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Synthesis of 5-[1-Hydroxy(or methoxy)-2-bromo(or chloro)ethyl]-2′-deoxyuridines and Related Halohydrin Analogues with Antiviral and Cytotoxic Activity

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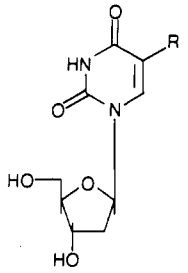
A series of new 5-(1-hydroxy-2-haloethyl)-2′-deoxyuridines (**3**, **6**, **8**) were synthesized in 60–70% yields by addition of HOX (X = Br, Cl, I) to the vinyl substituent of the respective 5-vinyl-2′-deoxyuridines (**2**, **5**, **7**). Treatment of **3a**,**b** with methanolic sulfuric acid afforded the corresponding 5-(1-methoxy-2-haloethyl)-2′-deoxyuridines (**4a**,**b**). The 5-(1-hydroxy-2-chloroethyl) (**3b**), 5-(1-methoxy-2-bromoethyl) (**4a**), 5-(1-hydroxy-2-bromo-2-(ethoxycarbonyl)ethyl) (**6a**), and 5-(1-hydroxy-2-iodo-2-(ethoxycarbonyl)ethyl) (**6b**) derivatives exhibited in vitro antiviral activity (ID_{50} = 0.1–1 μg/mL range) against herpes simplex virus type 1 (HSV-1). 5-(1-Hydroxy-2-bromo-2-(ethoxycarbonyl)-ethyl)-2′-deoxyuridine (**6a**) was the most active cytotoxic agent in the in vitro L1210 screen exhibiting an ED_{50} of 11 μg/mL relative to melphalan (ED_{50} = 0.15 μg/mL).

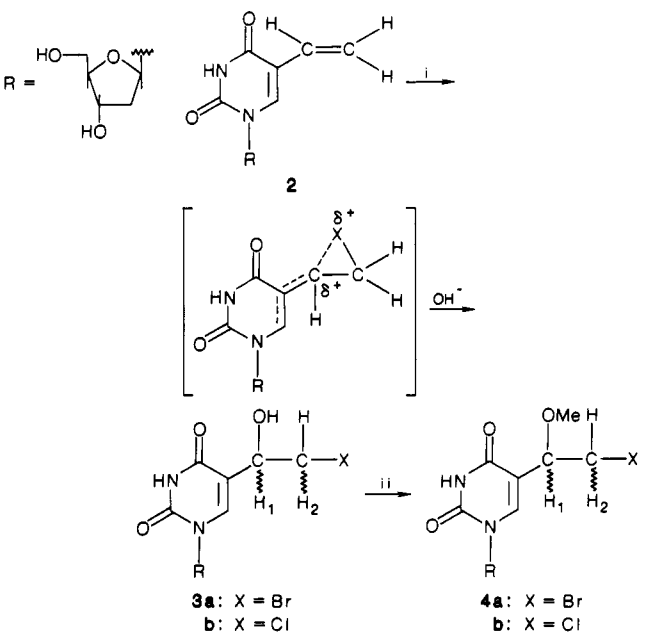
(*E*)-5-(2-Bromovinyl)-2′-deoxyuridine (**1a**, BVDU)[1] and 5-(2-chloroethyl)-2′-deoxyuridine (**1b**, CEDU)[2] are two of the most potent and selective antiviral agents from the large number of 5-substituted pyrimidine nucleoside analogues investigated.[3] CEDU was effective in vivo



1a: R = (*E*)-CH=CHBr
b: R = CH_2CH_2Cl
c: R = $CH=CH_2$
d: R = CH_2CH_2OH
e: R = CH_2CH_3

against systemic herpes simplex virus type 1 (HSV-1) infection and HSV-1 encephalitis in mice at a 5–15-fold lower dose than either BVDU or acyclovir (ACV).[2,4,5] 5-Vinyl-2′-deoxyuridine (**1c**, VDU) is a potent antiviral and antileukemic agent in tissue culture[1,6,7] but was inactive in vivo as an antiviral, antileukemic, or cytotoxic agent due to in vivo degradation by thymidine phosphorylase.[8] Although 5-(2-hydroxyethyl)-2′-deoxyuridine (**1d**, HEDU) is an inactive antiviral agent,[2] 5-ethyl-2′-deoxyuridine (**1e**, EDU) is a potent and selective inhibitor of herpes simplex virus type 1 (HSV-1) and 2 (HSV-2) replication that is being investigated for the topical treatment of HSV-1 and HSV-2 infections in humans. The selectivity of EDU is due to its preferential phosphorylation by the virus-infected cell and its preferential incorporation into viral DNA.[9] It was therefore of interest to investigate the effect of novel 5-halohydrin substituents attached to the C-5 position of 2′-deoxyuridine on biological activity. Halohydrins (**3**, **6**, **8**) could undergo selective phosphorylation,

