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5-(Dimethoxymethyl)-2′-Deoxyuridine: A Novel Gem Diether Nucleoside with Anti-Orthopoxvirus Activity

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Abstract

To provide potential new leads for the treatment of orthopoxvirus infections, the 5-position of the pyrimidine nucleosides have been modified with a gem diether moiety to yield the following new nucleosides: 5-(dimethoxymethyl)-2′-deoxyuridine (**2b**), 5-(diethoxymethyl)-2′-deoxyuridine (**3b**), 5-formyl-2′-deoxyuridine ethylene acetal (**4b**), and 5-formyl-2′-deoxyuridine propylene acetal (**5b**). These were evaluated in human foreskin fibroblast cells challenged with the vaccinia virus or cowpox virus. Of the four gem diether nucleosides, only the dimethyl gem diether congener showed significant antiviral activity against both viruses. This antiviral activity did not appear to be related to the decomposition to the 5-formyl-2′-deoxyuridine, which was itself devoid of anti-orthopoxvirus activity in these assays. Moreover, at the pH of the in vitro assays, **2b** was very stable with a decomposition (to aldehyde) half-life of >15 d. The anti-orthopoxvirus activity of pyrimidine may be favored by the introduction of hydrophilic moieties to the 5-position side chain.

Introduction

The events of September 11, 2001 forced terrorism to the forefront of national consciousness.[1-12] Contamination of government, press, and mail facilities, numerous infections, and several deaths from anthrax vividly demonstrated the potential[13-23] of bioterrorism.[12,24-32] However, smallpox represents an even more serious bioterrorist threat than anthrax [10,33-45] to the civilian and military populations[46-50] because of its high fatality rates and transmissibility. The lethality of the disease and its ease of transmissibility place the variola virus at the top of the CDC's list of high-threat (category A) agents. One drug, cidofovir (Vistide), is licensed to treat cytomegalovirus (CMV) retinitis in HIV-infected

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Supporting Information Available: The HPLC purity of new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

patients; however, it is available through an investigational new drug (IND) protocol to treat smallpox vaccine reactions (http://www.bt.cdc.gov/agent/smallpox/vaccination/cidofovir.asp) if the vaccine immune globulin (VIG) fails. Cidofovir might be used to treat generalized vaccinia, eczema vaccinatum, or progressive vaccinia.[51-60] Cidofovir, when intravenously administered, produces nephrotoxicity; however, it remains the only drug available (IND) to treat vaccination complications or, on a compassionate basis, to treat smallpox itself. Progress has been made on the development of oral dosage forms of Cidofovir[9,61-63,82], but these are not yet available in the clinic. Even though other candidates such as ST-246 that targets the smallpox virus core protein cysteine proteinase,[64] are in development, to date, there is no drug approved by the FDA to treat smallpox (variola) itself. It is, therefore, the stated role of the U. S. government to have available two anti-smallpox drugs possessing different mechanisms of action and to have two more such drugs in the pipeline.[65]

Herein we report on the first of our inquiries using the nucleoside scaffold as a point of departure in the search for antiviral drugs targeting orthopoxviruses.

Results

Strategy and Chemistry

Our cornerstone for the exploration of 5-substituted pyrimidine nucleoside chemical space has been the known 5-formyl-2′-deoxyuridine (**1b**), which recruits the rich and extensive chemistry of the aldehyde carbonyl to this undertaking and permits the introduction of electronegative hydrophilic substituents to the pyrimidine side chain.

The preparation of the pyrimidine nucleoside 5-substituted gem diether side chains began with the known 3′,5′-di-*O*-acetyl-5-formyl-2′-deoxyuridine (**1a**).[66] 3′,5′-Di-*O*-acetyl-5-formyl-2′-deoxyuridine dimethylacetal **(2a**) and 3′,5′-di-*O*-acetyl-5-formyl-2′-deoxyuridine diethylacetal (**3a**) were prepared by refluxing a methanol or ethanol solution of **1a** in the presence of an acidic resin used as a catalyst (Scheme 1). Ammonia/CH_3OH deprotection afforded 5-formyl-2′-deoxyuridine dimethylacetal (**2b**) (Scheme 1) and 5-formyl- 2′-deoxyuridine diethylacetal (**3b**).

The two cyclic acetal acetates, 3′,5′-di-*O*-acetyl-5-formyl-2′-deoxyuridine ethylene acetal (**4a**) (Scheme 2) and 3′,5′-di-*O*-acetyl-5-formyl-2′-deoxyuridine propylene acetal, (**5a**) (Scheme 2) were obtained by refluxing a benzene solution of **1a** and either ethylene glycol or propylene glycol in the presence of an acidic catalyst. These two acetal products were deacetylated by treating them with NH_3/MeOH to give **4b** and **5b**.

