In the 5-(1-azido-2-haloethyl) series of compounds (3a–c), the relative anti-HSV-1 activity order parallels that of 5-(2-haloethyl)-2'-deoxyuridines (Cl ≥ I > Br for both HSV-1 and HSV-2 activity),[3] whereas the relative activity order for 3a–c differs with respect to anti-HSV-2 activity (Br ≥ Cl > I). Although the 5-(1-azido-2-haloethyl) compounds 3a–c were inactive against human cytomegalovirus (HCMV), they exhibited appreciable antiviral activity against both VZV and EBV which compared favorably with that of the reference drugs 5-ethyl-2'-deoxyuridine (EDU, 1e) and acyclovir. The antiviral spectrum exhibited by 5-(1-azido-2-chloroethyl)-2'-deoxyuridine (3c) is similar to that of acyclovir, except it is much less active against HSV-2.

The observation that the 5-(1-azidoethyl) analog 4 is devoid of antiviral activity, except for weak activity against VZV, indicates that the halogen atom in the 5-(1-azido-2-haloethyl) moiety is an essential requirement for potent antiviral activity. The 5-(1-azidoethyl) compound 4 and 5-(1-aminoethyl)-2'-deoxyuridine·HI (5) were both inactive against HSV-1 and HSV-2. In contrast, the 5-(1-methoxyethyl) compound 6 exhibited similar antiviral activities against HSV-1 and HSV-2 to EDU (1c). The anti-HCMV activity of the 5-(1-methoxyethyl) compound 6 (EC_{50} = 5.9 μM) is about 7-fold and 12-fold less than that of EDU and ganciclovir, respectively. However, it is conceivable that 6 may possess a high *in vivo* stability, compared to EDU, since the methoxy substituent would obstruct metabolic hydroxylation at the C-1 position of the 5-substituent which results in the inactivation of EDU.[8] The anti-HSV-2 potency of 5-substituted 2'-deoxyuridines such as BVDU (1b) is usually much less than that against VZV. It has been suggested that if BVDU exerts its effect as the triphosphate, the inefficient phosphorylation of the monophosphate contributes to the insensitivity of the HSV-2 virus to BVDU. The observation that the anti-HSV-2 activity of the 5-(1-methoxyethyl) analog 6 is much greater than its anti-VZV activity was therefore unexpected. This result suggests that compound 6 may be a better substrate for the HSV-2 than the VZV deoxythymidine kinase.[13] In the 5-(1-substituted)ethyl group of

Table I. In Vitro Antiviral Activity of 5-Substituted-2′-deoxyuridines



no.	R	IC_{50}[a] (μM) cytotoxicity	EC_{50} (μM) HSV-1[c] (E-377)	HSV-2[c] (MS)	VZV[c] (Ellen)	EBV[d] (P3HR-1)	HCMV[c] (AD169)	IC_{50}[b] (μM) cell proliferation
3a	$-CH(N_3)CH_2I$	>118	0.5	13.2	12.3	4.0	>118	76
3b	$-CH(N_3)CH_2Br$	>130	6.4	2.9	4.0	9.3	>130	91
3c	$-CH(N_3)CH_2Cl$	>150	0.06[e]	6.6	4.5	11.1	>150	>300
4	$-CH(N_3)CH_3$	>170	>170	>170	>67	ND[f]	>170	81
5	$-CH(NH_2)CH_3 \cdot HI$	>125	>100	>100	>250	44.6	>125	>250
6	$-CH(OMe)CH_3$	>175	1.7	1.0	>87	ND	5.9	330
1e	$-CH_2CH_3$(EDU)	>195	0.6	1.5	66.4	12.1	0.9	115
IVDU[g]	(E)-CH=CHI	86	0.02	2.21	0.01	ND	24.8	4.4
acyclovir		>440	0.04	0.09	7.1	2.9	ND	>440
ganciclovir		>390	ND	ND	ND	ND	0.5	168