Scheme I[a]



[a] Reagents: i, *N*-bromosuccinimide (**3a**), *N*-chlorosuccinimide (**3b**), dioxane–water (3:7, v/v), acetic acid, 25 °C; ii, H_2SO_4–MeOH, 25 °C.

like BVDU[1] and EDU,[9] by HSV-1 virus-encoded thymidine kinase to exhibit selective antiviral activity and/or

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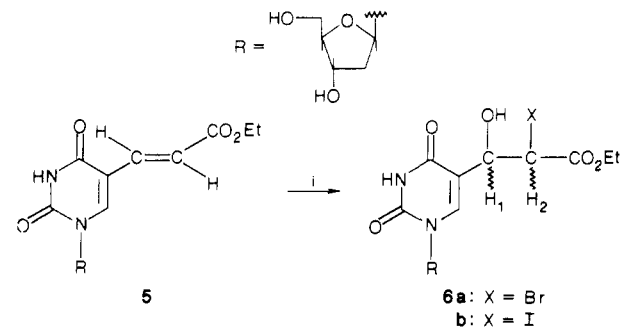
act as irreversible inhibitors of thymidylate synthetase or other enzymes in the DNA path to exhibit cytotoxic activity. We now report the synthesis and antiviral and cytotoxic activities for a new class of halohydrins (**3**, **6**, **8**) and related 5-(1-methoxy-2-haloethyl) analogues (**4**).

Chemistry

The target 5-(1-hydroxy-2-bromoethyl)- and 5-(1-hydroxy-2-chloroethyl)-2′-deoxyuridines (**3a** and **3b**) were synthesized by reaction of 5-vinyl-2′-deoxyuridine (**2**) with *N*-bromosuccinimide (NBS) and *N*-chlorosuccinimide (NCS) in aqueous dioxane in 70 and 60% yields, respectively, as illustrated in Scheme I. The ^{13}C NMR (*J* modulation) spectrum of **3a** provided conclusive evidence for the regiospecific addition of HOBr to the 5-vinyl substituent of **2**. The bromine atom is attached to a methylene carbon that showed dual resonances at δ 38.18 and 38.48, whereas the hydroxyl substituent is attached to a methine chiral carbon which exhibited dual resonances at δ 65.62 and 65.98. Compound **3a** is a mixture of two diastereomers which differ in configuration (*R* and *S*) at the 1-carbon atom of the 5-(1-hydroxy-2-bromoethyl) substituent. This regiospecific addition is consistent with the results of Dalton et al.[10] in which unsymmetrical olefins, capable of bromonium 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. Treatment of **3a,b** with methanolic sulfuric acid[11] afforded the corresponding 5-(1-methoxy-2-bromoethyl)- and 5-(1-methoxy-2-chloroethyl)-2′-deoxyuridines (**4a** and **4b**) in 93 and 98% yields, respectively. The reaction of (*E*)-5-(2-(ethoxycarbonyl)vinyl)-2′-deoxyuridine (**5**) with NBS yielded the 5-(1-hydroxy-2-bromo-2-(ethoxycarbonyl)ethyl) derivative **6a** (70%). A related reaction of **5** with iodine and potassium iodate afforded the 5-(1-hydroxy-2-iodo-2-(ethoxycarbonyl)ethyl) derivative **6b** (60%) as illustrated in Scheme II. The addition of HOBr to the vinyl group of **5** was regiospecific since the 2-dimensional INADEQUATE NMR spectrum of **6a** (two diastereomers) indicated that the two resonances at δ 68.07 and 68.58 due to the *C*HOH methine chiral carbon were connected to the C-5 carbon at δ 112.54, and that the two resonances at δ 46.92 and 47.14 due to the *C*HBr methine chiral carbon were connected to the CO_2 carbon at δ 168.14. In contrast, reaction of (*E*)-5-(2-iodovinyl)-2′-deoxyuridine (**7**) with NBS gave a mixture of regioisomers **8a** and **8b** in a ratio of 1:1 (64%) that could not be separated by column or TLC chromatography (see Scheme III). Each regioisomer **8a** and **8b** was a mixture of two diastereomers. The ^1H NMR spectrum (D_2O) of **8a** and **8b** exhibited overlapping multiplets for the C*H*OH resonances at δ 5.02, two separate sets of closely spaced doublets assigned to the C*H*Br resonances for the two regioisomers at δ 6.01 and 6.16, and four closely spaced singlets for H-6 at δ 8.05. Double-irradiation studies provided confirmation that the product was a mixture of two regioisomers **8a** and **8b**, and that each regioisomer was a mixture of two diastereomers since (1) irradiation of the resonance at δ 6.16 did not change the resonance at δ 6.01, but it did simplify the resonance at 5.02, (2) irradiation of the resonance at δ 6.01 did not alter the resonance at δ 6.16, but it did simplify the resonance at δ 5.02, (3) irradiation of the overlapping resonances at δ 5.02 resulted in the appearance of two closely spaced singlets at both δ 6.01 and 6.16 (viz. evidence that each regioisomer is a mixture of two diastereomers). The ^{13}C NMR spectrum provided further evidence for the assigned structures based on the resonances at δ 20.66 and 21.11 (*C*HBrI of **8a**), 51.90 and 52.26 (*C*HBr of **8b**), and 71.10, 71.43, 71.94, 72.37 (*C*HOH of **8a** and **8b**).