Finally, 5-formyluracil dimethylacetal (**7**) was prepared from 5-formyluracil (**6**).

Biological Activities. Anti-Orthopoxvirus Activity of Novel 5-Substituted Pyrimidine Nucleosides

The antiviral activities of compounds (Table 1) were determined in human foreskin fibroblast cells, and the challenge orthopoxviruses were either the vaccinia virus (VV) or the cowpox virus (CV). An initial evaluation was performed using a viral cytopathogenic effect

as the endpoint. A second confirmatory assay involved plaque reduction. The concentration of the agent that inhibited viral CPE or plaque formation by 50% was defined as the EC_{50} value. The effect of the potential antiviral agent on uninfected host cell viability was ascertained by neutral red uptake as a measure of toxicity. The concentration of the applied agent that reduced neutral red uptake by 50% was defined as the CC_{50} value. Neutral red toxicity assays were performed with confluent monolayers. None of the compounds described here had any significant cytopathic effect on uninfected cells under these conditions. All of the CC_{50} values were in excess of 300 μM.

Of all the compounds bioassayed, by far, the most active one was the gem diether (**2b**), which displayed EC_{50} values of 8.4 and 9.0 μM against VV as determined by the cytopathogenic effect and plaque reduction methods, respectively. Compound **2b** also was highly active against CV showing EC_{50} values of 11.7 and 7.4μM as determined by CPE and plaque reduction, respectively. Its 3′,5′-diacetate was devoid of significant activity. The other compound with significant, albeit much reduced, antiviral activity was the 3′,5′-diacetate ester of diethylacetal (**3a**); however, this activity was not increased when free nucleoside **3b** was evaluated. The extremely modest antiviral activity of the diethylacetal (**3a**) coupled with the lack of activity shown by its free nucleoside may imply a different mode of action from that of the dimethylacetal or some difference by which the infected cells process **3a** and **b** relative to **2a** and **b**, or their activities may not be related at all.

For the remaining congeners, there was a dramatic drop-off of antiviral activity when the alcoholic moieties of the nucleoside aldehyde acetal were combined in the form of either ethylene or propylene glycols. These five- and six-membered dioxolanes possessed no anti-orthopoxvirus activity either as free nucleo-sides or as their corresponding diacetates. In addition, the aglycone, 5-formyluracil dimethylacetal (**7**), was completely devoid of antiviral activity.

Chemical Stability of 5-(Dimethoxymethyl)-2′-Deoxyuri-dine (2b) Under Aqueous Conditions

Proton NMR was used to follow the rate of hydrolysis of the gem diether (**2b**) at (1) pH 7.5 in a phosphate buffer and (2) pH 6.0. From Figure 1a, it is clear that **2b** had a half-life for hydrolysis of approximately 16 days in the buffer at pH 7.5. As expected, the half-life significantly diminished (to approximately 18 h) in water at pH 6.0 (Figure 1b). In both instances, no other transformation product other than 5-formyl-2′-deoxyuridine was noted.

Stability of 5-(Dimethoxymethyl)-2′-deoxyuridine (2b) to Esterase

Shown in Figure 2 are experiments conducted to determine the efficiency of the conversion of **2a** to free unesterified nucleoside **2b** in the presence of purified porcine liver esterase to simulate the various protease-esterases that would be present in the cell-virus assay. From Figure 2, it is apparent that under the conditions employed, tdiacetate **2a** was rapidly degraded. However, an immediate product was produced, most probably the 3′-monoacetate (although we have not pursued this identification), and this was de-esterified relatively slowly to provide free nucleoside **2b**. Under these conditions, the half-life for the loss of the

presumed 3′-monoacetate was 1.5 h, which was the same as the half-life for the formation of free nucleoside **2b**.

