Synthesis and Antiviral Activity of Novel Acyclic Nucleoside Analogues of 5-(1-Azido-2-haloethyl)uracils

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We present the discovery of a novel category of 5-substituted acyclic pyrimidine nucleosides as potent antiviral agents. A series of 1-[(2-hydroxyethoxy)methyl] (**5**−**7**), 1-[(2-hydroxy-1-(hydroxymethyl)ethoxy)methyl] (**8**−**10**), and 1-[4-hydroxy-3-(hydroxymethyl)-1-butyl] (**11**−**13**) derivatives of 5-(1-azido-2-haloethyl)uracil were synthesized and evaluated for their biological activity in cell culture. 1-[4-Hydroxy-3-(hydroxymethyl)-1-butyl]-5-(1-azido-2-chloroethyl)uracil (**12**) was the most effective antiviral agent in the in vitro assays against DHBV (EC_{50} = 0.31−1.55 μM) and HCMV (EC_{50} = 3.1 μM). None of the compounds investigated showed any detectable toxicity to several stationary and proliferating host cells.

Introduction

Modified nucleosides have acquired an important role as therapeutic agents in the treatment of patients with devastating infections with viruses such as human immunodeficiency virus (HIV), hepatitis B virus (HBV), and herpes viruses. A promising class of nucleoside analogues for antiviral chemotherapy belongs to a group in which the cyclic carbohydrate moiety is replaced with open-chain "acyclic" sugar moieties.[1] Among purine acyclic nucleosides, Acyclovir (ACV, **1a**), 9-[(2-hydroxyethoxy)methyl]guanine, is a clinically useful antiherpes agent against herpes viruses including HSV-1, HSV-2, and VZV. The triphosphate form of ACV was shown to be a modest inhibitor of HBV DNA polymerase.[2] Ganciclovir (GCV, **1b**), 9-[(2-hydroxy-1-(hydroxymethyl)-ethoxy)methyl]guanine, is structurally similar to ACV. GCV is clinically used to treat HCMV infections and is a potent inhibitor of HBV.[3] However, long-term clinical use of GCV has been limited because of its severe dose-related toxicity.[4] Penciclovir (PCV, **1c**), 9-[4-hydroxy-3-(hydroxymethyl)-1-butyl]guanine, is an acyclic nucleoside closely related to GCV. PCV is an effective antiviral agent against HSV-1, HSV-2, and VZV and has shown potent anti-HBV activity in vitro and in vivo.[5] In contrast to GCV, PCV and ACV have little activity against HCMV.[6]

The selectivity of ACV and GCV against herpes viruses HSV and HCMV has been partially attributed to selective phosphorylation in virus-infected cells, to a greater extent than in uninfected cells,[7,8] by virus-encoded kinases (thymidine kinase and UL97, respectively) followed by inhibition of virus DNA polymerases by their triphosphate analogues.[9,10] Recent studies on the mode of action of PCV explain the potent and selective antiherpes, as well as anti-HBV, activities of PCV. For the inhibition of herpes DNA polymerase, a high concentration of PCV-TP in infected cells is necessary. In contrast, very low intracellular concentrations of PCV-TP can selectively inhibit HBV DNA polymerase.[5,11] Thus, the basis of potent and selective anti-HBV activity of PCV is at the HBV DNA polymerase level, whereas for herpes viruses, the selectivity is exerted at the levels of phosphorylation as well as DNA polymerase. These studies suggest that introduction of acyclic moieties contribute significantly to the potent antiherpes and anti-HBV activities.

In contrast to the purine acyclic nucleosides, 5-substituted pyrimidine acyclic nucleoside analogues have not shown significant antiherpes activity[1,12] and potential against HBV has not been explored.[13,14] However, it is interesting to note that the triphosphate derivative of acyclic pyrimidine nucleoside, 5-propyl-1-[(2-hydroxyethoxy)methyl]uracil (**1e**), inhibited HSV-1 DNA polymerase, whereas it was itself inactive against HSV-1 in cell culture, suggesting that either lack of phosphorylation or the very low level of phosphorylation may be partially responsible for the lack of antiherpes activity.[15] However, cidofovir (HPMPC, **1f**), which does not require activation to the monophosphate by the virus-induced kinase, is effective against HCMV.[16]