Scheme II[a]



[a] Reagents: i, *N*-bromosuccinimide, H_2O, 25 °C (**6a**); I_2, KIO_3, H_2O, H_2SO_4, 55 °C (**6b**).

Results and Discussion

The antiviral activities for this new class of 5-[1-hydroxy(or methoxy)-2-bromo(or chloro)ethyl]-2′-deoxyuridines (**3**, **4**) and related 5-halohydrin analogues (**6**, **8**), determined as the concentration to inhibit plaque formation by 50% (ID_{50}) in Vero cells infected with herpes simplex virus type 1 (HSV-1, strain JLJ), are summarized in Table I. The 5-(1-hydroxy-2-chloroethyl) compound (**3b**) exhibited an activity greater than 10-fold that of the corresponding bromo analogue (**3a**). This correlation is consistent with the report that CEDU (**1b**) is 100-fold more active than the corresponding bromo analogue (BrEDU) against HSV-2.[2] Elaboration of the 5-(1-hydroxy-2-haloethyl) substituents of **3a,b** to the corresponding 5-(1-methoxy-2-haloethyl) substituents (**4a,b**) resulted in an unexpected reversal in activity where the bromo compound (**4a**) exhibited an activity greater than 10-fold that of the corresponding chloro analogue (**4b**). The reason for this reversal in activity (**3b** > **3a** relative to **4a** > **4b**) is not known. The approximately equipotent halohydrins (**6a** and **6b**) exhibited an activity 10 times that reported for (*E*)-5-(2-(methoxycarbonyl)vinyl)-2′-deoxyuridine (MIC, 10 μg/mL).[12] The mixture of regioisomers 5-(1-hydroxy-2-bromo-2-iodoethyl) **8a** and 5-(1-bromo-2-hydroxy-2-iodoethyl) **8b**, like **3a**, was inactive, indicating that incorporation of an additional 2-iodo substituent does not improve activity. The most active compounds, **3b**, **4a**, **6a,b** (0.1–1 μg/mL range), exhibited weaker antiviral activity than acyclovir (ID_{50} = 0.01 μg/mL) or BVDU.[1]

The cytotoxic activities for compounds **3**, **4**, **6**, and **8** were determined by an in vitro L1210 assay, and the results are summarized in Table I. A comparison of cytotoxic activities at 50 μg/mL indicated that **3a** was equipotent with **3b**, whereas **4a** was more active than **4b** (P = 0.10).[13] Elaboration of the 5-(1-hydroxy-2-haloethyl) substituents of **3a,b** to the corresponding methoxy analogues (**4a,b**) increased cytotoxic activity for the bromo compound (**4a** > **3a**, P = 0.40) but decreased activity for the chloro com-

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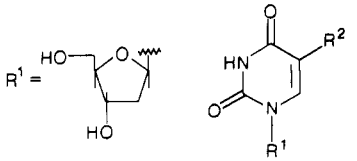
(13) The statistical significance was determined using the Student's *t* test.