[a] The drug concentration (μM) required to reduce the uptake of neutral red stain by uninfected cell monolayers to 50% of untreated uninfected controls. [b] The drug concentration (μM) required to reduce proliferation of human foreskin fibroblasts to 50% of untreated controls. [c] The drug concentration (μM) required to reduce the viral cytopathic effect (CPE) in infected cell monolayers to 50% of untreated, uninfected controls. [d] The drug concentration (μM) required to reduce antigen production in infected Raji cells to 50% of untreated infected controls. [e] Selectivity index (IC_{50}/EC_{50}) > 2500. [f] ND = not determined. [g] IVDU = (E)-5-(2-iodovinyl)-2′-deoxyuridine.

compounds (4–6, 1e), the substituent present at the C-1 position of the 5-ethyl moiety was a determinant of antiviral HSV-1 activity where the relative potency order was H (1e) ≥ OMe (6) > N_3 (4) and NH_2 (5).

All of the compounds investigated exhibited low host cell cytotoxicity (IC_{50} > 118 μM) and inhibited cell proliferation only at high concentrations (IC_{50} > 76 μM). The compounds (3–6) investigated are a mixture of two diastereomers (1:1 ratio) which differ in configuration (*R* or *S*) at the C-1 position of the 5-substituent that could not be separated by flash silica gel column, or multiple development TLC, chromatography. In an earlier study, we reported that 5-[(1*R*)-2,2-dichlorocyclopropyl]-2′-deoxyuridine was more active against HSV-1 than the 1*S*-diastereomer which was inactive.[14] Methodology to separate the diastereomers (3, 6) will be developed if *in vivo* studies indicate the testing of pure diastereomers is desirable.

Summary

The 5-(1-azido-2-haloethyl)-2′-deoxyuridines, which require a halogen atom (I, Br, Cl) for maximum activity, exhibited antiviral activity against HSV-1, HSV-2, VZV, and EBV. 5-(1-Azido-2-chloroethyl)-2′-deoxyuridine, which was highly selective (SI > 2500), was 10-fold more potent than EDU and equiactive to acyclovir against HSV-1. Although 5-(1-methoxyethyl)-2′-deoxyuridine (6) was 12-fold less active than ganciclovir against HCMV, it could serve as a useful lead compound for development of an improved anti-HCMV drug which is urgently required for antiviral chemotherapy.

Experimental Section

Melting points were determined with a Buchi capillary apparatus and are uncorrected. Nuclear magnetic resonance spectra (^1H NMR, ^{13}C NMR) were determined on a Bruker AM-300 spectrometer using Me_4Si as an internal standard (^1H NMR). The assignment of all exchangeable protons (OH, NH) was confirmed by the addition of D_2O. ^{13}C NMR spectra were acquired using the *J*-modulated spin echo technique where methyl and methine carbon resonances appear as positive peaks and methylene and quaternary carbons appear as negative peaks. Fast atom bombardment (FAB) mass spectra were determined on an AEI-MS-12 spectrometer. Microanalyses were within ±0.4% of theoretical values for all elements listed, unless otherwise indicated. Preparative thin-layer chromatography (PTLC) was performed using Whatman PLK5F plates, 1.0 mm in thickness, and silica gel column chromatography was carried out using Merck 7734 silica gel (100–200-μm particle size). 5-Vinyl-2′-deoxyuridine (2)[15] and 5-(1-methoxy-2-iodoethyl)-2′-deoxyuridine (1f)[5] were prepared using literature procedures.

