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Synthesis of 5-[1-hydroxy (or methoxy)-2,2-dihaloethyl]-2′-deoxyuridines with antiviral and cytotoxic activity

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Abstract

The 5-[1-hydroxy (or methoxy)-2,2-dihaloethyl]-2′-deoxyuridines (3–12, Cl, Br and/or I) were synthesized by the addition of HOX or CH_3OX (X = Cl, Br, I) to the vinyl substituent of the respective (*E*)-5-(2-halovinyl)-2′-deoxyuridines (1a–c). In vitro antiviral (HSV-1, HSV-2, HCMV, VZV, EBV) and cytotoxic (L1210) activities were determined. The 5-(1-hydroxy-2,2-dihaloethyl) series were generally more active than the 5-(1-methoxy-2,2-dihaloethyl) series against HSV-1, HSV-2, VZV and EBV. Anti-HSV-1 activity was dependent upon the steric orientation and/or hydrophobic properties of the halogen atom(s), with -CH(OH)CHBr(I) and -CH(OH)$CHBr_2$ C-5 substituents providing the most potent activity. 5-(1-Hydroxy-2-bromo-2-iodoethyl)-2′-deoxyuridine (6), which exhibited the most potent anti-HSV-1 activity, was 12-fold less active than acyclovir. In contrast, the halogen atom(s) were not determinants of anti-VZV activity, where the approximately equipotent 5-(1-hydroxy-2,2-dihaloethyl) compounds (3, 4, 5, 6) exhibited anti-VZV activity comparable to that of acyclovir. All of the 5-(1-hydroxy (or methoxy)-2,2-dihaloethyl) analogs (3–12) were inactive against HCMV. The 5-(1-hydroxy-2-chloro-2-iodoethyl) compound (4) was an active cytotoxic agent as determined in the in vitro L1210 screen. The compounds 3–12 were non-toxic to uninfected host cells. The inhibitory effect on cell proliferation diminished upon replacement of the 5-(1-hydroxy-2,2-dihaloethyl) substituents of 3–6 with the corresponding 5-(1-methoxy-2,2-dihaloethyl) substituents (7–12).

Key words: Antiviral; Nucleoside; Pyrimidine

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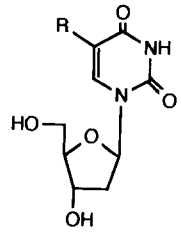
1. Introduction

(*E*)-5-(2-Bromovinyl)- (**1b**, BVDU) (De Clercq et al., 1979), (*E*)-5-(2-iodovinyl)- (**1c**, IVDU) (Perlman et al., 1985) and 5-(2-chloroethyl)-2′-deoxyuridine (**1e**, CEDU) (Griengl et al., 1985) are among the most potent and selective agents against herpes simplex virus type 1 (HSV-1), of the many 5-substituted pyrimidine nucleosides that have been investigated. BVDU (**1b**) and CEDU (**1e**) also effectively inhibit varicella-zoster virus (VZV) replication in vitro (De Clercq et al., 1979; Perlman et al., 1985; De Clercq et al., 1982; De Clercq and Rosenwirth, 1985; Rosenwirth et al., 1985). 5-(2-Fluoroethyl)-2′-deoxyuridine (**1h**, FEDU) showed activity against HSV-1 replication (Griengl et al., 1987) whereas the bromo (**1f**) and iodo (**1g**) analogs of CEDU were 30–100-fold less active (Griengl et al., 1985). In contrast, (*E*)-5-(2-fluorovinyl)-2′-deoxyuridine (**1d**, FVDU) was inactive (Reefschlager et al., 1984). Within the dihalovinyl class of compounds, 5-(2,2-difluorovinyl)-2′-deoxyuridine (**1i**, DFVDU) was active against HSV-1 (Bobek et al., 1987), whereas the dibromovinyl analog (**1j**, DBVDU) was virtually inactive (De Clercq and Walker, 1984; De Clercq, 1985).