Table I. In Vitro Antiviral Activity (HSV-1) and L1210 Cytotoxicity of 5-Substituted 2'-Deoxyuridines

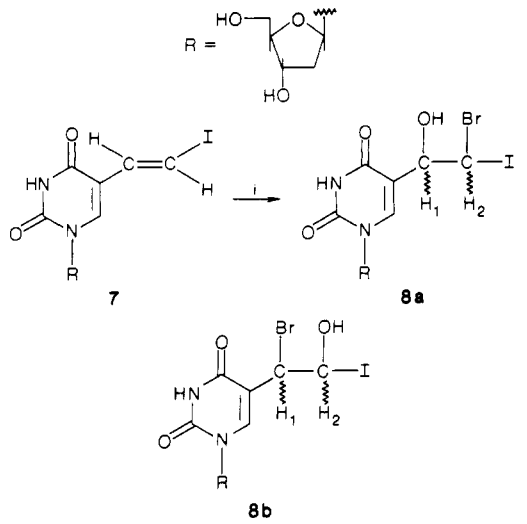


3, 4, 6, 8

no.	R^2	antiviral activity ID_{50},[a] μg/mL	L1210 cytotoxicity % survival ± SD[b] 10 μg/mL	50 μg/mL
3a	$CH(OH)CH_2Br$	>10	65.5 ± 8.7	23.7 ± 1.5
3b	$CH(OH)CH_2Cl$	0.1–1	74.0 ± 4.5	24.3 ± 2.1
4a	$CH(OMe)CH_2Br$	0.1–1	80.8 ± 3.4	11.7 ± 18.6
4b	$CH(OMe)CH_2Cl$	>10	75.0 ± 3.2	38.8 ± 1.1
6a	$CH(OH)CH(Br)CO_2Et$	1	49.7 ± 2.8	4.9 ± 0.3[c]
6b	$CH(OH)CH(I)CO_2Et$	0.1–1	73.2 ± 3.2	10.4 ± 1.7
8a:8b	CH(OH)CH(Br)I and CH(Br)CH(OH)I[d]	>10	79.2 ± 5.3	40.1 ± 1.3
acyclovir		0.01		
melphalan[e]			2.8 ± 0.68[f]	0.0

[a] The concentration required to inhibit plaque formation in monolayers of Vero cells by 50%. [b] The result is the mean value ± SD for three experiments. [c] ED_{50} = 11 μg/mL. [d] Mixture of regioisomers **8a** and **8b** in a ratio of 1:1. [e] 4-[*N*-Bis(2-chloroethyl)amino]phenylalanine. [f] Percent survival ± SD determined at 1 μg/mL, ED_{50} = 0.15 μg/mL.

Scheme III[a]



[a] Reagents: i, *N*-bromosuccinimide, dioxane–water (3:7, v/v) acetic acid, 25 °C.

pound (**3b** > **4b**, P = 0.005). A comparison of the activities for **6a** and **3a** (**6a** > **3a**, P = 0.001) indicated that the introduction of an ethoxycarbonyl group enhanced cytotoxic activity significantly whereas incorporation of an iodine substituent (**3a** > **8a:8b**, P = 0.001) decreased activity. The most active cytotoxic agent, **6a** (ED_{50} = 11 μg/mL), was 73 times less active than the reference compound melphalan (ED_{50} = 0.15 μg/mL). Compounds **3**, **4**, **6**, and **8a,b** were not evaluated in the P388 leukemia in vivo screen since compounds exhibiting an ED_{50} > 5 μg/mL in this in vitro screen are considered to have low cytotoxic activity.

A number of similar correlations were observed in both the antiviral and cytotoxic screens where the relative activities were **4a** > **4b**, **4a** > **3a**, **3b** > **4b**, and **6a** > **3a**.