As a part of our investigation of new antiviral agents, we have designed, synthesized, and evaluated a large number of 5-substituted pyrimidine nucleoside analogues.[17−20] Of these analogues, we reported that the 5-(1-azido-2-haloethyl) (**1g**) derivatives of 2′-deoxyuridine exhibited the most potent in vitro antiviral activity against HSV-1 (EC_{50} = 0.06−6.4 μM), HSV-2 (EC_{50} = 2.9−13.2 μM), and VZV (EC_{50} = 4.5−12.3 μM), but they were inactive against HCMV. In contrast, the 5-(1-azido-2-haloethyl) derivatives of arabinouridine (**1h**) showed marked inhibition of HSV-1 (EC_{50} = 0.46−1.8 μM), VZV (EC_{50} = 0.08−0.23 μM), and HCMV (EC_{50} = 1.45−10.4 μM) but were inactive against HSV-2.[21] Recently, we reported that the 5-(1-azido-2-haloethyl) (**1g**, X = Br) derivative of 2′-deoxyuridine also exhibited significant in vitro anti-DHBV activity (EC_{50} = 2.6−6.6 μM).[22] Tao et al.[23] reported that among the 5-alkyl-substituted 2′-deoxyuridines, the 5-(2-chloroethyl) (**1i**, CEDU) derivative, in its triphosphate form, was the most efficient inhibitor of DHBV DNA polymerase. Compounds **1a**−**1i** are shown in Chart 1.

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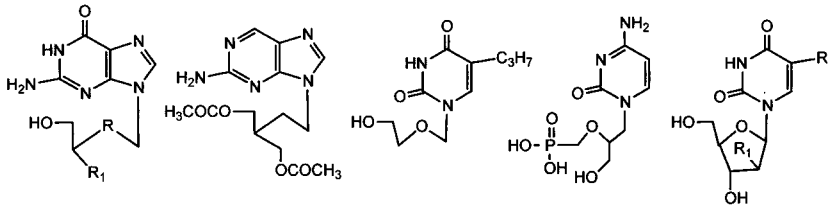
Chart 1

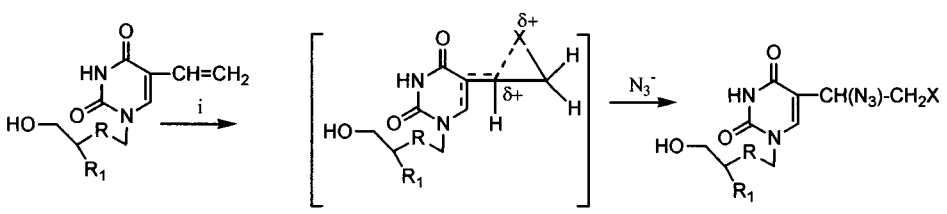


1a, R = O, R_1 = H
1b, R = O, R_1 = CH_2OH
1c, R = C, R_1 = CH_2OH

1d **1e** **1f**

1g, R = $CH(N_3)CH_2X$, R_1 = H
1h, R = $CH(N_3)CH_2X$, R_1 = OH
1i, R = CH_2CH_2Cl, R_1 = H

Scheme 1[a]



2, R = O, R_1 = H
3, R = O, R_1 = CH_2OH
4, R = C, R_1 = CH_2OH

5-13

5, X = Br, R = O, R_1 = H
6, X = Cl, R = O, R_1 = H
7, X = I, R = O, R_1 = H
8, X = Br, R = O, R_1 = CH_2OH
9, X = Cl, R = O, R_1 = CH_2OH
10, X = I, R = O, R_1 = CH_2OH
11, X = Br, R = C, R_1 = CH_2OH
12, X = Cl, R = C, R_1 = CH_2OH
13, X = I, R = C, R_1 = CH_2OH

[a] Reagents: (i) *N*-bromosuccinimide, sodium azide, DME−H_2O, 15−30 min (**5**, **8**, **11**); *N*-chlorosuccinimide, sodium azide, DME, H_2O, 0 °C, 2−3 h (**6**, **9**, **12**); iodine monochloride, CH_3CN, 0 °C, 30 min (**7**, **10**, **13**).

The 2-haloethyl side chain at C-5 of compounds **1g**−**i** and the acyclic moieties in guanosine analogues (**1a**−**c**) contribute to potent and selective antiviral activities. It was therefore of interest to us to evaluate the effect of combining these structural features and to investigate the hitherto unknown acyclic pyrimidine nucleosides containing novel 5-(1-azido-2-haloethyl) substituents against a variety of DNA viruses.