Discussion

Acetals have been employed as prodrug candidates;[67,68] therefore, the possibility exists that the acetals prepared in this study may owe their mode of action to the hydrolysis to 5-formyl-2′-deoxyuridine which, in contrast to the present study, has been reported to have some anti-vaccinia virus activity.[69] In the present situation, It seems reasonably certain that the antiviral potency of the gem diether **2b** cannot be due to its action as a prodrug of 5-formyl-2′-deoxyuridine (**1b**) because the latter is completely devoid of biological activity in these assay systems (Table 1) and because **2b** was stable at pH of 7.5, which is the pH employed for these assays. It might be argued that perhaps the pH within the regions of the virus-infected cells could provide a more acidic milieu. Indeed, in the case of the orthopoxviruses, cell-associated enveloped virions may enter cells through endocytosis followed by a low pH disruption of their outer members and subsequent fusion with endosomal membranes. Instead, in the absence of definitive mechanistic studies, it would be reasonable to argue that the gem diether or dimethylacetal (**2b**) may possess inherent antiviral activity by virtue of its particular structure. There was no activity associated with the aglycone (**7**); therefore, antiviral activity was not attributable to cleavage to the heterocyclic base. 5-Isopropyl-2′-deoxyuridine has been reported to possess anti-herpes virus activity,[70-72] and 5-cyclopropyl-4′-thio-2′-deoxyuridine possesses significant activity against both herpes simplex-1 and -2 as well VV.[73] The distantly related pyrimidine 5-dimethoxymethyl substituent of compound **2b** would be significantly less hydrophobic than 5-alkylpryimidine nucleo-sides of comparable chain length and configuration because it contains the electronegative hydrogen-bond-acceptor oxygen atoms capable of interacting with a more hydrophilic protein domain.

In contrast to results with the free nucleoside, dimethylacetal **2b** in the diacetate form (**2a**) does not provide antiviral activity (Table 1). The inactivity of the diacetate derivative becomes relevant in the context of potential prodrug formulations that may be envisioned in the future. Therefore, we sought to develop further information regarding this inactivity. We examined the conversion of **2a** to free unesterified nucleoside **2b** in the presence of purified porcine liver esterase to model the various protease-esterases present in the cell culture milieu. Although diester **2a** was rapidly transformed by esterase, free nucleoside **2b** was slow to form, most probably because of the intermediate formation of the esterase-resistant 3′-acetate. The facile cleavage of the primary alcoholic ester would be expected, as would the more difficult cleavage of the secondary alcohol ester. The hypothesis may be forwarded that the inactivity of diester **2a** may be related to the kinetics of this slower cleavage and the resultant failure to provide sufficient inhibitory concentrations of nucleoside **2b**. However, the long duration of the antiviral assays in the cell culture might have been expected to provide sufficient time for the esterase cleavage of even the secondary ester. Thus, the cellular milieu may not provide sufficient esterase activity to effect the required de-esterification.

Conclusions

The nucleoside acetal, 5-(dimethoxymethyl)-2′-deoxyuridine (**2b**) can be readily synthesized from 3′,5′-diacetate of 5-formyl-2′-deoxyuridine and displays potent in vitro antiviral activity against two representative orthopoxviruses, namely, VV and CV. Its activity against both viruses is comparable to the activity of cidofovir. This activity is strongly dependent on the nature of the pyrimidine 5-substituent. The replacement of the 5-dimethoxymethyl group by the diethoxymethyl group, the 1,3-dioxolan-2-yl moiety, or the 1,3-dioxan-2-yl substituent all resulted in the complete loss of anti-orthopoxvirus activity. Present evidence is consistent with the inherent activity of 5-(dimethoxymethyl)-2′-deoxyuridine itself as opposed to its action as an acetal prodrug form of 5-formyl-2′-deoxuridine. The potent anti-orthopoxvirus activity of compound **2b** is likely to be related to the pyrimidine 5-substituent oxygens, which enable polar and hydrogen bonding interactions, thereby permitting binding to more hydrophilic protein target domains than many previously described 5-substiuted pyrimidine nucleosides that bear hydro-phobic alkyl or alkenyl groups. At the least, the antiviral activity of 5-(dimethoxmethyl)-2′-deoxyuridine provides important clues to the design of anti-orthopoxvirus agents.

Experimental Section

Melting points were recorded with a Barnstead 1201D electro-thermal melting point apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian 400 MHz spectrometer. $CDCl_3$, CD_3OD, or DMF-d_7 is used as the solvent for different compounds. The chemical shifts of the deuterated solvent served as the internal standard. The mass spectra were performed on an HP1100 MSD spectrometer at the HT Laboratories, San Diego, CA. The HRMS (High-Resolution Mass Spectra) were performed on a JEOL HX 110A spectrometer at the Department of Chemistry, University of Arizona. Silica gel column chromatography was conducted with Sigma-Aldrich silica gel (70-230 mesh). 5-Formyl-2′-deoxyuridine (**1b**) was prepared essentially as described by protecting the carbonyl group in **1a** as the dimethylacetal (**2a**), which was then sequentially treated with NaOMe/MeOH and AcOH/H_2O to give **1b**.

Chemical Stability Studies

1. Compound **2b** (11.8 mg, 39.1 μmol) was dissolved in D_2O (1 mL). The pH of this final solution was 6.0. The solution was then monitored by ^{1}H NMR. 2. A second ^{1}H NMR study of 5-formyl-2′-deoxyuridine dimethylacetal (**2b**) was carried out in KD_2PO_4-D_2O buffer (pH) 7.5) at the same final concentration as that above (11.8 mg, 39.1 μmol in 1 mL buffer). The resulting solution was monitored by ^{1}H NMR.