Experimental Section

Melting points were determined with a Büchi capillary apparatus and are uncorrected. Nuclear magnetic resonance spectra (^1H NMR, ^{13}C NMR) were determined for solutions in pyridine-d_5, D_2O, or Me_2SO-d_6 with Me_4Si as internal standard (^1H NMR) with a Bruker AM-300 spectrometer. Chemical-ionization mass spectra (CIMS, NH_3) were measured on an AEI MS-12 spectrometer. Preparative thin-layer chromatography (PTLC) was performed with Whatman PLK5F plates, 1.0 mm in thickness. Silica gel column chromatography was carried out with Fisher S-704 (60-200 mesh) silica gel. Microanalyses were within ±0.4% of theoretical values for all elements listed, unless otherwise indicated. 5-Vinyl-2'-deoxyuridine (**2**)[14] and (*E*)-5-(2-(ethoxycarbonyl)vinyl)-2'deoxyuridine (**5**)[15] were prepared by using the literature procedures.

5-(1-Hydroxy-2-bromoethyl)-2'-deoxyuridine (3a). *N*-Bromosuccinimide (0.149 g, 0.844 mmol) was added slowly with stirring to a solution of **2** (0.208 g, 0.82 mmol) in dioxane–water (3:7, v/v, 10 mL) and glacial acetic acid (0.04 mL) during a period of 5 min. The reaction was allowed to proceed for 2 h at 25 °C with stirring and the solvent was removed in vacuo. The product was purified by elution from a silica gel column using chloroform–methanol (94:6, v/v) as eluant to yield **3a** (0.20 g, 70%) after recrystallization from methanol: mp 135 °C dec; ^1H NMR (pyridine-d_5) δ 2.7 (complex m, 2 H, H-2'), 3.85 (2 d, J = 9.6 and 7.2 Hz, 1 H, C*H*Br), 4.12–4.32 (complex m, 3 H, H-5', C*H*'Br), 4.58 (m, 1 H, H-4'), 5.05 (m, 1 H, H-3'), 5.25 (br s, 6 H, 3'-OH, 5'-OH, CHO*H*CH_2Br, $^3/_2H_2O$, exchange with deuterium oxide), 5.5 (2 d, J = 7.2 and 2.4 Hz, 1 H, C*H*OHCH$_2$Br), 7.1 (d, J = 6 Hz of d, J = 6 Hz, 1 H, H-1'), 8.89 (s, 1 H, H-6), 13.54 (s, 1 H, NH, exchanges with deuterium oxide); ^{13}C NMR (Me_2SO-d_6) δ 38.18 and 38.48 (*C*-Br), 39.49 (C-2'), 61.42 (C-5'), 65.62 and 65.98 (CHOHCH_2Br), 69.99 and 70.41 (C-3'), 84.18 (C-1'), 87.31 and 87.36 (C-4'), 114.16 (C-5), 137.47 (C-6), 149.99 (C-2), 162.06 (C-4); MS (CIMS, NH_3) m/z 369 (M^+ + NH_4). Anal. ($C_{11}H_{15}N_2O_6Br$·$^3/_2H_2O$) C, N; H: calcd, 4.35; found, 4.79.

5-(1-Hydroxy-2-chloroethyl)-2'-deoxyuridine (3b). *N*-Chlorosuccinimide (0.12 g, 0.90 mmol) was added slowly with stirring to a solution of **2** (0.221 g, 0.87 mmol) in dioxane–water (3:7, v/v, 10 mL) and glacial acetic acid (0.03 mL) during a period of 5 min. The reaction was allowed to proceed for 24 h at 25 °C. The product was isolated and purified by silica gel column chromatography, as described for **3a**, to yield **3b** as a white solid after recrystallization from methanol (0.16 g, 60%): mp 155 °C dec; ^1H NMR (pyridine-d_5) δ 2.7 (m, 2 H, H-2'), 3.98 (d, J_{gem} = 10.3 Hz of d, J_{vic} = 7.16 Hz, 1 H, C*H*Cl), 4.23 (complex m, 2 H, H-5'), 4.38 (d, J_{gem} = 10.3 Hz of d, J_{vic} = 2.38 Hz, 1 H, C*H*'Cl), 4.58 (m, 1 H, H-4'), 4.9–5.3 (br s, 7 H, 3'-OH, 5'-OH, CHO*H*CH_2Cl, $^3/_2H_2O$, H-3', hydroxyls exchange with deuterium oxide), 5.51 (2 d, J = 7.16 and 2.38 Hz, 1 H, C*H*OHCH$_2$Cl), 7.1 (d, J = 6 Hz of

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d, J = 6 Hz, 1 H, H-1′), 8.85 (s, 1 H, H-6), 13.52 (s, 1 H, NH, exchanges with deuterium oxide). Anal. ($C_{11}H_{15}N_2O_6Cl\cdot{}^3/_2H_2O$) C. Calcd: H, 4.75; N, 7.96. Found: H, 5.43; N, 8.39.