5-(1-Azido-2-iodoethyl)-2′-deoxyuridine (3a). Iodine monochloride (50 mg, 0.3 mmol) was added slowly during a 5-min period to a suspension of sodium azide (65 mg, 1.0 mmol) in dry acetonitrile (10 mL) at ice-bath temperature with stirring. This mixture was stirred for a further 5 min, a solution of 2 (62 mg, 0.24 mmol) in dry acetonitrile (25 mL) was added, and the reaction mixture was warmed to 25 °C and stirred for 30 min. The resulting red-brown colored reaction mixture was poured onto ice-cold water (25 mL), the mixture was extracted with ethyl acetate (3 × 50 mL), and the ethyl acetate extract was washed with 5% aqueous sodium thiosulfate (10 mL). Drying the colorless ethyl acetate fraction (Na_2SO_4), removal of the solvent *in vacuo*, and purification of the residue obtained by elution from a silica gel column using $CHCl_3$/MeOH (92:8, v/v) as eluent afforded **3a** (60 mg, 58%) as a viscous oil: ^1H NMR (MeOH-d_4) (mixtures of two diastereomers in a ratio of 1:1) δ 2.28 (m, 2H, H-2′), 3.58 and 3.66 (two m, 2H total, CH_2I), 3.82 (m, 2H, H-5′), 3.98 (m, 1H, H-4′), 4.44 (m, 1H, H-3′), 4.68 (m, 1H, CHN_3), 6.30 and 6.32 (two overlapping t, J = 6 Hz, 1H total, H-1′), 8.24 and 8.28 (two s, 1H total, H-6); ^{13}C NMR (MeOH-d_4) δ 6.4 and 7.0 (CH_2I), 41.7 and 41.8 (C-2′), 60.7 and 61.3 (CHN_3), 62.5 (C-5′), 72.0 (C-3′), 86.8 and 86.9 (C-1′), 89.1 (C-4′), 112.26 and 112.31 (C-5), 140.7 (C-6), 151.6 (C-2 C=O), 164.1 (C-4 C=O). Anal. ($C_{11}H_{14}IN_5O_5$) C, H, N.

5-(1-Azido-2-bromoethyl)-2′-deoxyuridine (3b). *N*-Bromosuccinimide (NBS) (80 mg, 0.45 mmol) was added in aliquots to a precooled (−5 °C) suspension prepared by mixing a solution of 2 (0.1 g, 0.39 mmol) in dimethoxyethane (10 mL) with a solution of sodium azide (104 mg, 1.6 mmol) in water (0.2 mL). The initial yellow color produced upon addition of each NBS aliquot quickly disappeared. When all the NBS had reacted, the reaction mixture was stirred for 40 min at 0 °C, poured onto ice–water (25 mL), and extracted with ethyl acetate (3 × 50 mL). The ethyl acetate extract was washed with cold water (10 mL) and dried (Na_2SO_4),

the solvent was removed *in vacuo*, and the residue obtained was purified by elution from a silica gel column using $CHCl_3$/MeOH (92:8, v/v) as eluent to give **3b** (76 mg, 51%) as a viscous oil: ^1H NMR (MeOH-d_4) (mixture of two diastereomers in a ratio of 1:1) δ 2.34 (complex m, 2H, H-2'), 3.75 (m, 2H, CH_2Br), 3.86 (m, 2H, H-5'), 4.04 (m, 1H, H-4'), 4.48 (m, 1H, H-3'), 4.82 (m, 1H, CHN$_3$), 6.35 and 6.36 (two closely spaced t, J = 6 Hz, 1H total, H-1'), 8.30 and 8.32 (two s, 1H total, H-6); ^{13}C NMR (MeOH-d_4) δ 33.8 and 33.9 (CH_2Br), 41.7 (C-2'), 60.8 and 61.1 (CHN$_3$), 62.6 (C-5'), 72.0 (C-3'), 86.9 (C-1'), 89.0 (C-4'), 111.4 (C-5), 140.9 (C-6), 151.6 (C-2 C=O), 164.2 (C-4 C=O). Anal. ($C_{11}H_{14}BrN_5O_5$) C, H, N.