In earlier in vitro studies, we reported the synthesis, antiviral (HSV-1) and cytotoxic (L1210 and P388 cells) activities of halohydrin and halomethoxy derivatives of 5-vinyl-2′-deoxyuridine (**1k**, VDU) (Kumar et al., 1989; 1990). 5-(1-Methoxy-2-iodoethyl-2′-deoxyuridine (**2a**, $EC_{50} = 0.1$ μg/ml) (Kumar et al., 1990) exhibited appreciable in vitro antiviral activity (HSV-1), relative to acyclovir ($EC_{50} = 0.01$ μg/ml) or IVDU ($EC_{50} = < 0.1$ μg/ml). 5-(1-Hydroxy-2,2-diiodoethyl)-2′-deoxyuridine (**2d**, EC_{50} 0.77 μg/ml) (Kumar et al., 1990) exhibited significant in vitro cytotoxic (L1210 cells) activity, relative to the reference drug melphalan ($EC_{50} = 0.15$ μg/ml). In the 5-(1-methoxy-2-haloethyl)-2′-deoxyuridine class of compounds (**2a–c**), the 5-(1-methoxy-2-iodoethyl) (**2a**, $EC_{50} = 0.7$ μg/ml) and 5-(1-methoxy-2-bromoethyl) (**2b**, $EC_{50} = 0.19$ μg/ml) analogs were recently found to exhibit potent in vitro activity against Epstein-Barr virus (EBV) (Kumar, Knaus and Wiebe, unpublished results) relative to the reference compound acyclovir ($EC_{50} = 7.4$ μg/ml). The 5-(1-methoxy-2-chloroethyl) analog (**2c**) was equipotent (EBV) to acyclovir. The selectivity indices IC_{50}/EC_{50}) of **2a** and **2b** were > 143 and > 526, respectively. Compounds **2a** ($EC_{50} = 4.1$ μg/ml) and **2b** ($EC_{50} = 5.4$ μg/ml) also effectively inhibited VZV replication in vitro (Kumar, Knaus and Wiebe, unpublished results) at concentrations comparable to acyclovir ($EC_{50} = 2.0$ μg/ml). These structure-activity correlations for 5-(1-hydroxy (or methoxy)-2-haloethyl)-2′-deoxyuridines indicate that a halogen substituent is an important determinant of antiviral and cytotoxic activities (Kumar et al., 1989; 1990; Kumar, Knaus and Wiebe, unpublished results). It was therefore of interest to extend these studies so as to acquire structure-activity correlations for the structurally related 5-(1-hydroxy (or methoxy)-2,2-dihaloethyl)-2′-deoxyuridines (**3–12**). We now report the synthesis, antiviral and cytotoxic (L1210) activities of the novel 5-[1-hydroxy (or methoxy)-2,2-dihaloethyl]-2′-deoxyuridines (**3–12**).



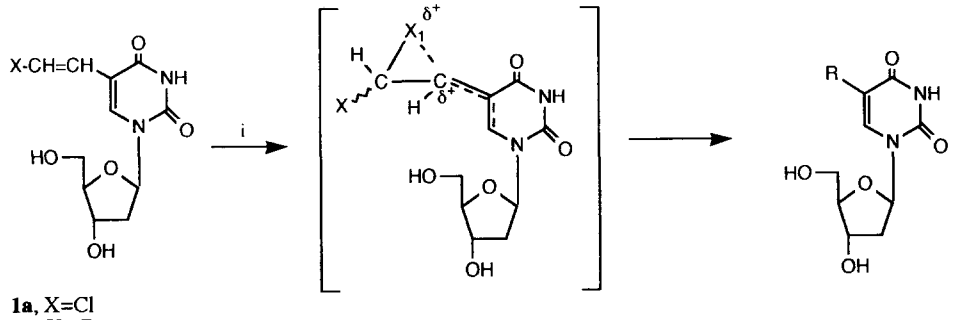
1a;R=(E)-CH=CH-Cl
1b;R=(E)-CH=CH-Br
1c;R=(E)-CH=CH-I
1d;R=(E)-CH=CH-F
1e;R=CH_2CH_2Cl
1f;R=CH_2CH_2Br
1g;R=CH_2CH_2I
1h;R=CH_2CH_2F
1i;R=$CH=CF_2$
1j;R=$CH=CBr_2$
1k;R=$CH=CH_2$
1l;R=CH(Cl)CH(OH)Cl

2a;R=$CH(OMe)CH_2I$
2b;R=$CH(OMe)CH_2Br$
2c;R=$CH(OMe)CH_2Cl$
2d;R=$CH(OH)CHI_2$
2e;R=$CH(OH)CH_2I$
2f;R=$CH(OH)CH_2Br$
2g;R=$CH(OH)CH_2Cl$

2. Results and discussion

2.1. Chemistry

The target 5-(1-hydroxy (or methoxy)-2,2-dihaloethyl)-2′-deoxyuridines (**3–12**) were synthesized by the regio-specific reaction of (*E*)-5-(2-halovinyl)-2′-deoxyuridines (**1a–c**) with an electrophilic halogen in aqueous dioxane or methanol, in 32–90% yields (see Scheme 1). For example, the ^{13}C-NMR spectrum of **3** supported a regio-specific addition of HOCl to the 5-vinyl substituent of **1a**, which showed methine resonances at δ75.84 and 75.98 ($CHCl_2$) and δ5 73.08 and 73.44 (*C*HOH). Compounds **3–12** are a mixture of two diastereomers which differ in configuration (*R* and *S*) at the 1-carbon atom of the 5-(1-hydroxy (or methoxy)-2,2-dihaloethyl)



