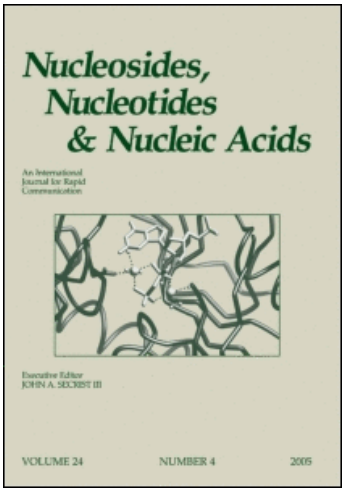

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Synthesis and Antiviral Activity of 1-β-D-Arabinofuranosyluracils and Uridines Containing 5-[2-Bromo-2-chloro (or Bromo)-1-Hydroxy (or Methoxy) Ethyl] Substituents

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SYNTHESIS AND ANTIVIRAL ACTIVITY OF 1-β-D-ARABINOFURANOSYLURACILS AND URIDINES CONTAINING 5-[2-BROMO-2-CHLORO (OR BROMO)-1-HYDROXY (OR METHOXY) ETHYL] SUBSTITUENTS

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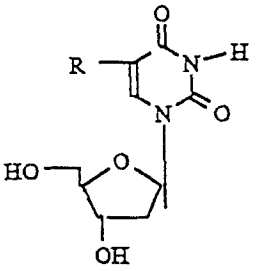
ABSTRACT: 1-β-D-Arabinofuranosyl-5-(-2,2-dibromo-1-hydroxyethyl) uracil (4) and 1-β-D-arabinofuranosyl-5-(2-bromo-2-chloro-1-hydroxyethyl) uracil (5) were synthesized by the regiospecific addition of HOX (X = Br or Cl) to the vinyl substituent of *(E)*-5-(2-bromovinyl)arabinouridine (2). A related reaction of 2 and its uridine analog (3) with bromine in methanol afforded the 5-(2,2-dibromo-1-methoxyethyl) derivatives (6 and 7). Of the newly synthesized compounds, 4 and 5 showed activity in vitro against HSV-1. The most active compound (4, $ED_{50} = 7.5$ μg/mL) was 25 times less active than acyclovir ($ED_{50} = 0.3$ μg/mL).

INTRODUCTION

(E)-5-(2-Bromovinyl)- (BVDU,1a), *(E)*-5-(2-chlorovinyl)- (1b) and *(E)*-5-(2-iodovinyl)-2′-deoxyuridine (IVDU 1c) are the most potent and selective antiherpetic agents from the large family of investigated 5-substituted pyrimidine nucleoside analogs.[1] However, these analogs are subject to rapid catabolism by pyrimidine nucleoside phosphorylases and possibly other enzymes.[2-4] Stabilization of these 2′-deoxyuridine nucleosides against phosphorylysis in vivo has been achieved by incorporation of a hydroxy group in the arabino

onfiguration[5] at C-2′ of the furanosyl ring without having any detrimental effect on their antiviral activities.[6]



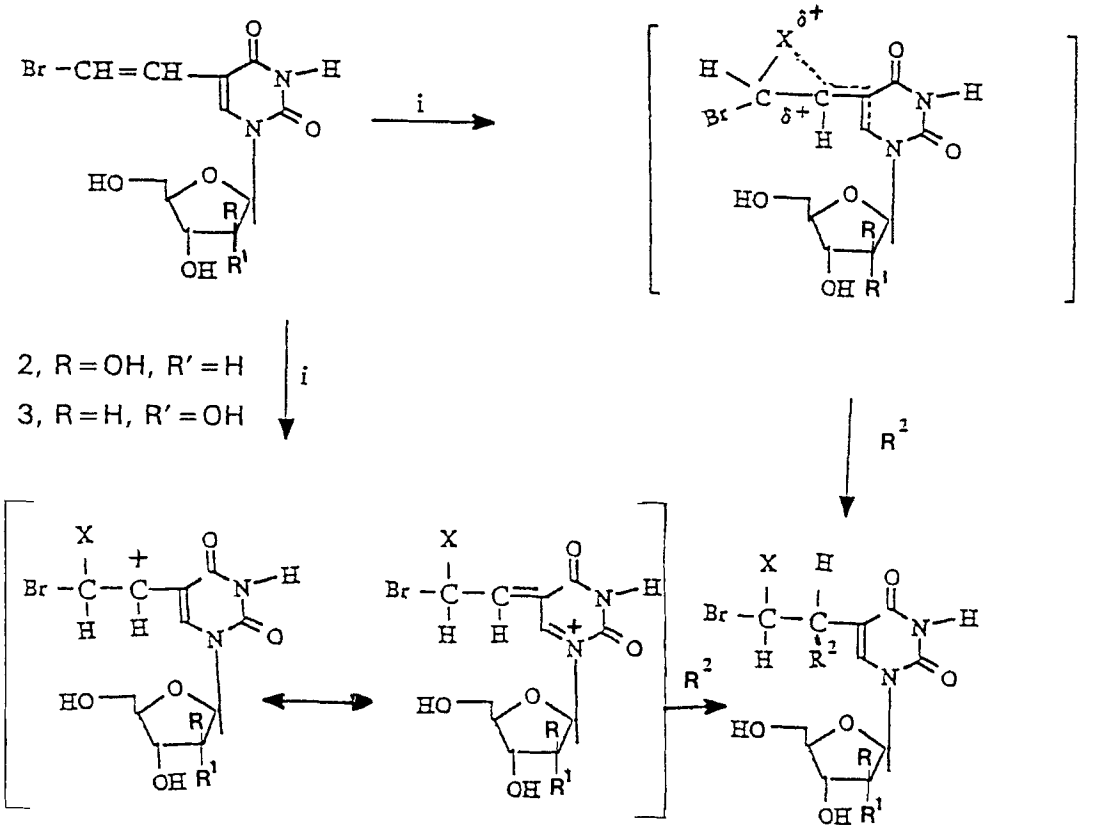
1a; R = (E)CH = CHBr
1b; R = (E)CH = CHCl
1c; R = (E)CH = CHI
1d; R = CH(OH)$CHBr_2$