We now report the synthesis and antiviral activities of this novel class of 5-(1-azido-2-haloethyl) derivatives (**5**−**13**) with various modified acyclic glycosyl moieties. The 5-substituted acyclic pyrimidine nucleosides are described here for the first time to display prominent anti-HBV activity.

Chemistry

The target 1-[(2-hydroxyethoxy)methyl]-5-(1-azido-2-haloethyl)uracils (**5**−**7**, 34−35%), 1-[(2-hydroxy-1-(hydroxymethyl)ethoxy)methyl]-5-(1-azido-2-haloethyl)uracils (**8**−**10**, 30−57%), and 1-[4-hydroxy-3-(hydroxymethyl)-1-butyl]-5-(1-azido-2-haloethyl)uracils (**11**−**13**, 21−69%) were prepared by the reaction of respective 5-vinyl analogues (**2**−**4**) with *N*-bromosuccinimide, *N*-chlorosuccinimide, or iodine monochloride and sodium azide (Scheme 1).

The ^{13}C NMR (*J* modulation) spectrum provided conclusive evidence for the regiospecific addition of XN_3 (X = Br, Cl, I) across the 5-vinyl substituent of **2**−**4**. For example, the bromine atom in **5** is attached to a methylene carbon that exhibited resonance at δ 34.0, whereas the azido substituent is attached to a chiral methine carbon that exhibited resonance at δ 60.9. 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 1, even in solvents having a high dipole moment.[24]

Results and Discussion

The antiviral activities for this new class of acyclic nucleoside analogues (**5**−**13**) were determined in culture against herpes simplex virus type 1 (HSV-1, strains KOS and E-377), herpes simplex virus type 2 (HSV-2, strain MS), varicella zoster virus (VZV, strain Ellen), and human cytomegalovirus (HCMV, strain AD-169) infected human foreskin fibroblast (HFF) or Vero cells. In addition, the compounds were also evaluated against thymidine kinase-deficient (TK^-) mutant HSV-1 strain (KOSSB) in Vero cells. Acyclovir or ganciclovir were used in all these assays as reference drugs, and the results are summarized in Table 1. None of the compounds were markedly inhibitory against TK^+ HSV-1 strains (KOS, E-377), TK^- HSV-1 strain (KOSSB), and HSV-2, which is not surprising in view of the inactivity of various other acyclic pyrimidine nucleosides.[1,12,15] In contrast, compounds **5**−**13** exhibited antiviral activity against VZV (EC_{50} = 2.4−52 μM), compared to the reference drug acyclovir (EC_{50} = 2.6 μM) (Table 1). Compound **10** showed the most potent activity against VZV (EC_{50} = 2.4 μM), approaching that of acyclovir. It is interesting that the acyclic derivatives of 5-(1-azido-2-haloethyl)uracil (**5**−**13**) also proved to be inhibitory to HCMV. The derivatives containing the 1-[4-hydroxy-3-(hydroxymethyl)-1-butyl] substituent (**11**, **12**), with the exception of compound **13**, were surprisingly more active against HCMV (EC_{50} = 2.7 and 3.1 μM, respectively) than the derivatives containing the 1-[(2-hydroxy-1-(hydroxymethyl)ethoxy)methyl] moiety (**8**−**10**). The anti-HCMV activity of 1-[4-hydroxy-3-(hydroxymethyl)-1-butyl]-5-(1-azido-2-bromoethyl)uracil (**11**) (EC_{50} = 2.7 μM) and 1-[4-hydroxy-3-(hydroxymethyl)-1-butyl]-

5-(1-azido-2-chloroethyl)uracil (**12**) (EC_{50} = 3.1 μM) is 12- and 13.5-fold less than that of the 1-[(2-hydroxy-1-(hydroxymethyl)ethoxy)methyl] analogue of guanosine (GCV, EC_{50} = 0.23 μM), a drug that is licensed for the treatment of HCMV infections. However, both of these compounds were less toxic (IC_{50} > 270 μM and IC_{50} > 310 μM) to proliferating HFF cells than ganciclovir (IC_{50} = 156 μM). It is interesting that the anti-HCMV activity of compounds **11**−**13** (EC_{50} = 2.7−14.4 μM) was significantly improved compared to their 2′-deoxyribosyl analogues (**1g**), which were inactive, but equivalent to their 2′-arabinosyl derivatives (**1h**) (EC_{50} = 1.45−10.4 μM).[19,21] The observation that compounds **5**−**13** exhibited appreciable anti-VZV and anti-HCMV activity and no toxicity to several host cells (Table 2) suggests that they may be selectively phosphorylated in virus-infected cells by VZV-TK or UL-97 and may inhibit viral DNA polymerase. Alternatively, compounds **5**−**13** may be phosphorylated by cellular kinases in both virus-infected and uninfected cells but may selectively inhibit viral DNA polymerase.