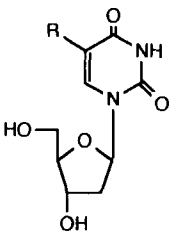
1a, X=Cl
1b, X= Br
1c, X= I

3-12

3, R = $CHOHCHCl_2$
4, R = CHOHCHICl
5, R = $CHOHCHBr_2$
6, R = CHOHCHBrI
7, R = $CHOMeCHCl_2$
8, R = CHOMeCHBrCl
9, R = CHOMeCHClI
10, R = $CHOMeCHBr_2$
11, R = CHOMeCHBrI
12, R = $CHOMeCHI_2$

Scheme 1. Reagents: i, *N*-Chlorosuccinimide (**3**,**4**) or *N*-bromosuccinimide (**5**), dioxane/water (3:7, v/v), acetic acid, 25°C; 12, KIO_3, dioxane/water (3:7, v/v), acetic acid, 25°C (6); *N*-chlorosuccinimide, MeOH, acetic acid (**7**,**8**); ICl, MeOH (**9**,**11**,**12**); Bromine, MeOH (**10**).

Table 1
In vitro antiviral activity and L1210 cytotoxicity of 5-substituted-2′-deoxyuridines



no.	*R*	IC_{50}[a] μM	EC_{50}[b,c] μM					IC_{50}	EC_{50}[g] (μM)
		Cytotoxicity	HSV-1[d] (E-377)	HSV-2[d] (MS)	HCMV[e] (AD-169)	VZV[e] (Ellen)	EBV, P3HR-1 (Raji cells)	Cell proliferation[f]	(L1210)
3	$CH(OH)CHCl_2$	>294	>73.5	>73.5	>294	6.0	22.3	94.1	73.5
4	CH(OH)CHCl(I)	>231	6.9	15.8	>231	6.9	ND	185	3.5
5	$CH(OH)CHBr_2$	>232	3.9	6.3	>232	6.3	>23	11	15.6
6	CH(OH)CHBr(I)	>210	0.7	3.3	>209	6.0	79.6	41	37.7
7	$CH(OMe)CHCl_2$	>282	22.9	>141	>282	13.8	>28.2	>282	>141
8	CH(OMe)CHBr(Cl)	>250	3.7	>125	>250	7.5	>25	>250	>125
9	CH(OMe)CHCl(I)	>214	>107	>107	>214	155	>214	>214	>53.5
10	$CH(OMe)CHBr_2$	>225	>56.3	>56.3	>225	>225	>22.5	>225	>56.3
11	CH(OMe)CHBr(I)	>204	>100	>100	>203	93	3.0	>203	>100
12	$CH(OMe)CHI_2$	>186	>93	>93	>186	91	26.4	>186	>93
Acyclovir		>440	0.13	0.26	ND	7.4	6.6	>440	
Ganciclovir		>390	ND	ND	10.1	ND	ND	168	
Melphalan[h]									0.49

[a]The drug concentration (μM) required to reduce uptake of neutral red stain by uninfected human foreskin fibroblasts (HFF) cell monolayers to 50% of untreated, uninfected controls after 7 days.
[b]The concentration (μM) required to reduce plaque formation (or antigen production for EBV) in infected cell monolayers to 50% of untreated, infected controls.
[c]Mean of two to four assays.
[d]CPE inhibition assay in human foreskin fibroblasts (HFF).
[e]Plaque reduction assay in human foreskin fibroblasts (HFF).
[f]The drug concentration (μM) required to reduce proliferation of human foreskin fibroblasts to 50% of untreated controls.
[g]The in vitro concentration required for a 50% reduction in the number of surviving L1210 cells in suspension.
[h]4-[*N*,*N*-bis-(chloroethyl)amino]phenylalanine.

substituent, that could not be separated by flash silica-gel column chromatography, or multiple development TLC chromatography.