Recently we reported the synthesis and antiviral activity of 5-(2,2-dibromo-1-hydroxyethyl)-2′-deoxyuridine (1d).[7] This nucleoside showed appreciable activity against HSV-1 in Vero cells (ED_{50} = 5 μg/mL) and in human foreskin fibroblast cells (ED_{50} = 1.7 μg/mL) as compared to (*E*)-5-(2-iodovinyl)-2′-deoxyuridine; (ED_{50} = 0.5 μg/mL and 0.06 μg/mL, respectively) and acyclovir (ED_{50} = 0.2 μg/mL and 0.03 μg/mL, respectively) in vitro. In addition, the cytotoxicity of 1d against uninfected Vero or human foreskin fibroblast (HFF) cells (ID_{50} = >100 μg/mL) was found to be minimal.

Now we report the synthesis and anti-HSV-1 activity of four related nucleosides, 1-β-D-arabinofuranosyl-5-[2-bromo-2-chloro(or bromo)-1-hydroxy (or methoxy)ethyl] uracils (4-6) and 5-(2,2-dibromo-1-methoxyethyl)uridine (7).

CHEMISTRY

The target compounds 5-(2,2-dibromo-1-hydroxyethyl) (4) and 5-(2-bromo-2-chloro-1-hydroxyethyl)-2′-deoxyuridine (5) were synthesized by the reaction of *(E)*-5-(2-bromovinyl)arabinouridine (2) with N-bromosuccinimide or N-chlorosuccinimide in aqueous dioxane, in 31% and 56% yields, respectively. The ^{13}C NMR spectrum of 4 supported a regiospecific addition of HOBr to the 5-vinyl substituent of 2, which showed methine resonances at δ 51.42 and 51.47 ($\underline{C}HBr_2$)



4; R=OH, R'=H, R^2=OH, X=Br

5; R=OH, R'=H, R^2=OH, X=Cl

6; R=OH, R'=H, R^2=OCH_3, X=Br

7; R=H, R'=OH, R^2=OCH_3, X=Br

Reagents: i = N-Bromosuccinimide (4), N-Chlorosuccinimide, dioxane-water (3:7, v/v), glacial acetic acid, 25°C (5); Bromine, CH_3OH, 25°C (6 and 7).

SCHEME 1

and δ 73.32 and 73.62 (CHOH). Compound 4 is a mixture of two diastereomers which differ in configuration (R and S) at the 1-carbon atom of the 5-(2,2-dibromo-1-hydroxyethyl) substituent; this mixture could not be separated by thin layer or column chromatography. The observed regiospecific addition is consistent with the results of Dalton et al[8] in which unsymmetrical olefins capable of halonium ion formation, were found to favor an unsymmetrical bridged intermediate of the type illustrated in Scheme 1, even in solvents having a high dipole moment. The regiospecificity can also be explained by assuming a resonance stabilized carbocation intermediate (Scheme 1). Reaction of *(E)*-5-(2-bromovinyl)- derivatives of 1-(β-D-arabinofuranosyl)uracil (2) and uridine (3) with bromine in methanol afforded 5-(2,2-dibromo-1-methoxyethyl)- analogs 6 and 7 in 91% yields.