Procedure for Porcine Liver Esterase Assay

Enzyme or control assay mixtures were prepared by adding 40 μL of a DMSO solution (1 mM) of the compound to be assayed to 1750 μL of H_2O plus 200 μL of KH_2PO_4 buffer (100 mM, pH 7.5). Reaction was initiated by the addition of 10 μL of enzyme, or for the control mix, 10 μL of H_2O. Enzyme reaction mixtures (or control) were incubated at 37 °C, and aliquots of 300 μL were removed at 0.08, 0.5, 1, 1.5, 2, and 2.5 h. These were flash-frozen in

a dry ice/acetone bath and stored at −80 °C until HPLC analysis at which time, individual time-point samples were rapidly thawed and injected into the HPLC together with a thymine solution (0.1 mM) as an internal standard. HPLC elution was with a stepwise elution of solvent A (50mM NH_4Oac, pH 7) into solvent B (1:1 CH_3CN/H_2O).

1. Preparation of 3′,5′-Di-*O*-acetyl-5-formyl-2′-deoxyuridine (1a)

To a solution of $K_2S_2O_8$ (16.6 g, 61.4 mmol) and $CuSO_4$' $5H_2O$ (3.0 g) in 110 mL H_2O was added a CH_3CN solution (100 mL) containing 3′,5′-di-*O*-acetyl-thymidine (10.0 g, 30.6 mmol) and 2,6-lutidine (12.2 mL). The mixture was stirred at 65 °C for 2 h. Upon completion, the mixture was concentrated to half of the initial volume, and the remaining solution was extracted with EtOAc. The organic layer was washed with H_2O. The aqueous layers were combined and back-extracted with $CHCl_3$. Then the organic layers were combined, dried over Na_2SO_4, and then concentrated. The residue was purified through silica gel column chromatography with a mixture of EtOAc and hexane (2:1, v/v) as eluant. The fractions were collected and concentrated. The solid product was crystallized from EtOAc to give **1a** as white crystals (3.68 g, 35.4%); mp 148-150 °C; ^{1}H NMR ($CDCl_3$): δ 2.12 (s, 3H, CH_3), 2.17 (s, 3H, CH_3), 2.23-2.26 (m, 1H, H2′-1), 2.58-2.64 (m, 1H, H2′-2), 4.31-4.42 (m, 3H, H4′, H5′), 5.25-5.27 (m, 1H, H3′), 6.30-6.34 (m, 1H, H1′), 8.45 (s, 1H, H6), 9.05 (br s, 1H, NH), 10.01 (s, 1H, CHO). ^{13} C NMR ($CDCl_3$) δ: 20.84, 21.04, 38.81, 63.79, 74.22, 83.25, 86.38, 111.81, 144.84, 149.67, 162.39, 170.60, 170.82, 186.16. FAB MS *m*/*e*: 341 (MH^+), 363 (MNa^+).

2. Preparation of 3′,5′-Di-*O*-acetyl-5-formyl-2′-deoxyuridine Dimethylacetal (2a)

In the presence of Amberlite IR-120 (100 mg), 3′,5′-di-*O*-acetyl-5-formyl-2′-deoxyuridine (**1a**, 340 mg, 1.0 mmol) in 20 mL of anhydrous methanol was refluxed with stirring for 2 h. The mixture was filtered to remove the solid acid, and the filtrate was concentrated. The residue was purified through silica gel column chromatography with a mixture of EtOAc and hexane (2:1, v/v) as eluant to give a colorless solid (**2a**, 327 mg, 84.7%). Compound **3a** was prepared in a similar manner from **1a** and anhydrous ethanol.

Compound 2a

Mp 120-122 °C; ^{1}H NMR ($CDCl_3$) δ: 2.04 (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 2.10-2.18 (m, 1H, H2′-1), 2.38-2.43 (m, 1H, H2′-2), 3.26 (s, 3H, CH_3), 3.33 (s, 3H, CH_3), 4.18-4.26 (m, 3H, H4′, H5′), 5.17-5.19 (m, 1H, H3′), 5.24 (s, 1H, CH), 6.27-6.30 (m, 1H, H1′), 7.66 (s, 1H, H6).^{13} C NMR ($CDCl_3$) δ: 20.71, 21.06, 37.91 54.16, 54.94, 64.29, 74.86, 82.57, 85.29, 98.45, 112.76, 137.61, 150.49, 162.32, 170.68. FAB MS *m*/*e*: 387 (MH^+), 409 (MNa^+). HRMS (ESI): calcd for $C_{16}H_{22}N_2NaO_9$, 409.1223 $(MNa)^+$; found, 409.1217.