5-(1-Methoxy-2-bromoethyl)-2′-deoxyuridine (4a). A solution of sulfuric acid in methanol (4 mL, 5 N) was added to a solution of **3a** (0.144 g, 0.41 mmol) in methanol (50 mL). The reaction was allowed to proceed for 48 h at 25 °C with stirring prior to neutralization with methanolic sodium hydroxide. Removal of the solvent in vacuo, dissolution of the residue in methanol (5 mL), adsorption on silica gel (2 g), removal of the solvent in vacuo, and application of this material to the top of a silica gel column followed by elution with chloroform–methanol (96:4, v/v) yielded **4a** (0.14 g, 93%) as a viscous oil. Trituration with cold ether gave **4a** as a white solid: mp 135 °C dec; ^{1}H NMR (Me_2SO-d_6) (mixture of two diastereomers in a ratio of 1:1) δ 2.15 (m, 2 H, H-2′), 3.25 and 3.28 (2 s, 3 H total, OMe), 3.45 (m, 1 H, C*H*Br), 3.6 (m, 2 H, H-5′), 3.76 (complex m, 1 H, C*H*′*Br*), 3.85 (m, 1 H, H-4′), 4.28 (m, 1 H, H-3′), 4.36 (m, 1 H, C*H*OMe), 5.08 and 5.3 (2 br s, 1 H each, 3′-OH, 5′-OH, exchange with deuterium oxide), 6.2 (2 overlapping d, J = 6 Hz of d, J = 6 Hz, 1 H total, H-1′), 7.92 and 7.95 (2 s, 1 H total, H-6), 11.54 (s, 1 H, NH, exchanges with deuterium oxide). Anal. ($C_{12}H_{17}N_2O_6Br\cdot{}^1/_2H_2O$) C, H; N: calcd, 6.94; found, 7.48.

5-(1-Methoxy-2-chloroethyl)-2′-deoxyuridine (4b). A solution of sulfuric acid in methanol (5.5 mL, 5 N) was added to a solution of **3b** (0.131 g, 0.43 mmol) in methanol (50 mL). The reaction was completed, and the product was isolated and purified as described for **4a**, to yield **4b** as a white solid (0.135 g, 98%): mp 157 °C dec; ^{1}H NMR (Me_2SO-d_6) (mixture of two diastereomers in a ratio of 1:1) δ 2.19 (m, 2 H, H-2′), 3.3 and 3.32 (2 s, 3 H total, OMe), 3.4 (m, 1 H, C*H*Cl), 3.64 (m, 2 H, H-5′), 3.72 (m, 1 H, C*H*′*Cl*), 3.88 (m, 1 H, H-4′), 4.3 (m, 1 H, H-3′), 4.36 (m, 1 H, C*H*OMe), 5.1 and 5.31 (2 m, 1 H each, 3′-OH, 5′-OH, exchange with deuterium oxide), 6.2 (2 overlapping d, J = 6 Hz each, 1 H total, H-1′), 7.92 and 7.95 (2 s, 1 H total, H-6), 11.52 (s, 1 H, NH, exchanges with deuterium oxide). Anal. ($C_{12}H_{17}N_2O_6Cl\cdot{}^1/_2H_2O$) C, H, N.