2.2. Antiviral activity

The antiviral activities of 5-(1-hydroxy-2,2-dihaloethyl) (**3–6**) and structurally related 5-(1-methoxy-2,2-dihaloethyl)-2′-deoxyuridines (**7–12**) against HSV-1, HSV-2, HCMV, VZV and EBV were measured in order to establish the effect of dihalogeno (Cl, Br and/or I) substituents at the 2-position of a 5-(1-hydroxyethyl) or 5-(1-methoxyethyl) moiety. Antiviral and cytotoxicity tests were performed by the National Institutes of Health, MD, USA, according to their established methods. These procedures are briefly explained as footnotes to Table 1. The results are summarized in Table 1. The 5-(1-hydroxy-2,2-dihaloethyl) series of compounds were generally more active than the 5-(1-methoxy-2,2-dihaloethyl) series against HSV-1, HSV-2, VZV and EBV. Exceptions to this generalization were observed in the following antiviral assays: [HSV-1; $CH(OMe)CHCl_2$ (**7**) > $CH(OH)CHCl_2$ (**3**); CH(OMe)-CHBr(Cl) (**8**) > (CH(OMe)CHBr(I) (**11**) > CH(OH)CHBr(I) (**6**)]. The relative anti-HSV-1 and anti-HSV-2 orders of potency for the 5-(1-hydroxy-2,2-dihaloethyl) compounds (**3–6**) were similar. These test results show that anti-HSV-1 activity is dependent upon the steric and/or hydrophobic properties of the halogen atom(s), where -CH(OH)CHBr(I) and $CH(OH)CHBr_2$ C-5 substituents provided the most potent activity; specifically CH(OH)CHBr2 (**5**) > $CH(OH)CHCl_2$ (**3**) and $CH(OH)CHI_2$ (**2d**) (Kumar et al., 1990); CH(OH)CHBr(I) (**6**) > CH(OH)CHBr2 (**5**) > CH(OH)CHCl(I) (**4**) > $CH(OH)CHCl_2$ (**3**) and $CH(OH)CHI_2$ (**2d**) (Kumar et al., 1990). 5-(1-Hydroxy-2,2-dibromoethyl)-2′-deoxyuridine (**5**, $EC_{50} = 3.90$ μM) exhibits superior anti-HSV-1 activity relative to 5-(2,2-dibromovinyl)-2′-deoxyuridine ($EC_{50} = 48.5$ μM) (Bobek et al., 1987). The most potent compound in this series was the 5-(1-hydroxy-2-bromo-2-iodoethyl) analog (**6**) which was about 12-fold less active than acyclovir. In contrast, the type of halogen atom(s) (e.g., Cl, Br, I) do not appear to be determinants of anti-VZV activity since the 5-(1-hydroxy-2,2-dihaloethyl) compounds (**3,4,5,6**), which were approximately equipotent ($EC_{50} = 6.0$–20.7 μM range), exhibited activity comparable to acyclovir ($EC_{50} = 7.4$ μM). All of the 5-[1-hydroxy (or methoxy)-2,2-dihaloethyl]-2′-deoxyuridines (**3–12**) were inactive against HCMV ($EC_{50} > 200$ μM). The 5-(1-methoxy-2-bromo-2-iodoethyl) (**11**) derivative exhibited significant activity against EBV ($EC_{50} = 3$ μM).

Compounds in which the two halogen atoms are the same possess a single chiral center at C-1 in the 5-[1-hydroxy (or methoxy)-2,2-dihaloethyl] substituent and exist as a 1:1 mixture of two diastereomers (**3,5,7,10,12**), whereas compounds **4,6,8** and **9** in which the halogen atoms are different also possess a second chiral center at C-2. Although the latter group of compounds possess two adjacent chiral centers, the ^{13}C-NMR spectra exhibited dual chemical shifts for these carbons suggesting the presence of two different diastereomers. The effect of chirality upon antiviral activity is not known since attempts to separate the two diastereomers by flash column chromatography or multiple development TLC in quantities sufficient for testing were unsuccessful and because the configuration at the C-1 and/or C-2 positions of

the 5-[1-hydroxy (or methoxy)-2,2-dihaloethyl] substituent were not established. In an earlier study, we reported that 5-[(1*R*)-2,2-dichlorocyclopropyl]-2′-deoxyuridine was weakly active against HSV-1, whereas the (1*S*)-diastereomer was inactive (Tandon et al., 1991). Methodology to separate the diastereomers (**3–12**) must be developed before any in vivo studies are initiated.

Among the new compounds tested, **3** (VZV), and **6** (HSV-1) showed the greatest antiviral activity. The concentrations required to impair the viability of human foreskin fibroblasts monolayers (uninfected host cells) generally exceeded the effective antiviral concentrations by at least 50-fold; as expected, proliferating cells were more sensitive to inhibition.

It is interesting to note that when the C-1 hydroxy substituent in the 5-substituted side chain of compounds **4**,**5** and **6**, which are active against HSV-1, HSV-2 and VZV, is replaced by the methoxy substituent (**9**,**10**,**11**), the anti-HSV- 1, -HSV-2 and -VZV activity decreases; however, the inhibitory effect on proliferating cells also decreases.

The cytotoxic activities for these two series of compounds (**3–6** and **7–12**) were determined by an in vitro L1210 assay (Table 1). 5-(1-Hydroxy-2,2-dihaloethyl) analogs (**3–6**) were more potent than the corresponding (inactive) methoxy derivatives (**7–12**). The 5-(1-hydroxy-2-chloro-2-iodoethyl) **4** ($EC_{50} = 3.5$ μM) is the most active cytotoxic agent, relative to the reference drug melphalan ($EC_{50} = 0.49$ μM).

Finally, these nucleosides are stable for 48 hours in aqueous solution. It is therefore reasonable to suggest that the observed biological activities are due to the described compounds and not to elimination products, although their biotransformation was not studied under in vitro assay conditions.