BIOLOGICAL RESULTS

The antiviral activity for the compounds 4-7 were determined as the concentration required to inhibit plaque formation by 50% (ED_{50}) in Vero cells infected with Herpes simplex virus type 1 (HSV-1, strain JLJ). 1-β-D-Arabinofuranosyl-5-(2,2-dibromo-1-hydroxyethyl)uracil (4) and 1-β-D-arabinofuranosyl-5-(2-bromo-2-chloro-1-hydroxyethyl)uracil (5) exhibited ED_{50} values of 7.5 and 45 μg/mL, respectively, relative to the reference drug acyclovir ($ED_{50} = 0.3$ μg/mL). In contrast, 5-(2,2-dibromo-1-methoxyethyl)- derivatives 6 and 7 were inactive. These test results indicate that the 5-(2,2-dibromo-1-methoxyethyl) substituent (compare 4 vs 6) is detrimental to anti-HSV-1 activity; anti-HSV activity would not be expected in the uridine nucleoside 7. No cytotoxicity toward uninfected Vero cells was observed at test compound concentrations of 100 μg/mL.

IN VITRO ANTIVIRAL ASSAY (HSV-1)

Monolayers of mycoplasma-free Vero cells in 60 mm plates were infected with HSV-1 (JLJ strain 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 4-7 and 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% (ED_{50}) was determined.

EXPERIMENTAL

Melting points were determined on a Buchi capillary apparatus and are uncorrected. Nuclear magnetic resonance spectra (^1H NMR, ^{13}C NMR) were recorded on a Bruker AM 300 spectrometer. Chemical shifts are given in PPM downfield from tetramethylsilane (^1H NMR) as internal standard. ^{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 determined in all instances. Thin layer chromatography was performed using Whatman MK6F silica gel microslides. Silica gel column chromatography was carried out using Merck 7734 silica gel (100-200 μ particle size). *(E)*-1-(β-D-arabinofuranosyl-5-(2-Bromovinyl)uracil 2)[6] and *(E)*-5-(2-bromovinyl)uridine (3)[9] were prepared using literature procedures.

1-β-D-Arabinofuranosyl-5-(2,2-dibromo-1-hydroxyethyl)uracil(4). N-Bromosuccinimide (13 mg, 0.073 mmol) was added to a solution of

2 (25 mg, 0.0716 mmol) in dioxane-water (3:7, v/v, 5 mL) and glacial acetic acid (0.05 mL) and the reaction was allowed to proceed for 2.5 h at 25°C. Removal of the soluent *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 4 as a foam (10 mg, 31%). M.p. 145-50°C (dec); ^{1}H NMR (CD_3OD), (mixture of two diastereomers in a ratio of 1:1) δ 3.82 (m, 2H, H-5'), 3.97 (m, 1H, H-4'), 4.06-4.18 (complex m, 2H, H-2' and H-3'), 4.90 (m, 1H, C<u>H</u>OH), 6.20 (m, 2H, H-1' and C<u>H</u>Br_2), 7.98 and 8.0 (2s, 1H total, H-6); ^{13}C NMR (CD_3OD) δ 51.42 and 51.47 (<u>C</u>HBr_2), 62.83 (C-5'), 73.32 and 73.62 (<u>C</u>HOHCHBr_2), 76.85 and 76.97 (C-3'), 77.97 and 78.02 (C-2'), 86.62 and 86.82 (C-4'), 87.78 and 88.04 (C-1'), 112.71 (C-5), 142.87 and 142.96 (C-6), 151.83 (C-2), 164.47 (C-4). Anal.Calcd for $C_{11}H_{14}Br_2N_2O_7.1H_2O$: C, 28.46; H, 3.47; N, 6.03. Found: C, 28.69; H, 3.0; N, 5.95.

1-β-D-Arabinofuranosyl-5-(2-bromo-2-chloro-1-hydroxyethyl)uracil (5).