The anti-DHBV activity for compounds **5**−**13** was assessed in confluent cultures of primary duck hepatocytes obtained from chronically infected Pekin ducks. In the new class of acyclic pyrimidine nucleosides, 5-(1-azido-2-haloethyl) analogues with 1-[(2-hydroxy-1-(hydroxymethyl)ethoxy)methyl] (**8**−**10**) and 1-[4-hydroxy-3-(hydroxymethyl)-1-butyl] (**11**, **12**) substituents at N-1 were found to exhibit in vitro activity against DHBV. In contrast, the compounds containing a 1-[(2-hydroxyethoxy)methyl] moiety (**5**−**7**) exhibited little (<50% inhibition) or no anti-DHBV activity at the concentration of 10 μg/mL, suggesting that anti-DHBV activity depends on the N-1 alkyl side chain in these derivatives, which is consistent with the corresponding acyclic purine nucleosides. Compounds **8**−**10**, **11**, and **12** possess anti-DHBV activity similar to or higher than the activities of their 5-(1-azido-2-haloethyl)-2′-deoxyribose analogues (**1g**, EC_{50} = 2.6−15 μM), whereas lower homologues **5**−**7** were significantly less inhibitory than the **1g** series.[22] This suggests that a hydroxyl group, at a position mimicking the 3′-OH group of the 2′-deoxyribose ring of nucleosides, is necessary for efficient activity of these compounds. In addition, the 1-[4-hydroxy-3-(hydroxymethyl)-1-butyl] moiety at N-1 appears to be an important determinant for potent anti-DHBV activity because compound **12** (EC_{50} = 0.31−1.55 μM) exhibited superior anti-DHBV activity than the corresponding 5-(1-azido-2-chloroethyl) derivative **9** (EC_{50} = 3.1−15.5 μM) and **1g** (X = Cl, EC_{50} = 15 μM).[22] It is noteworthy that anti-DHBV activity exhibited by compound **12** was 4−8 times higher than the most potent compound of the 5-(1-azido-2-haloethyl)-2′-deoxyuridine (**1g**, X = Br, EC_{50} = 2.6−6.6 μM) series.[22]

Among the compounds **8**−**10**, anti-DHBV activity exhibited by **9** (EC_{50} = 3.1−15.5 μM) and **10** (EC_{50} = 2.4−12 μM) is comparable to that of the corresponding 9-[(2-hydroxy-1-(hydroxymethyl)ethoxy)methyl] analogue of guanosine (GCV, EC_{50} = 4.0 μM), as measured in the DHBV-infected primary duck hepatocyte cultures.[25] Similarly, among compounds **11** and **12**, compound **12** showed potent activity against DHBV in vitro (EC_{50} = 0.31−1.55 μM) equal to that of the 9-[4-hydroxy-3-(hydroxymethyl)-1-butyl] analogue of guanosine (PCV, EC_{50} = 0.7 μM).[25] These observations suggest that the natural guanine base moiety in acyclo nucleosides (**1a**−**c**) can be replaced by a novel, unnatural 5-substituted uracil base to design and develop a new generation of antiviral agents. The precise mechanism of action of these compounds remains unclear. However, anti-DHBV activity of compounds **8**−**12** could be attributed to their phosphorylation by cellular kinases followed by selective inhibition of DHBV DNA polymerase by their triphosphate derivatives, as suggested for other antiviral nucleosides.[13,14]

Compounds **5**−**13** were selected by the National Cancer Institute (NCI) to determine their cytotoxic activities against 60 human tumor cell lines using an in vitro assay.[26] None of the compounds showed significant activity or selectivity in these assays up to the highest concentration tested (100 μM) (data not shown), indicating that novel compounds **5**−**13** do not have cytotoxicity against various human cell lines or any anticancer activity.