3. Materials and methods

3.1. Chemistry

Melting points were determined using a Büchi capillary apparatus and are uncorrected. Nuclear magnetic resonance spectra (^{1}H-NMR, ^{13}C-NMR) were determined for solutions in Me_2SO-d_6, D_2O or CD_3OD with Me_4Si as internal standard (^{1}H-NMR) with a Bruker AM-300 spectrometer. The assignments of all exchangable protons were confirmed by addition of deuterium oxide. ^{13}C-NMR (J modulated spin echo) spectra, where methyl and methine carbon resonances appear as positive peaks, and methylene and quaternary carbon resonances appear as negative peaks, were recorded for all compounds. The complete ^{1}H-NMR and ^{13}C-NMR description has been given for compound *3* as a representative; for other compounds only the relevant signals have been described. Silica-gel column chromatography was carried out using Merck 7734 (60–200 mesh) silica-gel. (*E*)-5-(2-chlorovinyl) (**1a**), (*E*)-5-(2-bromovinyl (**1b**) and (*E*)-5-(2-iodovinyl)-2′-deoxyuridine (**1c**) (Jones et al., 1979) were prepared according to the literature procedures.

5-(1-Hydroxy-2,2-dichloroethyl)-2'-deoxyuridine (3)

N-Chlorosuccinimide (30 mg, 0.224 mmol) was added slowly with stirring to a solution of **1a** (60 mg, 0.208 mmol) in dioxane/water (3:7, v/v, 4 ml) and glacial acetic acid (25 μl). The reaction was allowed to proceed for 24 h at 25°C. Additional aliquots of *N*-chlorosuccinimide (45 mg, 0.337 mmol) and glacial acetic acid (15 μl) were added to the reaction mixture and stirring was continued for another 24 h prior to neutralization with aqueous sodium hydroxide. Removal of the solvent in vacuo, dissolution of the residue in methanol (5 ml), adsorption on to silica-gel (2 g), removal of solvent in vacuo, and application of this material to the top of a silica-gel column followed by elution with chloroform/methanol (90:10, v/v) yielded **3** as a viscous oil (42 mg, 59%): ^{1}H-NMR (CD_3OD) δ 2.20 and 2.32 (two m, 1H each, H-2'), 3.77 (m, 2H, H-5'), 3.97 (m, 1H, H-4'), 4.40 (m, 1H, H-3'), 4.90 (m, 1H, C*H*$CHCl_2$), 6.20 and 6.22 (two d, J = 3.5 Hz, 1H total, $CHCHCl_2$), 6.30 (t, J = 6 Hz, 1H, H-1'), 8.10 (s, 1H, H-6); ^{13}C-NMR (CD_3OD) δ 41.33 and 41.50 (C-2'), 62.99 (C-5'), 72.27 and 72.36 (C-3'), 73.08 and 73.44 (*C*$HCHCl_2$), 75.84 and 75.98 (*C*HCl_2), 86.86 and 86.91 (C-1'), 89.02 (C-4'), 113.63 (C-5), 141.14 (C-6), 151.82 (C-2), 164.87 (C-4). Anal. Calcd. for $C_{11}H_{14}Cl_2N_2O_6$:C, 38.72; H, 4.13; N, 8.21. Found: C, 38.48; H, 4.0; N, 8.38.

5-(1-Hydroxy-2-chloro-2-iodoethyl)-2'-deoxyuridine (4)

A mixture of **1a** (28.8 mg, 0.1 mmol), iodine (15 mg, 0.11 mmol), potassium iodate (18 mg, 0.084 mmol), dioxane/water (3:7, v/v, 4 ml), and glacial acetic acid (15 μl) were stirred for 24 h at 25°C. This compound was obtained in a 46% yield as a syrup after purification; ^{1}H-NMR (CD_3OD) δ 4.96 (m, 1H C*H*CHClI), 6.18–6.30 (complex m, 2H total, H-1', CHC*H*ClI); ^{13}C-NMR (CD_3OD) δ 34.41 and 34.56 (*C*HICl), 74.31 and 74.92 (*C*HCHICl). Anal. Calcd. for $C_{11}H_{14}ClIN_2O_6 \cdot 1H_2O$: C, 29.31; H, 3.57; N, 6.21. Found: C, 29.43; H, 3.47; N, 5.87.

5-(1-Hydroxy-2,2-dibromoeethyl)-2'-deoxyuridine (5)

N-Bromosuccinimide (27 mg, 0. 151 mmol) was added slowly with stirring to a solution of **1b** (47 mg, 0.141 mmol) in dioxane/water (3:7, v/v, 2 ml) and glacial acetic acid (5 μl) during a period of 5 min. The reaction was allowed to proceed for 3 h at 25°C with stirring, after which the solvent was removed in vacuo. The product was purified by elution from a silica-gel column using chloroform/methanol (95:5, v/v) as eluent to yield 5 (49 mg, 80%) after trituration with hexanes: mp 128–131°C dec; ^{1}H-NMR (D_2O) 8 4.92 (m, 1H, C*H*CHBr2), 6.02 and 6.05 (two d, J = 3.28 Hz, 1H total, CHC*H*Br_2); ^{13}C-NMR (CD_3OD), δ 51.18 and 51.54 (*C*HBr_2), 73.19 and 73.63 (*C*$HCHBr_2$). Anal. Calcd. for $C_{11}H_{14}Br_2N_2O_6.1H_2O$: C, 29.48; H, 3.59; N, 6.25. Found: C, 29.40; H, 3.17; N, 5.89.