Compound 3a

Mp 100-102 °C; ^{1}H NMR ($CDCl_3$) δ: 1.11-1.17 (m, 6H, $2CH_3$), 2.05 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 2.10-2.19 (m, 1H, H2′-1), 2.39-2.44 (m, 1H, H2′-2), 3.42-3.72 (m, 4H, $2 \times CH_2$), 4.19-4.28 (m, 3H, H4′, H5′), 5.17-5.19 (m, 1H, H3′), 5.33 (s, 1H, CH), 6.25-6.29 (m, 1H, H1′), 7.73 (s, 1H, H6), 8.27 (br s, 1H, NH).^{13} C NMR ($CDCl_3$) δ: 15.36, 15.41, 20.98, 21.12, 38.02, 63.39, 63.86, 64.34, 74.86, 82.68, 85.60, 96.71, 114.13, 137.46, 150.24,

161.96, 170.61, 170.66. FAB MS *m*/*e*: 415 (MH^+), 437 (MNa^+). HRMS (ESI): calcd for $C_{18}H_{26}N_2O_9$, 437.1536 $(MNa)^+$; found, 437.1518.

3. Preparation of 3′,5′-Di-*O*-acetyl-5-formyl-2′-deoxyuridine Ethylene Acetal (4a)

To a flask containing 3′,5′-di-*O*-acetyl-5-formyl-2′-deoxyuridine (**1a**, 340 mg, 1.0 mmol) and Amberlite IR-120 (100 mg) were added 0.3 mL ethylene glycol and 20 mL anhydrous benzene. The mixture was then refluxed with stirring for 1 h. Upon completion, the solid acid was removed by filtration. The filtrate was washed with water (20 mL × 2). The aqueous phase was extracted with chloroform (10 mL × 2), and the combined organic phases were then dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified through silica gel column chromatography with a mixture of EtOAc and Hexane (2:1, v/v) as eluant to give a colorless solid (**4a**, 334 mg, 86.9%). Compound **5a** was prepared in a similar manner from **1a** and propylene glycol.

Compound 4a

Mp 176-178 °C; ^{1}H NMR ($CDCl_3$) *δ*: 2.11-2.21 (m, 7H, $2CH_3$, H2′-1), 2.50-2.55 (m, 1H, H2′-2), 3.96-4.07 (m, 4H, $-CH_2CH_2-$), 4.28-4.36 (m, 3H, H4′, H5′), 5.24-5.26 (m, 1H, H3′), 5.75 (s, 1H, CH), 6.28-6.32 (m, 1H, H1′), 7.76 (s, 1H, H6), 8.33 (br s, 1H, NH). ^{13} C NMR ($CDCl_3$) *δ*: 20.88, 21.12, 38.27, 64.22, 65.32, 65.46, 74.75, 82.85, 85.75, 98.10, 112.05, 137.58, 149.88, 161.45, 170.55. FAB MS *m*/*e*: 385 (MH^+), 407 (MNa^+). HRMS (FAB): calcd for $C_{16}H_{20}N_2O_9$, 385.1247 $(MH)^+$; found, 385.1261.

5a

Mp 172-173 °C; ^{1}H NMR ($CDCl_3$) *δ*: 1.39-1.44 (m, 1H, CH_2-1), 2.05-2.23 (m, 8H, CH_2-2, 2xCH_3, H2′-1), 2.46-2.51 (m, 1H, H2′-2), 3.91-3.98 (m, 2H, CH_2), 4.13-4.19 (m, 2H, CH_2), 4.28-4.38 (m, 3H, H4′, H5′), 5.24-5.26 (m, 1H, H3′), 5.52 (s, 1H, CH), 6.31-6.35 (m, 1H, H1′), 7.80 (s, 1H, H6), 8.42 (br s, 1H, NH). ^{13} C NMR ($CDCl_3$) *δ*: 20.88, 21.11, 38.16, 64.36, 67.67, 74.96, 82.77, 85.61, 95.46, 113.36, 138.13, 149.88, 161.22, 170.63. FAB MS *m*/*e*: 399 (MH^+), 421 (MNa^+). HRMS (FAB): calcd for $C_{17}H_{22}N_2O_9$, 399.1404 $(MH)^+$; found, 399.1390.