5-(1-Azido-2-chloroethyl)-2'-deoxyuridine (3c) and (*E*)-5-(2-Chlorovinyl)-2'-deoxyuridine (1c). *N*-Chlorosuccinimide (0.27 g, 2.0 mmol) was added slowly to a precooled suspension (−5 °C) prepared by mixing a solution of **2** (0.45 g, 1.77 mmol) in dimethoxyethane (25 mL) with a solution of sodium azide (0.45 g, 6.9 mmol) in water (0.25 mL). The reaction mixture was stirred for 2 h at 0 °C. Completion of the reaction, as described for the isolation of **3b**, gave a residue which was purified by silica gel column chromatography. Elution with $CHCl_3$/MeOH (95:5, v/v) as eluent yielded **3c** as a viscous oil (0.25 g, 43%); ^1H NMR (MeOH-d_4) (mixture of two diastereomers in a ratio of 1:1) δ 2.26 (m, 2H, H-2'), 3.70–4.0 (complex m, 5H, CH_2Cl, H-4', H-5'), 4.42 (m, 1H, H-3'), 4.74 (m, 1H, CHN$_3$), 6.28 (t, J = 6 Hz, 1H, H-1'), 8.22 and 8.23 (two s, 1H total, H-6); ^{13}C NMR (MeOH-d_4) δ 41.6 (C-2'), 46.0 and 46.1 (CH_2Cl), 61.1 and 61.3 (CHN$_3$), 62.6 (C-5'), 72.0 (C-3'), 86.9 (C-1'), 89.1 (C-4'), 110.8 (C-5), 141.0 and 141.1 (C-6), 151.7 (C-2 C=O), 164.2 (C-4 C=O). Anal. ($C_{11}H_{14}ClN_5O_5 \cdot 1/2H_2O$) C, H, N.

Further elution using the same solvent afforded **1c** (20 mg, 4%) which was identical (mp, ^1H NMR) to an authentic sample.[16]

5-(1-Azidoethyl)-2'-deoxyuridine (4), 5-(1-Aminoethyl)-2'-deoxyuridine Hydrogen Iodide Salt (5), and 5-Ethyl-2'-deoxyuridine (1e). Reaction of **3a** (0.13 g, 3.0 mmol) in ethanol (100 mL) with 10% Pd/C (50 mg) and H_2 gas at 35 psi for 5 h at 25 °C, filtration of the mixture through a Celite pad, and removal of the solvent *in vacuo* gave a viscous oil which was separated by silica gel column chromatography. Elution with $CHCl_3$/MeOH (19:1, v/v) gave a mixture of **4** and **1e** which was then separated by PTLC using $CHCl_3$/MeOH (9:1, v/v) as development solvent. Extraction of the band having R_f 0.67 yielded **4** as a viscous oil (8 mg, 9%): ^1H NMR (MeOH-d_4) (mixture of two diastereomers in a ratio of 1:1) δ 1.43 [d, J = 6.7 Hz, 3H, CH(N$_3$)CH_3], 2.26 (m, 2H, H-2'), 3.78 (m, 2H, H-5'), 3.96 (m, 1H, H-4'), 4.42 (m, 1H, H-3'), 4.58 [m, 1H, CH(N$_3$)CH$_3$], 6.30 and 6.32 (two overlapping t, J = 6 Hz, 1H total, H-1'), 8.20 (s, 1H, H-6); ^{13}C NMR (MeOH-d_4) δ 18.7 (CH_3), 41.7 (C-2'), 55.0 and 55.1 (CHN$_3$), 62.6 and 62.7 (C-5'), 72.1 and 72.2 (C-3'), 86.8 and 86.9 (C-1'), 89.08 and 89.12 (C-4'), 115.0 (C-5), 139.3 and 139.5 (C-6), 151.8 (C-2 C=O), 164.6 (C-4 C=O). Anal. ($C_{11}H_{15}N_5O_5$) C, H; N: calcd, 23.56; found, 22.94. Extraction of the band having R_f 0.60 afforded **1e** (6 mg, 7%) which was identical (mp, ^1H NMR) to an authentic sample.[17] Further elution of the silica gel column using $CHCl_3$/MeOH (7:3, v/v) as eluent gave impure **5** which was purified by elution from a Bio-gel column using water as eluent to yield **5** (45 mg, 38%) as a white solid: ^1H NMR (Me_2SO-d_6) (mixture of two diastereomers in a ratio of 1:1) δ 1.38 (d, J = 6.7 Hz, 3H, CHCH_3), 2.12 (m, 2H, H-2'), 3.56 (m, 2H, H-5'), 3.82 (m, 1H, H-4'), 4.15–4.32 (complex m, 2H, H-3, CHN$^+$H$_3$), 5.12 and 5.30 (two br s, 1H each, 3'-OH, 5'-OH), 6.18 and 6.23 (two overlapping t, J = 6 Hz, 1H total, H-1'), 7.92 (br s, 3H, N$^+H_3$), 11.80 (s, 1H, NH); ^{13}C NMR (MeOH-d_4) δ 17.60 and 17.66 (CH_3), 41.7 and 41.8 (C-2'), 46.8 and 46.9 (CHN$^+$H$_3$), 62.6 (C-5'), 72.0 (C-3'), 86.9 and 87.0 (C-1'), 89.1 and 89.2 (C-4'), 111.1 (C-5), 141.3 (C-6), 151.6 (C-2 C=O), 164.7 (C-4 C=O); MS (M$^+$ + Na, FAB) calcd for $C_{11}H_{17}N_3O_5Na$ 294, found 294; calcd for $C_{11}H_{16}IN_3O_5$-Na 422, found 422. Anal. ($C_{11}H_{16}IN_3O_5 \cdot 5H_2O$) C, N; H: calcd, 5.76; found, 4.98.