5-(1-Hydroxy-2-bromo-2-iodoethyl)-2'-deoxyuridine (6)

A mixture of **1b** (65 mg, 0. 195 mmol), iodine (26 mg, 0.204 mmol), potassium iodate (18 mg, 0.084 mmol), dioxane/water (3:7, v/v, 5 ml) and glacial acetic acid (30 μl) were stirred for 12 h at 25°C. Removal of the solvent in vacuo gave a residue which was purified by silica-gel column chromatography using chloroform/methanol (94:6, v/v) to yield **6** (35 mg, 37%) as a foam: mp 129–132°C dec; ^{1}H-NMR (CD_3OD) δ 4.94 (m, ^{1}H, C*H*CHBrI), 6.0 and 6.05 (two d, J = 3.37 Hz, 1H, CHC*H*BrI); ^{13}C-NMR (CD_3OD) 8 18.95 and 19.25 (*C*HBrI), 74.13 and 74.70 (*C*HCHBrI). Anal. Calcd. for $C_{11}H_{14}BrIN_2O_6$: C, 27.69; H, 2.95; N, 5.87. Found: C, 27.61; H, 3.0; N, 5.76.

5-(1-Methoxy-2,2-dichloroethyl)-2'-deoxyuridine (7)

N-Chlorosuccinimide (32 mg, 0.239 mmol) was added with stirring to a solution of **1a** (50 mg, 0. 173 mmol) in MeOH (5 ml) and glacial acetic acid (10 μl). The reaction was allowed to proceed for 15 h at 25°C. Additional aliquots of *N*-chlorosuccinimide (25 mg, 0.187 mmol) and glacial acetic (10 μl) were added to the reaction mixture and stirring was continued for another 78 h. The product was purified, as described for the preparation of **3**, to yield **7** as a foam (20 mg, 32%): mp 160–165°C dec; ^{1}H-NMR (CD_3OD) δ 3.40 (s, 3H, OMe), 4.48 and 4.50 (two d, J = 4.08 Hz, 1H total, C*H*CHCl$_2$), 6.12 and 6.15 (two d, J = 4.08 Hz, 1H total, CHC*H*Cl$_2$); ^{13}C-NMR (CD_3OD) δ 58.97 (O*C*H$_3$), 73.91 and 74.03 (*C*HCl$_2$), 82.41 and 82.75 (*C*HCHCl$_2$). Anal. Calcd. for $C_{12}H_{16}Cl_2N_2O_6$:C, 40.57; H, 4.50; N, 7.88. Found: C, 40.87; H, 4.83; N, 7.42.

5-(1-Methoxy-2-bromo-2-chloroethyl)-2'-deoxyuridine (8)

A solution of **1b** (55 mg, 0. 165 mmol), *N*-chlorosuccinimide (30 mg, 0.224 mmol), and glacial acetic acid (10 μl) in methanol (5 ml) was stirred at 25°C for 45 h. The product was purified as described for preparation of **6**, to yield **8** (25 mg, 38%): mp 150–155°C dec; ^{1}H-NMR (CD_3OD) δ 3.40 (s, 3H, OMe), 4.53 and 4.55 (two d, J = 3.75 Hz, 1H total, C*H*CHBrCl), 6.12 and 6.14 (two d, J = 3.75 Hz, 1H total, CHC*H*BrCl); ^{13}C-NMR (CD_3OD) δ 58.95 and 59.04 (O*C*H$_3$), 61.14 and 61.31 (*C*HBrCl), 82.39 and 82.84 (*C*HCHBrCl). Anal. Calcd. for $C_{12}H_{16}BrClN_2O_6$: C, 36.06; H, 4.03; N, 6.74. Found: C, 35.83; H, 4.07; N, 7.01.

5-(1-Methoxy-2-chloro-2-iodoethyl)-2'-deoxyuridine (9)

A solution of **1a** (31.7 mg, 0.11 mmol) and ICl (20 mg, 0.123 mmol) in methanol (3 ml) was stirred at 50°C for 30 min, at which time TLC indicated the reaction was completed. Removal of the solvent in vacuo gave a residue which was purified by silica-gel column chromatography. Elution with chloroform/methanol (92:2, v/v) afforded **9** (45 mg, 90%): mp 135–140°C dec; ^{1}H-NMR (CD_3OD) δ 3.45 (s, 3H, OMe), 4.56 and 4.57 (two d, J = 3.75 Hz, 1H total, C*H*CHClI), 6.12 and 6.14 (two

d, J = 3.75 Hz, 1H total, CHC*H*ClI); ^{13}C-NMR (CD_3OD) δ 30.78 and 31.02 (*C*HClI), 59.03 and 59.09 (O*C*H_3), 83.26 and 83.89 (*C*HCHClI). Anal. Calcd. for $C_{12}H_{16}ClIN_2O_6$: C, 32.30; H, 3.61; N, 6.28. Found: C, 31.99; H, 3.95; N, 6.25.