N-Chlorosuccinimide (18 mg, 0.13 mmol) was added slowly with stirring to a solution of 2 (21 mg, 0.06 mmol) in dioxane-water (3:7, v/v; 2 mL) and glacial acetic acid (10 μL) during a period of 5 min. The reaction was allowed to proceed for 48 h at 25°C with stirring and the solvent was removed *in vacuo*. The product was purified by PTLC using chloroform-methanol (80:20, v/v) as development solvent to yield 5 as a syrup (13 mg, 56%). ^{1}H NMR (CD_3OD) (mixture of two diastereomers in a ratio of 1:1) δ 3.80 (m, 2H, H-5'), 3.94 (m, 1H, H-4'), 4.04-4.15 (complex m, 2H, H-2' and H-3'), 4.94 (m, 1H, C<u>H</u>OH), 6.12 (m, 2H total, H-1' and C<u>H</u>Br_2), 7.93 and 7.95 (2s, 1H total, H-6); ^{13}C NMR (CD_3OD) δ 62.79 (C-5'), 63.87 (<u>C</u>HBrCl), 73.74 and 73.37

(CHOH), 76.96 and 76.86 (C-3'), 77.99 and 77.91 (C-2'), 86.80 and 86.82 (C-4'), 88.0 and 87.78 (C-1'), 112.19 and 112.11 (C-5), 143.04 and 142.87 (C-6), 151.85 (C-2), 164.52 (C-4). Anal.Calcd for $C_{11}H_{14}BrClN_2O_7$: C, 34.26; H, 3.65; N, 7.26. Found: C, 34.17; H, 3.25; N, 6.70.

1-β-D-Arabinofuranosyl-5-(2,2-dibromo-1-methoxyethyl)uracil (6). A solution of bromine in methanol was added dropwise to a solution of 2 (25 mg, 0.0716 mmol) in methanol (5 mL) at 25°C until TLC showed that no starting material remained, then neutralized with methanolic sodium hydroxide and evaporated to dryness. The residue was purified by PTLC using chloroform-methanol (80:20, v/v) as development solvent. Extraction of the ultraviolet visible spot with chloroform-methanol (85:15, v/v) yielded 6 as a foam (30 mg, 91%); M.P. 152-56°C (dec); ^{1}H NMR (CD_3OD) (mixture of two diastereomers in a ratio 1:1), δ 3.50 (2s, 3H total, OCH_3), 3.86 (m, 2H, H-5'), 4.02 (m, 1H, H-4'), 4.17 (m, 2H, H-2' and H-3'), 4.57 (m, 1H, C<u>H</u>OCH_3), 6.22 (2d, J = 3 Hz of d, 1H, H-1'), 6.10 (m, 1H, C<u>H</u>Br_2), 7.94 and 8.0 (2s, 1H total, H-6); ^{13}C NMR (CD_3OD) δ 47.50 and 47.58 (<u>C</u>HBr_2), 58.76 and 58.94 (O<u>C</u>H_3), 62.59 and 62.70 (C-5'), 76.97 (C-3'), 77.75 and 77.96 (C-2'), 82.51 and 83.02 (<u>C</u>$HOCH_3CHBr_2$), 86.65 (C-4'), 87.75 and 87.88 (C-1'), 109.26 and 109.44 (C-5), 143.50 and 143.56 (C-6), 151.86 (C-2), 164.70 (C-4). Anal. Calcd for $C_{12}H_{16}Br_2N_2O_7 \cdot \frac{1}{2}H_2O$: C, 30.72; H, 3.65; N, 5.97. Found: C, 30.91; H, 3.67; N, 5.51.

5-(2,2-Dibromo-1-methoxyethyl)uridine (7). A solution of bromine in methanol was added dropwise to a solution of 3 (25 mg, 0.0716 mmol) in methanol (5 mL) at 25°C until a light yellow color persisted. The reaction was completed, and the product

was isolated and purified as described for 6, to yield 7 as a foam (30 mg, 91%). M.P. 175-80°C (dec.); ^{1}H NMR (CD_3OD) (mixture of two diastereomer in a ratio of 1:1), δ 3.45 (s, 3H, OCH_3), 3.76 and 3.90 (two m, 1H each, H-5'), 4.06 (m, 1H, H-4'), 4.18 (m, 2H, H-2' and H-3'), 4.53 (m, 1H, C<u>H</u>OCH_3), 5.94 (2d, J = 4 Hz of d, 1H, H-1'), 6.10 (m, 1H, C<u>H</u>Br_2), 8.22 and 8.25 (2s, 1H total, H-6); ^{13}C NMR (CD_3OD), δ 47.38 and 47.68 (<u>C</u>HBr_2), 59.01 (OCH_3), 62.24 (C-5'), 71.41 (C-2'), 76.28 (C-3'), 82.63 and 83.03 -[<u>C</u>$HO(CH_3)CHBr_2$], 86.39 and 86.47 (C-4'), 91.08 and 91.26 (C-1'), 111.21 (C-5), 141.99 (C-6), 152.03 (C-2). Anal.calcd for $C_{12}H_{18}Br_2N_2O_7 . ½H_2O$: C, 30.72; H, 3.65; N, 5.97. Found: C, 30.86; H, 3.83; N, 6.50.

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