5-(1-Hydroxy-2-bromo-2-(ethoxycarbonyl)ethyl)-2′-deoxyuridine (6a). *N*-Bromosuccinimide (0.109 g, 0.61 mmol) was added in small aliquots to a solution of **5** (0.2 g, 0.615 mmol) in water (10 mL) and the reaction was allowed to proceed for 24 h at 25 °C. Removal of the solvent in vacuo gave a viscous oil, dissolution in methanol (5 mL), adsorption on silica gel (2 g), removal of the solvent in vacuo, and application of this material to the top of a silica gel column followed by elution with chloroform–methanol (95:5, v/v) afforded **6a** (0.18 g, 70%) as a white solid: mp 68 °C (sublimes); ^{1}H NMR (Me_2SO-d_6) (mixture of two diastereomers in a ratio of 1:1) δ 1.22 (t, J = 7 Hz, 3 H, CH_2CH_3), 2.12 (m, 2 H, H-2′), 3.6 (m, 2 H, H-5′), 3.8 (m, 1 H, H-4′), 4.2 (q, J = 7 Hz, 2 H, CH_2CH_3), 4.26 (m, 1 H, H-3′), 4.82 (2 overlapping m, 2 H, C*H*OHC*H*Br), 5.14 and 5.31 (2 m, 1 H each, 3′-OH, 5′-OH, exchange with deuterium oxide), 6.06 (m, 1 H, CHO*H*CHBr, exchanges with deuterium oxide), 6.21 (2 overlapping d, J = 6 Hz, of d, J = 6 Hz, 1 H total, H-1′), 8.04 and 8.06 (2 s, 1 H total, H-6), 11.54 (s, 1 H, NH, exchanges with deuterium oxide); ^{13}C NMR (Me_2SO-d_6) δ 13.71 (CH_2CH_3), 39.58 (C-2′), 46.92 and 47.14 (*C*HBr), 61.24 (C-5′), 61.39 (CH_2CH_3), 68.07 and 68.58 (*C*HOH-CHBr), 70.26 and 70.41 (C-3′), 84.16 and 84.34 (C-1′), 87.48 (C-4′), 112.54 (C-5), 139.51 and 139.36 (C-6), 149.84 (C-2), 162.18 and 162.24 (C-4), 168.14 (CO_2). Anal. ($C_{14}H_{19}N_2O_8Br$) C, H, N.

5-(1-Hydroxy-2-iodo-2-(ethoxycarbonyl)ethyl)-2′-deoxyuridine (6b). A mixture of **5** (49 mg, 0.15 mmol), iodine (19 mg, 0.15 mmol), potassium iodate (6 mg, 0.028 mmol), water (2 mL), and sulfuric acid (15 μL, 5 N) were stirred for 12 h at 55 °C. Removal of the solvent in vacuo gave a residue which was purified by PTLC using chloroform–methanol (90:10, v/v) as development solvent. Extraction of the ultraviolet visible spot with chloroform–methanol (88:12, v/v) yielded **6b** as a white solid (42 mg, 60%): mp 110 °C dec; ^{1}H NMR (Me_2SO-d_6) (mixture of two diastereomers in a ratio of 1:1) δ 1.22 (t, J = 7 Hz, 3 H, CH_2CH_3), 2.15 (m, 2 H, H-2′), 3.64 (m, 2 H, H-5′), 3.83 (m, 1 H, H-4′), 4.18 (q, J = 7 Hz, 2 H, CH_2CH_3), 4.3 (m, 1 H, H-3′), 4.75–4.9 (2 overlapping m, 2 H, C*H*OHC*H*I), 5.15 and 5.3 (2 m, 1 H each, 3′-OH, 5′-OH, exchange with deuterium oxide), 5.9 (m, 1 H, CHO*H*CHI, exchanges with deuterium oxide), 6.22 (2 overlapping d, J = 6 Hz each, 1 H total, H-1′), 8.0 and 8.02 (2 s, 1 H total, H-6), 11.52 (s, 1 H, NH, exchanges with deuterium oxide); ^{13}C NMR (Me_2SO-d_6) δ 13.53 (CH_3), 24.56 and 24.78 (*C*HI), 39.63 and 39.87 (C-2′), 61.01 (CH_2CH_3), 61.25 and 61.39 (C-5′), 68.79 and 69.51 (*C*HOHCHI), 70.26 and 70.41 (C-3′), 84.09 and 84.31 (C-1′), 87.49 (C-4′), 113.42 and 113.54 (C-5), 139.21 and 139.42 (C-6), 149.84 (C-2), 162.09 and 162.15 (C-4), 170.0 (CO_2). Anal. ($C_{14}H_{19}N_2O_8I$) C, H, N.