5-(1-Methoxy-2,2-dibromoethyl)-2'-deoxyuridine (10)

A solution of Br2 (0.5 ml) in methanol (2 ml) was added dropwise to an ice cold solution of **1b** (70 mg, 0.21 mmol) in methanol (10 ml). The reaction was vigrously stirred until TLC indicated that no starting material remained. The solvent was removed in vacuo and the product was purified by silica-gel column chromatography using chloroform/methanol (90:10, v/v) as eluent to afford **10** (55 mg, 58%): mp 158–160°C dec; ^{1}H-NMR (CD_3OD) δ 3.45 (s, 3H, OMe), 4.51 and 4.53 (two d, J = 3.5 Hz, 1H total, C*H*CHBr_2), 6.02 and 6.05 (two d, J = 3.5 Hz, 1H total, CHC*H*Br_2); ^{13}C-NMR (CD_3OD) δ 47.47 and 47.80 (*C*HBr_2), 59.03 (O*C*H_3), 82.44 and 82.87 (*C*HCHBr_2). Anal. Calcd. for $C_{12}H_{16}Br_2N_2O_6$:C, 32.45; H, 3.63; N, 6.30. Found: C, 32.22; H, 3.54; N, 6.21.

5-(1-Methoxy-2-bromo-2-iodoethyl)-2'-deoxyuridine (11)

A solution of ICl (40.5 mg, 0.25 mmol) in methanol (0.5 ml) was added to a solution of **1b** (60.1 mg, 0.18 mmol) in methanol (3 ml) and the mixture was allowed to react at 50°C for 1 h, with stirring. Removal of the solvent in vacuo and elution of the product from a silica-gel column using chloroform/methanol (19:1, v/v) as eluent gave **11** (70 mg, 78%) as a colorless foam: mp 145–148°C; ^{1}H-NMR (Me_2SO-d_6) δ 3.34 and 3.36 (two s, 3H total, OMe), 4.46 and 4.48 (two d, J = 3.8 Hz, 1H total, C*H*CHBrI), 5.83 and 5.85 (two d, J = 3.8 Hz, 1H total, CHC*H*BrI); ^{13}C-NMR (Me_2SO-d_6) δ 15.53 and 15.78 (*C*HBrI), 57.92 and 57.94 (O*C*H_3), 84.69 and 85.03 (*C*HCHBrI). Anal. Calcd. for $C_{12}H_{16}BrIN_2O_6$: C, 29.34; H, 3.28; N, 5.70. Found: C, 29.72; H,3.42; N, 5.70.

5-(1-Methoxy-2,2-diiodoethyl)-2'-deoxyuridine (12)

A solution of ICl (97.2 mg, 0.6 mmol) in methanol (3 ml) was added to a solution of **1c** (100 mg, 0.29 mmol) in methanol (3 ml) and the mixture was stirred at 25°C for 1 h. Removal of the solvent in vacuo, and elution of the product from a silica-gel column using chloroform/methanol (97:3, v/v) as eluent, yielded 12 (100 mg, 63%) as a white solid: mp 153–156°C dec; ^{1}H-NMR (Me_2SO-d_6) 5 3.35 and 3.37 (two s, 3H total, OMe), 4.02 and 4.06 (two d, J = 3.9 Hz, 1H total, C*H*CHI_2), 4.28 (m, 1H, H-3'), 5.35 and 5.37 (two d, J = 3.9 Hz, 1H total, CHC*H*I_2); ^{13}C-NMR (Me_2SO-d_6) δ-16.85 and -17.48 (*C*HI_2), 57.51 (O*C*H_3), 84.25 and 84.79 (*C*HCHI_2). Anal. Calcd. for $C_{12}Hi_6I_2N_2O_6$:C, 26.78; H, 2.99; N, 5.20. Found: C, 27.25; H, 3.11; N, 4.94.

3.2. Antiviral and antiproliferative assay

Antiviral activities were determined by the US National Institutes of Health

Antiviral Testing Program, using the in vitro procedures described previously (Norbeck et al., 1990).

Antiproliferative studies were conducted on L1210 cells using previously reported in vitro methodology (Kumar et al., 1990).

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References

Bobek, M., Kavai, I. and De Clercq, E. (1987) Synthesis and biological activity of 5-(2,2-difluorovinyl)-2′-deoxyuridine. J. Med. Chem. 30, 1494–1497.