Compounds **5**−**13** were tested in vitro for their toxicity against several other cell lines (Table 2). None of these compounds exhibited in vitro cytotoxicity against stationary-phase cells [Vero cells (CC_{50} > 260−620 μM), HFF cells (CC_{50} > 240−340 μM)] and proliferating cell lines [HFF (IC_{50} > 130−310 μM)] up to the highest concentration tested. In rapidly proliferating fresh human T lymphocyte cell culture, cellular DNA synthesis was also not affected by compounds **5**−**13** in concentrations up to 120−170 μM.

Summary

We present 5-substituted acyclic pyrimidine nucleosides, 1-[(2-hydroxy-1-(hydroxymethyl)ethoxy)methyl]-5-(1-azido-2-haloethyl)uracils (**8**−**10**) and 1-[4-hydroxy-3-(hydroxymethyl)-1-butyl]-5-(1-azido-2-haloethyl)-uracils (**11,12**), as a new class of potent and selective inhibitors of DHBV replication in cell culture with anti-DHBV activity comparable to those of other established nucleoside analogues. It is noteworthy that compounds **8**−**13** also exhibited potent activity against VZV and HCMV. The most potent compound 1-[4-hydroxy-3-(hydroxymethyl)-1-butyl]-5-(1-azido-2-chloroethyl)uracil (**12**) was 7.5-fold less potent than 3-TC against DHBV, 7-fold less active than acyclovir against VZV, and 13.5-fold less active than ganciclovir against HCMV and could serve as a useful lead compound. Additional in vitro and in vivo tests and studies on structure−activity relationships and the mechanism of action of the specific potent new agents are ongoing in our laboratories.

Experimental Section

^{1}H NMR and ^{13}C NMR spectra were determined for solutions in DMSO-d_6 or CD_3OD on a Bruker AM-300 spectrometer using Me_4Si as an internal standard (^{1}H 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. Microanalyses were within ±0.4% of the theoretical values for all the elements listed unless otherwise indicated.

1-[(2-Hydroxyethoxy)methyl]-5-(1-Azido-2-bromoethyl)uracil (5). *N*-Bromosuccinimide (NBS, 110 mg, 0.61 mmol) was added in aliquots to a precooled (−5 °C) suspension prepared by mixing a solution of **2** (130 mg, 0.61 mmol) in 1,2-

dimethoxyethane (10 mL) with a solution of sodium azide (160 mg, 2.46 mmol) in water (0.4 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 15 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 chloroform/methanol (97:3, v/v) as eluent to give **5** (71 mg, 35%) as a viscous oil.

1-[(2-Hydroxyethoxy)methyl]-5-(1-Azido-2-chloroethyl)uracil (6). *N*-Chlorosuccinimide (NCS, 88 mg, 0.65 mmol) was added slowly to a precooled (−5 °C) suspension prepared by mixing a solution of **2** (116 mg, 0.55 mmol) in 1,2-dimethoxyethane (20 mL) with a solution of sodium azide (143 mg, 2.2 mmol) in water (0.35 mL). The reaction was stirred for 3 h at 0 °C. Completion of the reaction, followed by the workup as described for the isolation of **5**, gave a residue, which was purified by silica gel column chromatography. Elution with chloroform/methanol (97:3, v/v) as eluent yielded **6** as a syrup (56 mg, 35%).

1-[(2-Hydroxyethoxy)methyl]-5-(1-azido-2-iodoethyl)-uracil (7). Iodine monochloride (92 mg, 0.56 mmol) was added slowly during a 5 min period to a suspension of sodium azide (129 mg, 2.0 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** (105 mg, 0.49 mmol) in dry acetonitrile (15 mL) was added, and the reaction mixture was stirred at 0 °C for 30 min. The resulting red-brown 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 (50 mL). The colorless ethyl acetate fraction (Na_2SO_4) was dried, the solvent was removed in vacuo, and the residue was passed through a silica gel column and eluted with chloroform/methanol (93:7, v/v). This yielded **7** (65 mg, 34%) as a viscous oil.

1-[(2-Hydroxy-1-(hydroxymethyl)ethoxy)methyl]-5-(1-azido-2-bromoethyl)uracil (8). Reaction of **3** with *N*-bromosuccinimide, using the procedure outlined for the preparation of **5**, and purification of the product by silica gel column chromatography using chloroform/methanol (92:8, v/v) as eluent provided **8** as a syrup (92 mg, 31.4%).