4. Preparation of 5-Formyl-2′-deoxyuridine Dimethylacetal (2b)

3′,5′-Di-*O*-acetyl-5-formyl-2′-deoxyuridine dimethylacetal (**2a**, 193 mg, 0.5 mmol) was dissolved in 2 mL of anhydrous methanol. To this solution was added 2 mL of 7 N NH_3/MeOH solution. The mixture was stirred at 0 °C for 3 h and then at ambient temperature for 5 h. Upon completion, the solvent was removed under high vacuum. The residue was purified through column chromatography (chloroform/methanol, 8:1, v/v) to give the corresponding product (**2b**, 137 mg, 91%). Compounds **3b**, **4b**, and **5b** were prepared in a similar manner.

1-((2*R*,4*R*,5*R*)-Tetrahydro-4-hydroxy-5-(hydroxymethyl)furan-2-yl)-5-(dimethoxymethyl)pyrimidine-2,4(1*H*,3*H*)-dione (2b)

Mp 144-145 °C; ^{1}H NMR (CD_3OD) *δ*: 2.21-2.30 (m, 2H, H2′-1, 2), 3.34 (s, 6H, $2CH_3$), 3.69-3.79 (m, 2H, H5′), 3.92-3.94 (m, 1H, H4′), 4.37-4.40 (m, 1H, H3′), 5.26 (s, 1H, CH),

6.26-6.29 (m, 1H, H1′), 8.11 (s, 1H, H6).[13] C NMR (CD_3OD) δ: 40.41, 53.81, 54.14, 61.94, 71.17, 85.18, 88.15, 98.56, 111.02, 138.970, 150.75, 162.53, 170.7. FAB MS *m*/*e*: 303 (MH^+), 325 (MNa^+). HRMS (ESI): calcd for $C_{12}H_{18}N_2O_7$, $(MNa)^+$ 325.1012; found, 325.1006.

1-((2*R*,4*R*,5*R*)-Tetrahydro-4-hydroxy-5-(hydroxymethyl)furan-2-yl)-5-(diethoxymethyl)pyrimidine-2,4(1*H*,3*H*)-dione (3b)

Mp 112-114 °C, ^{1}H NMR ($CDCl_3$) δ: 1.21-1.24 (m, 6H, 2 × CH_3), 1.77 (br s, 1H, OH), 2.32-2.46 (m, 2H, H2′), 2.88 (br s, 1H, OH), 3.56-3.91 (m, 6H, 2 CH_2, H5′), 4.01-4.03 (m, 1H, H4′), 4.56-4.59 (m, 1H, H3′), 5.39 (s, 1H, CH), 6.13-6.17 (m, 1H, H1′), 7.84 (s, 1H, H6). ^{13} C NMR ($CDCl_3$) δ: 15.40, 40.32, 62.61, 63.57, 64.60, 71.81, 87.48, 87.85, 96.82, 113.28, 139.65, 150.42, 162.10. FAB MS *m*/*e*: 331 (MH^+), 353 (MNa^+). HRMS (ESI): calcd for $C_{14}H_{22}N_2NaO_7$, 353.1325 $(MNa)^+$; found, 353.1313.

5-(1,3-Dioxolan-2-yl)-1-((2*R*,4*R*,5*R*)-tetrahydro-4-hydroxy-5-(hydroxymethyl)-furan-2-yl)pyrimidine-2,4(1*H*,3*H*)-dione (4b)

Mp 192-193 °C; ^{1}H NMR (CD_3OD) δ: 2.19-2.33 (m, 2H, H2′), 3.70-3.81 (m, 2H, H5′), 3.91-3.96 (m, 3H, CH_2, H4′), 4.03-4.07 (m, 2H, CH_2), 4.38-4.41 (m, 1H, H3′), 5.74 (s, 1H, CH), 6.25-6.27 (m, 1H, H1′), 8.24 (s, 1H, H6). ^{13} C NMR (CD_3OD) δ: 40.43, 61.47, 64.92, 64.96, 70.93, 85.67, 87.86, 98.50, 111.31, 139.83, 150.79, 163.20. FAB MS *m*/*e*: 301 (MH^+), 323 (MNa^+). HRMS (ESI): calcd for $C_{12}H_{17}N_2O_7$, 301.1037 $(MH)^+$; found, 301.1038.