De Clercq, E. (1985) In: Harnden, M.R. (Ed), Approaches to Antiviral Agents, pp. 57–99. MacMillan, New York.

De Clercq, E., Descamps, J., DeSomer, P., Barr, P., Jones, A.S. and Walker, R.T (1979) (*E*)-5-(2-Bromovinyl)-2′-deoxyuridine: A potent and selective anti-herpes agent. Proc. Natl. Acad. Sci. USA 76, 2947–2951.

De Clercq, E., Descamps, J., Ogata, M. and Shigeta, S. (1982) In vitro susceptibility of Varicella zoster virus to *E*-5-(2-bromovinyl)-2′-deoxyuridine and related compounds. Antimicrob. Agents Chemother. 21, 33–38.

De Clercq, E. and Rosenwirth, B. (1985) Selective in vitro and in vivo activities of 5-(2-haloalkyl) pyrimidine nucleoside analogs, particularly 5-(2-chloroethyl)-2′-deoxyuridine, against herpes simplex virus. Antimicrob. Agents Chemother. 28, 246–251.

De Clercq, E. and Walker, R.T. (1984) Synthesis and antiviral properties of 5-vinyl pyrimidine nucleoside analogs. Pharmacol. Ther. 26, 1–44.

Griengl, H., Bodenteich, M., Hayden, W., Wanek, E., Streicher, W., Stutz, P., Bachmayer, H., Ghazzouli, I. and Rosenwirth, B. (1985) 5-(Haloalkyl)-2′-deoxyuridines: a novel type of potential antiviral nucleoside analogue. J. Med. Chem. 28, 1679–1684.

Griengl, H., Hayden, W. and Wanek, E. (1985) 5-Haloethyl-deoxyuridine analogues: synthesis and antiviral activity. Nucleosides and Nucleotides. 4, 241.

Griengl, H., Wanek, E., Schwarz, W., Streicher, W., Rosenwirth, B. and De Clercq, E. (1987) 2′-Fluorinated arabinonucleosides of 5-(2-haloalkyl) uracil: synthesis and antiviral activity. J. Med. Chem. 30, 1199–1204.

Jones, A.S., Verhelst, G. and Walker, R.T. (1979) The synthesis of the potent anti-herpes virus agent, *E*-5-(2-bromovinyl)-2′-deoxyuridine and related compounds. Tetrahedron Lett. 45, 4415–4418.

Kumar, R., Wiebe, L.I., Hall, T.W., Knaus, E.E., Tovell, D.R., Tyrrell, D.L., Allen, T.M. and Fathi-Afshar, R. (1989) Synthesis of 5-[1-hydroxy (or methoxy)-2-bromo (or chloro)ethyl]-2′-deoxyuridines and related halohydrin analogues with antiviral and cytotoxic activity. J. Med. Chem. 32, 941–944.

Kumar, R., Xu, L., Knaus, E.E., Wiebe, L.I., Tovell, D.R., Tyrrell, D.L. and Allen, T.M. (1990) Synthesis and antiviral and cytotoxic activity of iodohydrin and iodomethoxy derivatives of 5-vinyl-2′-deoxyuridines, 2′-fluoro-2′-deoxyuridine and uridine. J. Med. Chem. 33, 717–722.

Norbeck, D.W., Kern, E., Hayashi, S., Rosenbrook, W., Sham, H., Herrin, T., Plattner, J.J., Erickson, J., Clement, J., Swanson, R., Shipkowitz, N., Hardy, D., Marsh, K., Arnett, U., Shannon, W., Broder, S. and Mitsuya, H. (1990) Cyclobut-A and cyclobut-G: broad-spectrum antiviral agents with potential

utility for the therapy of AIDS. J. Med. Chem. 33, 1281–1285.

Perlman, M.E., Watanabe, K.A., Schinazi, R.F. and Fox, J.J. Nucleoside 133 (1985) Synthesis of 5-alkenyl-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl) cytosines and related pyrimidine nucleosides as potential antiviral agents. J. Med. Chem. 28, 741–748.

Reefschläger, J., Barwolff, D. and Langen, P. (1984) Differential antiherpes activity of the (*E*)- and (*Z*)-isomers of 5-(2-fluorovinyl)-2′-deoxyuridine (FUVdR). Acta Virol. 28, 282–286.

Rosenwirth, B., Griengl, H., Wanek, E. and De Clercq, E. (1985) 5-(2-Chloroethyl)-2′-deoxyuridine: a potent and selective inhibitor of herpes viruses in vitro and in vivo. Antiviral Res. Suppl. 1, 21–28.

Tandon, M., Singh, S., Wiebe, L.I., Knaus, E.E., Gati, W.P. and Tempest, M.L. (1991) Synthesis of the diastereomers of 5-(2,2-dichlorocyclopropyl)- and 5-(2-chlorocyclopropyl)-2′-deoxyuridine and the antiviral and cytotoxic activity of these and bromo analogues, Drug Design Del. 7, 295–307.