1-[(2-Hydroxy-1-(hydroxymethyl)ethoxy)methyl]-5-(1-azido-2-chloroethyl)uracil (9). Reaction of **3** with *N*-chlorosuccinimide, using the procedure outlined for the preparation of **6**, and purification of the product by silica gel column chromatography using dichloromethane/methanol (90:10, v/v) as eluent yielded **9** as syrup (75 mg, 57%).

1-[(2-Hydroxy-1-(hydroxymethyl)ethoxy)methyl]-5-(1-azido-2-iodoethyl)uracil (10). Reaction of iodine monochloride (72.0 mg, 0.44 mmol) with **3** (95 mg, 0.39 mmol), using the procedure described for the preparation of **7**, and purification of the product by silica gel column chromatography using chloroform/methanol (90:10, v/v) as eluent afforded **10** as a syrup (48 mg, 29.6%).

1-[4-Hydroxy-3-(hydroxymethyl)-1-butyl]-5-(1-azido-2-bromoethyl)uracil (11). *N*-Bromosuccinimide (46 mg, 0.26 mmol) was added in aliquots with stirring to a precooled (−5 °C) suspension, prepared by mixing a solution of **4** (63 mg, 0.262 mmol) in 1,2-dimethoxyethane (25 mL) with a solution of sodium azide (68 mg, 1.04 mmol) in water (0.19 mL). The initial yellow color produced upon addition of each NBS aliquot quickly disappeared. After all of the NBS had been added, the reaction mixture was allowed to proceed for 30 min at 0 °C with stirring. Removal of the solvent in vacuo gave a residue, which was purified by silica gel column chromatography using chloroform/methanol (90:10, v/v) as eluent to afford **11** (66 mg, 69%) as a viscous oil.

1-[4-Hydroxy-3-(hydroxymethyl)-1-butyl]-5-(1-azido-2-chloroethyl)uracil (12). *N*-Chlorosuccinimide (50 mg, 0.37 mmol) was added slowly with stirring to a precooled (−5 °C) suspension, prepared by mixing a solution of **4** (75 mg, 0.31 mmol) in 1,2-dimethoxyethane (25 mL) with a solution of sodium azide (81 mg, 1.24 mmol) in water (0.23 mL). The reaction mixture was allowed to proceed for 2 h at 0 °C with stirring, and the solvent was removed in vacuo. The resulting residue was purified by silica gel column chromatography using chloroform/methanol (90:10, v/v) as eluent to afford **12** (46 mg, 47%) as a viscous oil.

1-[4-Hydroxy-3-(hydroxymethyl)-1-butyl]-5-(1-azido-2-iodoethyl)uracil (13). Iodine monochloride (50 mg, 0.3 mmol) was added slowly over 5 min with stirring to a suspension of sodium azide (72 mg, 1.1 mmol) in dry acetonitrile (10 mL) at ice-bath temperature. This mixture was stirred for a further 5 min, and a solution of **4** (66 mg, 0.275 mmol) in dry acetonitrile (40 mL) was added. The reaction mixture was maintained at 0 °C for 30 min with stirring. The resulting red-brown mixture was poured onto ice-cold water (25 mL) and extracted with ethyl acetate (3 × 50 mL), and the ethyl acetate extract was washed with 5% aqueous sodium thiosulfate (30 mL). The colorless ethyl acetate fraction (Na_2SO_4) was dried, the solvent was removed in vacuo, and the residue eluted from a silica gel column using chloroform/methanol (90:10, v/v) yielded **13** (24 mg, 21%) as a viscous oil.

In vitro Antiviral Assays [HSV-1 (E-377), HSV-2, HCMV, VZV]. Cytopathic effect (CPE) inhibition and plaque reduction assays for HSV-1, HSV-2, HCMV, and VZV were performed under the NIH Antiviral Research Branch Testing Program using standard procedures described previously.[19]

In vitro Antiviral Assays [HSV-1 (KOS, TK$^+$), (KOSSB, TK$^-$)]. Antiviral activity against HSV-TK positive and negative strains of HSV-1 were performed according to a previously reported procedure.[22]

In vitro Antiviral Assay (DHBV). Primary hepatocyte cultures obtained from congenitally infected ducks were used to determine the anti-DHBV activity of test compounds, as reported previously.[22]