5-(1-Hydroxy-2-bromo-2-iodoethyl)-2′-deoxyuridine (8a) and 5-(1-Bromo-2-hydroxy-2-iodoethyl)-2′-deoxyuridine (8b). A mixture of *N*-bromosuccinimide (91 mg, 0.51 mmol) and **7** (190 mg, 0.5 mmol) in dioxane–water (3:7, v/v, 8 mL) and glacial acetic acid (25 μL) was allowed to stir at 25 °C for 2 h. Removal of the solvent in vacuo and purification of the product by elution from a silica gel column using methanol–chloroform (6:94, v/v) as eluant yielded a mixture of regioisomers **8a** and **8b** in a ratio of 1:1, determined by integration of the C*H*Br resonances at δ 6.01 and 6.16 respectively, as a white solid (0.152 g, 64%): mp 88 °C (sublimes); ^{1}H NMR (D_2O) (each regioisomer **8a** and **8b** is a mixture of two diastereomers) δ 2.36 (complex m, 4 H total, H-2′), 3.76 (complex m, 4 H total, H-5′), 4.05 (m, 2 H total, H-4′), 4.45 (m, 2 H total, H-3′), 5.02 (m, 2 H total, C*H*OHCHBr), 6.01 (m, 1 H, C*H*Br in one regioisomer), 6.16 (m, 1 H, C*H*Br in one regioisomer), 6.28 (m, 1 H total, H-1′), 8.05 (4 closely spaced singlets, 2 H total, H-6); ^{13}C NMR (Me_2SO-d_6) δ 20.66 and 21.11 (*C*HBrI of **8a**), 39.65 and 39.95 (C-2′), 51.90 and 52.26 (*C*HBr of **8b**), 61.85 and 61.75 (C-5′), 70.59 and 70.71 (C-3′), 71.10, 71.43, 71.94 and 72.37 (*C*HOH of **8a** and **8b**), 84.61 (C-1′), 87.51 and 87.57 (C-4′), 113.13 and 113.63 (C-5), 138.88 and 138.94 (C-6), 149.99 (C-2), 162.21 (C-4). Anal. ($C_{11}H_{14}N_2O_6BrI\cdot1H_2O$) C, H, N.

In Vitro Antiviral Assay (HSV-1). Monolayers of mycoplasma-free Vero cells in 60-mm plates were infected with HSV-1 (strain JLJ isolated from a patient with herpes simplex encephalitis). After a 1-h adsorption period at 37 °C, the cells were overlaid with 1% agarose in Eagle minimum essential medium (E-MEM) containing 2% fetal bovine serum and known concentrations of the agents (0.1, 1, and 10 μg/mL for **3**, **4**, **6**, and **8**; 0.01, 0.1, and 1 μg/mL for acyclovir) being tested. Cells were stained with neutral red after incubation for 4 days at 37 °C in a 5% CO_2 incubator, and the number of plaques were counted. The experiment was performed once. The concentration of test compound required to inhibit plaque formation by 50% (ID_{50}) was determined.

In Vitro L1210 Cytotoxic Assay. Mouse L1210 leukemia cells were cultivated as a suspension in Fischer's medium supplemented with 10% heat-inactivated horse serum and incubated at 37 °C in a humidified 5% CO_2 atmosphere to prepare a cell stock solution. The number of cells/mL of medium was determined with a Model ZF Coulter Counter 48 h after incubation. The test compound was dissolved in ethanol–water (1:1, v/v, **3a,b**), water (**4a,b**), or ethanol **6a,b**, **8a:8b**) and 10 μL of this solution was added to test wells containing 2 mL of suspended L1210 cells (10^5 cells/mL) such that 2 mL of the cell suspension had a test compound concentration of 50, 10, and 1 μg/mL of medium, respectively. Control wells were identical, except that the test compound was absent. Compounds for which ED_{50} values were obtained had the following test compound concentrations (μg/mL of medium): **6a** (50, 25, 10, 5.0, 2.5, and 1) and melphalan (10, 1, 0.5, 0.25, 0.1, and 0.05). All tests and controls were grown in triplicate. The percent cell survival was calculated with the formula: percent survival = $(T_{48} - T_0)/(C_{48} - C_0) \times 100$; where T_{48} is the number of living cells/mL for each test drug concentration at 48 h, T_0 is the number for test wells at time zero (normally 10^5), C_{48} is the number for the control at 48 h, and C_0 is the number for the control at time zero (normally, $T_0 = C_0 = 10^5$ cells/mL). Compounds exhibiting an $ED_{50} > 5$ μg/mL are considered to be inactive in this screen.

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