5-(1-Methoxyethyl)-2'-deoxyuridine (6) and 5-Ethyl-2'-deoxyuridine (1e). Hydrogenation of **1f** (0.124 g, 0.3 mmol) in 98% EtOH (25 mL) in the presence of 10% Pd/C (0.2 g) and H_2 gas at 35 psi for 12 h at 25 °C and isolation of the product, as described for the preparation of **5**, gave a residue. Purification by silica gel PTLC using $CHCl_3$/MeOH (92:8, v/v) as development solvent yielded **6** (R_f 0.64, 22 mg, 26%) as a viscous oil and **1e** (R_f 0.60, 10 mg, 13%) which was identical (mp, ^1H NMR) to an authentic sample.[16] Compound **6**: ^1H NMR (MeOH-d_4) (mixture of two diastereomers in a ratio of 1:1) δ 1.36 (d, J = 6.7 Hz, 3H, CHCH_3), 2.30 (m, 2H, H-2'), 3.32 (s, 3H, OCH_3), 3.80 (m, 2H, H-5'), 3.98 (m, 1H, H-4'), 4.35 (m, 1H, CHOMe), 4.45 (m, 1H, H-3'), 6.34 (t, J = 6 Hz, 1H, H-1'), 8.04 and 8.05 (two s, 1H total, H-6); ^{13}C NMR (MeOH-d_4) δ 20.8 and 20.9 (CHCH_3), 41.4 and 41.5 (C-2'), 56.9 (OCH_3), 62.8 and 62.9 (C-5'), 72.3 (CHOCH$_3$), 73.6 (C-3'), 86.4 and 86.7 (C-1'), 88.9 and 89.0 (C-4'), 116.7 (C-5), 138.6 and 138.8 (C-6), 152.1 (C-2 C=O), 164.8 (C-4 C=O). Anal. ($C_{12}H_{18}N_2O_6 \cdot 1/2H_2O$) C, H, N.