5-(1,3-Dioxan-2-yl)-1-((2*R*,4*R*,5*R*)-tetrahydro-4-hydroxy-5-(hydroxymethyl)furan-2-yl)pyrimidine-2,4(1*H*,3*H*)-dione (5b)

Mp 186-188 °C; ^{1}H NMR (CD_3OD) δ: 1.39-1.44 (m, 1H, CH_2-1), 2.04-2.32 (m, 3H, H2′, CH_2-2), 3.69-3.79 (m, 2H, H5′), 3.90-3.95 (m, 3H, H4′, CH_2), 4.11-4.15 (m, 2H, CH_2), 4.36-4.39 (m, 1H, H3′), 5.45 (s, 1H, CH), 6.23-6.27 (m, 1H, H1′), 8.14 (s, 1H, H6). ^{13} C NMR (CD_3OD) δ: 25.54, 40.18, 61.63, 67.32, 67.36, 70.96, 85.66, 87.72, 95.70, 112.28, 139.92, 150.64, 162.85. FAB MS *m*/*e*: 315 (MH^+), 337 (MNa^+). HRMS (FAB): calcd for $C_{13}H_{18}N_2O_7$, 315.1193 $(MH)^+$; found, 315.1189.

5. Preparation of 5-Formyluracil Dimethylacetal (7)

5-Formyluracil (**6**, 280 mg, 2.0 mmol) was added to a flask containing 20 mL of anhydrous methanol and Amberlite IR-120 (200 mg). The mixture was then heated to 80 °C with stirring for 1.5 h. Upon completion (monitored by TLC), the solid acid was removed through filtration. The filtrate was allowed to cool to room temperature, whereupon the product precipitated. The solid so formed was collected by filtration to give **7** (338 mg, 91%).

Compound 7

^{1}H NMR (DMF-d_6) δ: 3.47 (s, 6H, 2 × CH_3), 5.41 (s, 1H, CH), 7.64 (s, 1H, H6), 11.08 (br s, 1H, NH), 11.29 (br s, 1H, NH).[13] C NMR (DMF-d_7) δ: 53.35, 98.70, 110.08, 140.33, 151.71, 163.55. FAB MS *m*/*e*: 187 (MH^+), 209 (MNa^+).

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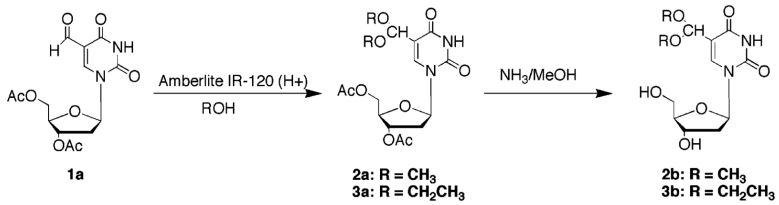
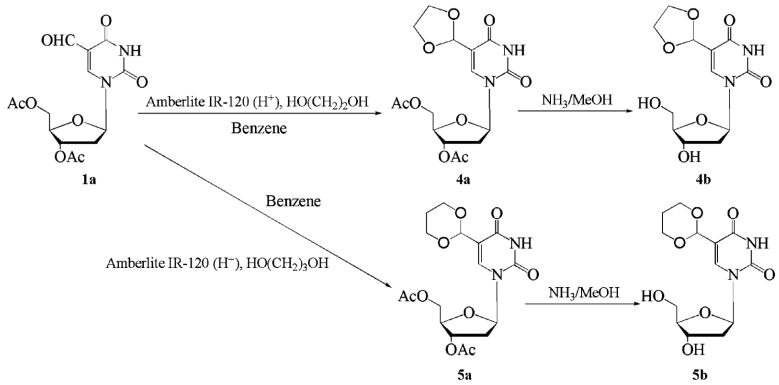


Scheme 1.



Scheme 2.

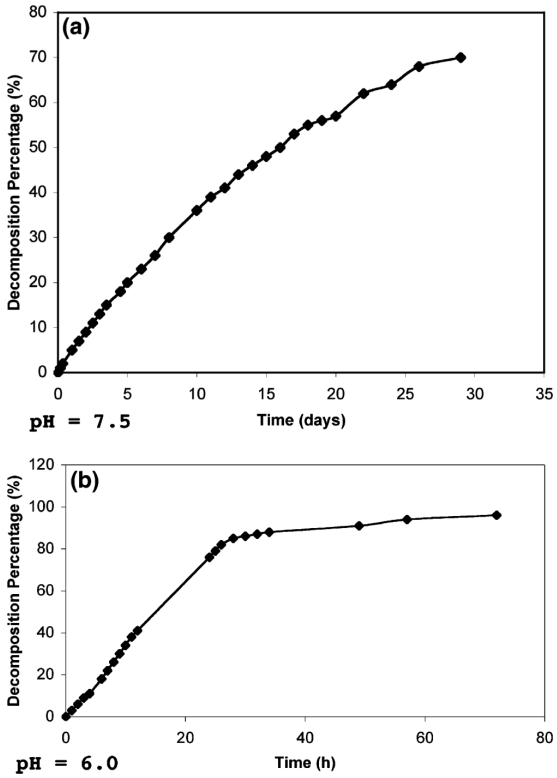


Figure 1.
Decomposition of Compound **2b** at pH 7.5 (a) and pH 6.0 (b).