Cell Cytotoxicity Assay. Cytotoxicities of test compounds on human foreskin fibroblasts (HFF) and Vero cells were determined using neutral red uptake and MTT assays, respectively, as described earlier.[22]

Cell Proliferation Assay. The effect of text compounds on proliferation of HFF cells and fresh human T lymphocytes was determined according to reported procedures.[19,22]

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Supporting Information Available: In vitro antiviral activity (Table 1), toxicity (Table 2), ^{1}H NMR, ^{13}C NMR, and elemental analysis of compounds **5**−**13**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Chu, C. K.; Cutler, S. J. Chemistry and Antiviral Activities of Acyclonucleosides. *J. Heterocycl. Chem.* **1986**, *23*, 289−319.
(2) Minuk, G. Y.; German, G. B.; Bernstein, C.; Benarroch, A.; Gauthiar, T.; Sekla, L. A Pilot Study of Steroid Withdrawal Followed by Oral Acyclovir in the Treatment of Chronic Type B Hepatitis. *Clin. Invest. Med.* **1992**, *15*, 506−512.
(3) Luscombe, C.; Pedersen, J.; Uren, E.; Locarnini, S. Long-Term Ganciclovir Chemotherapy for Congenital Duck Hepatitis B Virus Infection *in vivo*. Effect on Intrahepatic-Viral DNA, RNA and Protein Expression. *Hepatology* **1996**, *24*, 766−773.
(4) Chachoua, A.; Dieterich, D.; Krasinski, K.; Greene, J.; Laubenstein, L.; Wernz, J.; Buhles, W.; Koretz, S. 9-(1,3-Dihydroxy-2-propoxymethyl)guanine (Ganciclovir) in the Treatment of Cytomegalovirus Gastrointestinal Disease with the Acquired Immunodeficiency Syndrome. *Ann. Intern. Med.* **1987**, *107*, 133−137.