In Vitro Antiviral Assays. Cytopathic effect (CPE) inhibition assays for HSV-1, HSV-2, HCMV, and VZV were performed under the NIH Antviral Research Branch testing program using the following procedures: Low passage human foreskin fibroblast (HFF) cells were seeded into 96 well tissue culture plates 24 h prior to use at a cell concentration of 2.5 × 10^4 cells per mL in 0.1 mL of minimal essential medium (MEM) supplemented with 10% fetal bovine serum (FBS). The cells were then incubated for 24 h at 37 °C in a CO_2 incubator. After incubation, the medium was removed and 100 μL of MEM containing 2% FBS was added to all but the first row. In the first row, 125 μL of experimental drug was added in triplicate wells. Medium alone was added to both cell and virus control wells. The drug in the first row of wells was then diluted serially 1:5 throughout the remaining wells by transferring 25 μL using the Cetus liquid handling machine. After dilution of drug, 100 μL of the appropriate virus concentration was added to each well, excluding cell control wells which received 100 μL of MEM. For HSV-1 and HSV-2 assays, the virus concentration utilized was 1000 PFU's per well. For CMV and VZV assays, the virus concentration added was 2500 PFU's per well. The plates were then incubated at 37 °C in a CO_2 incubator for 3 days for HSV-1 and HSV-2, 10 days for VZV, or 14 days for CMV. After the incubation period, media was aspirated and the cells were stained with a 0.1% crystal violet solution for 30 min. The stain was then removed, and the plates were rinsed using tap water until all excess stain was removed. The plates were allowed to dry for 24 h and then read on a Skatron plate reader at 620 nm.

The immunofluorescence assay, using monoclonal antibodies, for Epstein–Barr virus (EBV) was performed as follows: Raji cells were infected with the P3HR-1 strain of EBV, and the test drug was added after adsorption (45 min at 37 °C) and washing of the cell cultures. These cultures were then incubated for 2 days in complete medium to allow viral gene expression. Following the 48-h incubation period, the number of cells in each sample were counted and smears were made. Monoclonal antibodies to the different early antigen (EA) components and VCA were then added to the cells, incubated, and washed. This was followed by the addition of a fluorescein conjugated rabbit anti-mouse Ig antibody. The number of fluorescence positive cells in the smears were counted, and the total number of cells in the cultures positive for EA or VCA were then calculated and compared.

Cell Cytotoxicity Assay. The following procedures were used under the NIH Antiviral Research Branch testing program. Twenty-four h prior to assay, HFF cells were plated into 96 well plates at a concentration of 2.5 × 10^4 cells per well. After 24 h, the media was aspirated and 125 μL of drug was added to the first row of wells and then diluted serially 1:5 using the automated Cetus liquid handling system in a manner similar to that used in the CPE assay. The plates were incubated for 7 days in a CO_2 incubator at 37 °C. At this time the media/drug was aspirated, and 200 μL/well of 0.01% neutral red in Dulbecco's phosphate buffered saline (DPBS) was added. This was incubated in the CO_2 incubator for 1 h, the dye was aspirated and the cells were washed using a Nunc plate washer. After the DPBS wash was removed, 200 μg/well of 50% EtOH/1% glacial acetic acid in water was added. The plates were rotated for 15 min, and the optical densities were read at 550 nm on a plate reader.

Cell Proliferation Assay. Twenty-four h prior to assay, human foreskin fibroblast (HFF) cells were seeded in 6-well plates at a concentration of 2.5 × 10^4 cells per well in MEM containing 10% FBS. On the day of the assay, drugs were diluted serially in MEM containing 10% FBS at increments of 1:5 covering a range from 100 μg/mL to 0.03 μg/mL. For drugs that have to be solubilized in DMSO, control wells received MEM containing

10% DMSO. The media from the wells was then aspirated, and 2 mL of each drug concentration was then added to each well. The cells were incubated in a CO_2 incubator at 37 °C for 72 h. At the end of this time, the media–drug solution was removed, and the cells were washed. One mL of 0.25% trypsin was added to each well and incubated until the cells started to come off of the plate. The cell–media mixture was then pipetted up and down vigorously to break up the cell suspension, and 0.2 mL of the mixture was added to 9.8 mL of Isoton III and counted using a Coulter counter. Each sample was counted three times with three replicate wells per sample.

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