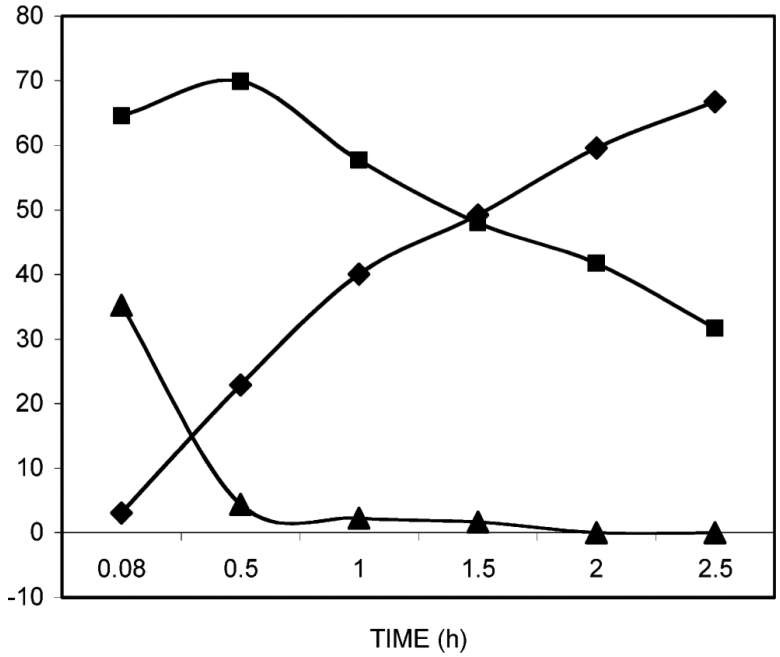


Figure 2.

Action of porcine liver esterase on 5-(dimethoxymethyl)-2′-deoxyuridine 3′,5′-diacetate (**2a**, ■) resulting in the production of the intermediate presumed to be 5-(dimethoxymethyl)-2′-deoxyuridine 3′-acetate (▲) and the ultimate fully deacetylated product 5-(dimethoxymethyl)-2′-deoxyuridine (**2b**, ◆).

Table 1

Anti-Orthopoxvirus Activities of Nucleosides[a]

compd	efficacy EC_{50}[b] (μM)				toxicity CC_{50}[c] (μM)
	VV[d] CPE	VV[d] PR	CV[d] CPE	CV[d] PR	neutral red uptake
Cidofovir	6.9–8.2	9–9.8	8.3–9	9–16	>300
1b	>300	NT	>300	NT	>300
1a	>300	NT	>300	NT	>300
2a	296	NT	>300	NT	>300
2b	8.4	9.0 ± 1.3[e]	11.7	7.4 ± 3.0[e]	±300 ± 0[e]
3a	38	79	52.4	25.8	>300
3b	>60	NT	>60	NT	>300
4a	>300	NT	>300	NT	>300
4b	>300	NT	>300	NT	>300
5a	>300	NT	>300	NT	>300
5b	>300	NT	>300	NT	>300
7	>300	NT	>300	NT	>300

[a] Assays were performed according to the procedures described previously for activity against the vaccinia virus (VV) and cowpox virus (CV) and for cytotoxicity (neutral red uptake assay) in human foreskin fibroblast (HFF) cells. Briefly, to determine efficacy, initial cytopathogenic effect (CPE) assays were performed in 96-well plates seeded with HFF cells. Varying concentrations of the drug were challenged with VV or CV at 1000 PFU/well (incubation at 37 °C for 7 days). Confirmatory assays involving plaque reduction (PR) were performed using HFF cells seeded in 6-well plates, 2 days prior to use and infected with either VV or CV by the addition of 20–30 PFU/well. Plates were incubated for 1 h, and various concentrations of the drug were then added to triplicate wells, and the plates were incubated at 37 °C for 3 days. Toxicity was evaluated using HFF cells seeded in 96-well plates incubated with various concentrations of the drug for 7 days at 37 °C. Neutral red toxicity assays were performed with confluent monolayers.

[b] EC_{50}: effective concentration to reduce viral cytopathogenicity or plaque formation by 50%; NT, not tested.

[c] CC_{50}: the concentration that causes a cytotoxic effect (as ascertained by neutral red uptake) on 50% of uninfected cells.

[d] Virus used for challenge: VV (Copenhagen) or CV (Brighton).

[e] Values are the mean (standard deviation of two or more assays. In instances where the means and standard deviations are provided, at least two or more assays have been done. If there is no such indication, then only one assay was performed.