(5) Korba, B. E.; Boyd, M. R. Penciclovir Is a Selective Inhibitor of Hepatitis B Virus Replication in Cultured Human Hepatoblastoma Cells. *Antimicrob. Agents Chemother.* **1996**, *40*, 1282−1284.
(6) Boyd, M. R.; Safrin, S.; Kern, E. R. Penciclovir: A Review of Its Spectrum of Activity, Selectivity, and Cross-Resistance Pattern. *Antiviral Chem. Chemother.* **1993**, *4*, Suppl.1, 3−11.
(7) Germershausen, J.; Bostedor, R.; Field, A. K.; Perry, H.; Liou, R.; Bull, H.; Tolman, R. L.; Carkas, J. D. A Comparison of the Antiviral Agents 2′-nor-2′-Deoxyguanosine and Acyclovir: Uptake and Phosphorylation in Tissue Culture and Kinetics of *in vitro* Inhibition of Viral and Cellular DNA Polymerase by their Respective Tri-phosphates. *Biochem. Biophys. Res. Commun.* **1983**, *116*, 360−367.
(8) Biron, K. K.; Stanat, S. C.; Sorrell, J. B.; Fyfe, J. A.; Keller, P. M.; Lambe, C. U.; Nelson, D. J. Metabolic Activation of the Nucleoside Analog 9-[(2-Hydroxy-1-(hydroxymethyl)ethoxy)-methyl]guanine in Human Diploid Fibroblasts Infected with Human Cytomegalovirus. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 2473−2477.
(9) Crumpacker, C. S.; Schnipper, L. E.; Zaia, J. A.; Levin, M. J. Growth Inhibition by Acycloguanosine of Herpes-Viruses Isolated from Human Infections. *Antimicrob. Agents Chemother.* **1979**, *15*, 642−645.
(10) Sullivan, V. C.; Talarico, C. L.; Stanat, S. C.; Davis, M.; Coen, D. M.; Biron, K. K. A Protein Kinase Homologue Controls Phosphorylation of Ganciclovir in Human Cytomegalovirus-Infected Cells. *Nature (London)* **1992**, *358*, 162−164.
(11) Shaw, T.; Mok, S. S.; Locarnini, S. A. Inhibition of Hepatitis B Virus DNA Polymerase by Enantiomers of Penciclovir Triphosphate and Metabolic Basis for Selective Inhibition of HBV Replication by Penciclovir. *Hepatology* **1996**, *24*, 996−1002.
(12) Beauchamp, L. M.; Serling, B. L.; Kelsey, J. E.; Biron, K. K.; Collins, P.; Selway, J.; Lin, J.-C.; Schaeffer, H. J. Effect of Acyclic Pyrimidines Related to 9-[(1,3-Dihydroxy-2-propoxy)methyl]-guanine on Herpes Viruses. *J. Med. Chem.* **1988**, *31*, 144−149.
(13) Hong, J. H.; Choi, Y.; Chun, B. K.; Lee, K.; Chu, C. K. Current Status of Anti-HBV Chemotherapy. *Arch. Pharm. Res.* **1998**, *21*, 89−105.
(14) Colacino, J. M.; Staschke, K. A. The Identification and Development of Antiviral Agents for the Treatment of Chronic Hepatitis B Virus Infections. In *Progress in Drug Research*; Jucker, E., Ed.; Birkhauser Verlag: Basel, Switzerland, 1998; Vol. 50, pp 263−310.
(15) Kelley, J. L.; Beauchamp, L. Antiviral Agents. *Annu. Rep. Med. Chem.* **1983**, *18*, 139−148.
(16) Hitchcock, J. M.; Jaffe, H. S.; Martin, J. C.; Stagg, R. J. Cidofovir, a New Agent with Potent Anti-herpesvirus Activity. *Antiviral Chem. Chemother.* **1996**, *7*, 115−127.
(17) Kumar, R.; Wiebe, L. I.; Hall, T. W.; Knaus, E. E.; Tovell, D. R.; Tyrrell, D. L.; Allen, T. M.; Fathi-Afshar, R. 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.* **1989**, *32*, 941−944.
(18) Kumar, R.; Xu, L. H.; Knaus, E. E.; Wiebe, L. I.; Tovell, D. R.; Tyrrell, D. L.; Allen, T. M. 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.* **1990**, *33*, 717−723.
(19) Kumar, R.; Wiebe, L. I.; Knaus, E. E. Synthesis and Antiviral Activity of Novel 5-(1-Azido-2-haloethyl) and 5-(1-Azido-, amino-, or methoxyethyl) analogs of 2′-deoxyuridine. *J. Med. Chem.* **1993**, *36*, 2470−2474.
(20) Kumar, R.; Knaus, E. E.; Wiebe, L. F.; Allen, T. M. Synthesis of 5-[1-Hydroxy(or methoxy)-2,2-dihaloethyl]-2′-deoxyuridines with Antiviral and Cytotoxic Activity. *Antiviral Res.* **1994**, *24*, 315−325.
(21) Kumar, R.; Wiebe, L. I.; Knaus, E. E. Synthesis of 5-(1-Azido-2-haloethyl)arabinouridines. *J. Heterocycl. Chem.* **1997**, *34*, 1369−1370.
(22) Kumar, R.; Rai, D.; Sharma, S. K.; Saffran, H. A.; Blush, R.; Tyrrell, D. L. J. Synthesis and Antiviral Activity of Novel 5-(1-Cyanamido-2-haloethyl) and 5-(1-Hydroxy(or methoxy)-2-azidoethyl) Analogs of Uracil Nucleosides. *J. Med. Chem.* **2001**, *44*, 3531−3538.
(23) Tao, P.-Z.; Lofgren, B.; Lake-Bakaar, D.; Johansson, N. G.; Datema, R.; Oberg, B. Inhibition of Human Hepatitis B Virus DNA Polymerase and Duck Hepatitis B Virus DNA Polymerase by Tri-phosphates of Thymidine Analogs and Pharmacokinetic Properties of the Corresponding Nucleosides. *J. Med. Virol.* **1988**, *26*, 353−362.
(24) Dalton, D. R.; Dutta, V. P.; Johns, D. C. Bromohydrin Formation in Dimethyl Sulfoxide. *J. Am. Chem. Soc.* **1968**, *90*, 5498−5501.
(25) Shaw, T.; Amor, P.; Civitico, G.; Boyd, M.; Locarnini, S. *In vitro* Antiviral Activity of Penciclovir, a Novel Purine Nucleoside, against Duck Hepatitis B Virus. *Antimicrob. Agents Chemother.* **1994**, *38*, 719−723.
(26) Boyd, M. R., De Vita, V. T., Hellman, S., Rosenberg. S. A., Eds. Cancer. In *Principles and practice of oncology update*; Lippincott: Philadelphia, PA, 1989; Vol. 3, pp 1−12.

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